Review Article

Can we predict and/or prevent type I diabetes?

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Summary

The highest risk for the development of type I diabetes resides with first-degree relatives of the diabetic proband, this risk being in the order of 2,9%, 6,6% and 4,9% for parents, siblings and children of the proband, respectively. The major genetic markers associated with the development of insulin-dependent diabetes mellitus (IDDM) is the possession of the HLA alleles DR3/DR4 and more recently the absence of aspartate in the 57th position on the β-chain of the HLA DQ gene (HLA DQ β Asp 57 negative). The most important auto-immune marker for predicting preclinical IDDM is the presence of high titres ($>$ 40 Juvenile Diabetes Foundation units) of islet cell antibodies (ICA), while the finding of insulin auto-antibodies (IAA) is a good predictive marker in children $<$ 5 years of age. The presence in a susceptible individual of ICA plus IAA is a better predictor of impending IDDM than the presence of either of these two markers alone. Antibodies which precipitate an islet membrane protein (MW 64K) are highly sensitive and specific markers of preclinical IDDM. The presence of 64K antibodies may well be the most important predictive marker of impending IDDM in the future. The progressive decline of the first phase of insulin secretion in response to an intravenous glucose challenge is associated with the onset of IDDM within 18 months. Of the immunotherapeutic agents at present used in clinically manifest IDDM, azathioprine has been shown to be ineffective in increasing the remission phase, while the value of nicotinamide is controversial. At present the agents showing the most promise in inducing and maintaining remissions are cyclosporin A or a combination of prednisone plus azathioprine; however, further studies are needed. As regards the prophylaxis of IDDM, the use of immunotherapy in minute doses during the preclinical phase or the administration of a vaccine (derived from attenuated autoreactive T cells) to all newborn infants comprise some of the strategies envisaged for use in the future.

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In order to answer the questions posed in the title of this article it is necessary critically to analyse the data available in published reports on the following issues: (*i*) the value of the markers at present available for the prediction of preclinical type I diabetes; (*ii*) the benefit of the immunotherapeutic agents at present in use in patients with clinically manifest insulin-dependent diabetes mellitus (IDDM); and (*iii*) the potential role of immune modulation in the prophylaxis of IDDM.

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Early detection of IDDM

Family studies

It is well known that the highest risk for the development of IDDM resides with first-degree relatives of the proband. In this regard, Tillil and Köbberling[1] recently calculated by age-corrected empirical genetic-risk estimates that the overall risks for parents, siblings and children of the proband were 2,9%, 6,6% and 4,9% respectively. However, as only 1 out of every 8 patients with confirmed IDDM has a positive family history, the need for alternative markers of impending IDDM is clear.

Genetic markers

The genetic markers in IDDM are to be found in the histocompatibility leucocyte antigen (HLA) system that resides on the short arm of the 6th chromosome. The genes predominantly associated with IDDM are found at the HLA DR and DQ loci.

Influence of the DR gene

It has been shown that the presence of the alleles DR3, DR4 or DR3/DR4 seems to be significantly associated with the development of IDDM. In this regard, retrospective studies have shown that for a given population ± 95% of newly diagnosed patients with IDDM possessed either DR3, DR4 or both.[2] However, since ± 40% of the non-diabetic population possess one or more of the above alleles, and since the incidence of IDDM is only 1 - 2% in these subjects, the possession of the HLA DR3/DR4 alleles is associated with, but *not* the cause of, increased susceptibility to the development of IDDM. It must be noted that protection from IDDM seems to be associated with the absence of DR3/DR4 or the possession of the DR2 allele. More recently, Tarn *et al.*[3] in a study of first-degree relatives of insulin-dependent diabetic subjects (IDDS), showed that the cumulative risks of becoming insulin-dependent by the age of 25 years were increased 24 times (16%) for siblings sharing two haplotypes (HLA identical), increased 2,5 times (9%) for those sharing one haplotype (haploid identical) and zero for those who shared none (HLA non-identical).

Influence of the DQ gene

Studies involving analysis of DNA fragments from lymphocytes by cDNA probes (restriction fragment length polymorphisms) have shown that linkage exists between the HLA DR and DQ loci. In this context, recent data suggest that the primary association of IDDM susceptibility may lie with the DQ rather than the DR gene. DR4 is usually associated with DQw3 specificity, which in turn has been subdivided into DQw3.1 and DQw3.2 subsets. Recently it has been shown that in DR4-positive IDDS, the DQw3.2 (DR4-DQR4) subset appears to be associated with IDDM susceptibility, while the DQw3.1 (DR4-DQR5) subset is associated with non-susceptibility.[4,5] Furthermore, amino acid sequence analysis of the β-

chain of the HLA DQ gene product suggested that a single amino acid of the β-chain at position 57 was uniquely important in determining susceptibility or resistance to IDDM. An aspartate residue at this position appears to confer resistance to IDDM, whereas the presence of the neutral amino acids leucine, serine and valine in the 57th position is associated with susceptibility to IDDM.[6] However, recently it has been shown that this finding is not applicable in all IDDM populations.[7,8]

Auto-immune markers

The auto-immune markers for predicting IDDM consist of the following humoral and cell-mediated components: islet cell antibodies (ICA); insulin auto-antibodies (IAA); antibodies to a human islet cell antigen of MW 64 000 (64KA); and the immune activated T lymphocytes (Ia T cells).

Islet-cell antibodies

The ICAs are IgG antibodies directed against an intracytoplasmic antigen present in all islet cell types, which has been shown to consist of a sialoglycoconjugate.[9] ICAs are detectable in approximately 55-80% of subjects with newly diagnosed IDDM, the prevalence progressively declining to about 20% 1 - 3 years after diagnosis.[10,11] In addition, ICAs have been detected in 3 - 6% of first-degree relatives and in less than 0,5% of the general population.[12] After three international workshops, it has now been possible to standardise the ICA assay where titres are expressed in terms of Juvenile Diabetes Foundation (JDF) units. There is general consensus that relatives of patients with type 1 diabetes whose sera contains > 40 JDF units/ml of cytoplasmic islet cell antibodies have a high risk of developing IDDM.[13] In this context, recent data have shown that the risk of developing IDDM in first-degree relatives, who were followed up for 8 years, was 76% in those with high titres (> 40 JDF units) of ICA, 3% with low ICA titres and 0,6% in those who were ICA negative.[3]

Insulin auto-antibodies

Since several studies have documented the presence of IAA in new-onset type I diabetics before insulin administration and in the relatives of overt type I diabetics before the onset of hyperglycaemia, it has been suggested that IAA could serve as an immunological marker for impending IDDM. It appears that the highest prevalence of pre-clinical IAAs is found in children,[14–16] especially those < 5 years of age,[17] while IAAs are rarely found in adult-onset type I diabetes.[18] In a recent follow-up study of ICA-positive non-diabetic first-degree relatives, the relatives with *both* ICA and IAA had more impaired insulin responses and were more likely to progress to IDDM compared with those possessing only one of these markers.[19]

64K islet-cell antigen antibodies

In contrast to the ICAs that are directed against an intracytoplasmic antigen in the islet cells, antibodies have been described that immunoprecipitate an islet membrane protein having a molecular weight of 64 000 (64KA).[20] With respect to prediabetes, two studies that recently followed the course of subjects deemed 'at risk' for developing IDDM have shown that 64KAs were present in 90% of the subjects who subsequently developed clinical IDDM. In these studies 64KAs were detected from 4 to 91 months before the clinical onset of IDDM.[21,22] Although it was claimed that 64KA demonstrated both high sensitivity and specificity for the beta cell, recent evidence has shown that the 64KAs can precipitate antigens in both somatostatin- and glucagon-containing islet cells. However, it appears that the 64K antigen concentration is 10 times higher in beta cells than in these non-beta-cell populations.[23] Until recently, the detection of 64KA involved a complicated and time-consuming technique confined to isolated research laboratories; however, a new simplified procedure for detecting 64KA using Western blotting with rat islet preparations has been described.[23] If, through these simplified techniques, detection of the highly sensitive and specific 64KA becomes more readily available, the presence in sera of 64KA could well become the most important predictive marker for impending IDDM in the future.

Immune activated T lymphocytes

Since Ia T cells (those expressing the DR antigen on their surface) are found in auto-immune diseases and since IDDM is considered to have a chronic auto-immune phase, several studies have documented the presence of increased circulating Ia T cells in patients with IDDM. Earlier studies showed the presence of increased Ia T cells at the onset of clinically manifest IDDM[24,25] while, more recently, several studies have shown the presence of these cells in normoglycaemic first-degree relatives of type I diabetic patients, many of whom on follow-up have developed clinical diabetes.[26–28] It was thus felt that Ia T cells might also represent an auto-immune marker for impending IDDM. It has been shown that, like ICAs, the Ia T cells disappear within 6 months - 1 year after clinical diabetes onset — possibly due to a reduction of the antigen to which these markers are directed.[24,28]

Metabolic markers

It has been previously documented during a study of monozygotic twins who were initially discordant for IDDM, that during the 'pre-diabetic' phase these twins demonstrated progressive decline in the first-phase insulin response (sum of 1 plus 3-minute values) during an intravenous glucose tolerance test (IVGTT).[29] These findings were subsequently confirmed in other patients deemed 'at risk' for developing IDDM.[30,31] Moreover, it was shown that 95% of subjects whose first phase of insulin secretion was < 25μU/ml developed overt IDDM within 12 months.[31] However, caution must be exercised in interpreting these insulin responses, since it has recently been shown that blunted insulin responses can be obtained in 'normal' non-diabetic subjects between the ages of 17 years and 25 years,[32] a finding which could be misinterpreted as a false-positive predictive indicator. Thus, age and pubertal status must be carefully considered when interpreting insulin responses to IVGTT in patients suspected of having early abnormalities of carbohydrate metabolism.

Future studies — early detection IDDM

In any epidemiological study it is important to identify the cohort of subjects who can be considered as 'high risk' (high yield of positives) and those considered 'low risk' (lower yield of positives). In this regard: (*i*) the 'high-risk' groups relative to the prediabetes state include: first-degree relatives of subjects with type 1 diabetes, patients with auto-immune disorders (i.e. Addison's disease, thyroiditis), gestational diabetics and clinical prediabetics with transient or intermittent hyperglycaemia; while (*ii*) the 'low-risk' group involves mass screening of general populations. One such study tested 5 000 children for the presence of ICAs and 21 positives were obtained, several of these children have subsequently progressed to overt IDDM.[33] It appears that the most reliable predictive marker for screening purposes is the presence of a high titre (> 40

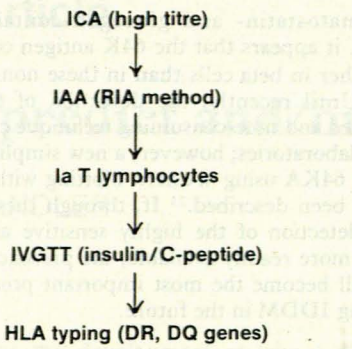

Fig. 1. Methodology involved in screening for and detection of subjects 'at risk' for impending IDDM.

JDF units) of ICAs. These individuals can then be further investigated by testing for IAA (fluid phase RIA method), Ia T cells, insulin/C-peptide response to IVGTT and HLA typing (DR/DQ genes) in that order (Fig. 1). Armed with the above information a reasonably accurate 'prediction profile' can be constructed. Recently, a dual parameter model for predicting the onset of type 1 diabetes has been proposed,[34] consisting of the following multiple linear regression equation derived from data of 14 first-degree relatives followed to onset of IDDM:

$$\text{years to diabetes} = 1{,}5 + 0{,}03\ (1 + 3\ \text{min IVGTT insulin}) - 0{,}008\ (\text{IAA})\ R^2 = 0{,}79\ (P < 0{,}0002).$$

Although the model appears to give a good correlation, it must be remembered that IAAs are predominantly detected in childr... < 5 years and insulin responses to IVGTT are known to be highly variable. Therefore, it might be prudent in future studies to consider the addition to this equation of ICA and Ia T-cell values in order to enhance the predictive value of such an equation.

Immunotherapy of IDDM

Current immunotherapy

Since it became widely believed that IDDM resulted from acute-on-chronic auto-immune destruction of the pancreatic beta cells, a number of pilot studies have investigated the use of immunotherapy at the onset of type I diabetes in humans. In the early trials no apparent benefits were reported with the use of levamisole,[35] prednisone or prednisolone,[36] human leucocyte interferon,[37] horse antithymocyte globulin plus prednisone[38] and plasmapharesis.[39] The later and ongoing trials at present include the use of the following drug regimens: azathioprine; azathioprine plus prednisone; cyclosporin A; and nicotinamide.

Azathioprine

Azathioprine, a purine analogue with suppressive effects on both T and B lymphocytes, has been widely used in children suffering from auto-immune disorders. The first randomised controlled trial using azathioprine at the onset of IDDM reported the development of remission in 50% of the young adult IDDS; however, these remissions were not maintained in the 1 - 2 years after azathioprine therapy was discontinued.[40] The initial success of this study prompted a larger randomised double-blind placebo-controlled trial of azathioprine in young adults with IDDM. At the end of 1 year, the results showed no difference in the remission rate of the IDDS compared with controls. It was therefore concluded that at the dosage used, azathioprine alone did not influence the remission phase in young adults with newly diagnosed IDDM.[41]

Azathioprine plus prednisone

Although it has been shown that prednisone alone[36] or azathioprine alone[40,41] have limited use in IDDM therapy, a recent study using a combination of azathioprine plus prednisone in patients with newly diagnosed IDDM showed preliminary results that were slightly more promising.[42] At 1 year half the immunosuppressed patients but only 15% of the controls were in good metabolic control, while 15% of the immunosupressed but none of the controls were insulin independent after 12 months.

Cyclosporin A

The most promising results with immunotherapy in IDDM have been achieved with cyclosporin A. Several studies to date have evaluated the use of cyclosporin A in increasing the remission phase of recent-onset IDDM.[43–47] These studies were randomised double-blind placebo-controlled in design and cyclosporin therapy was commenced between 6 and 14 weeks after the diagnosis of IDDM was made. Results of these studies showed that: (*i*) the highest percentage of remissions occurred if therapy was started within 6 weeks of the diagnosis of IDDM; (*ii*) remissions occurred in 25 - 68% of patients within 2 - 9 months of starting therapy; (*iii*) the relative chance of being in non-insulin-requiring remission at 1 year was approximately 5 times that of a placebo-treated patient; and (*iv*) cessation of therapy was almost always associated with a return to a state of glucose intolerance or frank IDDM within a few months.

One of the major reservations about the use of cyclosporin therapy is the presence of side-effects. The most common side-effects are transient hirsutism, gingival hyperplasia and paraesthesiae,[43–46] while the most serious side-effect, viz. the induction of lymphoma, has only been described using the high doses required for patients with auto-immune diseases or those receiving an organ transplant.[47] In the treatment of recent-onset IDDM, the most serious and controversial side-effect of cyclosporin is that of nephrotoxicity. The renal lesions described have included reduction of glomerular filtration rate,[46] glomerulosclerosis[44] and interstitial fibrosis with tubular atrophy.[45,46] Some authors have stated that the abnormal renal appearance on histological examination is completely reversible on withdrawal of cyclosporin,[4] while others claim that the lesions are ongoing despite cessation of therapy and lead to chronic nephropathy.[48] At present, cyclosporin has been the only drug shown to increase the rate and length of the early remission phase of type I diabetes. However, the dangers of long-term immunosuppression must be weighed against the well-known long-term complications of type I diabetes treated with conventional insulin therapy. Thus, at present cyclosporin therapy for recent-onset IDDM should only be used in controlled trials conducted in academic institutions with the necessary clinical and laboratory expertise.

Nicotinamide

The use of nicotinamide in the early diabetic state is based on the ability of this substance to inhibit nuclear (poly-ADP-ribose) synthetase. In IDDM, activation of this enzyme is triggered in response to auto-immune-induced breaks in the DNA strands of the beta cell. The nuclear (poly-ADP ribose) synthetase of these affected beta cells have been implicated in DNA repair using cytosolic nicotinamide adenine dinucleotide (NAD) as a substrate, resulting in reduced concentrations of intracellular NAD which, in turn, is thought to be the final step in the destruction of the beta cell.[49–50] Thus, several reports in animal studies have shown that nicotinamide, by

increasing the NAD content in beta cells, prevented the onset of spontaneous diabetes mellitus in non-obese diabetic (NOD) mice,[51] prevented the induction of diabetes by alloxan and streptozotocin in bio-breeding (BB) rats[52] and showed evidence of inducing beta-cell regeneration in rats that were partially depancreatised.[53] As regards human studies, several groups have evaluated the effect of oral nicotinamide on residual beta-cell function in patients with recent-onset IDDM.[54–57] These studies have shown that nicotinamide preserved the insulin secretory capacity in newly diagnosed patients with IDDM for periods ranging from 3 months to 12 months. Although endogenous insulin secretion was retained in these patients, controversy existed as to whether patients on nicotinamide required lower insulin dosage to maintain metabolic control and whether nicotinamide therapy induced non-insulin-requiring remissions. It appears that these discrepant results could be explained by variations of study methodology or design. In this regard, future studies will need to be standardised in terms of study protocols before any meaningful consensus is to be reached as to the value of nicotinamide therapy in patients with recent-onset IDDM.

Future studies with immunotherapy

Due to the varying responses obtained with immunosuppressive agents in recent-onset IDDM, it might be worth while noting the factors that have been associated with early remission in these subjects. In this regard, the study using a combination of prednisone and azathioprine[42] noted that the best response to immunosuppression correlated with older age, better initial metabolic status (determined by lower HBA_1 and higher stimulated C-peptide values) and post-immunosuppression lymphopenia (< 1 800 lymphocytes/cm^3). The French study[45] using cyclosporin A in children with recent-onset IDDM showed that the predictive markers associated with early remission included better metabolic control (assessed by HBA_1 and stimulated C-peptide values) and the least body-weight loss. A prospective study, which analysed the predictors of beta-cell survival in patients with recent-onset IDDM, concluded that the survival of functioning beta cells was significantly shortened the younger the subject was at disease onset, if ICAs were present at diagnosis, and if the subjects were male.[58] Thus, by combining the information obtained regarding the positive predictors associated with early remission, it may be possible to improve remission rates by the *selected* use of immunosuppressive therapy in subjects known to possess these positive predictive criteria (Table I). In addition, as no study to date has exceeded 1 year's duration, it is important that further studies of immunosuppressive therapy in IDDM should be designed to last at least 2 years before final conclusions are drawn.

TABLE I. FACTORS ASSOCIATED WITH A FAVOURABLE RESPONSE TO IMMUNOSUPPRESSIVE THERAPY

Clinical	Laboratory
Older age at onset of IDDM (> 12 years)	HBA_1 < 8%
Female sex	Stimulated C-peptide > 0,6 nmol/l
Minimal weight loss before clinical onset of IDDM	Lymphopenia (< 1 800 lymphocytes/cm^3) after immunotherapy

Prophylaxis of IDDM

Pre-clinical IDDM

All immunotherapy to date has been given to patients at the time of clinically manifest IDDM, a state known to be associated with the death of 80 - 90% of the beta-cell mass.[59] Thus, it is not surprising that, although initial remissions occurred, once immunotherapy was withdrawn most patients rapidly became insulin-dependent, since the remaining 10 - 20% of beta cells were unable to maintain a state of normoglycaemia. Thus, it may be more advantageous in future studies to identify the subjects 'at risk' for developing IDDM (via the aforementioned markers) and introduce immunotherapy at this 'preclinical' stage in an attempt to salvage a larger concentration of viable beta cells, which, after immunotherapy is withdrawn, will it is to be hoped have a greater chance of facilitating insulin-independent euglycaemia. At this early 'preclinical' stage of beta-cell destruction it should be possible to reduce drug toxicity by administration of immunotherapeutic drugs in minute or intermittent doses. In this regard it has been shown that spontaneous insulitis was delayed and in some cases prevented when BB rats were treated at an early age with *intermittent* administration of cyclosporin-A therapy.[60] In addition, a viable regimen of immunotherapeutic agents given in *minute* doses during this early 'preclinical' diabetic state may include a *combination* of the following drugs: (*i*) prednisone (to block islet macrophage-secreted interleukin-1 activation of resting helper T cells); (*ii*) cyclosporin A (to block Ia T-cell-secreted interleukin-2 recruitment of helper, cytotoxic and suppressor T cells); and (*iii*) nicotinamide (to increase intracellular NAD concentration by inhibition of nuclear (poly-ADP-ribose) synthetase activation in susceptible beta cells). However, it must be stressed that the administration of immunotherapeutic agents to subjects 'at risk', but not yet clinically diabetic, will be associated with the moral and ethical dilemmas of 'treating' subjects who are not yet patients. Such controversial issues will in future undoubtedly affect all clinicians involved in the treatment of subjects with 'preclinical' IDDM.

Immune modulation

Since the advent of *monoclonal anti-T lymphocyte antibodies* much has been written about their possible therapeutic use in preventing IDDM. Several studies have shown that administration of monoclonal antibodies directed against immune-activated helper T lymphocytes (CDT_4^+),[61] cytotoxic T lymphocytes (CDT_8^+)[62] or both[63] have prevented the onset of insulitis and IDDM in NOD mice, a strain of animal prone to the spontaneous development of IDDM. Can these results be extrapolated to man? Since the immune system of the NOD mouse and that of man is vastly different, and since monoclonal antibody therapy has been shown to be antigenic in man, this form of possible immune therapy for the prophylaxis of IDDM in man must be considered speculative and of limited value at the present time. However, the development of a *vaccine*, for administration to all newborn infants, may well be a feasible alternative. The basis of such a vaccine would rely on identifying the autoreactive T-cell population that is cardinal in the cell-mediated destruction of the beta cell. Once identified, these T cells can be modified (i.e. irradiated) and then constitute the basis of a vaccine consisting of modified non-reactive T cells that will be fundamental in blocking the helper T-cell interaction with the beta-cell antigen and consequent cytotoxic T-cell destruction of the beta cell. Studies in this direction have already enjoyed success in the animal models of IDDM.[64]

At the beginning of the 20th century, who would have believed it was possible to put a man on the moon, perform heart transplantation or begin a human life in a test tube? For all these events to have become a reality, enough committed

people had to want them to be done and believed them possible to do. Such will have to be the state of affairs regarding the future prediction and prevention of type 1 diabetes. However, before this can become reality, like all other new scientific truths, a diabetic vaccine will have to pass through the following three classic stages of recognition:

> 'Firstly, people would say that it conflicts with the Bible or other established doctrines or scientific dogma; next, they would say that it had all been discovered before; finally, as it became an established fact, they would say that they had always believed it could be done.'
>
> — Louis Agassiz, 1807-1873 — American naturalist

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Malaria at Johannesburg Hospital

A retrospective study

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Summary

A total of 43 patients diagnosed as having malaria were admitted to Johannesburg Hospital during 1988; 40 (94%) were infected with *Plasmodium falciparum*. Only 26 patients (60%) were recorded as having used prophylaxis of any kind; chloroquine alone and in combination was used as prophylaxis by 17. Patients were treated with quinine (alone or in combination) in 67% of cases. In 42% of patients chloroquine-resistant malaria was considered a possibility.

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Over the last 10 - 15 years the deterioration in the incidence of malaria in Africa has been partly due to the increasing spread of a *Plasmodium falciparum* population that is resistant to chloroquine and other drugs. There have been reports of chloroquine-resistant *P. falciparum* malaria from regions such as Ethiopia,[1] Kenya,[2] East Africa,[3] northern Malawi[4] and Mozambique[5] and chloroquine resistance has also been confirmed *in vitro* in southern Africa.[6,7]

The difficulty of malaria control in chloroquine-resistant parasites is further compounded by the toxicity of alternative drugs that make it difficult to propose effective and safe medication for chemoprophylaxis and therapy.

A selected group of malaria patients and the drugs used in their therapy is reviewed. It is important to note that Johannesburg Hospital is a tertiary referral centre and the patients reported may not be representative of the general population.

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Patients and methods

Johannesburg Hospital has 833 beds at present and during 1988 there were approximately 32 051 admissions. The hospital serves as a tertiary referral centre and is a teaching hospital of the University of the Witwatersrand. The records of the Haematology Laboratory of the South African Institute for Medical Research in Johannesburg, which serves the hospital, were studied to identify all patients with positive malaria smears admitted during 1988. The medical records of these patients were then obtained from the Medical Records Department.

Results

There were 43 positive blood smears from 32 male and 11 female patients (median age 30 years; range 3 - 66 years). The incidence of malaria in the different age groups is shown in Table I.

TABLE I. INCIDENCE OF MALARIA IN DIFFERENT AGE GROUPS

Age group (yrs)	No. of patients	%
0 - 10	2	5
11 - 20	5	12
21 - 30	20	46
31 - 40	7	16
41 - 50	3	7
51 - 60	4	9
61 - 70	2	5

Types of malaria

Infection with *P. falciparum* was the most common type of malaria seen and occurred in 40 patients (94%). Two patients were infected with *P. vivax* and *P. ovale*, respectively, and in 1 patient the type of malaria was not identified.