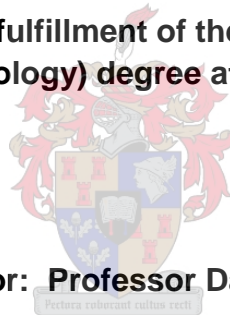


HISTOPATHOLOGY OF THE PLACENTA IN HIV POSITIVE WOMEN

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**Thesis presented in partial fulfillment of the requirements for the MMed
(Obstetrics and Gynaecology) degree at Stellenbosch University**



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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2015

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DEDICATION

Thank you to my heavenly Father for the strength to go on. This thesis is dedicated to my husband, mother, family and friends that kept me going when it seemed very dark.

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SUMMARY

Objectives

To describe the histopathological (macro- and microscopic) findings in placentae of mothers with HIV and to investigate the influence of maternal ARV therapy on histopathology, as well as to investigate the influence of immune suppression (CD4 count) on histopathology.

Methods

A retrospective audit of placentae collected between 2009 and 2010 from mothers with HIV disease was performed. Placentae were examined according to the standard protocol used by the Division of Anatomical Pathology (Appendix B). Data were extracted from the files and reports by the MMed student and loaded onto a database in a strictly anonymous fashion. Women with HIV plus co-morbidities specifically affecting placental function such as diabetes mellitus and pre-eclampsia were noted but not excluded from the study.

Results

There were 200 HIV cases that were identified during the study. Of the 200 cases 196 had histopathology results. The main placental findings in the study were that of maternal vascular under-perfusion (36%), ascending infection (29%) including chorioamnionitis and small placenta (15%). However, there was no significant difference in pathological diagnostic groups between the CD4 counts of the less than or greater than 350 cells/mm³.

Conclusion

Maternal vascular under-perfusion, ascending infection and small placenta may be linked to HIV placental infection, but further higher powered, controlled studies need to be done.

OPSOMMING

Mikpunte

Om die histopatologiese (makro- en mikroskopies) bevindinge in plasentas van moeders met HIV te omskryf en die invloed van moederlike ARV terapie op histopatologie te ondersoek, asook die invloed van immuun onderdrukking (CD4 telling) op histopatologie te ondersoek.

Metodes

'n Retrospektiewe oudit van plasentas wat gedurende 2009 en 2010 gekollekteer is van moeders met HIV is uitgevoer. Plasentas is ondersoek deur gebruik te maak van die standaard protokol wat deur die Afdeling Anatomiese Patologie gebruik word (Aanhangsel B). Data is deur die MMed student versamel uit lêers en verslae en op 'n databasis gelaai op 'n streng anonieme wyse. Vroue met HIV en ko-morbiditeite soos diabetes en preëklampsie, wat spesifiek plasentale funksie affekteer, is opgeteken maar nie uitgesluit van die studie nie.

Resultate

Daar is 200 HIV gevalle gedurende die studie geïdentifiseer. Uit 200 gevalle het 196 histopatologie resultate gehad. Die hoof plasentale bevindinge in die studie was moederlike vaskulêre onder-perfusie (36%), opstygende infeksie (29%) insluitende chorioamnionitis en klein plasenta (15%). Nietemin was daar geen merkbare verskil in patologiese diagnostiese groepe tussen die CD4 telling van minder as of meer as 350 selle/mm³.

Gevolgtrekking

Moderlike vaskulêre onder-perfusie, opstygende infeksie en klein plasenta mag verbind word met HIV plasentale infeksie, maar verdere groter gekontroleerde studies moet gedoen word.

CHAPTER 1: INTRODUCTION

Currently there are more than 33 million individuals who are living with the Human Immuno Deficiency Virus (HIV) worldwide ^[1].

HIV prevalence is estimated to be 29.4% to 30% amongst pregnant women in South Africa and Mother-to-Child Transmission rates (MTCT) range from 15 to 40 % if no antiretroviral medication (ARV) is administered. With the advent of ARV's, the MTCT rate has been reduced to less than 2% in some parts of the country ^[1].

Most transmission is believed to occur at birth and the various factors influencing this event include viral load, CD4 count, genotype of the virus, ARV's and obstetric factors such as prolonged rupture of membranes. Vaginal delivery has been associated with increased transmission of the virus as opposed to planned elective delivery by Caesarean section ^[2].

CHAPTER 2: LITERATURE REVIEW

2.1 HIV

The 2012 world UNAIDS day report highlighted that 30 million individuals globally are aware of their HIV status, with 2.5 million new cases of infection. Of these 30 million, 14.8 million people qualified for antiretroviral (ARV) treatment and 8 million were already on ARV medication [3].

Latest data regarding the global distribution of the disease, shows that 4.8 million individuals are infected with the virus in Asia, 2.3 million in North America, 1.4 million in Europe, 1.4 million in Latin America and 23.5 million in sub-Saharan Africa. South Africa currently has the highest prevalence with 5.6 million people living with the virus. There are approximately 1.7 million people on antiretroviral therapy in South Africa [3].

There are two subtypes of HIV, namely Type 1 and Type 2. HIV 1 is more prevalent globally whilst type 2 is more common in West Africa. Mother-to-child-transmission (MTCT) or vertical transmission may occur in utero during the intrapartum period due to exposure to maternal blood and fluids at delivery and post-partum during breastfeeding. HIV 1 has been closely linked to vertical transmission while HIV 2 has not been associated with this process [1].

Sub-Saharan Africa is plagued by the HIV 1 virus which is most commonly acquired heterosexually. It is however encouraging to note that in South Africa, the prevalence in the 15 to 24 year group has declined by 35% [3]. This may be attributed to an effective HIV awareness program as well as the availability of ARV's to the affected pregnant population, thereby decreasing the MTCT rate. Hence, more children are born HIV negative. Prior to antiretroviral therapy the achievement of uncomplicated pregnancies was challenging. Women with non-pregnancy related infections experienced increased mortality, which has since decreased by 25% compared to the period of 2002-2004 [4].

In South Africa in 2010, 30.2% of women attending antenatal clinics were living with the virus. In the Western Cape Province the prevalence amongst pregnant women was 18.5%. The overall HIV prevalence for the Western Cape in 2008 was 3.8%. (South African HIV statistics 2002 to 2008). Currently in South Africa more than 95% of HIV positive pregnant women are receiving ART (antiretroviral therapy). This has led to a decrease in MTCT. However, there were still 29 100 newly infected children in the year 2012. It should be noted that vertical transmission rates range between 25 to 45% in the untreated population [5].

With more women now being on antiretroviral treatment, clinicians are required to address the “cause and effect” picture of HIV - the effect of HIV on the placenta, the influence of the maternal CD4 cell count and viral load (surrogate markers), the effects of antiretroviral treatment on the placenta and also the outcome of the babies born to HIV positive mothers. A CD4 count of more than 400/mm³ of blood is associated with a low risk of vertical transmission. When the CD4 count is 200 to 400mm³, there is a moderate transmission risk and when it is less than 200 there is an associated higher risk of transmission and complications related to maternal HIV infection [6].

Most of the HIV transmission happens around the time of parturition via transplacental HIV transmission or during labour when the fetus comes into contact with maternal blood in the birth canal. Extensive work has been done to reduce MTCT during labour. Advanced HIV infection is associated with poor fetal and maternal outcomes. Some studies suggest that there is an association between advanced HIV infection and miscarriage, intrauterine foetal growth restriction, preterm delivery and perinatal mortality [7,8,9]. In certain protocols, Zidovudine (AZT) has been favoured as it inhibits HIV replication within placental cells and this has decreased placental/in-utero HIV transmission. Patients on AZT have been shown to have normal placentas with favorable fetal outcomes. The risk of chorioamnionitis, chorangiomas and chronic villitis was less in the population receiving AZT [10]

Studies suggest that an ailing immune system with a low CD4 count and a viral load of more than 1000 copies does increase MTCT rates ^[2]. On the other hand, a near normal CD4 count with a low viral load (less than 1000 copies), has a transmission rate of 1% ^[6]. This seems to be a protective factor and is the basis for the widespread use of ARVs in pregnancy. Preterm delivery, vaginal delivery, mixed feeding (formula and breast), antepartum invasive procedures, chorioamnionitis, hepatitis infection, illicit drug use especially cocaine have been postulated to increase the risk of vertical transmission. However, the advent of ARVs has brought significant improvements in maternal and neonatal outcomes ^[11]. ARVs help decrease the amount of the HI virus in the body; this decreases the risk of vertical transmission and also leads to improved maternal health.

There are six classes of antiretroviral medication, namely:

- **Entry inhibitors:** Interfere with the ability of the virus to bind to receptors in the outer surface of the cell; this prevents HIV from entering the cell.
- **Fusion inhibitors:** Prevent the virus from fusing with the cellular membrane.
- **Reverse transcriptase inhibitors– RTI:** Inhibitors to double stranded HIV DNA (deoxyribose nucleic acid).
 - **Nucleoside – NRTI:** these drugs are analogues to the deoxynucleotides needed to form the viral DNA. They however lack the hydroxyl group on the deoxyribose moiety and this leads to chain termination if there is incooperation with viral DNA, thus halting viral DNA formation.
 - **Non-nucleoside – NNRTI:** (bind to the enzyme reverse transcriptase and inhibits it from converting viral RNA to DNA).
- **Integrase inhibitors:** Block the enzyme HIV Integrase, which integrates the viral DNA to the host cell.
- **Protease inhibitors (PI):** These affect the protease enzyme which is responsible for assembling the new virion, and blocks HIV replication.
- **Multi class combination products:** The use of two or more of the classes is accepted as it reduces drug resistance ^[12].

The main classes of antiretroviral drugs used in pregnancy are nucleoside reverse transcriptase inhibitors (NRTI), e.g. Zidovudine. The main concern with this group is lactic acidosis which has a high mortality rate. Other side effects include anemia, gastrointestinal and dermatological manifestations such as skin rashes, skin colour changes (hyperpigmentation), hypertrichosis, pruritus, toxic epidermal necrolysis, bluish-brown coloured hands and nails and Stevens-Johnson syndrome. The non-nucleoside reverse transcriptase inhibitors (NNRTI) class e.g. Nevirapine (NVP) and Efavirenz (EFV) inhibits the reverse transcriptase enzyme. EFV has been associated with teratogenicity. Neural tube and central nervous system abnormalities have been shown to be the commonest abnormalities in the first trimester animal models as well as retrospective human case reports as neural tube closure occurs at day 25 of development ^[12]. This drug has since been classified as a category D drug by the Food and Drug Administration (FDA), which means that it is not safe or recommended in pregnancy ^[13]. “Nevirapine is associated with dermatological complications such as Stevens-Johnson syndrome and hepatic toxicity. Protease inhibitors (PIs) e.g. Kaletra have been associated with diabetes mellitus and lipodystrophy and have therefore fallen out of favour. The combination of Emtricitabine together with Tenofovir (TDF) (NRTIs) as a single dose regimen has also been associated with lactic acidosis and hepatomegaly.”^[12] In fact most ARVs have potentially serious side effects. For this reason the clinician must be able to triage patients appropriately to the various regimens in order to decrease these side effect profiles which, if neglected, may lead to poor compliance and/or complications.

Initially monotherapy with AZT was the regimen of choice for reducing vertical transmission of HIV. Thereafter, combination therapy was used because monotherapy was shown to pose a higher risk of drug resistance than combination therapy ^[5]. Combination therapy also showed a greater reduction in rates of transmission, even if started in the second or early third trimester. In South Africa there is an extremely high prevalence of HIV positivity in pregnancy. The public sector is not in a position to offer all HIV positive women an elective caesarean section to decrease vertical transmission. Combination therapy has in this sense “given” most women the option of a vaginal delivery with reduced risks of transmission especially in the group with undetected viral load ^[2].

The public sector can currently not afford to offer planned elective caesarean section to high risk HIV positive women due to cost.

In a large prospective study of 2 543 pregnant HIV infected women, monotherapy was compared to combination therapy. Tuomala et.al, found that combination therapy with PIs increased the risk of preterm delivery without differences in birth weight and still birth rates [14]. A similar study published by Thorne et.al, in 2012, investigated 4372 pregnancies exposed to HIV and HAART. They reported an increased rate of prematurity of up to 24.9% in the protease inhibitor group of ARVs and thus an increase in the mortality rate [15]. This raises concerns about the safety of various combinations of antiretroviral medications. Clinicians must constantly and systematically assess which agents and combinations give the best results in pregnancy. The goal is always to have the smallest number of adverse outcome for the mother and her fetus. However, Lambert et.al. found that exposed and treated women compared to non-exposed women had similar risk factors with respect to adverse pregnancy outcomes. They suggested that prenatal care and ARV medication would reduce adverse pregnancy outcomes [16]. There is much conflict of opinion displayed in the research arena with regards to antiretroviral treatment and this is fueled by the ever increasing amount of new information on the HIV.

Studies undertaken in Botswana investigated the association of HIV transmission in women on Highly Active Antiretroviral Therapy (HAART) and the risk of stillbirth. It was concluded that 'in-utero HIV infection was rarely associated with stillbirths and did not occur in those receiving HAART' [17]. The stillbirths in this study were mainly associated with hypertension and placental insufficiency. A further question that still needs to be definitively answered is whether there is a link between pre-eclampsia and treatment with HAART. In a prospective cohort study published by Hall and colleagues in 2014, pre-eclampsia and gestational hypertension was less common in HIV infected women being managed on mono or triple antiretroviral therapy [18].

2.2 The placenta

The placenta is a fetal organ which also affects the mother, providing functions such as gaseous exchange, excretion, maintenance of homeostasis, hormone secretion, hemopoiesis and hepatic metabolic functions [19]. The placenta comprises the parenchyma, membranes and the umbilical cord. It records all fetal and maternal changes and also serves as an endocrine gland and a gaseous exchange organ for the developing fetus.

The formation of the placenta and membranes is completed by the 12th to 13th week of pregnancy. Following implantation, the fetoplacental unit develops from hemangioblastic cells. These merge together to form primitive capillary vessels and under the influence of vascular endothelial growth factor (VEGF) and placental growth factor (PIGF), multiply to form an elaborate vessel network [20]. While it is clear that placental abnormalities may lead to fetal demise and maternal illness (as in the case of pre-eclampsia), the converse is also true. Maternal illness such as hypertension and auto-immune diseases may lead to placental dysfunction with associated adverse outcomes [18].

It is important to note that the effects of HIV on the placenta are still not well understood. The placenta is thought to have a protective role against HIV transmission to the fetus.^[21] 'Thus there is heightened interest in determining whether there are differences in the spectrum of placental lesions, particularly inflammatory lesions, between HIV-infected and -uninfected women and whether specific lesions are related to an increased risk of perinatal HIV transmission^[22]. Currently, there are no clear answers to these questions because the number of studies is small and findings on the effects of HIV on the placenta differ. The most common inflammatory lesion is chorioamnionitis observed in a third of cases. The most common non-inflammatory lesion is cytotrophoblastic hyperplasia, observed in about $\frac{3}{4}$ of the cases. Antiretroviral therapy does not diminish the incidence of these lesions^[23]. However, additional studies report either no or few placental differences to be associated with HIV. In a prospective study undertaken by Vermaak et.al, at Tygerberg Academic Hospital in the Western Cape Province of South Africa,

placentae from the HIV-positive pregnancies were characterized by decreased weight and an increased number of marginal infarcts in comparison to HIV-negative pregnancies. The most important microscopic finding in that study was the increased presence of villitis of unknown etiology (VUE) among the group of untreated HIV-positive women with CD4 cell counts of $< 200 \text{ cells/mm}^3$ [24].

A small prospective study was performed by D'Costa et al., in Mumbai (2006), where 51 placentae from HIV positive women were examined and compared with a matched control group from HIV negative women. The most common inflammatory lesion detected was chorioamnionitis (31.37%) while the dominant non-inflammatory lesion was cytotrophoblastic hyperplasia (76.47%). They concluded that, 'there was no significant decrease in the incidence of lesions following treatment with ARVs and there was no correlation between placental lesions and HIV in the newborn' [25].

Amirnessami-Aghili et al. showed that the trophoblastic cells of human placenta tissue express CD4 antigen and are susceptible to HIV-1 infection. They also demonstrated that the placental endocrine function is decreased in HIV-1 infection. Thus, the placenta may serve as a reservoir of HIV-1 infection during pregnancy, contributing to infection of the fetus, and decreased placental hormone production may result in impaired fetal development [26].

Indications for placental examination vary between sites, but there are published guidelines from the Royal College of Pathologists developed together with the British Paediatric Pathology Association (BRIPPA), Royal College of Obstetricians and Gynaecologists (RCOG), The Royal College of Paediatrics and Child Health (RCPCH) and the Royal College of Midwives. 'All placentas from stillbirths, intrauterine growth restriction (below 3rd centile), immaturity, cases of severe fetal distress requiring admission to a neonatal intensive care unit (NICU) and late miscarriages should be referred' [27]. These may be adapted to local needs prior to consultation with all stakeholders. The indications used at Tygerberg Academic Hospital based on these guidelines are included as Appendix C. It is currently uncertain whether placental

examination should be performed on uncomplicated HIV positive deliveries. This uncertainty exists due to the paucity of scientific information, but anecdotal cases and limited studies in South Africa suggest these mothers and babies might represent an “at risk” population.

In Tygerberg Academic Hospital, approximately 1400 HIV positive pregnancies are managed annually. In 2012, unpublished data showed that 1379 HIV positive patients were managed, of whom 731 were on HAART and 648 on other combinations to prevent vertical transmission. Patients not receiving HAART generally received AZT from 14 weeks gestation (or later) as well as Nevirapine and Truvada® (Emtricitabine and TDF) intrapartum. During the same year, there were 1343 live births and 107 stillbirths. This high stillbirth rate highlights the need for placental examination. It is important to ascertain whether the risk of stillbirth is independent or dependent on HIV infection. Another factor that must be kept in mind is that patients presenting to a tertiary academic institution such as Tygerberg Academic Hospital have other related or independent co-morbidities. For this reason it should be established whether the risk of stillbirth is related to the various complex maternal diseases alone or is associated with HIV infection as well. It has already been mentioned that a study performed in Botswana indicated that stillbirths had a greater association with maternal hypertensive disease rather than HIV infection of the mother [17]. These findings may be different in an urban academic hospital such as Tygerberg Academic Hospital. A study performed by Kennedy et.al.in an urban but secondary hospital located in the same city as Tygerberg Academic Hospital, found HIV infected pregnancies to be associated with significantly higher rates of stillbirth, antepartum hemorrhage, intra-uterine growth restriction and chorioamnionitis in the exposed group [28].

Finally, Chandwani and colleagues in a small prospective study of 20 placentae, examined the expression of HIV in placentas of seropositive women. They found that there was an increase in chorioamnionitis in HIV exposed placentas as opposed to the non-exposed placentae. However, they did not find villitis to be a significant finding. HIV antigens were found in 10% of placentae which harbored chorionitis, this was attributed

to the direct effect of HIV or co-existence with other infection ^[29]. This supports the postulate that chorioamnionitis is higher in the HIV exposed pregnancy.

There is paucity of scientific information in relation to the effect of HIV on the histopathology of the placenta. Tygerberg Academic Hospital has a high level of expertise and there is close co-operation between the Department of Obstetrics and Gynaecology and the Division of Anatomical Pathology. This team was therefore ideally placed to investigate this association further. The formal aim of this study was to investigate placental changes related to HIV infection and to correlate these with maternal characteristics and neonatal outcomes.

Chapter 3: Research methodology

3.1 Study setting

This retrospective, descriptive study was performed at Tygerberg Academic Hospital in the Western Cape province of South Africa. Patients requiring secondary and tertiary care from the Metro East region of Cape Town are referred to this centre for specialist management.

3.2 Aim

To investigate placental changes related to HIV infection and to correlate these with maternal characteristics and neonatal outcomes.

3.3 Objectives

- To describe the histopathological (macro- and microscopic) findings in placentae of mothers with HIV.
- To investigate the influence of maternal ARV therapy on histopathology.
- To investigate the influence of immune suppression (CD4 count) on histopathology.

3.4 Methods

A retrospective audit was performed on placentae collected between 2009 and 2010 (1400 cases) from mothers with HIV disease. Placentae were examined according to the standard protocol used by the Division of Anatomical Pathology (Appendix B). Data were extracted from the files and reports by the MMed student and loaded onto a database in a strictly anonymous fashion. Women with HIV and co-morbidities specifically affecting placental function such as diabetes mellitus and pre-eclampsia were noted but not excluded from the study. Maternal and neonatal data were extracted from the hospital files by Dr M Zungu (the primary investigator). This data was transferred to an anonymous

(only study numbers used) spread sheet by the primary investigator. The detailed pathology reports on the placentas were coordinated by Dr Schubert and then added to the data sheet by Dr Zungu.

Cases that were excluded included women with unknown HIV status, multiple pregnancies and stillborn babies at less than 26 weeks' gestation, as well as miscarriages. The data sheet is provided as Appendix C.

3.5 Sample size

One thousand, four hundred files were available. Of the available files 200 were eligible for review and 196 qualified for inclusion in the study.

3.6 Data analysis

The statistical analysis was performed together with Prof M Kidd of the Research and Support Division. STATA version 13 was used for data analysis ^[30]. Variables were tested for associations using cross tabulation and Chi square test. Summary statistics were reported as medians (with range) and frequencies with percentages. Where indicated descriptive statistics alone were used for data description. For summary purposes, the summary statistics including histograms, frequency tables, means and standard deviations were calculated.

Where indicated, the following tests were used:

- Mann-Whitney test for categorical variables
- Fisher's exact test for categorical variables less than ten
- Kruskal-Wallis ANOVA for categorical variables with more than two categories.

A p-value of $p < 0.05$ was used to determine statistical significance.

3.7 Ethical considerations

The protocol was submitted to the Research Ethics Committee of Stellenbosch University and thereafter Tygerberg Academic Hospital for approval (SU study No. S12/01/011). No patient identifying data were used on the data capture sheet. A waiver of consent was requested for this retrospective, anonymous audit. Only the primary investigator retained a patient identification log, as a separate document, in a secure location. This audit contains no deviation from standard clinical practice.

3.8 Anticipated benefits

The results should broaden our understanding of perinatal outcomes and of transmission of HIV disease.

CHAPTER 4: RESULTS

During the defined study period 1400 files were reviewed and 200 cases with documented maternal HIV seropositive status were identified with their placental histology reports. Three cases with twins were excluded due to difficulties in reporting various types of placentae accurately. The final number of placentae for the study was therefore 197. The descriptive data from the files of the HIV positive mothers is shown in Table 1.

Table 1: Maternal descriptive profile

Characteristic	Median (range) or n (%)
Age	27 (15-46)
Gravidity	2 (1-7)
Parity	1 (0-6)
BMI	28 (14-47)
CD4 count	306 (14-1108)
<350 n (%)	92 (46.9)
≥350 n (%)	104 (53.1)
Antiretroviral medication n (%)	
NIL	39 (19.8)
HAART	63 (32.0)
PMTCT	95 (48.2)
Smoking n (%)	24 (12.2)

CD4 = T-helper lymphocytes per cubic millimeter of blood; **BMI**= body mass index;

HAART = highly active antiretroviral therapy; **PMTCT** = prevention of mother to child transmission

The important clinical conditions identified in this group are shown in Table 2. Tuberculosis was found in four patients all of whom had a CD4 count of <350 cells/mm³. Two of these patients had not received antiretroviral medication while the other two received PMTCT and HAART respectively.

Table 2: Clinical conditions

Conditions	CD4 count/mm ³		P value	ARVs		
	CD4<350	CD4≥350		HAART	PMTCT	Nil
Pre-eclampsia	21	21	0.84	15 (35.7)	16 (38.1)	11 (26.2)
Diabetes mellitus	0	3	0.28	0	0	3 (100)
Tuberculosis	4	0		1 (25)	1 (25)	2 (50)
None	72 (78.3)	80 (76.9)	0.11	48 (31.6)	79 (52.7)	25 (16.4)

Data given as n (%); Patient may have > 1 condition. Statistical comparisons were performed on clinical conditions and CD4 counts (<350 vs ≥350/mm³) as well as clinical conditions and forms of therapy (HAART vs no therapy; HAART vs PMTCT; PMTCT vs no therapy and any therapy vs no therapy), but no statistically significant differences were found.

In the pre-eclampsia group shown in Table 2, 15 women received HAART, 16 PMTCT, while 11 received no treatment prior to delivery. With regard to the women in Table 2 where no significant clinical conditions (co-morbidities) were noted 48 received HAART, 79 PMTCT while 25 received no treatment prior to delivery. None of the groups, including HAART and PMTCT together were significantly different from each other in the “no co-morbidity” group in Table 2.

The specified cluster diagnoses noted on placental examination are shown in Table 3.

Table 3: Pathological diagnoses: macro- and microscopic features according to cluster diagnoses and CD4 count

Cluster diagnoses	CD4 count/mm ³		Total (%)	p-value
	CD4<350	CD4≥350		
Maternal vascular under perfusion (MVUP)	35 (50)	35 (50)	70 (36)	0.70
Placental insufficiency	1 (16.7)	5 (83.3)	6 (3)	0.26
Placental abruption*	6 (35.3)	11 (64.7)	17 (9)	0.43
Ascending infection	30 (51.7)	28 (48.3)	58 (29)	0.54
Hematogenous infection	5 (83.3)	1 (16.7)	6 (3)	0.36
Immunological damage	0	1 (100)	1 (0.5)	
Small placenta	11 (36.7)	19 (63.3)	30 (15)	0.28
Normal/ no pathology	6 (66.7)	3 (33.3)	9 (5)	0.44

Data given as n (%); Small placenta = placenta < 10th centile of weight for gestational age; P =0.05;

*macro- and/or microscopic diagnosis

The comparison of placental weights, CD4 counts and antiretroviral medications are shown in Table 4. The following applies to the antiretroviral treatment provided to the classes of cases in Table 4. In the category: placental weight <10th centile 32 cases (36.4%) received HAART, 38 (43.2%) received PMTCT while 18 (20.4%) received no therapy. In the category: placental weight >10th to 90th centile 17 (31%) cases received HAART, 31 (56.3%) received PMTCT while 7 (12.7%) received no therapy. None of these differences were significant. In the category: placental weight >90th centile 6 (31.6%) cases received HAART, 9 (47.4%) received PMTCT while 4 (21%) received no therapy. None of these differences were significant. There were four documented cases of tuberculosis. In three of these, the placental weight was below the 10th centile whilst the fourth placenta fell in the 10 to 90th centile category.

Table 4: Placental weight and ARV medication

Placental weight	CD4 count/mm ³		P value
	<350	≥350	
<10 th centile	37 (42.0)	51 (58.0)	0.12
10-90 th centile	33 (60.0)	22 (40.0)	0.03
>90 th centile	8 (42.1)	11 (57.9)	0.57

Data given as n(%)

The comparison of cluster diagnoses to antiretroviral treatment is shown in Table 5.

Table 5: Cluster diagnoses and ARVs

Cluster diagnosis	HAART	PMTCT	NIL	P value
Maternal vascular under perfusion (MVUP)	23 (32.8)	34 (48.6)	13 (18.6)	NS all
Placental insufficiency	1 (16.7)	4 (66.6)	1 (16.7)	NS all
Placental abruption*	5 (29.4)	12 (70.6)	0 (0)	NS
Ascending infection	13 (22.4)	32 (55.2)	13 (22.4)	NS all
Hematogenous infection	2 (33.3)	2 (33.3)	2 (33.3)	NS all
Immunological damage	0 (0)	1 (100)	0 (0)	
Small placenta	12 (40)	10 (33.3)	8 (26.7)	NS all
Normal	6 (66.7)	2 (22.2)	1 (11.1)	0.06

*macro- and/or microscopic diagnosis; p-value comparisons

The cluster diagnoses present in the cases with pre-term birth are shown in Table 6. There were 43 cases of pre-term birth at ≤ 32 weeks' gestation and nine cases from 33-36 weeks' gestation. The CD4 counts and antiretroviral treatment of the cases with pre-term birth are shown in Table 7.

Table 6: Cluster diagnoses and preterm delivery

Cluster diagnoses	Preterm delivery		P value
	≤32 weeks' gestation	33-36 weeks' gestation	
Maternal vascular under perfusion (MVUP)	11 (73.3)	4 (26.7)	0.44
Placental insufficiency	3 (100)	0 (0)	
Placental abruption*	3 (60)	2 (40)	0.26
Ascending infection	17 (89.5)	2 (10.5)	0.58
Hematogenous infection	3 (100)	0	
Small placenta	6 (85.7)	1 (14.3)	0.89

*macro- and/or microscopic diagnosis

Table 7: Preterm delivery, CD4 counts and antiretroviral therapy

Preterm delivery	CD4 cells/mm ³		Antiretroviral therapy		
	<350	≥350	HAART	PMTCT	NIL
≤32	22	22	13 (29.6)	22 (50)	9 (20.4)
33-36	4 (44.4)	5 (55.6)	4 (44.4)	4 (44.4)	1 (11.2)

Data given as in n(%); p-value comparisons. Statistical comparisons were performed on clinical conditions and CD4 counts (<350 vs ≥350) as well as preterm delivery and forms of therapy (HAART vs no therapy; HAART vs PMTCT; PMTCT vs no therapy and any therapy vs no therapy), but no statistically significant differences were found

The neonatal data for the study are shown in Table 8.

Table 8: Neonatal data

Characteristic	Median (range)
Gestational age at delivery (weeks)	32 (25–43)
Apgar score < 7 at 5min n (%)	14 (7.1%)
Delivery method	
Caesarean section	79 (40.3)
Normal vaginal delivery	117 (59.7)
Birth weight	1840 (180–3860)
≥ 2500g	61 (31.1)
1500- 2499g	66 (33.7)
<1500g	69 (35.0)

Data given as median (range) or n(%)

CHAPTER 5: **DISCUSSION**

The aim of this retrospective study was to investigate placental changes related to HIV infection and to correlate these with maternal characteristics and neonatal outcomes. The main histopathological (macro- and microscopic) findings demonstrated small placentae (15%) with maternal vascular under-perfusion (36%) and ascending infection (29%). No significant associations between histopathological cluster diagnoses findings and maternal ARV therapy or maternal CD4 count (as a marker of immune competence), were demonstrated.

The study population was chiefly comprised of young, non-obese, multiparous women of whom a surprisingly high percentage (20%) did not receive any form of antiretroviral medication. This is surprising for the metropolitan area of Cape Town as the service has been rigorously implemented. However, many such patients arrive unbooked or with intra-uterine deaths. In addition, ARV therapy may be delayed due to maternal illness or prescribed, but not taken by the patient due to unexpressed fears.

5.1 Placental pathology findings and clinical outcomes

As stated above, the main placental findings in this study were maternal vascular under-perfusion (MVUP) (36%), ascending infection (which includes chorioamnionitis) (29%) and small placenta (15%). However there were no significant differences between the CD4 counts of <350 and ≥ 350 / mm³ within the pathological cluster diagnosis groups.

In a prospective single-blinded pilot study conducted by Vermaak et al., (2012), where the study population comprised of 91 HIV infected patients, there were associations between villitis of unknown aetiology (VUE), decreased placental weights and an increase in the number of marginal infarcts [24].

Various hypotheses have been put forward to explain the increase in chorioamnionitis (CAM). In a prospective study undertaken by Ladner et al. in Kigali, examining the role of

chorioamnionitis in women treated for sexually transmitted infections in pregnancy, 561 women were followed up from 24-28 weeks. There was no difference between the study and control group, with the study group consisting of HIV seropositive pregnant women with low CD4 counts ($<200/\text{mm}^3$). It was concluded that CAM was not associated with treated sexually transmitted infections. Chorioamnionitis was strongly associated with poor pregnancy outcomes in HIV infected women and this was likely due to the deleterious effect of HIV in the marginal infarcts in the immunosuppressed group with CD4 count $<200/\text{mm}^3$ [31].

These findings highlight the paucity of and conflicting information in the body of evidence on the placental findings. Reporting of pathological diagnoses should be standardized to enable findings to be compared. In this regard the Tygerberg/Stellenbosch group has laid great emphasis on specific findings and cluster diagnoses [24]. In addition, more studies need to be performed, preferably prospective ones that include a control group. The ARV regimens have changed regularly over a relatively short period of years and the aim is that untreated pregnant women will be kept to an absolute minimum. Nonetheless, in low socio-economic circumstances there will always be women who are unaware of their HIV infected status and who do not book for care during their pregnancies. In 1998 Schwartz et.al, performed a prospective study in Thailand and demonstrated an increase in the rate of CAM in the HIV positive population compared to HIV negative women, translating to an odds ratio of 2:1. More villitis was also observed and they concluded that there was an increase in the inflammatory placental lesions in the HIV population. However, the findings did not translate to an increase in HIV transmission to the baby [22].

In 2010 Shapiro et.al. investigated the association of HAART and a possible increase in stillbirths in a moderately sized, prospective study performed in Botswana [17]. They found no association between HAART and stillbirths and postulated that the increased rate seen in tertiary centers was linked to the high prevalence of hypertensive diseases seen in tertiary care.

Another longitudinal follow-up study investigated the direct and indirect causes of maternal mortality among 499 patients admitted to certain hospitals in Uganda. HIV/AIDS

was noted to be the most significant independent predictor of maternal mortality with an OR of 5.1. This was attributed to the lack of antiretroviral medication. Other predictors of maternal mortality included lack of blood, not booking and living at a distance of more than 10km from the hospital in an area with poor infrastructure and transport [32].

In 2006 Cibulka, emphasised the importance of a viral load of < 1000 copies or undetectable in order to reduce transmission rates [33]. In the pre HAART era, transmission rates were as high as 25 to 30%. However, with the introduction of the single dose regimen with zidovudine, the transmission rate decreased to 8% [4]. Unfortunately this mode of therapy was complicated by an increase in viral mutations which lead to resistance to the drug. The recommendations were therefore changed to include two nucleoside reverse transcriptase inhibitors in addition to a protease inhibitor to ensure adequate suppression of viral replication. In the American population described by Cibulka, a CD4 count of less than 500 copies per cubic millimeter was viewed as being low and hence these patients were started on HAART to suppress the viral load thus improving maternal outcomes and also decrease perinatal HIV transmissions. The influence (both direct and indirect) of the various ARV medications on the histopathological findings has been poorly described. Although the index study sought to investigate the influence of such therapy on the histopathological findings, it is difficult to separate the placental findings from the effects of the stage of the disease itself or associated habits and infections. Substance abuse (smoking and cocaine use in particular) and other sexually transmitted infections have been shown to be associated with placental damage which translates into increased risk of HIV transmission. This in turn, results in increased perinatal morbidity due to preterm birth and chorioamnionitis [33]. An interesting finding in the index study was that 69% of the babies fell into the low or very low birthweight categories. This is probably because of a large number of pre-term births due to medical or iatrogenic reasons. Another interesting finding was that more placentae fell into the normal weight category in the <350/mm³ CD4 count compared to ≥ 350 CD4 count range/mm³.

A large recent retrospective South African study examined 18870 births, of which 3259 (17%) had HIV infected mothers. They observed a trend in increased perinatal mortality in the HIV positive group compared to the negative group. The main causes of morbidity were infections, intrauterine growth restriction (IUGR) and antepartum hemorrhage (APH) [28]. The findings of the study may have been strengthened by examination of the placentae. Histopathology may confirm or sometimes exclude clinical findings and as already stated insufficient attention has been given to placental examination in HIV infected pregnancies. Lack of expertise in histopathological analysis of the placenta and cost factors would often preclude this service.

The findings of the index study suggest a low prevalence of smoking (12%). Although it is difficult to substantiate this finding, the placental changes may not be attributed to smoking alone. At the time of the index study, most patients received mono- or dual antiretroviral therapy for prevention of mother-to-child transmission as the intended roll-out of HAART for patients with CD4 counts $\leq 350/\text{mm}^3$ was still to be in the pipeline. What is of concern is that a large percentage (20%) of cases received no ARV medications at all. This could be explained by patient-related factors such as non-attendance and birth before arrival as well as non-compliance with prescribed medication. In addition, if a patient presents with an intra-uterine death, prevention of mother-to-child transmission is not applicable. Finally, the failure to prescribe ARV's by doctors must be considered.

Although the finding of maternal vascular under-perfusion was more frequent in patients receiving ARV's than those not receiving treatment, this difference was not significant.

5.2 Co-morbidities

The commonest comorbidity was pre-eclampsia (42 cases) that was also seen to overlap with preterm deliveries, which accounted for 53% of cases. It should be emphasized that this study was performed in a tertiary institution. Pre-eclampsia is a common indication for referral and often leads to iatrogenic preterm delivery in order to optimize maternal and neonatal outcomes. What is of greater importance is the onset of "spontaneous" preterm

birth that may be initiated by ascending infection as noted in the cluster diagnoses of the pathology reports. In this study there was no demonstrated evidence of pre-eclampsia and HIV or with the various treatment regimens. Previous studies have found no link between HAART and pre-eclampsia, however they did find a risk between pre-eclampsia and low birth weight infants [34, 35]. In a prospective cohort study performed by Hall et al. in 2013, it was reported that pre-eclampsia and gestational hypertension are less common in HIV infected women being managed with mono- or triple (HAART) anti-retroviral therapy^[18]. A prospective observational study done by Landi et.al in 2014, found that pregnant women with HIV infection seem to be protected against gestational hypertension and pre-eclampsia and this protective effect also remains in a high risk population, such as African-American Black ethnic group. The effect is present independently from treatment received and virus copies. The changed immune response present in early pregnancy may explain the altered trophoblast invasion resulting in a better placentation [35].

Tuberculosis was observed in four of the placentae, and all patients were found to have CD4 counts $<350/\text{mm}^3$. Two patients were not exposed to ARVs. One was given PMTCT and the other received HAART. The combination of TB and HIV is well described [36]. Diabetes was observed in three of the patients. All of these had a CD4 count less than $350/\text{mm}^3$ and did not receive antiretroviral medication. Tuberculosis and HIV are intimately related. HIV is driving the TB epidemic in many countries [36]. Looking at the correlation between maternal mortality, HIV and TB, Jamison et.al. stated that maternal mortality was on the rise with the majority of maternal deaths being due to TB in combination with HIV and concluded that treatment of this condition would lead to improved maternal and neonatal outcome [37].

HIV infection carries morbidity and mortality risks for both the mother and her unborn child. The use of antiretroviral medication has decreased HIV transmission rates to 2%, which has resulted in improved neonatal and maternal outcomes in particular in the Western Cape Province of South Africa ^[4]. The histopathological (macro- and microscopic) findings of placentae from HIV infected pregnancies need to be strengthened by larger studies with controls. However in order to allow comparison, the categories should be standardized, as in this study.

CHAPTER 6: APPENDICES**Appendix A: Maternal Data**

Study no									
Age									
Gravidity				Parity					
BMI									
Smoking	YES				NO				
CD4 count									
ARV treatment	HAART				PMTCT			NIL	
Co-morbidity conditions									
	Pre-eclampsia								
	Diabetes mellitus								
	Rheumatoid disease								
	Other								
Placental function (Doppler)									
	>75 th centile								
	75-95 th centile								
	>95 th centile								
	*AEDF								
	*REDF								
Neonatal									
	Gestational age at delivery								
	Indication for delivery	Preterm labour			Pre-eclampsia			Other	
	Apgars								
	>7	YES				NO			
	Birthweight								
Delivery method									
	Normal vaginal delivery								
	Caesarean section								
	Instrumental								

*AEDF = Absent end-diastolic flow / *REDF = Reversed end-diastolic flow

Appendix B: Combinations of macroscopic and microscopic placental features used for cluster diagnoses

Maternal uteroplacental insufficiency (minimum of 3 criteria present)

- Small placenta (below 10th percentile for gestation)
- Extensive infarction microscopically
- Extensive infarction (>20% at term; >15% if premature)
- Accelerated maturation
- Distal villous hypoplasia
- Decidual vasculopathy
 - Increased syncytial knots
 - Increase perivillous fibrin
 - Increased extravillous trophoblast (islands)

Fetal thrombotic vasculopathy (at least 2 criteria present)

- Cord thrombosis
- Chorionic plate/stem villous thrombosis
- Avascular villi
- Villous-stromal karyorrhexis

Placental abruption

- Macroscopic abruption >15% adherent retroplacental hematoma
- Microscopic abruption (at least 2 criteria present)

Retroplacental hemorrhage

Overlying infarction

Intravillous hemorrhage

Treponemal infection (at least 2 criteria present)

- Diffuse villitis, chronic
 - Delayed maturation
 - Necrotizing funisitis
-

Appendix C: Indications for placental histology at Tygerberg academic Hospital

Placental histology must be requested in all the following cases of singleton and multiple pregnancies:

1. All unexplained stillbirths 34+ weeks or more than 2.0 kg. This excludes cases of abruptio placentae, cord prolapse and syphilis. In case of uncertainty, the placenta must be kept with the body for Prof de Jong's opinion.
2. Indications of asphyxia in a viable baby. This group consists of all neonates who required resuscitation, unless clearly due to abruptio placentae or prolapse.
3. Second or higher order midtrimester loss.
4. Idiopathic preterm labour (gestational age less than 34 weeks or birth weight less than 1800 g).
5. Suspected subclinical chorioamnionitis.
6. Cases of severe intrauterine growth restriction ***without*** antenatal work-up (Doppler and ultrasound).
7. Multiple pregnancies:
 - All applicable indications that would be relevant in singleton pregnancies.
 - All multiple pregnancies with uncertain chorionicity at time of birth.
8. Congenital abnormalities without prior diagnosis (unless otherwise requested by Prof de Jong or Prof Geerts).
9. Cases of severe pre-eclampsia if requested by Special Care Unit.

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