

HEPATITIS-B-ASSOCIATED GLOMERULAR DISEASE

A clinicopathological study of Hepatitis B virus associated Membranous Glomerulonephritis in Namibian and South African children 1974 – 2005 and a comparison with Hepatitis B associated Membranous Glomerulonephritis as well as Idiopathic Membranous Glomerulonephritis in adults.



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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously submitted it in its entirety or in part at any university for a degree.

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SUMMARY

Background and Objective: The most common cause of severe proteinuria/nephrotic syndrome (NS) in children worldwide is minimal change disease (MCD). This is also the pattern observed in white and Indian children in South Africa (SA). By contrast, black and mixed race/coloured children of Southern Africa in the 1960s to 1990s were shown to have a different pattern of NS. One of the main differences was the frequency of hepatitis B virus (HBV) associated glomerulonephritis, usually membranous glomerulonephritis (MGN). The objective of this project was a clinicopathological study of this subgroup of nephrotic children to document the disease further and in particular to seek correlations between pathological and clinical features including prognosis. A central focus was to document the detailed ultrastructural examination of the renal biopsies of these children and to correlate the spectrum of pathological features with demographic, clinical, laboratory and prognostic features.

The hypothesis was that the clinicopathological features of HBV MGN in children differed substantially from idiopathic MGN in general (children and adults) and also from HBV MGN in adults and that HBV MGN in children should be viewed as a distinct disease.

Patients and methods: The childhood (12 years and younger) patient cohort was 309 children with severe proteinuria/nephrotic syndrome who presented at Tygerberg Hospital (TBH) over a 21 year period from 1974-1995, including 67 children from Namibia. The study group was 71 children with HBV MGN who were followed up to 2005. The comparative adult group was 45 adults with MGN of whom 12 had HBV

MGN and 33 idiopathic MGN. (A comparison could not be made with idiopathic MGN in childhood as this centre only had 2 such patients during the study period.) Demographic, clinical, laboratory and renal pathology data were collected, compared and correlated.

Results: HBV associated MGN was the most frequent cause of NS in the Namibian subgroup, 25/67 (37%) and the third most frequent, 71/309 (23%) in the childhood cohort as a whole. The MGN group was 86% (71/83) of the total HBV childhood nephrotic cohort, by far the dominant subgroup.

The average age of the 71 children with HBV MGN was 6.0 years (range 2-12 years) at presentation and boys comprised 80% of the group. Hepatitis B envelope antigen (HBeAg) was identified in the serum of 87% of children tested. Laboratory features different from idiopathic MGN included more prominent haematuria, mildly raised serum transaminases and more frequently lowered serum C₃ and C₄ levels. Light microscopic examination of renal biopsies showed mesangial proliferation in all patients but with minimal glomerular sclerosis and interstitial disease. On ultrastructural examination mesangial and subendothelial deposits were common and prominent as was mesangial interposition. The MGN of HBV in children therefore frequently showed mesangiocapillary glomerulonephritis (MCGN) features in addition to the subepithelial deposits of MGN. The subgroup of 23 whose renal biopsies displayed severe mesangial interposition in addition to the subepithelial deposits of MGN were termed the mixed HBV MGN-mesangiocapillary GN group. Virus like bodies and tubuloreticular inclusion bodies were both found in more than 80% of biopsies of childhood HBV MGN. HBeAg was identified in the subepithelial deposits in the glomeruli. This was the first time this feature was demonstrated in Africa. The 46 South African children with HBV MGN showed a cumulative remission rate of 25%

at 2 years and 52% at 4 years. Seven of the children (10%) of the total cohort developed chronic renal failure (CRF). Age of 6 years and above at presentation and severe mesangial deposits on biopsy correlated with fewer remissions and poorer outcome. In 3 patients the interval between the diagnosis of HBV MGN and the onset of CRF was more than 19 years with the longest being 23 years. The 358 cases of childhood HBV MGN from Southern Africa constitute 37% of the reported childhood patients.

Comparative data

A comparison was made between the 71 children with HBV MGN, 12 adults with HBV MGN and 33 adults with idiopathic MGN. The main differences were that both HBV MGN groups included only coloured and black patients and were more predominantly male while the idiopathic MGN group included all races. In the HBV patients, haematuria was more frequent and severe, liver enzymes were frequently raised and C₃ more frequently reduced than in the idiopathic cohort. Both groups of adult MGN patients had normal C₄ levels while the childhood HBV MGN group had reduced C₄ levels.

The immune complex pattern in both of the HBV MGN adult and childhood groups on biopsy was similar with more mesangial and subendothelial deposits as well as mesangial interposition than the idiopathic group. Despite this similarity between the two HBV groups, both adult groups showed more glomerular sclerosis and interstitial disease than the childhood group. The clinical outcome of the children's cohort was better than the other 2 groups with remission (52%) more frequent at 4 years ($p < 0.01$) and better renal and patient survival.

Including the 83 cases from this series, at least 1243 renal biopsy proven cases of HBV MGN have been reported in the English literature; children (80%) and adults

(20%). The male gender predominance in both age groups for HBV MGN is similar (children 79%; adults 84%) and significantly greater than for idiopathic MGN.

Conclusions: The findings confirm that HBV MGN in children is a distinct form of GN which broadens the classical morphologic description of MGN by often including a number of mesangiocapillary GN features. The subgroup of renal biopsies with the most severe mesangiocapillary GN features was classified as the mixed HBV MGN-mesangiocapillary GN group. The MGN spectrum as a whole comprised 86% of the HBV positive childhood group. HBV MGN was the most frequent association with NS/severe proteinuria in the Namibian subgroup (37%) and the third largest group (19%) in the SA children. It showed a relatively high spontaneous remission rate but at least 10% of the children developed renal failure. Age of 6 years and above at presentation and severe mesangial deposits on biopsy correlated with fewer remissions and poorer outcome. Extended follow up (more than 15 years) was required to demonstrate renal failure in some patients in the poor outcome group.

Urbanisation, associated with lower HBV carrier rates, and HBV vaccination (initiated routinely in 1995 in SA), have already lead to a sharply decreasing incidence of this disease in SA. HBV MGN has been a valuable and possibly unique model of human GN and MGN in particular in that the HBeAg has been identified in both the serum and glomeruli enabling confirmation of the aetiological role of HBeAg.

OPSOMMING

Agtergrond en Doelwit: Die algemeenste oorsaak van erge proteïenurie/nefrotiese sindroom (NS) in kinders wêreldwyd is minimale veranderingsiekte. Hierdie patroon kom ook voor in blanke- en Indiër kinders in Suid-Afrika. In teenstelling hiermee is aangetoon dat swart en kleurling/gemengde ras kinders in Suider Afrika tussen die jare 1960s tot 1990s 'n ander patroon van nefrotiese sindroom gehad het. Een van die hoof verskille was die algemene voorkoms van hepatitis B virus (HBV) geassosieerde glomerulonefritis, gewoonlik membraneuse glomerulonefritis (MGN). Die doelwit van hierdie projek was 'n klinies-patologiese studie van hierdie subgroep van nefrotiese kinders ten einde die siekte verder te beskryf en veral om korrelasies te tref tussen patologiese en kliniese kenmerke insluitende prognose. Die gedetailleerde ultrastrukturele ondersoek van die kinders se nierbiopsies en die korrelasie van die spektrum patologiese kenmerke met demografiese, kliniese, laboratorium en prognostiese kenmerke was 'n sentrale fokusarea.

Die hipotese was dat die klinies-patologiese kenmerke van HBV MGN in kinders wesenlik van idiopatiese MGN in die algemeen verskil (in kinders en volwassenes) en ook van HBV MGN in volwassenes, en dat die beeld in kinders as 'n afsonderlike siekte beskou behoort te word.

Pasiënte en metodes: Die kinder kohort (12 jaar en jonger) was 309 kinders met erge proteïenurie/nefrotiese sindroom wie in Tygerberg Hospitaal (TBH) behandel was oor 'n 21 jarige periode vanaf 1974 tot 1995, insluitende 67 kinders van Namibië. Die studiegroep was 71 kinders met HBV MGN wie waar moontlik tot 2005 opgevolg

was. Die vergelykende volwasse groep was 45 volwassenes met MGN van wie 12 HBV MGN gehad het en 33 idiopatiese MGN. (’n Vergelyking met idiopatiese MGN in kinders kon nie gedoen word nie omdat hierdie sentrum net twee sulke pasiënte tydens die studietyd behandel het.) Demografiese, kliniese, laboratorium en nierpatologie inligting is versamel, vergelyk en gekorreleer.

Resultate: HBV geassosieerde MGN was die algemeenste oorsaak van NS in die Namibiese subgroep, 25/67 (37%) en die derde mees algemeen, 71/309 (23%) in die kinder kohort as geheel. Die MGN groep was 86% (71/83) van die totale HBV kinder nefrotiese kohort en verreweg die oorheersende subgroep.

Die gemiddelde ouderdom van die 71 kinders met HBV MGN by presentering was 6.0 jaar (reikwydte 2-12 jaar) en seuns het 80% van die groep behels. Hepatitis B omhullingsantigeen (*envelope antigen*- HBeAg) is aangetoon in die serum van 87% van die kinders wie daarvoor getoets is. Laboratoriumkenmerke wat van idiopatiese MGN verskil het, het ingesluit meer prominente hematurie, gering verhoogde serum transaminases en meer dikwels verlaagde serum C₃ en C₄ vlakke. Ligmikroskopiese ondersoek van die nierbiopsies het mesangiale proliferasie in elke pasiënt getoon, maar met minimale glomerulêre sklerose en interstisiële siekte. Met ultrastrukturele ondersoek was mesangiale en subendoteliële neerslae asook mesangiale interposisie algemeen. Die MGN van HBV in kinders het dus dikwels kenmerke van mesangiokapillêre glomerulonefritis getoon bo en behalwe die subepiteliële neerslae van MGN. Die ondergroep van 23 van wie die nierbiopsies erge mesangiale interposisie aangetoon het asook die subepiteliële neerslae van MGN is die gemengde HBV MGN-mesangiokapillêre GN groep genoem. Virustipe liggaampies en tubuloretikulêre insluitingsliggaampies is in meer as 80% van die biopsies bevestig. HBeAg was in die subepiteliële neerslae identifiseer. Dit was die eerste keer dat hierdie kenmerk in Afrika identifiseer is. Die 46 Suid-Afrikaanse kinders

het 'n kumulatiewe remissie koers van 25% teen 2 jaar en van 52% teen 4 jaar getoon. Sewe van die kinders (10%) van die hele kohort het kroniese nierversaking (KNV) ontwikkel. Ouderdom van 6 jaar en meer by presentasie en erge mesangiale neerslae in 'n biopsie het met minder remissies en 'n swakker uitkoms gekorreleer. Drie pasiënte het meer as 19 jaar na aanvanklike voordoening ooglopende KNV ontwikkel, waarvan 23 jaar die langste interval was. Die 358 gevalle van kinderjare HBV MGN van Suidelike-Afrika maak 37% uit van die gerapporteerde kinder pasiënte.

Vergelykende data

'n Vergelyking is getref tussen die 71 kinders met HBV MGN, 12 volwassenes met HBV MGN en 33 volwassenes met idiopatiese MGN. Die hoof verskille was dat beide HBV groepe net kleurling en swart pasiënte ingesluit het en meer oorwegend manlik was, terwyl die idiopatiese groep alle rasse ingesluit het. In die HBV pasiënte was hematurie meer algemeen en erg, lewer ensieme meer dikwels verhoog en C₃ meer dikwels verlaag as in die idiopatiese kohort. Beide groepe van volwasse MGN pasiënte het normale C₄ vlakke getoon terwyl die kindergroep met HBV MGN verlaagde C₄ vlakke bewys het. Die immuunkompleks patroon in biopsies van die HBV MGN volwasse en kindergroepe was soortgelyk met meer mesangiale en subendoteliële neerslae asook meer mesangiale interposisie as in die idiopatiese groep. Ten spyte van hierdie ooreenkoms tussen die twee HBV groepe, het die twee volwasse groepe meer glomerulêre sklerose en interstisiële siekte as die kindergroep vertoon. Die kliniese uitkoms van die kinderkohort was beter as die ander twee groepe met remissie (52%) wat meer algemeen was teen 4 jaar ($p < 0.01$) en met beter nier- en pasient oorlewing.

Ingeslote die 83 gevalle van hierdie reeks, is ten minste 1243 nierbiopsie bewysde gevalle van HBV MGN in kinders (80%) en volwassenes (20%) in die Engelse literatuur gerapporteer. Die manlike oorheersing in beide ouderdomsgroepe van HBV MGN is soortgelyk (kinders 79%; volwassenes 84%) en betekenisvol meer as vir idiopatiese MGN.

Gevolgtrekkings: Die bevindinge bevestig dat HBV MGN in kinders 'n afsonderlike vorm van GN is wat die klassieke beskrywing van MGN verbreed deur die algemene insluiting van 'n aantal mesangiokapillêre GN kenmerke. Die ondergroep van nier biopsies met erge mesangiokapillêre GN kenmerke is as die gemengde HBV MGN-mesangiokapillêre GN groep geklassifiseer. Die MGN spektrum in geheel het 86% van die HBV positiewe kindergroep behels. HBV MGN was die mees algemene assosiasie met NS/erge proteïenurie in die Namibiese subgroep (37%) en die derde grootse groep (19%) onder die SA kinders. Die siekte het 'n relatiewe hoë spontane remissiekoers getoon, maar ten minste 10% van die kinders het nierversaking ontwikkel. Ouderdom van 6 jaar en meer by presentasie en erge mesangiale neerslae in 'n nierbiopsie het met minder remissies en 'n slegter uitkoms gekorreleer. Uitgebreide opvolg (meer as 15 jaar) was nodig om nierversaking in sommige van die swak uitkomsgroep aan te toon.

Verstedeliking is geassosieer met laer HBV draersyfers en hierdie faktor saam met algemene HBV inenting in die kinderjare (wat in 1995 in SA begin was), het 'n skerp daling in die voorkoms van hierdie siekte in SA teweeg gebring. HBV MGN is 'n waardevolle en moontlik unieke model van menslike GN en MGN, veral omdat die HBeAg in beide die serum en glomeruli identifiseer kon word om die etiologiese rol van HBeAg te bevestig.

ABBREVIATIONS

ALT - alanine aminotransferase
ARP/AFIP - American Registry of Pathology/ Armed Forces Institute of Pathology
AST - aspartate aminotransferase
AuAg - Australia antigen
C₃ - complement 3
C₄ - complement 4
CI - Confidence interval
CIC - circulating immune complexes
CRF - chronic renal failure
DNA - deoxyribonucleic acid
EM - electron microscopy
FITC - fluorescein isothiocyanate
FSGS - focal segmental glomerulosclerosis
HBcAg - hepatitis B core antigen
HBeAg - hepatitis B envelope antigen
HBeAb - hepatitis B envelope antibodies
HIVAN - human immunodeficiency virus associated nephropathy
HBsAg - hepatitis B surface antigen
HBV - hepatitis B virus
IC - immune complexes
IFN - interferon
IL- interleukin
ISN - International Society of Nephrology
KNV – kroniese nierversaking
LCL - lymphoblastoid cell lines
Lam - Lamivudine
MCD - minimal change disease
MCGN - mesangiocapillary glomerulonephritis
MGN - membranous glomerulonephritis
No. - number
NS - nephrotic syndrome
RIA - radioimmunoassay
RPS - Renal Pathology Society
SLE - systemic lupus erythematosus
SA - South Africa(n)
TRIBS - tubuloreticular inclusion bodies
TBH - Tygerberg Hospital
vs. - versus, compared to
WHO - World Health Organization

DEDICATION

This work I dedicate to those most important to my life and its purpose.

My **parents, Monica and Brendon Bates** who encouraged me in my human and intellectual development and in particular in my medical studies. Sadly, they are no longer alive to see this project come to fruition.

My **wife Judy and children Lauren and David**, thank you for your support and for believing that I could and would finish this dissertation.

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The discovery of a well defined aetiology for MGN can be an intellectually satisfying experience.

RJ Glassock 1992 [1]

Chapter 1

INTRODUCTION

The introduction of needle biopsies of the kidney revolutionised the study of renal pathology. Until 1950 knowledge of the pathology of renal disease was based almost entirely on postmortem reports. Autopsy findings would have reflected the more severe and chronic examples of renal disease while mild, acute and usually reversible diseases would almost never have been seen. Consequently correlations between clinical findings and renal pathology could not be performed. The pioneers of the use of needle biopsies for the diagnosis of medical renal diseases were Iversen and Brun in Denmark (1951) [2], Alwall in Sweden (1952) [3] and Pardo et al in Cuba (1953) [4]. Within a short time many researchers in the United States and Europe in particular were reporting on ever increasing series of cases. In time the proportion of specimens adequate for diagnosis increased to more than 90% and the frequency of complications decreased. [5] Heptinstall's Pathology of the Kidney in 1966 (first edition) was the earliest book in which renal biopsy studies, including immunological and ultrastructural evaluation of the biopsy material, were fully acknowledged. [6] In 1975, "A Handbook of Kidney Nomenclature and Nosology", was published by an international group working through subcommittees on anatomy, immunology, pathology, physiology, radiology and clinical medicine. A further international combined study around that time, 'The International Collaborative Study of Kidney Disease in Children', emphasised the importance of the development of uniform criteria. They used a group of pathologists to review the renal biopsy material and this helped to develop shared terms and criteria for diagnosis. [7] Building on the foundational contribution of the abovementioned as

well as other efforts, the World Health Organization (WHO) established a Collaborating Centre for the Histological Classification of Renal Diseases in New York under the direction of Jacob Churg in 1974. The Centre worked with nephrologists and pathologists from at least 14 countries to evaluate and categorise the material to develop the WHO classification eventually published in 1982. They highlighted that international agreement on criteria for pathological diagnosis, standardized nomenclature and an internationally accepted uniform system for classification were needed for reliable comparative studies of renal diseases. It was recognised in the preface to the first edition that the classification reflected the present state of knowledge at the time and that modifications were almost certain to be needed as time passed and experience accumulated. [7] The fact that relatively few major modifications to that classification have occurred since 1982 may reflect that a successful, internationally representative classification process was achieved as well as that there have been relatively few major advances in understanding the aetiology and pathogenesis of many primary idiopathic forms of glomerular disease. HIV-associated nephropathy (HIVAN) is one of the few examples of a new glomerular disease, described first in 1984. [8]

Until the histopathology including ultrastructural features and immunopathology of MGN were established, the natural history, outcome and therapeutic responsiveness of MGN remained confused with other glomerular diseases that presented with proteinuria or the nephrotic syndrome. Thus to quote Schwartz, "...the pathologic identification of MGN is the starting point for a rational discussion of the disease. " [9]

Concerning this study of the HBV MGN-like variant, it is important to note that in the 1982 WHO classification, there was not a specific category under systemic renal diseases for HBV. By contrast the nephritis of systemic lupus erythematosus (SLE), also called lupus nephritis, had 6 different categories or classes available to deal with

the variety of morphological appearances including the combination of categories. HBV was however, by that time recognised as a cause of secondary MGN. It was mentioned that HBV may be associated with mesangial deposits in MGN. HBV was not mentioned in the mesangiocapillary GN (MCGN) description. [7]

This study documents from the literature and local experience the expanded understanding of HBV associated glomerulonephritis and in particular the MGN-like subtype in cohorts of both children and adults. The childhood HBV MGN group was the initial focus group, is larger and was the primary study cohort while the adult HBV MGN group and adult idiopathic group of MGN were comparative cohorts.

To begin to fully document, appropriately classify and more fully understand glomerular diseases a wide range of demographic, laboratory, renal biopsy, treatment and outcome data needs to be gathered, compared and correlated and this is what has been undertaken in this study.

The first case of HBV associated MGN ever reported was in 1971 [10] and as will be shown in this study the worldwide frequency of reported cases reached a peak around 1988-1994, a period during which about 300 cases were reported and has since been in decline largely linked to increasing HBV immunisation. [11] This project has therefore enabled both our cases to be added to the world experience and the detailed review, comparison and analysis of nearly 4 decades of reported experience of a disease apparently on the brink of disappearing.

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Chapter 2

LITERATURE SURVEY

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1. HISTORICAL SURVEY – LANDMARKS AND MILESTONES

Hepatitis B virus (HBV) has caused a worldwide pandemic with up to 350 million carriers. This is a serious infection which carries a significant morbidity and mortality not only in the acute phase with acute hepatitis but more significantly in its chronic carrier phase where it makes a major contribution to chronic hepatitis, cirrhosis and hepatocellular carcinoma. [1] This study focuses on a rarer complication, namely HBV immune complex GN and in particular membranous glomerulonephritis (MGN), affecting an unknown but very small proportion of HBV carriers. To place this relatively recently described disease in context a selected history of HBV and HBV associated glomerular disease in particular follows. Key events are summarised in Table 2-1.

The first outbreaks of viral 'serum' hepatitis were recorded in 1885 in Germany, when Lurman and Jehn reported the occurrence of jaundice in a significant percentage of the personnel of a Bremen shipyard and of the inmates of a Merzing insane asylum who had been vaccinated against smallpox. [2] Another devastating outbreak occurred in 1942 when 28,585 United States Servicemen inoculated with yellow fever vaccine developed jaundice and 62 died. [2]

In 1963, sera from patients with haemophilia who had received transfusions were tested for the presence of isoprecipitins using a panel of 24 sera from normal individuals, including sera from populations from all around the world and not just the USA where the studies were being carried out. Two of the haemophiliac sera formed a clearly defined precipitin line with one of the panel sera (from an Australian aborigine), but with none of the others. The serum protein was tentatively called Australia antigen (AuAg) and in time was identified as HBV. [3]

Table 2-1 *HBV and MGN – some of the landmarks and milestones*

Year	Author	Event	Reference
1885	Levy	Jaundice after smallpox vaccination in two German groups	[2]
1942	Levy	Outbreak jaundice US servicemen after yellow fever vaccine	[2]
1963	Blumberg	Discovers Australia antigen (AuAg - HBV)	[3]
1966	Chan	MGN frequent in proteinuric/nephrotic Chinese children Hong Kong 9/90-10% 80% male Most into remission - one CRF Probable first series of 'HBV' MGN - virus not yet tested for	[4]
1968	Ehrenreich, Churg	Classic description of idiopathic membranous glomerulonephritis	[5]
1970	Blumberg	AuAg linked to hepatitis Rates vary in different populations Moderately high in SA blacks	[6]
1970	Grobbelaar	Durban SA - carrier rate highest in blacks Rare in Asians/Indians and whites	[7]
1971	Combes	First patient - HBV MGN - adult in USA	[8]
1972	Cameron	Suggests link between high rate of MGN in Hong Kong children and HBV	[9]
1972	Meyers	HBV carrier rates in Western Cape SA Highest in blacks, then coloureds and then whites	[10]
1972	Brzosko	First series of HBV GN children – Poland Only 2/16 (12.5%) MGN Issue of proliferation, electron microscopy (EM) and interpretation	[11]
1973	Vos	SA link - high HBV rate in black renal patients and blood donors	[12]
1974	Van Buuren	First HBV MGN child diagnosed at Tygerberg Hospital	
1976	Mrozowicz	First HBV MGN case to mention mesangial deposits - Poland	[13]
1976	Adhikari	SA series of children with nephrotic syndrome MGN and mesangiocapillary GN (MCGN) patterns frequent in black children HBV testing not yet available	[14]
1978	Takekoshi	Larger groups of HBV MGN children - Japan - HBs negative in glomeruli - 5/11 remission	[15]
1979	Kleinknecht	Larger groups of HBV MGN children - France - 7/15 remission usually by 18 m - 1 renal failure First child with poor outcome	[16]
1979	Takekoshi	HBe identified in subepithelial deposits	[17]
1981	Ito	HBe glomerular resolution linked to HBe seroconversion - 2 patients	[18]
1983	Amemiya	Mesangial deposits, HBs and HBe demonstrated in glomeruli	[19]
1983	Collins	HBe and HBs identified in glomerulus as well as mesangial deposits	[20]
1983	Wiggelink-huizen	Larger group (10) of HBV MGN in remission linked to HBe seroconversion Mesangial interposition described and illustrated Emphasis that EM needed to distinguish MGN from mesangiocapillary GN	[21]
1985	Southwest	Renal failure after 105 months in HBV MGN child	[22]
1985	Garcia	Remission in a patient treated with interferon	[23]
1988	Elidrissy	Unusually poor outcome 2/3 HBV MGN in young boys progressed to renal failure	[24]
1989	Hsu	Correlations between HBV MGN features and outcome	[25]
1991	Lai	Renal pathology similar but outcome poorer in adults with HBV MGN	[26]
1996	Tomonaga	Renal failure in child with HBV GN after 17 years	[27]

In 1966 the first series of HBV MGN in children was probably described. Ten (10) cases of membranous glomerulonephritis (MGN) were diagnosed in Hong Kong including 9/90 (10%) in a biopsy series. This proportion of 10% of MGN was high by comparison with childhood nephrotic series worldwide. The age range was 4-10 years with a mean of 7.4 years and eight of the 10 were male. One renal biopsy showed cellular proliferation in addition to MGN. Half of the children were reported to have responded well to steroids. A 5 year old male developed chronic renal failure (CRF). At this stage tests for Australia antigen/HBV were not yet available. Most of the clinical and pathological features in the group above fit very well with the subsequently described HBV MGN from which the children almost certainly suffered. [4] There followed numerous reports on this disease from Hong Kong, Taiwan and China where HBV carrier rates were high. [25, 26, 28, 29]

In 1968, Ehrenreich and Churg described the clinical and pathological features of idiopathic MGN. Central features included adult dominance with most patients aged between 40 and 60 years and a male: female ratio of 60:40. Most presented with nephrotic syndrome. Renal biopsies showed subepithelial electron dense deposits and associated capillary wall thickening as the main abnormalities with no to minimal mesangial changes. Mesangial deposits, subendothelial deposits and mesangial interposition were not found in these cases of idiopathic MGN. Varying degrees of tubular, interstitial and blood vessel involvement were seen. [5]

By January 1970 there was great interest in Australia Antigen (called Au1 at that time) and it became clear that it was closely related to or was part of a hepatitis virus. It was shown that Au1 (HBV) was rare in healthy controls in the USA but occurred in high frequency in those with hepatitis as seen in Table 2-2. [6]

Table 2-2 *Percentage of various patient categories positive for HBV (Au1)*

Subjects USA	No. tested	Au1/HBV pos	Au1/HBV %
Controls: Non hospitalised 'normal'	2412	2	0.1
Patients with hepatitis acute viral	180	49	27.2
Most likely source of hepatitis			
Post transfusion	51	21	41.1
Infectious	129	28	21.7

[6]

By this time Blumberg and his group had studied more than 20,000 serum specimens of 'normal' subjects in populations from around the world establishing the ranges of what would later be viewed as essentially carrier rates of HBV in different populations. Table 2-3 highlights a few rates of interest and relevance to this present study. Blumberg concluded that Au1 (HBV) was rare in most North American and North European populations (including African Americans from Maryland, USA) but occurred with intermediate frequencies in Japanese and Mediterranean populations (about 1%) and in higher frequencies in the tropics and Southeast Asia (2-20%). The South Africans tested showed a rate of 2.8% in the black Africans and 0% in the coloured (mixed race) patient sample with no white sample reported. [6]

The first tests on South Africans reported from SA were in Natal (1970) showing the highest chronic carrier rates in blacks/Africans (3.2%); Indians (0.2%); Caucasian/white (0%). [7]

Table 2-3 *HBV carrier rates in various populations*

Population	Location	No. tested	No. positive	%
Americas				
White	Maryland USA	896	0	(0)
African Americans	Maryland USA	607	0	(0)
Black	Brazil	119	3	(2.5)
Eskimos	Alaska USA	394	1	(0.3)
Indians, Casinahua	Peru	89	18	(20.2)
Africa				
Black	South Africa	72	2	(2.8)
Coloured	South Africa	100	0	(0)
Ghanaians	Ghana	95	9	(9.5)
Asia				
Indians	South India	127	3	(2.4)
Japanese	Japan	1034	5	(0.5)
Chinese	USA Taiwan	100	0	(0)
Taiwanese	Taiwan	23	3	(13.0)
Vietnamese	Vietnam	128	8	(6.3)
Europe				
Finns	Finland	924	1	(0.1)
Greeks	Greece	857	15	(1.8)
Oceania				
Aborigines	Australia	1807	38	(2.1)
Micronesians	Marshall Islands	474	34	(7.2)

[6]

The first suggestion of the aetiological role of HBV in GN was reported in 1971. A 53 year old USA man developed HBV infection after a blood transfusion and became nephrotic a year later. Renal biopsy showed MGN. The authors suggested "that Australia antigen was involved in the formation of immune complexes whose glomerular deposition initiated the pathologic process leading to the development of diffuse membranous glomerulonephritis". [8]

The British nephrologist JS Cameron made an astute observation and suggested a hypothesis at an International Symposium in 1972. In the discussion on aspects of membranous GN he said, "I have wondered whether if this is a model, as Dr Heptinstall has suggested, of soluble complex disease in man, we should be looking in these patients for soluble antigens that might underlie it. Now Hong Kong has two things in particular, one is the high incidence of true epimembranous nephropathy (an earlier name for MGN) and the other is a very large incidence of carriers of Australia antigen and it would be well worthwhile for someone in Hong Kong to look at both the Australian antigen carriers for proteinuria and in those with membranous nephropathy for Australian antigen." [9] This idea of HBV MGN as a model of GN in humans may be one of the reasons for the strong interest in this disease over the last 4 decades although the number of documented cases is relatively small. Cameron showed he was aware of this disease possibility when he described a child in London in 1978 with HBV MGN, a young white girl who appeared to have become HBV positive in Zambia. [30] This was one of the earliest cases of HBV MGN reported in a child with an African connection.

Meyers et al in 1972 reported on the prevalence of Australia Antigen in Western Cape blood donors, hospitalised patients and healthy black/African subjects and found the carrier rates to be highest in the black donors (4.11%) and lowest in the white donors (0.47%), with the coloured donors having intermediate carrier rates (2.22%). In donors from prisons or reform schools the rates were higher but maintained the relative frequencies. Table 2-4 [10].

Table 2-4 Prevalence of hepatitis B infection in Western Cape blood donors

Patient groups	No. Tested	Positive HBsAg No. (%)	Positive HBsAb No. (%)
Non prison			
White	8712	41 (0.5)	10 (0.1)
Coloured	3641	81 (2.2)	33 (0.9)
Black	413	17 (4.1)	8 (1.9)
Total	12766	139 (1.0)	51 (0.4)
Prison			
White	42	0	0
Coloured	1537	80 (5.2)	15 (0.9)
Black	148	12 (8.1)	3 (2.0)
Total	1727	92 (5.3)	18 (1.0)
Total both groups	14493	231 (1.6)	69 (0.5)

[10]

The first childhood HBV GN series was published in 1972 by Brzosko from Poland. On the basis of positive serum and glomerular HB surface antigen (HBsAg) and morphology, in a series of 16, only 2 (12.5%) were classified as MGN, ten as mesangiocapillary GN, two as endo- and extracapillary proliferative and two as endocapillary proliferative. The frequently proliferative features of HBV associated glomerular disease and the need for extensive ultrastructural examination to try to distinguish potentially overlapping appearances, were illustrated by this series but possibly not fully appreciated by the authors at the time. [11]

Vos et al in 1973 were the first to suggest a relationship between the HBV carrier state and renal disease in South African patients. They reported an HBsAg carrier rate of 20.3% in 182 black adults with chronic renal disease compared to a 9.8% carrier rate among 5130 black blood donors in Durban. Both these carrier rates were high but the rate in those with renal disease was significantly greater. [12] (The

difference between rural and urban carrier rates which may have impacted on these figures is discussed later in this chapter on page 2-37.) [31]

In 1974 the first child with HBV MGN was diagnosed at Tygerberg Hospital. He was a 5 year old coloured boy who presented with nephrotic syndrome and was suspected initially to have acute post-infectious glomerulonephritis. The nephrotic syndrome did not respond to steroids and a renal biopsy was performed. Features of MGN were shown in the biopsy. Australia Antigen was positive in the serum leading to a diagnosis of HBV MGN. He went into remission after 41 months and was followed a total of 19 years. Last seen in 1992 at age 23, he was still HBsAg positive, HBeAg negative and in remission.

In 1976 Mrozowicz from Poland was the first to document mesangial deposits on ultrastructure in cases of HBV MGN. [13]

In 1976, Adhikari et al published the first clinicopathological description of nephrotic syndrome in children in South Africa. In the group of 30 black children, 12 cases of MGN and 12 cases of MCGN (each 40%) were the most frequent subtypes of GN while minimal change disease was most frequent in the 21 Indian children (11/21-52%). Electron microscopy was not available and immunofluorescence was done on 12 of the cases. One black child with MCGN was noted to be serum HBV positive but routine testing was not yet available. (It is not stated how many children were tested for HBV.) This series strongly suggested that in South Africa black (African) children had a different nephrotic syndrome pattern to the white and Indian children. [14]

Subsequent series in 1978 and 1979 of HBV MGN children from France and Japan further highlighted several points. [15, 16] Both reported a relatively favourable prognosis. Spontaneous remission occurred in 50% in both groups. Renal failure was unusual with just one 13 year French boy in renal failure 4 years after presentation out of a total of 26 children in the two groups. This was the first patient reported to develop renal failure from HBV MGN. In the seven patients in the French series who remitted, nephrotic syndrome disappeared within 5 months and proteinuria within 18 months. None of the 7 relapsed with follow up from 9 months to 7 years after remission. In the Japanese series (Antibody from Hoechst, West Germany) and French series (Behringwerke Laboratories) HBsAg could not be detected in any of 9 biopsies tested in each series. [15, 16] The relevance of mentioning the specific antibodies used should become clearer later in the dissertation.

In 1979, Takekoshi demonstrated the presence of HBeAg by fluorescent antibody technique in the subepithelial membranous deposits of two children. In these children two forms of HBeAg activity had been identified in the serum: small molecular (free) and large molecular, associated with IgG. [17]

Ito et al added a further central piece to the puzzle in 1981 when their group studied 6 children with HBV MGN. Two in remission still had HBsAg in the serum but tested negative for HBeAg in serum and glomeruli. The 4 with active disease were positive for HBeAg in both serum and glomeruli. The authors concluded that HBeAg played the central role in the development of HBV MGN and that resolution of the HBeAg deposits in the glomeruli and clinical remission were linked to HBe seroconversion. [18]

In 1983 Amemiya reported a 10 year old child with not only HBeAg in the serum and also predominantly in the subepithelial deposits in glomeruli, but also with mesangial deposits on ultrastructure. HBsAg was demonstrated in both mesangial and subepithelial areas. On electron microscopy subepithelial, subendothelial and mesangial deposits were seen. [19] This was the second report of mesangial deposits in HBV MGN after that reported by Mrozowicz. [13] Collins in 1983 also documented mesangial deposits in HBV MGN, staining for HBsAg in addition to subepithelial deposits staining with anti-HBe. [20]

In 1983 Wiggelinkhuizen et al from Red Cross Hospital, Cape Town reported that remission in 10 of 25 children with HBV MGN was associated with HBe seroconversion. They also described and illustrated mesangial interposition in a biopsy categorised as HBV MGN for the first time, noting that it was frequently seen. It was emphasised that ultrastructure was needed to show and distinguish the MGN and mesangiocapillary glomerulonephritis (MCGN) features. [21]

A USA multicentre series of 11 HBV MGN children in 1985 confirmed many features seen by then including the generally good prognosis. At least 11 centres were involved in this study confirming the relative rarity of the disease in the USA. No larger series of North American children with HBV MGN has ever been assembled. They documented that one child had developed renal failure 105 months after presentation. This emphasised the need for long term follow up. [22]

In 1985 Garcia reported on the use of human leucocyte interferon in treating HBV MGN in an adult and a child. They illustrated that interferon could be associated with

both changes in viral markers and remission (temporary, partial or complete) of nephrotic syndrome. [23]

Elidrissy reported an unusual series from Saudi Arabia in 1988. Three of 4 HBV positive children biopsied, all boys aged between 3 and 5 years, had MGN and two of these developed chronic renal failure. Two other HBV positive children, one with a mesangial proliferative GN and one not biopsied also developed renal failure. This small group, particularly of such young boys, with very poor outcome is unique. No clear explanation was given at the time or has emerged since. [24]

In 1989, Hsu from Taiwan reported a series of 52 children and found that younger age and earlier stage of subepithelial deposits correlated with earlier remission. The 7 year cumulative follow up reported a remission rate of 92%, one of the best described. [25]

Lai from Hong Kong reported a group of 21 adults with HBV MGN with detailed clinical and pathological data in 1991. Although this was not a direct comparative study with childhood HBV MGN, they reported that the glomerular pathology was similar but outcome poorer than in children with HBV MGN with no spontaneous remission and 29% development of chronic renal failure. [26]

Tomonaga et al in 1996 described a child who presented with probable HBV associated GN (no renal biopsy) at age 12 and progressed to end stage renal failure 17 years after the clinical onset, in spite of seroconversion with HBe antibodies. This case again emphasised the need for long follow up to more accurately assess the outcome of HBV GN. [27]

2. THE HEPATITIS B VIRUS (HBV)

a) Introduction

It was estimated that HBV had infected over 2 billion of the individuals alive in 2004 at some point in their lives but that only a minority, 350 million (17.5%) of these people had become HBV carriers. Seventy five percent of all chronic carriers lived in Asia and the Western Pacific rim. The global prevalence of chronic hepatitis B infection, before large scale immunisation began, varied from high (>8%) in Africa, Asia and the Western Pacific to intermediate (2-7%) in Southern and Eastern Europe to low (<2%) in Western Europe, North America and Australia. [1] This is illustrated in Figure 2-1 from a 2001 WHO report. [32]

In addition, in individual countries, major differences in serum HBsAg prevalence may occur between ethnic or other groups, as in Southern Africa (Schoub 1992). [33] A recent review by Kew highlights the burden of HBV in South Africa, again emphasising that HBV infection, both acute and chronic, occurs commonly in the black African population of South Africa and that chronic infection and its sequelae of cirrhosis and hepatocellular carcinoma are major public health threats. Chronic HBV infection is rare in other population groups, with the exception of the very small Chinese community and the coloured community. (This latter group, found predominantly in the Western Cape and the largest group using Tygerberg Hospital, is central to this current study.) Prevalences of chronic carriage of HBV in SA blacks as measured between 1974 and 1989, were 5-16% in rural males, 8-9% in urban males, 4-12% in rural females and 2.7-4% in urban females. [31, 34-39] (The preceding figures and following description would have pertained especially in the

Geographic Distribution of Chronic HBV Infection

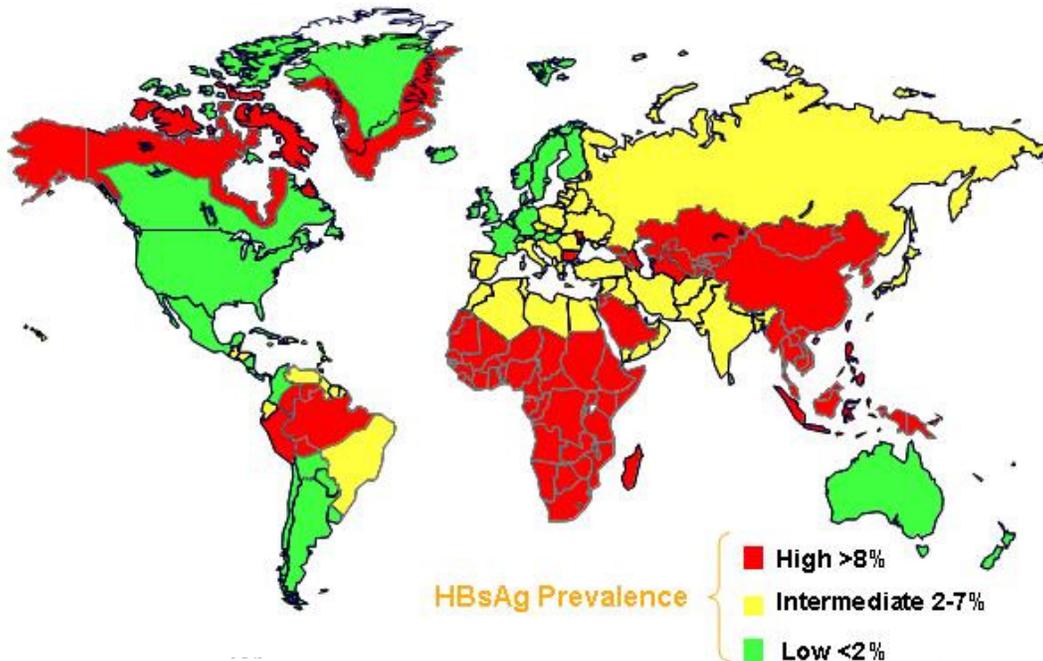


Fig 2-1 Geographic distribution of chronic HBV infection in 2001 [32]

pre HBV immunisation phase, before 1995, by which time most of the patients in this current study developed their HBV related diseases and presented for medical attention.)

There are currently three to four million SA blacks who are chronically infected with HBV as reported by Kew in 2008. [39] The overall male to female ratio is 2.6:1.0. In rural black populations chronic HBV was acquired very early in life, predominantly as a result of horizontal transmission of the virus, and by the age of 5 years adult carrier rates were approached. A further small increase occurred at school going age and a greater increase at the time of becoming sexually active. Urban black carrier rates

were significantly lower and the infection was acquired later in life. The lower urban HBV rates appeared mainly in the first generation born in the urban environment. In 1986 the Kew group reported a low carrier rate in black children from Soweto, a large black township in Johannesburg. The black boys had a carrier rate for HBsAg of 1.5% and girls 0.6% in contrast to accepted figures of 15% HBsAg carrier rates for rural black SA children. [40] There has been no convincing explanation for the lower urban carrier rates. Kew emphasises the serious and frequent liver consequences of this disease but makes no mention of the extrahepatic consequences such as HBV GN in this review, most likely reflecting their rarity and therefore relatively small contribution to overall disease burden. [39]

b) The virus

HBV is a member of the Hepadnaviridae, a family of DNA (deoxyribonucleic acid) containing viruses that cause hepatitis in multiple animal species. The mature HBV virion is a 42 nm, spherical double layered 'Dane particle' that has an outer surface envelope of protein, lipid and carbohydrate surrounding an electron dense, 28 nm slightly hexagonal core. [1] **(Figures 2-2a, 2-2b, 2-3a and 2-3b)**

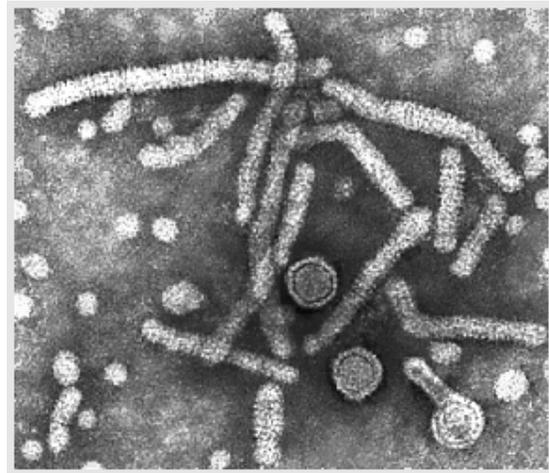
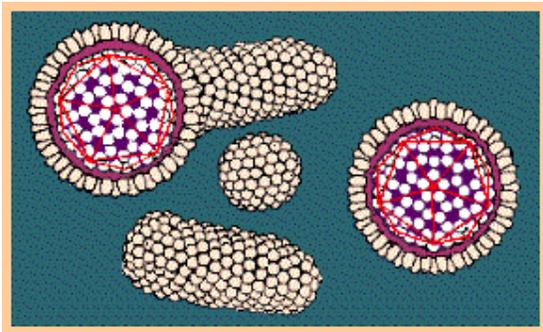


Fig 2-2a *Cartoon of Hepatitis B Virus (HBV)* **Fig 2-2b** *Electron Micrograph of HBV*

The virus is 42nm in diameter and possesses an isometric nucleocapsid or "core", surrounded by an outer coat. The protein of the virus coat is termed "surface antigen" or HBsAg. It sometimes extends as a tubular tail on one side of the virus particle. The surface antigen is usually produced in vast excess, and is found in the blood of infected individuals in the form of filamentous and spherical particles. Filamentous particles are identical to the virion "tails"; they vary in length and have an average diameter of about 22nm. (Courtesy of Linda Stannard, Department of Medical Microbiology, University of Cape Town. Unpublished.)

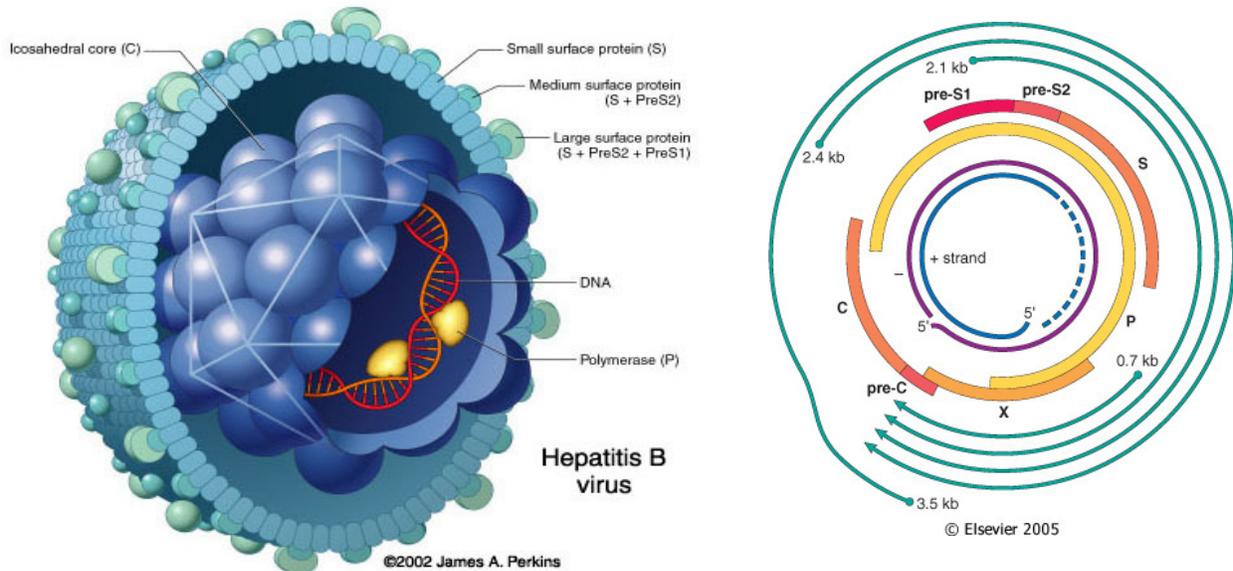


Illustration from internet - unpublished

Fig 2-3 (a) A depiction of the surface (HBs) antigen, core (HBc) antigen and DNA

Figure 2-3 (b) Diagrammatic representation of genomic structure of the hepatitis B virion. The innermost circles represent the DNA (+) strand and the DNA (-) strand of the virion. The thick bars labeled "P," "X," "pre-C," "C," "pre-S1," "pre-S2," and "S" denote the peptides derived from the virion. The outermost lines denote the mRNA transcripts of the virion. [1]

The genome of HBV is a partially double stranded circular DNA molecule having 3200 nucleotides. All regions of the HBV genome encode protein sequences:

An envelope glycoprotein Hepatitis B surface antigen (HBsAg). Infected hepatocytes are able to synthesise and secrete large quantities of noninfective surface protein over and above HBcAg synthesis. HBsAg appears in cells and the serum as spheres and tubules approximately 22 nm in diameter.

A nucleocapsid 'core' protein Hepatitis B core antigen (HBcAg) which remains in hepatocytes for the assembly of complete virions.

A longer polypeptide transcript than the above one with a precore and core region designated **Hepatitis B e antigen (HBeAg)**. The precore region directs the HBeAg toward secretion into blood.

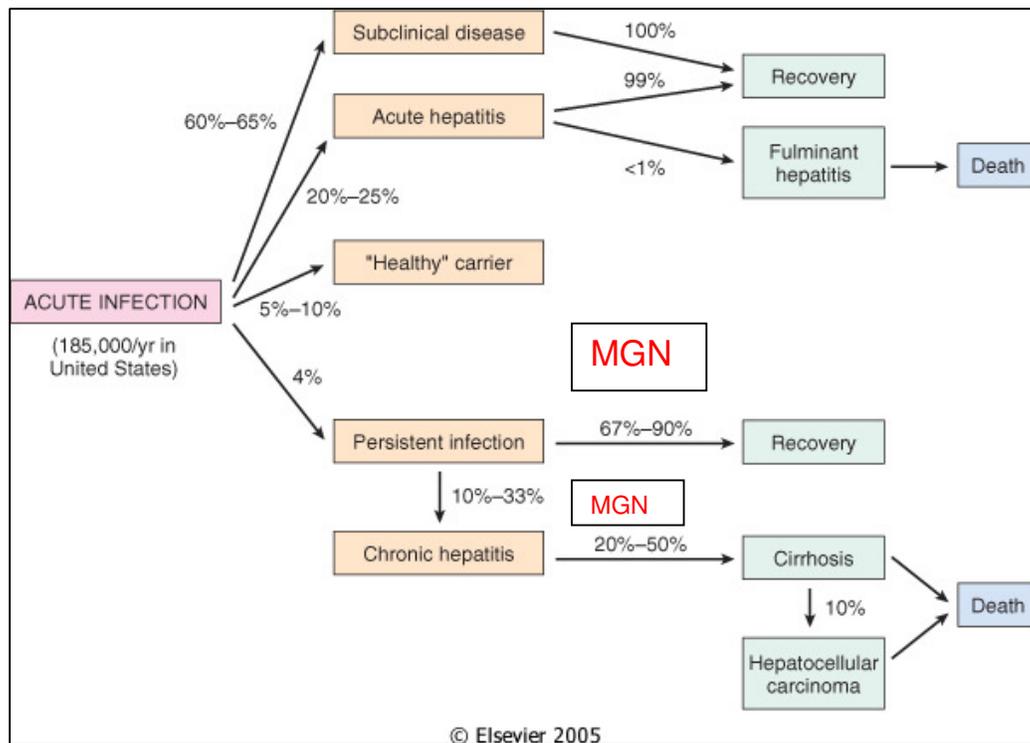
A DNA polymerase that exhibits reverse transcriptase activity.

A protein from the X region, HBx, which is necessary for virus replication. This may play a role in the development of hepatocellular carcinoma.[41]

Those antigens of HBV of central importance in this study include HBsAg, HBeAg and HBcAg together with their antibodies.

c) Clinical consequences

Hepatitis B virus (HBV) infection can produce a variety of clinical consequences including acute hepatitis with resolution, the most frequent outcome; fulminant hepatitis with massive necrosis which occurs rarely; 'healthy carriers' who show no or minimal signs of disease; those with persistent infection, most of whom finally recover and the group with chronic hepatitis which can lead to cirrhosis and hepatocellular carcinoma. An estimate of the relative frequencies of these outcomes is illustrated for the USA in Figure 2-4. [1]



[1]

Fig 2-4 Schematic of the potential outcomes of hepatitis B infection in adults, with their approximate frequencies in the United States. [1] The red MGN boxes have been placed where MGN occurs, in the HBeAg positive carriers, particularly in the seroconversion phase. This would fit in particular with the persistent group on the way to recovery; therefore represented by the larger sized MGN.

d) Epidemiology

Exchange of blood and body fluids are the primary modes of transmission; the body fluids and secretions would include semen, saliva, sweat, tears, breast milk and effusions. Transfusion of blood or blood products, haemodialysis, needle stick accidents among health care workers, intravenous drug abuse and sexual activity are the primary risk categories. In up to one third of patients, the source of infection is unknown. [41] In endemic regions such as Africa and Southeast Asia, spread from an infected mother to a neonate could occur during birth (vertical transmission). A Namibian study suggested transmission there was mainly horizontal from infected family members or infected playmates. It was shown that black (African) children acquired chronic HBV infection in early childhood and that most who become chronic carriers become HBsAg positive after the age of 11 months. [42] (These findings are of particular relevance to this study as this survey was conducted in the early 1980s in Namibia among the community from where the majority of the Namibian HBV MGN children in this study cohort came.) These findings contrast with the experience in Southeast Asia, where perinatal transmission accounted for the majority of children infected. [42] The risk of vertical transmission is related to maternal HBeAg and HBV DNA status. These neonatal infections more often than usual lead to the carrier state, sometimes for life as noted in Table 2-5. [2]

Table 2-5 *Relationship between age at which HBV infection occurs and the risk of developing HBV carrier state*

Age	Risk (%)
Less than 1 year	70 – 90
2-3 years	40 – 70
4-6 years	10 – 40
Over 7 years	5 -10

[2]

Males are at greater risk than females of becoming chronic carriers. In most population studies the chronic HBsAg carrier state is 1.5 to 2 times more frequent in males than females. [2] Kew in a recent review pertaining to SA reported 2.6:1 HBV chronic carrier rates in black males to females. [39]

e) Serologic course and diagnosis

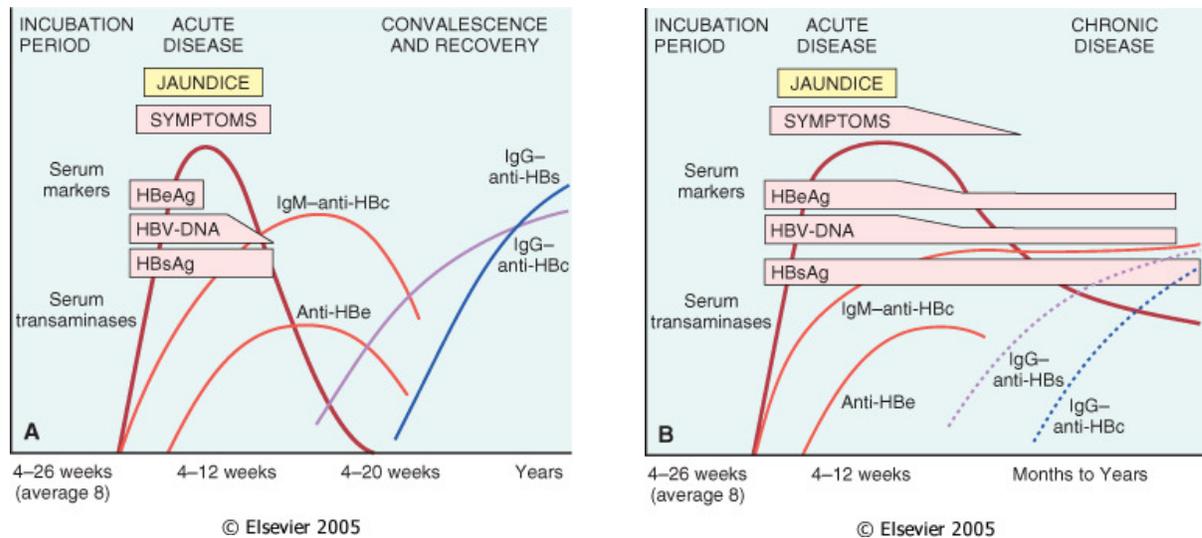
After exposure to HBV, the long asymptomatic incubation period (average 6-8 weeks) is followed by acute hepatitis lasting many weeks to months. Most patients, particularly adults, experience a self limited illness. (Figure 2-5A)

In the serum, HBsAg is present before the onset of symptoms, peaks during overt disease and then decreases to undetectable levels in 3 to 6 months as shown in Figure 2-5A. HBeAg, HBV DNA and DNA polymerase appear in the serum soon after HBsAg and all indicate active viral replication. Anti-HBcAb IgM appears in the serum shortly before the onset of symptoms, at the same time as the rise in serum aminotransferases. Over the months, IgG HBcAb replaces the IgM antibody. [1] Anti-HBe becomes detectable shortly after the disappearance of HBeAg, indicating that

the acute infection has peaked and the disease is on the wane. IgG HBsAb is usually not detectable for a few weeks to several months after the disappearance of HBsAg.

The hepatitis B viral carrier state is defined by the presence of HBsAg in the serum for 6 months or longer after initial detection (Figure 2-5B). The presence of serum HBsAg by itself does not necessarily indicate replication of complete virions and patients may be asymptomatic and without liver damage. By contrast, chronic replication of HBV virions is usually associated with the persistence of circulating HBsAg, HBeAg and HBV DNA, often with anti-HBc antibodies and sometimes with anti-HBs antibodies. Progressive liver damage may occur in these patients. A schematic representation of the potential outcomes of hepatitis B infection in adults, with their approximate frequencies in the United States was shown above (Figure 2-4). [1]

This group of chronic carriers of HBV is also the one in whom immune complex GN, usually MGN, develops rarely. This usually occurs at the stage when both HBeAg and anti-HBe antibodies are present and immune complexes can form in the subepithelial location of susceptible patients. Of note those who develop the GN are almost always in the process of becoming HBeAg negative; therefore they do not tend to develop further liver complications like cirrhosis and cancer. (Figure 2-4). HBV MGN can also occur in the group developing cirrhosis but the frequency of this group is uncertain.



[1]

Figure 2-5 A and B Sequence of serologic markers for hepatitis B viral hepatitis demonstrating (A) acute infection with resolution and (B) progression to chronic infection.

f) HBV infection and MGN - Evidence for the association

Epidemiological, clinical and immunopathological evidence points to a causal relationship between the HBV carrier state and the development of MGN and some other forms of GN. [43, 44] The pathology of HBV MGN clearly indicates the immunological nature of the disease. The presence of IC, the presence of IgG and C₃ in deposits support this. The antigen responsible was initially thought to be HBsAg [8] but a number of subsequent studies showed HBeAg to be the most important antigen in glomerular disease, and in particular associated with granular subepithelial staining. [15, 18, 45-47]. Supporting this finding is that in many of the children with HBV MGN, the seroconversion from HBeAg to anti-HBe has been associated with remission of the nephrotic syndrome. [48] In addition, the reduction in the number of

MGN cases in areas where vaccination has been implemented is strong evidence for the link between the two conditions. [29, 49]

g) Pathogenesis of HBV MGN

Both viral and host factors are involved in the pathogenesis of HBV MGN. [50] An association with HLA genes has been reported, highlighting the role of genetic predisposition. [51] The main pathogenetic mechanism in HBV related GN is through the deposition of immune complexes in the glomerulus. After HBV infection the antigens HBsAg and HBeAg appear in the serum while the HBcAg remains in hepatocytes and the host raises antibody in the following order: anti-HBc, anti-HBe and anti-HBs. [1, 51] As a result immune complexes (IC) involving different antigens (particularly HBeAg and HBsAg) are produced in the circulation and may induce a variety of extrahepatic disorders, including glomerular diseases. An immunologically competent host can eliminate HBV antigens readily, while a persistent supply of antigen might lead to chronic immune complex diseases in a host who cannot mount an appropriately intense antibody response. [52] The immune complexes then activate complement and glomerular injury occurs via the formation of membrane attack complex and other downstream events such as the induction of proteases, oxidation injury and disruption of the cytoskeleton. [53]

HBsAg has been occasionally identified in the glomeruli of HBV MGN (Table 2-14 page 2-80) usually in mesangial areas but rarely also in the subepithelial deposits. There is not yet final clarity on the role of HBsAg in HBV MGN.

As Bhimma and Coovadia concluded in their comprehensive discussion on the pathogenesis of HBV nephropathy in their review, further studies are needed to confirm or clarify the various hypotheses. They indicated that immune complexes

containing varying combinations of the HBV antigens and antibodies may be responsible for other HBV linked forms of GN such as IgA nephropathy and MCGN. [43, 54] Fig 2-6 below from Bhimma and Coovadia with minor modifications, [43] depicts an hypothesis regarding the pathogenesis of HBV GN.

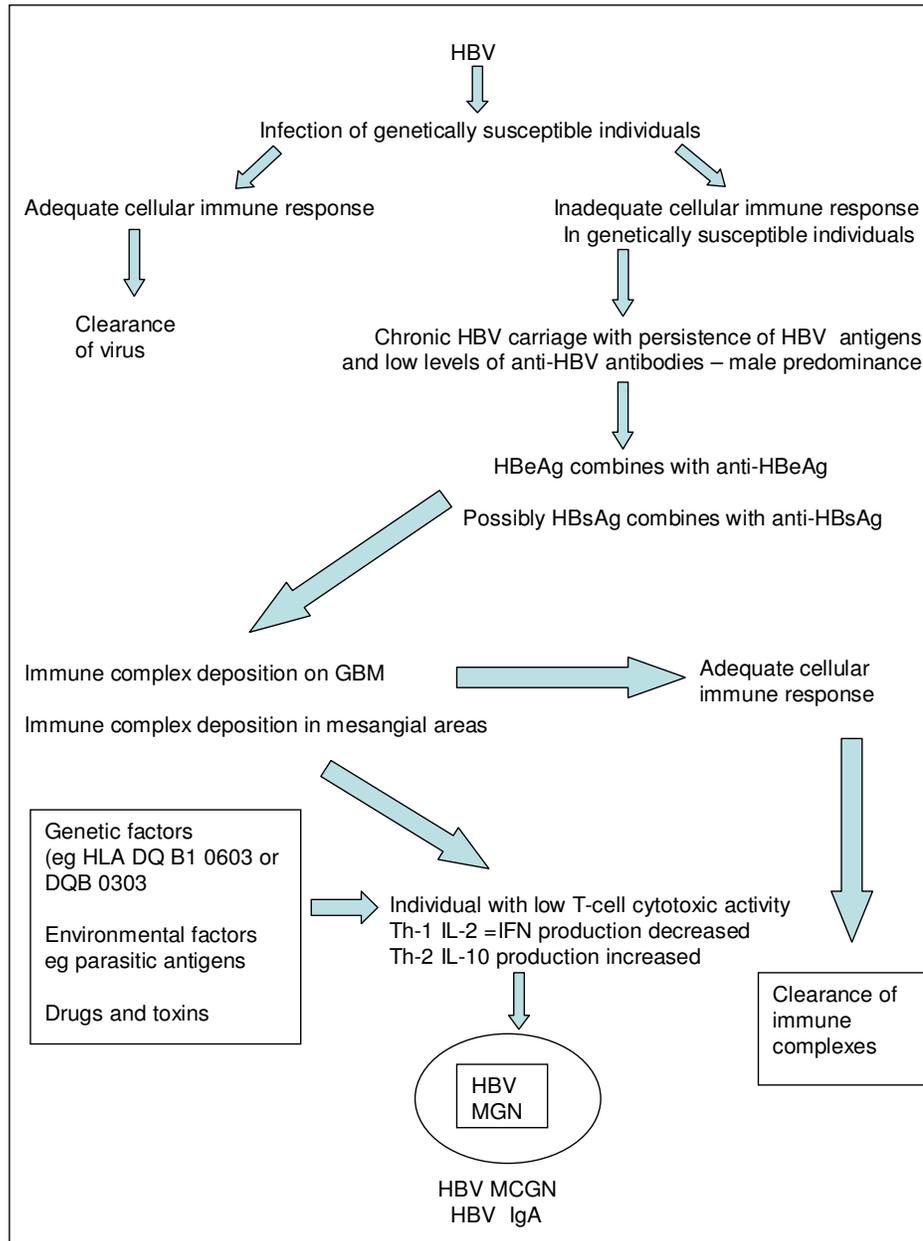


Fig 2-6 Hypothesis for the pathogenetic mechanisms in the development of HBV MGN

From Bhimma and Coovadia Fig 4 [43] with modifications

h) Frequency of HBV MGN and why the disease occurs in some carriers only

An intriguing feature of this disease is that it is restricted to a small number of persistent HBsAg and HBeAg carriers. Estimates regarding incidence of HBV MGN are difficult. Bhimma et al estimated the average annual rate ratio as 0.25 per 100,000 children, equivalent to 2.5 children per million per year presenting with HBV MGN in the KwaZulu-Natal province of South Africa. These calculations were made for the 18 year period from January 1984 to December 2001 during which time 119 children with HBV MGN between ages 0-14 were documented. [49] If on average about 10% of the children of KwaZulu-Natal during that time (1984-2001) were HBV carriers, then the incidence would be 1 per 40,000 HBV childhood carriers per year. The 10% is probably an underestimate. Figures from a study by Karim et al in 1988 reported that black children aged 6-14 in rural areas had an HBsAg carrier rate of 18.5%, urban children in the same age group 10% and institutionalised children 25.1%. [37]

In a large study in Alaska, a total of 1400 serum hepatitis B surface antigen positive Alaska natives, 824 men and 576 woman of all ages, were followed up prospectively over a period of 7815 carrier years (over an 11 year time period) for the development of sequelae related to chronic hepatitis B infection. During the observation period (1975 – 1986), only a single case of MGN, in a child, was seen while 20 cases of hepatocellular carcinoma, 14 cases of chronic active hepatitis and 8 cases of cirrhosis were identified,. [55] This is equivalent to 1 case of HBV MGN per 7815 HBV carrier years acknowledging the complicating factor of mixtures of childhood and adult carriers.

HBV MGN is a rare complication of the HBV carrier state as substantiated by the relative small total number of reported cases (Table 2-6) in relation to the estimated 350 million carriers in 2004. [1]

Why then does HBV MGN occur in only some HBV carriers? The impact of viral genetics on the development of HBV MGN has been investigated in a study which included 2 paediatric and 4 adult Japanese patients. The investigators did not find any association between HBV MGN and HBV genome mutations. [56] Although a high prevalence of HBV genotype A has been reported by some investigators, Chan in a 2010 review, states that this has not yet been established and further evidence is required. [50]

Animal studies by Germuth et al and previous findings that HBeAg is highly related to the subepithelial deposit in HBV MGN led Lin et al (1997) to hypothesise that the development of HBV MGN requires two major determinants. [57] Firstly, persistent HBe antigenaemia in the sera of patients and secondly, trace amounts of anti-HBeAb which are unable to neutralise HBeAg. Lin postulated that excess circulating HBeAg is filtered and deposited in the glomerular basement membrane and then joined in situ with filtered anti-HBe antibodies resulting in the HBV MGN. [57] One of the difficulties with this theory has been that anti-HBe antibodies were usually undetectable by radioimmunoassay (RIA) in the sera of patients with HBV MGN during the early acute nephrotic stage. [58] Lin suggested that this was due to HBeAg:anti-HBeAb immune complex formation which made the anti-HBeAb undetectable by RIA. The level of HBe circulating immune complexes (CIC) was low in HBV MGN patients and absent both in HBsAg+/HBeAg+ patients without HBV MGN and HBs+/HBeAg- asymptomatic virus carriers. [57]

Lin et al also characterised the cellular immune response to HBV in patients with HBV MGN and compared it to that of unaffected carriers. They established a model system by using autologous HBcAg expressing Epstein-Barr virus immortalised lymphoblastoid cell lines (LCL) as stimulator/target cells. Both proliferative response after stimulation with HBcAg and cytotoxic activity against autologous HBcAg expressing LCL of the peripheral blood T cells obtained from HBV MGN patients and asymptomatic HBsAg carriers could be measured. HBV MGN patients had lower cytotoxic activity than HBV carriers and immune children. In vitro cytokine production of peripheral blood T cells after HBc stimulation showed that T-helper cell-1-related Interleukin(IL)-2 and Interferon-gamma production were very low in HBV MGN patients but that T-helper cell-2 related IL-10 production was higher in HBsAg+/HBeAg+ patients with HBV MGN than in those without MGN. Based on these findings they concluded that HBV MGN children seem to have an inadequate cellular response to HBcAg. [57]

i) Immunization and the incidence of HBV MGN

Within 20 years of the discovery of HBsAg by Blumberg, a vaccine against HBV had been developed. [3, 59] The first vaccine contained HBsAg that had been purified from plasma of chronic carriers of serum HBsAg. Later, a vaccine was produced in an organism, *Saccharomyces cerevisiae*. The efficacy of vaccination against HBV was clearly shown in Taiwan where in 1984 a mass vaccination campaign was started. A decrease in childhood carrier rates from 10% to less than 1% occurred as well as reductions in mortality rates from fulminant hepatitis and decreased annual incidence of childhood hepatoma. [60]

The Global Advisory Group of the Expanded Programme on Immunization recommended in 1991 that HBV vaccine be integrated into national immunization programmes: those with an HBsAg prevalence of 8% or greater by 1995 and all countries by 1997. This was endorsed by the WHO in 1994 and although not immediately achieved, considerable progress has been made. By 2004 HBV vaccine had been introduced into routine infant immunization programmes in 153 (79.7%) of the 192 WHO member states increasing to 193 by 2006. Coverage grew in the worldwide infant target population from 1% in 1990 to 55% in 2005. [61]

The HBV vaccine was introduced into the South African Expanded Programme on Immunisation on April 1, 1995. Vaccine coverage rates for children for the first, second and third doses were 85.4%, 78.2% and 62.0% respectively. Bhimma et al [49] examined the impact of the HBV vaccine on the incidence of HBV MGN in their centre and by extension, the province of KwaZulu-Natal in SA. Between January 31, 1984 and December 31, 2001 the average annual rate ratio was 0.25 per 100,000 children; the overall rate ratio decreased to 0.12 for 2000-2001 compared to the pre-immunisation period 1984 -1994. No cases of HBV MGN were seen in their centre in children from birth to the age of 4 after 1998. Children aged 5 to 10 years showed a significant decrease in 2000-2001 in the incidence of HBV MGN compared with pre-vaccination years. (Incidence rate ratio 0.19) The authors concluded that the HBV vaccine, even at relatively low coverage, reduced the hospital incidence of HBV MGN over a six year period. They intended to monitor the situation to ensure that the decline was permanent and would also be reflected in the 10-14 year age group. [49]

A study from China also evaluated the effect of hepatitis B vaccination on the incidence of childhood HBV MGN and other HBV associated GN. Of the 727 renal biopsies, 64 were HBV associated and 46 (72%) of the 64 were MGN. There were

6/231 cases of HBV MGN (2.6%) in the vaccinated group while 40/381 (10.5%) cases of HBV MGN occurred in the non vaccinated group ($p < 0.01$). Of interest and concern were 6 cases of HBV MGN occurring in vaccinated children. In the 2 mothers tested, infection with HBV was found. Mothers with HBV can transmit intra-uterine infection and diagnosing and treating them during pregnancy may reduce this possibility. The children should also have special follow up. With routine vaccination, they believed the incidence of MGN should decrease. Their series showed a downward trend from 8.53% in the pre-vaccination era to 4.11% in 2000-2002 but this failed to reach statistical significance. (The decrease in the broader HBV-GN group, 64 as opposed to 46 MGN patients was statistically significant.) All the cases of MGN in this series were found in HBV positive children. [29]

In 2005, 20% of all unvaccinated children lived in sub-Saharan Africa demonstrating that a challenge still remains. Namibia was the only country in Southern Africa which had not implemented routine HBV vaccination by 2008. The SA HBV immunization programme began in 1995 and by 2005 had 94 % coverage. [61]

Zimbabwe, from where Seggie reported 8 children with HBV MGN in 1984, gathered in 18 months from Harare hospitals, began universal vaccination of infants in 1994 and by 2005 had 90% coverage. [61, 62]

In summary, as a public health measure HBV immunisation has reduced the risk of cirrhosis and hepatocellular carcinoma in high risk areas. Vaccination also has the effect of reducing the incidence of HBV GN and MGN. This has been documented in children in China and South Africa. [29, 49]

3. REPORTED CASES OF HBV MGN

In Table 2-6, all the reported HBV MGN cases have been included in chronological order of publication. Children, generally defined as aged 12 years or younger (although this definition varies between publications), have been separated from adults where the information was available. Information on gender has been included where available. The criteria applied for inclusion in the table and to be included in the statistics for HBV MGN were the same as for the current study, namely serum HBsAg positivity and a renal biopsy diagnosis of MGN without other possible causes of MGN such as SLE, syphilis or malignancy. Some reports are included for historical perspective, because they are from Africa as well as two earlier reports from this hospital (TBH) and group. In these instances where studies are in the table but not included in the HBV MGN statistics a superscript denotes with an explanation at the bottom of the page. Where groups had more than one report, it was difficult to ensure that cases were not counted more than once. There is therefore a margin of error in the overall summary statistics but this is unlikely to undermine the broad findings.

Note for Table 2-6 page 2-31 and following:

Child - Less than or equal to 12 years of age.

Adult – 13 years of age or older.

This information as well as gender data was not always available in detail and therefore some inferences were made.

Table 2-6 Cases of HBV MGN reported in the literature

Author	Year	Child No.	Child m:f	Adult No.	Adult m:f	Country	Ref.
Combes	1971	-	-	1	1:0	USA	[8]
Knieser	1974	-	-	1	1:0	USA	[63]
Ainsworth	1974	-	-	1	1:0	USA	[64]
Brzosko	1974	2	-	-	-	Poland	[11]
Ertugrul	1974	2	2:0	1	1:0	Turkey	[65]
Kohler	1974	-	-	1	1:0	USA	[66]
Blaker	1974	2	-	-	-	Germany	[67]
Bajtai	1975	-	-	1	1:0	Hungary	[68]
Mrozowicz	1976	7	-	-	-	Poland	[13]
Ozawa	1976	1	1:0	-	-	USA	[69]
Moriyama	1976	-	-	1	0:1	Japan	[70]
Cogan	1977	-	-	1	1:0	USA	[71]
Cameron	1978	1	0:1	-	-	Zambia,UK	[30]
Takekoshi	1978	11	9:2	-	-	Japan	[15]
Kleinknecht	1979	15	13:2	-	-	France	[16]
Nagy	1979	-	-	4	-	Hungary	[72]
Thomas	1979	-	-	1	1:0	UK	[73]
Silver	1979	1	1:0	-	-	Canada	[74]
Takekoshi	1979	2	2:0	-	-	Japan	[17]
Sluzarczyk	1980	21	-	-	-	Poland	[75]
Hirsch	1981	1	1:0	-	-	USA	[76]
Rashid	1981	-	-	1	1:0	UK	[77]
Nagata	1981	1	1:0	-	-	Japan	[45]
Ito	1981	4	5:1	-	-	Japan	[18]
Furose	1982	6	6:0	-	-	Japan	[46]
Kim	1982	9	8:1	-	-	Korea	[78]
Glassock	1982	-	-	1	1:0	USA	[79]
Abdurrahman ^a	1983	9 MCGN	-	-	-	Nigeria	[80]
Amemiya	1983	1	0:1	-	-	Japan	[19]
Adhikari ^b	1983	31	-	-	-	SA, Durban	[81]
Wiggelinkhuizen	1983	25	24:1	-	-	SA, Cape Town	[21]
Collins	1983	-	-	1	1:0	USA	[20]
Hsu	1983	13	12:1	-	-	Taiwan	[82]
Vecchio-BI	1983	9	8:1	-	-	Italy	[83]
Dreyer ^c	1984	2	2:0	19	12:7	SA, Pretoria	[84]

^a Included as from Africa. No ultrastructure done. Some cases likely to have been MGN. Not included in MGN statistics.

^b HBV testing began during this series. Not included in overall figures.

^c There is uncertainty with these figures as the inclusion criteria for HBV MGN were primarily glomerular HBV staining usually without serum HBsAg testing and in some cases despite negative HBsAg serum tests. (Serum HBsAg tested in 11 patients, positive in only 3) Included as a SA study with a pathology emphasis. Not included in the HBV MGN statistics but children included in Thomson report. [85]

Author	Year	Child No.	Child m:f	Adult No.	Adult m:f	Country	Ref.
Akinsola ^a	1984	-	-	11 MCGN	-	Nigeria	[86]
Awunor-Renner ^b	1984	-	-	3	-	Nigeria	[87]
Chow	1984	5	5:0	-	-	Hong Kong	[88]
Via	1984	-	-	1	1:0	USA	[89]
Seggie	1984	7	3:4	1	1:0	Zimbabwe	[62]
Hirose	1984	10	4:6	6	6:0	Japan	[47]
Wyzynska ^c	1984	34	26:8	-	-	Poland	[90]
Cadrobbi	1985	1	1:0	-	-	Italy	[91]
Southwest	1985	11	11:0	-	-	USA	[22]
Sham	1985	-	-	5	4:1	Hong Kong	[92]
Garcia	1985	1	1:0	1	1:0	USA	[23]
La Manna	1985	9	8:1	-	-	Italy	[93]
Yoshikawa	1985	16	13:3	-	-	Japan	[94]
Magil	1986	-	-	1	1:0	Canada	[95]
Gregorek ^d	1986	19	14:5	-	-	Poland	[96]
Ikeda	1986	-	-	1	1:0	Japan	[97]
Esteban	1986	1	1:0	-	-	Spain	[98]
Dreyer ^e	1986	6	6:0	13	-	SA, Pretoria	[99]
Zacchello	1986	6	-	-	-	Italian	[100]
Lai ^f	1987	15	-	10	-	Hong Kong	[28]
Wiggelinkhuizen	1987	46	-	-	-	SA, Cape Town	[101]
Takekoshi ^g	1987	16	13:3	-	-	Japan	[102]
Guerra	1987	-	-	1	1:0	USA	[103]
Mizushima	1987	-	-	1	1:0	Japan	[104]
Nammalwar	1987	2	-	-	-	India	[105]
Hattori	1988	3	3:0	-	-	Japan	[106]
Chen	1988	2	1:1	3	3:0	Taiwan	[107]
Lee	1988	8	5:3	10	9:1	Korea	[108]
Elidrissy	1988	3	3:0	-	-	Saudi Arabia	[24]
Milner	1988	14	-	-	-	SA, Joburg	[109]
Ishihara	1988	1	1:0	2	2:0	Japan	[110]
Akano	1989	5	1:4	1	1:0	Japan	[111]
Lai KN ^h	1989	17	15:2	11	-	Hong Kong	[112]

^a Included as from Africa. No ultrastructure done. Some cases likely to have been MGN. Not included in MGN statistics.

^b From Africa. No ultrastructure. Again possible that more cases categorised as proliferative GN (19) had MGN elements.

^c Some uncertainty about exact numbers. Element of estimation as information not complete for MGN subgroup.

^d Similar to above. Uncertainty about exact numbers. Element of estimation as information not complete for MGN subgroup.

^e Uncertainty with these figures as the inclusion criteria for HBV MGN were primarily glomerular HBV staining usually without serum HBsAg testing and in some cases despite negative HBsAg serum tests. (Serum HBsAg tested in 14 patients – positive in only 5) Included as a SA study with a pathology emphasis. Not included in the HBV MGN statistics but children included in Thomson report. [85]

^f Some uncertainty about exact numbers for gender in each age group. Overall gender m:f 22:3. See ref 112.

^g Review including the cases from 1978 in reference 15. Therefore only this series used for statistical purposes of HBV MGN cases.

^h Appears to be the same patient group as Lai 1987 Ref 28 above. Only the 1989 group used for statistical purposes.

Author	Year	Child No.	Child m:f	Adult No.	Adult m:f	Country	Ref
Thyagarajan	1989	-	-	4	-	India	[113]
Zhang	1989	-	-	9	-	China	[114]
Lisker-Melman	1989	-	-	4	4:0	USA	[115]
Lee	1989	-	-	1	1:0	Korea	[116]
De Man	1989	1	1:0	-	-	Netherlands	[117]
Hsu	1989	52	40:12	-	-	Taiwan	[25]
Lin ^a	1990	34	25:9	-	-	Taiwan	[118]
Abdurrahman ^b	1990	1	-	-	-	Nigeria	[119]
Venkatase	1990	6	4:2	4	3:1	USA	[120]
McMahon	1990	1	1:0	-	-	USA Alaska	[55]
Lai KN	1991	-	-	21	17:4	Hong Kong	[26]
Wrzolkowa	1991	77	58:19	-	-	Poland Gdansk	[121]
Madalinski	1991	51	41:10	-	-	Poland Warsaw	[122]
Hong SY	1991	-	-	8	2:6	Korea	[123]
Schectman	1991	-	-	1	1:0	USA	[124]
Ozen	1992	5	5:0	-	-	Turkey	[125]
Wong	1992	2	2:0	-	-	Hong Kong	[126]
Coovadia	1993	57	-	-	-	SA Durban	[127]
Giboa	1993	1	1:0	-	-	USA	[128]
Wong	1993	18	15:3	-	-	Hong Kong	[129]
Chen	1993	-	-	2	1:1	France	[130]
Lidman	1993	-	-	1	1:0	Sweden	[131]
Nakopoulou	1994	5	5:0	2	2:0	Greece	[132]
Gilbert	1994	70	59:11	-	-	SA Cape Town	[48]
Conjeevaram ^c	1995	-	-	10	8:2	USA	[133]
Mouthon	1995	-	-	1	1:0	France	[134]
Kavukcu	1995	1	1:0	-	-	Turkey	[135]
Shapiro	1995	-	-	1	1:0	Canada	[136]
Tomonaga ^d	1996	1	-	-	-	Japan	[27]
Levy	1996	1	0:1	-	-	France	[137]
Bates ^e	1996	1	0:1	-	-	SA, Cape Town	[138]
Chung	1997	-	-	2	2:0	Korea	[139]
Bhimma	1997	81	51:30	-	-	SA, Durban	[140]
Ohba	1997	-	-	1	0:1	Japan	[141]
Thomson ^f	1997	87	58:29	-	-	SA, Gauteng	[85]
Bhimma	1998	70	54:16	-	-	SA, Durban	[142]

^a Lin's cases may be included in Hsu's report above. Reference 25.

^b HBV again reported as more associated with proliferative GN. Ultrastructure was carried out on 26 of the 98 renal biopsies in this series of 100 Nigerian childhood nephrotics.

^c Gender breakdown not available for MGN subgroup. This estimate includes the 2 females in the total group of 15.

^d Unusual case with renal failure after 17 years follow up. No biopsy to prove HBV MGN, therefore excluded from statistics.

^e Report from this centre (TBH) and group. Not included in the literature review HBV MGN statistics.

^f This survey reports summarised data without detail which required assumptions and estimates to produce these figures. Therefore some uncertainty regarding the numbers. The report is dealt with in more detail in the next section on Southern Africa page 44.

Author	Year	Child No.	Child m:f	Adult No.	Adult m:f	Country	Ref.
He	1998	30	-	-	-	China	[143]
Lopes	1998	-	-	1	1:0	Brazil	[144]
Gonzalo	1999	1	0:1	-	-	Spain	[145]
Van Buuren ^a	1999	25	22:3	1	1:0	Namibia	[146]
Taskapan	2000	-	-	1	1:0	Turkey	[147]
Cronje	2000	3	1:2	-	-	SA Bloemfontein	[148]
Lai FMM	2000	-	-	22	18:4	Hong Kong	[149]
Pena	2001	12	11:1	-	-	Spain	[150]
Miller ^b	2002	8	5:3	-	-	Jamaica	[151]
Bhimma	2002	22	-	-	-	South Africa	[152]
Connor	2003	1	1:0	-	-	Australia SA child	[153]
Ozdamar	2003	6	5:1	-	-	Turkey	[154]
Xu ^c	2003	46	40:6	-	-	China	[29]
Filler	2003	1	0:1	-	-	Canada Vietnamese	[155]
Park	2003	-	-	11	10:1	South Korea	[51]
Bhimma ^d	2003	119	101:18	-	-	South Africa	[49]
Azad	2004	-	-	4	-	Pakistan	[156]
Rihova	2005	-	-	3	2:1	Czech	[157]
Sayarlioglu	2005	-	-	1	1:0	Turkey	[158]
Tang	2005	-	-	10	7:3	Hong Kong	[159]
Izzedine	2005	-	-	1	0:1	France	[160]
Gan	2005	-	-	2	1:1	Canada Chinese	[161]
Dang	2006	9	-	-	-	China	[162]
Kanaan	2006	-	-	1	1:0	Belgium	[163]
Kim	2006	7	-	-	-	South Korea	[164]
Panomsak	2006	-	-	6	4:2	Thailand	[165]
Okuse	2006	-	-	1	1:0	Japan	[166]
Kusakabe	2007	1	1:0	4	4:0	Japan	[56]
Chuang	2007	-	-	1	1:0	Taiwan	[167]
Rodrigues	2007	1	1:0	-	-	USA Thai	[168]
Mesquita	2008	-	-	1	1:0	Belgium	[169]
Zeng	2008	-	-	47	41:6	China	[170]

^a Report from this centre (TBH) and group. Not included in the literature review HBV MGN statistics.

^b Gender breakdown not available for MGN subgroup. This estimate includes the 3 females in the total group of 10 HBV GN.

^c Gender breakdown not available for MGN subgroup. This estimate includes the 6 females in the total group of 64 HBV GN

^d Overall number and gender breakdown used from Durban studies.

There were at least 1160 reported cases of HBV MGN in the English accessible literature by December 2009: Childhood HBV MGN 918 (79%) and adult 242 (21%). (Fig 2-7 a) In the 1053 where gender could be ascertained the proportion was 81% male. In the childhood HBV MGN group the male proportion was 79% and 83% in the reported adult HBV MGN patients . (Fig 2-7 b)

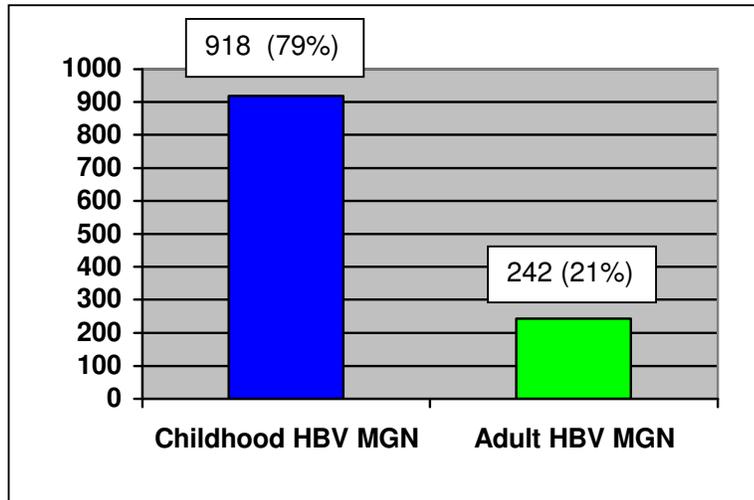


Fig 2-7 (a) Total numbers of reported HBV MGN patients 1971 -2009

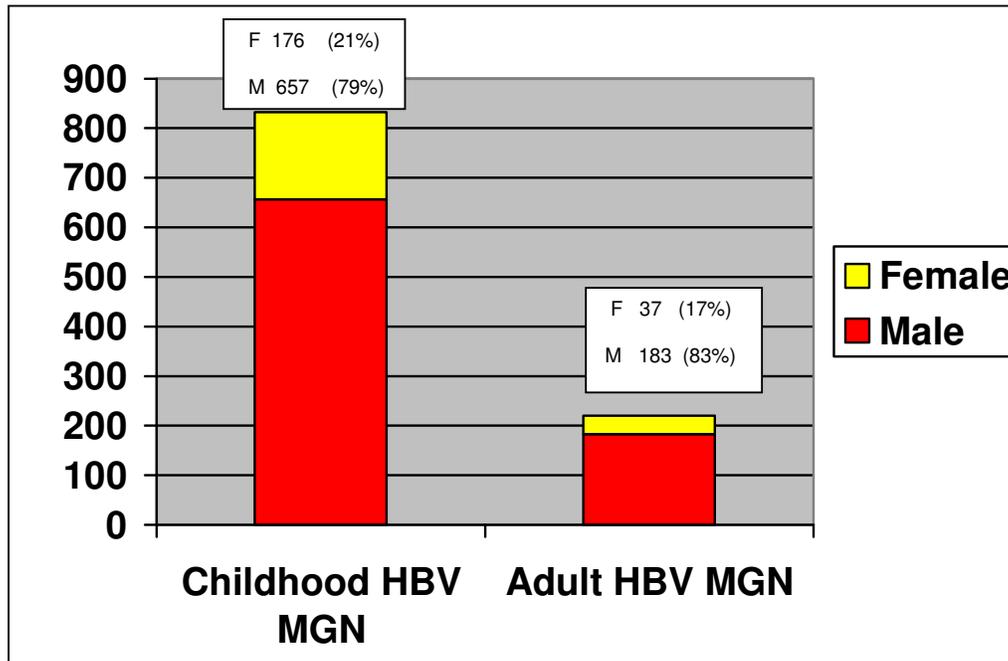


Fig 2-7 (b) Gender proportions in reported childhood and adult HBV MGN 1971-2009

4. HBV MGN in Southern Africa

Despite the universally high prevalence of serum HBsAg in Africa, Fig 2-1 [32], especially sub-Saharan Africa, there are relatively few reported cases or series of HBV associated renal disease, except from Southern Africa. Reports from Nigeria lacked ultrastructural examination of biopsies making the diagnosis of MGN and the distinction from MCGN and overlaps between them difficult if not impossible to make. [80, 86, 87]

Table 2-7 *Nephrotic syndrome and HBV MGN in Southern African children*

	Study period	Nephrotic cohort		MGN in nephrotic group		HBV MGN of MGN group		HBV MGN Gender		HBV MGN Renal failure		Ref
	Years	No.	race	No.	(%)	No.	(%)	No. male (%)	No.	(%)		
South African												
Kwazulu-Natal Durban	20	236	Black	94	(40)	81	(86)	51/81	(63)	5	(6)	[140, 142]
		286	Indian	7	(2,4)	0						
		23	Coloured	6	(26)	0						
		545		107	(20)			101/119	(85) ^a			[49]
Gauteng Johannesburg Pretoria	10 11.5	720	Black	97	(13)	87	>(90)	± 58/87	(67)	No follow up		[85]
								2,86:1 for < 10 years				
Western Cape Cape Town Red Cross	21	559	all races	92	(16)	70	(76)	59/70	(84)	2	(3)	[48]
Freestate Bloemfontein	4	57	Black	2	(3,5)	2	(100)	1/3	(33)	No follow up		[148]
		3	Coloured	1	(33)	1	(100)					
		6	White	0	(0)							
		66		3	(4,5)							
Zimbabwe	1.5	23	Black	8	(35)	8	(100)	4/8	(50)	1	(12.5)	[62]
Total		1913		307	(16)	249		173/249	(69)	8	(3)	
						Of MGN	(81)	223/287	(77) ^b			
						Of nephrotics	(13)					

^a These are the gender proportions from this 2003 article by Bhimma et al [49]

^b This is the gender ratio using the Bhimma et al 2003 article which has the largest number of childhood HBV MGN patients reported by the group and raises the overall reported proportion to 77% male in childhood HBV MGN from Southern Africa.

Vos et al were the first to suggest a relationship between persistent hepatitis B antigenaemia and renal disease in Southern African blacks. They reported an HBsAg carrier rate of 20.3% in 182 black adults with chronic renal disease compared to a 9.8% carrier rate among 5130 black blood donors in Durban, KwaZulu-Natal and suggested that the HBsAg carrier state may predispose to the development of chronic renal disease. [12] Vos et al subsequently documented a difference in HBsAg carrier rate between rural (15.5%) and urban populations (7.4%). In this epidemiological study the rural subjects were mothers and their children from the Transkei, Eastern Cape and the urban patients again adult male and female blood donors from Durban. [31] It is likely that the blood donors were a self-selected urban population while the chronic renal patients in the initial study reflected both urban and rural populations, accounting in part for the carrier rate discrepancy, known to be lower in urban populations. [39]

A number of other Southern African studies documented the relatively high incidence of MGN and more specifically HBV MGN in Southern African black and coloured children with nephrotic syndrome or severe proteinuria. [14, 44, 53, 68, 73, 76, 90, 93, 102, 120, 133, 135, 165, 166] The Southern African data is summarised in Table 2-7. The MGN group constituted 16% of the total of 1913 nephrotic children. HBV MGN was shown in 249 of the 307 (81%) children with MGN, most of whom were black African, with a substantial group of coloured children from the Cape Town series. Of note is the variation in proportions of MGN as cause of nephrotic syndrome (and by extension HBV MGN) in different studies as highlighted by Thomson in 1997. [85] This proportion ranges from 40% in the black children in the KwaZulu-Natal group, 35% in the Zimbabwe series to 16% in Cape Town, 13% in the Gauteng region and 4.5% in the Bloemfontein series. [48, 62, 85, 142, 148] Some of these

aspects are more fully dealt with in the Southern African section of the childhood HBV MGN discussion, page 6-31.

In the following section of the literature review, (page 2-47), the features of HBV MGN are reviewed systematically and many contributions from the Southern African series will be mentioned there, integrated into the appropriate topics. This section includes a general overview of the South African series highlighting issues that may not fit easily in the systematic review.

CHRONOLOGICAL REVIEW OF SOUTHERN AFRICAN CASES AND SERIES OF CHILDHOOD NEPHROTIC SYNDROME AND HBV MGN

KWAZULU-NATAL In 1976, Adhikari et al published the first clinicopathological description of nephrotic syndrome in children in South Africa. In the group of 30 black children from KwaZulu-Natal, MGN and MCGN were the most frequent patterns while minimal change disease was most frequent in the Indian children. Electron microscopy was not available and although one black child with MCGN was noted to be HBV positive, routine testing for HBV was not yet available. (No white children were reported in this series.) [14]

ENGLAND/ZAMBIA One of the earliest cases of HBV MGN with a Southern African connection was reported in 1978 in England. The patient was a 9 year old white girl who had almost certainly become an HBV carrier after her mother developed clinical jaundice while they were both in Zambia. To our knowledge, no other case has ever been documented in a white child in Southern African. [30] Of interest Cameron, who suggested a possible link between MGN and HBV in children from Hong Kong, before it became established, contributed to this publication. [9] This single case,

diagnosed in the UK, is not included in the table above as it is unlikely to be representative of HBV MGN in children in Zambia.

In 1983, two substantial series of children with MGN were published from South Africa.

WESTERN CAPE Wiggelinkhuizen et al reported 28 cases of MGN in children of whom 25 were HBsAg carriers. Of this latter group 24 were boys. They made several novel observations and confirmed other relatively new ideas which have subsequently been confirmed. [21]

Firstly, a central ultrastructural renal biopsy observation was that in addition to the subepithelial deposits of MGN, massive thickening of the basement membrane and at times striking interposition of mesangial cytoplasm and matrix between the basement membrane and endothelium of the glomerular capillary loops were seen. Wiggelinkhuizen et al emphasised that as late HBV associated glomerulonephritis may resemble mesangiocapillary glomerulonephritis on examination by light microscopy, electron microscopy was needed in order to make the correct diagnosis of MGN with further associations. On review, it appears striking, that although they made and illustrated this novel finding, they did not sufficiently emphasise this anomaly or note how it broadened the spectrum of MGN and indeed questioned the very categories on which GN classifications were based. Also while focusing on mesangial interposition they did not specifically mention mesangial deposits, an unusual feature in idiopathic MGN. [21]

Secondly, like some other authors, they failed to detect HBsAg in the glomerular wall of renal biopsies, [15, 16] but did not test for other antibodies such as HBe. Thirdly,

they noted that while HBsAg persisted in 80% of patients after recovery from glomerulonephritis, the remission of proteinuria correlated well with seroconversion to anti-HBe. In this series full recovery took 20 months on average and about 66% of the patients (10 children) followed 3 years or longer were in remission. This confirmed the findings of Ito et al who had reported two patients in 1981 in remission from HBV MGN who had seroconverted to HBeAb. [18]

KWAZULU-NATAL Adhikari et al, also in 1983, reported that membranous nephropathy was the most frequent histological category among 104 black children with nephrotic syndrome. [81] Thirty-one (29.8%) showed MGN predominantly evaluated on light microscopy as only 3 cases were also examined ultrastructurally. Males predominated (77.4%) with a peak age range of 4 to 11 years. They noted that the incidence of this histological category and clinical outcome in the African children were similar to that in adults with idiopathic MGN. Hypertension (19.3%), the low remission rate (33.3%) and the persistence of proteinuria were similar to the disease in adults. Surprisingly serum HBsAg was present in only three of six children tested but serum HBsAg testing appears only to have begun during the course of the study. In a subgroup with proliferative changes on histology five of eight patients tested were HBsAg positive. They concluded that a direct association of HBV with MGN was not justified but added that, since 1981, however, a further 12 patients with MGN and nephrotic syndrome had been investigated by this group of whom ten were serum HBsAg positive. [81] These findings and the subsequent reports by this group, [127, 142] suggest that technical difficulties with HBV serum testing and the fact that HBV MGN can produce a proliferative appearance (leading to misclassification especially in the absence of ultrastructural examination of renal biopsies) may have prevented the association of MGN with HBV from being clarified earlier by these authors.

Reporting from **ZIMBABWE** in 1984, Seggie et al [62, 171] reviewed all cases of patients with nephrotic range proteinuria over 18 months and found that the major patterns were post-streptococcal GN in young adults and HBV MGN in children. In their study, 8 of 23 (35%) children with nephrotic syndrome had MGN and all were serum HBsAg positive. Serum HBe was absent in all 8 patients. The equal number of male 4/8 (50%) and female is unusual but the series is small. One child developed renal failure, only the second child documented with HBV MGN and renal failure. [62]

KWAZULU-NATAL The same group as mentioned above reported on 178 African children with nephrotic syndrome from 1981 to 1988 (Coovadia et al 1993). [127] Membranous nephropathy was the most common histological lesion occurring in 60 (34%). Fifty-seven (95%) of the latter children were serum HBsAg positive and 52 of these 57 were serum HBeAg positive. The 52 represent 87% of the MGN group as a whole and 91% of the HBsAg positive group. HBsAg was detected in only 6 (5%) and HBeAg in the serum of one (0,8%) of the 118 patients with non membranous lesions. The relative risk of membranous nephropathy was 35 and 15 times higher in the 178 African children with nephrotic syndrome who had serological evidence of HBsAg and HBeAg, respectively. [127]

The sensitivity of the test used at the time was 95% for HBsAg and 87% for HBeAg, specificity, positive and negative predictive values were above 90% for each antigen. The strength of such an association in this particular community, it was proposed, permitted reliable prediction of MGN from serological tests for HBsAg, thereby avoiding the need for renal biopsy. [127]

GAUTENG – PRETORIA In the biopsy series of Dreyer (MD thesis Pretoria University 1986) Gauteng Province, HBV positive staining in glomeruli was found in 22/41 (53.6%) cases of MGN and 17/39 (43.6%) cases of MCGN. (The overall study was of 342 renal biopsies from children and adults with GN, most were stained for HBsAg and HBcAg in the glomeruli and were examined ultrastructurally, but HBeAg was not stained for in the glomeruli.) All the children with MGN were black boys and 6/7 had glomerular staining for HBsAg and/or HBcAg. Cases identified as HBV MGN had significantly more mesangial deposits, 18/22 vs 4/19 ($p = 0,0001$). (The group of 19 cases was negative for HBs and HBc in the glomeruli – viewed as idiopathic MGN.) Subendothelial deposits (5 cases) were also more common in the HBV group ($p = 0,0351$) and 2 of these cases also showed mesangial interposition. No viral-like particles were seen but no mention was made of tubuloreticular bodies being looked for or identified. [99] Testing for HBV in the serum was incomplete. Thirty-one of the 43 in the MGN group had been tested for HBV serum markers. Only 5 were HBsAg positive. Nine in the group who were serum HBsAg negative tested positive in glomeruli for HBsAg and/or HBcAg and were included in the HBV MGN group. These findings call in to question the reliability of both serological testing and glomerular staining, the latter in particular.

WESTERN CAPE - CAPE TOWN Wiggelinkhuizen et al reported on 63 (16%) of 388 nephrotic children followed between 1969 and 1985 in Cape Town with MGN. They were either black or coloured children but none was white or Asian. (There were white and Asian children in the nephrotic cohort as a whole.) Of the 53 MGN patients tested, 46 (86,7%) were serum HBsAg positive and 80% serum HBeAg positive whereas the prevalence of HBsAg in patients with glomerular disease other than MGN was 10,8%. [101] The racial breakdown for the MGN group of 63 was 20

black (32%) and 43 (68%) coloured. [101] The initial report of 25 HBV MGN patients by this group, noted 8 (32%) black and 17 (68%) coloured children. [21] It appears that proportion has remained similar over time. One reason to emphasise this racial subdivision is that race may be a direct or indirect prognostic factor. Wiggelinkhuizen et al noted that the prognosis of coloured children with MGN was better than for blacks with 35/41 (85%) of the coloured children achieving remission compared to 8/14 (57%) for the black children. [101]

GAUTENG, JOHANNESBURG Milner et al reported (1988) that 14/59 (23.7%) black children with nephrotic syndrome, seen between 1981 and 1985 were serum HBsAg and HBeAg positive, and one half had circulating HBV DNA. All these children had MGN. In comparison to the other 45 children with idiopathic nephrotic syndrome the C₃ levels were depressed and ALT and AST levels were elevated. The gender of the children was not reported. [109]

WESTERN CAPE, CAPE TOWN The series of Gilbert et al (1994) on the clinical course of HBV associated GN is comprehensive and expands the earlier work of Wiggelinkhuizen et al. [48] The clinical features confirmed previous descriptions. Gilbert et al noted that some degree of glomerular basement membrane splitting was observed in 42 of 70 initial renal biopsies. In 8 this was circumferential in the majority of capillary loops. There was increased mesangial matrix in all biopsies, which was severe in 7. Mesangial cellular proliferation was present in 93%. HBsAg was demonstrated in 15 of 24 and core antigen in 16 of 22 renal biopsies. There was no correlation between the interval from onset of symptoms to biopsy and grade of MGN, or between grade, degree of mesangial interpositioning, tubular atrophy, interstitial cellular infiltrate and interval to remission. The renal histopathological

features associated with HBV infection they believed were characteristic, showing a combination of features of MGN and MCGN. In most cases features of MGN dominated. Thirty-seven (37) of the children went into remission associated with HBeAg seroconversion. Two developed CRF, one of whom underwent renal transplantation. [48]

DURBAN KWAZULU-NATAL In 1998 the Durban group described 70 children with biopsy proven HBV MGN. There were 54 boys (77%) and 16 girls (23%) with mean age of onset being 7.1 years. Co-existing liver disease occurred in 18 (25.7%) and hypocomplementaemia C₃ (47.1%) and C₄ (11.4%) respectively. HBV MGN was clinically indistinguishable from 24 children with idiopathic MGN although some biochemical features were different. In particular, complement levels were significantly lower in the HBV children but liver enzymes were not significantly different in the two groups. Twelve children remitted, 10 of these clearing both serum HBsAg and HBeAg while the remaining 2 cleared serum HBeAg. Five showed varying degrees of renal impairment. [142]

Thomson summarised much of the South African experience of renal problems in black children in a 1997 survey which included the patients in the abovementioned Bhimma et al reports. [140, 142] The reports of SA authors comprising 954 black children with nephrotic syndrome were reviewed. In this report pooling SA experience, HBV MGN was not the most frequent cause of nephrotic syndrome in black children but in fact third in the consolidated data: Focal segmental glomerulosclerosis [FSGS]- 30.5%; MCD-20.7%; MGN-20.0% (mostly but not exclusively HBV associated.) [85] In KwaZulu-Natal (Durban) MGN was the predominant category with 40.2% but the Gauteng figure was much lower at 13.5%.

Thomson stated 'Some adult nephrologists in South Africa are still under the misconception that membranous nephropathy is the commonest cause of nephrotic syndrome in black South African children. FSGS was not even mentioned in the reference'. (Of YK Seedat) [172] The contention of Thomson can be disputed because for many decades published series such as from Durban did show that MGN and in particular HBV MGN was the most common cause of nephrotic syndrome in the children they treated. [81, 85, 140, 142]

The surprisingly low carrier rate of HBV in black children in urban areas like Soweto, long before vaccination was initiated – boys 1.5%, girls 0.57% in 1986 compared to KwaZulu Natal (rural 18.5%, urban 10.0% in 1988) and Northern Namibia; 13% in 1984, seem largely to account for these variations in frequency of HBV MGN. [37, 40, 42]

Thomson's report provides some difficulties. Firstly the age of the children for the Johannesburg and Pretoria data is 'under 21' which is older than for most childhood series making comparison more problematic. Secondly, while the Durban data of Bhimma et al seems to be essentially that published in their 1997 and 1998 reports [140, 142], the Johannesburg and Pretoria data has not been published in detail elsewhere. (Subgroups have been documented by Dreyer [84, 99] and Milner [109]) This means that some of the important detail of the data such as exact HBV numbers, gender and ages of patients eg those 13 – 20 years, clinical features, biopsy detail, course and outcome, is not available. This precludes further interrogation of the data and means that some figures in Table 2-7 are estimates based on Thomson's data. [85]

These Southern African series include more than 30% of the documented cases of HBV MGN in children and have been of special interest and relevance to those working in Southern Africa. Childhood HBV MGN is discussed later in chapter 6 where our experience at TBH is integrated and the Southern African experience as a whole further discussed . (Chapter 6 pages 33-37 - Tables 6-3 and 6-4.)

5. LITERATURE REVIEW BY FEATURES PERTAINING TO CHILDHOOD HBV MGN, ADULT HBV MGN AND IDIOPATHIC MGN

The documentation and more complete understanding of different forms of glomerular disease require a wide range of detailed demographic, laboratory, renal biopsy, treatment and outcome data to be gathered, compared and correlated to establish clinicopathological entities. [173, 174] Comparisons enable the similarities and differences between possible entities to be established. In this section, the features of childhood HBV MGN, adult HBV MGN and idiopathic MGN are summarised and contrasted.

a) DEMOGRAPHIC DATA

AGE

Children with HBV MGN typically presented at 2-12 years (mean: 6 years). [175] In the series of Hsu (1989), the 52 children with HBV MGN ranged from 2-16 years with a mean of 7.6 years. [25] (Note that the limit used to define children will affect the means and ranges.) The series of 77 children with HBV MGN, from Poland reported by Wrzolkowa (1991) did not provide complete demographic data but it was noted that the disease was observed mainly in young children, 90% of them being under 6 years of age. [121]

The age range of the 70 patients of Gilbert (1994) at presentation was from 13 months to 13 years with a mean age at onset of nephrotic syndrome of 6.6 years. [48] The largest series from a single centre was from Durban, SA with 119 children over a 17 year period with a range of 1-14 years and a mean of 7 years. [49]

The Hong Kong series of 21 adults with HBV MGN had an age range from 15-53 years with a mean of 30 years. [26]

Idiopathic MGN, although seen throughout adult life has a peak incidence from 30 – 50 years. [176] Ehrenreich and Churg had 60 patients ranging in age from 3 to 79 with 6 under 15 years. Half of the patients were between 41 and 60 years of age. [5]

In summary, there is a bimodal age distribution of HBV MGN with mean ages of 6 to 7 years in children and 30 years in adults. Idiopathic MGN in adults has a mean age a decade later of 40 years.

GENDER

In most human populations there is a greater prevalence of chronic carriers of HBV among males than females. Females are more likely to produce anti-HBs in response to infection. Diseases associated with HBV carrier state such as chronic hepatitis, post necrotic cirrhosis, primary hepatocellular carcinoma, polyarteritis nodosa, cryoglobulinaemia and glomerulonephritis such as MGN all have a higher prevalence in males. [39, 177]

In HBV MGN as many as 80 to 100% of children are males, compared to the 60-70% of idiopathic MGN noted below. [175] In the series of Hsu (1989), 40 of the 52 (77%) were boys. [25] Boys comprised 75% of the cohort in a series of 77 Polish children. [121] Gilbert et al had 59 boys (84%) and 11 girls with HBV MGN [48] and the Bhimma et al series of 119 had 101 boys (85%) and 18 girls (15%). [49]

In the Lai et al Hong Kong series of HBV MGN adults 17/21 were males (81%). In this report, in the initial section of the discussion the statement is made 'males are less apt to predominate among adults than children with this disorder.' [26] In the two

references given there is no information about adults with HBV MGN and gender so that this statement, which has subsequently been repeated by others for example Bhimma and Coovadia in a review on Hepatitis B Virus-Associated Nephropathy [43], appears to have no evidence to support it and is contradicted by the 81% male preponderance in their own series. [26, 72, 178] The proportion of males in adults with HBV MGN from the reported cases is 183/220 (83%), as dominant as in the childhood group. (Table 2-6) There is no current explanation for this consistent finding in both childhood and adult HBV MGN of extreme male preponderance.

In adult idiopathic MGN, males are more commonly afflicted. [176] Three large databases report the proportion of males as 66%, 72% and 62% [176] and Ehrenreich and Churg as 60%, lower than the HBV MGN male proportions. [5]

RACE, ETHNICITY, GEOGRAPHIC AREAS

HBV MGN is most common in children and adults where carrier rates of HBV in the background population are highest. In these populations, the vast majority of cases of MGN in children are associated with HBV. [175]

In the United States, predominance of HBV MGN in the black population as well as immigrants from HBV endemic areas has been noted. [22, 120] In Southern Africa HBV MGN has been reported most often in children and adults from communities known to have high carrier rates namely, black (African) and coloured groups and rarely in white and Indian patients in line with the relative prevalence of HBV in the populations. [21, 39, 101] The incidence of serum HBsAg positivity in children and adults with MGN in different countries and regions of some countries is shown in Tables 2-8 and 2-9.

Table 2-8 Serum HBsAg positivity in children with MGN according to underlying prevalence of serum HBsAg positivity in reporting region

Country	Rate	No.	(%)	Year	Reference
A. HBsAg carrier rate 0.1-1.0%					
USA		11/55	(20)	1985	[179]
Western Europe					
France		14/33	(42)	1979	[16]
Italy		9/14	(64)	1985	[93]
B. HBsAg carrier rate 1-5%					
Eastern Europe					
Poland		21/23	(91)	1980	[75]
Poland		34/37	(92)	1984	[90]
C. HBsAg carrier rate 2-20%					
Orient					
Japan		16/28	(57)	1985	[94]
Japan		13/16	(81)	1987	[102]
South Korea		8/10	(80)	1988	[108]
Hong Kong		15/16	(94)	1987	[28]
Hong Kong		5/5	(100)	1984	[88]
Taiwan		52/54	(96)	1989	[25]
China		46/46	(100)	2003	[29]
Africa					
Zimbabwe		8/8	(100)	1984	[62]
South Africa		71/81	(88)	1994	[48]

Table 2-9 Incidence of serum HBsAg in adults with MGN

Country	HBV MGN/MGN Cases/Total	(%)	HBsAg Carrier Rate (%) in that population	Year	Reference
Great Britain	1/28	(4)	<1	1981	[77]
Scotland	0/54	(0)	<1	1986	[180]
Hungary	3/17	(18)	1-5	1979	[72]
Hong Kong	5/15	(33)	5-10	1985	[92]
Hong Kong	10/23	(43)	5-10	1987	[181]
Korea	10/50	(20)	5-10	1988	[108]

Tables 2-8 and 2-9 modified from Johnson et al. [175]

In idiopathic MGN different genetic markers in different populations suggest genetic susceptibility unique in each. For example in Caucasians, HLA B8 and B18 and DR3 are more frequent in MGN. In the USA, MGN is the most common cause of nephrotic syndrome in Caucasians while in African Americans it is FSGS. MGN itself does not appear to have specific racial predilection. [176]

b) CLINICAL FEATURES AND LABORATORY FINDINGS

VIROLOGY- HBV SEROLOGY STATUS

Serum HBsAg is by definition always present in patient cohorts presenting with HBV associated MGN. Approximately 60-80% of patients also have serum HBeAg and the remainder will usually have serum anti-HBe antibodies. [175] In two large childhood series the proportion of patients with serum HBeAg was higher (93%) and (98%) respectively. [25, 48] Rarely patients may be positive for both serum HBeAg and serum anti-HBe or for both serum HBsAg and serum anti-HBs. (74). Occasionally HBsAg has been found in the glomeruli of patients who are serum HBsAg negative [72, 84, 99]. (This raises questions regarding the specificity and sensitivity of serum tests and in particular glomerular HBV immunofluorescent or immunohistological tests, a topic which will be covered in detail later.) Circulating immune complexes (CIC) have been reported in up to 80% of cases of childhood HBV MGN whereas CIC were rarely found in idiopathic MGN [46, 96]. HBsAg immune complexes were found in 55% and HBeAg immune complexes in 44% of children with HBV MGN. [96]

There are few adult HBV MGN cohorts. The Lai et al Hong Kong adult HBV group reported serum HBeAg in 17/21 (81%) [26] and the Lee et al Korean adult HBV MGN study 6/9 (67%). [108]

The presence of HBs antigen in the serum generally precludes the diagnosis of idiopathic MGN. It is however possible that some of the numerous serum HBsAg carriers may have developed idiopathic MGN. This would be a sustainable diagnosis if the patient were serum HBeAg negative and if anti-HBeAg staining in glomeruli were negative with adequate tissue and controls. For practical purposes, a diagnosis of idiopathic MGN in this study required absent serum HBsAg.

CLINICAL FEATURES

The characteristic clinical features of all forms of MGN include proteinuria, often but not always with the other elements of the nephrotic syndrome. Nephrotic syndrome (NS) is defined as proteinuria of more than 3.5 grams per 24 hours and usually includes oedema, ascites, lowered albumin as well as hypercholesterolaemia. Additional features can include hypertension and haematuria. [176]

RENAL DISEASE AT PRESENTATION

Heavy proteinuria with or without nephrotic syndrome (NS) and microscopic or rarely macroscopic haematuria are the usual presentation of HBV MGN. [175] In the childhood HBV MGN series of Hsu (1989), nephrotic syndrome occurred in 43 (83,3%), while seven had proteinuria alone and two had haematuria alone. [25] Among the 77 patients of Wrzolkowa (1991), the mode of onset was that of nephrotic syndrome in 74% and less frequently that of non-nephrotic proteinuria in 26%. This was usually accompanied by haematuria not further quantified. [121] All 70 patients reported by Gilbert (1994) were defined as nephrotic; 50 (71%) were noted to have microhaematuria and 15 (21%) to have macrohaematuria. [48]

In the series from Lai et al of 21 adults with HBV MGN 12 (57%) had nephrotic syndrome at presentation, 7 (33%) asymptomatic proteinuria and 2 (10%) had chronic renal failure (CRF). Haematuria was not mentioned specifically in either of the two descriptions of these patients. [26, 149]

Idiopathic MGN usually presents as NS. Ehrenreich and Churg reported that 53 out of the 60 had nephrotic syndrome either initially or later. [5] In idiopathic MGN microscopic haematuria occurs in 25% to 85% but gross haematuria is less common (range, 1 – 17%). [176]

Oedema and ascites are frequent in the heavy proteinuria and/or nephrotic syndrome usually found with all forms of MGN. [176] This was the case for example in the childhood HBV MGN series of Gilbert (1994) : 70/70 (100%) for oedema and 50/70 (71 %) for ascites. [48]

Hypertension was present in fewer than 25% of childhood HBV MGN cases. [175] Hypertension was not specifically noted by Hsu (1989) and Wrzolkowa (1991) commented that it was rarely seen. [25, 121] At presentation in the patients of Gilbert, (27%) were noted to have hypertension. [48] In Bhimma's series, (10%) showed hypertension initially and (33%) showed hypertension initially and persistently, a total of 43%. [142] The Lai adult HBV MGN series showed 7/21 (33%) with hypertension at presentation. [26] In the review by Schwartz, 17-50% of adult idiopathic MGN patients had hypertension when initially diagnosed. [176] The frequency of hypertension in the 3 groups is similar.

RENAL FAILURE

Johnson and Couser reported that only rarely do children with HBV MGN manifest renal insufficiency at presentation. [175]

Adults with HBV MGN present with renal failure more frequently. In the Lai et al adult HBV series, two patients were initially referred for investigation of chronic renal failure and 5/21 (24%) in total showed raised creatinine at presentation. [26] In the Lee et al Korean series, one of 10 adults with HBV MGN had renal failure and 6/28 (21%) of the HBV MCGN group. [108]

Schwartz collated data from 8 series and reported that approximately one half of idiopathic MGN patients have abnormal serum creatinine when MGN is initially diagnosed. [176]

LIVER DISEASE

Children with HBV MGN usually have no history or clinical evidence for ongoing liver disease. [65] Adults with HBV MGN are more likely than children to have a history of acute hepatitis, usually 6 months to several years before the onset of the kidney disease. [175] Despite the absence of a history of hepatitis, liver enzymes especially transaminases, are often mildly deranged in children with HBV MGN in contrast to idiopathic MGN where normal levels are present. [15, 16, 21, 22, 94, 120]

The liver pathology in children with HBV MGN described by Johnson et al (1990) was usually chronic persistent hepatitis or minimal abnormalities but unusual cases had been reported in children with chronic active hepatitis, cirrhosis and even fulminant hepatitis. By contrast, the most frequent liver pathology in adults with HBV MGN was chronic active hepatitis although cases had been reported in patients with other forms

of acute and chronic HBV-induced liver disease. [175] The terminology of these liver reactions has undergone changes and currently 'chronic persistent hepatitis' would be classified as 'chronic HBV associated hepatitis without interface hepatitis' while 'chronic active hepatitis' is now 'chronic HBV associated hepatitis with interface hepatitis' with grades and stages applied to both groups according to accepted criteria. (Scheuer 2006). [182]

In the Lai et al adult HBV MGN series, 3/21 (14%) had a raised ALT level, a relatively low level of liver involvement but one of the very few available for comparison with the current series. [26] Three of the 4 adults with HBV MGN reported by Venkateshan had liver biopsies documented as chronic persistent hepatitis, chronic active hepatitis and submassive necrosis respectively. Only one of these four had raised AST levels reported. [120]

Patients with idiopathic MGN would not be expected to have liver disease and would therefore be predicted to have normal liver enzyme levels. The Bhimma SA series was unusual in that the mean AST level in a group of 70 children with HBV MGN was marginally but not significantly higher than the mean in 25 children with idiopathic MGN (47.54 vs 42.16). This appears to reflect raised levels in the idiopathic group and is not a finding reported by any other group. [142]

Of some interest is that as far as is known, no cases of hepatocellular carcinoma have been reported in the 1160 cases of HBV MGN, although few have long term follow up. It seems logical that HBV MGN, which is usually an expression of antibody formation in the process of seroconversion to HBeAg negativity, may be a rare marker of a process (HBe seroconversion) which to a large extent protects patients from

complications which are more frequently associated with the continued presence of HBeAg such as cirrhosis and hepatocellular carcinoma. [41, 183]

SERUM COMPLEMENT

Serum C₃ and C₄ levels have been depressed in 15% to 67% of HBV MGN cases, in data almost exclusively from childhood cases. [17, 20, 22, 46, 48, 62, 94, 120] In the series of Gilbert et al serum total complement was reduced in 71%, C₃ in 67% and C₄ in 47% of a group of 70 patients. This pattern suggested mainly alternate pathway complement activation [48]. In other series complement levels were said to be normal. [15, 16, 82]

Concerning adult HBV MGN, the Lai et al comparative series on SLE and HBV MGN from Hong Kong gives a mean serum C₃ value of 970 mg/L in 22 cases of HBV MGN. This is contrasted with the significantly lower mean of 511mg/L of 26 SLE MGN. The authors do not comment on this, therefore it is uncertain whether the HBV group values (mean 970mg/L) were also low or in the normal range for their laboratory. [149] Venkateshan et al reported that all 4 of the adults with HBV MGN had reduced C₃. [120] The adult patients in the Korean study showed hypocomplementaemia (C₃ and/or C₄) in 2/10 (20%) with MGN and 11/26 (42%) in the MCGN group. [108] In summary, many patients, adults and children with HBV MGN had lowered complement levels

Schwartz reports that complement levels are usually normal in idiopathic MGN. [176]

c) RENAL PATHOLOGY OF MGN - HBV AND IDIOPATHIC

The main features of MGN are capillary wall thickening on light microscopy, IgG and C₃ positivity on immunofluorescence of glomeruli and subepithelial deposits on electron microscopy. [5, 176, 184] In the following section the pathological features of renal biopsies in HBV MGN and idiopathic MGN will be described with a focus on differences between the groups and on some of the controversial and as yet unresolved issues. Illustrations from our material are found predominantly in Chapter 5 where comparisons between the 3 groups are made.

I) LIGHT MICROSCOPY

MESANGIAL PROLIFERATION

In HBV MGN the mesangium sometimes shows minor changes but appreciable proliferation may be noted in some cases. [5] The first reported case of HBV MGN (Combes 1971) described focal mesangial hypercellularity of glomeruli as well as the diffuse thickened capillary basement membranes characteristic of MGN. [91] Wiggelinkhuizen et al (1983) noted mild mesangial cell proliferation with an increase in mesangial matrix in the glomeruli of their HBV membranous cases. [21] Venkateshan et al [120] noted that although the occurrence of mesangial proliferation and deposits in idiopathic MGN was rare, as documented by Ehrenreich and Churg [5] these occurred in secondary forms of MGN such as lupus nephritis and HBV MGN. [75] In the series reported by Venkateshan, all 6 cases of childhood HBV MGN as well as all 4 cases of adult HBV MGN showed mesangial proliferation which was variously described as mild, moderate, focal and diffuse. [120] Well defined mesangial hypercellularity is uncommon in idiopathic MGN. [5, 176, 184]

GLOMERULAR SCLEROSIS

Glomerular sclerosis is not prominent in childhood HBV MGN as implied by the fact that the feature is not addressed in the large reviews dealing with childhood HBV MGN. [120, 175] In the Lai adult HBV MGN group, 8/22 (36%) showed glomerular sclerosis suggesting this may be a more prominent finding in adults. [149]

In idiopathic MGN, global glomerular scarring is widespread in advanced cases with renal insufficiency. [176] Segmental glomerular scars are seen in approximately 20% of biopsy specimens, but their significance was unknown until recently. Wakai and Magil have shown that the presence of segmental scars in MGN identifies patients with an adverse prognosis. [185]

Glomerular sclerosis appears to be more frequent in both adult groups than the childhood HBV MGN cohorts.

CRESCENT FORMATION

Crescents are accumulations of cells in Bowman's space composed of proliferating parietal epithelial cells, macrophages and infiltrating leucocytes. They are associated with severe glomerular injury and when prominent are associated with the clinical syndrome of rapidly progressive GN. [41] Crescents are unusual in idiopathic MGN with only 3.2% of cases showing any crescents and fewer than 0.1% displaying more than 50% crescents. [186]

No specific reference to crescents and HBV MGN in children has been found.

Although various glomerular lesions had been described between (1971 and 1992) in HBV carriers, crescentic GN had not, prompting F Lai et al to document their experience of two adult patients, who had 56% and 64% crescents. The underlying

glomerular pathology is important in such cases both for diagnosis and prognosis. In glomeruli not affected by crescents, both of these two cases had a proliferative type of GN. One had an endocapillary hypercellularity reminiscent of post-infectious GN while the second progressed from a predominantly MGN pattern only to what the authors called a 'mixed MGN and membranoproliferative GN' with crescentic transformation. This development took place over a period of 2 to 3 months between the two renal biopsies during which time renal failure had developed. [187]

In the comparison between HBV MGN and SLE MGN by the Lai group, 2/22 (9%) of the HBV cases had crescents compared to 9/26 (35%) of SLE MGN. [149] (The 2 HBV MGN cases are possibly the same 2 reported in reference 187 above.)

A further case was reported in 2000 by Taskapan et al from Turkey of a 23 year old man with HBV MGN which progressed to crescentic transformation after immunosuppressive treatment withdrawal. Despite aggressive treatment of the crescentic GN he developed end stage renal failure. [147]

INTERSTITIAL INFLAMMATION, FIBROSIS, TUBULAR ATROPHY and VESSEL CHANGES

Venkataseshan et al [120] noted that tubulo-interstitial changes were rare even in longstanding cases of HBV MGN. In general, few reports specifically mention the interstitium, tubules and blood vessels in HBV MGN, especially in children, probably suggesting that they seldom show abnormalities. The report of Venkataseshan et al was a pivotal one despite the small numbers, (12) because the pathological material was examined in great detail. [120] In the adult Lai et al HBV MGN series, tubular atrophy was noted as infrequent but not further discussed. [26]

In adult idiopathic MGN the renal tubules atrophy as the glomerular lesions progress. Interstitial inflammatory infiltrates are not a prominent feature of idiopathic MGN. In uncomplicated MGN, diffuse fibrosis tends to occur early without prominent inflammation or tubular atrophy. Arteriosclerosis may be seen in the larger vessels, reflecting the older age of the patients with idiopathic MGN. [176]

II) ELECTRON MICROSCOPY

SUBEPITHELIAL DEPOSITS

The classical paper of Ehrenreich and Churg emphasised that the fundamental change of MGN is thickening of capillary walls. On ultrastructural examination electron dense deposits are present on the epithelial side of the basement membrane, beneath the foot processes (Stage I). In addition to and often between the deposits projections of the basement membrane can be laid down ("spikes") (Stage II). With time the projections extend laterally incorporating the deposits (Stage III). The last stage involves a loss of the electron density of the incorporated deposits, usually leaving the wall thickened (Stage IV). [5] Fig 2-8 and 2-9

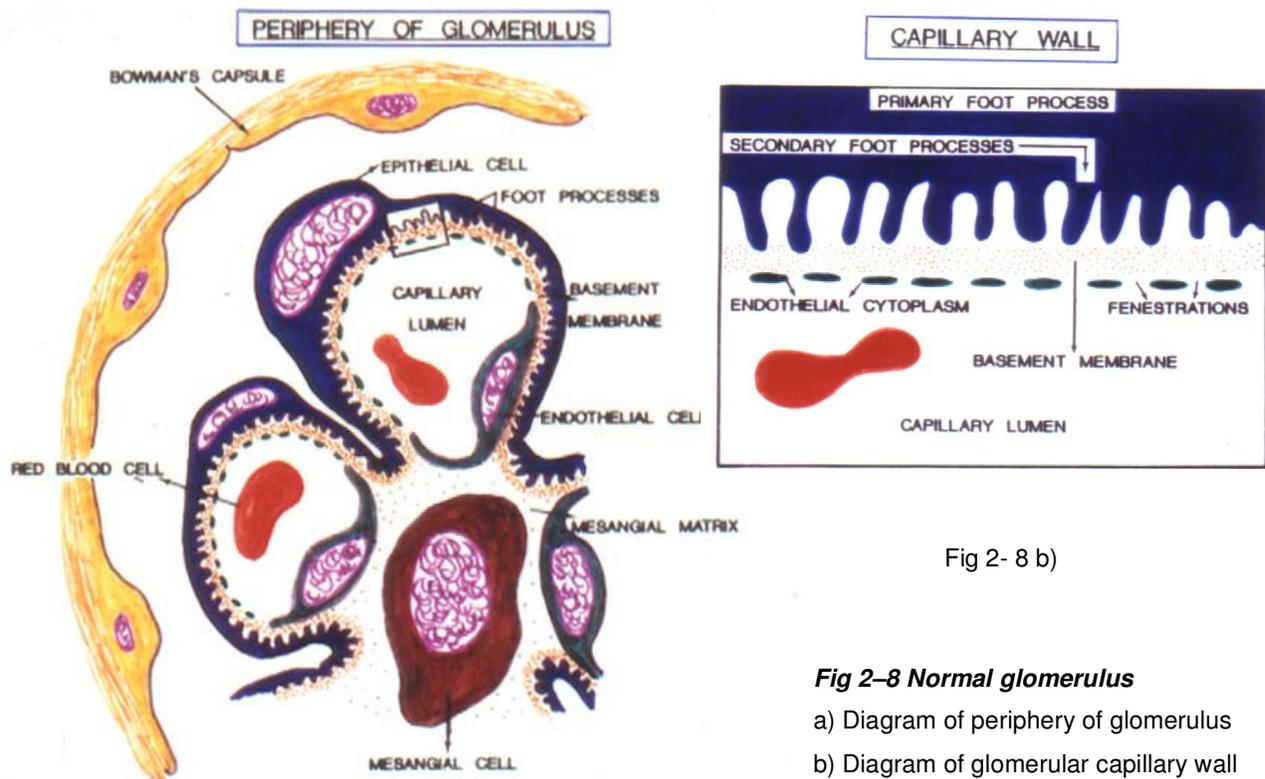


Fig 2- 8 a)

Fig 2- 8 b)

Fig 2-8 Normal glomerulus

- a) Diagram of periphery of glomerulus
- b) Diagram of glomerular capillary wall

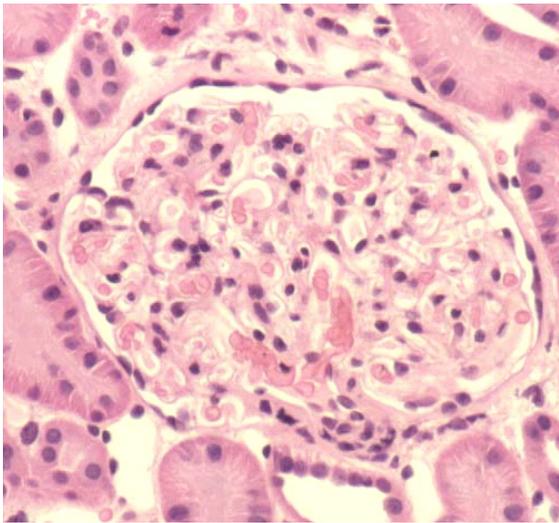


Fig 2-9 (a) Normal glomerulus
Haematoxylin and eosin stained section of normal glomerulus

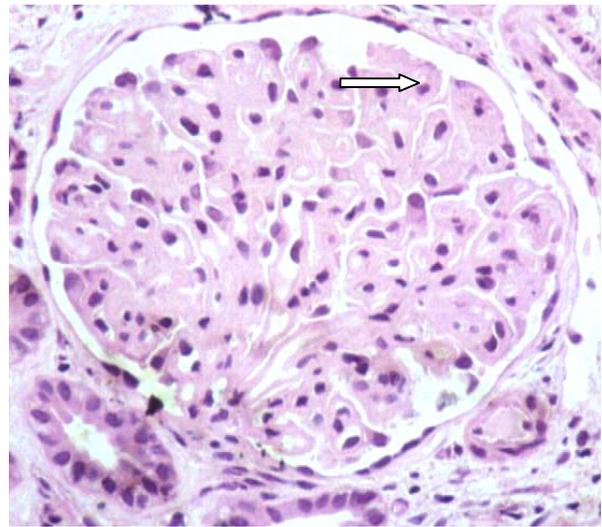


Fig 2-9 (b): Membranous GN. Capillary wall thickening
Indicated by the arrow (→)

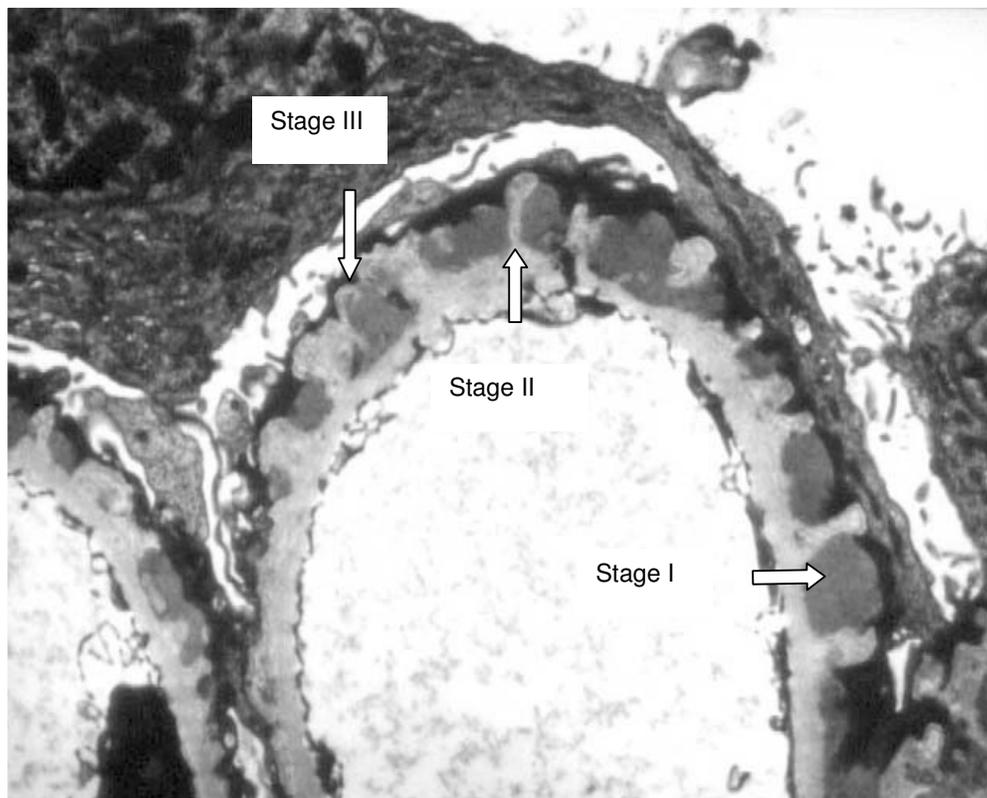


Fig 2-9 (c): Membranous GN. Capillary wall thickening
See text for details

In a comprehensive comparative study, Yoshikawa et al (1985) contrasted laboratory and pathological findings in 16 children with membranous GN associated with HBV to 12 children with idiopathic MGN. Ultrastructural material was available in 14 of the 16 children with HBV MGN and 9 with idiopathic MGN for this comparison. [94] Electron dense deposits in the glomerular basement membrane were the most constant and prominent feature. The biopsies from 3 patients with idiopathic MGN were graded as stage I, 8 with HBV-associated MGN and 2 with idiopathic MGN as stage II, 5 with HBV MGN and 4 with idiopathic MGN as III and 1 with HBV-associated MGN as IV. There was usually overlapping of stages, but one stage generally predominated and biopsies were graded on the morphology of the majority of capillary loops observed. [94]

Hsu's 52 Taiwanese children documented in 1989 with HBV MGN showed 4 (8%) in stage I, 19 (36%) in stage I-II, 16 (31%) in stage II, 10 (20%) in stage II-III, 3 (6%) in stage III and 0 in stage IV. The majority (75%) had the earlier stages I to II changes. This system of classifying the 4 stages into 6 groups recognises that the stages are often combined and uses the predominant stage or combination. (70)

Venkateshan et al also reported on the ultrastructural findings on 12 cases of HBV associated GN, 10 of which were classified as MGN or combinations including MGN. [120] The glomerular capillary basement membranes in MGN were irregularly thickened, showing numerous granular subepithelial deposits and spikes unevenly involving capillary walls and sparing occasional loops or short sections of the basement membrane. Five cases of the seven 'pure' MGN cases were in stage II and 2 were in stage III, with children and adults not being distinguished. [120] Both HBV and idiopathic MGN have subepithelial deposits in common by definition and there are

not any reported clear differences on ultrastructural level. Both groups can show the four stages of deposit evolution.

MESANGIAL DEPOSITS

Mrzokowicz was the first to document mesangial deposits on ultrastructure in childhood HBV MGN in 1976. [188] Amemiya in 1983 was the first to document all three of the distinctive features of HBV MGN namely mesangial deposits as well as HBeAg in the subepithelial region and mesangial interposition in a single case report.

[19] Wiggelinkhuizen et al reported an increase in mesangial matrix but not specifically electron dense deposits in mesangial areas of their initial series of 25 children with HBV MGN. [21] In the ultrastructural comparison of Yoshikawa et al 1985, in 13 of the 14 patients with HBV associated MGN, small mesangial electron dense deposits were found. Although the mesangial deposits were usually sparse or moderate, numerous mesangial deposits were seen in 2 patients. In the biopsy of one patient with HBV MGN graded as stage IV, which showed regression of capillary wall changes with disappearance of basement membrane deposits, mesangial dense deposits were also observed. In idiopathic MGN, mesangial deposits were seen in only 2 of 9 patients ($p < 0.01$) and they were small and rare. In both types of MGN, there was little or no increase in mesangial cells or matrix. [94] Table 2-10 on page 70 summarises the Yoshikawa data after the various ultrastructural features have been described individually. In the biopsy series of Dreyer (1986 MD thesis) the 22 renal biopsies identified as HBV MGN on the basis of HBs and/or HBc staining in glomeruli, had more mesangial deposits, 18 (81%) versus 4 (21%) in the 19 MGN cases negative for HBV staining in glomeruli. [99]

Venkateshan et al reported that, in contrast to the idiopathic form, all 4 of the children's cases of 'pure' MGN and 2 of the 3 adult cases of 'pure' HBV MGN had

variable mesangial deposits and 5 of these 7 also showed mesangial proliferation suggesting further a link between the two features. [120]

Wrzolkowa in a large and comprehensive ultrastructural study of 77 cases of childhood HBV MGN in 1991 noted that the mesangial areas were slightly enlarged. The electron dense deposits seen between and adjacent to the mesangial cells were similar to those seen in the capillary loops. [121]

In the Lai et al adult group of HBV MGN, 11/22 (50%) showed mesangial deposits. [149] The Korean study of Lee et al included ultrastructural findings on 17 of their 18 cases of HBV MGN (8 children and 10 adults). The report did not separate childhood and adult data. Small amounts of mesangial deposits were reported in 12 of the 17 biopsies. [108]

Mesangial deposits have only rarely been reported in idiopathic MGN. [5, 176]

SUBENDOTHELIAL DEPOSITS

In the series of Yoshikawa, in 4 children with HBV associated MGN, small subendothelial deposits were seen in addition to numerous subepithelial and intramembranous deposits. In 3 of these 4 cases, subendothelial deposits were present together with subepithelial and/or intramembranous deposits in the same capillary loop while in the other one only subendothelial deposits were present without subepithelial/intramembranous deposits in several loops. Subendothelial deposits were not found in the children with idiopathic MGN. [94]

The subendothelial deposits in the series of HBV MGN in children reported by Wrzolkowa were less abundant and frequently took the form of small elongated 'concentrations'. The texture of the electron-dense deposits was somewhat heterogeneous, but clearly distinct from the texture of the lamina densa itself. [121]

In the biopsy series of Dreyer, Pretoria SA, subendothelial deposits (5 cases) were also more common in the HBV MGN group than the idiopathic MGN patients ($p = 0,0351$) [99]. Note that children and adults were not distinguished in these figures. [99]

In the Lai et al Hong Kong adult HBV MGN series of 22, 15 (68%) showed subendothelial deposits. [149] The Korean series reported a few isolated tiny subendothelial deposits in 4/17 (23%) biopsies of HBV MGN with adults and children not distinguished. [108]

Subendothelial deposits have seldom been reported in idiopathic MGN. [5]

MESANGIAL INTERPOSITION

Wiggelinkhuizen et al (1983) in a series of 25 children first reported and illustrated at ultrastructural level the occurrence of mesangial interposition in association with HBV MGN. They emphasised that in addition to the subepithelial deposits which are the diagnostic hallmark of this disease, that at times striking interposition of mesangial cytoplasm and matrix was seen between the basement membrane and endothelium of the glomerular capillary loop. [21] Between 1971 and 1983 both membranous and mesangiocapillary patterns had been described in HBV associated glomerular disease. Until the Wiggelinkhuizen et al report, however, the existence and indeed probable frequency of mixtures or combinations of the two classical idiopathic glomerular morphologies had not been noted or emphasised. A minor degree of mesangial cell interposition into capillary loops was observed in 4 children with HBV associated MGN and one with idiopathic MGN in the 1985 Yoshikawa et al report. [94]

In the biopsy series of Dreyer, 2 of the 5 cases with subendothelial deposits also showed mesangial interposition. Unfortunately, children and adults are not separated in the data. [99]

Of the 7 'pure' HBV MGN cases of Venkateshan, 2 showed focal or early mesangial interposition. In two of these 7 cases progression over 2 and 5 years from a membranous to a mesangiocapillary pattern was noted on a second biopsy. [120] In addition 2 of the 3 further cases they described as membranous in combination with another GN pattern, also showed mesangial interposition. Therefore a total of 4 of the 10 cases (40%) this group described as MGN with or without a second GN pattern showed mesangial interposition. [120]

In Wrzolkowa's series of 77 children with HBV MGN, the mesangial areas were reported as slightly enlarged. The cells and their processes were slightly increased in number and contained abundant rough endoplasmic reticulum and free ribosomes; their processes were at times seen penetrating into the glomerular basement membrane. However, this penetration was usually limited to the paramesangial areas, not further than to one eighth of the capillary loop circumference. [121]

In the Lee et al Korean HBV GN series of adults and children, the 28 classified as MCGN and studied ultrastructurally all showed mesangial interposition and mesangial deposits but in addition 22 (79%) also showed subepithelial/intramembranous deposits. This highlights an HBV group with combined MGN and MCGN features. [108] In their HBV MGN cases mesangial interposition was not noted. [108] Mesangial interposition is not a feature of idiopathic MGN. [5, 176, 184]

In summary, mesangial interposition has been reported in HBV MGN but with prominent variation in frequency while it is not found in idiopathic MGN,

Two further ultrastructural features have been described with varying regularity in cases of HBV GN including MGN. It was therefore decided to specifically look for and document their presence in renal biopsies in the current series. The structures are tubuloreticular bodies and virus-like particles.

TUBULORETICULAR BODIES

Tubuloreticular bodies, variously also known as microtubuloreticular complexes, tubuloreticular structures and undulating tubules, amongst other terms, are arrays of loosely intertwined and branched interconnecting tubules that are found in the endoplasmic reticulum. [189] These structures show variations in shape and form. They appear to consist of two components: a tubular component of microtubular structures with a diameter between 20nm and 30nm and a membranous component in which profiles suggest undulating membranes associated with only sparse microtubules. Their occurrence has usually been reported in lymphocytes and vascular endothelial cells but they also occur occasionally in mesenchymal cells and epithelial cells. It appears that their formation is produced by the effect of an unusual acid labile alpha - interferon. The presence of tubuloreticular bodies in glomerular endothelial cells was initially strongly associated with systemic lupus erythematosus (SLE). This is of interest because the presence of an unusual acid labile interferon has been established in the serum of patients with SLE. Elevated levels of serum acid labile alpha-interferon and the frequent presence of tubuloreticular bodies occurring as lymphocyte inclusions have been recorded in these patients. [189]

In a South African study by Mills et al, all 29 children with HBV MGN had tubuloreticular bodies. [190] High percentages of smaller groups of other patients with a variety of glomerular disease also demonstrated these structures.

Dreyer reported 41 cases of MGN which were studied ultrastructurally including 22 cases of possible HBV MGN but no mention was made of tubuloreticular bodies. [99] Further, contrary to the experience of Mills et al reported above, [190] Wrzolkowa et al in 1991 failed to find these bodies in their study. [121] In the FM Lai Hong Kong adult HBV MGN series 3/22 (13%) showed tubuloreticular structures, a significantly lower

frequency than the proportion of the SLE group with which they were compared. (19/26-73%) [149]

It may well be that some of these apparently contradictory findings are not truly comparable as these small structures need to be specifically searched for at high magnification for a substantial time by an electron microscopist familiar with their appearance in order to identify and document them. These variable findings suggest that further comprehensive examination of a large series of HBV MGN in children and adults with an idiopathic MGN group as control would be warranted.

VIRUS-LIKE PARTICLES

Focal collections of extracellular spherical particles of 5 to 30 nm believed to be of viral origin by several investigators have been recognised within the glomerular deposits of HBV MGN and other immune complex diseases. The exact composition of these particles is not known, but they may be aggregated viral antigens or immune complexes (Venkateshan 1990). [120] In many of the studies, the morphology and size of these particles are not consistent with the 22nm or 42nm HBV particles (Johnson 1990). [175] No viral like particles were reported in the biopsy series of Dreyer. [99] In the Hong Kong HBV and SLE MGN comparison 18% of the 22 HBV MGN cases showed viral like particles on EM. [149] These extracellular spherical particles have been described in the glomeruli in a number of cases. [15, 25, 62, 63, 191, 192] The morphology and size of the particles (50-300 angstroms) are not the same as the 22nm or 42nm HBV particle and identical structures occur in occasional cases of MGN not associated with HBV. [176] No mention is made of these structures in a recent review of the pathology of idiopathic MGN although as mentioned above they are seen rarely. [176]

INTEGRATED COMBINED DESCRIPTIONS OF ULTRASTRUCTURAL FEATURES OF HBV MGN FROM THE LITERATURE

Yoshikawa et al were the first to document in detail differences between idiopathic and HBV MGN in children with a focus on ultrastructure. Therefore it seems appropriate to summarise their findings in the table below to highlight and integrate these features. Included below are some of their own comments on the findings as well as a critique of their assessment. [94] (Table 2-10) In the next section an updated summary of ultrastructural features of HBV MGN and idiopathic MGN will be presented. (Table 2-12)

Table 2-10 Ultrastructural features: HBV MGN compared to idiopathic MGN. (Yoshikawa) [94]

Feature	HBV MGN (n = 14)		Idiopathic MGN (n = 9)	
	No.	%	No.	%
Mesangial deposits	13	(93) most mild or moderate, 2 severe	2/8	(25) mild
Subendothelial deposits	4	(29)	0	(0)
Subepithelial deposits				
Stage I	0	(0)	3	(33)
Stage II	8	(57)	2	(22)
Stage III	5	(36)	4	(44)
Stage IV	1	(7)	0	(0)
Mesangial interposition	4	(29)	1	(11)

Yoshikawa et al (1985) noted that mesangial electron dense deposits, subendothelial deposits and reduced C₃ levels mentioned elsewhere in their article, were indicators not normally found in idiopathic membranous. [94] (Of interest is that they did not include the finding of mesangial interposition in this combined description of their findings although it was as frequent as subendothelial deposits.) They further pointed

out that although there were many reports of the electron microscopy findings in HBV MGN and other HBV associated GN by that time, the observations were mainly concentrated on glomerular capillary walls (i.e. subepithelial deposits) and there was little published literature relating to mesangial deposits. Although an increase in mesangial cells and matrix, and diffuse circumferential interposition typical of mesangiocapillary GN (MCGN), were not observed, most of the children with HBV MGN in their series, showed some features of MCGN (low C₃, mesangial and subendothelial deposits). One patient, also described in the article, who was not included in this particular series was seropositive for HBeAg and HBsAg, and had reduced serum C₃ values and characteristic biopsy features of MCGN type III. There were numerous subepithelial and subendothelial deposits associated with prominent mesangial interposition and moderate increase in mesangial cells and matrix associated with deposits. Both HBsAg and HBeAg were detected in the glomeruli of this case. These observations led them to conclude that HBV infection may induce a spectrum of glomerulopathies from typical MGN to typical MCGN. [94] This was a pivotal study that contributed to clarifying the initially confusing spectrum of ultrastructural morphology seen and raised the further question of whether the morphological spectrum related to clinical and prognostic factors. [94]

The Venkateshan group also found that there was a difference between idiopathic MGN and the 7 cases of 12 they categorized as pure or predominantly HBV MGN, including both children and adults. They emphasised that unlike the idiopathic form, 5 of 7 cases showed mesangial proliferation, 6 of 7 cases variable mesangial deposits, 5 of 7 cases focal subendothelial deposits and 2 of the 7 focal or early mesangial interposition. [120]

Table 2-11 below from Wrzolkowa et al illustrates the major influence of ultrastructural examination on the final diagnosis of 98 children with glomerular disease and serum HBsAg positivity. [121]

Table 2-11 Children with serum HBsAg: Comparison of initial diagnoses based on paraffin sections versus final diagnosis after electron microscopic examination. [121] (n=98)

Type of GN	Paraffin section diagnosis		Electron microscopy diagnosis	
	(n=98)	%	(n=98)	%
MGN	12	(12)	77	(79)
MCGN	50	(51)	3	(3)
Mesangial GN	28	(29)	13	(13)
Minimal change disease	5	(5)	2	(2)
Endocapillary GN	3	(3)	3	(3)

It is clear from Table 2-11 that ultrastructural examination radically altered in particular the initial diagnoses of the proliferative GN categories of mesangiocapillary GN and mesangial GN in this serum HBsAg positive childhood GN series. In the majority of cases, 77 (79%) the final diagnosis was secondary HBV MGN. This form of MGN usually had at least some of the additional features emphasised in the preceding section such as mesangial deposits, mesangial proliferation, subendothelial deposits and mesangial interposition in addition to the 'core' feature of widespread subepithelial deposits. [121]

SUMMARY OF ULTRASTRUCTURE OF 3 GROUPS OF MGN

Table 2-12 below is a summary of the ultrastructural features of HBV MGN in adults and children and idiopathic MGN from the literature and particularly the following references. [5, 13, 19, 21, 25, 85, 90, 113, 114, 142, 171, 172, 184, 185]

Table 2-12 *Main ultrastructural features of HBV MGN and idiopathic MGN –*

Summary of reported literature

Feature	Idiopathic MGN	Childhood HBV MGN	Adult HBV MGN
Subepithelial Deposits	Stages I-IV	Stages I-IV	Stages I-IV
Mesangial Deposits	Rare – seen in secondary forms like SLE	Frequent Not always documented ^a	Not well described
Subendothelial Deposits	Rare	Occasional Not always documented	Not well examined
Mesangial Interposition	Rare	Well described Much variation Not usually documented	Seldom reported Suggested overlap between MGN and MCGN
Tubuloreticular Bodies	Not often reported	Seen frequently when carefully searched for Not often documented	Occasionally seen Not often documented
Virus like structures	Not often reported Only rarely seen	Not often documented	Not often documented

^a Not always documented means that neither the presence nor absence of a feature is reported. This leaves uncertainty as to whether the feature was absent or not specifically searched for. The favoured interpretation of this author is that usually features not mentioned were not specifically looked for.

III) IMMUNOFLUORESCENCE

In the evaluation of glomerular disease, immunohistology (immunofluorescence or immunohistochemistry) is directed at the identification of pathogenic immunoglobulin and complement molecules as well as other antigens. [174] These techniques determine the distribution, pattern and composition of glomerular immune deposits which occur in many forms of GN and form an integral part of the pathological evaluation of renal biopsies. Most of the attention on immunofluorescent and immunohistochemical staining of renal biopsies of HBV MGN has focused on the various hepatitis B antigens but here the conventional antigens will be discussed first.

IMMUNOGLOBULINS AND COMPLEMENT

The glomeruli generally stain diffusely or segmentally for several immunoglobulins in both the childhood and adult HBV MGN groups, the most intense being IgG with weaker staining for either IgA or IgM. [120] Venkateshan et al also reported considerable amounts of complement (C₃) and variable amounts of C1q and C₄ in a few cases in a granular pattern. [120]. In a review of predominantly children's series, Johnson reported that HBV MGN immunofluorescent staining of the capillary walls was usually positive for IgG (100%), C₃ (75%), IgM (50%) and IgA (10%). [175]

The Lai et al adult HBV MGN series demonstrated diffuse granular deposition of IgG and C₃ along the capillary walls in all 21 specimens. [26] Adult idiopathic MGN is characterised by IgG and C₃ positive deposits with IgA and IgM variably positive; (IgA:1% to 54% and IgM:1% to 58%). The variability may be related to the inconsistent/nonstandardised reporting of low levels of staining. [176]

HBV ANTIGENS AND ANTIBODIES

Although the first case of HBV MGN appeared to show HBsAg in the glomeruli, [8] a number of later studies showed HBeAg as the most important antigen in HBV MGN, associated with granular subepithelial staining. [15, 17, 18, 45-47] Supporting the pivotal role of this antigen in the pathogenesis of the disease is that in many of the children with HBV MGN, the seroconversion from HBeAg to anti-HBe has been associated with remission of the nephrotic syndrome. [48]

Dreyer undertook a comprehensive pathology focussed examination which provided valuable data and highlighted some of the difficult issues of this disease group of HBV MGN. [99] In this study 342 renal biopsies from GN patients were examined and classified histologically. Two hundred and seventy four (274) of the biopsies were tested for HBsAg and 255 for HBcAg; 21% of biopsies were positive for HBsAg and 8% for HBcAg. Unusual findings included that 8 (36%) of the 22 SLE biopsies also tested positive for HBsAg. HBsAg serum testing was incomplete in this series making interpretation difficult. For example only 5 of 22 cases of MGN with HBV antigens were serum HBsAg positive, 9 were negative and 8 were not tested. This raises concerns about the reliability of serum and glomerular tests and the conclusions drawn from this data. [84, 99] These findings highlight the issue of the specificity and reliability of immunological staining techniques in glomeruli as well as the reliability of serum HBV tests.

HBsAg

There have been conflicting reports regarding the involvement and location of HBsAg in the glomeruli in HBV MGN. Some, using monoclonal antibodies, have not found

HBsAg at all, [82] while others showed a small proportion with HBsAg in the mesangium. [193] An early comprehensive case study from the Massachusetts General in Boston, suggested HBsAg in the mesangium with HBeAg along the capillary walls (Collins et al 1983) [20] as did Amemiya et al from Japan in the same year. [19]

Immunofluorescence studies are clearly potentially subject to technical artefacts. Concerning the staining for HBV antigens, false positive HBsAg staining had already been reported by Maggiore et al in 1981 from IgM deposits with antiglobulin activity in mixed cryoglobulinaemia or lupus nephritis. [192]

A cross reacting reagent in use was the DAKO anti-HBs. A high incidence of false positive staining resulting from contaminating antibodies to plasma proteins in this commercial goat polyclonal anti-HBs antiserum (DAKO B 560) was reported by Goodman in 1988. [194] This anti-HBs stains for HBsAg but also plasma proteins in the tissue. Therefore in idiopathic membranous with serum negative for HBsAg, it would be possible to have anti-HBsAg staining in glomeruli. Therefore also in MGN in HBV positive patients the anti-HBsAg staining could be falsely positive.

HBcAg and HBeAg

It remained uncertain until 1989 whether the observation of glomerular deposition of different HBV antigens in HBV GN resulted from a genuine difference in antigens, variation in host response in different geographic areas, false positive staining or the difference in antisera being studied. [195] Lai et al demonstrated HBeAg in 4 out of 6 HBV MGN renal biopsies along capillary walls in a granular pattern that would correlate with subepithelial deposits on ultrastructure, an incidence similar to that reported by Hirose (1984) using the same F(ab)₂ fragments of HBeAg monoclonal antibody. [47] Using a monoclonal anti-HBs, glomerular capillary wall HBsAg could

not be identified in this study. Half the biopsies showed capillary deposits of HBcAg by polyclonal antiserum with a distribution pattern similar to those of HBeAg. As part of their study, this group checked the possible cross-reactivity between commercial polyclonal anti-HBcAg antibodies with anti-HBe antibody by enzyme immunoassay for anti-HBe with the use of HBeAg derived from recombinant DNA origin. Table 2-13 [195]

The commercial polyclonal rabbit antihuman anti-HBcAg antiserum (Dakopatts B 586) was demonstrated to contain both anti-HBcAg and anti-HBeAg activities by enzyme immunoassays, suggesting that it had reactivity to antigenic components derived from both HBcAg and HBeAg. Similar cross reactivity was not observed with the monoclonal antibodies. [195]

Table 2-13 *Reactivity of HBV antibodies used in immunological studies of renal biopsies of HBV MGN. Table from Lai [195]*

Antisera/Antibodies	Anti-HBcAg activity	Anti-HBeAg activity
Rabbit human HBsAg (Behringwerke)	—	—
HBcAg (Dakopatts)	+	+
Monoclonal HBcAg (Jichi Medical School)	+	—
Monoclonal HBeAg (Jichi Medical School)	—	+
Monoclonal HBcAg (St. Mary's Medical School)	+	—
Monoclonal HBeAg (St. Mary's Medical School)	—	+

This cross-reactivity can be explained by the fact that HBeAg is an integral part of the core of Dane particles and is also contained in HBcAg particles prepared from nuclei of infected hepatocytes. Lai KN et al in 1989 concluded that HBeAg is the HBV antigen deposited in HBV associated membranous nephropathy and cautioned that careful testing and evaluation of each antibody would therefore be necessary to prevent misinterpretation. [195] An important point from Table 2-13 is that the Dakopatts polyclonal antiHBc in common use stains for HBe antigen too.

Trying to resolve the confusion concerning HBV antigens in the glomeruli

Lai et al noted that although HBV appeared to be implicated in many types of GN, the characterization of putative HBV antigens in various types of GN had been inconsistent because of conflicting findings. The difficulties encountered in characterizing HBV-related glomerulopathies, they contended, were due in part to the small numbers examined and in part to the poor specificity of antibodies used. [193] Lai FM et al investigated the glomerular pathology and hepatitis B virus (HBV) antigens in renal tissue in 100 consecutive patients with both primary glomerulonephritis and positive serology for HBsAg. Of the 100 patients, 92 were adults and 8 patients were aged 15 or younger. [193]

Glomerular HBV antigens including HBsAg, HBcAg and HBeAg were tested for in frozen tissue using both polyclonal and monoclonal antibodies. HBV serology and glomerular HBV antigens were correlated. Using monoclonal antibodies, at least one of the three HBV antigens was detectable in glomeruli in 39% of the cases. These findings corresponded mainly to detectable glomerular HBsAg and HBeAg in 22,3% and 28,4% of cases respectively. [193] A good correlation was found between glomerular and serum HBeAg but not for HBsAg. Serum HBcAg was not examined

and not correlated with glomerular staining. HBcAg is essentially confined to liver cells though antibodies to HBcAg are found in serum and their measurement and type is of importance in understanding and managing HBV disease. [1]

When the diagnosis of HBV-related glomerulonephritis was based strictly on detectable glomerular antigens, three distinctive morphologies were identified: membranous nephropathy (MGN), mesangiocapillary glomerulonephritis and mesangial proliferative glomerulonephritis with IgA deposits (IgA nephropathy). Each of these lesions was seen in pure form or occasionally in overlapping form leading to double glomerulopathies. [193] In MGN, glomerular HBeAg and HBsAg were associated with subepithelial and mesangial immune complexes respectively, a very important finding from a large series confirming earlier case reports such as Amemiya [19], Collins [20] and Venkateshan's small series. [120] Rare overlap between membranous and IgA nephropathy further emphasised the distinctive pathology of HBV-related glomerulonephritis and the independent aetiological roles of HBeAg and HBsAg. In other glomerulonephritides, which rarely demonstrated glomerular HBV antigens, they maintained that the pathogenic role of chronic HBV infection remained to be proven. [193]

In order to localise HBeAg and HBsAg precisely in the kidney of HBV associated GN, a Japanese group (Ohba et al 1997) examined a biopsy from an affected adult by immunohistochemistry at light and electron microscopic levels. At light microscopic and ultrastructural level the HBe antigen was predominantly along the capillary walls with the HBsAg mostly in mesangial areas. [141] Although this is a single case, it was carried out with proven monoclonal antibodies and seemed to confirm existing data. No further data has emerged to contradict this description. [141]

Localisation of HBeAg and HBsAg in HBV MGN (Table 2-14)

Twenty-six reports have shown HBeAg in glomeruli with monoclonal anti-HBe antibodies. (Table 2-14) Only a few have shown HBsAg in the mesangium although only a small proportion of authors have reported on the presence or absence of HBsAg in their HBV MGN renal biopsy series. The differential deposition of HBsAg and HBeAg could be attributed to the difference in size and/or charge of the immune complexes. Ohba 1997 [141] In experimental glomerulonephritis in rats, albumin-antibody complexes with a size of 1,000,000 Daltons or less were shown to be precipitated in the subepithelial space and larger immune complexes were localised primarily in the mesangial area. [196]

Table 2-14 Localisation of HBeAg and HBsAg in HBV MGN - after Ohba 1997 [141]

Author	Year	Child	Adult	Country	HBs Mesangial	HBe Capillary wall	Reference
Takekoshi	1979	2		Japan	-	+	[17]
Ito	1981	4		Japan		+	[18]
Furose	1982	6		Japan		+	[46]
Amemiya	1983	1		Japan		+	[19]
Collins	1983		1	USA	1/1	+	[20]
Hirose	1984	5	5	Japan	-	+	[47]
Yoshikawa	1985	16		Japan		+	[94]
Ikeda	1986		1	Japan		+	[97]
Zacchello	1986	6		Italian		+	[100]
Takekoshi	1987	16		Japan		+	[102]
Hattori	1988	3		Japan		+	[106]
Ishihara	1988	1	2	Japan		+	[110]
Akano	1989	5	1	Japan		+	[111]
Zhang	1989		18	China		+	[114]
Lai KN	1989		8	Hong Kong	3/8	+ 5/7	[195]
Lee	1989		1	Korea		+	[116]
De Man	1989	1		Netherlands		+	[117]
Hsu	1989	52		Taiwan	-	+ 41/43	[25]
LIn	1990	34		Taiwan		+	[118]
Venkatase	1990	6	4	USA		+	[120]
Lai KN	1991		21	Hong Kong		+	[26]
Lai	1994			Hong Kong		+	[193]
Ohba	1997		1	Japan	+ 1/1	+ 1/1	[141]
He	1998	30		China		+	[143]
Lai FMM	2000		22	Hong Kong		+	[149]
Zeng	2008		47	China		+	[170]

Child – Less than or equal to 12 years of age Adult – 13 years of age or older

The molecular size of the HBeAg and antibody complex is approximately 300,000 Daltons; thus, the complex is thought to be deposited in the glomerular basement membrane and to induce MGN. [197] In contrast to the HBeAg and antibody complex, the molecular size of HBsAg is much larger at 3,000,000 Daltons even without antibody; thus it is possibly deposited in the mesangial area. [198]

SUMMARY OF DISTINCTIVE HBV MGN PATHOLOGY

Table 2-15 consolidates morphologic features that have proved to be distinctive of HBV MGN. In addition to the capillary wall thickening as a result of subepithelial deposits that are necessary for diagnosing MGN, 47 (36%) of the 132 reports in the literature also documented at least one of mesangial deposits, mesangial interposition and HBeAg in the subepithelial location. It has become clear that these 3 features are very characteristic of HBV MGN yet for various reasons they have not often/always been documented and seldom all 3 in the same biopsies. Two are ultrastructural features and not all biopsies appear to be studied with the same thoroughness or awareness of what may be present beyond the subepithelial deposits required to diagnose MGN. Regarding the HBe antigen, the monoclonal HBe antigens were available from the 1980s but not widely used due to cost considerations and limited commercial availability.

Of the 47 publications that mentioned at least one of these 3 features, 24 (51%) noted mesangial deposits, 24 (51%) HBe staining and 10 (21%) mesangial interposition. Only 5 reported all 3 to be present in HBV MGN. [19, 94, 102, 120, 149] This suggests that the pathological description of HBV MGN could still be enhanced by a further 71 cases of childhood HBV MGN and 12 cases of adult HBV MGN where detailed ultrastructural studies have been carried out reporting and semi-quantifying a

range of ultrastructural features including the above two distinctive ones in particular and where HBe antigen has been tested for and demonstrated in at least some of the cases.

Table 2-15 *HBV MGN cases in the literature - Selected pathology*

Author	Year	Child No.	Adult No.	Country	HBeAg	Mesangial Deposits	Mesangial Interposition	Ref
Mrozowicz	1976	7		Poland		+		[13]
Takekoshi	1979	2		Japan	+			[17]
Ito	1981	4		Japan	+			[18]
Furose	1982	6		Japan	+			[46]
Glasscock	1982		1	USA		+		[79]
Amemiya	1983	1		Japan	+	+	+	[19]
Wiggelinkhuizen	1983	25		SA, Cape Town			+	[21]
Collins	1983		1	USA	+	+		[20]
Dreyer	1984	2	19	SA, Pretoria		+	+	[84]
Via	1984		1	USA		+		[89]
Hirose	1984	5	5	Japan	+			[47]
Wyzynska	1984	34		Poland Warsaw		+		[90]
Southwest	1985	11		USA		+		[22]
Yoshikawa	1985	16		Japan	+	+	+	[94]
Magil	1986		1	Canada Chinese		+ IgA		[95]
Ikeda	1986		1	Japan	+	+		[97]
Dreyer	1986	6	16	SA, Pretoria		+	+	[99]
Zacchello	1986	6		Italian	+			[100]
Takekoshi	1987	16		Japan	+	+	+	[102]
Guerra	1987		1	USA		+		[103]
Hattori	1988	3		Japan	+			[106]
Lee	1988	8	10	Korea		+		[108]
Ishihara	1988	1	2	Japan	+	+		[110]
Akano	1989	5	1	Japan	+			[111]
Zhang	1989		18	China	+			[114]
Lee	1989		1	Korea	+			[116]
De Man	1989	1		Netherlands	+			[117]
Hsu	1989	52		Taiwan	+			[25]
LIn	1990	34		Taiwan	+			[118]
Venkatase	1990	6	4	USA	+	+	+	[120]
Lai KN	1991		21	Hong Kong	+			[26]
Wrzolkowa	1991	77		Poland		+	+	[121]
Gilbert	1994	70		SA Cape Town			+	[48]
Bhimma	1997	81		SA Durban				[140]
Ohba	1997		1	Japan	+	+		[141]
Bhimma	1998	70		SA Durban		+		[142]
He	1998	30		China	+			[143]
Lai FMM	2000		22	Hong Kong	+	+	+	[149]
Miller	2002	8		Jamaica		+		[151]
Connor	2003	1		Australia/ SA		+		[153]
Zeng	2008		47	China	+	+		[170]
Total					24/47 (51%)	24/47 (51%)	10/47 (21%)	

IV) MOLECULAR TECHNIQUES

HBV DNA and RNA have been detected in kidneys from HBV related GN. Lai et al from Hong Kong detected HBV DNA by in situ hybridisation as well as polymerase chain reaction followed by in situ hybridisation in most of the HBV MGN renal biopsies tested. HBcAg RNA was detected by in situ hybridisation in both tubules and glomeruli. The authors concluded that these findings indicated the presence of viral transcription in glomerular cells and renal tubular epithelium, supporting an aetiologic role of HBV in chronic HBsAg carriers who develop GN such as HBV MGN. [44]

A 2008 report in Chinese with an English abstract, concluded that with the multiplication of HBV-DNA in the serum, the pathological renal lesion was aggravating from stage I to stage III in MGN with increasing deposition of HBV Ag in glomeruli. (The words multiplication and aggravating are not entirely clear to this author in the context but the abstract is understood to imply that with increasing levels of HBV DNA in the serum the stage of deposits increases from I towards III with deposition elsewhere in the glomerulus as well.) [199]

D) COURSE, TREATMENT AND CLINICOPATHOLOGICAL CORRELATIONS

The primary role of the renal biopsy is to provide a diagnosis that allows lesion specific therapy to be assigned and to inform regarding prognosis. [174]

Table 2-16 summarises HBV MGN cases/series which gave information on treatment and/or outcome. Of the 565 children reported, 314 went into remission (55%), many spontaneously. Only 15 (3%) had a poor renal outcome namely renal failure or death from renal disease. Of 70 adults, 35 (50%) remitted (many with treatment) and 8 (11%) had renal failure. (The remaining patients in these reports were still in other categories such as nephrotic syndrome or proteinuria.)

I) CHILDHOOD HBV MGN

From the first two series with follow up data in 1978 and 1979, one of the most encouraging aspects of this 'new disease' of HBV MGN in children was the relatively high remission rate of 12/26 (46%) with a further two showing incomplete remission in the combined series. [15, 16]

The study of Hsu [25] was one of the first and remains one of few available describing the outcome in a large group and correlating clinicopathological features with prognosis in children with HBV MGN. Hsu used the time to remission as an index of outcome. The children had received varied treatment; 38 (73%) had prednisolone therapy but most failed to respond. Adenine arabinoside and thymic extract had been used in 10 patients; 4 received no special therapy, all of whom remitted within 2 years. The patients with a mean follow-up of four years had a cumulative remission rate of 92% in seven years. Only one patient had mild renal function impairment. [62]

Table 2-16 *HBV MGN cases in the literature with outcome*

Author	Year	Child <12 n=565	Adult >13 n=70	Country	Remission	Poor renal outcome ^a	Ref
Kleinknecht	1979	15		France	7	1	[16]
Ito	1981	4		Japan	2		[18]
Furose	1982	6		Japan	3		[46]
Chow	1984	5		Hong Kong	3		[88]
Seggie	1984	8		Zimbabwe	4	1	[62]
Wyzynska	1984	34		Poland Warsaw	20		[90]
Cadrobbi	1985	1		Italy	1		[91]
Southwest	1985	11		USA	2	1	[22]
Garcia	1985	1	1	USA	1 IFN (child)		[23]
Gregorek	1986	19		Poland Warsaw	8		[96]
Esteban	1986	1		Spain	1 Adenine arabinoside		[98]
Zacchello	1986	6		Italian	4		[100]
Takekoshi	1987	16		Japan	10		[102]
Mizushima	1987		1	Japan	1 IFN		[104]
Elidrissy	1988	3		Saudia Arabia		2	[24]
De Man	1989	1		Nether-lands	1 IFN		[117]
Hsu	1989	52		Taiwan	35	1	[25]
LIn	1990	34		Taiwan	16	1	[118]
Venkatase	1990	6	4	USA	1 (child)	1 (adult)	[120]
Lai KN	1991		21	Hong Kong	4	6	[26]
Wrzolkowa	1991	77		Poland Gdansk	59		[121]
Madalinski	1991	51		Poland Warsaw	36		[122]
Schectman	1991		1	USA	1		[124]
Ozen	1992	5		Turkey	4 (2 steroids)		[125]
Wong	1992	2		Hong Kong	1 (IFN)		[126]
Giboa	1993	1		USA	1 (IFN)		[128]
Wong	1993	18		Hong Kong	10	1	[129]
Lidman	1993		1	Sweden	1 (IFN)		[131]
Gilbert	1994	70		SA Cape Town	37	2	[48]
Conjeevaram	1995		10 MGN		8 (IFN)		[133]
Shapiro	1995		1	Canada	1 (IFN)		[136]
Bhimma	1998	70		SA Durban	12	5	[142]
Gonzalo	1999	1		Spain	1		[145]
Taskapan	2000		1 (crescentic)	Turkey		1	[147]
Pena	2001	12		Spain	12		[150]
Miller	2002	8		Jamaica	4		[151]
Bhimma	2002	17		South Africa	10 (IFN)		[152]
Connor	2003	1		Australia/ SA	1 (Lam)		[153]
Ozdamar	2003	6		Turkey	4		[154]
Filler	2003	1		Canada Vietnamese	1 (Lam)		[155]
Rihova	2005	-	3	Czeck	1 IFN	-	[157]
Tang	2005		10	Hong Kong	7 (Lam)		[159]

^a Poor renal outcome: Renal failure or death from renal failure IFN – Interferon Lam - Lamivudine

Table 2-16 continues on the next page

Table 2-16 HBV MGN cases in the literature with outcome (cont)

Author	Year	Child	Adult	Country	Remission	Poor renal outcome ^a	Ref
Izzedine	2005	-	1	France	1 (Lam)	-	[160]
Gan	2005	-	1	Canada	1 (Lam)	-	[161]
Kanaan	2006	-	1	Belgium	1 (Lam)	-	[163]
Panomsak	2006	-	6	Thailand	3 (1:3TC 2 Steroids)	-	[165]
Okuse	2006	-	1	Japan	1 (Lam)	-	[166]
Kusukabe	2007	1	4	Japan	1 child (IFN) 2 adults (IFN Lam)	-	[56]
Chuang	2007	-	1	Taiwan	1 (Lam)	-	[167]
Rodrigues	2007	1	-	USA (Thai)	1 (IFN)	-	[168]
Mesquita	2008	-	1	Belgium	1 (Lam)	-	[169]
Total		565	70	Children Adults	314 (55%) 35 (50%)	15 (3%) 8 (11%)	

^a Poor renal outcome: Renal failure or death from renal failure IFN – Interferon Lam - Lamivudine

From Table 2-16, of 565 children included in reports where outcome data is available, 314 (55%) showed remission and 15 (3%) had a poor renal outcome. (Renal failure or death from renal failure) Remission tended to take place within 4 years while renal failure may take more than 10 years to develop. Both of these figures are likely to be underestimates, the poor outcome more so than the remission as longer follow up is required for poor outcome to be revealed. (The remaining 42% of patients were still nephrotic or showed proteinuria and/or haematuria when last assessed.)

This first patient (child) to develop renal failure from HBV MGN was reported in 1979 and is noted in Table 2-1 on page 2-3 of the literature survey. By 1989 there was a total of at least six (6) cases of renal impairment/failure in HBV MGN in children. [16, 22, 24, 25, 62]

The findings of Hsu et al suggested that HBV associated MGN in children had a good outcome, even better than idiopathic MN in children. [25] It is generally accepted that

idiopathic MN in children has a better prognosis than in adults (Cameron 1990). [200] Hsu et al acknowledged, however, that prolonged course and renal failure do occur. Their assertion that little was known about the natural course of the disease has been partially answered by their publication and other reports since then. [25] Nevertheless, possible variations in outcome in different populations in different parts of the world and in particular the outcome of the small group who do not remit early, usually before 4 years, remained unclear and provided further motivation for this study.

Hsu (1989) found in their 52 patients that a number of clinical, renal biopsy and viral parameters were related to the disease course. Remission tended to occur more often and earlier in children of younger age, with a shorter disease course, those with fewer and less severe glomerular deposits and without focal glomerular sclerosis. The significantly longer course and reduced remission rate in children with more advanced sub-epithelial deposits (stages II-III and III) and/or focal sclerosis suggested that irreversible glomerular damage had already occurred in children with prolonged persistent proteinuria. [25] HBeAg seroconversion to anti-HBe was frequently accompanied by remission confirming the findings of others. [18, 21] Remission, however, usually occurred before HBeAg seroconversion and even in the presence of HBeAg. In addition, they found that remission was related more closely to the HBsAg seroconversion as all the patients (100%) with HBsAg seroconversion to HBsAg negative and anti-HBsAb positive remitted within two years. These findings suggest that circulating HBeAg may be necessary for the maintenance of the immunopathologic process in the kidney, but its presence does not preclude the development of a complete remission. [25]

On meta-analysis Venkateshan et al in 1990 estimated the following spontaneous remission rates; one year, 65% ; two years, 85% ; five to seven years, 95%. [16, 18, 22, 25, 62, 90, 120, 201, 202] Venkateshan et al reported that most of the patients who have received corticosteroids as treatment for the nephrotic syndrome of HBV MGN have had no beneficial effect or have experienced only transient or partial remissions. They noted that there was also evidence that corticosteroids could delay or prevent seroconversion, thereby making remission of HBV MGN less likely. [120]

A large South African series of 70 childhood HBV MGN patients with extensive follow-up (mean 46.3 months; range 2-139 months) was reported by Gilbert in 1994. Of the 70 patients in this series, ten received steroid therapy for 4-8 weeks of whom 6 were in remission at last follow-up, but none went into remission while on steroids and only one remitted within 6 months of treatment. The other 31 children in remission (total 37) did not receive steroids and there was no significant difference in the duration of nephrotic syndrome between those who received steroids and those untreated. [48] Two patients received steroids and cyclophosphamide before the relationship between HBV infections and MGN was recognised. Both went into remission at 6 and 38 months. The overall remission rate of this childhood HBV MGN cohort was about 10% at one year, 30% at two years, 64% at 4 years and 84% at 10 years. Of the 37 patients in remission, 33 had become serum HBeAg negative at the last visit. Of the 7 with asymptomatic proteinuria, 5 were serum HBeAg negative. The average time from clearance of the HBeAg to remission was 5 months (range: remission 24 months before seroconversion to 31 months after seroconversion). [48]

In most cases renal function was well preserved. However 9 patients (13% of total cohort) had a decline in estimated GFR of more than 25% between presentation and the last follow-up visit. (Mean follow up interval not reported). Three patients whose

proteinuria had resolved completely had estimated GFR's of between 60 and 80ml/min per 1,73m². Two patients progressed to end stage renal failure within 1 and 10 years respectively. One had been successfully transplanted and had normal renal function 2.5 years later. A renal graft biopsy performed during an acute rejection episode 6 months post transplant showed no evidence of recurrent MGN in the graft, although he remained serum HBeAg positive. [48]

In the Gilbert et al series, there were no significant differences between those whose renal function remained stable and those who developed progressive renal failure in terms of age, plasma albumin, total protein, degree of proteinuria, proteinuria selectivity index, total complement, C₃ or C₄ at presentation. (It is of interest that age did not seem to be a prognostic factor in this group as Hsu had found.) [25, 48] There was also no correlation between the stage of MGN and either the interval from the onset of symptoms or interval from biopsy to remission. Tubulo-interstitial changes also did not correlate with outcome. They were unable to confirm the finding of Hsu et al that patients with focal glomerular sclerosis had a longer duration of symptoms than those without, and this feature was also unhelpful in predicting ultimate renal function. There were no females in the group whose renal function declined but this was not statistically significant on multivariate analysis. [25, 48]

Gilbert et al concluded that the tendency for HBV associated MGN to resolve with clearance of HBeAg from the circulation was confirmed by their study. A cumulative remission rate of 85% was similar to the 92% remission rate at 9 years observed by Hsu et al and the 95% calculated by Ventakaseshan (1990) in a review of the subject. [25, 120]) The early remission rates (1 and 2 years) observed in the Gilbert study were somewhat lower than those calculated by Venkataseshan 1990. [48, 120]

Of the 70 HBV MGN children reported by Bhimma et al in 1998, at last hospital visit, 12 were in remission, 38 in partial remission and 20 in relapse. Sixty-five had normal renal function and 5 had varying degrees of renal failure. Excluding those followed less than one year (12 months), 10 of 46 patients (21.7%) had cleared both serum HBsAg and serum HBeAg, whilst 16 (34.8%) had cleared only HBeAg. The mean period for the clearance of both HBsAg and HBeAg in the 10 patients was 32 months (range: 12-72 months) whilst that for HBeAg only was 26 months (range: 12-56 months). The 12 who were in remission had all cleared serum HBeAg and 10 had also cleared serum HBsAg. Expressed differently, all ten who had cleared serum HBsAg were in remission. [142]

This same group subsequently reported a further 24 black South African children with biopsy proven HBV MGN compared to 20 historical controls. Those treated with interferon alpha 2b for 16 weeks had accelerated clearance of HBeAg with remission of 10 patients within 40 weeks. [152]

ADULT HBV MGN

In the Venkateshan et al USA series reported in 1990 only one of 5 adults with HBV MGN received specific treatment in the form of steroids and one of the others developed CRF. [120] The first substantial series of adults with HBV MGN and follow up, 21 patients from Hong Kong, was reported by Lai et al in 1991, 20 years after the first case report. [26] In contrast to the generally good outcome of HBV MGN in children, in this report of HBV MGN in adults, 6/21 (29%) patients had progressive deterioration of renal function during an average follow up of 73 months. Two (10%) required dialysis, and in one dialysis was imminent. Hypertension was more common in these 6 patients than in those who had stable function (67% vs. 20%) but this was not statistically significant. All patients were persistently seropositive for HBsAg. Of the

17 who were positive for serum HBeAg, only 2 seroconverted to anti-HBe antibody after treatment with either prednisolone or alpha-interferon. Spontaneous complete remission of the nephrotic syndrome after anti-HBe seroconversion was not reported among these adult patients with HBV-related membranous nephropathy. Among the 11 who received neither interferon nor prednisolone, none had attained complete remission and all of this subgroup who were initially serum HBeAg positive (9/11) remained so despite prolonged follow up. [26]

Contrary to the findings in children, spontaneous remission of proteinuria or the nephrotic syndrome did not occur in this adult HBV MGN group of 21, although proteinuria tended to decrease with time. Complete remission occurred after prednisolone treatment in three (14%) patients and after interferon treatment in one (5%). Complete remission was associated with seroconversion to anti-HBeAb in both those in remission and followed for more than 12 months. There was progression of chronic renal failure in 29% of the 21 patients after an average of six years; 10% of patients required dialysis. There appeared to be a discrepancy between the clinical response of patients with HBV related membranous nephropathy from areas where HBV is endemic and the response of those from non endemic areas who did better. [115] Lai et al suggested that this could be due to the integration of HBV DNA into host-cell chromosomal DNA in patients who acquired the infection in early childhood thus rendering interferon less effective.

In summary, Lai et al they found that spontaneous remission was uncommon among adults with HBV MGN who lived in an area where HBV infection was endemic and that the disease followed a slow, relentless course often leading to end stage chronic renal failure. [26]

Conjeevaram et al reported fifteen adult patients with HBV related GN who were

referred to a National USA facility for alpha-interferon therapy. [133] Ten were categorised on morphology as MGN (71%) and 4 as MCGN (29%). Of the 15 who were treated with alpha-interferon, 8 were long term responders, all with the MGN pattern. All 8 became serum HBe negative and 5/8 (62.5%) became HBsAg negative. Two of these patients developed chronic renal failure, both with MCGN patterns. Comparison of the patients who had a response to alpha-interferon with those who did not, showed the responders to be younger, to have shorter known courses of liver and kidney disease, higher mean initial aminotransferase values and lower levels of serum HBV DNA, DNA polymerase and serum HBeAg than non-responders. All of those showing good response had MGN while all 4 of the non-responders had MCGN.

The response to interferon therapy found in the patients with GN (both HBV MGN and MCGN combined) was higher than that seen in patients with typical chronic HBV (8/15-53% vs. 30-40%). The reason for this was not clear but Conjeevaram et al suggested that patients with extrahepatic manifestations may have an increased immunologic responsiveness and this could expose them to both increased spontaneous remission, especially in children and better response to interferon therapy in both adults and children. In summary 8/10 (80%) HBV MGN adults responded to alpha-interferon and went into remission. [133]

In 2005 KN Lai et al from Hong Kong demonstrated that lamivudine treatment improves renal outcome in adult HBV carriers with MGN and evidence of liver disease. Cumulative 3 year renal survival was 10/10 (100%) with lamivudine and 7/12 (58%) without its use. [159]

A meta-analysis of anti-viral therapy of HBV associated GN, predominantly MGN identified 84 unique patients from 6 clinical trials; three had controlled design. The overall estimate for proteinuria remission was 65.2%, serum HBeAg clearance 62.0%

and drop out rate 12.7%. Meta-regression analysis reported a significant link between serum HBeAg clearance and the reduction in proteinuria from the 5 trials using alpha-interferon. This report supports the role of HBV in the pathogenesis of the disease and confirms the efficacy of alpha-interferon and Lamivudine. [203]

The introduction of the nucleoside analog, lamivudine, has greatly improved and indeed revolutionised the treatment of chronic HBV infection. Lamivudine is the (-)-enantiomer of 3/-thiacytidine and it inhibits DNA synthesis by terminating the nascent proviral DNA chain through interference with the reverse transcriptase activity of HBV. [159, 204] As has been reported above it has been shown to induce remission in a high proportion of patients with HBV MGN. [159] Table 2-16 shows that a high proportion of the adults with HBV MGN reported in the last decade have remitted while on Lamivudine treatment. With the relative success of childhood vaccination in many parts of the world, [49] it is in adult HBV carriers that HBV MGN and other HBV linked GNs will be present for a while still.

IDIOPATHIC MGN

Row et al reviewed 12 series of idiopathic MGN patients published before 1975 containing 435 adults (over 15 years) and 82 children. Adults had a worse outcome than children. Two hundred and seven adults and 27 children were followed for 5 years or until death and 82 adults (19%) and 4 children (4%) died of renal failure. Remissions with complete loss of proteinuria were more frequent in children (49%) than in adults (16%) and 50% of the remissions in adults and 67% in children were spontaneous. [205]

Schwartz reports on the clinical course of untreated primary MGN and summarises a wealth of data by noting that MGN is usually an indolent disease that progresses

slowly in most cases so that even when renal insufficiency develops, it takes years to reach end stage kidney disease. Some patients, however, progress more rapidly. Approximately 25% of patients will experience complete or partial remission but this may take years to occur. As many as 50% will suffer significant loss of renal function during follow up. [176]

Bhimma et al described a cohort of 24 black South African children with biopsy proven idiopathic MGN and compared them with their series of 81 children with HBV MGN. Although their follow up was limited (mean of 2.9 years, range 1.5 - 5.2 years), 22 (91.7%) at last visit had normal renal function and 2 (8.3%) chronic renal failure. The outcome was not markedly different between the groups. [142]

6) CLASSIFICATION of HBV GN in general and HBV MGN in particular

This review of HBV MGN and to an extent HBV GN in general and in particular the renal biopsy pathology has highlighted issues of GN classification.

The 1982 WHO classification of glomerular disease had a section entitled 'Glomerulonephritis of Systemic Diseases' but while this included such entities as lupus nephritis, glomerular lesions in certain systemic infections and a group of parasitic nephropathies, it did not have a specific category for any of the viral diseases. There was however a group of three chapters under the general heading of 'Glomerular lesions in Metabolic Diseases', where in chapter 16 a section was devoted to 'Nephropathy of Liver Disease'. This section made reference to mesangial, mesangiocapillary and MGN patterns and reported that in patients with Hepatitis B virus that HBsAg and HBeAg had been demonstrated in some cases. [206]

Therefore at that time a patient who was HBV positive with a predominantly MGN morphology would be categorised as secondary MGN. For the pathologist finding features in these cases not usually associated with MGN and idiopathic MGN in particular such as prominent mesangial deposits, frequent subendothelial deposits and striking mesangial interposition, the situation presented a dilemma. Should the immune complex GN be called something else such as a combination of MGN and mesangiocapillary GN or more specifically mesangiocapillary type III, a category not widely accepted or used? Was the appearance a unique combination or spectrum like SLE that warranted subtypes, possibly unique new ones, or at least recognition of combinations of current patterns? This was the situation that practicing renal pathologists faced, including this author from 1986, on commencing responsibility for the service in this centre. Ultrastructural pictures of HBV MGN were taken across the city to Dr C Sinclair Smith, who was more experienced and had been the pathologist in the Red Cross Hospital HBV MGN publication in children and it was confirmed that this was indeed what HBV MGN cases in children looked like. [21]

Many of the pathological issues involved were well set out by a Korean report from 1988 covering children and adults with HBV associated GN. [108] In a group of 732 consecutive renal biopsy patients, 560 adults and 172 children (14 years and younger), 87 were found to be serum HBsAg positive.

Of the 87 they discounted 40 of the patients whose biopsies were classified in categories of glomerular or renal disease (46%) viewed as being co-incidental including 6 with minimal change disease, 6 with FSGS, 16 with IgA nephropathy (although this is a group others have viewed as at least partially HBV related) [54, 207, 208] , 4 post infectious GN, 5 mild focal non-specific GN, one SLE MGN and 2 end stage kidney disease. Of the remaining 47 serum HBsAg positive patients, 9 were children and 38 were adults. The numbers and proportions of these 47 patients

reported to have MGN and MCGN patterns and the percentages these then comprised of the respective child and adult GN subgroups is shown below in table 2-17.

Table 2- 17 *Korean HBV MGN and MCGN as a proportion of each GN subgroup*
Serum HBsAg positive GN aetiologically linked (n= 47)

Age group	MGN	HBV MGN	HBV MGN%	MCGN	HBV MCGN	HBV MCGN%
Children	10	8	(80)	4	1	(25)
Adults	50	10	(20)	32	28	(88)

HBV MGN was prominent among children constituting 80% of the MGN cases while HBV MCGN was prominent among adults with 88% of the MCGN cases being HBV positive. In fact without the HBV associated patients, MCGN would have been rare in adults in this biopsy series. (4/560 adults – less than 1%) Table 2-17

The description of the HBV MGN cases included the observation that all children showed microscopic haematuria but only 40% of the adults. Concerning the pathology, as expected all 18 showed diffuse thickening of capillary walls. The ultrastructural studies, done on 17 of the adults and children, showed diffuse glomerular subepithelial and/or intramembranous deposits in all patients and small amounts of mesangial deposits in 12. In addition, a few isolated tiny subendothelial deposits were noted in 4 biopsies. (The ultrastructural table did not distinguish adults and children.)

The pathological features of the 29 MCGN cases included prominent subendothelial and mesangial deposits in all 28 cases studied by EM and subepithelial and/or intramembranous deposits in 22 (79%) These descriptions are confirmation of the spectrum of changes found in HBV GN often with both MGN and MCGN features as

emphasised by the frequency (79%) of subepithelial deposits in the cases described as MCGN. [108]

In the absence of an established international classification, each pathologist or unit classifying these cases must develop their own cut off points for MGN and MCGN or produce a mixed group with variable definitions. An example of an International classification would be the WHO or Renal Pathology Society/International Society of Nephrology (RPS/ISN) Lupus nephritis guidelines. [209, 210] (Even then the application by different pathologists of these guidelines is unlikely to be identical.)

When both subepithelial deposits and mesangial interposition, for example, are present which feature should take precedence? [108]

The assessment of Lee et al of their own findings included confirmation of the already well accepted notion by that stage (1988) of the high incidence of HBV MGN in children. They held that their correlation of HBV with MCGN in their adult group was remarkable and that this was a new finding that had not been emphasised before although there had been some case reports. [63, 72] Their frequency of non HBV MCGN was very low. They reported that the morphological features showed no characteristic pattern in HBV MCGN but it appears that the prominence and frequency of subepithelial deposits and the consequent frequent combination of MCGN and MGN features is distinctive though not pathognomonic. Similarly they downplayed the distinctiveness of HBV MGN where mesangial deposits and subendothelial deposits in their series and even mesangial interposition in many other cases and series are often present, challenging the boundary between MGN and MCGN. [108]

A 1994 Hong Kong series of 100 serum HBsAg positive renal biopsy patients (referred to in detail in the immunofluorescence section of this literature review on page 2-78) provided valuable perspectives on the frequency of HBV associated GN and the nature and spectrum of the associated forms of GN. Using monoclonal antibodies, at

least one of the 3 HBV antigens was detectable in glomeruli in 39% of cases. When the diagnosis of HBV related glomerulonephritis was based strictly on detectable glomerular antigens, 3 distinct morphologies were identified in Hong Kong patients; MGN, MCGN and mesangial proliferative with IgA (IgA nephropathy). These were seen mostly pure but also in overlapping forms. Glomerular HBeAg and HBsAg were associated with subepithelial and mesangial immune complexes respectively. [193]

Table 2-18 *Hong Kong adult HBV series (n=100)*

MGN		MGN/IGA		MGN/MCGN		MCGN		IgA/HSP ^a		CGN ^b		OTHER		END STAGE	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
20	(20)	2	(2)	4	(4)	3	(3)	34	(34)	2	(2)	35	(35)	4	(4) (excluded)

^a Henoch Schonlein Purpura ^b Chronic Glomerulonephritis

MGN was diagnosed in a total of 26 patients Hong Kong patients, two cases showed IgAN in addition and 4 cases were mixed with MCGN. The remaining 20 were classified as 'pure' but they also showed some MCGN features as will be detailed below. Twenty of the 24 tested in this group (83%) showed glomerular granular HBeAg staining, the diagnostic hallmark of the group. Seven of the 24 (29%) also showed monoclonal HBsAg, 5 in mesangial areas and 2 subepithelial. Eleven of the 20 'pure' MGN group, showed mild to severe mesangial sclerosis and severe cases were associated with extensive tubular loss or atrophy and interstitial fibrosis.

Ultrastructural features revealed subepithelial deposits in all 23 of the 26 examined with subendothelial deposits in 7, including all 4 mixed with MCGN. (Therefore 3/20 -

15%- 'pure MGN' also showed subendothelial deposits) Mesangial deposits on ultrastructure were also seen in 9 (39%) and 8 (35%) also showed virus like particles. The 3 with pure MCGN also showed sparse subepithelial deposits in 2 cases together with small mesangial and subendothelial deposits. Four patients were categorised as mixed with both spikes and mesangiocapillary interposition.

In summary, 7 cases of MCGN were recognised in this series, three essentially pure and 4 mixed with prominent MGN components.

IgA nephropathy was diagnosed in 36 patients, 3 with clinical features of Henoch-Schonlein purpura and 2 others mixed with MGN. Most, 22, showed mild mesangial hypercellularity. Others showed segmental sclerosis, segmental necrotising lesions and cellular crescents. The diagnosis was established by predominant IgA complexes on immunopathology and ultrastructure. Mesangial HBsAg was detected in between 35 and 47% with monoclonal and polyclonal HBsAg respectively. [193]

An earlier paper from this group found the frequency of HBsAg carriers to be 21/122 (17.2%) in Hong Kong patients with primary IgA nephropathy. This is significantly higher than the prevalence in the general population of 9.5%. Two glomerular entities; mesangial proliferative glomerulonephritis with predominant mesangial IgA deposits and a mixed picture of MGN with capillary IgG and mesangial proliferative with mesangial IgA deposits were observed. Glomerular deposits of HBsAg and/or HBcAg were observed in 12/21 (57%). Their study at that point suggested that HBV may play a significant role in the development of IgA nephropathy in areas of high HBV endemicity. [54, 207, 208] From the South African perspective, it might be added that an HBV carrier population strongly susceptible to IgA nephropathy is also needed. This is not an entity which has been strongly suggested from many other studies except Hungary and another from Hong Kong. [72, 92]

Venkateshan et al in their series of 12 children and adults with HBV GN also included a category, as Lai et al above had, to describe a mixture of features which they designated as 'Mesangiocapillary and membranous GN' and which was used to categorise 4 renal biopsies, 3 from children and one from an adult. [120, 193] Yoshikawa et al made reference to a biopsy in HBV GN which they described as mesangiocapillary Type III. [94] The use of these categories acknowledged the HBV MGN-mesangiocapillary spectrum of changes seen and the absence of an existing classification to accommodate the variety of biopsy appearances.

By 1996, the revised WHO classification had a more complete list of 'Glomerulonephritis of Systemic Diseases' with about 4 subgroups and 20 entities. Here HBV was dealt with together with HCV and HIV. Most of the main features of the pathology of HBV associated GN had become better recognised with both MGN and MCGN patterns and features and the overlap between the two being reported.

It was noted that in addition to the subepithelial deposits required to diagnose MGN that the HBV variety usually showed mesangial deposits with increased matrix and cells. Although it was noted that the MCGN lesion often had focal subepithelial deposits, the frequency of combinations of subendothelial deposits and mesangial interposition in the predominant MGN subgroup was not specifically emphasised. [210]

A comparison in 2000 between HBV MGN and SLE MGN highlighted many similarities and some differences between these 2 groups. [149] The glomerular lesions in both clinical entities were similar and at times indistinguishable, demonstrating polyclonal immunoglobulins and complement in similar ranges of subepithelial, subendothelial and mesangial deposits. In HBV associated GN the glomerular lesions linked to the virus included MGN, mesangiocapillary GN, a subset of IgA nephropathy and

importantly the morphologic overlaps. The range of reaction patterns in HBV GN bears many similarities to lupus nephritis (mesangial proliferative, mesangiocapillary, membranous, diffuse sclerosing and frequent combinations.) Lupus nephritis can not be adequately described by being a subset of MGN and MCGN and similarly neither can HBV MGN. [149, 209]

In the light of the above development in the 1996 WHO Atlas and the many similarities between SLE and HBV glomerular involvement referred to in the Lai comparison, [149, 210] it is surprising and of some concern that the 2005 Atlas of Non-Neoplastic Kidney Disease [184], does not follow the 1996 classification. This book deals with HBV associated GN as HBV MGN and HBV MCGN under the respective GN sections rather than on its own as a GN of a systemic infection with a range of morphological features. This atlas is part of the series of the American Registry of Pathology/Armed Forces Institute of Pathology (ARP/AFIP) Atlases of NonTumour Pathology and is therefore viewed as a worldwide reference text. [184]

To summarise, there is a lack of uniformity in the way HBV GN morphology has been reported. This fundamental issue regarding classification of GN in general and the difficulty of making consistent, distinctive and reproducible categories of biological continuums, such as that of HBV MGN and MCGN in particular, is one of the focus points of this study.

7) MOTIVATION FOR AND AIMS OF THIS STUDY

The above literature review confirms that much information has been gathered on HBV MGN since the first case was reported in 1971. Nevertheless, there are still fewer than 1000 documented cases in the English literature in children and fewer than 250 in adults. (Table 2-6) More than 250 of the children with HBV MGN have been

documented from Southern Africa but no adult series of HBV MGN with follow up. [48, 49, 62, 85, 140, 142, 148] Therefore, this study providing detailed clinicopathological information on 71 children and 12 adults with HBV MGN contributes substantial numbers to both groups. There are also a number of areas where data is still minimal or contradictory. This study aims to contribute additional information to add to the body of knowledge of HBV MGN both clinically and pathologically.

Areas of particular focus in this study are the pathology of HBV MGN especially the ultrastructural spectrum including the frequency and severity of mesangial and subendothelial deposits as well as mesangial interposition. The role of HBeAg in the glomerulus is also a focus area and as no other African centre has reported HBeAg glomerular staining; this would be a unique contribution. The range of data documented enables clinicopathological correlations to be made including with outcome. A unique feature of this study is the long follow up enabling the establishment of the long term outcome of HBV MGN in the children who fail to remit early. Another contribution is the comparison of a range of features in childhood and adult HBV MGN as well as idiopathic MGN in adults from a single centre which has not been documented before.

With long-term follow up in the cohort of 46 SA children with HBV MGN in particular, together with the literature review, results and critical discussion of these cohorts and the world literature, a comprehensive overall perspective on HBV MGN worldwide from 1971 - 2010 can be constructed. It is unusual for a new form of GN to be identified, have its pathogenesis substantially unravelled and then be observed to decline within 4 or 5 decades. In this case, this latter possibility would be due largely to urbanisation and a vaccine. [41, 59]

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Chapter 3

PATIENTS, MATERIALS AND METHODS

This was both a retrospective and prospective descriptive study with a comparison between the study cohort and two control/comparative cohorts. The hypothesis of this study was that HBV MGN in childhood was distinctive and therefore compared to an adult HBV MGN group and an idiopathic MGN group. A further desirable comparative group namely childhood idiopathic MGN was not feasible as only 2 such patients were seen in the study period. The project was conducted at Tygerberg Hospital (TBH) a teaching and referral hospital in Cape Town, Western Cape Province in SA. The hospital, in addition to serving the Western Cape and parts of the Northern Cape also serviced Namibia until 1988.

The three groups that are compared in this study are the following:

GROUP 1 – Study group: Childhood HBV MGN (n=71)

This group consisted of seventy-one (71) children below the age of 12 (inclusive) who presented between 1974 and 1995 with follow-up until June 2005. The children were part of a cohort of 309 children with severe proteinuria/nephrotic syndrome who were managed at Tygerberg Hospital over this period. They tested positive for serum HBsAg and had a membranous GN pattern on renal biopsy. The majority, 242 children, were from the Western and Northern Cape Provinces of South Africa and 67 were from Namibia.

GROUP 2 – Comparative group: Adult HBV MGN (n=12)

This group comprised twelve (12) adults, defined as 13 years and older who were serum HBsAg positive and had a membranous GN morphology on renal biopsy and

no other cause for MGN. This group was identified from a series of consecutive renal biopsies carried out between 1986 and 2000. In this adult group of 1196 patients who had an adequate native renal biopsy between 1986 and 2000 (excluding repeat biopsies and renal transplant biopsies), 126 patients were identified with MGN while 37 of the whole group of 1196 were serum HBsAg positive. Fifteen were both serum HBsAg positive and demonstrated membranous GN morphology. Three of this group were eliminated from the final statistical analysis as they had other possible associations with membranous GN namely carcinoma of the lung, positive VDRL for syphilis and positive anti-nuclear factor (ANF). All 3 had at least one of these and one had 2 risk factors. The 1991 Lai et al Hong Kong adult HBV MGN series had reported differences between the outcome of HBV MGN in adults and children but not by direct group comparison. [1] This study incorporated direct comparison between this adult HBV MGN group and the childhood HBV MGN cohort in order to reveal similarities and/or differences between patients with an MGN reaction pattern linked to the same association but in different age cohorts.

GROUP 3- Comparative group: Adult idiopathic MGN (n=33)

The third group was 33 adults with idiopathic MGN selected as a consecutive group from the same adult biopsy cohort referred to above. These 33 presented to Tygerberg Hospital between 1986 and 1993. This was not the whole group of 55 seen in the series but the minimum number viewed sufficient for statistical purposes. Group 3 was chosen to compare the study group to an idiopathic group of MGN with the same general glomerular morphology but no known cause.

a) DEMOGRAPHIC, CLINICAL INFORMATION AND ROUTINE LABORATORY TESTS

Clinical and routine laboratory data were obtained from patient records including hospital summaries and the renal clinic records. Demographic data collected included the **age**, **gender** and **race**. In the case of the childhood HBV MGN cohort the **town**, **suburb** (in the case of greater Cape Town or the Cape Metropolitan area, or **area of origin/ethnicity** in the case of the **Namibian children**).

The following clinical and laboratory data was documented; **Oedema** - absent, mild or severe, **Ascites** - present or absent , **Blood pressure** – adjusted for age, **Proteinuria** - measured by dipstix, **Haematuria** - measured by dipstix, serum **Haemoglobin**, serum **Urea**, serum **Creatinine**, serum **Total Protein**, serum **Albumin**, serum **Cholesterol**, **Liver enzymes** – serum **Aspartate-Aminotransferase (AST)**, serum **Alanine-Aminotransferase (ALT)**. For each of the liver enzymes of the HBV MGN children two values were noted, an initial level and the highest recorded for each patient during the study period. For the adults often only an initial value was available and therefore only the presentation values were recorded. Serum **Complement** : Complement testing began at Tygerberg Hospital in 1976, shortly after the start of the study period. Different methods and normal values applied during the study period. Values for C₃ and C₄ were recorded as normal or low according to normal values at the time of testing.

Other tests included serum electrolytes, VDRL, ASOT and antinuclear factor as well as tests for any infections suggested on clinical examination.

b) HEPATITIS B SEROLOGICAL TESTS

The method and system of testing varied over the years. Serum HBsAg was measured using radioimmunoassay (RIA) (Ausria-Abbott) 1973-1986; Enzyme linked

immunoassay (ELISA Abbott Laboratories) 1987-1990; Organon Microelisa 1990-1992; Abbott IMX 1992-1996 and Abbott AxSYM until the end of the study in 2005.

During 1991 testing for serum HBe antigen on then current HBsAg positive sera of the patients and stored sera of study group patients was undertaken.

Shortly after this, routine testing for serum HBe antigen positivity was initiated for HBs antigen positive patients using similar commercial kits to those used for the serum HBsAg and HBsAb tests.

c) RENAL BIOPSIES - LIGHT MICROSCOPY

All patients had renal biopsies performed. These were performed percutaneously with open biopsies in 2 children. All biopsies were performed by trained nephrologists or paediatric nephrologists.

Biopsy material was not handled uniformly over the study period. From 1973 until 1991 children's renal biopsy material for light and electron microscopy was initially fixed in 2.5% glutaraldehyde in 0, 1 M phosphate buffer and then sectioned under a dissecting microscope. Tissue for light microscopy was subsequently fixed in formalin and processed routinely. For light microscopic examination serial 2-3 micrometer sections were stained with haematoxylin and eosin, Alcian blue periodic acid - Schiff, periodic acid-methenamine silver, Masson trichrome and Verhoeff-van Gieson stains. Because glutaraldehyde fixation negatively influenced subsequent immunocytochemical testing, renal biopsy material from children was divided into 3 in the laboratory by the medical technologist using the dissecting microscope from 1991. Portions were prepared for light microscopy (then fixed in 10% buffered formalin) for immunofluorescence and ultrastructure. (Biopsy material from adults had always been handled in this way.) The light microscopic examination was carried out

in a routine fashion with all available slides on each case reviewed by the author and further sections cut and stained where necessary.

The following features were examined for, documented and scored where indicated.

(In all cases the features scored for each renal biopsy were assessed while reviewing all the available material on each biopsy enabling correlation and integration to take place. In all cases the light microscopy features were based predominantly on the wax block and toluidine blue sections, the ultrastructural features on the EM sections and pictures but the latter also including integration with light microscopy sections of the wax block and toluidine blue sections where appropriate as emphasised in the ultrastructural section page 3-9.)

1. The **number of glomeruli** in the biopsy
2. The **number of crescents** expressed as a percentage (%) of the total number of glomeruli.
3. The **number of sclerosed glomeruli**. For simplicity both segmental and global sclerosis counted as a sclerosed glomerulus which was then expressed as a percentage (%) of the total number of glomeruli in the biopsy.

4. **Blood vessel involvement**

0 - No abnormality; 1- mild; 2 - moderate; 3 - severe

Mild described hyalinosis or mild vessel wall thickening in up to 25% of vessels.

Severe would imply fibrinoid necrosis or severe luminal narrowing in many vessels, with moderate in between.

5. **Interstitial fibrosis**

6. **Tubular atrophy**

7 **Interstitial inflammatory infiltrate**

Categories 5, 6 and 7 scored according to the scale

0 - absent; 1- mild; 2 - moderate; 3 - severe

In this classification severe implied prominent change affecting more than 50% of the biopsy, mild less than 25% and moderate in between.

8. Mesangial proliferation [2] with further subdivision

1. Mild: 4 nuclei per mesangial area
2. Moderate: 5 or 6 nuclei per mesangial area
3. Severe: 7 or more nuclei per mesangial area

The scales of categories 4-8 are not standard but were developed by the author to semi-quantify the features. Similar approaches have been used by other workers evaluating renal biopsies for example lupus nephritis. [3]

d) ELECTRON MICROSCOPIC EXAMINATION

Processing

Prior to processing, tissue was divided into 1 mm cubes and placed in phosphate buffer. By viewing the tissue under the dissecting light microscope, small blocks most likely to contain glomeruli could be cut with a sharp blade. These small pieces of tissue were then placed in 1.5% osmium tetroxide for one hour as part of post-fixing or secondary fixation. The osmium tetroxide fixes mainly lipids which reduce the osmium leaving an electron dense deposit. The blocks were then taken through ascending concentrations of alcohol (70% -100%) and a mixture of alcohol and resin, and then embedded in resin placed in predried gelatine capsules. The capsules with the embedded tissue were left overnight at 60°C for the resin to polymerise. The resin block with the tissue at the tip of the capsule was then ready for trimming. The trimmer used was a Reighert TM60.

Toluidine Blue Sections

Sections were stained with toluidine blue and the most appropriate blocks and glomeruli selected for subsequent thin sections and electron microscopy. On average, 12 blocks were made of each renal biopsy. Until 1990, 5 of these blocks were trimmed to produce toluidine blue sections. One or two blocks with glomeruli would then be selected for further sectioning. As part of this study, all toluidine blue sections of each biopsy, as well as the available electron micrographs were reviewed. It was ensured that at least one glomerulus from each of no less than two blocks had been examined. From 1991 until 2000 (when the latest renal biopsy in the series was studied) all 12 blocks of all renal biopsies were sectioned for toluidine blue staining.)

Cutting of thin sections

The selected blocks were trimmed into a trapezium with a diamond bit. The ultra-microtome cut automatic thin sections of 70-90nm. These were lifted off the surface of the water bath onto a copper grid and allowed to dry. They were stained on a drop of saturated uranyl acetate in 50% alcohol and subsequently on a drop of lead citrate. The sections were then examined under the electron microscope. From 1973 to 1983 the electron microscope used was a Zeiss EM 952 and from 1984 till the end of the study a Hitachi 600/2 with both scanning and transmission capacity. At least 5 photomicrographs of each of the two sections were available for evaluation.

The following morphologic features on ultrastructure were assessed and quantified where possible.

Stage of subepithelial deposits

Stages as described by Ehrenreich and Churg [4] were used including combinations or overlaps following Hsu [5] and scored as below.

1. Stage I - subepithelial deposits only.
2. Combinations of Stage I and II. Some subepithelial deposits with no reaction, some showing basement membrane 'spike' formation.
3. Stage II. Subepithelial deposits with widespread spike formation.
4. Stage II and III. Subepithelial deposits with spike formation and areas of incorporation of deposits.
5. Stage III. Predominantly incorporated subepithelial deposits.
6. Stage IV. Any signs of intramembranous lucent areas. Usually seen in combination with earlier stages.

Mesangial deposits

0: None 1: Mild 2: Moderate 3: Severe

Mild described the presence of any mesangial deposits as assessed predominantly by ultrastructure. Severe implied large widespread deposits impinging to an extent on capillary lumina with moderate in between.

Subendothelial deposits

0: None 1: Mild 2: Moderate 3: Severe

A definite subendothelial deposit seen on one of the ultrastructural photos was staged as mild, severe implied that many of the capillary loops showed subendothelial deposits, at least 4 per glomerulus, and moderate was 2 or 3 per glomerulus.

Mesangial interposition

0: None 1: Mild 2: Moderate 3: Severe

As with the previous 3 categories a combination of light microscopy with special stains, toluidine blue sections and particularly ultrastructural examination was used to differentiate between these 4 categories.

Mild implied at least one clear example of mesangial interposition, severe described 4 or more examples of mesangial interposition per glomerulus and moderate fell between the two, that is 2 or 3 per glomerulus.

Virus like bodies

A heterogeneous collection of "virus like bodies" was identified usually in or near deposits. They were noted as either absent or present.

Tubuloreticular bodies

These bodies were also noted as either being present, usually in glomerular endothelial cytoplasm, or absent. In the initial documentation of the biopsies a small number had been photographed. As part of this study they were specifically searched for, for about 10 minutes, on each of the two glomeruli available in each case, by trained and experienced electron microscopic technologists.

IMMUNOFLUORESCENCE AND IMMUNOHISTOCHEMICAL METHODS

From 1977 until the end of the study, immunofluorescent staining was routinely carried out on renal biopsies. Between 1986-1988 immunoperoxidase techniques were sporadically tried on renal biopsies which formed part of the study cohort. From 1988 more systematic immunoperoxidase testing was begun. By 1990 a monoclonal antibody to Hepatitis Be antigen had become available and was imported from

Japan. The antibodies were produced by the Institute of Immunology Co., Ltd Tokyo. The specific monoclonal antibodies were HBe (a) (IgG2a) Hyb-904 and HBe (b) (IgG1) Hyb-905. (Institute of Immunology Specification sheet) As part of the study, systematic testing of stored and prospective frozen tissue as well as paraffin blocks of formalin fixed tissue of the study group renal biopsies was carried out. Later similar procedures were followed with tissue from some of the renal biopsies of the control groups.

Tissue Preparation

Frozen tissue

Segments of unfixed renal tissue were placed on saline impregnated blotting paper and then frozen using liquid nitrogen. Cryostat sections of 3 micrometers were cut, fixed in acetone and air dried.

Immunofluorescence

There have been a number of changes in routine methods since 1977. Initially from 1977 to 1983, a direct method was used with Fluorescein isothiocyanate (FITC) linked primary antibodies. The polyclonal anti-HBsAg (goat anti-HBsAg from Dako Laboratories) has only been available for use in an indirect method since the commencement of the study. In 1983 a two stage indirect method was introduced necessitating different primary antibodies and a secondary FITC linked antibody. From 1986 a three step process was used with a biotinylated antibody of the appropriate species with visualisation by a streptavidin-FITC. In 1991, more specific immunoglobulins were selected. Once the samples had been prepared, the specimens were examined with a fluorescent microscope.

Immunofluorescent testing was scored as negative or 1+ to 3+. For this study 1+ to 3+ was simplified to positive. As illustrated in Fig 5-41 on page 5-38 this staining was

usually granular, predominantly along capillary walls and was almost always 2+ or 3+ in intensity. Therefore 3 categories were possible:

0: Never done or no glomeruli available 1: Positive 2: Negative

The following antigens were so evaluated:

IgA - Dako Corporation FITC- Conjugated Rabbit Anti-human IgA Code F0204

IgG – Dako Corporation FITC- Conjugated Rabbit Anti-human IgG Code F0202

IgM - Dako Corporation FITC- Conjugated Rabbit Anti-human IgM Code F0203

C₃ - Dako Corporation FITC- Conjugated Rabbit Anti-human C3c Code F0201

Fibrinogen - Dako Corporation FITC- Conjugated Rabbit Anti-human Fibrinogen Code F0111

HBs - Dako Corporation Polyclonal Goat Anti-HBsAg Code B560

HBs - Dako Corporation Monoclonal Mouse Anti-HBsAg Code M3506

HBc - Dako Corporation Polyclonal Rabbit Anti-HBcAg Code B586

HBe (a) - Institute of Immunology Co., Ltd Tokyo. Mouse monoclonal anti-HBe (a) (IgG2a) Hyb-904

HBe (b) - Institute of Immunology Co., Ltd Tokyo. Mouse monoclonal anti-HBe (b) (IgG1) Hyb-905.

MORPHOLOGIC EXAMINATION OF THE LIVER

Most of the HBV MGN children, who were all serum HBsAg carriers, had abnormal liver function tests at times. In view of the degree of abnormality of liver function tests or other clinical indications, ten children underwent liver biopsy and 9 had sufficient tissue for diagnosis.

FOLLOW UP

South African HBV MGN children

From the clinic follow-up records the clinical status (nephrotic syndrome, proteinuria, haematuria, remission, renal failure or death) of each child at 2 and 4 years and when last seen was documented. Every effort was made to contact all previous patients via letters to their last known home address. This resulted in a number of patients visiting the paediatric nephrology clinic.

Namibian patients

Where known, renal failure and death from renal failure were noted. An attempt was made via a Windhoek paediatrician to get further follow-up but this proved unsuccessful.

Adult membranous groups

From the clinical records, outcome at 2 and 4 years where available was noted in the same categories as set out above regarding the children. The years chosen were determined by the follow up available on the adult groups.

STATISTICAL ANALYSIS

Continuous measurements were compared between the different groups using Analysis of Variance (ANOVA). Bonferroni posthoc tests were done in cases with significant differences between the groups. Categorical variables were compared using the Chi-square test. For continuous variables means and 95% confidence intervals are given while for categorical variables numbers and percentages are provided. A p value < 0.05 was viewed as significant.

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Chapter 4

RESULTS a) CHILDHOOD HBV MGN

The renal biopsies of all 71 patients in the study group were examined by a single observer (the author) and confirmed to have at least the minimum inclusion criteria morphological features of MGN namely widespread subepithelial deposits on ultrastructure. (Note again that many biopsies also had features not usually found in MGN and these additional elements are among the main focal points of this thesis.) The parameters of this group of 71 will be presented in the form of tables and then selected features in various figures such as histograms, maps and photographs. For some parameters, the results are further highlighted by figures or a summary of key results. Findings are at presentation in the childhood HBV MGN group unless otherwise specified.

Table 4-1 Demographic and clinical data of children with HBV MGN (n=71)

Parameter	Result	Further Information
	Mean or No. (95% CI) or (%)	
<i>Demography</i>		
Age - years	6.0 (3.8;8.2)	
Gender - male	57 (80.3)	14 female (19.7%)
Race Black	31 (43.7)	25 Namibian:6 Western Cape
Coloured	40 (56.3)	40 Western and Northern Cape
White	0 (0)	
Serum HBsAg positive	71 (100)	Part of definition of group
Serum HBeAg positive	33 (87)	Total number tested = 38 (33/38)
<i>Clinical</i>		
Oedema - Present	70 (99)	
Mild	39 (55)	
Severe	31 (44)	
Ascites - present	34 (48)	
Hypertension	25 (35)	
Proteinuria – stix mean	2.87 almost +++	Scored as from key in parameter column
Absent = 0	0 (0)	
+ = 1	2 (2.8)	
++ = 2	15 (21.1)	
+++ = 3	44 (61.9)	
++++ = 4	10 (14.0)	
Haematuria – stix mean	3.0 = ++	Scored as from key in parameter column
Absent = 0	4 (5.6)	
Trace = 1	3 (4.2)	
+ = 2	16 (22.5)	
++ = 3	17 (23.9)	
+++ = 4	23 (32.3)	
++++ = 5	8 (11.2)	

Table 4-2 Laboratory data of children with HBV MGN (n=71)

Parameter	Result Mean or No. (95% CI) or (%)		Further Information
Laboratory			
Haemoglobin g/dL	11.5	(11.1;12.0)	
Urea mmol/L	5.1	(3.9;6.4)	
Creatinine micromol/L	45.8	(25.9;65.7)	5 had age adjusted raised levels
Total protein g/L	46.3	(44.4;48.3)	
Albumin g/L	16.4	(15.9;17.9)	
Cholesterol mmol/L	10.7	(9.9;11.5)	
AST U/L (Normal 0-40)	57.0	(46.7;67.2)	44/71 (62%) above normal ;8 above 100, outlier at 412
AST Highest	102.6	(82.0;123.2)	60/71 (84.5%) above normal. 23 above 100
ALT U/L (Normal 0-53)	57.0	(43.4;70.6)	24/71 (31%) above normal. 8 above 100, same outlier as AST above at 560
ALT Highest	114.6 (87.3;142.1)		48/71 (67.6%) above normal. 29 above 100
C3 low	41/65	(63.1)	
C3 low after 6 months	20/31	(64.5)	
C4 low	22/65	(33.9)	
C3 low after 6 months	10/31	(32.3)	

The children ranged from 2-12 years. The relatively small group of 6 year olds (close to both the mean and median of the group) is striking and has no obvious explanation. Of the whole group, 37 were 5 years or younger and 34 were 6 years and older. (Fig 4 -1)

The Namibian group of 25 black children had a similar mean age at presentation to the group as a whole namely 5.9 years.

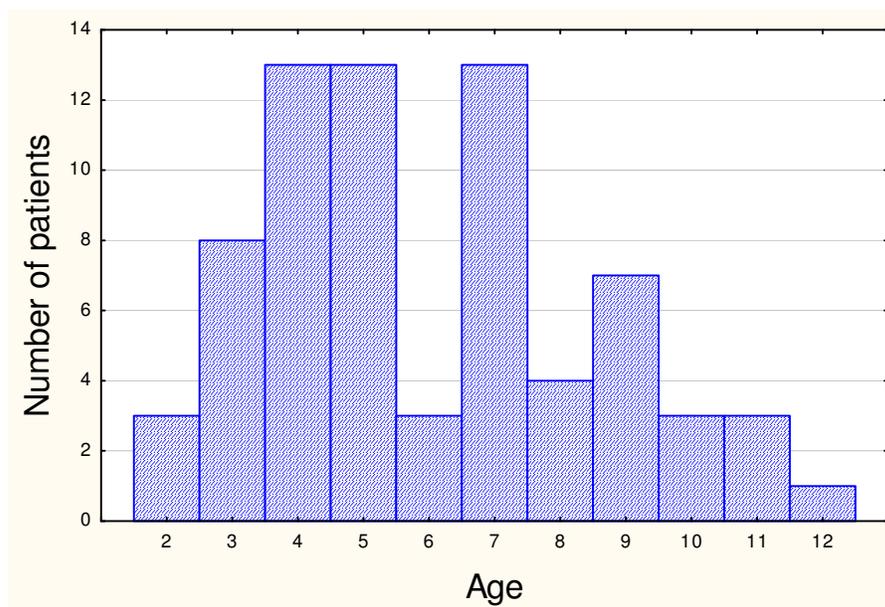


Fig 4-1 Age distribution of the HBV childhood cohort

The disease occurred significantly more commonly in boys than in girls.

(57:14 - 80%: 20%).

All the children were coloured or black as noted in Table 4 -1. The origins of the children show that rural areas are well represented. (Fig 4-2 and Table 4-3)

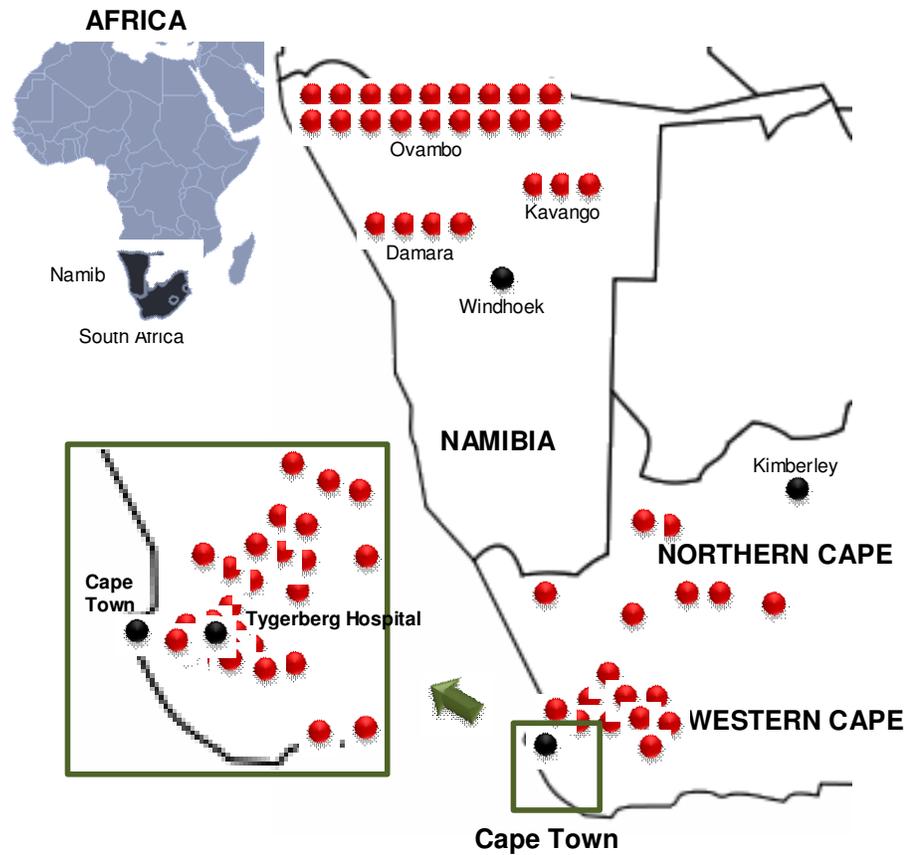


Fig 4-2 Map of Africa and enlargement of Southern Africa to show the origin of the 71 HBV MGN children. Each red dot represents a child with HBV MGN.

Table 4-3 *Detail of home suburb, town, city and province of SA children*

Data base Number	Home	Urban Greater Cape Town (CT)	Peri urban ;To 100 km from CT	Rural - More than 100 km
76	Muldersvlei		WC Peri-urban	
77	Eerste River		WC Peri-urban	
78	Keimoes			NC Rural
79	Porterville			WC Rural
80	Bellville South	WC Urban		
81	Carnarvon			NC Rural
82	Nyanga East	WC Urban		
83	Faure		WC Peri-urban	
84	Langebaan			WC Rural
85	Elsies River	WC Urban		
86	Groot Drakenstein		WC Peri-urban	
87	Carnarvon			NC Rural
88	Moreesburg			WC Rural
89	Victoria West			NC Rural
90	Worcester		WC Peri-urban	
91	Tulbach			WC Rural
92	Stanford		WC Peri-urban	
93	Ravensmead	WC Urban		
94	Laingsburg			WC Rural
95	Paarl East		WC Peri-urban	
96	Paarl		WC Peri-urban	
97	Ravensmead	WC Urban		
98	Keimoes			NC Rural
99	Williiston			NC Rural
100	Mitchell's Plain	WC Urban		
101	Durbanville	WC Urban		
102	Elsies River	WC Urban		
103	Hermanus		WC Peri-urban	
104	Wellington		WC Peri-urban	
105	Bellville South	WC Urban		
106	Paarl East		WC Peri-urban	
107	Elsies River	WC Urban		
108	Porterville			WC Rural
109	Kuils River		WC Peri-urban	
110	Elgin		WC Peri-urban	
111	Ceres		WC Peri-urban	
112	Robertson			WC Rural
113	Wellington		WC Peri-urban	
114	Nababeep			NC Rural
115	Durbanville	WC Urban		
116	Stellenbosch		WC Peri-urban	
117	Belhar	WC Urban		
118	Ladismith			WC Rural
119	Calitzdorp			WC Rural
120	Worcester		WC Peri-urban	
121	Prince Albert			WC Rural

Key: WC - Western Cape, NC – Northern Cape

As detailed in Table 4-3, twelve of the 46 South African children came from greater Cape Town – suburbs like Bellville, Ravensmead, Mitchell’s Plain; 17 from towns within about 150 kms from Cape Town such as Paarl, Worcester and Hermanus – categorised as periurban - and 17 from farms and towns further than 150kms from Cape Town grouped as rural to deep rural. This data confirms that many children came from rural and periurban areas and fits with the finding that HBV carrier rates were higher in rural areas. [3, 4] A large group came from Namibia, especially the north (Ovambo). The children from Namibia came from the following tribes/areas: 18 Ovambo, 4 Damara and 3 Kavango.

Serum HBeAg positivity was frequent in the childhood HBV MGN patients (87%). Concerning clinical features, almost all showed oedema, while ascites (48%) and hypertension (35%) were commonly reported. Proteinuria and haematuria were usually present on Labstix testing and were reported as 76% showing 3+ or 4+ for protein and 67% displaying 2+-4+ for haematuria with only 6% showing no haematuria. (Table 4-1)

Five children had age adjusted raised creatinine levels but only one reported a level over 100micromol/L. (Table 4-2) Almost all displayed features of nephrotic syndrome/severe proteinuria such as lowered total protein and albumin levels as well as raised cholesterol levels. Increased levels of the liver enzyme AST were present in 62% of the children at presentation with 85% demonstrating raised values when follow up was included. Another liver enzyme ALT demonstrated a similar pattern with slightly lower percentages. More than 60% demonstrated lowered C₃ values which remained low for at least 6 months. C₄ patterns were similar but the initial proportion with lowered levels was 34%. (Table 4-2)

Table 4-4a Renal biopsies of childhood HBV MGN : Light microscopy (n=71)

Parameter	Result	
	Mean or No.	(95% CI) or (%)
Number Glomeruli	26.8	(21.9;31.6)
Glomerular sclerosis mean %	4.5	(0.8;8.3)
None	48	(68)
< 10%	12	(17)
> 10%	11	(15)
Crescents Present in biopsies	4	(6)
Mean of % per biopsy	0.52	(Four values %: 2, 4, 14, 17.)

Table 4-4b Renal biopsies of childhood HBV MGN : Light microscopy (n=71)
Semi-quantitative biopsy scoring

Parameter	Mesangial	Interstitial	Tubular	Interstitial Fibrosis	Blood vessels
	Proliferation	Inflammation	atrophy		
	Mean (95% CI) or No. (%)				
	1.78 (1.01;1.20)	0.60 (0.44;0.76)	0.15 (0.02;0.28)	0.38 0.22;0.53)	0.05 (-0.05;0.16)
Absent	0 (0)	34 (48)	61 (86)	45 (63)	67 (94)
Mild	64 (90.1)	32 (45)	9 (13)	25 (35)	4 (6)
Moderate	6 (8.5)	4 (6)	1 (1.5)	1 (1.5)	0 (0)
Severe	1 (1.4)	1 (1.5)	0 (0)	0 (0)	0 (0)

Key to semi- quantitative scoring : Absent = 0; Mild = 1; Moderate = 2; Severe = 3. Criteria described in Chapter 3 pages 3-5 and 3-6

Glomerular sclerosis was rare with a mean of only 4.5% and most biopsies (68%) reporting no sclerosis. Crescents were also rare (0.5%). (Table 4-4a) Glomerular mesangial proliferation was present in all biopsies, usually mild (90%), and interstitial damage was rare. Where interstitial inflammation and fibrosis were present the degree was almost always mild. (Table 4-4b)

Table 4 - 5a Renal biopsies of childhood HBV MGN: Ultrastructure (n=71)

Ultrastructural parameter	Result Mean or No. (95% CI) or (%)	
Stage of subepithelial deposits ^a		
1 = Stage I	1	(1.5)
2 = I + II	2	(3)
3 = II	5	(7)
4 = II + III	35	(49)
5 = III	23	(32)
6 = IV	5	(7)
Mean score for group	4.29	(4.0; 4.6) Between stages II and III
Virus-like bodies	59	(83.1)
Tubuloreticular bodies	62	(87.3)

^a Hsu 1989 [5]

Table 4-5b *Renal biopsies of childhood HBV MGN: Ultrastructure (n=71)*
Semi-quantitative biopsy scoring

Parameter	Mesangial deposits		Subendothelial deposits		Mesangial interposition	
	Mean No.	(95% CI) or (%)	Mean No.	(95% CI) or (%)	Mean No.	(95% CI) or (%)
Absent	0	(0)	38	(53)	8	(11)
Mild	22	(31)	26	(37)	22	(31)
Moderate	33	(46)	4	(6)	18	(25)
Severe	16	(23)	3	(4)	23	(32)

Key to semi- quantitative scoring : Absent = 0; Mild = 1; Moderate = 2; Severe = 3. Criteria described in Chapter 3 pages 3-8 to 3-9

Subepithelial deposits, the hallmark of MGN, were usually in stages II or III or combinations thereof (88%). (Table 4-5a) Virus like bodies (83%) and tubuloreticular-inclusion bodies (87%) were present in the majority of renal biopsies. Further features, not usually reported in idiopathic MGN, were mesangial deposits in 100% of biopsies, subendothelial deposits in (47%) and mesangial interposition in (89%). The group of (23%) with severe mesangial deposits and the (32%) reported with severe mesangial interposition are highlighted as these changes were shown to have prognostic and other correlations. (Table 4-5b) The group of 23 with severe mesangial interposition were categorised as a group then called mixed HBV MGN-mesangiocapillary GN for this dissertation. There is not a generally accepted definition for such a group and many of the remaining cases also showed some features found in mesangiocapillary GN as well as HBV MGN features. Two of the groups (Venkateshan et al and Lai et al) have employed such a category (HBV

mixed MGN-mesangiocapillary GN) as well as HBV MGN and HBV MCGN in categorizing their series. They have not provided clear definitions of this group but a reasonable inference would be that the biopsies displayed sufficient features to have been placed in both categories. [6, 7]

Positive immunofluorescent staining for IgG was reported in 98% of biopsies tested while for C₃ the proportion was 82%. These high figures would be expected in MGN. Lower figures were reported for IgA, IgM and fibrinogen. A polyclonal antibody against HBsAg was positive in 65% of biopsies while a more specific one was negative in the 3 cases tested. The 2 monoclonal anti-HBe antibodies proved positive

Table 4-6 *Renal biopsies of childhood HBV MGN : Positive Immunofluorescence in glomeruli tested for the following antigens (n=71)*

(Note number able to be tested varies and is shown after the antigen tested for or antibody used.)

Antigen/Antibody	No. glomeruli positive (%)	
IgA (52)	29	(55.7)
IgG (54)	53	(98.1)
IgM (53)	38	(71.7)
C₃ (51)	42	(82.3)
Fibrinogen (49)	13	(26.5)
HBs (Polyclonal) (40)	26	(65.0)
HBs (Monoclonal) (3)	0	(0)
HBc (20)	19	(95.0)
HBe(a) (20)	15	(75.0)
HBe(b) (20)	16	(80.0)
HBe(a) or (b) or HBc (20)	19	(95.0)

IgA Immunoglobulin A

IgG Immunoglobulin G

IgM Immunoglobulin M

C₃ Complement 3

HBs Hepatitis B Virus surface Antigen

HBc Hepatitis B Virus core Antigen

HBe(a) Hepatitis B Virus envelope Antigen subtype (a)

HBe(b) Hepatitis B Virus envelope Antigen subtype (b)

The positivity was predominantly granular along capillary walls of ++ or +++ intensity as illustrated in Fig 5-41 on page 5-38

in over 75% of cases with a combined figure of 95% similar to that of the HBc antibody. (Table 4-6)

LIVER BIOPSIES

Nine successful liver biopsies were undertaken in the 71 patients, usually at times when their liver enzymes were significantly elevated. Five had mild portal inflammatory changes, a morphology which was originally diagnosed as chronic persistent hepatitis. Currently these appearances would be classified as chronic HBV associated hepatitis, without interface changes; grade 1; stage 0 or 1 depending on the degree of structural change and fibrosis. [8] (Fig 4 - 3)

Four had mild or moderate portal inflammation with interface hepatitis, previously called piecemeal necrosis and the biopsies were originally diagnosed as chronic active hepatitis. In most cases architecture was essentially retained but early bridging fibrosis was present in one case. These would fit with grades 2-4 of chronic HBV associated hepatitis. No severe architectural changes such as cirrhosis were seen in any cases. [8] HBsAg and HBcAg were confirmed in these liver biopsies by immunohistochemical staining.

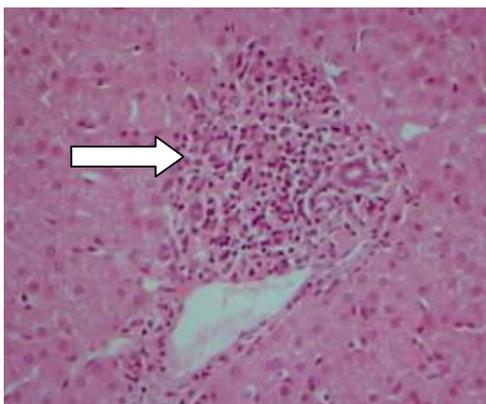
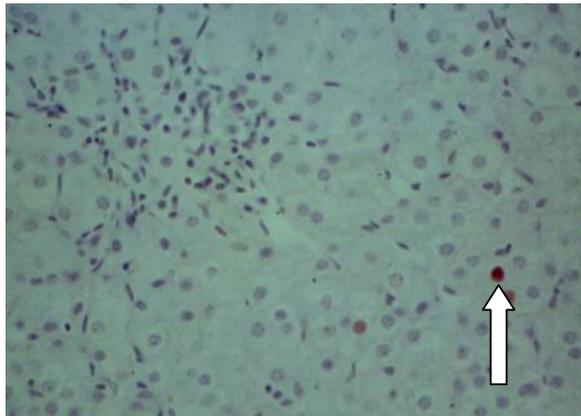


Fig 4-3 Liver biopsy from one of the HBV MGN children with chronic hepatitis (HBV associated). Grade 1 without interface changes. Originally diagnosed as chronic persistent hepatitis. Note chronic inflammatory infiltrate (→) in portal area without interface changes.

Haematoxylin and Eosin stain (H and E) x200



Immunohistochemistry for HBcAg x 200

Fig 4-4 Liver biopsy showing chronic hepatitis in an HBV MGN child. HBe staining (brown) in some nuclei noted. (↑)

OUTCOME

Table 4-7 Childhood HBV MGN: Outcome of entire cohort (n=71)
(43 had no 2 year follow up; 24 Namibian and 19 South African)

Outcome	At 2 years n=28		At 4 years n=23	
	No.	(%)	No.	(%)
Remission	7	(25.0)	12	(52.2)
Haematuria	0	(0)	1	(4.4)
Proteinuria	6	(21.4)	3	(13.0)
Nephrotic syndrome	13	(46.4)	5	(21.7)
Renal failure	2	(7.1)	1	(4.4)
Death	0	(0)	1	(4.4)
Remission	7	(25.0)	12	(52.2)
Other	21	(75.0)	11	(47.8)

Outcome was known for 28 children at 2 years and 23 at 4 years. At 4 years 12 patients (52%) were in remission and 2 had a poor renal outcome of failure or death (9%).

OUTCOME OF 46 SOUTH AFRICAN CHILDREN WITH HBV MGN

The forty-six (46) South African children were followed up for a mean of 89.7 months (range 0-356 months.) The outcome is shown in table 4-8. The one who had been in renal failure but had become HBsAg negative has been transplanted and two were known to have died of renal failure. While the features of patients in each group were not entirely uniform, there were similarities within most groups.

Table 4-8 Renal status/outcome and serum HBV status at last clinic visit or admission of SA HBV MGN Children. Follow up: Mean of 89.7 months (range 0-356 months.) (n=46)

OUTCOME	No. (%)	HBs Pos No. (%)	HBs Neg No. (%)	HBe Pos No. (%)	HBe Neg No. (%)	HBe not tested No. (%)
Remission	14	10 (71)	4 (29)	2 (14)	10 (71)	2 (14)
Proteinuria	6	6 (100)	0 (0)	2 (33)	3 (50)	1 (17)
Haematuria	1	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)
Nephrotic	19	19 (100)	0 (0)	10 (53)	3 (16)	6 (32)
Renal Failure	4	3 (75)	1 (25)	1 (25)	3 (75)	0 (0)
Death CRF	2	2 (100)	0 (0)	1 (50)	1 (50)	0 (0)
TOTAL	46 (100)	41 (89)	5 (11)	17 (37)	20 (43)	9 (20)

REMISSION (n=14)

Fourteen children (30.4%) of the total SA group were in remission when last seen. The mean time to remission was 31.2 months (range: 15 – 89 months) in the 13 where this could be determined. Seven of 13 (53.8%) were earlier than 24 months and 12/13 (92%) before 48 months.

Ten of the children (71%) who went into remission were followed for substantial periods and in all but one case remained in remission. This child was in clinical remission at 32 months after first presentation but remained positive for serum HBeAg as well as HBsAg. Over the course of the following few years this boy developed persistent haematuria and intermittent proteinuria associated with a marked elevation in liver enzymes. At a time of markedly raised liver enzymes he had a liver biopsy which showed HBV chronic hepatitis. Within a further year his liver enzymes settled and he became HBeAg negative but remained HBsAg positive. His renal disease remitted again, ninety-three months after his initial renal remission. Such an example of recrudescence of HBV MGN symptoms after initial remission has not been previously reported. (The patient died of unrelated causes at age 17 years and the kidney was not examined microscopically at the subsequent forensic autopsy.)

At the last visits of the 14 children in remission (Table 4-8), 4 patients (29%) were negative for both serum HBV antigens, 6 patients (43%) were positive for serum HBsAg but negative for serum HBeAg, 2 (14%) were positive for serum HBsAg with serum HBeAg unknown, one (7%) was still serum HBsAg and HBeAg positive and one (7%) untested. In summary in this group 4 (29%) had become serum HBsAg negative and only 2 (14%) had remained serum HBeAg positive. (The one child who had presented later as a young adult for an unrelated problem, but in remission from

HBV MGN was not tested for HBV status and so an updated serum HBV status cannot be included.)

PERSISTENT PROTEINURIA (n = 6)

The six in this group had follow up times ranging from 20-245 months (mean 96 months). All were still serum HBsAg positive but 3 (50%) had become serum HBeAg negative, 2 remained serum HBeAg positive and in one the status was unknown.

HAEMATURIA (n=1)

One child who would otherwise have been in remission showed persistent haematuria after 56 months follow up. This child was still HBs and HBe antigen positive.

NEPHROTIC SYNDROME (n=19)

This was the largest single group. Included in this group were 3 children with no follow up and 8 with fewer 12 months of follow up. All 19 (100%) were still serum HBsAg positive and 10/13 (76.9%) still HBeAg positive. The mean follow up time was 15 months (range 0-88 months).

RENAL FAILURE and DEATH (n = 6) (Table 4 – 9 includes the Namibian child)

This subset of 46 South African children includes the largest group (6 patients) reported with a poor outcome following HBV MGN from a single centre. They were all coloured males aged 4 - 11 years at presentation. Only one, patient 118, developed renal failure within 2 months of initial presentation, while the others developed CRF 7 to 23 years after diagnosis. The mean time to failure of this latter group of 5 is 197 months; in three CRF developed over longer than 19 years and

Table 4-9 Selected characteristics of children with HBV MGN and a poor outcome (n=7)

Patient	Age ^a Years	Gender	Race ^b	Initial creatinine umol/L	Time to raised creatinine Months	Serum HBsAg	Serum HBe Ag	Outcome
77	11	male	c	44	233	+	-	CRF
78	8	male	c	61	94	+	-	CRF-died age 21
81	10	male	c	53	253	-	-	CRF-tx
90	4	male	c	21	291	+	-	CRF
109	9	male	c	62	117	+	-	CRF- died age 20
118	9	male	c	63	2	+	+	CRF
63 Namibian	6	male	b	133	0	NA	NA	CRF- died within 4 years

Key: ^a Age at presentation ^b Race : c-coloured b-black CRF- Renal failure

tx – Renal transplant NA – Not available

one patient developed renal failure 23 years after initial presentation. This last mentioned child was the only one from the younger group of under 5s.

Concerning HBV antigen status, this group confounds some of the general tendencies that HBe seroconversion is associated with better outcome. Five of the six remained serum HBsAg positive when last tested and 3 of those with extended follow up had become serum HBeAg negative. (The child who developed renal failure within two months was followed only 5 months and was still HBsAg and HBeAg positive.) The 6th patient became HBsAg and HBeAg negative, which made the possibility of transplantation greater under SA conditions. He was also unusual as he became HBsAg negative within 3 years yet developed renal failure 20 years later.

This group of patients warrants individual case reports which can be found in the appendix.

Of significance is that all 7 (including the Namibian boy) who fared poorly were male and only one was under 5 years of age.

THOSE WITH AT LEAST 24 MONTHS FOLLOW UP

To try to compensate for incomplete follow up and therefore get a more accurate assessment of outcome those followed fewer than 24 months could be excluded unless they were in remission or failure. (The latter 2 being viewed as usually 'permanent' states.)

In this group of 29, remission occurred in 14, proteinuria 4, haematuria 1, nephrotic syndrome 4, renal failure 4 (one has been transplanted) and renal failure and known death 2. In summary therefore, good outcome in 14/29 (48.2%), poor renal outcome for 6/29 (20.6%) – although one transplanted - and uncertain outcome in 9/29 (31.0%) of the SA children. The final complete outcome would then depend on the number with persistent proteinuria, haematuria and nephrotic syndrome (the uncertain group of 9 – 31%,) as well as the other 17 followed fewer than 24 months who either went into remission or eventually developed renal failure.

CLINICOPATHOLOGICAL CORRELATIONS

Three features, one demographic (age), and two pathological (severe mesangial deposits and severe mesangial interposition) were chosen to test against all the other findings with results below. (**Tables 4-10 to 4-12**) Other features were also tested in this way such as gender and race. In addition a previous investigator had found some correlations with age and outcome. [5] Mesangial interposition seemed a natural one to correlate further as some investigators had found HBV

mesangiocapillary GN to have a poorer response to treatment and outcome than HBV MGN. [9] Age and severe mesangial deposits showed the most interesting and significant differences.

The younger patients had more frequent HBe positivity, less frequent hypertension, lower creatinine levels, less severe renal biopsy changes and more frequent remission. (Table 4-10)

Table 4-10 *Clinicopathological comparison of groups aged five and under versus six and over (n=71)*

Parameter	Age ≤5 n=37	(95% CI)	Age ≥6 n=34	(95% CI)	p value
	Mean or No.	or (%)	Mean or No.	or (%)	
HBe serum positivity	19/19	(100)	14/19	(73.6)	<0.01
Hypertension	9	(24.3)	16	(47.1)	0.04
Haemoglobin	11.1	(10.6;11.6)	12.0	(11.5;12.5)	0.01
Creatinine	40.6	(34.7;46.4)	51.5	(45.5;57.6)	0.01
Mesangial deposits	1.70	(1.5;1.9)	2.14	(1.9;2.4)	<0.01
Subendothelial deposits	0.40	(0.2;0.7)	0.82	(0.6;1.1)	<0.02
Mesangial interposition	1.51	(1.2;1.8)	2.08	(1.7;2.4)	0.02
Remission 2 years	7/15	(47)	0/13	(0)	<0.01
Remission 4 years	10/14	(71)	2/9	(22)	0.02
Tendency					
Cholesterol	11.4	(10.3;12.7)	9.8	(8.6;11.0)	0.05
Glomerular sclerosis%	2.5	(-0.8;5.9)	6.7	(3.2;10.3)	0.09
Interstitial fibrosis	0.27	(0.1;0.4)	0.50	(0.3;0.7)	0.06
Blood vessels	0.027	(-0.05;0.1)	0.088	(0.009;0.17)	0.09
Stage subepithelial deposits	4.1	(3.8;4.4)	4.5	(4.2;4.8)	0.07

Table 4-11 Mesangial deposits in Childhood HBV MGNNon severe versus severe subgroups. ^a

Parameter	Non Severe		Severe		P value
	n=55		n=16		
	Mean or No.	(95% CI or (%))	Mean or No.	(95% CI or (%))	
Age (years)	5.5	(4.9;6.2)	7.5	(6.4;8.7)	0.01
Gender-female	14	(25)	0	(0)	<0.01
Crescents %	0.25	(-0.4;0.9)	1.43	(0.1;2.7)	0.02
Sclerosis %	3.27	(0.5;6.0)	8.95	(3.8;14.0)	0.01
Interstitial inflammation	0.50	(0.3;0.7)	0.92	(0.6;1.2)	0.02
Tubular atrophy	0.07	(-0.03;0.17)	0.43	(0.3;0.6)	<0.01
Interstitial fibrosis	0.27	(0.14;0.40)	0.75	(0.51;0.98)	<0.01
Blood vessels	0.01	(-0.04;0.07)	0.18	(0.07;0.3)	<0.01
C ₃ immunofluorescence	35/38	(92)	7/13	(54)	<0.01
Subendothelial deposits	0.4	(0.2;0.6)	1.31	(0.9;1.6)	<0.01
Mesangial interposition	1.47	(1.2;1.7)	2.87	(2.4;3.3)	<0.01
Outcome Remission 2 years	(7/22)	(32)	0/6	(0)	0.05
Outcome Remission 4 years	(12/20)	(60)	0/3	(0)	0.03
Tendency					
Race - black	20/55	(36)	11/16	(69)	0.07
HBe serum positivity	27/29	(93)	6/9	(67)	0.06
Cholesterol	11.13	(10.2;12.1)	9.1	(7.3;10.9)	0.06
Mesangial proliferation	1.07	(1.0;1.2)	1.25	(1.1;1.4)	0.08
Stage subepithelial deposits	4.18	(3.9;4.4)	4.6	(4.2;5.1)	0.05

^a Defined in Materials and Methods Chapter 3

The patients with the most severe mesangial deposits (Table 4-11 above) were older, exclusively male, showed more severe renal biopsy changes in most categories and none went into remission. This group also showed a tendency to include more black patients and included 9 Namibian children. (The lack of follow up on the Namibian children leaves an outcome shortcoming as these 9 would be predicted to have done poorly)

Table 4-12 Mesangial interposition in Childhood HBV MGNNon severe versus severe (mixed HBV MGN-mesangiocapillary GN) subgroups. ^a

Parameter	Non Severe n=48		Severe n=23		P value
	Mean or No.	(95% CI) or (%)	Mean or No.	(95% CI) or (%)	
Race - black	17/48	(35)	14/23	(61)	0.04
Hypertension	12/48	(25)	13/23	(57)	<0.01
Mesangial proliferation- moderate	1/48	(2)	5/23	(22)	<0.01
Tubular atrophy	3/48	(6)	6/23	(26)	0.02
Interstitial fibrosis	13/48	(27)	12/23	(52)	0.02
Stage subepithelial deposits	4.10	(3.85;4.35)	4.69	(4.32;5.06)	<0.01
Mesangial deposits - severe	2/48	(4)	14/23	(61)	<0.01
Subendothelial deposits- Moderate and severe	1/48	(2)	6/23	(26)	<0.01
C ₃ immunofluorescence	31/34	(91)	11/17	(65)	0.02
Tendency					
Gender-female	12/48	(25)	2/23	(9)	0.09
Cholesterol	11.2	(10.2;12.3)	9.4	(8.0;10.9)	0.05
Blood vessels	1/48	(2)	3/23	(13)	0.07
Not significant					
Haematuria	3.2	(2.6;3.4)	3.2	(2.6;3.7)	NS
C3 low	31/47	(66)	10/18	(56)	NS
C4 low	16/47	(34)	6/18	(33)	NS
Outcome Remission 2 years	(6/18)	(33)	1/10	(10)	NS
Outcome Remission 4 years	(10/16)	(63)	2/7	(29)	NS

^a Defined in Materials and Methods Chapter 3 NS Not significant

This is a key comparison as this group, assessed as having severe mesangial interposition on light and electron microscopy, has been classified as the mixed HBV MGN-mesangiocapillary GN group where the degree of the features would be sufficient to make each diagnosis separately. Some of the significant differences include the severe mesangial interposition group having more black children, a higher proportion of hypertension, more children with subendothelial deposits, more

severe mesangial deposits and a greater degree of mesangial proliferation. More advanced subepithelial deposits and more severe interstitial disease are also associated with the more severe mesangial interposition subgroup of 23. The females (girls) show a tendency to less severe mesangial interposition. Concerning outcome, it may have been predicted that the severe mesangial interposition group of HBV MGN (the mixed HBV MGN-mesangiocapillary GN group as defined for purposes of this dissertation) would show a poorer outcome but while that appears broadly true (Table 4-12), the differences are not statistically significant. The 2 children with severe mesangial interposition who went into remission by 4 years, one even by 2 years, render the outcome correlation non significant.

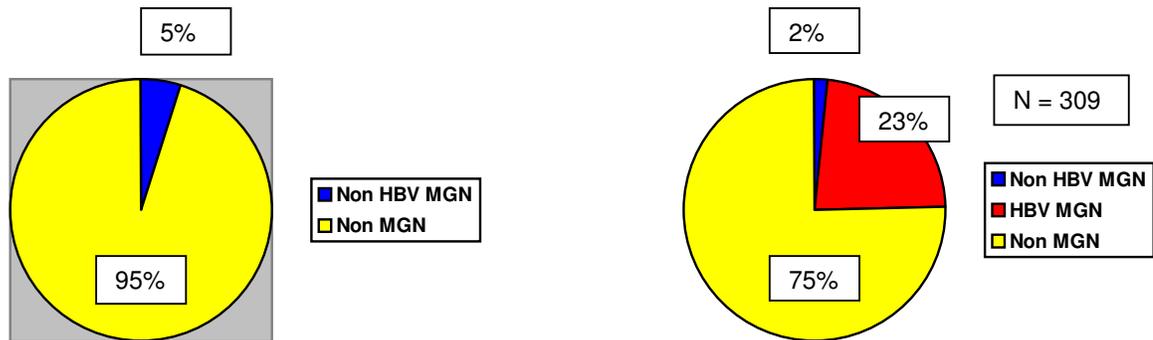
Table 4-13 Features of 14 HBV MGN children in remission compared to 7 children with poor outcome (n=21)

Feature/Category	Remission (n=14) Mean (95% CI) or No. (%)		Poor Outcome (n=7) Mean (95% CI) or No. (%)		P Value
Age - years	4.2	(3.1;5.4)	8.1	(5.9;10.4)	<0.01
Gender - male	10	(71)	7	(100)	0.05 T
Hypertension	6	(43)	6	(86)	0.05 T
Creatinine	37.7	(25.4;50.1)	62.4	(45.0;79.9)	<0.05
Renal Biopsy					
Stage deposits	4.1	(3.6;4.5)	5.3	(4.6;5.9)	<0.01
Tubular Atrophy	0.07	(-0.2;0.3)	0.6	(0.2;1.0)	<0.05
Mesangial deposits	1.6	(1.3;2.0)	2.3	(1.8;2.7)	<0.05
Subendothelial deposits	0.4	(-0.1;0.8)	1.4	(0.8;2.0)	<0.05
Mesangial interposition	1.4	(0.8;2.0)	2.3	(1.5;3.1)	<0.1 T

The most significant differences between these key groups (Table 4-13) were that the children with poor outcome were older children and their renal biopsies had more advanced stages of subepithelial deposits as well as higher creatinine levels and more renal changes such as tubular atrophy, mesangial deposits and subendothelial deposits.

GROUP OF NEPHROTIC SYNDROME/SEVERE PROTEINURIA CHILDREN AT TYGERBERG 1974-1995

Between 1974 and 1995, 309 children 12 years of age and below were seen and all except 65 were biopsied at TBH for severe proteinuria / nephrotic syndrome. The patients who were not biopsied but who were classified in this series were those who presented with features most likely to represent minimal change disease (MCD), who responded to immunosuppression and had a clinical course compatible with MCD. If they had an unusual course or some initial features less suggestive of MCD they would have been biopsied. Of this group of 309, 76 (24.6%) were found to have MGN; this proportion is much higher than that found in parts of the world where HBV carrier rates are low. (Fig 4-5) (71 had HBV MGN and of the remaining 5; 2 had SLE, 1 was associated with syphilis and 1 had never been tested for HBV leaving 1, possibly 2, as idiopathic.)



Western pattern or any country with low HBV carrier rates 5% non HBV MGN [1]

TBH Western Cape South Africa and Namibia 1974 -2005 HBV carrier rates 2% - 13 % [2, 3]

Fig 4-5 MGN as a cause of nephrotic syndrome in children

Table 4-14 *The Namibian nephrotic children – histopathological classification (n=67)*

Histopathological category	No.	%
Minimal change	5	(7.5)
Focal segmental glomerulosclerosis (1HBV)	11	(16.4)
Mesangial proliferative GN	15	(22.4)
Endocapillary proliferative GN	1	(1.5)
Mesangiocapillary GN (1 HBV positive)	3	(4.5)
Membranous GN (25 HBV, 1 syphilis – 1 eliminated as over 12 years of age)	26	(38.8)
Diffuse sclerosing GN	6	(8.9)

In the original clinical series of 70 children, 68 histological specimens were evaluated (67 biopsies and one autopsy). One child in advanced renal failure was not biopsied and one with steroid resistant, relentless nephrotic syndrome died before biopsy could be performed. Twenty-nine (41.4%) of these 70 children were HBV carriers of whom 25 (86.2%) were male. Of the 29, 26 had predominantly MGN, one mesangiocapillary (MCGN), one focal segmental glomerulosclerosis and one was in advanced failure and not biopsied. Three of the eight who are known to have died were HBV carriers in end stage renal failure. Only one child with MGN in this series was not HBV positive but had syphilis. Of the 26 with HBV MGN in this reported series, the one child of 15 years was not included in this present comparative series where the childhood cutoff age was 12. The remaining 25 are included with the 46 from the Western and Northern Cape. This series of Namibian children with nephrotic syndrome, confirmed previous findings that black children from Southern Africa, have very different patterns to most other children. Minimal change disease was very uncommon, 5/68 (7.4%) while HBV MGN was the single largest group 26/68 (39.7%) [10].

Table 4-15 South African children with nephrotic syndrome - TBH (n=242)

Histopathological category	HBV	No.	%
Minimal change ^a	3	87	(36.0)
Focal segmental glomerulosclerosis (1HBV)	1	8	(3.3)
Mesangial proliferative GN	5	61	(25.2)
Endocapillary proliferative GN	1	21	(8.7)
Mesangiocapillary GN		6	(2.5)
Membranous GN (46 HBV, 1 non HBV, 1 not tested, 2 SLE also included with SLE)	46	48	(19.8)
Total MGN 50 = 20.66%			
SLE (2 MGN)		6	(2.5)
Diffuse sclerosing GN		3	(1.2)
Tubulointerstitial		2	(0.8)

^a (22 biopsied 65 clinical and therapeutic response diagnosis)

In the series of SA children with severe proteinuria/nephrotic syndrome presenting from 1974 -1995 (Table 4-14) MCD is the single largest group (36%) including 65 who were managed without a renal biopsy. Mesangial proliferative GN (MPGN) was the next most common cause of NS. The HBV MGN group was the main subgroup of the MGN category which with the 2 SLE MGN children constituted nearly 21% of the cohort (50 children). The MGN group as a whole was the third largest category in the TBH SA series. Ten of the SA children who were HBV positive in the serum did not show MGN but a variety of other patterns; 3 MCD, 5 mesangial proliferative GN and one each of FSGS and post infectious (endocapillary proliferative) GN. As 2 of the 27 HBV positive Namibian children did not show MGN (one FSGS and the other MCGN), the overall proportion of MGN in the 83 HBV positive children in the total cohort of 309 was therefore 71/83 (85%), by far the dominant glomerular pattern.

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Chapter 5

RESULTS b) COMPARISON BETWEEN HBV MGN IN CHILDREN, ADULTS WITH HBV MGN AND IDIOPATHIC MGN

The HBV MGN and idiopathic MGN adult groups were drawn from a consecutive series of renal biopsies performed on adults during a 15 year period from 1986 to 2000. The 126 patients with MGN represent (10.5%) of the total of biopsied cases (n=1196); 69 (55%) were idiopathic; 29 (23%) SLE; 15 (12%) HBV and 13 (10%) had other (see page 5-42) associations. (Fig 5-1)

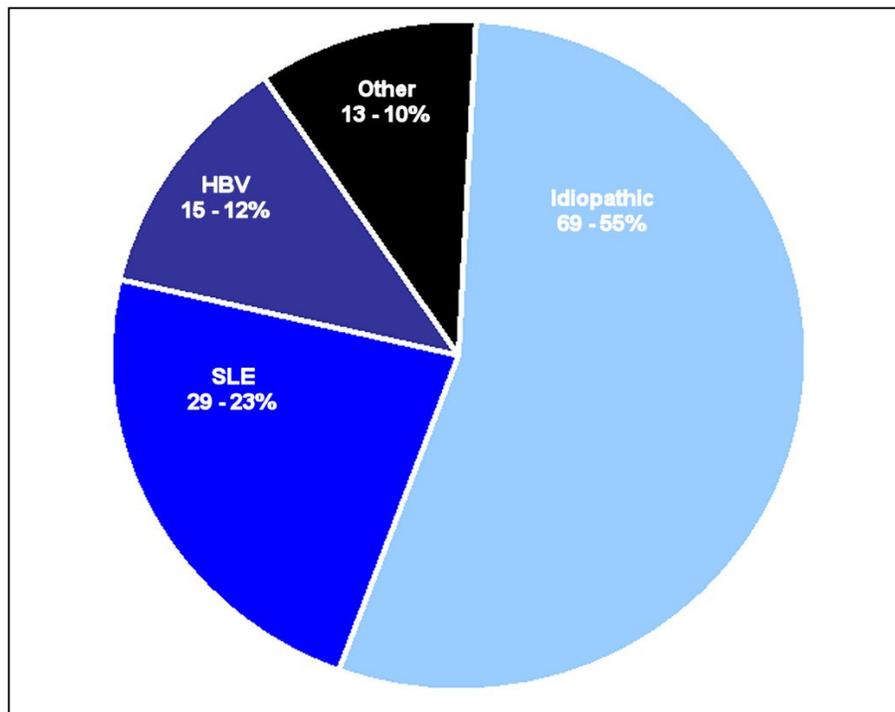


Fig 5-1 The aetiology of MGN in adults (n=126)

The following section compares the study group of children with HBV MGN and the two adult comparative cohorts.

Table 5-1 Comparison of demographic, serum HBV status and clinical data between the study group of childhood HBV MGN (n=71) and comparative groups; adult HBV MGN (n=12) and idiopathic MGN (n=33)

Parameter	Children HBV MGN (n=71)		Adults HBV (n=12)		Adults idiopathic (n=33)		P value
	Mean No.	(95% CI) or (%)	Mean No.	(95% CI) or (%)	Mean No.	(95% CI) or (%)	
Demography							
Age - years mean	6.0	(3.8;8.2)	25.7	(20.4;30.9)	38.4	(35.2;41.6)	<0.01 ^a
Male	57	(80.3)	11	(92)	21	(64)	0.07
Race Black	31	(43.7)	7	(58.3)	2	(6.1)	<0.01
Coloured	40	(56.3)	5	(41.7)	21	(64.6)	
White	0	(0)	0	(0)	10	(30.3)	
HBs positive	71	(100)	12	(100)	0	(0)	Definition
HBe positive	33/38	(86.8)	6/10	(60)	Not tested		0.07
Clinical							
Oedema– Present	70	(99)	12	(100)	32	(97)	0.26
Mild	39/70	(55)	8	(67)	23/32	(70)	
Severe	31/70	(44)	4	(33)	9/32	(27)	
Ascites - present	34	(47.9)	4/9	(44.4)	8	(24.4)	0.06
Hypertension	25	(35.2)	8	(66.7)	13	(39.4)	0.12
Proteinuria – stix	2.87 ^b		3.08		3.21		0.04
Absent = 0	0	(0)	0	(0)	0	(0)	
+ = 1	2	(2.8)	0	(0)	0	(0)	
++= 2	15	(21.1)	1	(8.3)	2	(6.1)	
+++ = 3	44	(61.9)	9	(75)	22	(66.7)	
++++ = 4	10	(14.0)	2	(16.7)	9	(27.2)	
Haematuria – stix	3.0 ^b		3.0		2.0		<0.01
Absent=0	4	(5.6)	0	(0)	11	(33.3)	
trace=1	3	(4.2)	1	(8.3)	3	(9.1)	
+ =2	16	(22.5)	2	(16.7)	3	(9.1)	
++=3	17	(23.9)	5	(41.7)	9	(27.2)	
+++ =4	23	(32.3)	4	(33.3)	5	(15.2)	
++++=5	8	(11.2)	0	(0)	2	(6.1)	

^a Comparison between adult groups ^b Mean score derived from key on left

Table 5-2 Comparison of laboratory data between the study group of childhood HBV MGN and comparative groups; adult HBV MGN and Idiopathic MGN

Parameter	Children HBV (n=71)		Adults HBV (n=12)		Adults idiopathic (n=33)		P value
	Mean No.	(95% CI) or (%)	Mean or No.	(95% CI) (%)	Mean No.	(95% CI) or (%)	
Laboratory							
Haemoglobin g/dL	11.5	(11.1;12.0)	13.0	(11.9;14.0)	12.5	(11.9;13.1)	0.04
Urea mmol/L	5.1	(3.9;6.4)	8.5	(5.6;11.4)	7.6	(5.8;9.3)	0.08
Creatinine micromole/L	45.8	(25.9;65.7)	132.1	(83.7;180.5)	121.9	(92.8;151.1)	<0.01
Total protein g/L	46.3	(44.4;48.3)	44.0	(39.3;48.7)	48.6	(45.8;51.5)	0.20
Albumin g/L	16.4	(15.0;17.9)	18.1	(14.6;21.6)	20.2	(18.1;22.4)	0.02
Cholesterol mmol/L	10.7	(9.9;11.5)	9.9	(7.9;11.9)	10.1	(8.9;11.3)	0.61
AST U/L	57.0	(46.7;67.2)	68.9	(43.8;94.0)	23.9	(8.1;39.8)	<0.01
Normal 0-40							
ALT U/L	57.0	(43.4;70.6)	63.8	(30.8;96.7)	18.2	(-2.6;39.1)	<0.01
Normal 0-53							
C3 low	41/65	(63.1)	6/12	(50.0)	4/30	(13.3)	<0.01
C4 low	22/65	(33.9)	0/12	(0)	1/30	(3.3)	<0.01

Further features of this adult cohort of MGN (*Fig 5-1*) as well as the total group of 37 HBV positive biopsies are given at the end of this chapter. (page 5-42, Fig 5-44 and Table 5-7)

DEMOGRAPHICS (Table 5-1)

AGE

The mean age of the HBV MGN childhood study cohort was 6.0 years (2-12 years).

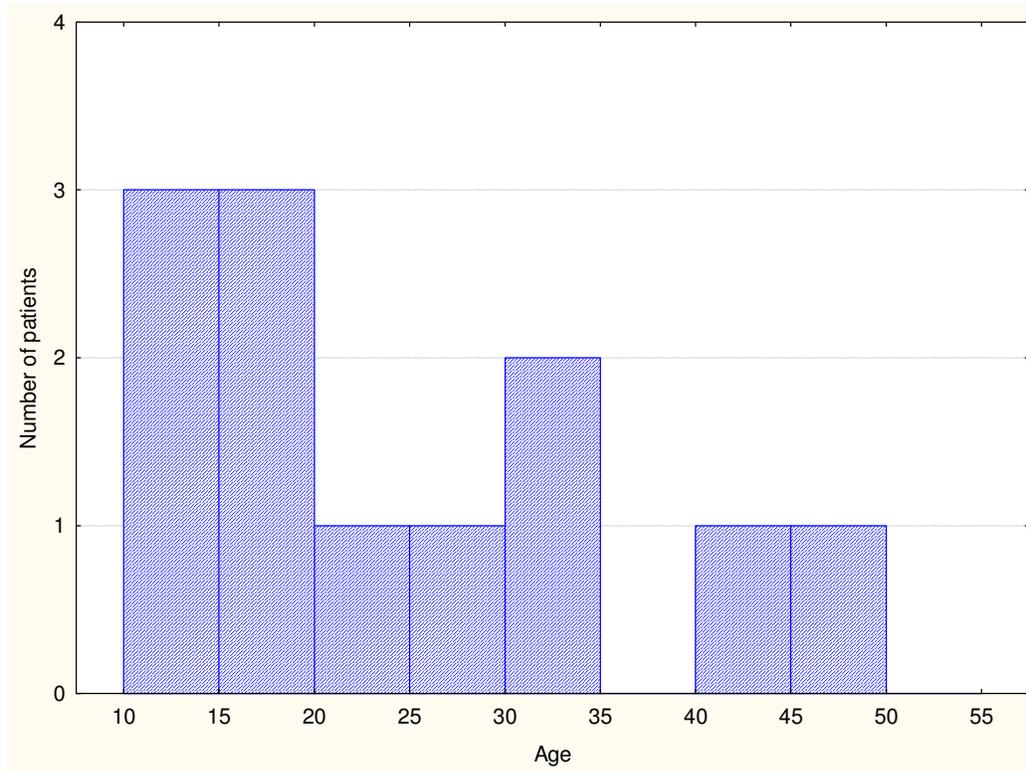


Fig 5-2 Adult HBV group by age in 5 year subgroups

The mean age of the HBV MGN adult group was 25.6 years with a range from 14-49.

This was significantly younger than the other control cohort, the adult idiopathic MGN group with a mean of 38.4 years and a range from 13 -74. (Figs 5-3 and 5-4)

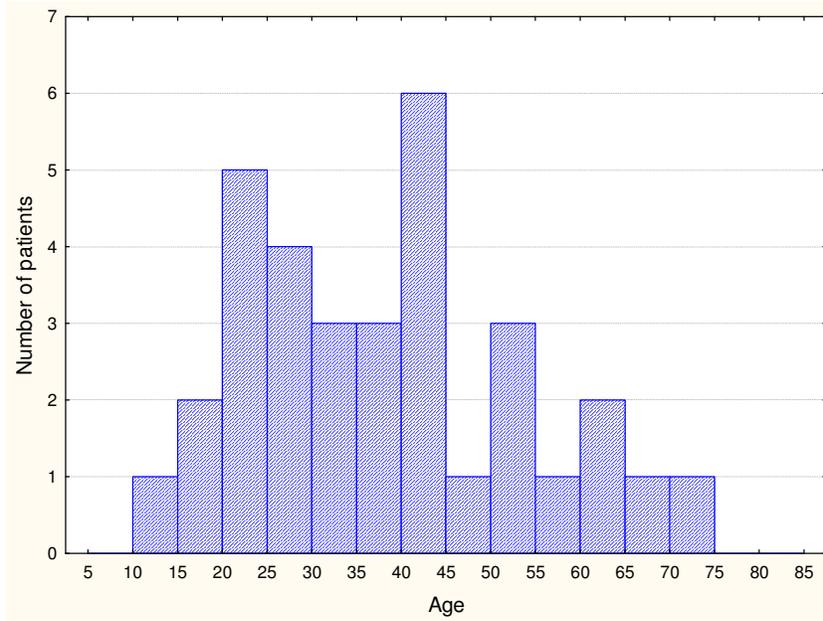


Fig 5-3 Adult idiopathic group by age in 5 year subgroups

The 33 adult idiopathic (non HBV group) included a range from 13 – 74 years with a mean of 38.4 years.

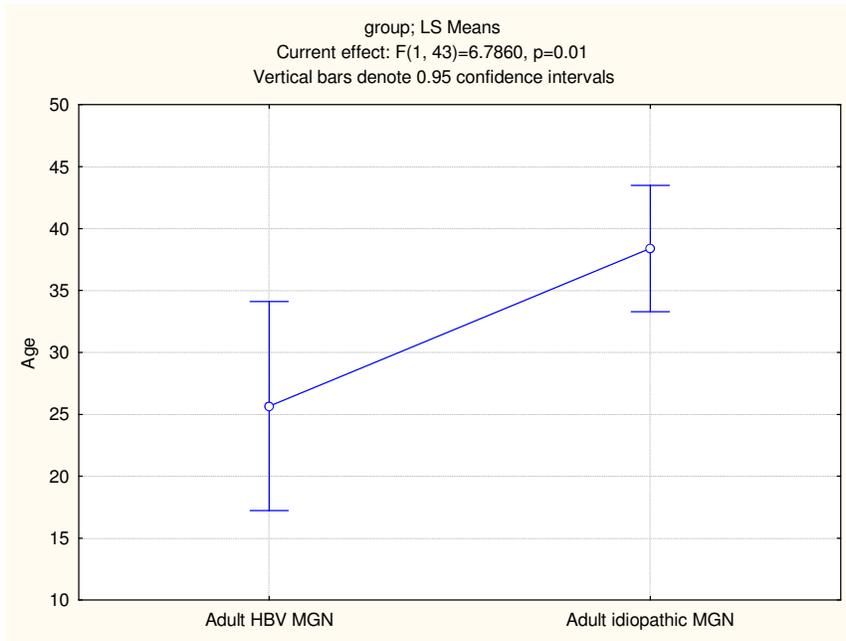


Fig 5-4 The age ranges and differences between the two adult MGN groups.

GENDER

In all 3 groups males predominate with the highest percentage in the adult HBV group 11/12, (92%); next the childhood HBV study group with 57/71 (80%) being male while in the idiopathic MGN control group, the male preponderance was 21/33 (64%). With a p value of 0.072 these differences are not statistically significant, but do show a tendency to differ. When all the HBV MGN patients (children and adults) are combined and compared to the idiopathic group the gender difference is significant as displayed below. This aspect is further dealt with in the discussion. (Chapter 7 page 7-4, Fig 7-1)

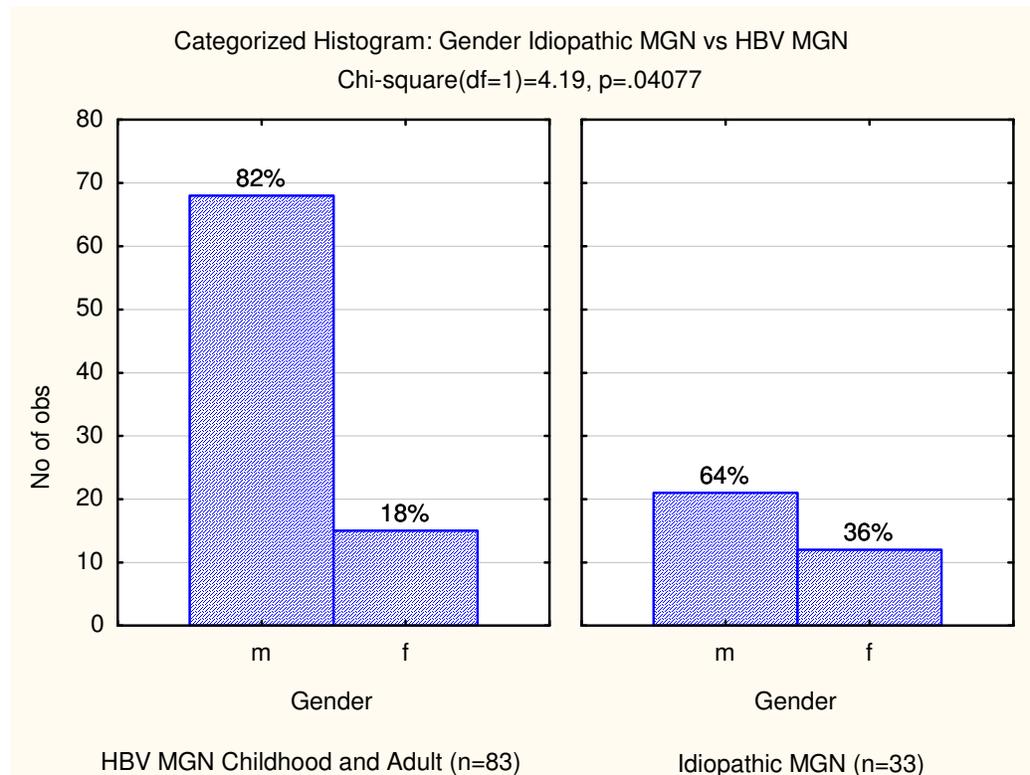


Fig 5-5 Comparison of gender of all the HBV MGN patients (children and adults) compared to the idiopathic MGN group

HAEMATURIA

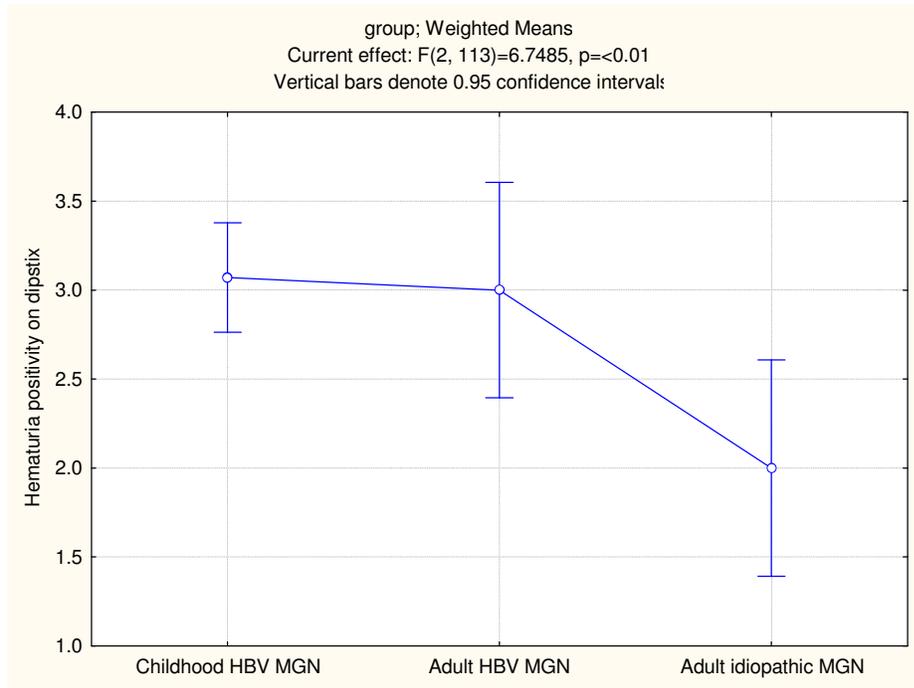


Fig 5-6 Comparison of mean measurement of haematuria on Labstix

Differences in the frequency and degree of haematuria are prominent. The two HBV groups are similar in displaying haematuria more prominently with a mean value of 2+ on dipstick while the idiopathic group has a mean of 1+.

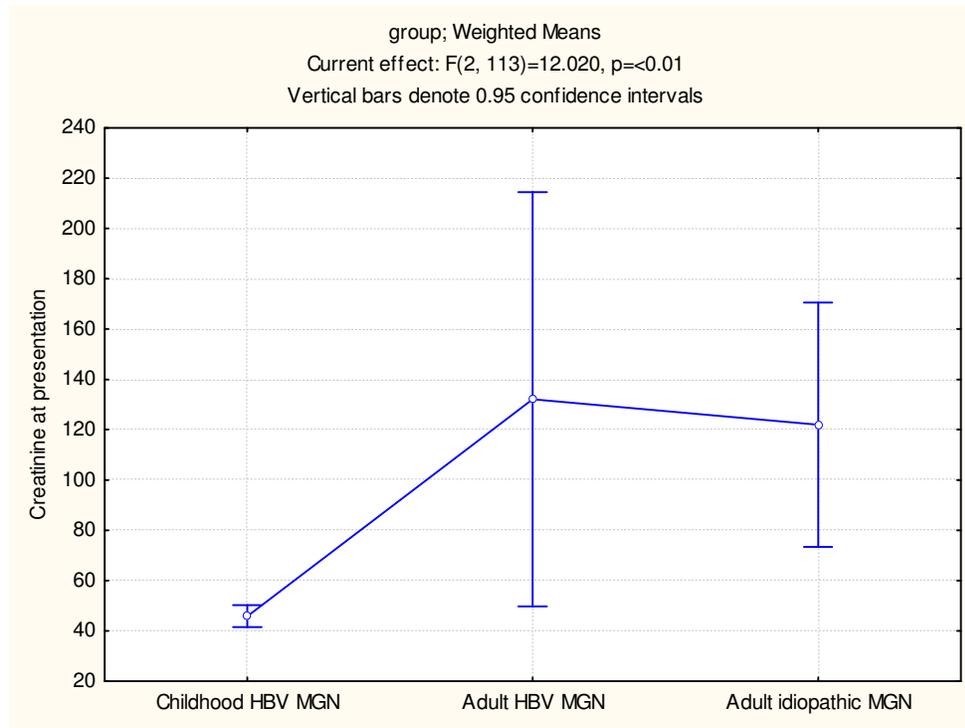
CREATININE

Fig 5-7 Comparison of serum creatinine levels at presentation in the 3 groups

The adult MGN groups had similar serum creatinine means a small amount above the upper limit of normal while the childhood HBV MGN group had a significantly lower mean as would be expected.

Five of the children had age adjusted raised creatinines at presentation: a 3 year old with level 62 micromole/L (Normal range 2-4 years: 30-56), two 5 year olds with values 80 and 88 (Normal range 4 – 6 years: 35 – 65), and two 6 year olds with values 78 and 133 (Normal range 6 – 8 years: 36 – 70). Of the 5, only one appears substantially raised, the 133 micromole/L. This was the child with a urea value of 22. Including all 5 the proportion is still low 5/71 (7.0%).

In summary, only 1/71 had a creatinine level above 120 micromole/L, reflecting renal failure, although 5 (7%) had raised age adjusted levels, while in the adult groups

presentation with raised creatinine was more frequent. In the adult HBV group 3/12 (25%) were above 120 micromole/L (179 – 504), very similar to the adult idiopathic MGN group with 8/33 (24%;135 – 725 micromole/L).

LIVER ENZYMES

ASPARTATE AMINOTRANSFERASE (AST) Normal 0 – 40 U/L

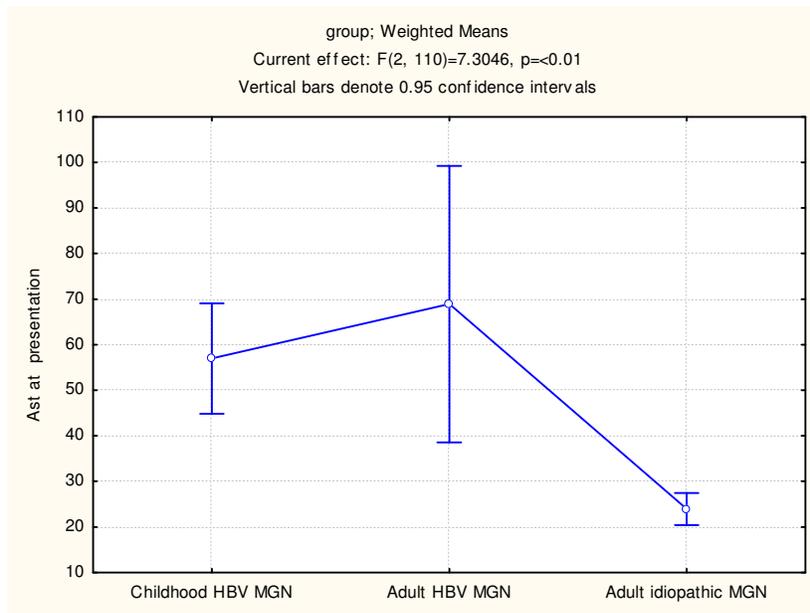


Fig 5-8 Comparison of serum AST at presentation between the groups

The idiopathic adult group showed normal liver enzymes while the HBV patients had mildly raised means. At presentation 44/71 (62%) of the childhood HBV group had AST levels above normal, with 8 above 100 and an outlier at 412. Similarly, at presentation 8/12 (67%) of the adult HBV group had raised levels with 3 above 100 and a highest level of 150. In the idiopathic group only 1/30 (3%) was raised – 44U/L.

ALANINE AMINOTRANSFERASE (ALT) Normal 0-53 U/L

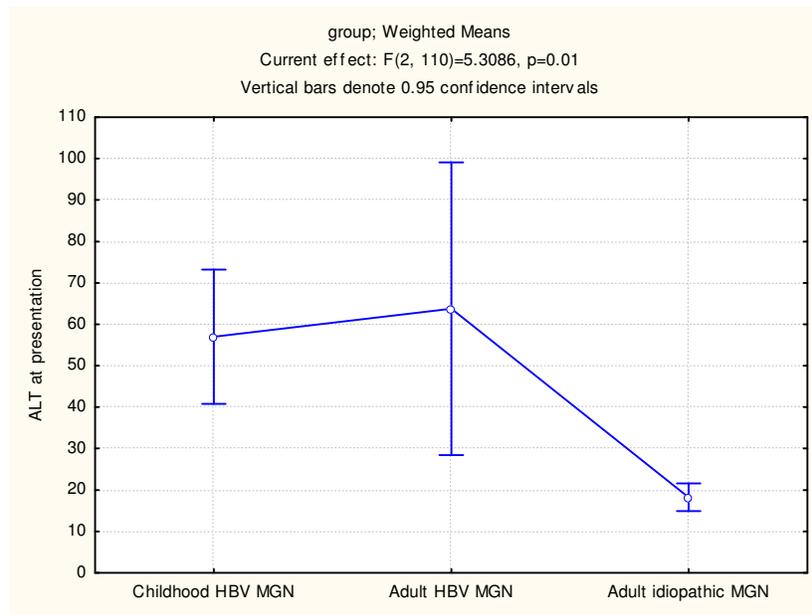


Fig 5-9 Comparison of serum ALT at presentation between the groups

The HBV MGN groups had a mean serum ALT above normal, but not markedly raised, while the idiopathic MGN group of patients had normal liver enzymes.

At presentation 24/71 (34%) of the childhood HBV MGN study group had ALT levels above normal, with again 8 above 100 and the same outlier at 560 as for AST.

Similarly, at presentation 5/12 (42%) of the adult HBV group had raised levels with 4 above 100 and a highest level of 147. As might be expected, raised levels were usually found in the same patients with similarly increased levels of ALT.

In the patients in the idiopathic MGN control group no ALT levels were raised above normal.

SERUM COMPLEMENT 3 – PROPORTION WITH LOW VALUES

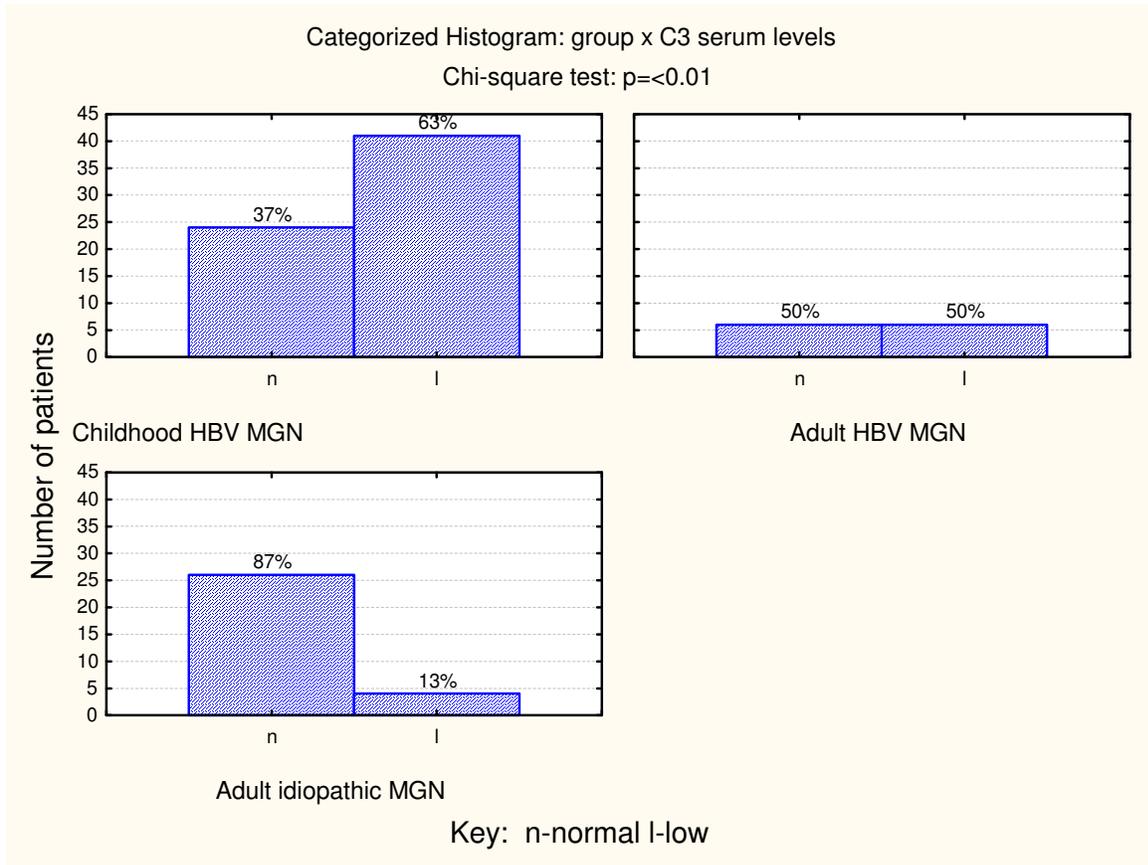


Fig 5-10 Comparison of C_3 levels at presentation between the groups

Many values below normal in the HBV groups but fewer in the idiopathic group. This finding has been made by some but not all investigators and is highlighted in the discussion.

SERUM COMPLEMENT4 IN SERUM – PROPORTION WITH LOW VALUES

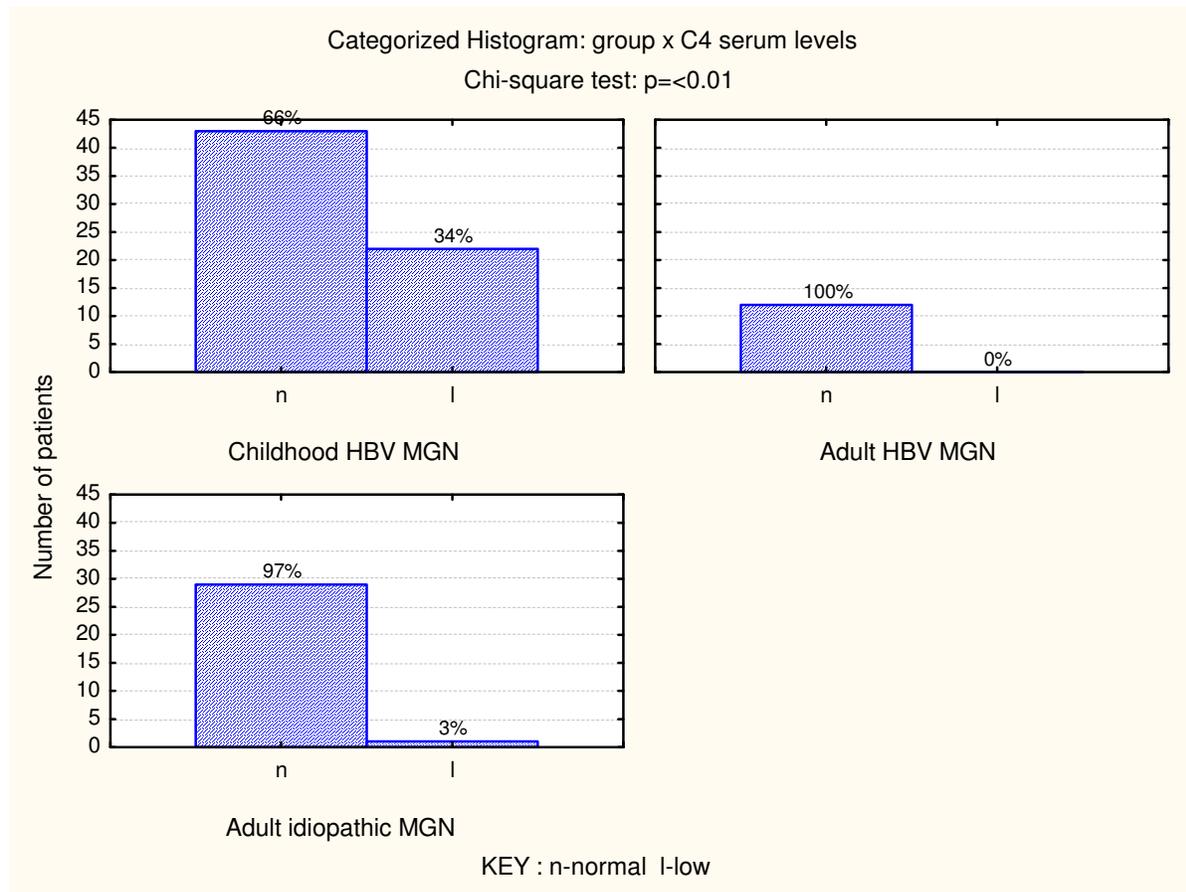


Fig 5-11 Comparison of serum C₄ levels at presentation between the groups

The serum C₄ levels were significantly lower in the childhood HBV MGN study group but were not lowered in the adult HBV MGN group. This is one of the very few differences found in this study between the pathological features of HBV MGN in adults and children in this study and is further covered in the discussion page 7-12. The difference points to the possibility of different forms or pathways of immune complex GN.

PATHOLOGY - LIGHT MICROSCOPY

Table 5-3 Renal pathology: Light microscopy. Comparison between the study group and the two comparative groups.

Parameter	Children HBV n= 71		Adults HBV n=12		Adults idiopathic n=33		P value
	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)	
Number of glomeruli per biopsy	26.8	(22.0;31.7)	19.3	(7.5;31.0)	20.6	(13.5;27.7)	0.24
Sclerosis (%)	4.5	(0.8;8.3)	24.3	(15.1;33.5)	17.0	(11.4;22.5)	<0.01
Crescents (%)	0.52	(-0.1;1.2)	1.1	(-0.5;2.7)	0.9	(>-0.1;1.9)	0.69
Mesangial Proliferation (0-3)^a See below	1.78	(1.0;1.2)	1.58	(1.1;1.6)	0.21	(0.2;0.4)	<0.01
Interstitial Inflammation (0-3)^a	0.60	(0.4;0.8)	1.00	(0.6;1.4)	1.03	(0.8;1.3)	<0.01
Tubular atrophy (0-3)^a	0.15	(<0.1;0.3)	0.58	(0.3;0.9)	0.69	(0.5;0.9)	<0.01
Interstitial Fibrosis (0-3)^a	0.38	(0.2;0.5)	1.16	(0.8;1.5)	1.03	(0.8;1.3)	<0.01
Blood vessels (0-3)^a	0.05	(-0.1;0.2)	0.50	(0.2;0.8)	0.39	(0.2;0.6)	<0.01

^a Key to semi quantitative scoring : 0 = absent, 1 = mild, 2 = moderate and 3 = severe

Defined in Chapter 3 Patients, Materials and Methods pages 3-4 to 3-6

The photographs on the following page (Fig 5-12 to 5-14) illustrate some of the characteristic features seen in HBV MGN including capillary wall thickening, mesangial proliferation, subepithelial and mesangial deposits as well as mesangial interposition.

LIGHT MICROSCOPY AND ULTRASTRUCTURE OF HBV MGN

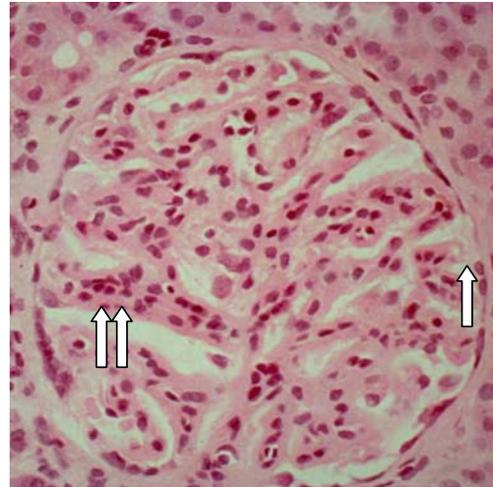
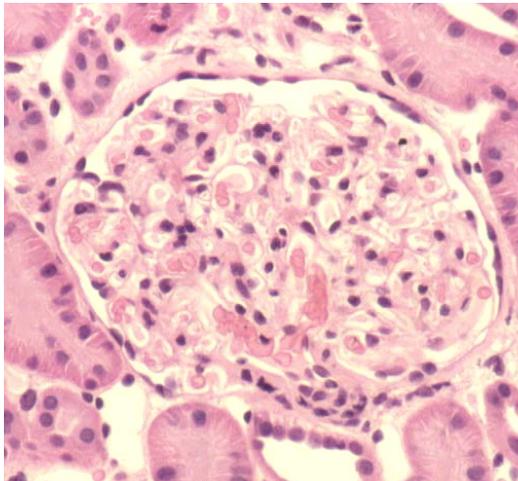


Fig 5-12 Left- Normal glomerulus. Right- HBV MGN Capillary wall thickening (↑) and moderate mesangial proliferation (↑↑) in childhood HBV MGN. (Haematoxylin and eosin stain x 400)

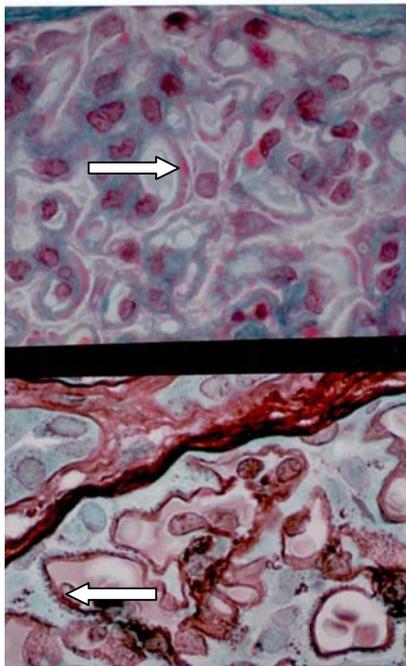


Fig 5-13 Subepithelial deposits - red. (Masson trichrome stain above) Spike formation. (Methenamine silver below) Both x 1000



Fig 5-14 Subepithelial deposits. (↑) Mesangial deposits. (↑↑) Mesangial interposition. (↑↑↑) Ultrastructure x 4500

MESANGIAL PROLIFERATION (Table 5 – 3 and Fig. 5-12)

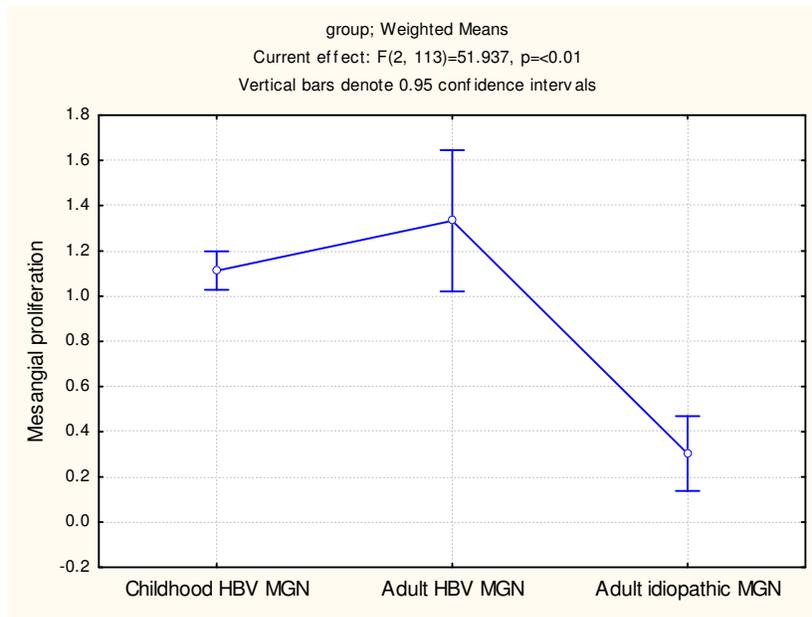


Fig 5-15 Comparison of degree of mesangial proliferation in glomeruli

A clear difference is present with both HBV groups showing more glomerular mesangial proliferation than the adult idiopathic group.

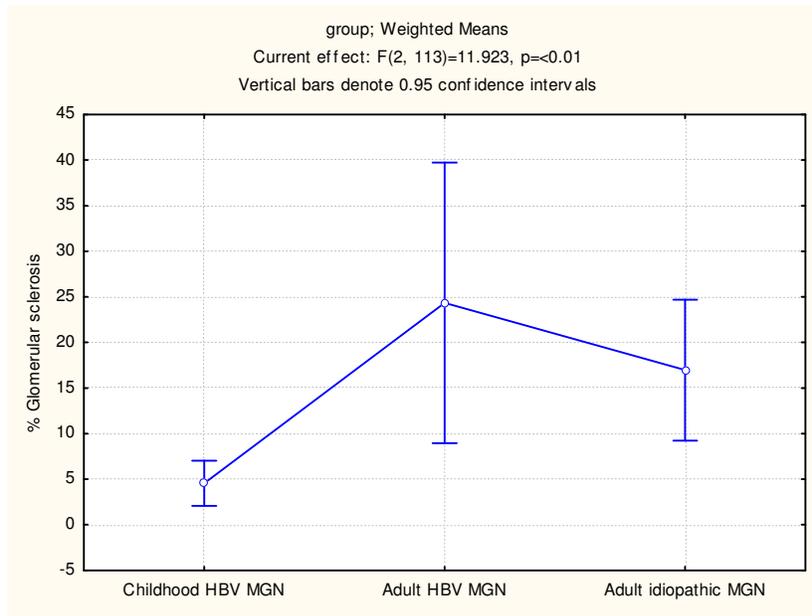
GLOMERULAR SCLEROSIS (Table 5 – 3 and Fig. 5-17)

Fig 5-16 Comparison of the percentage glomerular sclerosis

In the childhood group the extent of glomerular sclerosis was significantly less than in the adult groups. The differences are statistically highly significant and possibly one of the most important contributions of this comparative study. As far as is known such a finding has not previously been documented in a comparison like this. These changes reflect the end point of numerous morphologic processes and are known to correlate with outcome. [1]

On the following page (Fig 5-17) global and segmental sclerosis are displayed in one of the adult HBV MGN biopsies.

ADULT HBV MGN BIOPSY

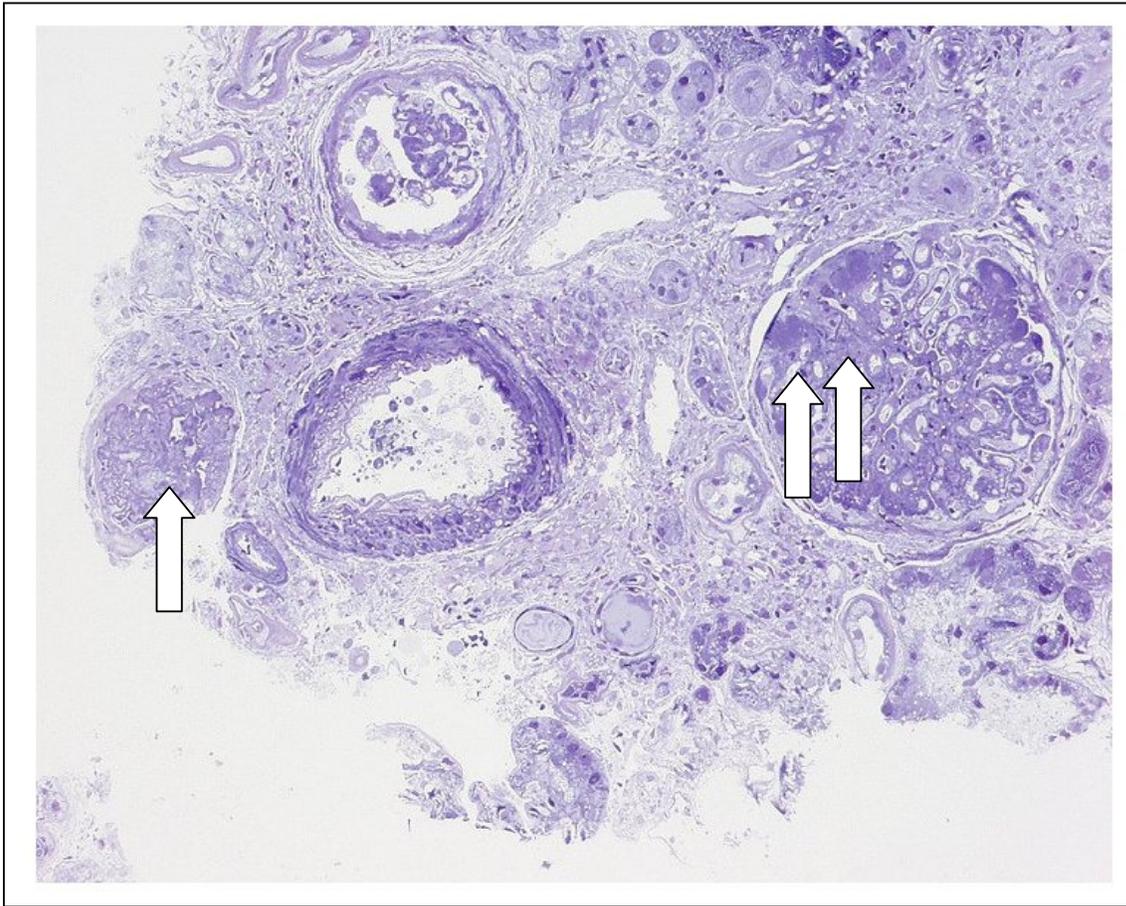


Fig 5-17 This field is from a renal biopsy of an adult with HBV MGN. On the left a sclerosed glomerulus (↑) is seen while the glomerulus on the right shows areas of segmental sclerosis (↑↑). Tubular atrophy and interstitial fibrosis are also present confirming that glomerular and interstitial damage often occur together. (Toluidine blue stain x 200)

CRESCENTS (Table 5 – 3 and Fig 5-18)

Crescents are rare in MGN and showed means from 0.5% to 1.1% in the three groups with the differences not statistically significant.

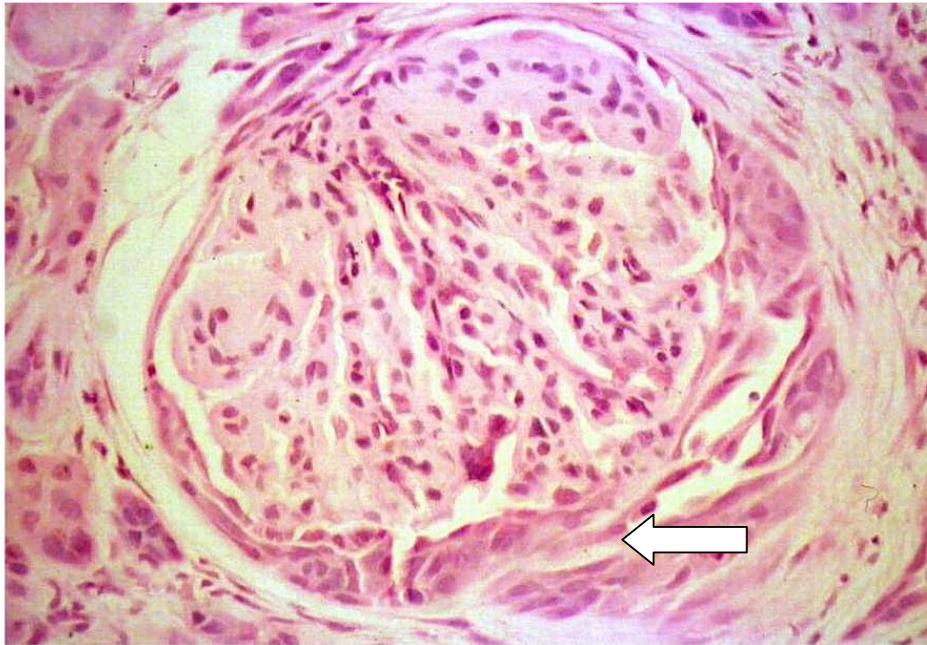


Fig 5-18 A cellular crescent (←) in a childhood HBV MGN renal biopsy.

(Haematoxylin and eosin x 400)

Using a definition of at least 30% of glomeruli being involved, none of the cases in these cohorts qualified as crescentic GN. Only 9 cases out of the total of 116 (8%) showed any crescents. In the HBV MGN children 4/71 (6%) had crescents with glomerular involvement being (2, 4, 14 and 17%) in the 4 cases respectively; in HBV MGN adult 2/12 cases (17%), with (5 and 8%) crescents in the 2 cases and in idiopathic MGN 3/33 (9%); (8, 8 and 14%) of the glomeruli in the 3 affected cases.

INTERSTITIAL INFLAMMATION (Table 5-3)

The two adult groups both had significantly more interstitial inflammation than the childhood group and were similar to each other. In the childhood group 34 (48%) showed no inflammation, while 32 (45%) showed mild inflammation, 4 (6%) moderate and 1 (1.4%) severe inflammation. In the adult groups, fewer biopsies, 3 (25%) in the HBV group and 8 (24%) in the idiopathic showed no inflammation and more showed moderate or severe inflammatory changes.

TUBULAR ATROPHY (Table 5-3)

The two adult groups both displayed considerably more tubular atrophy than the childhood group. In the children's biopsies 61 (86%) showed no tubular atrophy, 9 (13%) had mild involvement only and 1 (1.5%) moderate tubular atrophy. In the adult groups, again fewer biopsies, 7 (58%) in the HBV group and 14 (42%) in the idiopathic showed no tubular atrophy and more or similar proportions had moderate tubular atrophy changes.

INTERSTITIAL FIBROSIS (Table 5-3)

The children had significantly less fibrosis than the two adult groups. In the children (63%) showed no interstitial fibrosis and (35%) had mild involvement with only 1 (1.5%) displaying moderate fibrosis. In the adult groups 3 (25%) in the HBV group and 9 (27%) in the idiopathic showed no fibrosis and considerably more had moderate or severe interstitial fibrosis; (33%) HBV MGN and (27%) idiopathic MGN.

BLOOD VESSELS (Table 5-3)

The adults showed more damage than the children. Blood vessel changes such as hyalinosis and wall thickening were infrequent in all three cohorts, but again significantly rarer in the children's HBV group. In the children's biopsies 67 (94%) showed no blood vessel changes while the other 4 (6%) showed only mild involvement. In the adult groups, fewer biopsies, (67%) in both the HBV MGN and idiopathic MGN groups had no blood vessel changes and at least some had moderate or severe vessel pathology; (8%) HBV and (6%) idiopathic MGN cohorts.

In summary, concerning the last 4 parameters compared, the childhood group shows considerably less interstitial damage than the 2 adult groups.

ULTRASTRUCTURE**Table 5-4** Comparison of the Electron Microscopic features of MGN in the study group and 2 comparative cohorts

Parameters	Children HBV (n=71)		Adults HBV (n=12)		Adults idiopathic (n=33)		P value
	Mean (95% CI) or No.	(%)	Mean (95% CI) or No.	(%)	Mean (95% CI) or No.	(%)	
Stage of subepithelial deposits^a	4.3	(4.0;4.6)	3.9	(3.3;4.6)	4.1	(3.7;4.5)	0.51
I	1	(1.5)	0	(0)	2	(6)	
I + II	2	(3)	2	(17)	4	(12)	
II	5	(7)	0	(0)	6	(18)	
II + III	35	(49)	8	(67)	6	(18)	
III	23	(32)	1	(8)	6	(18)	
IV	5	(7)	1	(8)	9	(27)	
Mesangial deposits (0-3)^b	1.9	(1.8;2.1)	1.7	(1.3;2.1)	0.36	(0.1;0.6)	<0.01
Subendothelial deposits (0-3)^b	0.60	(0.4;0.8)	1.0	(0.6;1.4)	0.03	(-0.2;0.3)	<0.01
Mesangial interposition (0-3)^b	1.78	(1.6;2.0)	1.58	(1.1;2.1)	0.21	(-0.1;0.5)	<0.01
Virus-like bodies (%)	59	(83.1)	4	(33.3)	13	(39.4)	<0.01
Tubuloreticular Bodies (%)	62	(87.3)	5	(41.6)	4	(12.1)	<0.01

^a Hsu Stages of subepithelial deposits with combinations; 1=I, 2=I + II, 3=II, 4=II + III, 5=III, 6=IV [2]

Means derived from scores according to keys.

^b Semi-quantitative scoring - (0-3) : 0=absent, 1=mild, 2=moderate, 3=severe Defined in Chapter 3 pages 3-7 to 3-9

STAGE OF SUBEPITHELIAL DEPOSITS (Table 5-4 and Fig 5 -19 to 23)

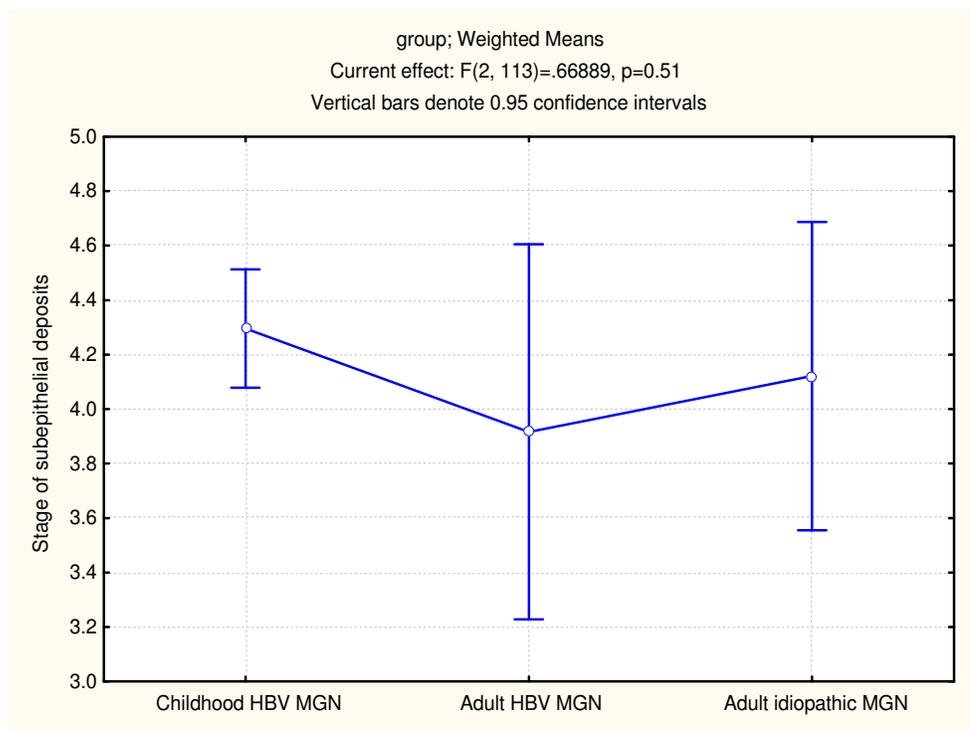


Fig 5-19 Comparison of stages of subepithelial deposits based on the 4 stages but using the grouping of Hsu [2]

These were means using the categorisation of Hsu [2] and were not significantly different between the three groups. All had means showing quite an advanced stage. (4 = stage II-III and 5 = stage III). The stages of subepithelial deposits are illustrated below at ultrastructural level from the childhood HBV MGN biopsy material. (Fig 5-20 to 5-23)

STAGES OF SUBEPITHELIAL DEPOSITS FROM HBV MGN IN CHILDHOOD

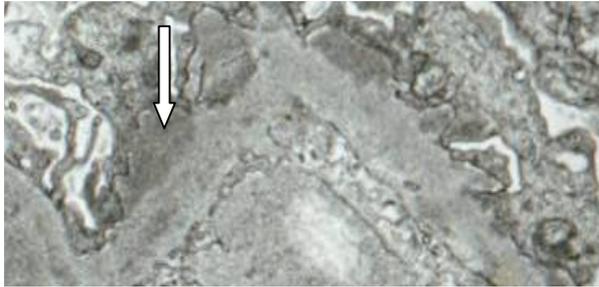


Fig 5-20 Stage I - Subepithelial deposit (↓) x 10,000

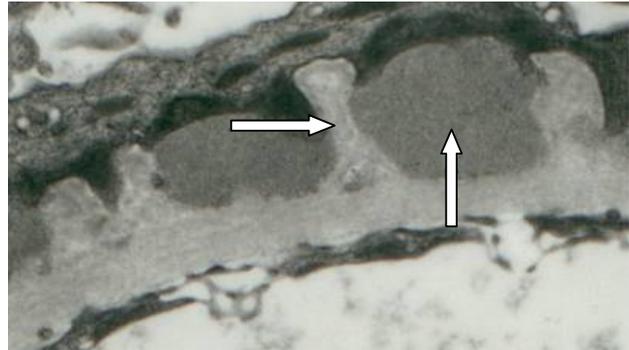


Fig 5-21 Stage II - Subepithelial deposit (↑) with spike formation (→) x 12,000



Fig 5-22 Stages II and III - Subepithelial deposits with spike formation (↓) and incorporation (↓↓) x 10,000

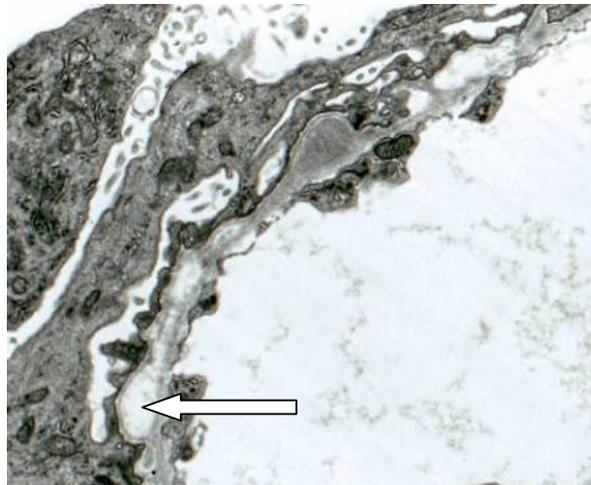


Fig 5-23 Stage IV - Lucent areas where subepithelial deposits had been (←) x 8,000

MESANGIAL DEPOSITS IN GLOMERULI (Table 5-4 and Fig 5-24 to 26)

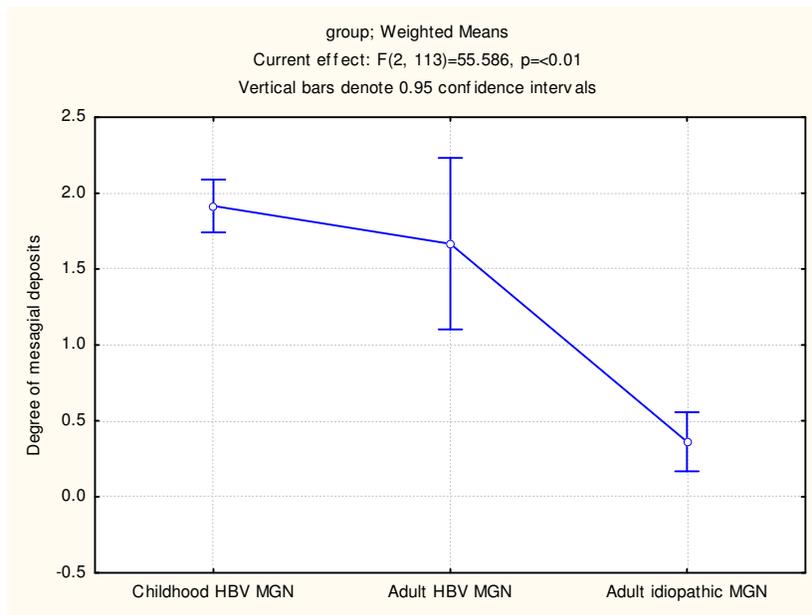


Fig 5-24 Comparison of severity of mesangial deposits in glomeruli in the study group and 2 control groups

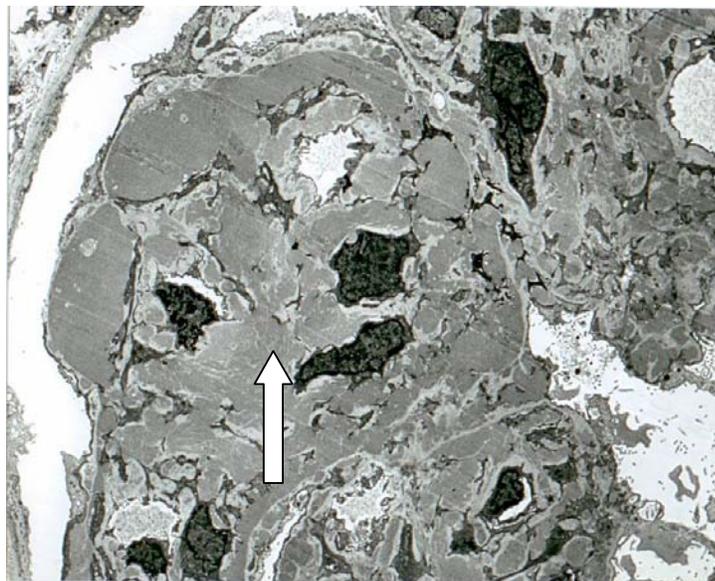


Fig 5-25 An example of severe mesangial deposits (↑) in a child with HBV MGN x 3000

MESANGIAL DEPOSITS IN GLOMERULI (Table 5-4)

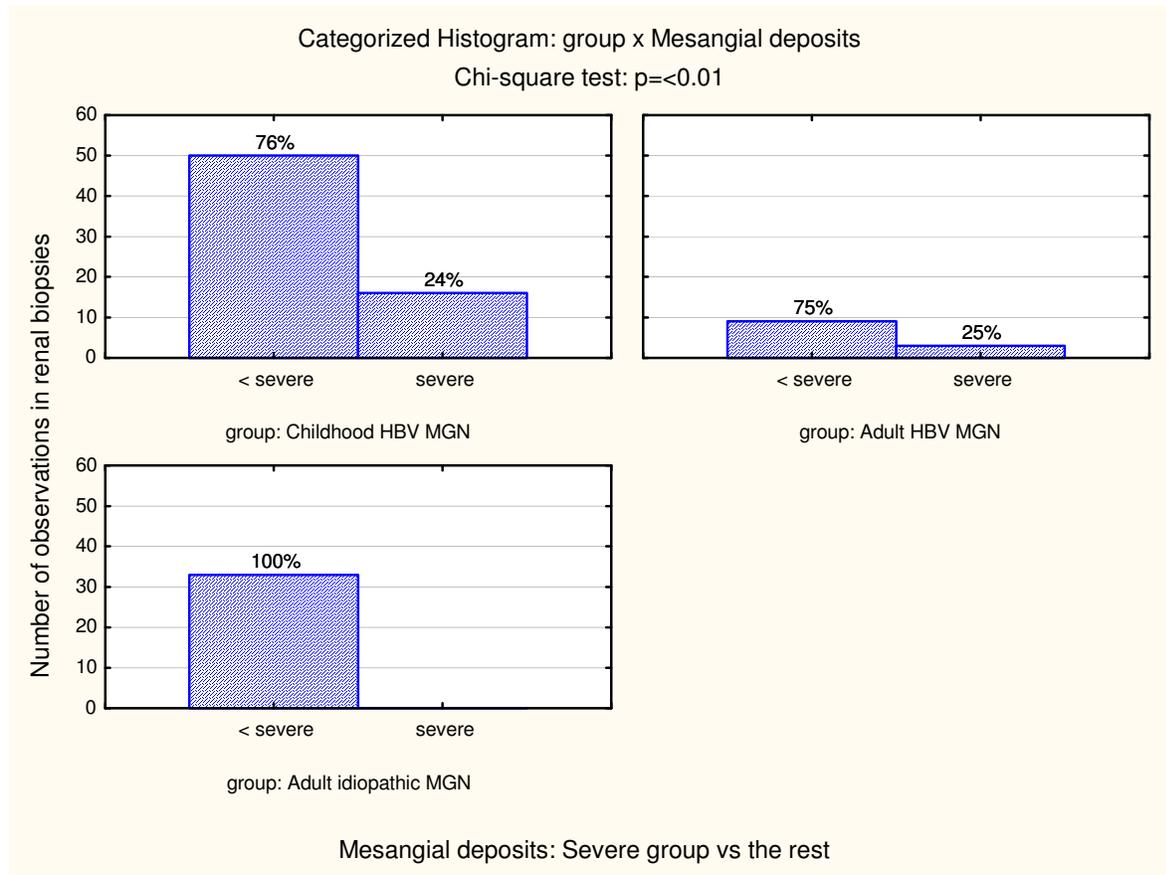


Fig 5-26 Comparison of severity of mesangial deposits in glomeruli – those biopsies with severe changes versus the rest in each group

The differences in frequency and severity of mesangial deposits between the 3 groups are represented in two ways above; one Fig 5-24 comparing the means of the semi-quantitative evaluations and the other Fig 5-26 contrasting those in the severe subgroup within the study group of childhood HBV MGN and the 2 comparative cohorts. Mesangial deposits were rare in the idiopathic form of adult MGN (Fig 5-24) and never severe (Fig 5-26), while in the HBV MGN groups they were significantly more common and severe in 25% of cases. The childhood patients with HBV MGN all had mesangial deposits; 22 (31 %) mild, 33 (46%) moderate and

16 (24%) severe. This was similar to the adult HBV group where all cases showed mesangial deposits; 7 mild (58%), 2 moderate (17%) and 3 (25%) severe. These 2 groups differed markedly from the adult idiopathic group where 10 (30%) were mild and only 1 moderate (3%) – a total of 11/33 (33%).

It has been shown in this study that severe mesangial deposits in the childhood group correlated with a number of demographic, pathological and outcome features. (Chapter 4 Tables 4-11 and 4-13)

SUBENDOTHELIAL DEPOSITS (Table 5-4 and Fig 5-27 to 30)

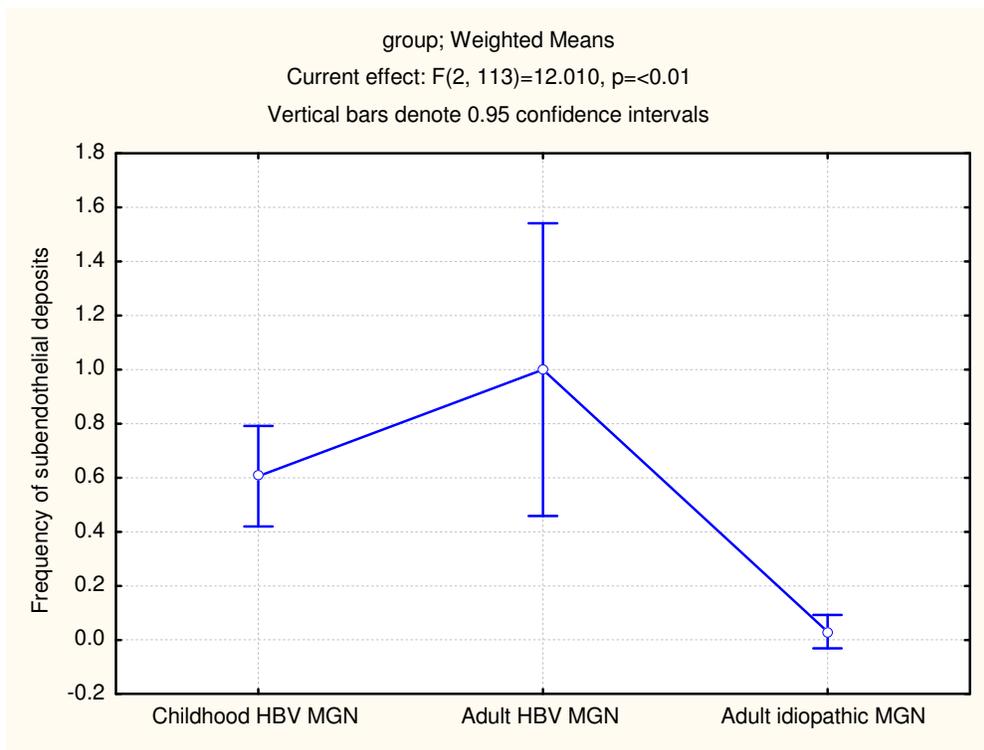


Fig 5-27 Comparison of frequency of subendothelial deposits in glomeruli

SUBENDOTHELIAL DEPOSITS (Table 5-4)

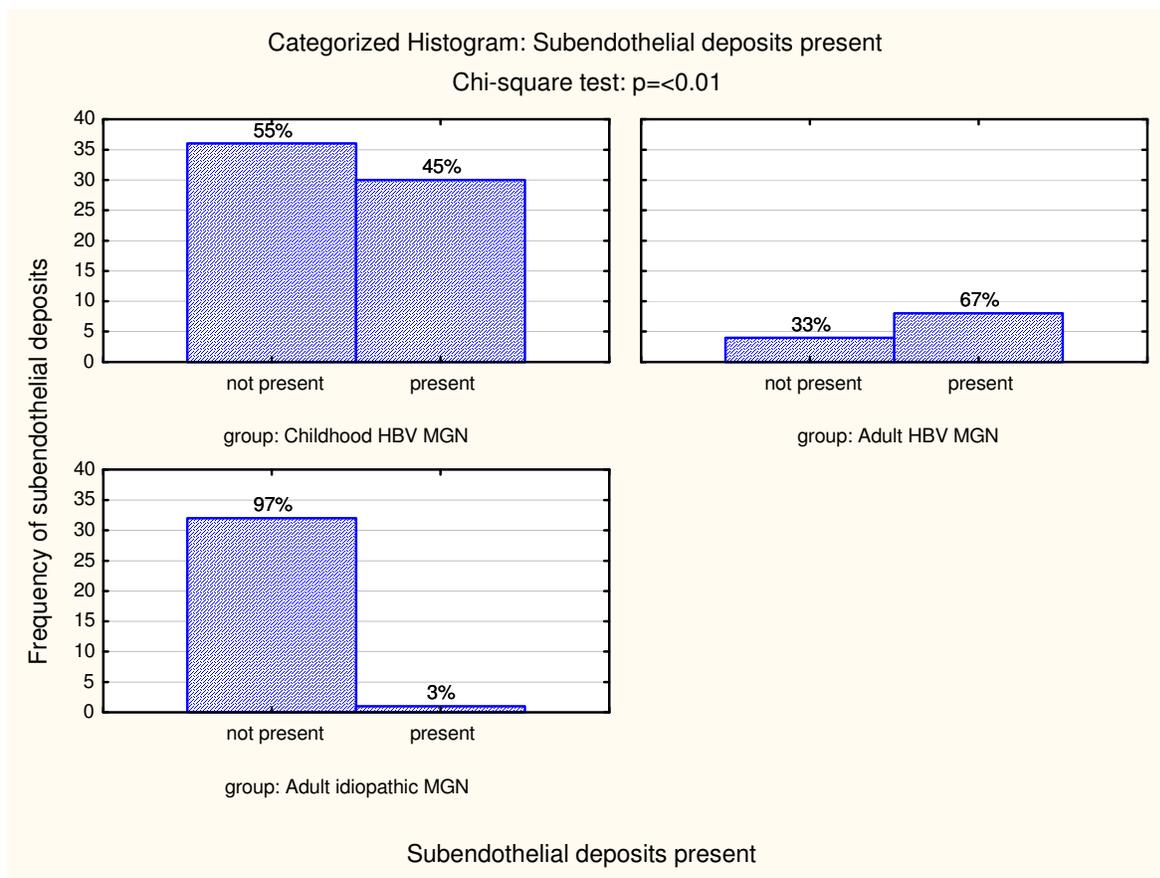


Fig 5-28 Comparison of frequency of subendothelial deposits in glomeruli between the childhood group and 2 adult control groups

Subendothelial deposits, not normally part of the MGN morphology, are very rare in the idiopathic form but frequent and often moderate or severe in the two HBV groups. Below ultrastructural examples of subendothelial deposits from the childhood HBV MGN renal biopsies are shown. (Fig 5-29 and 5-30)

EXAMPLES OF SUBENDOTHELIAL DEPOSITS FROM CHILDHOOD HBV MGN



Fig 5-29 Subepithelial deposits (↓) Subendothelial deposits (↑) x 10,000

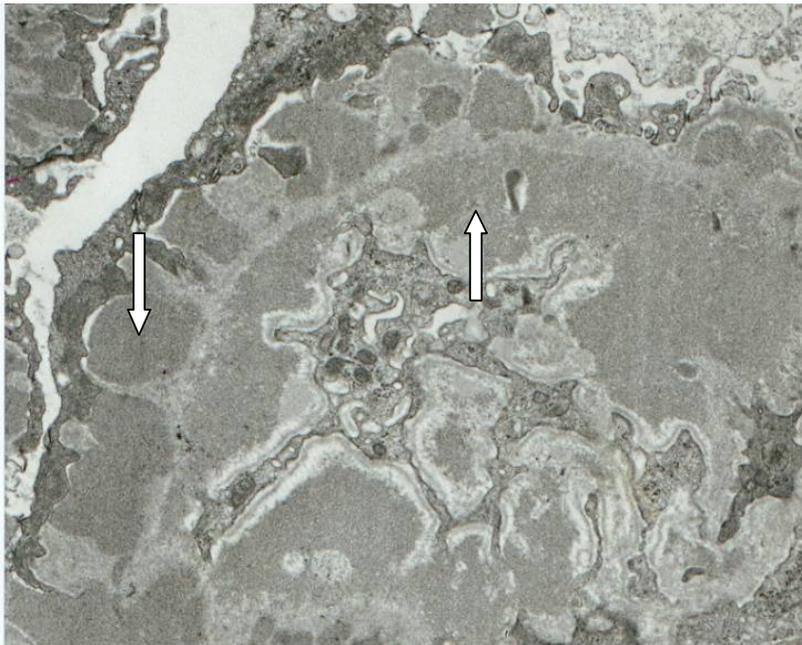


Fig 5-30 Subepithelial deposits (↓) Subendothelial deposits (↑) x 15,000

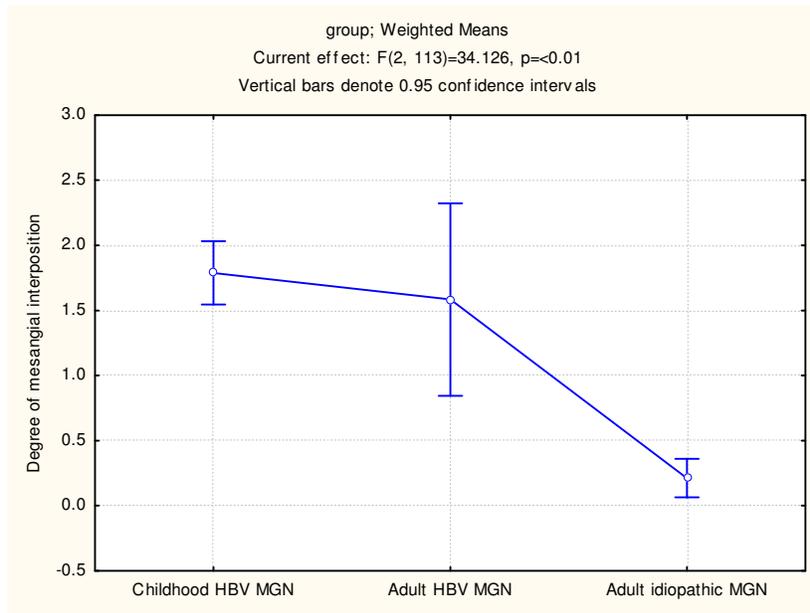
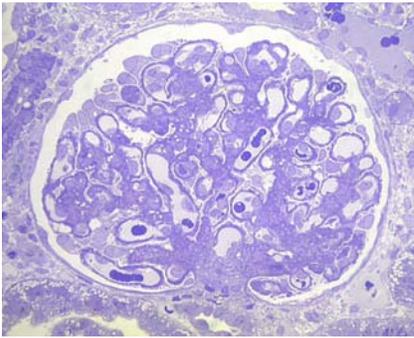
MESANGIAL INTERPOSITION (Table 5-4 and Fig 5-31 to 35)

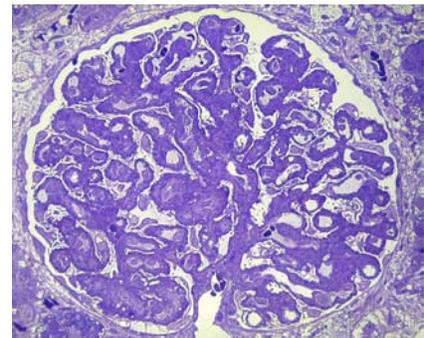
Fig 5-31 Comparison of frequency and severity of mesangial interposition in glomeruli

Mesangial interposition is a further feature where there is a significant difference between the idiopathic MGN group where mesangial interposition is rare and the two HBV MGN groups where it is frequent. (Fig 5-31) This is a feature more associated with a mesangiocapillary GN reaction pattern. The figure below, 5-32, contrasts the more classical 'idiopathic MGN' appearance with the mesangiocapillary GN side of the HBV MGN spectrum. The presence of mesangial deposits, subendothelial deposits and mesangial interposition in the group on the right side (Fig 5-32 b, d and f) is highlighted.

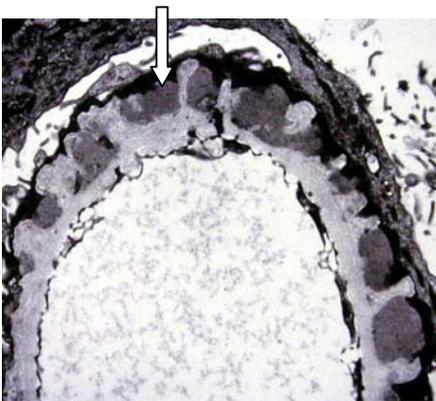
Fig 5-32 Comparison between the classical 'idiopathic MGN' appearance (left a, c and e) and the more 'severe' group of the spectrum of HBV MGN (right b, d and f)]



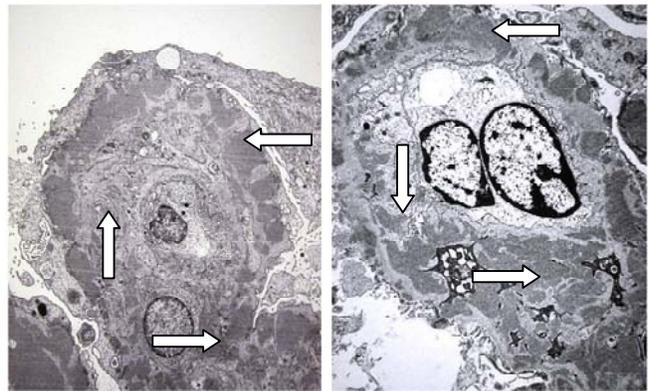
a) Toluidine blue section of HBV MGN with features close to idiopathic MGN x400



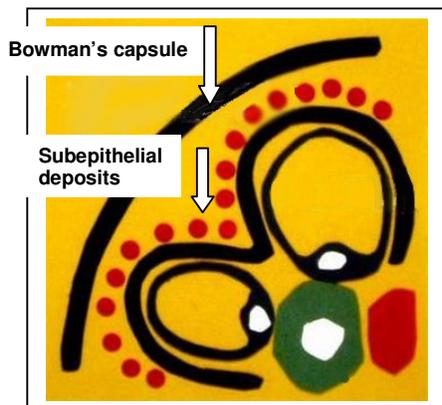
b) Toluidine blue section of HBV MGN with widened mesangial areas and prominent thickening of capillary walls x 400



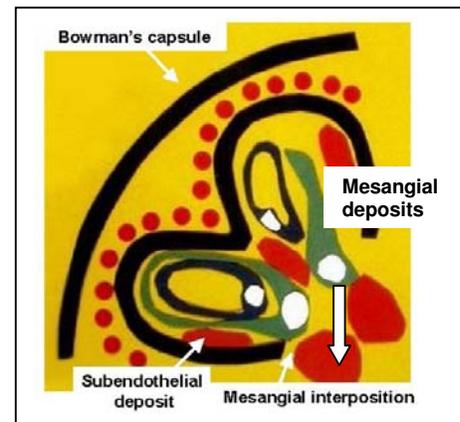
c) Capillary loop with subepithelial deposits (↓) x 9000



d) Mesangial (→), subendothelial (↓) and subepithelial (←) deposits in a child (left) and adult (right) with HBV MGN. Prominent mesangial interposition (↑) also seen. Original both 15000



e) Idiopathic or classical MGN pattern – can be seen in HBV MGN



f) Diagram of characteristic HBV MGN with subepithelial, subendothelial and prominent mesangial deposits as well as mesangial interposition

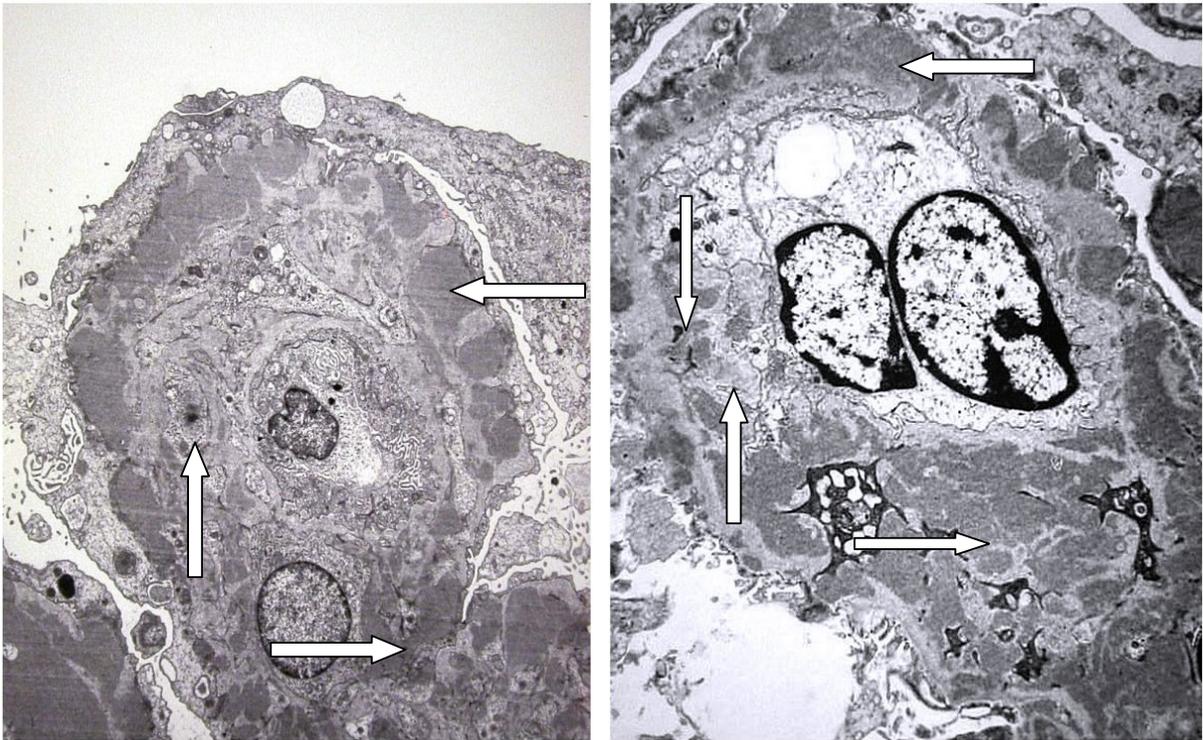


Fig 5- 33 Mesangial (→), subendothelial (↓) and subepithelial (←) deposits in a child (left) and adult (right) with HBV MGN on the renal biopsy. Prominent severe degrees of mesangial interposition (↑) are also seen in both peripheral capillary loops. These pictures highlight the ultrastructural similarity of HBV MGN in adults and children. (Originals both X 15000)

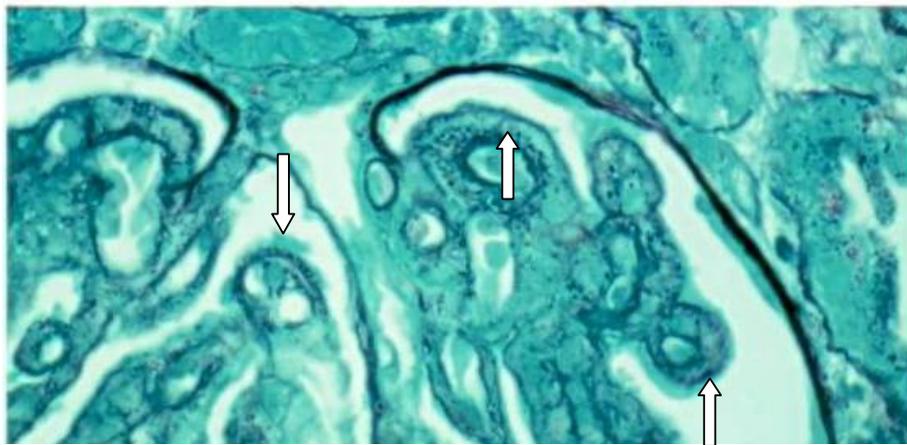


Fig 5- 34 Spikes and small double line in the same capillary loop. (↓) Prominent tramlines in a nearby area of the same glomerulus (↑) in a biopsy from a child with HBV MGN. Methenamine silver stain. X 1000

MESANGIAL INTERPOSITION (Table 5 - 4 and Fig 5-32 to 5-35)

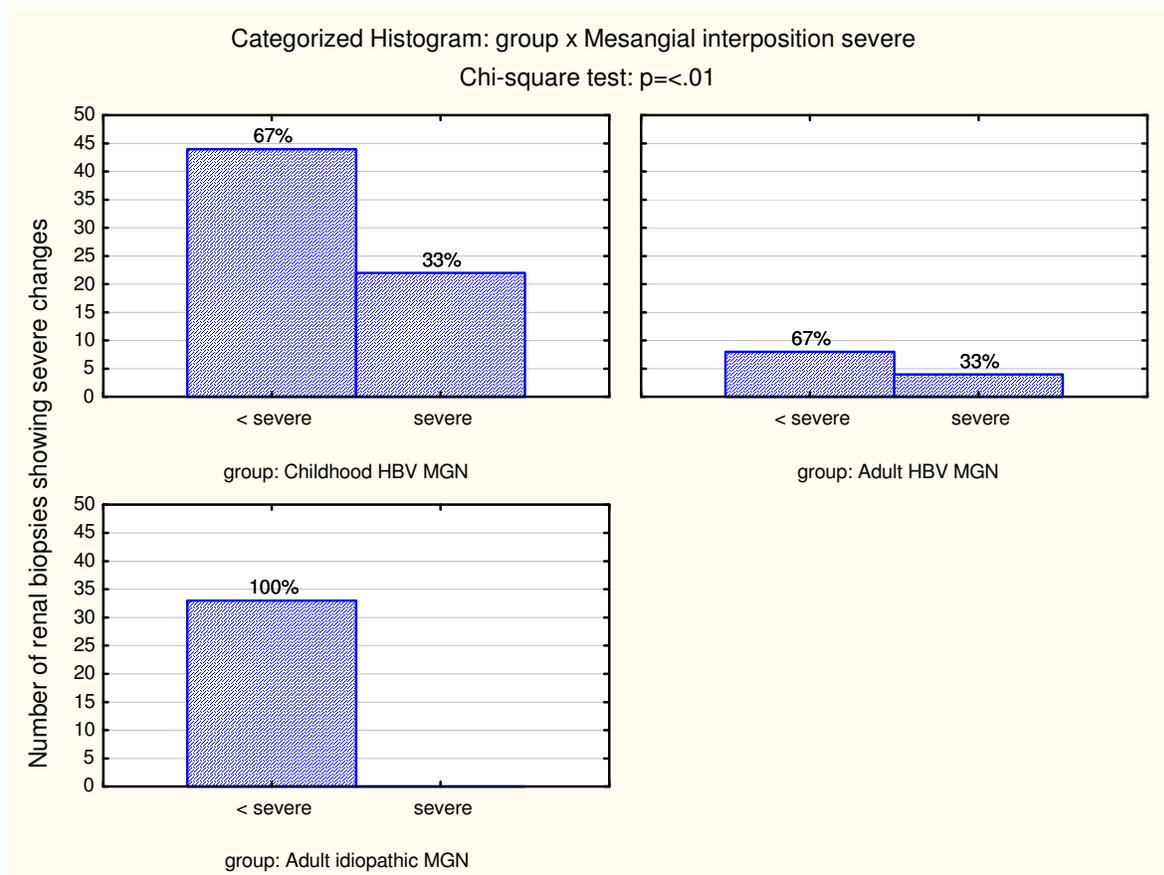


Fig 5-35 Comparison of degrees of mesangial interposition in glomeruli between the three groups – those biopsies with severe changes versus the rest.

Another way of representing the mesangial interposition differences between the 2 groups is shown above Fig 5-35. Those with severe or category 3 mesangial interposition are separated from the rest and are seen to be present only in the HBV groups, in similar frequency of 33% in each group. This is the subgroup classified as mixed HBV MGN-mesangiocapillary in this study.

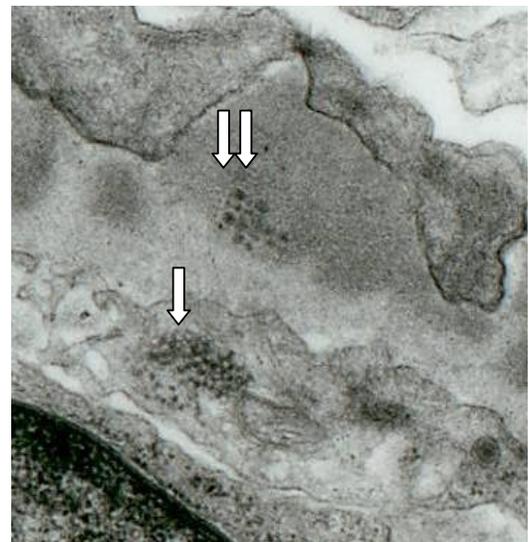
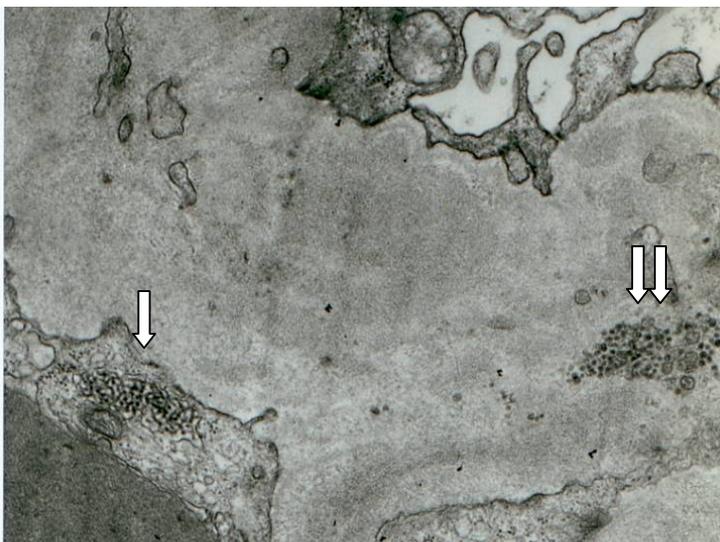
VIRUS LIKE BODIES (Table 5-4 and Fig 5-36 and 37)

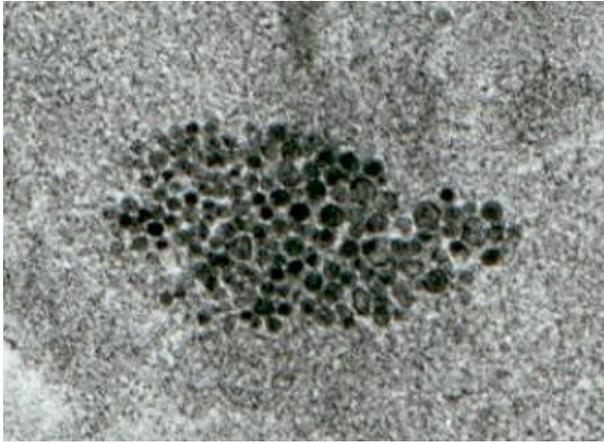
These were most frequent in the HBV MGN children and similarly uncommon in the two adult groups.

TUBULORETICULAR BODIES (Table 5-4 and Fig 5-36 and 38)

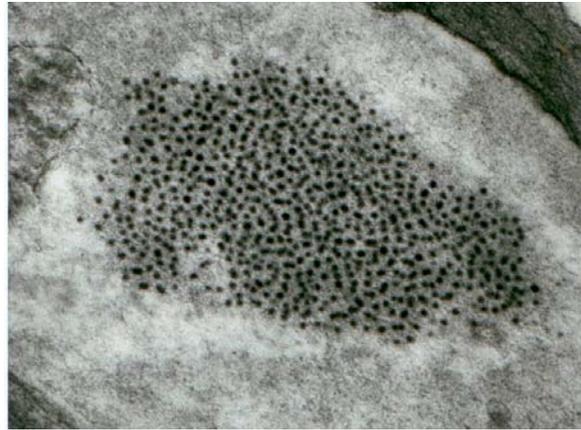
These ultrastructural bodies were seen most in childhood HBV MGN (87%), then the adult HBV MGN group (42%) and seldom (12%) in the idiopathic MGN group.

Fig 5-36 Tubuloreticular inclusion bodies (↓) and virus-like structures/bodies (↓↓) in one field from two different renal biopsies from children with HBV MGN x 50000



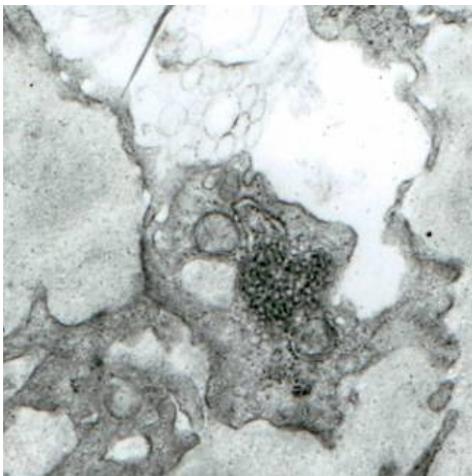


x 100000

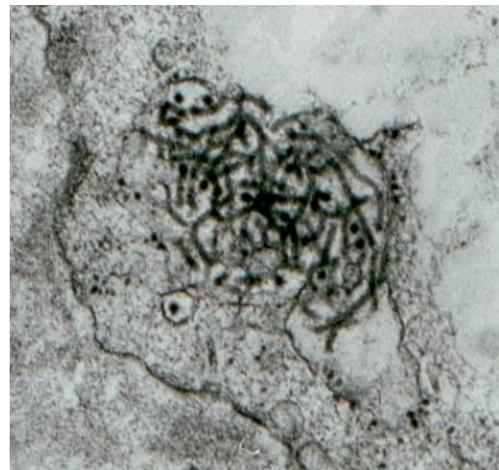


x 80000

Fig 5-37 *Two examples of virus like bodies from childhood HBV MGN*



x 50000



x 75000

Fig 5-38 *Two examples of tubuloreticular bodies in the endothelial cytoplasm from renal biopsies of childhood HBV MGN*

IMMUNOFLUORESCENCE**Table 5-5** Immunofluorescence positivity^a in glomeruli. A comparison between the study group of HBV MGN in childhood and the two adult groups.

Antigen	Children HBV (n=71 unless otherwise shown)		Adults HBV (n=12 unless otherwise shown)		Adults idiopathic (n=33 unless otherwise shown)		P value
	No.	(%)	No.	(%)	No.	(%)	
	IgA	29/52	(56)	9/11	(82)	21/25	
IgG	53/54	(98)	10/11	(91)	24/26	(92)	0.35
IgM	38/53	(72)	11/11	(100)	22/26	(85)	0.03
C3	42/51	(82)	10/11	(91)	23/26	(88)	0.64
Fibrinogen	13/49	(27)	5/11	(45)	17/23	(74)	<0.01
HBs (Polyclonal)	26/40	(65)	7/9	(77)	6/11	(55)	0.54
HBc	19/20	(95)	9/11	(82)	0/11	(0)	<0.01
HBe(a)	15/20	(75)	7/10	(70)	0/11	(0)	<0.01
HBe(b)	16/20	(80)	7/10	(70)	0/11	(0)	<0.01

^aThe predominant staining was along the capillary wall in a granular pattern (usually initially described as ++ or +++ with varying additional mesangial staining)

Most of the antigen categories included only some of the renal biopsies. Reasons for this included that the immunofluorescence test was not available at TBH until 1977, after the first 11 children had undergone a renal biopsy; the lack of frozen renal tissue; the lack of glomeruli on the frozen tissue and that some of the tests (HBe and HBc) were carried out long after the biopsy was done, on stored tissue where available.

IMMUNOFLUORESCENCE C3 STAINING IN GLOMERULI (Table 5-5)

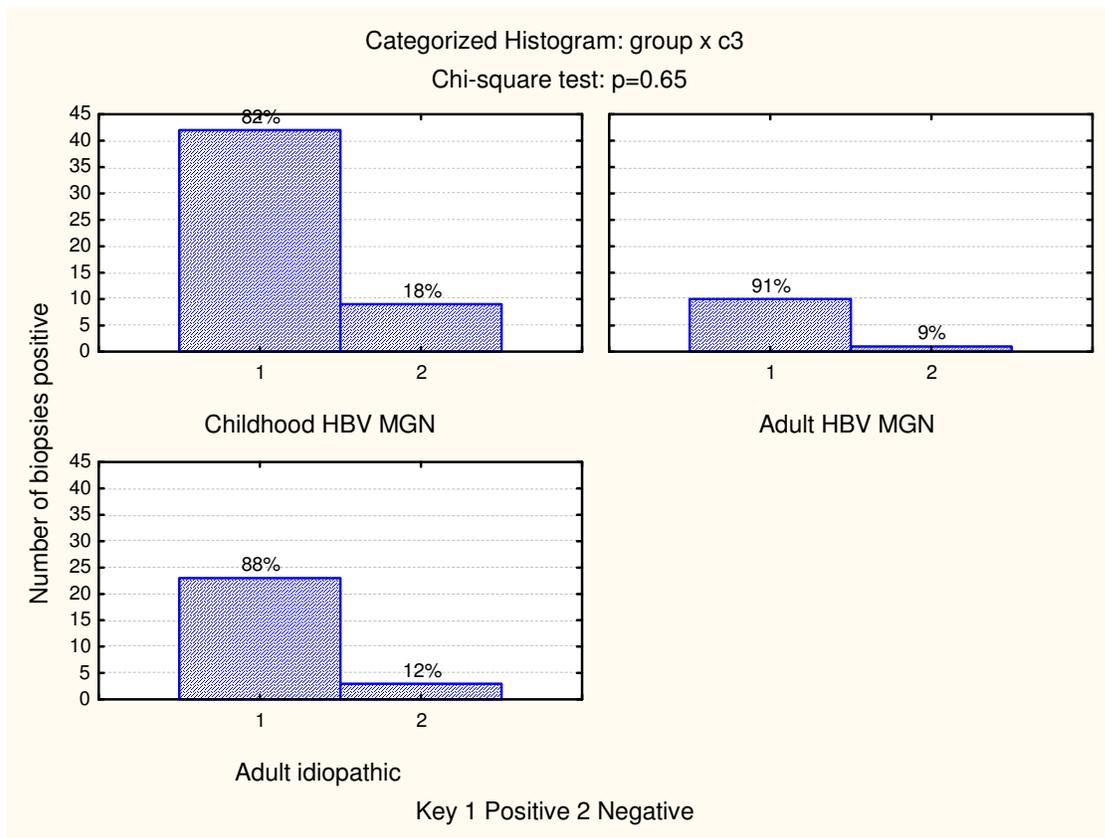


Fig 5-39 Comparison of positive immunofluorescence for C₃ in renal biopsies.

All three groups show high levels of positivity.

IMMUNOFLUORESCENCE HBsAg STAINING IN GLOMERULI

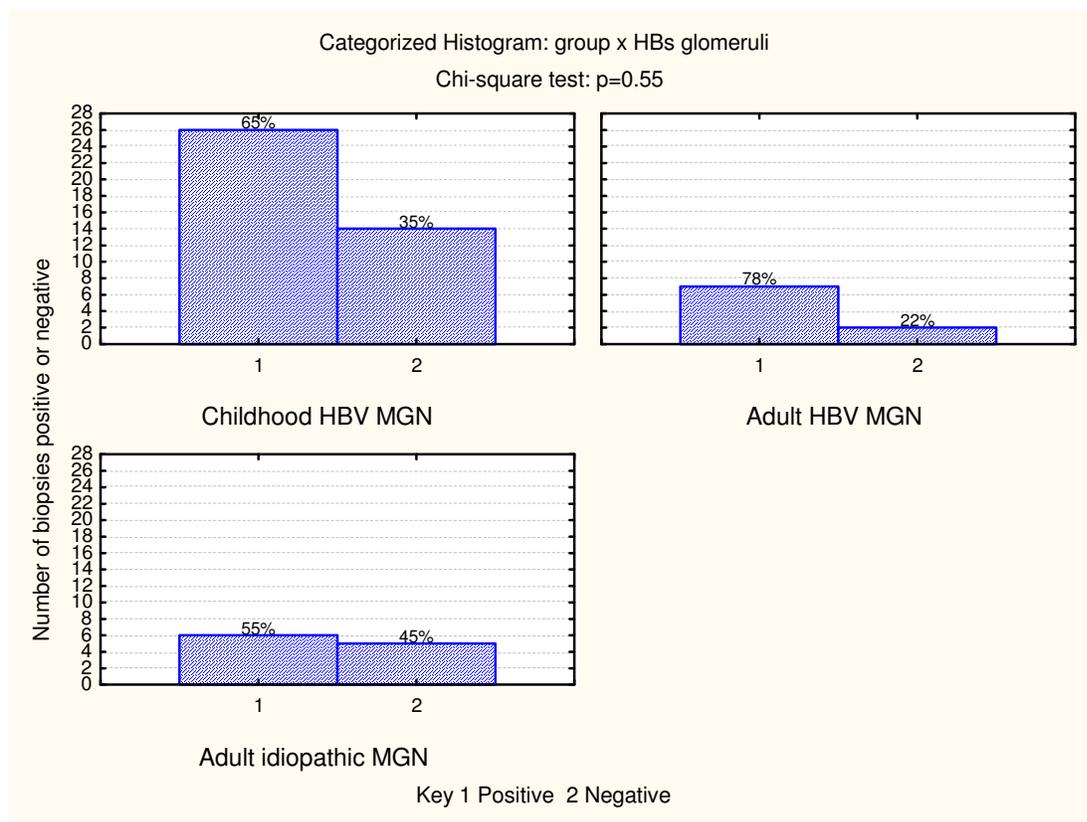


Fig 5-40 Comparison of positive immunofluorescence for HBsAg (Polyclonal DAKO) in renal biopsies

Although the HBsAg staining was positive in many of the HBV cases it also stained many of the idiopathic cases. This anomalous situation was also noted by others [3] and it will be further addressed in the discussion.

In Fig 5-41 below the characteristic granular capillary wall staining of MGN can be seen confirming the association between HBcAg, the monoclonal anti-HBeAg stains and the subepithelial deposits in the renal biopsies of HBV MGN patients in these series.

Fig 5-41 HBsAg, HBcAg and HBeAg immunofluorescent and immunohistochemical stains

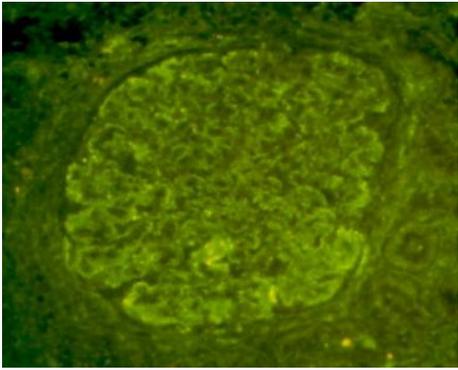


Fig 5-41a – Positive immunofluorescent HBsAg stain x 400

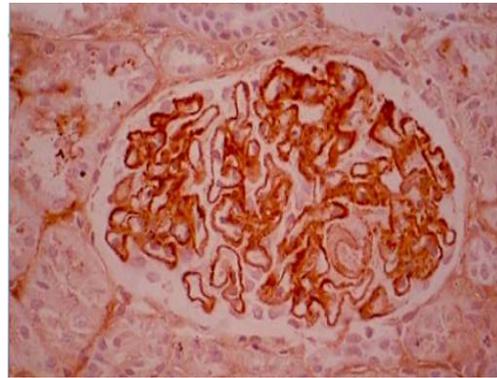


Fig 5-41b – Positive immunohistochemical HBsAg stain x 400

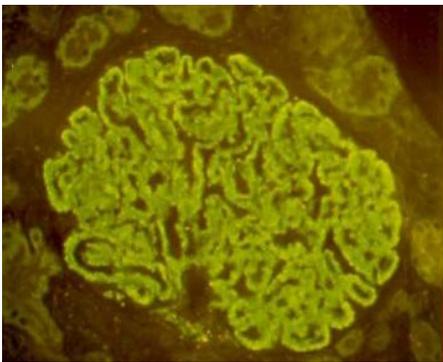


Fig 5-41c – Positive immunofluorescent HBcAg stain x 400

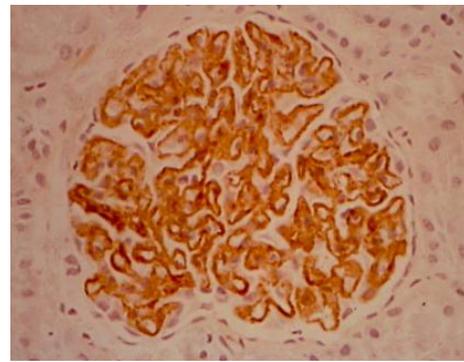


Fig 5-41d – Positive immunohistochemical HBcAg stain x 400

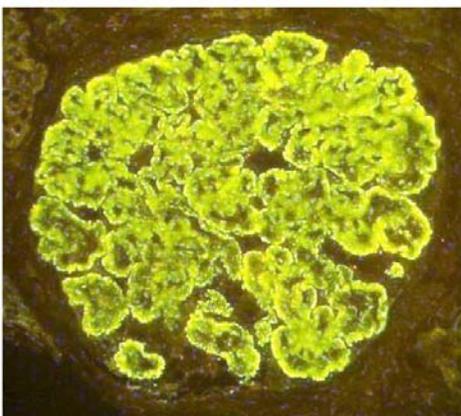


Fig 5-41e - Positive immunofluorescent HBeAg stain x 400

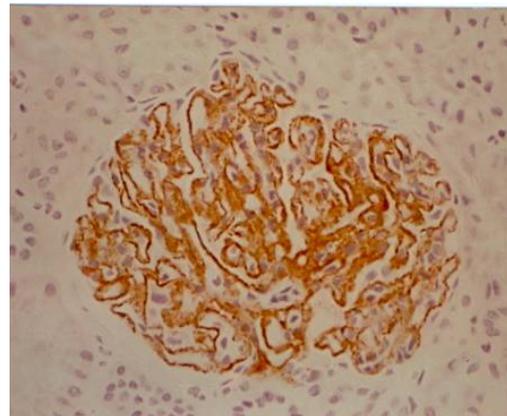


Fig 5-41f – Positive immunohistochemical HBeAg stain x 400

OUTCOME**Table 5-6** Outcome: Renal and patient outcome and survival over different time periods of follow up

Outcome at 2 years	Children HBV (n = 28)		Adults HBV (n = 8)		Adults idiopathic (n = 18)		P value
	No	(%)	No	(%)	No	(%)	
	28/71 (39% of total)		8/12 (67% of total)		18/33 (55% of total)		
	27/46 (59% of SA)						
Remission	7	(25.0)	0	(0)	0	(0)	<0.01
Proteinuria	6	(21.4)	5	(62.5)	4	(22.2)	
Nephrotic syndrome	13	(46.4)	0	(0)	5	(27.8)	
Renal failure	2	(7.1)	2	(25.0)	5	(27.8)	
Death	0	(0)	1	(12.5)	4	(22.2)	
Remission	7	(25.0)	0	(0)	0	(0)	<0.01
No Remission	21	(75.0)	8	(100)	18	(100)	
Outcome at 4 years	Children HBV (n = 23)		Adults HBV (n = 6)		Adults idiopathic (n = 15)		
	23/71 (32% of total)		6/12 (50%)		15/33 (45%)		
	22/46 (48% of SA)						
Remission	12	(52.2)	0	(0)	0	(0)	<0.01
Proteinuria	3	(13.0)	2	(33.3)	2	(13.3)	
Haematuria	1	(4.3)	0	(0)	0	(0)	
Nephrotic syndrome	5	(21.7)	0	(0)	1	(6.6)	
Renal failure	1	(4.3)	3	(50.0)	4	(26.6)	
Death	1	(4.3)	1	(16.7)	8	(53.3)	
Remission	12	(52.2)	0	(0)	0	(0)	<0.01
No Remission	11	(48.8)	6	(100)	15	(100)	

There were significant differences in the outcomes of the 3 groups at 2 years and 4 years. Remissions were found only in the HBV MGN childhood group while renal failure and death were more frequent in the adult comparative groups.

OUTCOME 2 YEARS

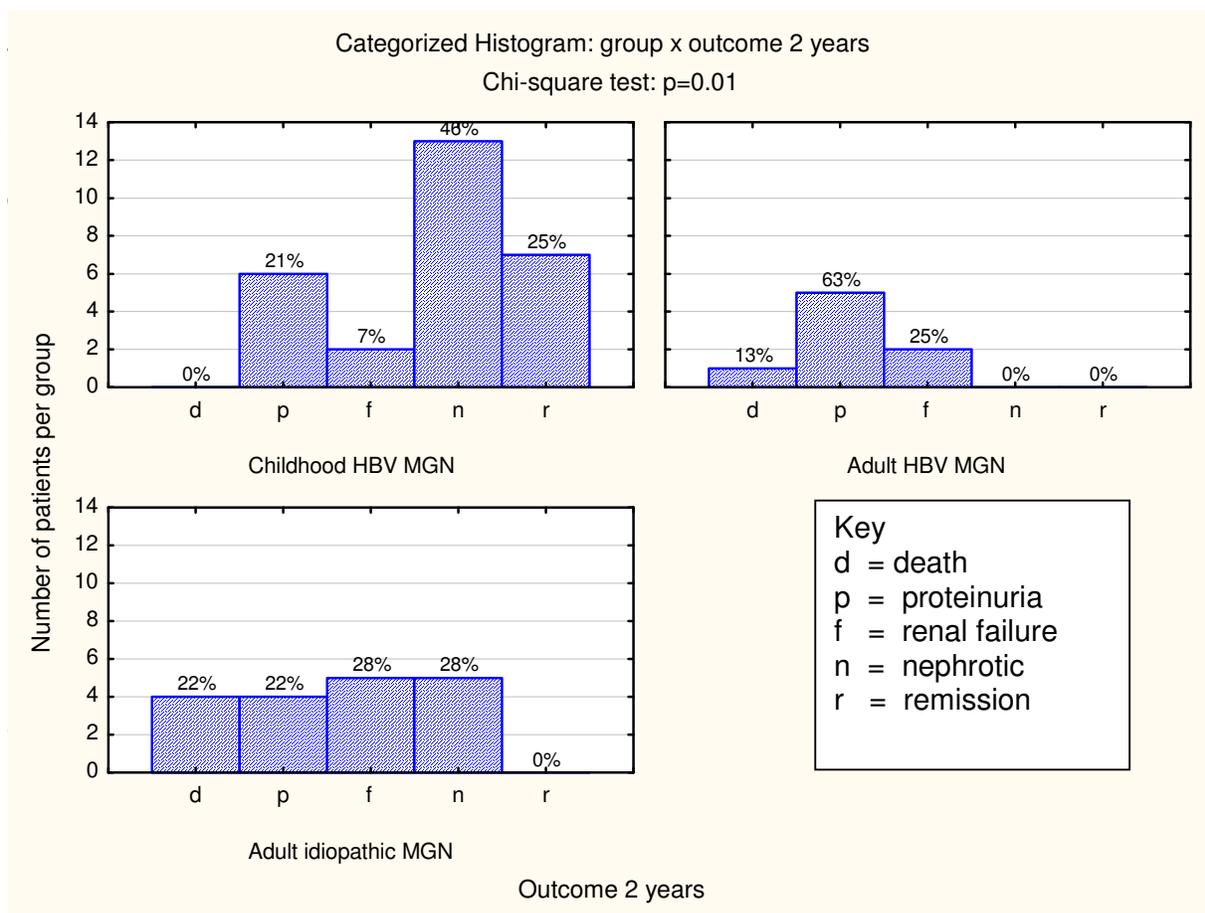


Fig 5-42 Outcome of patients from the 3 groups at 2 years

The childhood HBV MGN group has a better outcome at 2 years than the adult groups with 25% of the children in remission.

OUTCOME 4 YEARS

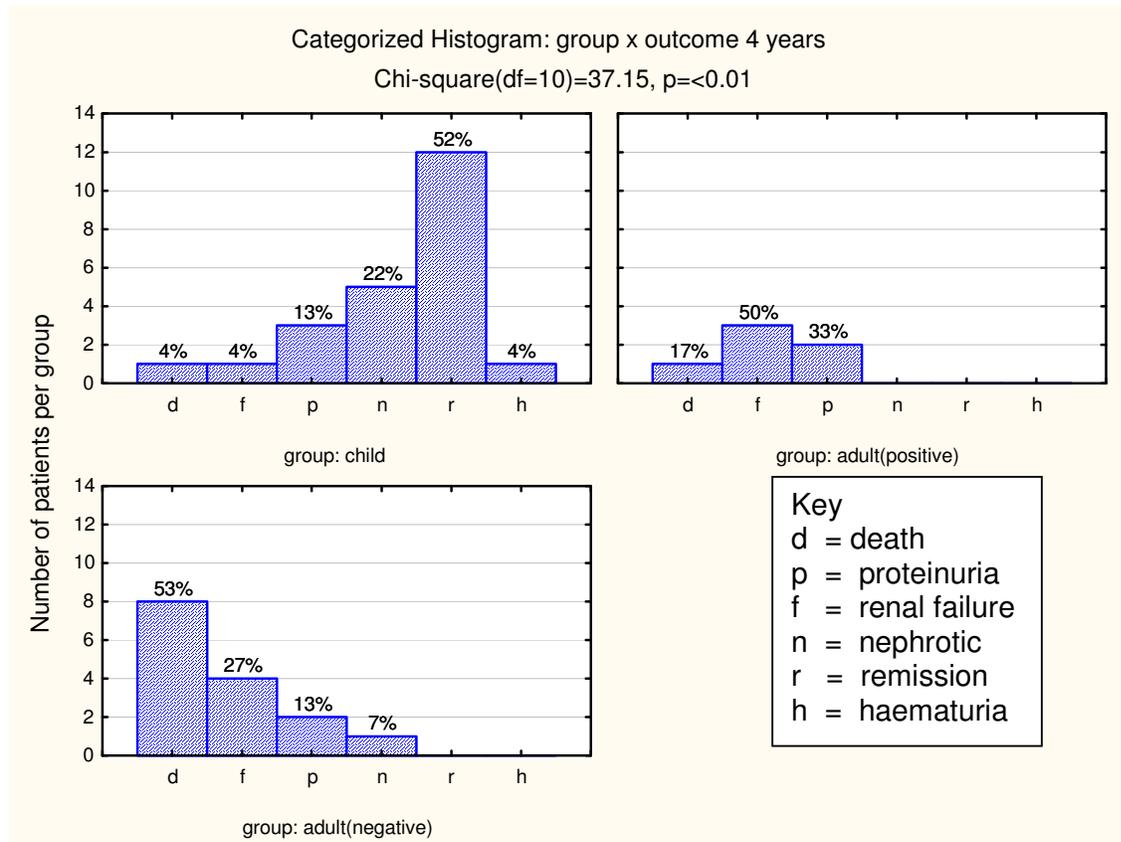


Fig 5-43 Outcome of the patients from the 3 groups at 4 years

By 4 years remission had only occurred in the childhood HBV MGN group 52% vs 0% in both adult groups, a significantly better outcome.

FEATURES OF ADULT MGN COMPARATIVE GROUPS - HBV AND IDIOPATHIC

The 12 HBV MGN adults who were compared to the childhood HBV MGN group and the adult idiopathic MGN cohort of 33 patients were subgroups of the 126 MGN adults. The adult HBV MGN cohort was also a subgroup of the 37 adult HBV patients. In order to gain greater perspective on where the two adult MGN cohorts fit in, these two larger groups are described in more detail. (All these adult patients were part of the total renal biopsy group from 1986-2000 inclusive of 1196 native renal biopsies.)

Gender and race varied considerably in the MGN subcategories as can be seen from the pie charts. (*Fig 5-44*) All 29 SLE patients were female (23 coloured, 4 black and 2 white).

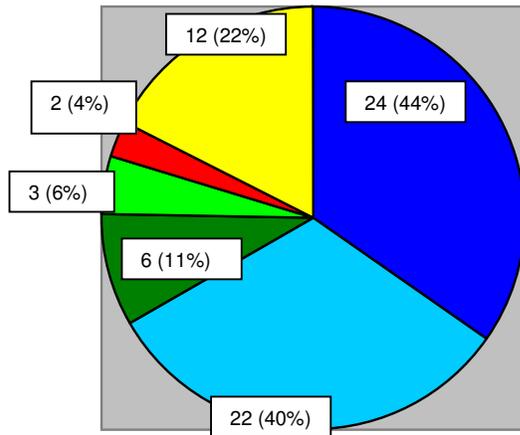
Thirteen of the 15 HBV patients were male and none was white. All were either black (African) (8) or coloured/mixed race (7).

The idiopathic membranous GN group was most representative of this hospital's patient population as a whole including 14 white patients. Males were just over half (54%) of the patients with idiopathic MGN.

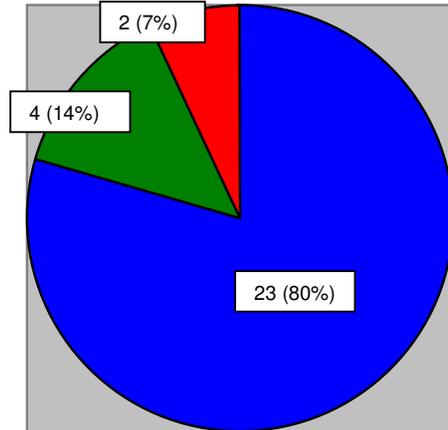
The associations of the 13 in the group headlined 'other' were: 2 neoplasms (Pharyngeal carcinoma and acinic cell tumour), 2 diabetes mellitus, 3 with positive serology for syphilis, 1 post-transplant, 1 HIV positive, 1 also with granulomatous inflammation, one with hypothyroidism, one with gout on medication and a final with suspected but not proven lung malignancy.

Fig 5-44 RACE AND GENDER IN THE ADULT MGN COHORT

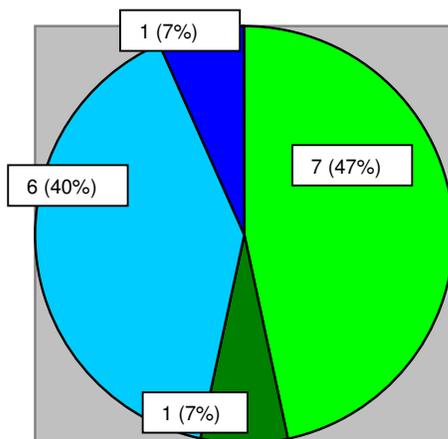
a) IDIOPATHIC MGN n=55



b) SLE MGN n=29



c) HBV GN n=15



Key

- **CF** : Coloured female
- **CM** : Coloured male
- **BF** : Black female
- **BM** : Black male
- **WF** : White female
- **WM** : White male

ADULT HBV PATIENTS

The 37 patients who were serum HBsAg positive constituted 3.1% of the total renal biopsy cohort of 1196 adult patients. They were divided into four (4) groups based on their biopsy morphology and the likelihood of association with HBV as an aetiological factor. The MGN subgroup of this HBV cohort constituted 15/37 (41%) of the group including the 12 in the comparison 12/37 (32%). HBV MGN is a much smaller proportion of the HBV associated GN in adults compared to children 15/37 (41%) to 71/83 (86%) and a smaller proportion of the adult and childhood renal disease groups as a whole 15/1196 (1.2%) to 71/309 (23%). There were 15 cases of adult HBV MGN in the 15 years from 1986-2000 compared to 71 children over 23 years who had HBV MGN. The renal biopsy incidence of HBV MGN was thus 3 times greater in children than adults. The 4 groups of adult HBV renal/glomerular disease are discussed below. (Table 5-7)

GROUP 1- HBV MGN (n=15)

Group 1 consists of the 15 patients with an MGN pattern and this includes the 12 patients who are part of this comparison. The group comprised 13 males and 2 females, 8 black patients and 7 coloured patients, mean age 28.1 years with a range from 15-49 years and a mean creatinine of 166 micromol/L. Six out of 12 (50%) of the whole subgroup who were tested for serum HBe showed positivity and the proportion rose to 60% when the three eliminated from the comparative statistical analysis were removed.

When these 15 HBV MGN patients were evaluated closely, it was recognised that 3 of them had other possible associations with MGN besides the HBV and they were

therefore excluded from the detailed statistical comparison. The first patient was a 39 year coloured male who was also VDRL positive and had squamous carcinoma of the lung diagnosed around the time of his presentation. He died within 2 months of presentation. Both of these are possible further risk factors for MGN. The second patient was a 41 year old coloured male who presented in renal failure with a creatinine of 717 and was also VDRL positive. The third patient was a 33 coloured female who was also ANF positive and the possibility of SLE was also considered but neither the HBV link nor SLE could be confirmed with certainty. She developed renal failure with a creatinine of 652 micromoles/L 21 months after presentation and was referred to another centre for renal replacement therapy.

The exclusion of these 3 patients from the group for purposes of the statistical analysis left 12 HBV MGN adults without another known association.

Table 5-7 Summary of 4 HBV GN groups (n=37)

Subgroup	No. (%)	Male (%)	Race C ^a (%)	Age Mean years	Creatinine(micromole/L)		Mean	Above 120	(%)
MGN^c	15 (40)	13 (87)	7 (47)	28.1	132		4>N		(27)
MCGN^d	8 (22)	7 (87)	6 (75)	37.8	403		7>N		(88)
OTHER	10 (27)	8 (80)	4 (40)	34.7	656		7>N		(70)
END STAGE	4 (11)	3 (75)	2 (50)	25.0	1459		4>N		(100)
TOTAL	37	31 (84)	C19 (51) B18^b (49)	31.9	490		22>N		(59)

^a C: Coloured ^b B: Black

^c MGN – Membranous glomerulonephritis ^d MCGN- Mesangiocapillary Glomerulonephritis

GROUP 2. HBV associated Mesangiocapillary glomerulonephritis.

(MCGN) (n=8)

This group comprised 8 (22%) of the HBV biopsy cohort. There were 7 males and 1 female, 6 coloured and 2 black with a mean age of 37.8 years (range 25-55 years). The mean creatinine on presentation was 403 micromoles/L and 7/8 (88%) presented with a raised creatinine. One patient was also HIV positive and another had crescentic GN. Two out of 4 (50%) tested had serum HBeAg at presentation. This proportion was similar to the MGN group while none of the four in the HBV 'other' group 3 showed HBeAg. This supports the notion of the link between serum HBeAg positivity and aetiologically related GN in HBV carriers.

GROUP 3. Other glomerular diseases probably not linked to HBV

(n=10)

This group consisted of 10 (27%) patients with a range of diagnoses not usually clearly linked aetiologically to HBV. Similar to the other groups and cohort as a whole, males predominated 8:2, with 4 coloured patients and 6 black. The mean age was 34.7 years with a range from 25 to 57 years. All 4 who were tested for serum HBeAg were negative. Three presented with normal range creatinines and 2 of the 7 with raised levels had very high levels above 1500micromoles/L. The mean was therefore high at 656. Four showed a post-infectious GN pattern, one also with crescents. Two were HIV positive and showed HIVAN. Two showed FSGS, one showed diabetic nephropathy changes and one was classified as mesangial proliferative. This last mentioned category has sometimes been categorised as one with a more specific link to HBV, sometimes also with IgA. [4-6]

GROUP 4. HBV End stage kidney disease (n=4)

The final 4 (11%) all presented end stage morphology. There were 3 males, one female: two were coloured and 2 black. The mean age was 25 years (range 14 – 43 years). Two of the youngest in the whole cohort at 14 and 16 years were in this group. The mean creatinine was predictably high at 1459 micromoles/L. One patient was also HIV positive and another VDRL positive.

IDIOPATHIC MGN (n=33)

The idiopathic MGN group for statistical comparison was a subgroup of 33 of the larger idiopathic MGN group of 55 patients from the adult biopsy group 1986-2000. They were 33 adults (13 years or older) who presented consecutively with proteinuria/nephrotic syndrome between 1986 and 1993. They were selected after other associations such as HBV, SLE, syphilis or drug reactions had been excluded. The dates were chosen in 1995 to include a statistically useful group on whom it was most likely to have at least 4 years worth of follow-up by the time of analysis. The most recent cohort fulfilling these criteria was selected.

The data on this group is presented in detail in the comparison tables (5-1 to 5-6) and discussed as part of the comparisons. Briefly, the idiopathic MGN group comprised 33 patients with a mean age of 38.4 years (range 13-74), 21 (64%) male with 2 (6%) black (African), 10 (30%) white and 21 (64%) coloured; the only comparative group with any white patients. The patients presented with clinical and laboratory features of nephrotic syndrome/severe proteinuria. In particular 42% showed no blood or only a trace, liver enzymes were normal and reduced

complement levels rare. Renal biopsies showed classical MGN features with no or minimal mesangial proliferation, prominent subepithelial deposits but seldom if ever mesangial or subendothelial deposits, or mesangial interposition. The outcome was relatively poor although the follow up was short. At neither 2 nor 4 years was any patient in complete remission, and death and renal failure were prominent in those with follow up 11/14 (79%).

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Chapter 6

DISCUSSION OF CHILDHOOD HBV MGN

This study cohort of 71 children with HBV MGN is one of the largest ever documented from a single centre and is unusual, if not unique, in having such a detailed pathological description and analysis correlated with demographic, clinical and outcome data. The study is also broad and comprehensive in scope both geographically and in terms of study time period. The cohort includes 46 children from two provinces in SA, the Western Cape and Northern Cape, and 25 children from Namibia. The South African children came from greater Cape Town, peri-urban as well as deep rural areas of the 2 SA provinces and the Namibian children predominantly from the far north, the Owambo region. The study has extended over a 32 year period from 1974 when the first child in the series presented to Tygerberg Hospital until 2005 when the series was closed to allow extended follow up. This extended time period has enabled a better understanding of the natural history of HBV MGN in children.

This study confirmed a number of facets of the disease in the demographic and clinical areas, such as marked male predominance, the risk of the disease in populations with high background HBV carrier rates, the prominent haematuria, the presence usually of HBeAg as well as HBsAg in the serum at presentation, the frequently, but moderately raised liver enzymes, the frequency of reduced C₃ and C₄ and the usually normal serum urea and creatinine. These in addition to the usual features of severe proteinuria/nephrotic syndrome such as oedema, ascites, lowered

serum albumin and lowered total serum protein levels as well as hypercholesterolaemia.

The renal biopsies showed membranous features (subepithelial deposits) usually in stage II-III but, in addition, substantial mesangial and subendothelial deposits as well as mesangial interposition were frequently noted. These latter 3 features had on occasion been previously noted but have seldom been documented together and almost never analysed in such detail and correlated with other features in any series of this size. The Yoshikawa series which compared 16 HBV MGN children with 9 idiopathic MGN children and the Wrzolkowa series of ultrastructural examination of 70 Polish children have similarities and have been used for comparison. [1, 2] The former is, however, a smaller series with no follow up and the latter, while having extensive ultrastructural data does not correlate this with other data which is in any case limited and again limited follow up data is included. The interstitium of the kidney was shown usually to be minimally involved and glomerular sclerosis rare in the renal biopsies from HBV MGN in children as others have seen but seldom documented in such detail in a series this large. [3] The cardinal MGN deposit has been confirmed to be HBeAg for the first and still only time on the African continent and only 26 (20%) cases or cohorts of the 132 reports on HBV MGN have shown this with Takekoshi in Japan in 1979 being the first. [4-6] (See Table 2 -14, page 80 in the literature review for other cases documenting this feature.)

The comprehensive tables compiled of all the cases in the literature of both children and adults in the literature review, pages 2-30 to 2-35, make it possible to place this series in context and to develop an overall perspective on this disease of HBV MGN since the first case was documented in 1971. [7] The information in this study on 3 of the central pathological features of HBV MGN namely mesangial deposits, mesangial

interposition and HBeAg staining of subepithelial deposits in the glomeruli, marks this study out as one of only 6 out of 132 reports which have provided positive information on all 3 of these features and the only one outside of Japan, Hong Kong and the USA. (Subendothelial deposits could be included as a fourth unusual glomerular feature but these are less frequent than the others and less comparative data is present in the literature.)

There are a number of areas where the primary study group, childhood HBV MGN, data is more complete or extensive than the adult comparative data. The additional information available on the children included geographical (where they lived regarding the South African children; the ethnic origins of the Namibian children); follow up values of serum HBsAg and HBeAg, follow up serum complement (C₃ and C₄) values at least 6 months after initial presentation and follow up liver enzymes, AST and ALT. More comprehensive long term follow up was also gathered on the 46 South African children in particular including, where possible, correlation with serum HBsAg and serum HBeAg status. This extended follow up included a group of 21 followed more than 5 years; 14 of these with follow up over 10 years, 5 of whom have follow up over 15 years and a further 4 who have been followed more than 20 years. This represents an unusual if not unique subgroup which is important because it shows that some of those who do not go into remission early, can develop renal failure years later.

The more detailed discussion will follow the same topic outline as the literature review and results.

DEMOGRAPHIC, CLINICAL AND LABORATORY FEATURES

AGE

The mean age of the children in the current study was 6.0 years (range 2-12 years) which was identical to the figures in the review of Johnson and Couser. [8] As has been shown by the current data as well as that of others, age at presentation appears to be an important variable related to morphology and outcome and therefore the age range of children's series is an important feature to consider; comparisons between childhood series may otherwise be less reliable. The series of 52 of Hsu et al [9], with 60% of the of 5 years or below was similar to this studies proportion of 52%, even though their upper limit of the age of children was older than in this series, namely 14 years. By contrast, a large Polish series in 1991 noted that this disease was observed mainly in young children, 90% of them less than 6 years of age compared to the 52% of the current series who were 5 years of age or less. [2] Another Polish series also demonstrated a preponderance of young children. (Wyszynska et al 1984)[10] In their series of 49 children with HBV GN, where 34 out of 44 who were biopsied showed a membranous pattern, 36 (73%) children were 5 years or below at presentation with 24 (49%), 3 years or less. [10] These different series suggest different age experiences in the various countries. It is known that younger children are more likely to become carriers when exposed to HBV. [11] It may be that in Poland, for example, at that time, many younger children, from birth to 3 years were exposed to HBV, a high proportion became carriers and then most seroconverted within a few years. If patients developed carrier status at a later age they were more likely to remain carriers longer. [11] The disease in the younger children with almost universally good prognosis was behaving more like a once off 'post infectious' GN pattern with the antigen causing the disease resolving

spontaneously within 6 months or a year in many and within 2 years in most, while in the older children, adults and those with a more chronic pattern the disease fitted better with a chronic MGN picture.

GENDER

The present study has an 80% male preponderance in line with other reports. [9, 12] Johnson and Couser (1990) reported that, although idiopathic MGN was associated with a slight male predominance of ratio 1,4:1 (58% male), in HBV MGN as many as 80% to 100% of children were males. [8] Boys are more likely to be HBV carriers. [13, 14] Nevertheless the exact reasons for the degree of male preponderance remain speculative.

RACE

There were two nationalities and 2 race groups of patients in this study; twenty-five (25) Namibian children who were all black (African) while of the 46 SA children, 40 were coloured and 6 black – 87%:13%. There were no white children present in either group. In the past (before the 1990s) black families and children were largely restricted from permanent residence in the Western Cape, almost certainly contributing to the lower proportion even though the HBV carrier rate among black children was higher. [14] Red Cross Children's Hospital (also in Cape Town), however, saw a larger proportion of black children with HBV MGN than this hospital did. As noted in the literature review (2-42 and 2-43) race may be a direct or indirect prognostic factor; Wiggelinkhuizen reported that the prognosis of coloured children with MGN was better than for black children. [15, 16] It is of interest that in this present series black (African) children were more likely to have severe mesangial deposits, a feature linked to poorer outcome, a finding discussed more fully

elsewhere. Unfortunately, in this study black patients had poorer follow up reducing the opportunity to make statistically significant prognostic correlations between these groups. There is almost no follow up of the 25 black Namibian HBV MGN children (except for the one who presented in renal failure and died within 4 years), and the 6 black SA children had a mean follow up of only 20 months compared to 99 months for the 40 coloured children.

CLINICAL AND LABORATORY FEATURES

SERUM HBsAg, HBeAg and ANTIBODY STATUS

In reported series, the prevalence of serum HBeAg ranged from 60-93%. [8, 9] Local prevalence were reported as 98% (Cape Town - Red Cross Children's Hospital) and 70% (Durban). [9, 12, 17] In this cohort 33 (87%) tested showed HBeAg in the serum at presentation or first testing. This supports the accepted hypothesis that HBe immune complexes are involved in the MGN pattern. Factors such as time to presentation and technical quality of laboratories may play a role in the variation of frequency of this finding. Serum HBeAg status and its evolution during the course of the disease is one of the interesting aspects of this disease. The central immune complex producing the membranous pattern has been shown to be HBe antigen – antibody complex rather than involving HBsAg as initially surmised. Also, remission of the renal disease is associated with seroconversion of HBeAg with the development of HBe antibodies and sometimes loss of serum HBsAg. [4, 5, 16] In this series, those in remission when last tested were 10/14 (71%) negative for serum HBeAg and 4 (31%) negative for serum HBsAg, fitting with the general pattern.

CLINICAL PRESENTATION

The childhood HBV MGN patients presented almost universally with severe proteinuria/nephrotic syndrome. Oedema was frequent in the patients, 70/71 (99%) and in keeping with the series of Gilbert where oedema was documented in all 70 HBV MGN children. [12] The same Gilbert series reported (71%) for ascites compared to this series with 48%. [12] Hypertension was reported in less than 25% of cases, [8] while in this study the frequency was marginally higher at 35%. At presentation in the patients of Gilbert, 19/70 (27%) were noted to have hypertension while in Bhimma's series 7 (10%) showed hypertension initially and 23 (33%) showed hypertension initially and persistently, a total of 43%. [12, 17].

All the study patients showed some proteinuria on dipstix and most, over 75%, showed 3+ or 4+. The majority (68%) also had prominent haematuria (2+ to 4+) with only (10%) showing no blood or a trace. The Johnson review suggested that children present typically with microscopic or rarely macroscopic haematuria. [8] Gilbert documented that (71%) had micro-haematuria and (21%) had macro-haematuria. [12] This is one of the features where HBV MGN differs from idiopathic having more frequent and severe haematuria as discussed more fully in the comparison discussion. (Chapter 7)

LABORATORY FINDINGS

Renal failure is very rare at presentation in children with HBV MGN. [8] This cohort is therefore in line with the literature in only rarely showing raised urea and creatinine levels.

Only one child of the 71 (1.5%) with HBV MGN presented with established renal failure. The group had a urea mean within normal limits. Children are known to have much lower creatinine levels than adults, which then rise with age due to increase in muscle bulk; the mean of 45.8 micromole/L was therefore not surprising.

In keeping with the clinical presentation of severe proteinuria/nephrotic syndrome, the mean serum albumin and total protein levels were usually lowered while the serum cholesterol was elevated. (Table 4-2)

The group showed mean total protein levels well below normal at 46.3 g/L. Ten values were below 40 and one outlier was actually above normal at 83g/L. The mean albumin level was very low at 16.4 g/L. Six showed values below 10g/L. These values are in line with other similar series. [12]

At presentation, 34% of the childhood HBV MGN group had raised AST levels and 62% elevated ALT levels. These proportions rose with follow up and repeated testing to 68% and 85% respectively. This confirms that liver enzymes demonstrate damage linked to hepatitis in almost 90% of children despite the literature summary that children with HBV MGN generally have no history or clinical evidence for ongoing liver disease. [8, 18] Liver biopsies almost invariably demonstrate what was previously called chronic persistent or chronic active hepatitis; however, most of these patients do not develop clinical hepatitis. [3] As noted on pages 4-12 and 4-13 of the childhood HBV MGN results, 5 of the 9 liver biopsies performed on these children, usually for persistently and markedly raised enzymes, demonstrated mild HBV associated chronic hepatitis while 4 showed moderate changes. [19] As was

suggested in the literature review on page 21 of chapter 2, the association of this disease (HBV MGN) with the formation of HBeAg – HBeAb complexes in the phase of HBe seroconversion, especially in the childhood HBV MGN, implies that the liver will from the time of the HBV MGN be less likely to manifest liver complications usually associated with ongoing HBeAg presence such as cirrhosis and hepatocellular carcinoma. [20] This study observed that both the serum C₃ and C₄ levels were reduced in the HBV MGN children; the C₃ (63%) more frequently than the C₄ (34%). Johnson et al summarised the situation in children noting that in most studies until that time, serum C₃ and C₄ levels had been depressed in 15 – 64% of cases although some series had found complement to be normal. [8] The Southwest study group found C₃ low in 7/11 (64%). [18] Bhimma et al found low C₃ in 33/70 (47%) of cases, more frequently than in both HBV associated non MGN renal disease (26%) and idiopathic MGN in children (0%). The lower complement levels in HBV MGN probably reflect the sequence of actively replicating HBV, antibody production, formation of circulating and/or in-situ immune complexes, and consequent consumption of complement proteins. [17]

The frequently lowered C₃ level in HBV MGN does not appear to return to normal within 6 months as 20/31 (64.5%) still showed low C₃ values after 6 months.

This childhood HBV MGN group also showed frequent low levels of C₄ 22/65 (34%), but less so than with C₃. The Southwest group study showed one very low C₄ value and three borderline low out of 7, making 4/7 (57%) low. [18] The Durban group found C₄ low in 8/70 (11%) of HBV MGN children. [17]

Again this studies' data shows that the proportion of low C₄ values remained very similar after 6 months with 10/31 (32%) showing low values, very similar to the 34% initial value.

These findings suggest that HBV MGN is a C₃ and C₄ consuming immune complex disease that tends to persist for at least 6 months, often at least a year and frequently longer from time to remission data. There does not appear to be other data in the literature highlighting this feature of persistent complement consumption.

RENAL BIOPSY PATHOLOGY - LIGHT MICROSCOPY

On light microscopy, HBV MGN in childhood has a typical though not diagnostic appearance. Only the glomeruli tend to be abnormal with mild to moderate mesangial proliferation and prominent thickening of capillary walls. The interstitium appears normal with no or few signs of fibrosis, inflammation, tubular or vascular changes. Special stains such as the Masson trichrome can highlight subepithelial and mesangial deposits in the glomeruli while the silver stain confirms membranous features by showing spikes (stage II) and incorporation (stage III) and often tramlines (double lines), features of mesangial interposition and the characteristic combination of MGN and mesangiocapillary GN reaction patterns. Glomerular sclerosis and crescents are rare. [12, 21] Each feature is discussed in more detail in the following section.

GLOMERULAR FEATURES

MESANGIAL PROLIFERATION

In this current series, the glomeruli of all 71 biopsies from the HBV MGN children showed some degree of mesangial proliferation and 7 (10%) showed moderate to severe proliferation. Ehrenreich and Churg reported that the only significant change in membranous nephropathy (as they preferred to call this entity at that time) was thickening of the glomerular capillary walls. Rarely, other changes were seen such as mesangial widening or hypercellularity of mesangial areas. [22] Schwartz described the subepithelial deposits but noted that other pathologic changes described in MGN included glomerular lobulation, mesangial hypercellularity and segmental scars but that these were more frequent in secondary forms of MGN than idiopathic. [21]

Wiggelinkhuizen et al (1983) noted mild mesangial cell proliferation with an increase in mesangial matrix in their HBV MGN cases. [16] Venkateshan et al (1990) reported that although the occurrence of mesangial proliferation and deposits in idiopathic MGN was rare (Ehrenreich and Churg 1968), they had been reported in secondary forms of MGN like SLE and HBV. [3, 22, 23] The presence of prominent mesangial proliferation was almost certainly one of the features leading Brzosko et al in 1974 to categorise HBV associated GN in proliferative categories such as mesangiocapillary GN rather than MGN where there was a combination of MGN and mesangiocapillary type changes. [24]

This current series confirms these findings with all biopsies showing some degree of mesangial proliferation and nearly 10% either a moderate or severe degree.

GLOMERULAR SCLEROSIS

Glomerular sclerosis is a sign of advanced damage to the glomerulus that was present in only a small number of patients. [25] This is in keeping with the clinical finding that renal failure was uncommon at presentation. The childhood group had a mean of 4.5% of the glomeruli sclerosed but the majority, 48 (68%) showed no glomerular sclerosis and a further 12 (17%) biopsies which showed sclerosis, included involvement of fewer than 10% of the glomeruli. Put another way, only 11/71 (15%) showed more than 10% glomerular sclerosis. This is viewed as a central finding as it differs prominently from the adult groups.

Glomerular sclerosis is not addressed in the large reviews dealing with HBV MGN, predominantly summarizing childhood data, reflecting its rarity. [3, 8]

CRESCENTS

In this cohort of children with HBV MGN, none had crescentic nephritis (defined as more than 50% crescents). Only 4/71 (6%) showed crescents with glomerular involvement per biopsy having a maximum of 17%. Crescents are not mentioned in the childhood reviews, reflecting their rarity. [8, 11] True crescentic GN with at least 50% crescents has not been described in childhood HBV MGN.

INTERSTITIAL INVOLVEMENT

Interstitial involvement, (inflammation, tubular atrophy, fibrosis and vascular changes), was not prominent in this cohort of childhood HBV MGN where more than 90% of biopsies had no changes, or if present only mild degrees of change in the various components. Venkateshan et al noted that tubulointerstitial changes were seldom found, even in cases of long duration. [3]

ULTRASTRUCTURE

SUBEPITHELIAL DEPOSITS

Few series have categorised the stages of subepithelial deposits in childhood HBV MGN and correlated them with other features including outcome. In Table 6-1 below the stages of three series' subepithelial deposits are compared to the study cohort. [1, 9, 12, 22]

Table 6-1 *Stages of subepithelial deposits on ultrastructure*

Stage ^a	I	I-II	II	II-III	III	IV
	No. (%)					
Yoshikawa (n=14) [1]	0 (0)	-	8 (57)	-	5 (36)	1 (7)
Hsu (n=52) [9]	4 (8)	19 (37)	16 (32)	10 (20)	3 (6)	0 (0)
Gilbert n=69 [12]	3 (4)	-	34 (49)	-	27 (39)	5 (7)
Current n=71	1 (1.5)	2 (3)	5 (7)	35 (49)	23 (32)	5 (7)

^a The data of Yoshikawa and Gilbert are added to Table 6-1 for completeness and comparison. These groups used the 4 stages without the overlap groups included by Hsu, hence gaps in the table. The figures are therefore not fully comparable. [1, 9, 12]

It was partly because of the availability of this Taiwanese series of Hsu and its data that this study used similar categories and combined groups, so that data could be readily compared. [9] This current group is of more advanced stage compared to the Taiwanese group with (88%) showing stage III and IV features compared to only (26%). In their series the presence of stage III changes was associated with significantly slower remission by 2 years. Yoshikawa and Gilbert's series are similar with intermediate figures of 43% and 46% stages III and IV.

Our study showed that the stage of subepithelial deposits emerged as one of 6 parameters which showed statistical significance and as one of the 2 factors, with age, with a p value below 0.01 when the group of 14 in remission was compared to

the 7 with poor outcome (Table 4-12). The group of Gilbert et al could not confirm a correlation between stage of subepithelial deposits and outcome. [12] One could question the idea that the stage of subepithelial deposits by itself may be a prognostic factor, as the stages are usually viewed as an inexorable chronological progression which all biopsies will undergo. [21, 22] (Unless for example stage I deposits were to disappear before spikes had even formed.)

MESANGIAL DEPOSITS

The severity of mesangial deposits emerged as a prognostic factor with correlations with age, gender and race and other parameters in this study. (Table 4-11) In addition, in the comparison between the 14 children in remission and the 7 who did poorly (Table 4-13), the extent of mesangial deposits was significantly more severe in the poor outcome group.

It is difficult to compare the extent of a feature like mesangial deposits on ultrastructure from descriptions and a few illustrations in published material but it seems highly likely our patients, especially in the severe deposit group, had far more prominent deposits than the majority of the Japanese (see description below), Polish and Taiwanese children described. [1, 2, 9]

In the series of Yoshikawa et al where mesangial electron dense deposits were found in 13 of the 14 (93%) children with HBV MGN, the deposits were described as usually sparse or moderate while 'numerous deposits' were seen in only 2 patients (14%).

Evidence for the assertion that some of these Southern African HBV MGN children showed more severe deposits than the Polish children include their statement that mesangial areas were 'slightly enlarged', their illustration of mesangial deposits where the deposits were not prominent and the lack of prominent associated

mesangial interposition. [2] The more severe glomerular changes in the current study of childhood HBV MGN also clustered with other severe glomerular changes as shown by the finding that 14 (88%) of those with severe mesangial deposits also showed severe mesangial interposition, while the other (12%) had moderate mesangial interposition.

SUBENDOTHELIAL DEPOSITS

In this study 33 (46%) of the childhood HBV group had subendothelial deposits of which 7 (10%) were moderate or severe. Yoshikawa et al 1985 also noted small subendothelial deposits in 4 of 16 (25%) of the HBV childhood patients while none of the 9 children in the idiopathic group had them. [1] In the series of Wrzolkowa et al (1991) the subendothelial deposits were described as less abundant than the subepithelial ones, frequently taking the form of small elongated concentrations. They were not, however, further quantified. [2] In the comparison of the 14 HBV MGN in remission and 7 with poor outcome, subendothelial deposits emerged as a significant factor associated with poor outcome. (Table 4-13) This is a new finding that has parallels with SLE and its management and prognosis. Subendothelial deposits in lupus nephritis are one of the features defining class III and IV (the focal and diffuse proliferative classes with poorer outcome) and are also part of the activity index which contributes to the management of lupus nephritis. [26]

MESANGIAL INTERPOSITION

In this present study, 63 (89%) of the childhood HBV MGN group showed at least mild mesangial interposition with 41 (58%) demonstrating moderate or severe mesangial interposition. Although both the moderate and severe categories of mesangial interposition displayed 'mesangiocapillary' features at light microscopic

level, the ones with severe changes 23/71 (32%) looked at light microscopic level strongly like MCGN and one might have predicted that they would constitute a distinct clinical and prognostic subset. (In this study this subgroup of 23 with severe mesangial interposition were categorised as mixed HBV MGN-Mesangiocapillary GN to acknowledge that they showed clear-cut features of both forms of GN.) By contrast in a Japanese series only a minor degree of mesangial interposition was observed in 4 of 14 (29%) of the HBV MGN group of Yoshikawa et al in 1985. [1]

Compared to the current series, the 77 biopsies of Wrzolkowa et al (1991) also reported less severe involvement by mesangial interposition. It was noted that mesangial cell processes at times penetrated the glomerular basement membrane; however, this penetration was usually limited to the paramesangial areas, to less than one eighth of the capillary loop circumference. What Wrzolkowa et al described would probably have qualified as mild to moderate mesangial interposition in this current series. [2]

It seems almost certain that the present Southern African subgroup of 23 of the HBV MGN patients with severe mesangial interposition often associated with severe (61%) or moderate mesangial deposits (26%) represent a subgroup of children with HBV MGN not seen often, if at all, in the large Polish series or in the Japanese children studied by Yoshikawa et al (1985). [1, 2]

The morphological differences referred to above pose the intriguing question whether these variations reflect different underlying immune responses to HBV including especially mesangial and subendothelial deposits, possibly linked to factors such as age, race, overall nutritional and immunological factors amongst others. In turn it seems likely that this more severe glomerular involvement in Southern African cases

including those in this series, is linked to slower resolution of the disease with a greater likelihood of chronic renal failure. More severe mesangial interposition in this series was associated with a tendency to correlate with poorer outcome. (Table 4-13)

TUBULORETICULAR BODIES AND VIRUS LIKE PARTICLES

Wrzolkowa et al (1991) reported in their 77 membranous HBV cases, that endothelial cell processes only rarely contained cellular organelles which included reticulo-tubular structures. [2] In this current series it was only when there was clarity as to what was being looked for plus the realisation that at least one tubuloreticular body should probably be seen in the majority of the biopsies of childhood HBV MGN (Mills et al 1988) [27], that a thorough specific search by a trained and motivated ultrastructural medical technologist lead to the finding of at least one in 62 (87%) of the childhood HBV MGN group.

The childhood group also showed many virus-like particles in 59 (83%) of cases but they do not appear to be representative of virus or correlate with outcome or other features. [21]

SUMMARY OF ULTRASTRUCTURE

The ultrastructural diversity of HBV membranous GN as first observed by this author on commencing responsibility for the TBH renal biopsy service in 1985 was one of the initial motivations for this study. There was already evidence of this spectrum and the differences from classical idiopathic MGN but they were not widely known or readily accessible in general surgical pathology texts. [1, 16] This study has confirmed and in many areas extended the documentation of those features.

The HBV MGN childhood group showed in addition to the subepithelial deposits (which defined the group), frequently prominent mesangial deposits, subendothelial deposits and mesangial interposition. This data has shown that the presence and severity of these 3 features tended to be associated. (Table 4 -11) These 3 are features of mesangiocapillary GN and confirm that morphologically many if not most cases of HBV MGN share significant features of mesangiocapillary GN. It will also be seen in the comparative data that the adult HBV MGN group also shares many of these features.

The ultrastructural parameters not only show a prominent and consistent overlap between two accepted GN categories namely MGN and MCGN but in addition this study (Table 4 -13) has demonstrated a significant correlation between poor outcome and more advanced subepithelial deposits as well as more severe subendothelial and mesangial deposits. In the next chapter the significant ultrastructural differences between this study group and the idiopathic MGN control group will be highlighted while the ultrastructural similarities to adult HBV MGN will be shown.

IMMUNOFLUORESCENCE

The childhood HBV MGN study group findings of 98% positivity for IgG and 83% for C₃ were consistent with the literature and reviews where the hallmarks of MGN immunofluorescent staining are almost universal staining for IgG and C₃ in a granular pattern along capillary walls with varying positivity for other immunoglobulins and fibrin. [8, 21, 28] The children's renal biopsies in this series showed a positivity of 56% for IgA. Johnson and Couser gave a figure of 10% for IgA, based on two references. [8] Venkateshan et al documented their mixed group of adult and childhood HBV MGN biopsies in some detail and IgA was noted in 3/6 (50%) of the childhood HBV MGN. [3]

The current childhood group showed 38/53 (72%) for IgM. Johnson and Couser gave a figure of 50% for IgM in HBV MGN [8]. Venkateshan's series however, showed 13/13 (100%) positive for IgM which varied from 1+ to 3+ on their scoring system. [3] The childhood group showed 13/49 (26.5%) for fibrin/fibrinogen. Fibrinogen is not mentioned in Schwartz's detailed discussion of the pathology of MGN and in only one case in 13 in Venkateshan's series. [3, 21] There is wide variation in these latter 3 stains (IgA, IgM and fibrinogen) making evaluation and interpretation difficult.

HBV ANTIGENS and ANTIBODIES

A special focus of this dissertation was the immunological staining for HBV antigens in the glomeruli of the study and comparative groups. This included importing monoclonal antibodies to HBeAg that were not readily available via usual commercial channels. It is unusual to be able to stain for specific associated antigens in glomeruli in GN and to seek to correlate the findings with similar serum antigens. This was one of the aims of this study.

The strongest evidence for HBeAg association with the membranous pattern was the staining with the monoclonal antibodies where 18/20 (90%) cases were positive for either HBe(a)Ag or HBe(b)Ag in a granular pattern along capillary walls. (65% were positive for both.) (Fig 5-41 page 5-38) The Hong Kong figures of 20/24 (83%) for HBeAg IgG2a subclass are similar to this study. [29]

In the childhood study group, 19 (95%) of the biopsies were positive in a predominantly subepithelial location for HBcAg (polyclonal antibody) in relation to the expected location of MGN immune deposits. As noted earlier, (Lit Survey Table 2-13, page 77) the Lai Hong Kong group tested 6 antisera /antibodies for anti-HBc and anti HBe activity and found that the monoclonal antibodies produced at Jichi and St Mary's Medical schools both had appropriate activity but that Dakopatts anti-HBcAg showed both anti-HBcAg and anti-HBeAg activity. [30] The implication therefore is that in this context, the polyclonal anti-HBcAg used in this study is almost always staining for HBeAg.

All cases staining for either of the HBe antigens also stained for HBcAg. This confirmed the important findings of KN and FM Lai et al. [29, 31] The more affordable anti-HBcAg polyclonal has 2 antibodies in one (to both HBcAg and HBeAg) but if there is uncertainty as to the Ag being identified then that is confusing. Cases

showing anti-HBeAg staining in glomeruli are included in Table 2-14 (page 2-80) of the literature review.

Another confusing finding was the polyclonal anti-HBsAg staining where in the childhood HBV MGN renal biopsies 26(65%) were positive. This was the same Dakopatts polyclonal antibody that others had shown during the study period tended to cross-react and therefore this finding should be discounted as false. [30, 32] This was referred to in the literature survey and will be further covered in the comparative discussion. (Chapter 7.)

In an attempt to clarify this situation, a newer monoclonal anti-HBsAg antibody was used on three HBV MGN cases and all were negative. There is however insufficient reliable data on HBsAg in the glomeruli in HBV MGN to draw firm conclusions about its frequency or location. [31]

SUMMARY OF IMMUNOFLUORESCENCE

The study cohort confirmed other findings showing high positivity (more than 75%) for IgG, C₃, HBe (monoclonal) and HBc (polyclonal) antigens usually in a granular pattern along capillary walls. The HBeAg staining in HBV MGN stands out as an unusual and unique contribution of this study as the first time this has been shown in patients from Africa and one of only 28 reports worldwide.

IgA, IgM and fibrinogen showed mild to moderate positivity in this cohort, positivity ranging from 26% to 72% of cases. Comparative figures were rare and the interpretation of partial positivity and the absence of universally agreed scoring systems complicate evaluation and comparison.

HBsAg (polyclonal antibody) was present in 65% of biopsies but was viewed as false staining. This will be fully discussed in the comparative section where non specific cross reactivity was confirmed.

TREATMENT

The majority of children in this current series were not given specific treatment for their HBV MGN in the form of either steroids, interferon or other antiviral agents as growing experience of its usually good outcome made this appear unnecessary. Their course therefore reflects the natural history of the disease with supportive treatment for hypertension, severe oedema and ascites as well as other complications.

Most of the patients from the literature who have received corticosteroids as treatment for the HBV MGN induced nephrotic syndrome have had no beneficial effect or have experienced only transient or partial remissions. [3] In the series of Gilbert et al, for example, of their 70 patients, ten received steroid therapy for 4 -8 weeks; 6 were in remission at last follow-up, but none went into remission while on steroids and only one did so within 6 months of treatment. There was no significant difference in the duration of nephrotic syndrome between those who did and those who did not receive steroids. [12] Two patients received steroids and cyclophosphamide before the relationship between HBV infections and MGN was recognised. They went into remission 6 and 38 months after onset respectively. [12] Specific antiviral therapy with interferon has also been administered to children with HBV MGN. In some cases this produced remission with complete HBV seroconversion. [33-35] See Table 2-16, Chapter 2 Literature survey, pages 2-85 and 2-86.

OUTCOME : COURSE, REMISSIONS AND CORRELATIONS

It is generally accepted that idiopathic MN in children has a better prognosis than in adults although prolonged course and permanent renal damage do occur. [36] HBV associated MGN in children has run a favourable course worldwide, even better than idiopathic MGN in children. The assertion by Hsu et al in 1989 that little was known about the natural course of the disease of HBV MGN has been partially altered by publications since then but nevertheless still provided part of the motivation for this study. [9]

In the current series 14 (30%) of the total group of 46 SA children went into spontaneous remission confirming the generally favourable outcome of HBV MGN in childhood as outlined above. At 2 years of follow up, 25% from the childhood HBV MGN group of this study still being followed had shown remission (7/28). At 4 years of follow up, remission increased in the childhood HBV group where 52% with known outcome had shown remission. Renal failure or death were present but not very frequent in the children in the early stages, with 2/23 (8.7%) at 4 years in this series. In total, 6 of the 46 (13%) of the SA children have had a poor renal outcome and one of the 25 Namibian children is known to have developed renal failure and died within 4 years of presentation. (It needs to be emphasised that follow up on the Namibian children once they returned to Namibia was unfortunately very limited.) This group of 7 with poor outcome is one of the largest from a single centre and warrants further analysis and discussion. (See below)

In the patients available for follow up, remission in this SA group of HBV MGN children occurred by a mean of 31.2 months (range 15-89). Remission appears usually to be a stable permanent state as Gilbert et al noted in their 37 patients in

remission followed for an average of 27 months after remission with none showing relapse. [12] This current series also confirmed that remission was usually permanent with 10/11 (91%) of those showing remission and who were subsequently followed, remaining in remission. One child however, who went into remission initially at 32 months but still with HBeAg in the serum subsequently relapsed with proteinuria, haematuria and elevated liver enzymes. This patient 5 years after the initial remission and 8 years after initial presentation went into remission again, this time also seroconverting to HBeAg negative. (Also described more fully on page 4-15)

In comparison with the group from Taiwan, it can be seen that the current group, while having a remission rate of 52% at 4 years, had a slower and lower remission rate than the Taiwanese patients (64% at 2 years and 83% at 5 years) and a higher rate of renal failure/death (9%) at 4 years and at least 10% overall compared to 2% for the series of Hsu. [9] Some in this SA group of childhood HBV MGN may still have gone into remission after they were last seen but this and other studies' figures suggest that if it had not occurred by 48 months it was less likely. [12] A proportion of those with persistent proteinuria do go on to renal failure but that figure is also unknown.

Hsu 1989 found in their 52 patients that several clinical, pathologic and virologic parameters were related to the disease course. Remission occurred more often and significantly earlier in children of younger age, with a shorter disease course, with smaller amounts of glomerular deposits and without focal glomerular sclerosis. The significantly longer course and lower remission rate in children with more advanced disease (stages II-III and III) and/or focal sclerosis suggested that permanent glomerular damage had already occurred in children with prolonged persistent proteinuria. [9] A number of parameters of the current study childhood HBV MGN

cohort were tested against all the other parameters to seek significant correlations. Three with the most prominent differences, age, mesangial deposits and mesangial interposition are discussed below. (Tables 4-10, 4-11 and 4-12)

The patients in the younger group (Table 4-10) in this present series were always serum HBeAg positive (100%) as opposed to usually (74%) in the older ones, were less often hypertensive, had lower haemoglobin and creatinine levels (as would be expected with age linked features) and showed less severe biopsy features: fewer and less severe mesangial and subendothelial deposits, and less frequent and severe mesangial interposition. Similar to Hsu's findings the group 5 years and under were significantly more likely to be in remission at both 2 and 4 years than the older group. [9] The other SA series did not document age as a prognostic factor. [12, 17]

Mesangial deposits (Table 4-11) are one of the distinguishing features of HBV MGN from idiopathic MGN and no children with severe mesangial deposits were known to have gone into remission in this series. Mesangial deposits are a parameter likely to affect the extent of mesangial proliferation and mesangial interposition in glomeruli. It would be a reasonable hypothesis that the extent of mesangial deposits may differ in various subgroups and affect severity of disease and influence remission and other outcomes. The extent of mesangial deposits and mesangial interposition are likely to be risk factors for the development of glomerular sclerosis and interstitial damage. The findings of this study support this suggestion. Those with severe mesangial deposits were significantly older, were exclusively male and tended to be black rather than coloured. Biopsy findings that clustered highly significantly with severe mesangial deposits were increased mesangial interposition, more subendothelial deposits, more crescents, more sclerosis and more severe interstitial involvement in all 4 components. Significantly, although the numbers were small, the severe

mesangial deposit group was also less likely to go into remission at 2 or 4 years than the remaining 55. In fact, as stated above, no children with severe mesangial deposits were known to have gone into remission in this series. Of these 16 children with severe mesangial deposits, 8 were Namibian without further follow up (8/25-32% of the Namibian children, a substantial subgroup) and of the 8 RSA children 2 developed renal failure at 118 and 309 months; one still showed proteinuria when last seen and the remaining 5 were still nephrotic at last visit, 2 under 1 year of follow up but one at 88 months.

The subgroup with severe mesangial interposition on light and electron microscopy, has been classified as the mixed HBV MGN-mesangiocapillary GN group where the degree of the features would be sufficient to make each diagnosis separately. (Table 4-12) A number of significant differences include the severe mesangial interposition group having more black children and a higher proportion of hypertension. The renal biopsies of these children show features associated with mesangial interposition namely more subendothelial deposits, more severe mesangial deposits and a greater degree of mesangial proliferation as well as more advanced subepithelial deposits and more severe interstitial disease in this subgroup of 23. The females (girls) show a tendency to less severe mesangial interposition. Concerning outcome, it may have been predicted that the severe mesangial interposition group of HBV MGN (the mixed HBV MGN-mesangiocapillary GN group as now defined) would show a poorer outcome than the HBV MGN cases with biopsies closer to the idiopathic MGN appearance end of the HBV MGN spectrum. At least 2 reasons could be put forward for this prediction: the pure MGN morphology biopsies in general tend to have a better prognosis than the mesangiocapillary GN reaction pattern [37] and more specifically HBV MGN was shown (in an adult group admittedly) to respond

better to interferon treatment than HBV MCGN. [38] While the better outcome for the HBV MGN group without severe mesangial interposition in this series appears broadly true (Table 4-12), the differences are not statistically significant.

The criteria for the categories of mesangial interposition and subendothelial deposits (absent, mild, moderate and severe) as applied in this study identified severe mesangial interposition which defined the mixed HBV MGN-mesangiocapillary GN biopsy subgroup. This subgroup resolved uncertainties pertaining to renal biopsies with prominent features of both MGN and mesangiocapillary GN. It furthermore displayed a set of clinicopathologic correlations different to the remaining HBV MGN biopsies. Mesangial interposition and subendothelial deposits also displayed statistically significant correlations with each other. (Fig 4-12)

Despite the value of the criteria for categorising the presence and severity of mesangial interposition and subendothelial deposits, the criteria for the mild group of mesangial interposition were set too 'low' leading to some renal biopsies being labelled as mild mesangial interposition that should have been assessed as showing no significant features of interposition. In addition the criteria for the subendothelial deposits were too stringent with few cases being in the moderate and severe categories. Application of revised criteria for the grading of these two features would lead to a frequency and severity correlation closer to the 1:1 ratio that may be expected between subendothelial deposits and mesangial interposition in the mesangiocapillary GN pattern spectrum associated with HBV immune complexes. [39]

PARAMETERS OF 14 CHILDREN IN REMISSION COMPARED TO 7 CHILDREN WITH POOR OUTCOME (Table 4 – 13)

This comparison was a unique way of interrogating the prognostic significance of the parameters. These findings have also been integrated into the discussion elsewhere where appropriate. Age differences and the stage of subepithelial deposits emerged as the most significant differences while creatinine levels, tubular atrophy, mesangial deposits and subendothelial deposits also are significantly different. Male gender, hypertension and increased mesangial interposition also show a tendency to be associated with poorer outcome. Although the table is unique, findings such as older age within childhood and more advanced stage of deposits confirm factors Hsu et al found associated with slower remission of HBV MGN. [9]

OUTCOME WHEN LAST SEEN CORRELATED WITH HBV STATUS IN THE SA GROUP OF 46 CHILDREN (Table 4-8)

In the current study 91% of those children who developed remission of HBV MGN became HBeAg negative. The development of remission was more frequently associated with clearance of HBeAg (91%) than clearance of HBsAg (31%). Four of the 5 who became HBsAg negative within 4 years went into remission. The one exception was the child who despite becoming HBsAg negative within 32 months of presentation, remained proteinuric, developed renal failure much later and is currently alive aged 44 years in June 2010, having had a renal transplant in 2000. This unusual patient is documented in detail in the appendix. (fj /81 page 10-4) There was a tendency for the HBeAg seroconversion to coincide broadly with the onset of remission as has been found by others. [12]

The 19 children who were nephrotic included 15 with fewer than 24 months follow up

and the longest follow up of a child still nephrotic was 88 months. All 6 black SA children were still nephrotic when last seen and the longest follow up was this last mentioned child. (Mean overall follow up of the 6 black children was 27.1 months compared to 99.1 months for the 40 coloured children.) It can only be speculated that the overall shorter follow up of the black children was based on socio-economic factors affecting access to medical care and possible relocations to rural areas amongst other possibilities. The short follow up of the black children limited comparisons between the racial groups regarding outcome.

The 6 South African children with poor outcome (renal failure with or without known death) showed 1 with serum HBsAg negative, the other 5 positive and 2 serum HBeAg positive with the others negative. Those who were HBeAg negative and in failure had very long follow up times. An interpretation of this situation could be that permanent damage had been caused to the glomeruli and other components of the kidney while serum HBeAg was present that subsequent HBeAg seroconversion could not reverse.

This current studies' proportion of HBeAg negativity in association with remission (91%) is similar to the (89%) of the Gilbert et al Cape Town series. [12] Of the 7 with asymptomatic proteinuria in the Gilbert series, 5 were HBeAg negative. The average time from clearance of the HBeAg to remission was 5 months (range: remission 24 months before seroconversion to 31 months after seroconversion). [12]

POOR OUTCOMES IN THE SOUTH AFRICAN GROUP AND POOR OUTCOMES IN CHILDREN WITH HBV MGN IN GENERAL

Of the forty-six South African children, 6 (13%) are known to have had renal failure and/or death from their renal disease. If children followed fewer than 24 months who still had nephrotic syndrome or proteinuria are excluded, (on the grounds that they had not yet had a chance to show whether they would go into spontaneous remission or not) the denominator of the equation reduces to 29 and the proportion of poor outcomes rises to (20.6%) with remissions (48.2%). The poor outcome figure is higher than reported in the literature and the remission figure lower. Two possible contributory factors to this poorer outcome are the more severe renal biopsy involvement in these children, especially prominent mesangial deposits and mesangial interposition and the long follow up ensuring that those developing renal failure many years later are included. (The evidence for the more severe biopsy involvement was presented on pages 6-10 to 6-18 of this chapter.)

Table 6-2 shows the children with HBV MGN with known poor outcome reporting age, gender, race/ethnicity and other available detail on this relatively small group.

Table 6-2 *Childhood HBV MGN – Reported cases of poor outcome including the current series*

Author	Year	No.	Country	Poor outcome	More detail	Ref
Kleinknecht	1979	15	France	1	M – 13 y : 2 y to CRF, 4 y to HD First child with HBV and CRF – though 13y	[40]
Seggie	1984	8	Zimbabwe	1	F - 11 years	[41]
Southwest	1985	11	USA	1	M - Age unknown 105m to develop CRF	[18]
Elidrissy	1988	3	Saudia Arabia	2	M - 3 and 4 y. HBeAg positive. RF within 5 years. High proportion of group of 3. No easy explanation.	[42]
Hsu	1989	52	Taiwan	1	One with renal impairment. (Not certain whether the two Taiwanese series may include the same children.)	[9]
LIn	1990	34	Taiwan	1	No age or gender details. Repeat biopsy showed more severe glomerular sclerosis and interstitial fibrosis.	[43]
Wong	1993	18	Hong Kong	1	Male 3 y at presentation. CRF at 15 treated with peritoneal dialysis	[44]
Gilbert	1994	70	SA Cape Town	2	9 had a decline of more than 25% in GFR. All boys. Age did not seem to be a factor.	[12]
Tomonaga	1996	1	Japan	1 (Not counted as no renal biopsy)	Presented age 12 with GN and HBV. No biopsy done. Became HBeAg negative but renal failure at 29 years. Emphasised 17 year course to RF. Gender not known.	[45]
Bhimma	1998	70	SA Durban	5	No age or gender detail	[17]
Current series	2008	71	SA Cape Town	7	All still HBsAg and HBeAg positive All M. Ages 4,6,8,9,9,10,11. Three of the seven took longer than 19 years to develop renal failure.	This study
Total				22	14 male: 1 female 7 gender not known	

One of the most prominent features of HBV MGN in children is that it has been shown to have a high spontaneous remission rate, confirmed by this present study. It is also acknowledged that renal failure does occur though rarely. This current study has documented the largest group from a single centre with a poor renal outcome implying renal failure or death from renal failure. (Table 6-2)

This total group is in the Table 6-2 above. In summary 22 children 13 years and below with biopsy proven HBV MGN have gone on to develop renal failure or to die of renal failure. The age at presentation and gender information on these patients from the reports is not complete but 14 were noted to be male and one female. One of the two RCCH patients (not reported which of the two) who developed renal failure and one TBH patient (number 81/fj described in appendix Chapter 10 page 4) are known to have had renal transplants. [12] The TBH patient was HBsAg negative at the time of transplant but the RCCH patient was still serum HBsAg and HBeAg positive.

HBV MGN IN SOUTHERN AFRICAN CHILDREN

One of the focus areas of this study has been HBV MGN in Southern Africa and the contribution made by the region to a better understanding of this disease. This history is summarised in Table 6-3 and placed into the broader context by including

Table 6-3 *HBV and MGN*

Landmarks and milestones. The overall history with focus on Southern Africa.

Year	Author	Landmark/milestone	Reference
1963	Blumberg	Discovers Australia antigen (HBV).	[46]
1970	Blumberg	AuAg (HBV) linked to hepatitis. Rates vary in different populations. Moderately high in SA blacks.	[47]
1970	Grobbelaar	Durban SA: High HBV carrier rates in blacks, rare in Asians/Indians and whites.	[48]
1972	Meyers	HBV carrier rates high in Western Cape SA. Highest in blacks, then coloureds and then whites.	[49]
1973	Vos	SA link - higher HBV rate in black renal patients than blood donors – rural and urban factor also plays a role.	[50, 51]
1974	Van Buuren	First HBV MGN child diagnosed at Tygerberg Hospital.	This study
1976	Adhikari	SA series of children with NS. MGN and mesangiocapillary GN patterns frequent in black children. Rare in Indian children. Routine serum HBV testing not yet available.	[52]
1983	Wiggelinkhuizen	Large group (10 of 25 with HBV MGN) in remission linked to serum HBe seroconversion. Mesangial interposition described and illustrated. No HBsAg in glomeruli, HBeAg not tested for. Emphasis that EM needed to distinguish MGN from mesangiocapillary GN.	[16]
1983	Adhikari	MGN most frequent group in black nephrotic children.	[53]
1984	Seggie Zimbabwe	8 children with HBV MGN over 18 month study period. One developed renal failure. Suggests many more undiagnosed - at least 4-6 per year in Zimbabwe.	[41]
1986	Dreyer	Mesangial deposits, subendothelial deposits and mesangial interposition more frequent in HBV MGN. HBcAg and HBsAg staining in glomeruli – some issues with serum correlation	[54]
1987	Wiggelinkhuizen	Remission rate better in coloured than black MGN children.	[15]
1988	Milner	14 black children HBV MGN compared to 45 idiopathic NS – HBV: C ₃ lower, serum AST and ALT raised, all serum HBe positive, 54% HBV-DNA in serum.	[55]
1993	Coovadia	HBeAg in serum of nephrotic child – high specificity for HBV MGN. Biopsy may be unnecessary.	[56]
1994	Gilbert	Large follow up series – 37 of 70 in remission.	[12]
1996	Bates	First pregnancy reported in a female who had had HBV MGN.	[57]
1997	Bhimma	HBV MGN frequent in KwaZulu/Natal children with nephrotic syndrome; only reported in black children.	[58]
1998	Bhimma	70 black HBV MGN children documented in more detail. Remission 12: Poor outcome 5.	[17]
1999	Van Buuren	Namibian children HBV MGN 25 cases -1975-1988. 37% of childhood nephrotic series. HBeAg glomerular positivity in subepithelial location. Mesangial deposits, subendothelial deposits and mesangial interposition characteristic of HBV MGN.	[59]
2002	Bhimma	Interferon shown to accelerate HBe clearance and remission in black SA children with HBV MGN.	[35]
2003	Bhimma	HBV vaccination 1995 reduced hospital incidence of HBV MGN in children within 6 years.	[60]
2011	Bates	HBV MGN in children and adults - similar glomerular morphology but better outcome in children. Fewer mesangial deposits and age under 6 had better outcome. Long term follow up needed to identify renal failure.	Current study

a few of the general landmarks in the HBV MGN saga. Table 6-4 collates the information from the Southern African series. The 358 children with biopsy proven HBV MGN from Southern Africa constitute one of the largest groups from any region of the world. This group constitutes 36% (358/989) of the childhood HBV MGN patients reported by 2010 including the current series. (Figure 7-2 in the following chapter displays the total numbers of childhood and adult HBV MGN cases reported to 2010) Table 6-4 highlights that MGN constituted between 4.5% and 39% of the various series of nephrotic/proteinuric children from Southern Africa. The dates when these series were collected or reported is also noted as both the time and location of the series have influenced the ratios. For example concerning different areas; between 1986 and 1996 the MGN frequency in NS in black children in Gauteng was 13% compared to 40% in black children at the same time in KwaZulu-Natal. [17, 58, 61] Concerning time variation, post widespread HBV immunisation that rate of 40% in KwaZulu-Natal has fallen significantly. [60] Where ethnic details are available from regions, black children have the greatest proportions of MGN in childhood nephrotic syndrome/proteinuria series with the black African subgroup in KwaZulu-Natal showing an MGN proportion of 40% during that study period compared to 0% in the Indian and coloured children. [17, 58] The total number of coloured/mixed race children of the 320 with HBV MGN was around 89 (28%). This number is derived from 40 from this study (Tygerberg Hospital), 1 from Bloemfontein and 48 from the Red Cross group of 70. (Note the final Red Cross report did not give racial numbers but the 68% proportion of the original first 25 was applied to the group of 70 as a whole to give an estimate.) [12, 16, 62]

Table 6-4 Nephrotic syndrome and HBV MGN in Southern African children (1969-2005)

Country Centre	Study period Years	Nephrotic cohort No. race	MGN		HBV MGN		HBV MGN	HBV MGN	HBV MGN	Ref	
			No. NS	(%)	No. of MGN group	(%)	M : F No. male (%)	Remission	Renal failure No. (%)		
South African											
Kwazulu-Natal Durban	20 1976-1996	236 black	94	(40)	81	(86)	51/81 (63)	12	5	(6)	[58]
		286 Indian	7	(2,4)	0	(0)					
		23 colour	6	(26)	0	(0)					
		545 total	107	(20)							
	1997-1999 Treatment study					17		10			[35]
	2003 Vaccination							101/119 (85) ^a			[60]
Gauteng Joburg	10 1986-1996	720 black	97	(13)	87	> (90)	± 58/87 (67)	No follow up	No follow up		[61]
Pretoria	11,5 1985-1996						2,86:1 for < 10 years				
Western Cape Cape Town Red Cross	21 1969-1990	559 all races	92	(16)	70	(76)	59/70 (84)	37	2 (3)		[12]
Bellville Tygerberg	31 1974-2005	242 all races	50	(21)	46	(92)	35/46 (76)	14	6 (14)		This study
Freestate	4 1996-2000	57 black 3 colour 6 white 66 total	2 1 0 3	(3,5) (33) (0) (4,5)	2 1 0 3	(100) (100) (0) (100)	1/3 (33)		No follow up		[62]
Namibia	14 1975-1988	67 black	26	(39)	25	(96)	22/25 (88)		1 (4) Minimal follow up		[59] This study
Zimbabwe	18 months Published 1984	23 black	8	(35)	8	(100)	4/8 (50)	5	1 (12.5)		[41]
Total	Range from 18 months - 31 years 1969-2005	2222	383	(17)	320	(84)	230/320 (72) ^c 280/358 (78) ^b	78/337 (23) ^d	15 (5%) of HBV MGN cohort: Could be (10-15%) with follow up		

^a These are the gender proportions from this 2003 article by Bhimma et al [60]

^b This is the gender ratio using the Bhimma et al 2003 article which has the largest summary number of childhood HBV MGN patients from their group and raises the overall proportion to 80% male in childhood HBV MGN in the overall total of 361 patients from Southern Africa (1974-2005).

^c The gender proportion using the 1987 Nephrotic Syndrome in children publication. [58] Not used in other calculations

^d Remission rate including the Bhimma et al 2002 treatment series. [35] Not used in other calculations.

The difference in frequency of HBV MGN as a cause of nephrotic syndrome between black children from different regions is also striking, ranging from 3.5% in Bloemfontein to just below 13% in Gauteng but 34% in Kwazulu/Natal, 35% in Zimbabwe and 37% in Namibia. The differences in carrier rate among the different populations of for example 1% in Soweto in 1986 compared to 13% in Namibia (1984) and 10-18% in Kwazulu (1988) must be a major factor. [63-65] Column 5 in the table emphasises that HBV is the most frequent (84%) association with MGN over the study period. The male predominance (72% or 78%) is highlighted in column 6. Some of the studies were short term and others have no outcome data therefore making both the remission rate (23%- column 7) and poor outcome proportion (5%-column 8) likely to be considerably underestimated. Using Gilbert et al's comprehensive remission data, the remission rate is likely to be at least 60%. [12] Using this current study's long term follow up with a poor outcome rate minimum of 6/46 (13%) in the SA children (all coloured children likely to have a better outcome than black children) [15], the number with chronic renal failure may be closer to 10% to 15% of the total group of Southern African childhood HBV MGN.

Evidence from the two short term studies in neighbouring Namibia (13 years) and Zimbabwe (18 months) strongly suggests that many more children in those 2 countries developed HBV MGN between 1973 and 2005. Recent publications noted that Namibia had still not begun a comprehensive HBV vaccination programme by 2008 while Zimbabwe was documented as having begun in 1994 with 90% coverage in 2005. [66] HBV MGN is likely to be an ongoing disease in children in Namibia and any other similar African or indeed any other country with high HBV carrier rates until at least 5 -10 years after a well established effective universal HBV vaccination programme. [60, 66]

A Global Alliance for Vaccines and Immunisation (GAVI) was launched in 2000. [66]

This is an association between national governments, UNICEF, WHO, the Bill and Melinda Gates Foundation, the World Bank, vaccine manufacturers, non-governmental organisations and research institutes. This fund supports HBV vaccination and is available only to the poorest countries. Many African countries are GAVI eligible and most have implemented HBV vaccination programmes whose coverage is being monitored, an encouraging development concerning the frequent severe consequences of chronic HBV disease of cirrhosis and liver carcinoma as well as our focus, the rarer HBV MGN. [66]

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Chapter 7

DISCUSSION OF COMPARISON

As highlighted in the results, these 3 groups, the study cohort of childhood HBV MGN and the 2 comparative groups, showed numerous differences as well as a smaller group of similarities. In addition one of the most interesting findings was that usually 2 of the 3 groups were similar but the outlier varied, often being the childhood HBV MGN group or the idiopathic MGN cohort but never the adult HBV MGN group. Put simply, where the 3 were not similar, the childhood HBV group always differed from the adult idiopathic MGN cohort. This finding supports the overall hypothesis of this thesis that HBV MGN in childhood differs substantially from idiopathic MGN and in particular adult idiopathic MGN. The adult idiopathic group in this study is also representing idiopathic MGN in general, including the childhood form as there were not sufficient childhood idiopathic MGN cases seen at our centre to constitute a comparative group. This aspect of the comparison is known to have limitations as acknowledged in numerous places in this study and partially compensated for by comparisons with childhood idiopathic MGN from the literature. [1, 2]

DEMOGRAPHIC, CLINICAL AND LABORATORY FEATURES

Of the 20 features compared in this section, 4 did not show significant differences namely oedema, hypertension, total protein and cholesterol. (Table 5 -1) Four parameters showed a tendency to differ; gender, HBeAg in the serum, ascites and urea.

Twelve showed significant differences but with notable variation. With regard to five categories the two HBV groups were similar and differed from the idiopathic group – race, haematuria, serum AST and ALT levels, as well as serum C₃ levels. These then have been demonstrated to be distinctive features of both adult and childhood HBV MGN in comparison to idiopathic MGN. Of the remaining seven, age differences were part of the definitions to create groups but the two adult groups differed significantly. Haemoglobin and creatinine would be expected to be lower in children than adults as they were, and the differences were compounded in the case of creatinine by the higher frequency of renal failure in both of the adult MGN cohorts. The proteinuria and albumin differences were not easy to understand or explain and are further explored in the discussion on page 7-10. Lowered levels of C₄ were found in the childhood HBV MGN group but almost never in the 2 adult groups. This is an original observation further dealt with on page 7-12 of this discussion. Finally, the serum HBsAg differences were part of the definition of the 3 cohorts.

DEMOGRAPHIC PARAMETERS

Comparing the 2 adult groups, the idiopathic group with a mean age at presentation of 38.4 years was significantly older than the HBV group mean of 25.7 years. Idiopathic MGN, although seen throughout adult life has a peak incidence in the 4th and 5th decades, from 30 – 50 years. [3] The group of adult idiopathic MGN in this study with a mean age of 38.4 years (range: 13 – 74) fits this usual pattern.

The HBV adult group had a mean of 25.7 years (range: 14 – 49). Five out of 12 of this group (42%) were below the age of 20 years compared to just 2/33 (6%) of the adult idiopathic group. The Hong Kong series of 21 adults with HBV MGN had a mean of 30 years (range: 15 – 53 years). [4] The cohorts in this study confirmed the age ranges, mean ages and difference between the two groups of adults with MGN;

the younger HBV group and the older idiopathic group. This age difference reflects the earlier age of exposure of the HBV MGN adult cohort to the causative serum antigens HBe and HBs than the generally unknown antigens responsible for idiopathic MGN.

Males predominated in all three groups with a tendency for the HBV MGN groups to show a higher proportion than the idiopathic group. It is well accepted that males are more commonly afflicted in adult idiopathic MGN. [3] Three large databases report the proportion of males as 66%, 72% and 62%. [5] The proportions in the series of Ehrenreich and Churg 1968 (which included 6 children under 15) were 36 males to 24 females - 60%:40%. [6] This appears to be a reasonable summary of a number of series. The comparative series in this study showed an average figure of 64% males in the adult idiopathic MGN group.

The study childhood HBV MGN group showed a higher male dominance at 80%. This proportion is borne out by the other large children's series as noted in the previous childhood HBV MGN comparative description. The overall male proportion in the 358 Southern African children with HBV MGN was 78%. (Table 6-4) The Johnson review does not seek to explain or even speculate further on this issue and no evidence has been reported since to clarify this finding beyond the higher male carrier rates. [7, 8]

The adult comparison HBV MGN group in this study had 11/12 males (92%), an even greater preponderance than in the childhood group (80%). The Hong Kong series had 17/21 males (81%). In the discussion in this Lai et al Hong Kong article as was noted in the literature review on page 2-48, the statement is made 'males are less apt to predominate among adults than children with this disorder.' as part of the discussion about HBV MGN in adults on page 1461 of their report. [4] In this author's assessment there was no evidence provided for this statement, their own series had

a male proportion of 17/21 (81%) and the present conclusion is that the available evidence from their own report, this study and the literature supports a similar male preponderance around 80% in adult HBV MGN groups as in childhood HBV MGN. (See Fig 7-1 where the proportion is shown; 194/232 (84%). In the Lai et al Hong Kong publication on the following page 1462, they themselves stated that ‘We found that the sex ratio among adult patients with HBV-related membranous nephropathy was similar to that among children.’ [4]) As noted in the results of this current study, when the two HBV MGN groups of the present study were combined and compared to the adult idiopathic cohort, (Fig 5-5) the gender tendency became statistically significant with male dominance clearly greater in the HBV groups compared to idiopathic MGN.

A recent Chinese study also noted this significantly greater male predominance between adult groups of HBV MGN and idiopathic MGN. [9]

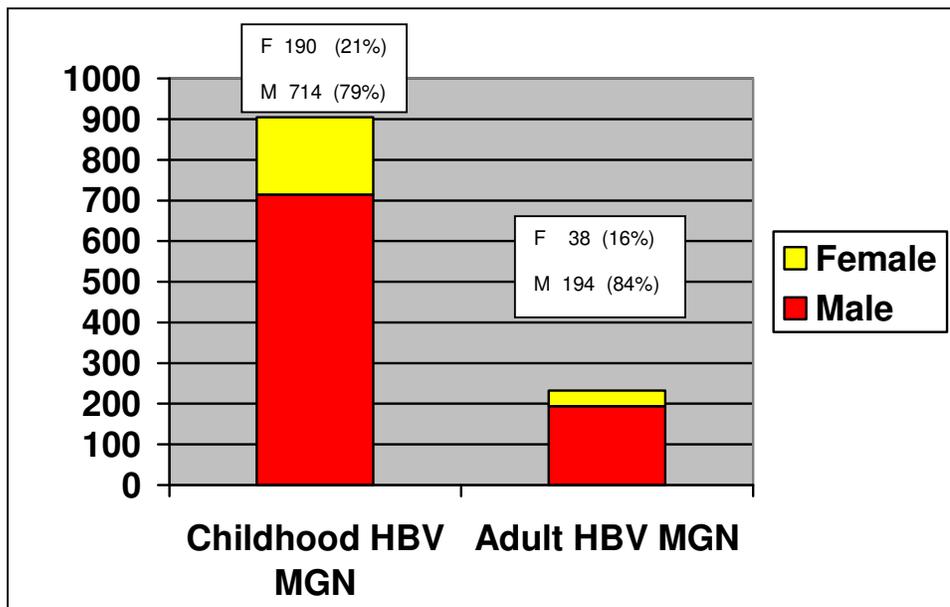


Fig 7-1 Gender proportions of all HBV MGN cases reported 1971- 2010 including the current series

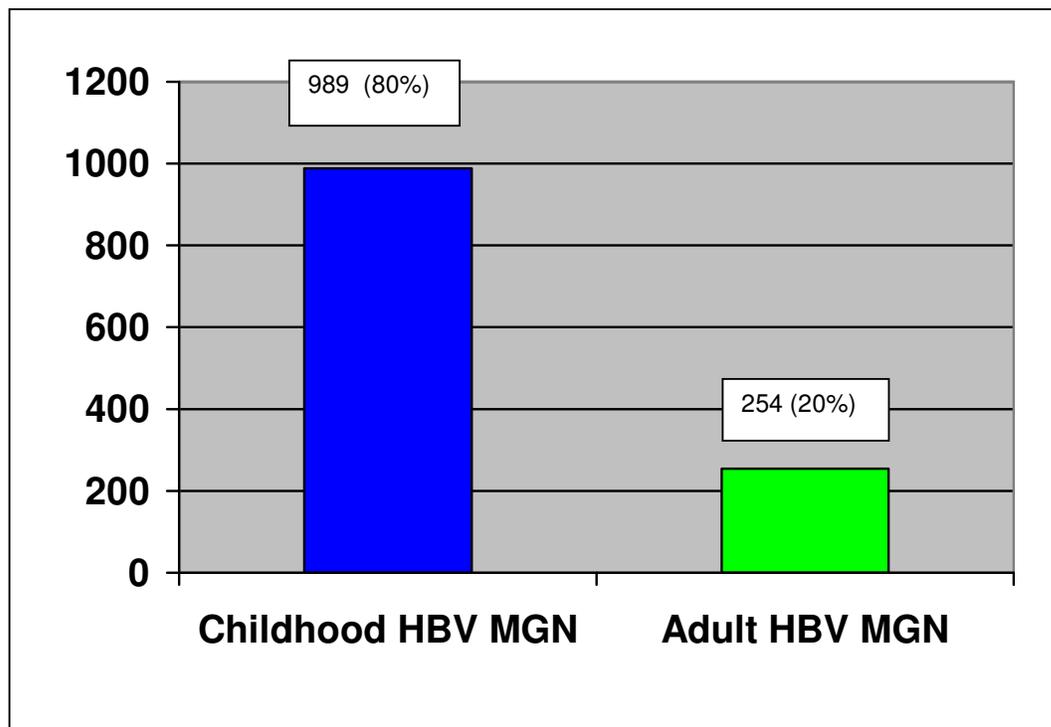


Fig 7-2 Total numbers of HBV MGN patients reported 1971 -2010 including this series n=1243

Some emphasis has been placed on demonstrating the similar male predominance of HBV MGN in adults compared to children as the contrary has been repeated in the literature since the statement first appeared in the Lai adult series in 1991, although they themselves had high male predominance of 81% in their own adult series. [4] For example it appears in Table 3 of an in depth review of Hepatitis B Virus-associated Nephropathy by Bhimma and Coovadia in 2004. [10] The table compares and contrasts a spectrum of differences in clinical presentation of HBV-associated nephropathy between children and adults from their experience and the literature. [10] This table of Bhimma and Coovadia has been expanded and further developed with the data of this current study and the intervening years of world literature as Table 7-4 near the end of this discussion. (Page 7-34)

There are statistically significant differences between the racial compositions of the three MGN cohorts in this study. The two HBV MGN groups contained no white patients while 30% of the idiopathic MGN group was white. The figure of 30% reflects approximately the proportion of white patients seen at Tygerberg Hospital during the study period. The absence of white patients in the HBV groups reflects the very much lower HBV carrier rates in this group in the Western Cape and South Africa . [8, 11]

The differences in the proportions of black and coloured patients in the different groups are confounded by a number of factors. The adult group of 12 HBV MGN had 7 (58%) black patients. This reflects the higher carrier rate among black adults than coloured adults outweighing the greater coloured population in this hospital's (TBH) drainage area. [8, 11]

The children's group had in addition to 46 SA children, 25 Namibian children who were all black and therefore increased the black proportion. The SA children showed a ratio of 40 coloured to 6 black – 87%:13%. Some racial issues concerning the children were addressed in the discussion on childhood HBV MGN. (Pages 6-5 and 6-6)

CLINICAL AND LABORATORY FEATURES

Serum HBs and HBe ANTIGEN and ANTIBODY STATUS

The groups were defined by their serum HBsAg status and therefore these differences were part of the study design. The difference between the serum HBeAg status of the childhood (87%) and adult (60%) groups, although not reaching statistical significance (p value = 0.07), were of interest. A review of many series,

predominantly children, stated that 60 – 80% of patients had serum HBeAg and the remainder usually had anti-HBe antibodies in their serum. [7] It was seen in this study that the younger children (≤ 5) had a significantly higher rate of HBeAg positivity (100%) than the ≥ 6 year group (74%). (Table 4-10) There appears to be a pattern of higher HBe positivity in younger HBV MGN patients decreasing with age into adulthood.

The Hong Kong adult group reported a serum HBeAg positivity of 17/21 (81%). [4] This group had set the requirement for the study cohort that HBV antigens be demonstrated in the glomeruli which may have increased the likelihood that HBeAg was still in the serum of their patients when tested.

CLINICAL PRESENTATION

The patients in all three groups had the features of nephrotic syndrome / severe proteinuria with no significant differences in the degree of oedema, hypertension or total protein and cholesterol levels. Variation in the degree of haematuria at presentation provided one of the most notable differences in clinical presentation. Haematuria was one of a group of features where the 2 HBV groups were similar (Mean ++ blood on labstix = score of 3) and differed significantly from the adult idiopathic cohort (Mean + blood on labstix = score of 2). The differences are better appreciated with more detail as reported in Table 5-1. In the childhood group, only 4/71 (6%) showed no blood on labstix while 3/71 (4%) showed a trace. In the adult HBV group all patients had at least some degree of haematuria with 1/12 (8%) showing a trace. In the idiopathic group, 11/33 (33%) had no blood while another 3/33 (9%) showed a trace. Johnson et al suggested that children present typically with microscopic or rarely macroscopic haematuria. [7] Gilbert documented figures of 50/70 (71%) for micro-haematuria and 15/70 (21%) for macro-haematuria. [12]

The summary situation from the literature regarding idiopathic MGN is that microscopic haematuria is seen in varying proportions of patients (range, 25% to 85%) but gross haematuria is less common (range:1– 17%). [3]

In summary in this current studies' cohorts, most patients in the HBV groups – 50/71 (70%) in the children and 9/12 (75%) in the adults showed prominent haematuria (++, +++ or ++++ on labstix) compared to 16/33 (48%) in the idiopathic group with correspondingly smaller HBV groups, (10% or less), showing no or only a trace of haematuria on labstix while a more substantial group, around 40% of the idiopathic MGN patients, showed no haematuria or only a trace. The reason for these differences is uncertain but the higher frequency of mesangial and subendothelial deposits, mesangial interposition and mesangial proliferation in the HBV MGN groups may play a role.

As noted in the introduction to this section, it was to be expected that almost all of the patients would have oedema so that to find no statistical differences between the groups was not surprising. The series of Gilbert documented oedema in all 70 HBV MGN children. [12] There was however a statistical tendency for the idiopathic group to show ascites less frequently in the current series. Like oedema, ascites is a soft feature, especially in a retrospective study relying, in the adult groups at least, on numerous observers working without standardized definitions. The Gilbert et al figure on ascites was 50/70 (71%) in their childhood HBV group. [12] Ascites is not specifically mentioned in the Hong Kong adult HBV MGN group. [4] The validity and reliability of this apparent difference is uncertain. In summary, oedema occurs in almost all the patients and ascites in 20 – 70% of all of the patients in these 3 MGN cohorts.

Hypertension was present in all 3 groups, affecting between 35 – 67% of patients and not showing statistically significant differences. This is in line with other series. [3, 4, 7]

All 3 groups showed a mean around 3+ for proteinuria on labstix. The differences between the groups are statistically different (Childhood HBV MGN vs Adult idiopathic MGN Bonferroni $p = 0.03$) but it is uncertain what the significance and meaning of this finding may be.

The childhood group's mean haemoglobin is significantly lower than both the adult groups in keeping with the physiological expectations of age. The adult means are near the lower limits of normal, not surprising as many patients were already chronically ill with degrees of renal failure. [3]

RENAL FUNCTION

The childhood HBV MGN study group showed a statistically significant much lower mean for serum creatinine than the two adult groups. At least two factors accounted for this. Children are known to have much lower creatinine levels which rise with increasing muscle mass and more of the adults presented with degrees of renal failure than the children. The childhood creatinine levels were dealt with more fully in the childhood HBV MGN section.

Only 1/71 (1.5%) of the childhood HBV MGN study group had a creatinine level above 120 micromole/L on presentation, reflecting renal failure, although 5 (7%) had raised age adjusted levels, while in the adult groups presentation with raised creatinine was more frequent. In the adult HBV MGN group 3/12 (25%) with values of 179 - 504 micromole/L presented with raised values, very similar to the idiopathic group with 8/33 (24%) with individual values ranging from 135 – 725 micromole/L.

The review of Johnson and Couser summarised that from the data to 1990 only rarely do children with HBV MGN manifest renal insufficiency at presentation. [7] The study cohort of childhood HBV MGN with 1.5% renal failure at presentation fits this description. In the Hong Kong adult HBV series, two patients were initially referred for investigation of chronic renal failure and 5/21 (24%) in total showed raised creatinine at presentation. [4] This current adult HBV MGN series with 25% raised creatinines is similar. Schwartz collated data from 8 series and stated that approximately one half of idiopathic MGN patients have abnormal serum creatinine when MGN is initially detected. Therefore the figure of 24% of patients with idiopathic MGN with raised creatinines in this study is in line with the literature. [3, 13] These findings correlate well with the increased glomerular sclerosis and interstitial damage present in the renal biopsies of the adult groups compared to the childhood HBV MGN study cohort. (Table 5-3)

TOTAL PROTEIN, ALBUMIN AND CHOLESTEROL

The three groups showed similarly reduced total protein levels. This reduction largely reflects the reduced serum albumin levels which are an integral part of the nephrotic syndrome/severe proteinuria with which most of these patients presented.

The serum albumin level means were all severely reduced but the childhood group was significantly lower. A confounding variable was introduced into the study which may have influenced these figures. In the children immuno-electrophoresis was usually done to get a 'more accurate' albumin level and these often seemed even lower than those done 'routinely' at similar times. The immuno-electrophoresis values were usually used as the childhood HBV albumin level. What is clear is that all that all three cohorts showed low mean albumin and total protein levels. The 3 groups all

show similarly raised levels of serum cholesterol as would be expected in severe proteinuria/ nephrotic syndrome, the clinical syndrome affecting all of the patients.

LIVER ENZYMES - AST (Aspartate-aminotransferase) and ALT (Alanine-aminotransferase)

The 2 HBV groups both displayed raised serum levels of AST, with a mean about 50% above the upper limit of normal at 57 U/L for the children and a mean of 69 U/L, 70% above the upper limit of normal for the adults. As expected the idiopathic MGN group showed a normal range mean of 23.9 U/L (one value above slightly above normal) significantly lower than both the HBV group's values. Attention is drawn again to the unusual Durban, KwaZulu/Natal, SA series where the mean serum AST level in 25 children with idiopathic MGN was not significantly different from the mean in the 70 HBV MGN children. (42.16 vs 47.54). The HBV MGN group did show the higher mean and the normal range was not reported for comparison. [1] This situation has not been reported by any other group. In the discussion of this data this finding was not specifically addressed. The finding implies there was some other liver disease in at least some of the idiopathic MGN patients including the possibility of medication side effects. It may also have been that the mean of the liver enzymes were influenced by a few very high values.

The serum ALT results in the current study were similar to the AST with both HBV groups raised and significantly different from the idiopathic group with normal values namely a mean of 18 and no raised values. The Hong Kong adult HBV group showed 3/21 (14%), with raised ALT levels. [4]

HYPOCOMPLEMENTAEMIA

Both HBV MGN groups had lowered C_3 significantly more frequently (63% children and 50% adults) while only 13% of the idiopathic group demonstrated this feature ($p < 0.01$). Johnson summarized the situation in children noting that in most studies, serum C_3 and C_4 levels had been depressed in 15 – 64% of cases although some series had found complement to be normal. [7] Information on adult HBV MGN and complement is not prominent. The comparative series from Hong Kong on SLE MGN and HBV MGN gave a mean C_3 value of 970 mg/L for 22 cases of HBV MGN. This was contrasted with the significantly lower mean of 511 mg/L of 26 SLE MGN. The authors failed to comment on this observation and it is uncertain whether the HBV cases were in the normal range of their laboratory. In their study it was emphasised that the SLE group's levels were significantly lower. [14] Complement levels are standardized in each laboratory and therefore difficult to compare. (Personal communication N. Nel Local Immunology Laboratory Manager.) Schwartz in the recent review does not mention lowered levels of serum C_3 and C_4 in the clinical summary on idiopathic MGN. [3] The childhood HBV MGN group also had the most frequent low levels of C_4 22/65 (34%) compared to the 2 adult groups, but less so than with C_3 . The adults with HBV MGN showed reduced serum C_4 levels in 0/12 (0%) and 1/30 (3%) idiopathic MGN, therefore almost always normal. The Hong Kong HBV MGN 22 showed a mean of 229 mg/L for C_4 , presumed within normal limits as it is not specifically mentioned in the discussion. [14]

This difference of C_4 levels between adults and children with HBV MGN in the current study may suggest a different pathway of complement consumption reflecting a different GN mechanism between adult and children with the disease.

RENAL PATHOLOGY COMPARISONS

The combined group of 116 renal biopsies from the primary study group of HBV MGN in childhood (n=71) and two control groups: Adult HBV MGN (n=12) and idiopathic MGN (n=33) were the central element of this dissertation. The establishment of a diagnosis of MGN characterised by widespread subepithelial deposits on ultrastructural examination made the renal biopsy and patient eligible for the study or comparative groups and the parameters evaluated in the biopsies were the central feature of the descriptions and clinico-pathological correlations. [3, 15]

LIGHT AND ELECTRON MICROSCOPY

GLOMERULI

The mean number of available glomeruli for evaluation, between 19 and 28, were quite acceptable by international standards and did not differ significantly between the groups. (Table 5-3) An acceptable number would be 10, preferably 20 glomeruli in a renal biopsy but in a diffuse disease such as MGN even fewer than 5 could be acceptable provided ultrastructure is available and performed. [16] This latter situation may enable a categorisation to take place (eg MGN) but if there is little cortex available for study the reliability of assessment of interstitial disease could be compromised.

Medical renal biopsies are one of very few biopsies routinely subjected to ultrastructural study. [17] The detailed study of renal ultrastructure in these three cohorts revealed interesting and significant differences as well as some similarities.

Subepithelial deposits

Stage II-III subepithelial deposits on ultrastructure represented the mean, or at least close to the mean, in all 3 of these groups and there was not a significant difference. As noted in the childhood HBV MGN discussion regarding the stage of subepithelial deposits, SA series such as the current one and that of Gilbert et al show more advanced MGN stages by comparison with the Taiwanese series of Hsu et al. The adult HBV MGN group in this series is similar to the SA children with HBV MGN in this series but comparison is limited as the Hong Kong adult series did not report the stage of subepithelial biopsies. [4, 12, 18] The proportion in each group in the current study showing stage IV changes, (lucent areas), was childhood HBV 5/71 (7%), adult HBV 1/12 (8%) and idiopathic 9/33 (27%). Ehrenreich and Churg reported 12 in stage I (18%), 26 in stage II (39%), 26 in stage III (39%) and 3 in stage IV (4%). [6] This suggests some of the patients in this study may have been biopsied later in the course of disease than the worldwide mean, probably having been symptomatic for many months and even years. It may also suggest that the disease in the SA setting seldom resolves before the spike and incorporation stage but that is difficult to prove in a situation without frequent rebiopsies. As noted previously, the later stage of subepithelial deposits correlated with poorer outcome in childhood HBV MGN in this study. (Table 4-13) It is not yet resolved from reported series overall whether the stage of subepithelial deposits is a prognostic factor in idiopathic MGN. [3]

Mesangial deposits

Mesangial deposits were more frequent and more severe in both of the childhood and adult HBV MGN groups than the idiopathic MGN group. This is a very important category of difference, not only between idiopathic MGN and HBV MGN in general

but also possibly between HBV MGN in different regions of the world including different age, gender and race groups. Evidence for this suggestion from this study is that the extent of mesangial deposits correlates with other pathological changes such as mesangial interposition and glomerular sclerosis and affects outcome adversely. (Table 4-11)

Mesangial deposits are conceptually accepted as the "first" abnormality in the mesangial area which then help "stimulate" mesangial proliferation and contribute to mesangial interposition together with the subendothelial deposits. The differences in outcome between, for example age, gender and race groups, need to be mediated through some different effect in the glomeruli and/or other areas of the kidney and the frequency and extent of mesangial deposits seems at least one likely candidate. In idiopathic MCGN, for example mesangial deposits also have a strong association with poor outcome. [19]

The Hong Kong adult HBV MGN group showed (50%) mesangial deposits but qualified this finding by adding that the deposits were usually small and scattered. [14] (Without directly comparing the biopsy material it is difficult to draw firm conclusions but the description of the deposits in the adult Hong Kong biopsies suggests the mesangial deposits of this current series' adult HBV MGN patients were probably larger and more severe.)

Mesangial electron-dense deposits are uncommon in idiopathic MGN. [20-22] Schwartz emphasises that the presence of mesangial deposits suggests that the patient has the membranous form of SLE or some other secondary form of MGN. [3] In their unpublished series of 64 patients with primary MGN, in whom the diagnosis of SLE was excluded clinically and serologically, 6 patients (9%) had small mesangial electron-dense deposits. [3] When mesangial electron dense deposits are seen in patients without clinical or serological evidence of SLE, lupus may still become

manifest after a latent period of several years, [21] but Shearn et al [22] found mesangial deposits in 9 of 107 cases (8.5%) of MGN in which SLE was not detected during a follow up period of mean, 9.8 years (range 2 to 16.5 years). In a series of 53 children with idiopathic MGN followed for a mean of 53 months (range, 2 to 167 months) without evidence of SLE, the Southwest Pediatric Nephrology Study Group [21] reported an incidence of mesangial electron dense deposits of 31%. This is a higher incidence than in most reported adult series and is unexplained since lupus and hepatitis B antigenaemia, two diagnoses associated with mesangial electron dense deposits, were specifically excluded.

This series is of relevance to the present study as there is not a comparative group of idiopathic MGN in children in this study. (Only 2 children with idiopathic MGN have been seen in this hospital in the study period.) It may be argued that the idiopathic MGN group in adults is not a valid comparison group for HBV MGN in children because of, for example, this difference between idiopathic MGN in adults and children. As noted in these findings, however, all the childhood HBV MGN renal biopsies (100%) showed mesangial deposits and the majority were either moderate (46%) or severe (23%). Therefore even if childhood idiopathic MGN tends to show more mesangial deposits than adult idiopathic MGN, childhood HBV MGN still displays significantly more mesangial deposits than idiopathic childhood MGN.

Mesangial proliferation

There were significant differences between the degrees of mesangial proliferation present, with the 2 HBV groups showing more frequent and severe proliferation than the idiopathic group. Ehrenreich and Churg stated that the fundamental and only significant change in membranous nephropathy was thickening of the capillary walls in the glomeruli. Only rarely were other changes seen such as mesangial widening or

hypercellularity of mesangial areas. [6] Schwartz confirmed the continued broad acceptance of this view. [3] Venkateshan et al [23] noted that although the occurrence of mesangial proliferation and deposits in idiopathic MGN was rare, they had been reported in secondary forms of MGN like SLE and HBV. [6, 24] Concerning adult HBV MGN, the Hong Kong group documented mesangial hypercellularity, defined as more than 3 cells per area, in 16/22 (73%). [14] This finding confirms a link between the presence of mesangial deposits in the HBV MGN groups and more prominent mesangial proliferation in these two cohorts than the idiopathic MGN group.

Subendothelial deposits

Although the hallmark of MGN is widespread subepithelial deposits, Yoshikawa et al 1985 noted small subendothelial deposits in (29%) of the renal biopsies of the childhood HBV MGN patients compared to their complete absence (0%) in the childhood idiopathic MGN group. [25] (Table 7-1) The current study also found that the HBV associated MGN groups showed more subendothelial deposits than the idiopathic form; (46%) in the childhood HBV group, (67%) in adult HBV group but only 1/33 (3%) of the adult idiopathic group. In the series of Wrzolkowa et al (1991) the subendothelial deposits were described as less abundant than the subepithelial ones, frequently taking the form of small elongated concentrations. They were not, however, further quantified. [26] Dreyer noted (23%) subendothelial deposits in adults and children with HBV MGN. [27] The first Lai et al Hong Kong report on HBV MGN in adults only mentioned that the ultrastructural features included subepithelial deposits with no mention of the presence or absence of subendothelial deposits, mesangial deposits or mesangial interposition. [4] A later (2000) contribution highlighted that the secondary forms of MGN associated with HBV and SLE share

many morphological features, at both light microscopic and ultrastructural level. Subendothelial deposits were seen in (68%) of the Hong Kong HBV MGN series but were described as usually small and scattered, unlike the broad, heavy subendothelial complexes seen in proliferative lupus nephritis, with which they were being compared. [14] Concerning idiopathic MGN, subendothelial deposits are very rare or non-existent and not mentioned in the ultrastructural description of MGN. [3] The finding of 1/33 in this study showing a mild degree of subendothelial deposit confirms its rarity.

Mesangial interposition

Mesangial interposition is a prominent feature of the HBV MGN spectrum. In this study in both adults and children with HBV MGN about 50% showed moderate or severe mesangial interposition with no patients with idiopathic MGN in these categories. Although both the moderate and severe categories of mesangial interposition show 'mesangiocapillary' features, the ones with severe changes, childhood HBV (32%) and adult HBV (33%) appear at light microscopic level usually strongly like MCGN and one might predict they would constitute a distinct clinical and outcome subset. The adult HBV MGN series from Hong Kong described 6 (27%) of 22 cases as mixed MGN-mesangiocapillary GN without defining clearly the criteria for inclusion in the category. It is uncertain whether there were features of mesangial interposition in other HBV MGN cases in their series. There were clearly other features not expected in idiopathic MGN in more cases than these 6 as 15/22 (68%) showed subendothelial deposits and 11/22 (50%) had mesangial deposits as well. [14] In the current study the biopsies with severe mesangial interposition have defined a group called mixed HBV MGN-mesangiocapillary GN which includes 23/71 (32%) of the HBV childhood cohort, 4/12 (33%) of the adult HBV group but no

patients from the idiopathic MGN cohort. The proportions of 32% and 33% are similar to the Hong Kong 6/22 (27%) proportion. [14] Another group who used the term mixed HBV MGN-Mesangiocapillary GN (again not specifically defined) was the Venkateshan et al group who also noted transformation from what they described as MGN to mesangiocapillary GN in the rebiopsies of one child and one adult with HBV GN. [23] In idiopathic MGN, mesangial interposition would not be expected and is not mentioned in a review on MGN. [3]

Table 7-1 includes the features from the three groups in this study together with the Yoshikawa findings in HBV MGN in children and idiopathic MGN in children. Differences regarding the HBV MGN cohorts include more prominent mesangial deposits in these SA series, more frequent and severe interposition and more advanced stages of subepithelial deposits overall in the local HBV MGN renal biopsies compared to the Japanese group of HBV MGN children. The idiopathic MGN groups are both characterised by the rarity of mesangial deposits, subendothelial deposits and mesangial interposition.

Glomerular sclerosis

One of the most striking differences between the childhood HBV cohort and the two adult groups was the degree of glomerular sclerosis. This is an original and significant finding as glomerular sclerosis is an end point of many pathological processes and is a marker of adverse prognosis. [3] Sclerosis was similar in the glomeruli of the two adult groups; HBV MGN (mean 24.3%) and idiopathic MGN (mean 17.0%) while the childhood HBV MGN group by contrast, had a mean of 4.5%, and the majority of renal biopsies (68%) showed no glomerular sclerosis at all. Glomerular sclerosis is not addressed in the large reviews dealing with HBV MGN, predominantly summarising childhood data, reflecting its rarity. [7, 10, 23]

Table 7-1 Comparison of Ultrastructure - Current series and Yoshikawa [25]

Feature	This study		This study		Yoshikawa		This study		Yoshikawa	
	HBV child n=71		HBV adult n=12		HBV child n = 14		Idiopathic adult MGN n=33		Idiopathic childhood MGN n = 9	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Subepithelial Deposits										
Stage I	1	(1.5)	0	(0)	0	(0)	2	(6)	3	(33)
Stage II	7	(10)	2	(17)	8	(57)	11	(33)	2	(22)
Stage III	58	(81)	9	(75)	5	(36)	12	(36)	4	(44)
Stage IV	5	(7)	1	(8)	1	(7)	8	(24)	0	(0)
Mesangial Deposits										
Absent	0	(0)	0	(0)	1	(7)	22	(67)	6/8	(75)
Mild	22	(33)	7	(58)	11	(79) ^a	10	(30)	2/8	(25)
Moderate	33	(46)	2	(17)			1	(3)	0	(0)
Severe	16	(23)	3	(25)	2	(14)	0	(0)	0	(0)
Subendothelial Deposits										
Absent	38	(53)	0	(0)	10	(71)	32	(97)	9	(100)
Mild	26	(37)	4	(33)	4	(29)	1	(3)	0	(0)
Moderate	4	(6)	4	(33)	0	(0)	0	(0)	0	(0)
Severe	3	(4)	4	(33)	0	(0)	0	(0)	0	(0)
Mesangial Interposition										
Absent	8	(11)	2	(17)	10	(71)	26	(79)	8	(89)
Mild	22	(31)	5	(42)	4	(29)	7	(21)	1	(11)
Moderate	18	(25)	1	(8)	0	(0)	0	(0)	0	(0)
Severe	23	(32)	4	(33)	0	(0)	0	(0)	0	(0)

^a 11 described as mild and moderate in one subgroup [25]

Both adult MGN groups have been reported previously to show significant glomerular sclerosis [3, 4, 28] but not in a direct comparison with a childhood HBV MGN cohort from a single centre.

These differences confirm the hypothesis of this study that childhood HBV MGN is a distinct form of MGN, usually with spontaneous remission before a variety of processes of uncertain nature can lead to glomerular sclerosis. What is intriguing

from a morphologist's viewpoint is that the initial glomerular morphology, especially ultrastructural features, of both the childhood and adult HBV MGN groups is similar yet the associated glomerular sclerosis and interstitial damage vary so greatly. This emphasizes that other factors such as the likelihood of spontaneous serum HBsAg and especially HBeAg seroconversion are likely to be involved by reducing the ongoing deposit burden in glomeruli in the childhood group of HBV MGN.

Crescents

Crescents were infrequent and crescentic GN was absent from all 3 groups which is in line with other reports. [29] Small mean percentages of crescents, usually less than 1%, were seen in all three groups (0.52% Childhood HBV, 1.1% Adult HBV and 0.9% Adult idiopathic) and there was not a statistical difference. Using a definition of at least 30% of glomeruli being involved, none of the cases in these cohorts qualified as crescentic GN. At least 3 distinct crescentic HBV MGN or GN cases have been reported in adults but none in children with HBV MGN. [30, 31] Jennette reports that crescents are unusual in MGN unless there are co-existing factors such as anti-GBM antibodies or ANCA which have been well described. [29]

Tubuloreticular inclusion bodies

Tubuloreticular inclusion bodies (TRIBS), usually in glomerular endothelial cells, were found on ultrastructural examination in (87%) of the childhood HBV MGN group, markedly more frequent than the adult groups. The difference between the adult groups [HBV MGN (42%)] and [Idiopathic MGN (12%)] was not statistically significant. The Hong Kong group in their HBV and SLE MGN comparison found 3/22 (13%) in the adult HBV MGN group but far more positive biopsies 19/26 (73%) in the SLE group.[14] Although no attempt was made to quantify TRIBS in the HBV

MGN cases (the presence of at least one made a case 'positive'), in many cases of SLE and HIV associated renal biopsies studied from the 1990s until the present in this centre, TRIBS have been quantified per grid space and are sometimes very frequent compared to the HBV MGN cases where usually only one was found. For example in 67 cases of HIVAN seen in TBH between 1992 and 2008, a mean of 5 TRIBS per grid space was seen per biopsy. The first case of HIVAN seen in our centre in 1992 was identified partly because of a high TRIB count of 18 per grid space on ultrastructure. [32] It is very difficult if not impossible to know whether most ultrastructural laboratories knew about these structures and routinely looked for them. The findings of Ishihara et al in 1988 in a paper entitled 'Ultrastructure of HBe Nephropathy' reported finding tubuloreticular structures in all 3 of their renal biopsies from a male child and two male adults with HBV MGN. [33] This correlates with the findings of this study of the relative frequency of these structures in HBV MGN in children in particular as also reported by Mills et al. [34] These TRIBS are not mentioned in the ultrastructural description of idiopathic MGN in Heptinstall. [3] The present finding of 4/33 (12%) indicates these bodies can be seen occasionally in idiopathic MGN in adults.

Virus-like particles

The childhood group showed virus-like particles in 83% of cases, more than the other 2 adult control groups which were similar, being present in between 30 – 40% of biopsies. The Hong Kong group of Mac-Moune Lai found 4/22 (18%) of these structures in adult HBV MGN patients and 5/26 (19%) in SLE MGN. [14] The consensus appears to be that these structures do not correlate with viral particles but represent unusual deposit patterns. [3]

INTERSTITIAL FEATURES

The frequency and extent of inflammation in the childhood HBV MGN group in this study was significantly lower than in the two adult groups which were similar. Venkateshan et al noted that tubulointerstitial changes were seldom found in HBV MGN, even in cases of long duration. [23] The adult HBV MGN series from Hong Kong noted interstitial inflammatory cells in 45% of cases but the intensity was not defined. [14] In idiopathic MGN interstitial inflammatory cell infiltrates are not a prominent feature. [3] Tubular atrophy, although not prominent in the biopsies of any of the three cohorts, was nevertheless significantly less common in the children. The Hong Kong adult HBV MGN group showed tubular atrophy in 36% of biopsies. [14] Renal tubules atrophy as the glomerular lesion progresses and in advanced idiopathic MGN with glomerular sclerosis and hypertension a significant component of atrophy results from ischaemia. [3] The biopsies of the children with HBV MGN had significantly less fibrosis than the two adult groups. The minimal interstitial fibrosis in the childhood group fits in with overall experience. [23] Concerning idiopathic MGN, in uncomplicated MGN, diffuse interstitial fibrosis tends to occur early without prominent inflammation or tubular atrophy. [3] Blood vessel changes were infrequent in all three study MGN cohorts but rarer in the childhood HBV group. Blood vessel changes are seldom, if ever, mentioned in HBV MGN in children in the literature. [7, 23] Blood vessel differences were not documented in the comparison of HBV and SLE MGN, suggesting they are rarely seen. [14] In idiopathic MGN in adults, Schwartz notes that, reflecting the generally older age of patients with MGN, arterial sclerosis may be seen in larger vessels and that with hypertension arterial sclerosis and arteriolosclerosis may be prominent. [3]

Number of ultrastructural photographs

The mean numbers of ultrastructural photographs taken per renal biopsy of between 11 and 17 confirm that numerous images were taken of all cases in the three groups of renal biopsies studied in order to document ultrastructural features. More photographs were taken of the children but this was the first group studied in detail and was part of the learning curve. The number of pictures taken does not imply that more time was spent scoping the children or that more glomeruli were examined. The numbers do help confirm that all the cases were examined and documented thoroughly and in some detail looking for specific features.

SUMMARY OF LIGHT AND ELECTRON MICROSCOPY

Six of the 7 light microscopic features compared showed significant differences between the 3 groups. In one category, mesangial proliferation, the two HBV MGN groups show the feature more prominently to a similar degree, almost certainly in response to the more prominent immune-complex deposits found in mesangial areas in the HBV MGN biopsies. In five further categories, the two adult group biopsies show significantly more severe damage than the childhood HBV MGN group. These include changes such as glomerular sclerosis, tubular atrophy and interstitial fibrosis, well known to correlate with poorer renal prognosis. [3] These differences have not been documented before in a comparative study of these three groups from one centre.

Regarding ultrastructure, the two HBV MGN groups both show far more mesangial deposits, subendothelial deposits and mesangial interposition than the idiopathic MGN group. These 3 are features of mesangiocapillary GN and confirm that morphologically many cases of HBV MGN in these series share significant features

of mesangiocapillary GN. To accommodate this spectrum, a category of mixed HBV MGN-mesangiocapillary GN has been made for those MGN biopsies also displaying severe mesangial interposition together with other mesangiocapillary GN features.

Virus-like bodies were seen more frequently in childhood HBV MGN than the other 2 groups which were similar. Tubuloreticular bodies were most seen in childhood HBV MGN (87%), next most frequently in adult HBV MGN (42%) and least, but still occasionally, in 12% of idiopathic MGN. The stage of subepithelial deposits was the only ultrastructural category showing statistical differences.

Table 7- 2 Ultrastructure of HBV MGN – Literature summary and this series

Parameter	Idiopathic MGN	Childhood HBV MGN	Adult HBV MGN	References
Subepithelial Deposits	Stages I-IV	Stages I-IV	Stages I-IV	[27] + this study
Mesangial Deposits	Rare	Frequent	Frequent	[14, 23, 25, 27] + this study
Subendothelial Deposits	Rare	Occasional	Frequent	[14, 23, 25, 27] + this study
Mesangial Interposition	Rare	Well documented Much variation	Frequent	[14, 23, 25, 27] + this study
Tubuloreticular Bodies	Not often examined Present but rare	Frequent	Common	This study
Virus-like structures	Rare	Frequent	Occasional	[25] + this study

The stage of deposits in all 3 of our cohorts is more 'advanced' than most of the other series with this kind of detail with which comparisons have been made. This may simply reflect a later time of biopsy in the course of disease but could reflect something more significant such as more severe, prolonged and less reversible disease. In the outcome comparison between the 14 children in remission and the 7 who did poorly, stage of subepithelial deposits was significantly different. (Table 4-13 with discussion in Chapter 6 page 27) In idiopathic MGN some reports have found favourable outcomes related to early stages (I and II) of membranous transformation while several large studies of idiopathic MGN have not reported a relationship between glomerular stage and outcome. [3]

A summary from the literature and this series of the main ultrastructural features of childhood and adult HBV MGN contrasted to those of idiopathic MGN is reflected in Table 7-2 above. (Page 7-25)

IMMUNOFLUORESCENCE

As emphasised previously, the central features of MGN on immunofluorescent staining for involved antigens is the almost universal prominent presence of IgG and C₃ in a granular pattern along the capillary walls in a subepithelial location. [3, 7] The study cohort and both control groups had similar high proportions of positivity for both of these antigens; IgG and C₃ - Childhood HBV MGN (98%) and (82%); Adult HBV MGN (91%) and (91%) and Idiopathic MGN (92%) and (88%) respectively in similar locations as would be expected. In the Lai et al Hong Kong 2000 series of adult HBV MGN IgG and C₃ were both positive in (100%) of their 22 renal biopsies and the adult HBV MGN group in the current SA study in similar. [14]

The findings of the other 3 routine antigens are more variable and difficult to interpret. The children with HBV MGN had lower positivity (56%) for IgA than the two adult groups, which were similar at 82% and 84%. Johnson and Couser reported 10% for IgA. [7] In the Lai et al series of HBV MGN, IgA was positive in 16/22 (73%). [14] (It is not clear what the age range of this group was though the mean was 30 years.) Venkateshan et al documented their mixed group of adult and childhood HBV MGN biopsies in some detail and IgA was noted in 6/10 (60%) – 3/6 (50%) of the childhood group and 3/4 (75%) of the adult HBV MGN group. [23]

In idiopathic MGN, Schwartz summarises IgA to have a range of 17% to 54% with the variation possibly related to the varied reporting of low levels of staining. [3] This statistical difference between the groups in this study for IgA cannot at present be explained. Others have not mentioned it and the range of IgA positivity seems so wide that subjective factors in evaluation possibly account in part for the differences.

In the adult HBV MGN group, IgM was present in 100% of biopsies but less frequent in the idiopathic group (85%) and childhood HBV study group (72%). Johnson and Couser reported 50% positivity for IgM in HBV MGN and Lai IgM positivity of (73%). [7, 14] Venkateshan reported (100%) positive for IgM including 6 children and 4 adults with HBV MGN which varied from 1+ to 3+ on their scoring system. [23] The figure for idiopathic MGN quoted by Schwartz gave 58% as the upper end of the range quoted. It is difficult to explain this series' figures which are generally higher than expected. [3] The Hong Kong and USA series are, however, similar to the current HBV figures. [14, 23]

The 3 groups showed wide variation in the mean positivity of fibrin/fibrinogen which was present in 74% of the idiopathic MGN group (significantly more common than the HBV MGN groups) but the relevance is uncertain. Fibrinogen is not mentioned in Schwartz's detailed discussion of the pathology of MGN [3] and in only (8%) in

Venkataseshan's HBV GN series and (18.2%) in the Hong Kong adult HBV MGN series. [14, 23]

HBV ANTIGENS and ANTIBODIES

The monoclonal antibodies used were directed against HBeAg and were raised to recognize two different antigens of HBe giving two monoclonal antibodies; anti-HBeAg(a) and anti-HBeAg(b). (Institute of Immunology Co. Ltd. Tokyo, Japan) The HBV MGN groups were usually positive for both HBeAg(a) and HBeAg(b); children 75% and 80% respectively and adults 70% for both while the idiopathic MGN group as the negative control showed 0% in 11 biopsies tested. This compares well with 83% reported for the HBeAg IgG2a subclass by Lai et al. [35]

The 2 HBV MGN groups showed similar prominent positivity for the polyclonal anti-HBcAg; children 95% and adults 82% in a predominantly subepithelial location in relation to the expected location of MGN immune deposits. The idiopathic MGN group provided an appropriate negative control with 0/11 – (0%) showing positivity. The important point about this polyclonal Dako antibody showing both anti-HBcAg and anti-HBeAg activity was made in the literature review and childhood HBV MGN immunofluorescent discussion. [35, 36]

Disturbingly, the three groups did not show a significant difference in immunofluorescent staining with the polyclonal anti-HBs antibody. The childhood group had 26/40 (65%) positive, the adult HBV MGN group 7/9 (78%) and the control idiopathic MGN group 6/11 (55%). This was the same Dakopatts polyclonal antibody that others had shown during the study period tended to cross-react. [36, 37] This study confirmed the lack of specificity of this antibody and the serious possibility that

it could cause confusion by staining idiopathic MGN and other forms of GN positively in a patient negative for HBsAg in the serum.

A monoclonal anti-HBsAg (Dako code M3506) stain was performed on three HBV MGN cases and all were negative. Of importance too was that all 11 of the idiopathic MGN cases tested with the replacement monoclonal anti-HBsAg stain were negative. In the Hong Kong series of HBV MGN 8/22 (35%) were positive for monoclonal anti-HBsAg. [14] As mentioned previously, the frequency and precise role of HBsAg in HBV MGN remains elusive and unresolved.

SUMMARY OF IMMUNOFLUORESCENCE COMPARISON

This comparison confirms that the 2 antigens most frequently associated with MGN, namely IgG and C3, were found in more than 90% and 80%, respectively, of the cases of all three groups. The other 3 'usual' antigens tested for, IgA, IgM and fibrinogen, did show differences with IgA less frequent in the childhood group than the other 2 groups, IgM in all the adult HBV MGN biopsies while still frequent in the other 2 groups and fibrinogen markedly more frequent in the adult idiopathic MGN group. It is not clear whether the statistical significance of differences in IgA and fibrin staining are matched by significant differences in pathogenesis or other features. The predominant location and form of this positive staining was along capillary walls in a granular pattern. Varying degrees of mesangial staining were also present.

The HBV antigen testing confirmed the cross reactivity of the Dako Polyclonal HBsAg stain with similar proportions of all 3 groups, 55-78%, showing positivity. (This inherently contradictory situation of apparent glomerular staining in patients negative for HBsAg in the serum has been noted by other workers). [37]

The most obvious differences were seen with the monoclonal HBe antibodies and polyclonal anti-HBc which distinguished the HBV groups from the idiopathic cohort. The monoclonal HBe antibodies from Japan were necessary to clarify the confusion caused by the cross reacting polyclonal HBs antibodies from Dako and highlight the specific mediator of this form of MGN. The Dako anti-HBcAg also caused some confusion as it cross-reacted for HBeAg.

CLINICAL OUTCOME OF PATIENTS

Although the follow up in all 3 of the groups was suboptimal (32%-50% follow up to 4 years), there were significant differences between them. The central observation was that the prognosis was much better in the childhood HBV MGN study group than in the two adult control groups. At 2 years of follow up 25% of the childhood HBV group had shown remission contrasted with none in the 2 adult groups. Renal death or failure were more frequent in the adult groups with HBV MGN (37.5%) and idiopathic MGN (50%) at 2 years compared to (7%) in the childhood HBV MGN group. At 4 years of follow up, remission had again only been seen in the childhood HBV group where 52% had recovered completely. Adverse outcomes of renal failure or death were more marked in the adult MGN groups at 4 years with HBV MGN (67%) and idiopathic MGN (80%); both more frequent than in the children (8.7%). (Table 5-6, Fig 5-42, Fig 5-43) Contrary to the findings in children, spontaneous remission of proteinuria/nephrotic syndrome did not occur in the Lai et al Hong Kong adult HBV MGN series, although proteinuria tended to decrease with time. Complete remission occurred after prednisolone treatment in three patients and after interferon-alpha treatment in one.

There was a slow and relentless progression to chronic renal failure in 29% of patients after an average of six years and 10% of patients required dialysis. [4] The

outcomes of the adult HBV MGN control group in the current study appear more adverse than the above Lai series with no examples of remission and a renal failure/death rate of 37.5% at 2 years and 66.7% at 4 years.

The idiopathic MGN cohort in this present study had a particularly poor outcome compared to the literature where MGN is reported as usually an indolent disease that progresses slowly in most cases so that even when renal insufficiency develops in up to 25%, it takes years to reach end stage. Approximately 25% of patients will experience a complete or partial remission and 50% will suffer no significant loss of renal function during follow up. [3] In this comparative cohort of idiopathic MGN, 12 had an adverse outcome by 4 years with 4 in renal failure and 8 dead. Even if these 12 represent all those with a poor outcome, they constitute 12/33 (36%) of the group and the process has occurred faster than usual. In addition, it seems likely the adverse group is really larger as only 15 were followed to 4 years or had already displayed renal failure earlier. Of the 15, this group forms 12/15 (80%). This adult idiopathic group had a particularly poor outcome making the contrast with the childhood HBV group even more marked. The possible reasons for this poor outcome were not a focus area of this study. The mean creatinine level of the group of 33 adult idiopathic MGN at presentation of 122 micromoles/L and the mean (%) sclerotic glomeruli of 17 appeared within normal ranges for adult idiopathic MGN. This group warrants further study to see whether other factors played a role in this poor outcome. The outcomes of the two adult groups were similar and conformed with their similarities in glomerular sclerosis and interstitial damage, known correlates of prognosis. [3]

Bhimma et al reported a cohort of 24 black South African children with biopsy proven

idiopathic MGN. Although their follow up was limited (2.9 year mean – range 1.5 – 5.2 years), their initial prognosis was good; 22 (91.7%) at last visit had normal renal function and 2 (8.3%) chronic renal failure. [1] The period of follow up was not sufficient to draw firm conclusions for comparison. It may be that both childhood HBV MGN and childhood idiopathic MGN while different in a range of ways to each other, (demographically, clinically and pathologically) sometimes have a similar prognosis with both the childhood forms of MGN having a better outcome than their adult MGN counterparts. [2, 38]

This current study did not have the groups to directly address this issue.

In the childhood HBV MGN discussion, (chapter 6) the patients with poor outcome were tabulated. (Table 6-2) The same is done below for adult HBV MGN. (Table 7-3)

Table 7-3 *Adult HBV MGN – Prognosis: Remission and Poor outcome*

Author	Year	Adult >13 No.	Country	Poor outcome	Reference
Venkatase	1990	4	USA	1	[23]
Lai KN	1991	21	Hong Kong	6	[4]
Taskapan	2000	1	Turkey	1	[31]
Bates	2010	12	South Africa	5	This study
Total	Total	48		13	

Thirteen adults with HBV MGN have been reported to have a poor renal outcome. (Renal failure/ death from renal failure - Table 7-3) In the general outcome 35 adults were reported to display remission from HBV MGN. (Table 2-16 page 2-85)

As an indication of the relatively better outcome of the childhood HBV MGN spectrum compared to the adult HBV MGN group, including all known outcomes, current study as well, the proportion of poor outcome to remission (with or without treatment) in the childhood group is 7% (22:328) in contrast to 37% for the adult HBV MGN group. (13:35) Data from Table 2-16, Table 4-8, Table 6-2, Table 7-3.

The Table 7-4 below summarises many of the key findings of the literature and this study on HBV MGN in children and adults and how these forms of GN differ from idiopathic MGN. The table is based on Table 3 in the review of Bhimma and Coovadia [10] with additions from the literature of the intervening years and the findings of the current comparative study.

Table 7-4 Summary of features of the 3 groups HBV MGN and HBV GN in general in children and adults and idiopathic MGN in adults

Parameter	Childhood HBV MGN	Adult HBV MGN	Adult Idiopathic MGN
Aetiology	Vertical transmission HBV in Far East Horizontal transmission in USA, Africa and Europe	Often unknown Horizontal transmission in areas of low endemicity often associated with drug abuse or sexual transmission	Unknown
Age at presentation	2-12 years	15-40 years	30-50 years
Gender	Male dominance 80:20	Male dominance 80:20	Male dominance 60:40
Clinical presentation	Asymptomatic – detected by routine urine and serological screening Proteinuria and nephrotic syndrome	Proteinuria and nephrotic syndrome	Proteinuria and nephrotic syndrome
Acute hepatitis	Low incidence	More often present in adults from non-endemic areas	Not applicable
Renal Histology			
Common diagnostic characteristics	Subepithelial deposits on EM IgG and C ₃ on IF	Subepithelial deposits on EM IgG and C ₃ on IF	Subepithelial deposits on EM IgG and C ₃ on IF
Light microscopy	Usually minimal glomerular sclerosis and interstitial damage	Variable glomerular sclerosis and more frequent interstitial damage	Variable glomerular sclerosis and more frequent interstitial damage
Ultrastructure	Often mesangial deposits, subendothelial deposits and mesangial interposition	Often mesangial deposits, subendothelial deposits and mesangial interposition	Usually no additional elements
Immunofluorescence	Specific HBV antigens detectable especially HBeAg in subepithelial deposits and sometimes HBsAg in mesangial deposits	Specific HBV antigens detectable especially HBeAg in subepithelial deposits and sometimes HBsAg in mesangial deposits	No HBV antigens detected If specific antigens detected, makes MGN secondary.
Other GN patterns	MCGN Possibly less frequent than in adult HBV GN. IgA not as well documented as in adults	MCGN, IgA	
Renal function	Preservation of renal function 60-80% without specific treatment. Even better with IFN, Lamivudine and other antivirals	Progression to renal failure in 20-40%. Encouraging response to IFN, Lamivudine and other antivirals	Progression to renal failure in 20%-40%. Variable response to treatment

Based on Table 3 in a 2004 review by Bhimma and Coovadia with expansion and updating. [10]

CLASSIFICATION OF HBV MGN and MCGN – Wider issues

A recent report on a new pathologic classification of diabetic nephropathy highlights an issue raised by this study which is of relevance to pathologists and nephrologists in the management of patients. The report notes that although pathologic classifications exist for several renal diseases including IgA nephropathy, focal segmental glomerulosclerosis and lupus nephritis, a uniform classification for diabetic nephropathy is lacking. [39] An international group of renal pathologists, commissioned by the Research Committee of the Renal Pathology Society, had the aim of developing a consensus classification combining Types 1 and 2 diabetic nephropathies. The aim of the classification would be to discriminate lesions by varying degrees of severity that would be easy and useful in clinical practice worldwide. The fact that such a large international exercise was deemed necessary, shows that even if criteria exist to make the initial diagnosis of an entity, in this case diabetic nephropathy, there are more levels of standardization and correlation required to ensure features are similarly viewed and understood by different pathologists worldwide and then to distinguish between different elements and develop a useful clinically appropriate classification. Such a process never took place for the GNs associated with HBV. Therefore while there is likely to be reasonable agreement on the classification of cases showing exclusively or almost only features of either MGN or MCGN, those with mixtures, as highlighted a few times in this study, are likely to have been categorised differently by different pathologists.

The current adult HBV GN series, for example, had 41% MGN and 22% MCGN in comparison to 15% MGN and 42% MCGN from a Korean adult HBV GN series. (Table 7-5) The Korean study from 1988 provided comparative data for both adult and childhood studies of HBV associated renal/glomerular disease and was discussed in the literature review. (Chapter 2 page 2-95) [40]

The Table 7-5 below contrasts the Korean adult HBV GN patients with this SA series of 37 adults with serum HBsAg and GN, collected over 15 years. The Korean series reported 66 adults with serum HBsAg (21 children in the group of 87) collected in 30 months.

Table 7-5 *HBV Renal biopsy series in adults – Korean Tygerberg comparison*

HBV GN	MGN No. (%)	MCGN No. (%)	OTHER No. (%)	END STAGE No. (%)
Korean (n= 66)	10 (15)	28 (42)	26 (39)	2 (3)
Tygerberg (n=37)	15 (41)	8 (22)	10 (27)	4 (11)

There were almost certainly differences in the way cases with either combined MGN and MCGN features or features predominantly of either alone were assigned to a category so that the combined percentage 57% and 63 % for the two groups (HBV MGN and HBV MCGN) as respective proportions of all HBV positive adults may reflect a greater similarity between the series than the MGN and MCGN figures as represented above, suggest. In this SA series, the emphasis was on the subepithelial deposits and cases were placed in the MGN group even with prominent mesangial interposition (although a subgroup of mixed HBV MGN-mesangiocapillary GN was created to accommodate biopsies with clear feature of both categories) while in the Korean series prominent mesangial interposition, even with subepithelial deposits, appears to have been given a diagnosis of MCGN. [40] The pathological features of the 28 adult HBV MCGN Korean cases included prominent subendothelial and mesangial deposits in all 27 cases (100%) studied by EM and subepithelial and/or intramembranous deposits in (79%). [40] It is strongly suspected that using the criteria of this present study, at least some if not most of the Korean MCGN cases

with prominent subepithelial deposits would have been classified as MGN with moderate to severe (these then in the 'mixed' subgroup) mesangial interposition giving an MGN: MCGN ratio of about 32:7 rather than the 10:29 Lee et al produced, an apparently very different 'result' for the same biopsies.

One way to deal with the HBV MGN to mesangiocapillary GN spectrum is to have a mixed category in the middle to include biopsies that display sufficient features of each category to have been placed in both groups (the mixed HBV MGN-mesangiocapillary GN group) and on either side the HBV MGN group and HBV mesangiocapillary group. It would be expected that both of the last two mentioned groups would often show some of the features of the other group but not sufficient to warrant placement in the mixed category. This is the categorisation that has been followed in this thesis which is similar to the approach of Lai et al and Venkataseshan et al. [14, 23]

Management of many glomerular diseases includes renal biopsy features and classification is a factor in decision making. It is therefore essential that those reporting renal biopsies recognize and evaluate features in a similar reproducible way. The international collaboration referred to above and another recently completed regarding IgA nephropathy are aimed to assist this process. [39, 41]

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Chapter 8

CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

It has been demonstrated that the original hypothesis is correct, namely that HBV MGN in children differs substantially from idiopathic MGN in adults as well as HBV MGN in adults and could be viewed as a distinct subgroup of MGN.

It has been shown that HBV MGN in children differs from both of the comparator groups but in distinct ways. The two HBV forms of MGN shared many demographic, clinical and renal biopsy morphological features in contrast to idiopathic MGN. In the demographic and clinical categories, these included marked male predominance, presence in populations with known high HBV carrier rates, prominent haematuria, raised liver enzymes and frequently lowered C₃. (An interesting observation from this study was that C₄ was reduced in children but not adults with HBV MGN suggesting possibly different pathways and pathogenesis of the HBV MGN pattern at different ages.)

Pathologically, mesangial proliferation, mesangial and subendothelial deposits as well as mesangial interposition were more frequent in the HBV MGN groups compared with the idiopathic MGN group. Although many of the HBV MGN group showed some of the features of mesangiocapillary GN such as mesangial deposits, subendothelial deposits and mesangial interposition, the subgroup showing severe mesangial interposition, which on light microscopy also resembled mesangiocapillary

GN as well as displaying the widespread subepithelial deposits of MGN, was termed mixed HBV MGN-mesangiocapillary GN. This subgroup was also present in the adult HBV MGN spectrum 4/12(33%) but absent from the adult idiopathic MGN group in this series and the Yoshikawa childhood idiopathic MGN series. [1] Specific stains for HBcAg and HBeAg were seen only in the glomeruli of the renal tissue of the two HBV MGN groups.

Where the two adult MGN groups were similar and contrasted strongly with the childhood group was in showing a substantially higher frequency of glomerular sclerosis and interstitial damage. This was associated with the absence of spontaneous remission and an overall poorer outcome over 4 years of follow up. The two HBV MGN groups (children and adults) therefore had similar glomerular immune complex deposition patterns but different degrees of glomerular sclerosis, interstitial damage and outcome. This finding emphasised the lack of consistent correlation between aspects of the appearance of a single renal biopsy and the long term outcome of the patient. The need therefore for clinico-pathological correlation including demographic and other information at the time of a renal biopsy was again confirmed.

Renal biopsies are unique among routine surgical pathology specimens in that besides a panel of 'special' histochemical stains, immunofluorescent or immunohistochemical staining and ultrastructural examination are accepted as part of the international standard of routine examination. [2] This study raised important issues concerning the application of the latter two techniques to renal biopsies in HBV MGN specifically but with broader implications. Many of the ultrastructural studies in the literature focused essentially on the subepithelial deposits, the presence of which was used to make a diagnosis of MGN. The ultrastructural

diversity including mesangial deposits, occasional subendothelial deposits and mesangial interposition, was often not reported. Unless there was specific information that documented that these features were present or absent it would be difficult to establish whether they were sought. This raises the possible need for a check list approach where relevant positive and negative findings are noted routinely. If ultrastructural examination focuses only on limited features in order to categorise a biopsy in a known category, ultrastructural diversity such as has been clearly shown in HBV MGN cohorts can easily be missed. There is a natural tendency to see and report only what is known about and what is enough to make a 'diagnosis' or place a biopsy in a known category. The danger is that findings that do not fit or appear to be unnecessary or extraneous at the time might get ignored and diversity within a group and indeed new diagnoses and entities could be missed.

As Rosai emphasises, there is probably no other method that has so revolutionized the field of anatomical pathology during the past 50 years as the immunohistochemical and immunocytochemical techniques of which immunofluorescence was a particular forerunner. The advantages include remarkable sensitivity and antigenic specificity as well as correlation with traditional morphology. [3] The immunofluorescent stains in the study cohorts highlight both the positive value of the techniques and potential pitfalls. The immunofluorescence stains emphasised the frequency of IgG and C₃ in MGN in a form and location that correlated with the subepithelial deposits, namely granular along capillary walls. The finding that a monoclonal HBe antibody identified HBeAg as the main exogenous component of this MGN immune complex deposit clarified the pathogenesis to a more specific level. The findings of others that HBsAg with reliable monoclonal antibodies was localized more frequently in mesangial areas helped explain the

variety of deposit locations. [4, 5] The morphological similarities between the glomerular spectrum of changes in SLE and HBV especially the MGN and MCGN overlap forms suggests that in SLE the variety of deposit locations may also reflect different antigen-antibody complexes with varying propensities for different locations. Although HBV MGN particularly in children from countries with HBV vaccination programs seems fortunately to be a disease largely of the past, SLE certainly is not and lessons and ideas from one disease may well contribute to research studies leading to better understanding and eventually management of another disease such as SLE. [6]

The possibility of identifying HBeAg in both the serum and glomeruli enabled a correlation to be made between the frequencies of the presence of the antigen in the 2 locations at the time of initial presentation and to track the loss of HBeAg in the serum and its relationship to remission. Although not as controlled or precise as a laboratory animal study might be, where numerous repeat biopsies could be undertaken, this disease has provided one of the best, if not the best, models of human GN as was suggested by Heptinstall and Cameron in 1972. [7] In this disease there is an antigen HBeAg that can be identified in the serum and glomerulus.

Like any other technique, however, immunofluorescence / immunohistochemistry for HBV antigens in the glomeruli presented pitfalls that needed to be acknowledged and where possible avoided to prevent the techniques from being misleading. [3] Both the Dako anti-HBsAg polyclonal antibody and the anti-HBcAg polyclonal antibody were shown to be deficient. The anti-HBsAg stain was confirmed by controls to be non specific in this study as others had shown while the anti-HBcAg stain others had

shown by rigorous testing to cross-react with HBeAg. [8] Although not proving this conclusively, nevertheless this studies' findings did fit that likelihood with all 19 cases positive for either or both of the monoclonal HBeAg antibodies (Institute of Immunology Co. Ltd. Tokyo, Japan) also being positive for the Dako HBcAg polyclonal antibody. One of the reasons why the anti-HBeAg antibodies that were imported from Japan for this study have seldom been used was the expense necessary to offset the cost of rigorous laboratory testing to ensure it was specific.

Limitations

There were a number of limitations to this study, many of which have been referred to within the dissertation but are consolidated here. This was not a well defined prospective and comparative study of three groups of patients. It was a detailed comprehensive descriptive cohort study primarily of 71 childhood HBV MGN patients seen over a 31 year period (1974-2005) with 2 adult comparative groups. The absence of a childhood idiopathic MGN group has been referred to previously and does constitute a limitation regarding the comparative component of this study. As this was not a well defined prospective study, there were therefore not consistent precise definitions in particular of various clinical and laboratory parameters such as oedema and ascites. The evaluations of the adult patients were done by numerous clinicians (consultant nephrologists and medical registrars in the renal unit under supervision of the consultants) inevitably leading to more variation in criteria. Regarding a feature such as proteinuria, not many 24 hour urine proteins were available and therefore Labstix readings with their limitations were used. Serum HBeAg and antibody testing only began at TBH in 1991 initially on patients from this study with research funding. There is therefore a gap in data even though stored sera

where available were also tested. The renal biopsy evaluations, however, were all done by the author of the thesis giving an element of consistency.

The childhood cohort while not strictly a prospective study group was managed by the same paediatric nephrologist from 1974 until his retirement after 2000. He developed a particular interest in this disease and these patients and made early presentations on the initial patients in the cohort at a Paediatric Association Congress in 1976, local Academic Year Day (1977) and an MRC research meeting in 1982. By 1982 he reported that the HBV MGN subgroup was 20% of the then series of 110 children with nephrotic syndrome. [9-11] There was therefore a strong element of consistency of examination criteria and good follow up data especially ongoing complement and liver enzyme testing enabling data to be gathered and conclusions to be drawn. Therefore while this was not an official single investigator prospective series, it had many of the good features of such an investigation. Despite extensive efforts over a long period of time follow up data remained a limitation especially severe regarding the Namibian children. An opportunity to evaluate whether race might be an independent or linked variable for outcome was therefore not possible because of very limited follow up of the black Namibian children and the 6 black SA children.

Recommendations

One of the obvious recommendations to flow from this study, namely to encourage interventions to reduce or prevent this particular form of HBV associated GN, has fortunately already been implemented via widespread hepatitis B immunisation in South Africa (1995) and many other parts of the world. [12] In this regard HBV MGN is one of the few success stories regarding GN. It must however be acknowledged that to an extent this positive renal outcome has been fortuitous. The core problem of

HBV is its more frequent and serious liver effects namely cirrhosis and carcinoma and not this rare renal disease. These liver complications were the major motivating factors for the development of vaccines and the implementation of national vaccination programmes such as in South Africa in 1995. [13] Nevertheless the reduction and hopefully imminent disappearance of HBV MGN in many countries is a fortunate result of increasing urbanisation (associated with lower HBV carrier rates) [14, 15] as well as worldwide vaccination programmes.[16]

Preceding the introduction to this study, there is a quote from RJ Glassock, “The discovery of a well defined aetiology for MGN can be an intellectually satisfying experience. “ [17]

This study of local experience of one of those well defined aetiologies with a review of the worldwide experience of HBV MGN has indeed been an intellectually satisfying and enriching endeavour.

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Chapter 9

GRANTS, AWARDS, PRESENTATIONS and PUBLICATIONS

RESEARCH GRANTS AND AWARDS ASSOCIATED WITH THIS STUDY

1990	MRC short term research grant (1990 - 1992)
1990	Harry Crossley research grant
1993	MRC short term research grant renewed (1993-1995)
1994	A J Brink trophy for best clinically relevant presentation at University of Stellenbosch Medical Faculty Academic Year day

PRESENTATIONS

1994	<p>Congress of South African Societies of Pathology, Cape Town. Poster. Hepatitis-B-Associated Glomerulonephritis. A clinicopathological study in Namibian and South African children - 1973 - 1993 (with AJ van Buuren, N Muller, DH Geiger and DJ Rossouw)</p> <p>Academic Year day of Stellenbosch Medical Faculty. Hepatitis-B-Associated Glomerulonephritis (with AJ van Buuren, N Muller, DH Geiger and DJ Rossouw) This paper was awarded the A J Brink trophy for the best clinically relevant presentation.</p> <p>Congress of the South African Renal Society, Pretoria. Hepatitis-B-Associated Glomerulonephritis (with AJ van Buuren, N Muller, DH Geiger and DJ Rossouw)</p>
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- XX International Congress of the International Academy of Pathology, Hong Kong. Hepatitis-B-Associated Glomerulonephritis. A spectrum of Membranous and Mesangiocapillary features (with AJ van Buuren, N Muller, DH Geiger and DJ Rossouw)
- 1996 Pathological Society of Great Britain and Ireland, 173rd Meeting, Southampton, UK. Membranous glomerulonephritis - adult idiopathic compared to HBV associated in children (with N Muller, AJ van Buuren, DH Geiger, MR Moosa and JJ Joubert).
- 2001 Congress of South African Societies of Pathology, Cape Town (July) Stellenbosch Medical Faculty Academic year day (August). HBV renal biopsy pathology in adults. 15 year review from Tygerberg Hospital, Western Cape. WD Bates, N Muller, MR Moosa.
- 2002 Congress of SA Renal Society, Bloemfontein (September). HBV renal biopsy pathology in adults. 15 year review from Tygerberg Hospital, Western Cape. WD Bates, N Muller, MR Moosa, R van Zyl-Smit.
- 2003 Academic year day, Stellenbosch Medical Faculty (August).
- Membranous glomerulonephritis – a comparison between the idiopathic form in adults and HBV associated in adults and children. WD Bates, AJ van Buuren, N Muller, JW Schneider, MR Moosa. (Poster)
- 2004 Congress of South African Societies of Pathology, Stellenbosch (July). Membranous glomerulonephritis- a comparison between the idiopathic form in adults and HBV associated in adults and children. WD Bates, AJ van Buuren, N Muller, JW Schneider, MR Moosa. (Poster)
- South African Renal Society Congress. Johannesburg (August). Membranous Glomerulonephritis – a comparison between the

idiopathic form in adults and HBV associated in adults and children. WD Bates, AJ van Buuren, N Muller, JW Schneider, MR Moosa.

2008. IAP Congress Athens October. (Poster) HBV MGN in children; a subgroup with renal failure. WD Bates, MR Moosa, N Muller, JW Schneider
- 2009 Congress of South African Societies of Pathology, Durban September. HBV MGN in children; a subgroup with renal failure. WD Bates, MR Moosa, N Muller, JW Schneider (Poster)
- 2010 South African Renal Society Congress. Cape Town (April) HBV MGN in children; a subgroup with renal failure. WD Bates, MR Moosa, N Muller, JW Schneider

PUBLICATIONS

1. Bates WD, Muller N, van Buuren AJ, Steyn DW
Pregnancy in partially remitted hepatitis-B-associated membranous glomerulonephritis.
Int J Gynecol Obstet 1996; 52: 163-165.
2. Van Buuren AJ, Bates WD, Muller N.
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Moosa MR, Hattingh TL, Bates WD. Viral infections and the Kidney.

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1. Van Buuren AJ, Bates W.
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2. Bates W, Van Buuren AJ.
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4. Bates WD, Moosa MR, Muller N, Schneider JW
HBV MGN in children; a subgroup with renal failure
Histopathology 2008; 53 Supplement 1: 373 (856)

Chapter 10

APPENDIX

RENAL BIOPSIES OF CHILDHOOD HBV MGN BY YEAR

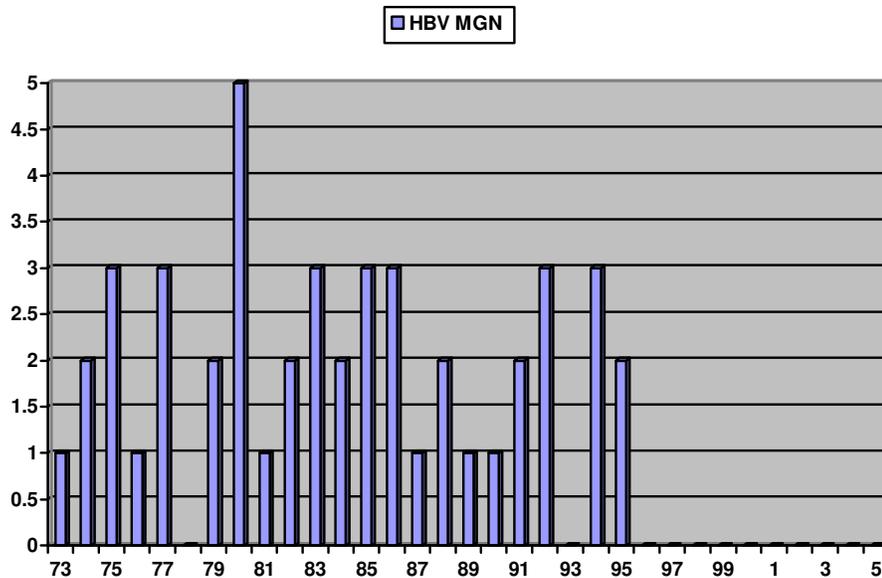


Fig 10-1 Cases by year of renal biopsy of the RSA children

South African cases: 46 – Over the 23 year period mean of 2 cases biopsied per year. Two years with no biopsies; 1978 and 1993. Maximum 5 in 1980. Note no biopsied cases since 1995, the year HBV vaccination began.

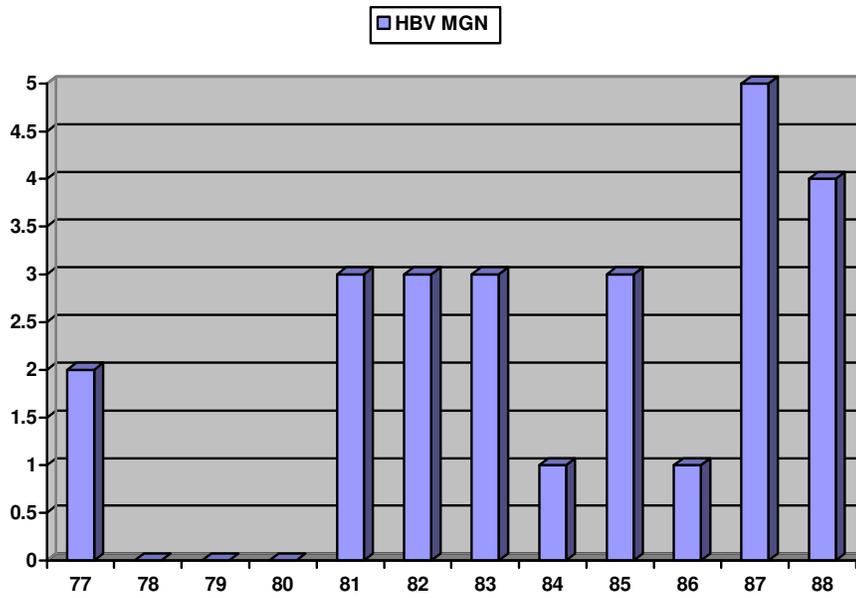


Fig 10-2 Cases by year of renal biopsy of the Namibian children

Namibian cases 25 – 2.1 cases per year 1977-1988 and 2.8 cases per year when there was at least one case seen. Note service stopped at Namibian independence.

CASE STUDIES OF HBV MGN CHILDREN WITH POOR OUTCOME

Case 77/jz (1974-2000) ^a

Born 2/1963^b

8/74 Presented to Tygerberg Hospital (TBH) at age 11. Previous history of kidney problems. Not clear whether nephritic or nephrotic. Possibly seen previously at Red Cross Children's Hospital. HBsAg positive.

6/76 Mother also HBsAg positive.

3/81 Followed until this time regularly at TBH paediatric nephrology clinic.

Age 18 years. Still HBsAg positive, proteinuria 2+, trace of blood, albumin 31 and oedema not noted. Creatinine 58

4/92 Returned to clinic as part of this study. 29 years old when seen at paediatric nephrology clinic. HBsAg positive, HBeAg negative, proteinuria ++, hypertension, creatinine 117 with clearance of 64. Indication of renal damage. Referred to adult nephrologists.

94 Creatinine 139 First clearly raised creatinine in 1/ 1994

98 Creatinine 181

2000 Proteinuria Hypertension

^a Dates indicate period of follow up at TBH

^b 2/1963 =2nd month February 1963

Case 78/pl (1975-1987)

Born 5/1966

Presented Keimoes 8/1974 as acute GN – sick and oedematous.

Urine protein 4+ and blood 3+ BP 150/110 Followed regularly despite the distance (1000 km)

Blood pressure often a problem, persistent proteinuria and haematuria. Normal serum creatinine.

7/82 Age 16 Creatinine raised for the first time 141. Persistently serum HBsAg positive.

5/87 Creatinine 1827. Sent home to Keimoes for terminal care. Remained serum HBsAg positive but had become serum HBe negative by this stage.

Case 81/fj (1975-2008)

Born 11/1965

8/75 At age 9 presented in Carnarvon, Northern Cape with dysuria. Found to have oedema of feet. Urine showed 4+ albumin. Nephrotic syndrome suspected.

10/75 Seen in TBH. MGN diagnosed on renal biopsy. Found to be serum HBsAg positive.

4/78 Noted to be serum HBsAg negative but still 4+ protein in urine and serum albumin 19.3, creatinine 63 and cholesterol 6.65. Unusual to be HBsAg negative but not in remission.

Over these years of follow up by Dr van Buuren there were episodes of peritonitis.

1/81 Last seen at paediatric nephrology – Doing well in general but BP 150/80 and +++ proteinuria in urine.

1988 X-ray at TBH for hand injury at work at quarry – indicates he was working at age 23 years.

1995 Presented to urology at age 29 with problem of impotence. Found to have hypertension, proteinuria 3+ and raised creatinine 246. A cystic lesion on kidney imaging lead to a biopsy which showed diffuse renal damage. More than 50% of glomeruli displayed global sclerosis while in the interstitium prominent damage was present with moderate chronic inflammation fibrosis as well as severe tubular atrophy and blood vessel wall thickening. Glomeruli no longer showed features of MGN including on ultrastructure.

11/98 Accepted onto long term renal program

8/2000 Renal transplant

7/2005 Creatinine 220 – renal transplant for 5 years

1/2008 Conjunctival lesion severe dysplasia /carcinoma in situ most likely linked to immunosuppression. Creatinine 318.

6/ 2010 Creatinine 448 Probable chronic allograft nephropathy. Not yet on dialysis.

Case 90/ws (1980-2004)

Born 12/75 Worcester

9/76 At 9 months presented with oedema of face and hands. Not clear that underlying cause was identified.

6/80 Presented to TBH with nephrotic syndrome. Serum HBsAg positive and MGN on renal biopsy.

8/81 Presented to RCCH with similar story. Biopsy repeated again showed MGN with serum HBsAg, HBeAg positive and normal creatinine.

2/85 Serum HBeAg negative but persistent proteinuria.

2/89 Last seen at RCCH with proteinuria 2+ and haematuria 2+.

7/04 Referred from Worcester with hypertension, proteinuria, haematuria and raised creatinine 137. HBsAg positive but HBeAg negative.

11/04 Creatinine 146 and clearance 53

Case 109/mb (1988-1999)

Born 11/1978

10/88 Presented TBH with hypertension, oedema, proteinuria 4+, haematuria 2+.

Serum HBsAg positive and creatinine 62.

3/92 HBsAg positive, HBeAg negative and creatinine 52.

10/92 Severe hypertension and creatinine clearance decreased though creatinine normal.

8/98 BP 170/120, creatinine 580, creatinine clearance 13.5.

Referred to GSH as still serum HBsAg positive, also again serum HBeAg positive (Note change since 3/92 – reversal from HBeAg negative back to HBeAg positive)

9/98 GSH Second year BA student at Stellenbosch. Active swimmer.

Liver biopsy. Chronic active hepatitis – Hepatitis B associated chronic hepatitis. Mild grade II with early bridging fibrosis – stage II or III.

6/99 Creatinine 1142. Had not been accepted as long term renal replacement candidate. Died.

Case 118/nj (1994)

Born 01/1985 Ladismith

5/94 Presented to TBH via Oudtshoorn with oedema, proteinuria 3+, creatinine 63, albumin 9.4, serum HBsAg and HBeAg positive.

6/94 Creatinine 216

8/94 Creatinine 686

Transferred home for terminal care

The one Namibian child known to have developed renal failure is also included in this section.

Case 63/mf (1985-1989)

6 year old Owambo boy. Only child in series of 71 who presented with raised creatinine 133. Open biopsy had 170 glomeruli of which 65% sclerosed. Severe interstitial disease. In renal failure from the start and died within 4 years.

Of some initial interest is that all 7 were male and only one presented at under 5 years of age, namely at 4 years (patient 90/ws).

TWO UNUSUAL CHILDHOOD HBV MGN PATIENTS

Two unusual patients in the series of children warrant special documentation.

1. A girl who first presented at age 11 became, to the best of our knowledge, the first patient with biopsy proven HBV MGN to become pregnant and deliver [1]. The birth occurred when she was 19, while in partial remission with normal albumin and + proteinuria on labstix. At this time she was still HBsAg positive but HBeAg negative, making perinatal transmission to the infant less likely.

Four years later she presented with an ectopic pregnancy.

2. The second patient was a 3 year old when he presented with nephrotic syndrome in 1991. He was found to have a unilateral multicystic dysplastic kidney which was removed at the same time that an open biopsy of the other kidney was performed. Of interest was the documentation of membranous features and hepatitis antigens in the

few glomeruli of the dysplastic kidney. Within 2 years he had gone into remission from the nephrotic syndrome but remained HBsAg and HBeAg positive up to nearly 5 years of follow-up. Later developed Hodgkin's lymphoma which has been successfully treated. He was last seen at TBH in 2008 in remission, a 19 year old working as an electrician in the Paarl area.

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