REVIEW OF PLACENTAL HISTOLOGICAL CHANGES IN STILLBIRTHS

AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

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Research report for Master of Medicine in Obstetrics and Gynaecology

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Declaration

The research is submitted to the University of the Witwatersrand by Dr Pearl Maseko to faculty of Health Sciences, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in Obstetrics and Gynaecology.

I, Pearl Maseko hereby declare that the research report is my own work. It does not contain any work from another source. I declare that this research has not been submitted to any other institution.

Signature: PKM.

Date: 6 December 2022

Dedication

I dedicate this study to my late loving father Mr Ncinti Mhlongo for getting me where I am today and my supportive husband Mr Nkosi Maseko.

Abbreviations

Abbreviation	Explanation
ARV	Antiretrovirals
AZT	Zidovudine
CEO	Chief Executive Officer
CD4	Cluster of differentiation 4
CHBAH	Chris Hani Baragwanath Academic Hospital
DNA	Deoxyribonucleic Acid
FPR	Feto Placental Ratio
FSB	Fresh Stillbirth
GDM	Gestational Diabetes Mellitus
HAART	Highly Active Anti-Retroviral Therapy
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HPT	Hypertension
HREC	Human Research Ethics Council
IUGR	Intrauterine growth restriction
MSB	Macerated Stillbirth
MTC	Mother to child transmission
NHLS	National Health Laboratory Service
PASS	Prenatal alcohol in sudden infant deaths and stillbirth
PCR	Polymerase chain reaction
PPIP	Perinatal Problem Identification Programme
Rh	Rhesus status
RPR	Rapid Plasma Reagin
WHO	World Health Organisation

Abstract

Introduction

Placental histology plays a vital role in determining the cause of stillbirths. A universal classification system has helped to standardise the interpretation of placental histological findings. The purpose of this study was to determine the placental lesions identified in stillbirths and their significance. The study describes histopathological changes in placentas of stillbirths, the association between placental findings and cause of fetal death and impact of maternal HIV infection on placental findings.

Methods

A retrospective cross-sectional study was performed at Chris Hani Baragwanath Academic Hospital in Soweto to identify placental lesions found in placentas of stillbirths. Data were abstracted from placental histology reports and case records from January to December 2017.The Amsterdam classification was used to classify placental findings

Results

A total of 625 stillbirths occurred at Chris Hani Baragwanath Academic Hospital between January and December 2017. In all the placentas from stillbirths sent for histology, 204 of the placentas met the inclusion criteria for the study. Maternal vascular malperfusion (MVM) was the most common finding on placental histology affecting 36.3% of cases, and the majority of placentas with MVM were from hypertensive mothers.

Histological changes in keeping with chorioamnionitis were the second most common lesions, found in 24.5% of cases. There were 62% of the total number of placentas with chorioamnionitis that had acute chorioamnionitis suggesting a recent infection. Hypertension in pregnancy and its complications was associated with 31.3% of placental abruption. Placental infarction was found in 40.6% of placentas and the majority of the mothers with infarction had hypertension in pregnancy and its complications.

There was no association between stillbirths and extremes of maternal age. HIV positive pregnant women who were not virally suppressed had the same number of stillbirths

compared to virally suppressed mothers. Women with maternal comorbidities had an increased risk of having a stillbirth. Hypertensive disorders complicated 29% of pregnancies in this study.

Conclusion

Maternal vascular malperfusion (MVM) was the most common finding on placental histology of stillbirths followed by chorioamnionitis. Hypertension was the commonest finding in mothers with stillbirths and maternal vascular malperfusion.

Acknowledgements

I am thankful to Chris Hani Baragwanath Academic Hospital record department for all their assistance, the NHLS (National Health Laboratory Services) for their help and granting me permission to utilize the histology reports, as well as the pathology department for their assistance, and for the extra effort of re-reporting the cases for the sake of this study. I would also like to express my gratitude to my two supervisors, Dr Maswime and Dr Wise, for their assistance and patience.

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1.1.1.1 Chapter 1: Extended Literature Review

1.1 Introduction

Globally 7200 stillbirths occur daily, and there is a deficit of data on the causes of stillbirth in low and middle income countries[1].The number of unexplained stillbirths is a public health concern, reflecting the lack of resources to investigate stillbirths in some settings. In the Saving Babies report in South Africa, an estimated 32 662 stillbirths were reported between the year 2012 – 2013[2]. An estimate of 2.6 million stillbirths occurred worldwide in 2015, the vast majority occurring in developing countries[2]. The Every Newborn Action Plan strategy by the World Health Organisation (WHO) aims to reduce the stillbirth rate from 32 per 1000 in Sub Saharan counties to less than ten stillbirths per 1000 by 2035[3]. Stillbirths have decreased at a rate of 2-3% annually from 2010 to 2016[4] .These rates need to double in order to achieve the Every Newborn Action Plan goals.

WHO defines a stillbirth as a baby born with no signs of life at or after 28 weeks gestation[5]. Increased survival of live infants born between 24 and 27 weeks especially in high income countries has led to the inclusion of infants born in this category in the stillbirth definition in some countries[6]. Lack of information on causes and patterns leading to stillbirths creates a challenge when explaining the cause of death to women who have delivered a stillbirth[3].

Histopathological examination of the placenta has reduced the number of unexplained stillbirths. Understanding of placental findings and interpretation promises better results and hope in preventing preventable stillbirths and recognising the cases where recurrence is most likely. Studies done comparing placentas of live babies and stillbirths, has shown that most of the lesions found in stillbirths also occur in placentas of live healthy babies[4,10]. However the extent of the lesions and the involvement of the placenta was increased in stillbirths compared to livebirths. Maternal infection and comorbidities are associated with an increased risk of stillbirth[4].

1.2 Classification systems

1.2.1 A review of various classification systems was published in 2016[9]. This review was conducted prior to adoption of the Amsterdam criteria. A total of 81 classification systems were compared against 17 characteristics identified by experts as required to establish an effective universal classification system for stillbirths and neonatal deaths[9]. A good classification system was required to have at least 9 of the 17 characteristics. These included 8 structural and 9 functional characteristics. None of the 81 classification systems were aligned with more than 9 of the 17 characteristics, this led to ongoing development of new classification systems.

The 8 structural characteristics for a global system include being able to use rules to ensure valid assignment of cause of death categories, be able to work with all levels of data (from both low-income and high-income countries), including minimal levels, must ensure cause of death categories are relevant in all settings, must require associated factors to be recorded and clearly distinguished from causes of death, distinguish between antepartum and intrapartum conditions, should record the level of data available to assign the cause of death, must have multiple levels of causes of death, with a small number of main categories and lastly include a sufficiently comprehensive list of categories to result in a low proportion of deaths classified as "other. Functional characteristics for a global system must be easy to use, and produce data that are easily understood and valued by users, have clear guidelines for use and definitions for all terms used, must produce data that can be used to inform strategies to prevent perinatal deaths, must require neonatal deaths to be clearly distinguished from stillbirths, must have high inter- and intra-rater reliability (there should be consistency between observers and different types of measures) must be available in different formats including inexpensive e-health and m-health options, and in multiple languages, must allow easy access to the data by the end-users, incorporate both stillbirths and neonatal deaths and must require the single most important factor leading to the death to be recorded. The classification system that was most aligned of the 81 systems was Frøen 2009-Codac followed by Korteweg 2006-Tulip.

Non of the systems aligned with more than 9 of the characteristics of a globally accepted system. This led to an unmet need for an internationally accepted system.

Amsterdam Criteria

The protocols for analysing the placenta and definition of placental lesions differed between institutions and this impeded in improving perinatal care and outcome. Specialists including

perinatal pathologist, neonatal pathologists and fetal medicine specialists were invited to a one day workshop in Amsterdam to derive recommended standards, where international definitions for placental lesions were described and controversies were discussed. In order to create uniformity in placental sampling and diagnostic criteria, a sampling and definition of placental lesions workshop group consensus statement was made[7]. In this workshop in 2015, placental, neonatal pathologists as well as maternal fetal medicine specialists developed a consensus statement for the classification for placental histology. The classification criteria describes maternal vascular malperfusion (MVM), Fetal vascular malperfusion (FVM), delayed villous maturation, ascending intrauterine infection and villitis of unknown aetiology[7].

1.2.1.1 Maternal vascular malperfusion

Maternal vascular malperfusion occurs due to defective spiral artery remodelling during implantation[7]. Features of MVM include placental hypoplasia, any percentage of infarction in preterm placentas and more than 5% non-peripheral and non-marginal infarction in term placentas. The last feature of MVM was retroplacental haemorrhage. Microscopic features include distal villous hypoplasia and accelerated villous hypoplasia. A minimum of 3 features, preferably 4 needed to fulfil the classification criteria or presence of a small placenta and 2 to 3 other features.

1.2.1.2 Fetal vascular malperfusion

Fetal vascular malperfusion lesions are caused by obstruction of fetal blood flow which could be from umbilical cord lesions, sequele of fetal cardiac dysfunction, hypercoagulable states and multiple other conditions[7]. Lesions indicative of FVM include thrombosis, villous stromal vascular karyorrrhexis and segmental avascular villi. Other markers included vascular ectasia, vascular intramural fibrin deposition and fibromuscular sclerosis.

1.2.1.3 Delayed villous maturation

Delayed villous maturation occurs after 34 weeks and it is characterised by repetitive villous with reduced vasculosyncytial membranes, capillaries placed at the centre and a continuous cytotrophoblast layer[7]. Villous maturation is variable, either accelerated or delayed. It can be graded as focal or diffuse.

1.2.1.4 Ascending intrauterine infection

Histologic chorioamnionitis does not equal to clinical chorioamnionitis. Identification of inflammation is essential more than grading of the inflammation[7]. In order to separate maternal and fetal inflammation, describing the topography of the inflammation is necessary since fetal inflammatory response is associated with fetal outcomes. Acute and chronic inflammation should be distinguished[7].

1.2.1.5 Villitis of unknown origin

Villitis of unknown aetiology can only be diagnosed histologically and it includes only cases where the cause of a villitis is unknown[7]. Diagnosis is made when there is 62% of villitis in 3 parenchymal blocks with a maximum of 6 to 7 parenchymal blocks with 85% villitis. It is associated with lymphohistiocytic infiltrates and may have a few plasma cells. Villitis of unknown aetiology is graded as low or high grade, with high grade more associated with fetal growth restriction, neurodevelopment impairment and recurrence. In Amsterdam, other placental grading systems were not looked at in detail. Lesions like infarction, looking at the size of infarction was recommended.

More than one classification criteria can be met in one placenta. The Amsterdam criteria recognizes that with improvement in knowledge of placental pathophysiology these definitions and categories will be revised.

1.2.2 PASS study

Knowing the aetiology of stillbirths can be used to predict risk factors for future pregnancies. The PASS study (Prenatal alcohol in Sudden Infant Death Syndrome and Stillbirth) was done to[8] illucidate the relationship between cigarette smoking, prenatal alcohol consumption and poor outcome and stillbirths[8].The PASS classification has been proposed to enhance the international classification system for stillbirths[8]. PASS used 5 sites of origin to find the cause of death (fetal, placental, maternal, external and undetermined). It also categorised causes of death by the mechanism of action leading to a stillbirth and whether sporadic or recurrent[8]. The aim was to form a classification system that would use both the clinical and pathological evidence to determine the cause of death and clearly recognise the cases where the cause of death was not determined. This would allow for the conclusion of whether the cause of death was recurrent or isolated. The secondary aim was to create an algorithm that could be used

even when the pathology and clinical data is insufficient to determine the cause of death. This formula can be used worldwide, including low income areas where resources are limited to try to establish the cause of death. Stillbirths were classified into aetiology, associated diseases/predisposing factors, amount of information attainable and recurrent risk. In this study, causes of stillbirth were classified as fetal (26%), placental (53%), external (5%) and undetermined (16%). Stillbirths caused by placental abnormalities were attributed to maternal disorders mostly acute on chronic perfusion failure[8].These carried an inheritant risk leading to a high risk of recurrence. The most common underlying fetal cause of death was from external substances e.g. infection introduced into the fetus. These findings emphasized the significance of external, maternal and environmental factors affecting the fetus and alternately leading to a stillbirth[8]. PASS classification was further compared to INCODE and ReCoDe systems since these classification systems were the recent and most frequently used for stillbirths[8]. INCODE (Initial causes of fetal death) created by Stillbirth Collaborative Research Network was found to be the most user friendly system, identifying specific and allowed grading to probable and possible cause of death. ReCoDe effectively identified conditions that were present during the process of fetal demise. The PASS system demonstrated the underlying pathophysiology compared to INCODE and ReCoDe, easy to use and could be implemented with little data[8].

1.3 Placental findings

Previous studies done to identify placental findings in stillbirths included a study done by The Institute of Child Health and Human development, comparing placental histology in stillbirths and live births[10]. The placental lesions disorders were classified into three groups; developmental, inflammatory and circulatory. Frequently observed placental abnormalities included inflammatory, thrombotic and retro placental hematomas[10]. Acute chorioamnionitis and inflammatory lesions predominantly caused by infection were common before 24 weeks gestation in both live and stillbirths. Retroplacental hematoma had a higher prevalence in stillbirths and preterm labour when compared to live births (23.8% vs 4.2%) except in gestational age less than 24 weeks, in which case retroplacental clot on placental examination was similar at 36%. This association may imply a contribution to preterm births and stillbirths. Parenchymal infarction was more prevalent in stillbirths compared to live births. The occurrence was greater than 35% at 24 to 31 weeks gestation and 32 to 34 weeks gestation compared to 6.4% in live births. Maternal circulation lesions were more frequent in stillbirths

than live births. The outcome from this study indicated than inflammation and retro placental hematoma are predominant in early stillbirths and live births whilst parenchymal infarction and thrombosis were common in later gestation in stillbirths[10].

1.4 Causes of stillbirth

A systematic review by Amina et al., described the causes of stillbirths and contributing factors to stillbirths in low and middle income countries[11]. The results showed that in low and middle income countries maternal disease were predominantly associated with stillbirths, as well as asphyxia, trauma and intrapartum infection[11]. Maternal diseases included hypertension in pregnancy, diabetes, syphilis and HIV. In high income countries where the cause of death was known, placental cause was the most common cause of death. Risk factors included advanced maternal age, gestational age of birth, parity, maternal comorbidities and poor antenatal clinic follow up. Improvement of screening and adequate antenatal care of pregnant mothers would markedly lower the stillbirth rate in low and middle-income countries[11].

1.5 Prediction and prevention of stillbirths

A case control study was done in 2011 in the United States of America to identify the relationship between the abnormalities associated with fetal growth, placental abnormalities and stillbirths[12]. The study included stillbirths and a control group of livebirths placentas. A total of 25 placental findings were investigated. Ten placental findings were associated with both stillbirths and fetal growth abnormalities. Placental infarction was found in stillbirths and live births with fetal growth anomalies but with a higher percentage in stillbirths compared to live births[12]. Stillbirths had a lower gestational age and birth weight compared to live infants. These findings suggest that placental function supports fetal growth and development and its impairment results in fetal growth affectation due to vascular malperfusion consequently leading to stillbirths.

This study also show that placental abnormalities can lead to stillbirth without fetal growth affectation suggesting a different mechanism of placental impairment[12]. In stillbirths were the fetal weight was not affected, a more acute and severe insult led to fetal death more rapidly.

Five relationships between placental findings, fetal growth anomalies and stillbirth were observed[12]. These included stillbirth with fetal growth anomalies, stillbirth without fetal growth anomalies, live births with fetal growth anomalies, live birth and stillbirth with fetal growth anomalies in the same manner and lastly stillbirth and live birth with fetal growth anomalies in a different manner. The birth weight to placental weight ratio could be estimated during the prenatal period using estimated fetal weight to placental volume ratio obtained using ultrasound[12]. The indication for delivery would be an abnormal ratio which carries an increased risk for stillbirth therefore preventing a stillbirth in a situation where there is fetomaternal malperfusion affecting fetal growth. The placental findings were categorised into maternal, fetal vascular malperfusion and chorioamnionitis[12].

Causes of IUGR can be classified into placental, fetal and maternal. Sixty percent of stillbirths are unexplained by fetal, placental, maternal or obstetric causes[10, 11]. Abnormal placentation is associated with development of preeclampsia, preterm labour, miscarriages, IUGR and intrauterine fetal death. Placental abnormalities can produce contrasting results in different pregnancies, fetal demise in one pregnancy and IUGR in another[12].

1.6 Association between IUGR fetuses and Stillbirths

The saving babies 2012-2013 report found that most unexplained stillbirths are undiagnosed IUGR fetuses, post term pregnancies, fetuses with congenital infection and congenital abnormalities[2].

Normal growth of the fetus is dependent on adequate supply of oxygen and nutrients from maternal circulation [13]. Adequate and proper functioning of the placenta is of vital importance for a healthy pregnancy. Placental pathology and inadequate oxygenation lead to adaptive changes in the fetus and placenta to compensate for the dysfunction[12, 13]. Failure of adaptive changes ultimately lead to fetal demise. In a study done in 2011 to investigate changes in placental histology in pregnancies of IUGR fetuses and stillbirths of unknown cause, placentas were collected in pregnancies with IUGR, unexplained stillbirths and live healthy fetuses[13]. Placental infarction was observed in 58% of fetuses with IUGR, 62% in intrauterine fetal death and 4% in live fetuses. Intervillous thrombosis was found only in intrauterine fetal

deaths and it may be a feature of intrauterine death. No major villous stem occlusions were found in placentas of IUGR fetuses and stillbirths. Chorionic vessel occlusion was found in IUGR and intrauterine fetal death. This outcome was not found in placentas of healthy babies. It was concluded from the study that infarction, chronic villitis, intervillous thrombosis, haemorrhagic endovasculitis, placental intravascular thrombi, perivillous fibrin deposition, fibrinoid necrosis, erythroblastosis and villous edema were the abnormalities causing a normal fetus to become growth restricted and die, but the magnitude of these lesions and clinical effect could not be defined. It was concluded that a relationship exists between changes in the placentas of IUGR fetuses and intrauterine fetal death[13].

1.7 Stillbirths and Preeclampsia

A sub analysis of a population-based case control study of stillbirths was performed to compare placental abnormalities in women with and without pre-eclampsia[14]. A comparison of placental pathology between livebirths and stillbirths in preeclamptic women was also conducted. Placental abnormalities were responsible for 23.6% of stillbirths, with obstetric conditions at 29.3%, fetal abnormalities at 13.7%, infections 12.9%, umbilical cord abnormalities 10.4%, hypertensive disorders 9.2% and other maternal conditions at 7.8%. Parenchymal infarction was the lesion predominantly found in women with pre-eclampsia in preterm deliveries[14,13] . This group had an increased number of histologic placental abnormalities and more severe abnormalities compared to term pregnancies. Placental lesions observed were predominantly lesions of maternal malperfusion[13] . There was an increased amount of placental hypoplasia and placental vascular lesions in deliveries before 34 weeks gestation compared to term deliveries[14].

IUGR was associated with decreased fetoplacental ratio implying a poorly effective placenta. Preeclampsia was associated with higher fetoplacental ratio when compared with stillbirths unaffected by preeclampsia[14].This finding is likely to be due to decreased fetal perfusion[12,13].

1.8 Infarction and stillbirths

Infarction is defined as an area of necrosis with ischaemic villi causing placental insufficiency [15]. This occurs commonly from disruption in maternal blood flow. Infarction can occur anywhere in the placenta but most commonly on the placental periphery[15]. It is associated with postdates, hypertension in pregnancy, abruptio placenta, chronic nephritis, systemic lupus erythematosus, diabetic microangiopathy and antiphospholipid syndrome. Infarction is not frequently visualized on ultrasound, when visualized it appears as hypoechoic regions with an enhancing rim[14]. The sequelae of placental infarction includes IUGR, fetal death and recurrent miscarriages[13]. In the Amsterdam classification system, infarction is one of the criteria under maternal vascular malperfusion[7].

1.9 Infections and stillbirths

A systematic review was conducted in 2003 to determine the connection between different perinatal infections and stillbirths[16]. Four mechanisms of infection were recognised. These included direct infection through intact membranes, ruptured membranes, placental injury and severe maternal illness. Organisms causing infections include bacteria, viruses and protozoa. Infection was responsible for 10-25% of stillbirths in developed countries. This incidence is much higher in developing countries[15]. It was concluded from this study that reduction of maternal infections may significantly decrease the stillbirth rates in developing countries. Syphilis, Malaria and intrauterine ascending infections (*E. coli, Group B Streptococcus and Urea plasma Urealyticum*) were found to be the most frequent causes of stillbirth in developing countries. The two most common viral infections were *Parvo virus* and *Coxsackie* virus. In developed countries bacterial ascending infection is the most frequent cause of intrauterine infection causing stillbirths[16].

The pathways of infection in stillbirths can be categorised into three including maternal infection leading to a systemic infection and poor oxygenation of the fetus without the infection reaching the fetus, secondly by direct placental infection leading to placental damage reducing blood flow to the fetus and thirdly by infection of the fetus through the placenta or membranes leading to organism damaging fetal organs and fetal death [16]. The most common pathway is by secondary fetal infection caused by chorioamnionitis.

Ascending infections from the vagina occur during or before pregnancy and stay in the uterus without causing disease due to low virulence[16] . During pregnancy these organisms pass through ruptured or intact membranes to infect the amniotic fluid and the fetus. The first organ affected are the lungs since the fetus breaths in infected amniotic fluid leading to a pneumonitis seen frequently in autopsies. Amniotic fluid infection occurs more before 28 weeks gestation and less in late preterm and term pregnancies[16].

Transplacental infection pass through membranes and infect the fetus via haematogenous spread e.g. *Listeria Monocytogenes,* leading to stillbirth. The fetus is infected directly and becomes growth restricted. The organ initially infected is the liver since the blood reaches the liver first via the umbilical vein. Other organs infecting the fetal liver include *Anthrax, Typhoid and Francisella tularensis[16].*

Treponema Pallidum infection occur via transplacental transmission and cross the placenta to the fetus. Syphilis infects the fetus after 14 weeks gestation and there is an increasing risk of infection thereafter [15, 16]. A percentage of 40-45% of the fetuses die in utero, 30-40% are born alive with clinical signs of congenital syphilis. Other spirochete infections also occur during pregnancy and lead to fetal death but syphilis is most common. Screening for syphilis in early pregnancy leads to early diagnosis and treatment. This has led to a 82% reduction in stillbirths, especially in Sub Saharan countries [16].

Viruses including *Parvovirus B19, enteroviruses, influenza* are associated with stillbirths. Protozoal infections such as Malaria and Chagas's disease have been associated with a small percentage of stillbirths [16].

Studies have demonstrated that infection in low income countries is high compared to developed countries and strategies which would improve the stillbirth rate should concentrate on reducing infection and improving the status of maternal nutrition[3, 4, 16]. Malaria

prophylaxis with chloroquine was given in malaria endemic area due to the fact that women who acquired malaria for the first time in pregnancy were found to have a high risk of stillbirths which is mostly associated with Plasmodium Falciparum [16]. Malaria in pregnancy is associated with a vast variety of adverse effects but more commonly fetal growth restriction, preterm labour and moderate to severe anaemia in malaria endemic areas.

Research has demonstrated the need to focus on improving maternal nutritional status by giving nutritional supplements, vitamins and minerals to assist in improving the mother and fetus wellbeing[16]. Adoption of strategies used in developed countries which include improving access to emergency services and caesarean section, where appropriate, o decrease complications related to prolonged labour and ascending infections which lead to stillbirth or early neonatal death. [16].

1.10 Stillbirth, HIV and HAART

HIV infection in pregnant women during the pre HAART period significantly increased maternal morbidity and led to poor fetal outcome and HIV infection of the neonate[17].

The HIV pandemic has significantly affected women and infant health predominantly in Sub Saharan Africa[17]. Introduction of HAART for all HIV positive pregnant women markedly improved PMTCT and pregnancy outcomes in HIV positive mothers but there are still gaps when HIV positive pregnant women are compared to HIV negative mothers[17, 18, 19]. In Mozambique a cohort of 1183 infected women was compared to 561 HIV negative women to establish the effect of HIV infection on maternal health, birth outcome and infant health[17]. The women were followed up from the first antenatal visit until one month post-delivery. ARV's were given based on CD4 count and T cell count. The infected women developed anaemia, had increased hospital admissions during pregnancy and double the number of stillbirths. Neonatal anaemia was also increased in babies born to HIV infected women[17]. This study shows that HIV continued to affect neonatal outcome and maternal morbidity with selection according to CD4 count in HIV positive mothers who received ARV's during pregnancy[18].

In a study done in India to evaluate the effect of HAART in HIV infected women, they compared HIV positive pregnant women on HAART to HIV women on AZT[18]. In the HIV infected women on HAART, there was an increased rate of preterm birth, IUGR and anaemia compared to women on AZT. There was no difference in the incidence of PIH, DM, and intrahepatic cholestasis in both groups[18]. The neonatal mean birth weight was lower and more admissions to ICU in neonates born to mothers on HAART compared to mothers on AZT. It was concluded from this study that even though benefits of HAART are necessary, gaps still needs to be filled. Good antenatal care and a multidisciplinary approach can improve pregnancy outcomes in HIV infected women[18].

A study done at Stellenbosch University in South Africa, identifying placental histopathology associated with HIV and effect of ARV's on placental histopathology[19]. Histopathological findings associated with HIV infection included maternal vascular malperfusion in 36% of cases, ascending infections including chorioamnionitis in 15% of cases and small placentas in 15% of cases. There were no differences in findings in women with a CD4 count below or above 350 cells/mm3 [19].

Studies from the pre-HAART era showed an increase in stillbirth rates among HIV infected women[19]. A study was done in Gaborone Princess Marina Hospital in November 2010 with the aim of identifying the causes of death in HIV infected women[19]. A comparative group of HIV negative women with stillbirths was used. Specimen taken included the placenta for histology, HIV DNA and PCR for stillbirths and verbal autopsies. Hypertension was found predominantly on stillbirths. To acquire information about the pregnancies, obstetrics records, maternal medical records, history of antiretroviral use and delivery were obtained. Placental insufficiency suggestive of hypertension was found predominantly on stillbirths in both HIV positive and HIV negative women, but with a higher percentage in patients on HAART in the HIV positive group. There was evidence of hypertension in 71% of patients with placental insufficiency before delivery. There was no association found between intrauterine HIV infection and stillbirth. There was no vertical HIV transmission in patients on HAART and very low transmission in HIV infected women not on treatment and the

group on AZT. HIV transmission was found in severely immunocompromised women. In this study HAART appeared to increase the risk of placental insufficiency. This finding was also observed in European studies[19].

Chapter 2: Journal article

Title: A review of placental histological changes in stillbirths at Chris Hani Baragwanath Academic Hospital

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Conflict of interest:

PK Maseko- Nil

AJ Wise and S Maswime- Nil

2.1 Abstract

Introduction

Placental histology plays a vital role in determining the cause of stillbirths. A universal classification system has helped to standardise the interpretation of placental histological findings. The purpose of this study was to determine the placental lesions identified in stillbirths and their significance. The study describes histopathological changes in placentas of stillbirths, the association between placental findings and cause of fetal death and impact of maternal HIV infection on placental findings.

Methods

A retrospective cross-sectional study was performed at Chris Hani Baragwanath Academic Hospital in Soweto to identify placental lesions found in placentas of stillbirths and classified using the Amsterdam classification system. Data were abstracted from placental histology reports and case records from January to December 2017.

Results

A total of 625 stillbirths occurred at Chris Hani Baragwanath Academic Hospital between January and December 2017. In all the placentas from stillbirths sent for histology, 204 of the placentas met the inclusion criteria for the study. Maternal vascular malperfusion (MVM) was the most common pathological finding affecting 36.3% of cases, and the majority of placentas with MVM were from hypertensive mothers. Histological changes in keeping with chorioamnionitis were the 2nd most common lesions, found in 24.5% of cases. There were 62% of the total number of placentas with chorioamnionitis had acute chorioamnionitis suggesting a recent infection. In placentas with an abruptio,31.3% of placental abruption cases were associated with hypertension in pregnancy and its complications. Placental infarction was found in 40.6% of placentas and the majority of the mothers with infarction had hypertension in pregnancy and its complications.

There was no association between stillbirths and extremes of maternal age. HIV positive pregnant women who are not virally suppressed had equal numbers of stillbirths when compared to virally suppressed mothers. Women with maternal comorbidities had an increased risk of having a stillbirth. Hypertensive disorders complicated 29% of pregnancies in this study.

Conclusion

Maternal vascular malperfusion (MVM) was the most common finding on placental histology followed by maternal infections. Hypertension was the commonest finding in mothers with stillbirths and maternal vascular malperfusion. Maternal morbidity was often associated with stillbirth.

2.2 Introduction

Stillbirths worldwide were estimated at 2.6 million in 2015 with the majority occurring in low and middle income countries[1]. A stillbirth is an emotional and traumatic experience to the mother and her family[2, 3]. It is more traumatic for the grieving mother when there is insufficient information about the cause of stillbirth[2, 3]. A stillbirth rate of 2 per 1000 in high

income countries compares to a rate of 34 per 1000 in Sub Saharan Africa, portraying a marked difference in the quality of perinatal care[2, 3]. Studies have shown that improving the quality of antenatal care, recognising the risk factors, contributing factors and avoidable factors can improve the outcome of the infant and the mother[4].

Placental histological examination has been considered to be one of the most reliable method of determining the cause of stillbirth, and placental abnormalities account for the leading cause of stillbirths[5]. In the last decade, strategies like 'making every baby count, every women every child' initiative to prevent preventable stillbirths and deaths of children under five years, have been put in place to try decrease the number of stillbirths and deaths of children under 5 years of age[6,7]. Extensive research has been conducted but interpretation of the placental findings has been a great challenge due to different classification systems.

Histological assessment is important to predict the outcome of future pregnancies, diagnose placental lesions that led to stillbirth, and possibly to treat or manage underlying conditions that lead to stillbirth. In studies comparing placental lesions of live babies and stillbirths, most lesions were present in both live and stillbirth placentas[5]. However, the extent of the lesions and the involvement of the placenta was worse in stillbirths compared to live births[5, 8, 9]. The aim of this study was to determine the placental causes of stillbirth in a South African tertiary hospital.

2.3 Methods

The study was conducted at Chris Hani Baragwanath Academic Hospital in Soweto Township in Gauteng. A tertiary hospital that serves as a referral centre for greater Soweto, Orange farm, Eldorado Park and Lenasia. It is the referral hospital for three hospitals, Bheki Mlangeni District Hospital, Sebokeng Hospital and Thelle Mogoerane Hospital. The maternity department delivers more than 20 000 babies per year. The HIV prevalence rate in pregnant women had increased to 29% from 2007 to 2017. The crude prematurity rate for all pregnant women at this institution was at 15% in 2017, and recently between 22 to 24%

A retrospective audit was conducted on all women who had a stillborn and placental histology assessment performed by pathologist between January and December 2017. The objectives of this study was to determine the placental lesions found in women with a stillbirth and the placental causes of stillbirth. The contributing factors and risk factors associated with stillbirths was also described. The secondary aim was to determine the association with HIV and placental lesions in stillbirths and placental infarction. We extracted data from the histology reports obtained from the National Health Laboratory Services (NHLS) and clinical data from the case records. A stillbirth was defined as an intrauterine fetal death with an estimated gestational age of 24weeks or more or a weight of 500g or more[12]. The placental lesions were classified using the Amsterdam criteria. The Amsterdam criteria is an international criteria for uniformity in gross and microscopic description of placental lesions. Placental lesions according to the Amsterdam criteria are classified into maternal vascular malperfusion, fetal vascular malperfusion, villitis of unknown aetiology, delayed villous maturation and ascending intrauterine infection.[(13)] ,

The study population included all mothers who had stillbirths who had a gestational age of 24 weeks or more, or a fetal weight of over 500g and fit the inclusion criteria. We included all stillbirths with placentas sent for histology to determine the cause.

Permission for the study was obtained from the University of the Witwatersrand, Human Research Ethics committee – medical (approval reference number M171121), Chris Hani Baragwanath Academic Hospital and the National Health Laboratory Services. See Appendix C and D

2.4 Results

- There was a total of twenty thousand nine hundred and twenty-nine (20 929) deliveries in the year 2017, with six hundred and twenty-five (625) stillbirths. The stillbirth rate was 33.4 per 1000. Out of the 625 stillbirths, 204 stillbirths met the inclusion criteria for the study. Histology reports were available in all 204 patients and clinical data from the maternity files were available in 124 cases (60%).

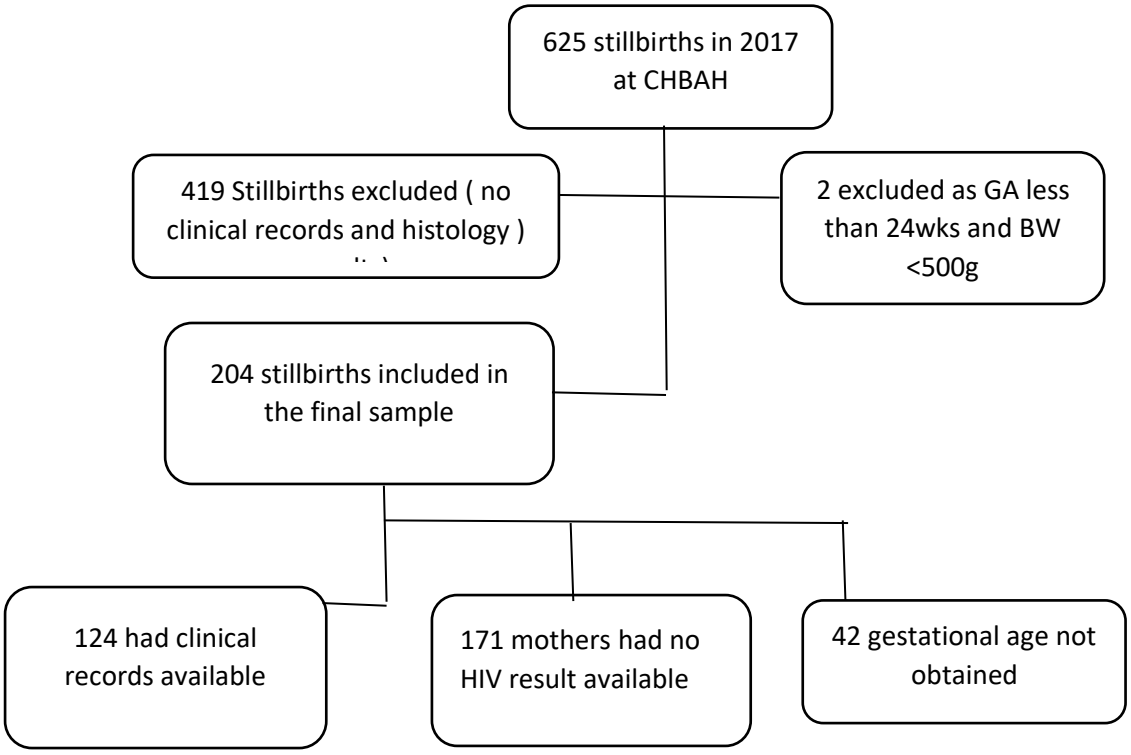

Figure 2.1: Flow diagram of participants and exclusions

The mean maternal age was 27.6 years (SD± 7.9). The youngest mother being 15 years and the oldest was 43 years of age. There were 29 mothers (14.6%) with age above 35 years. Eight mothers (3.92%) were below 18 years. A total of 88 (54.3%) out of a total of 204 stillbirths were in the second trimester whereas 74(36.3 %) occurred in the third trimester. The mean gestational age for stillbirths was 31wks and 4 days (SD 4.9). (See Figure 2.4.1) In 42 mothers (20.5%) the gestational age of the pregnancy was not recorded in the histology report and the patients clinical records could not be obtained. These mothers were included in

the final sample since they had histology reports available and the fetal weights were 500grams and more.

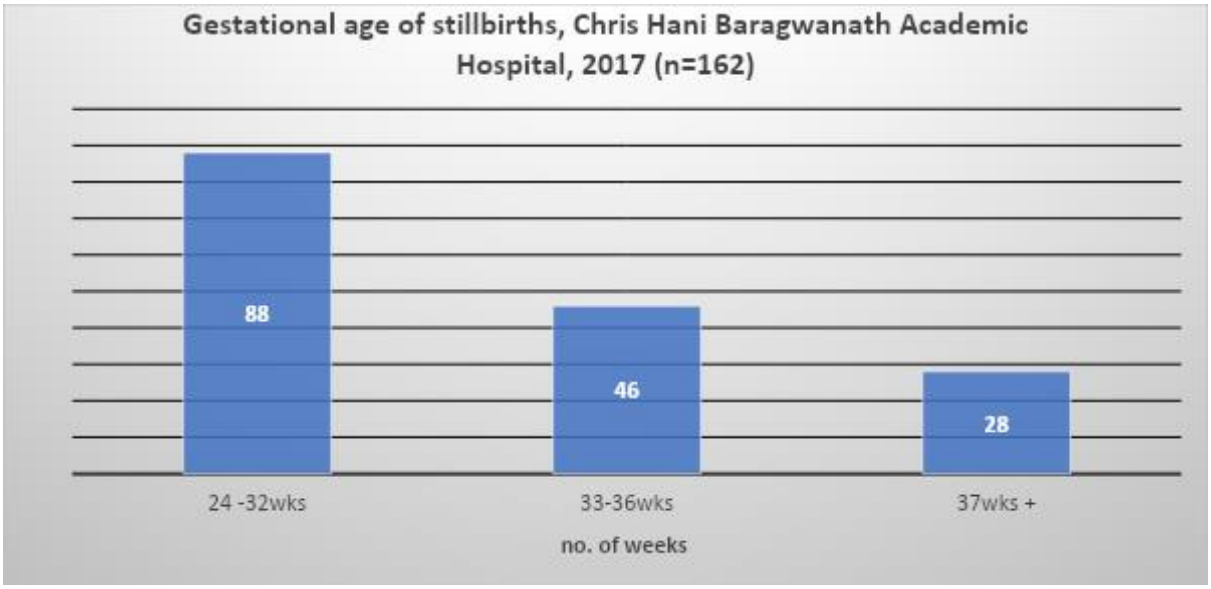

Figure 2.2: Gestational age of stillbirths , Chris Hani Baragwanath Academic Hospital, 2017

On placental examination, 154 (82.7%) stillbirths were macerated and 32 (17.2%) were fresh stillbirths. The fetal description was not recorded in eighteen (8.8%) stillbirths.

Table 2.1: Maternal demographics, Chris Hani Baragwanath Academic Hospital, 2017 (n =204)

Categories					
Age (n = 204)	**No. <18yrs**	**No. >35yrs**	**18 – 35yrs**	**Unknown**	**Mean**
	8 (3.92%)	29 (14.6%)	162 (79.4%)	5 (2.45%)	27y 6m (SD±6.7)
HIV(n=204)	**Pos**	**Neg**	**Unknown**	**Prevalence**	–
	41(20.1%)	130 (63.7%)	33(16.2%)	(130.4/1000	–
Viral load (n= 41)	**<200 copies/ml**	**>200 copies/ml**	**Unknown**	–	–
	10 (24.4%)	10 (24.4%)	21 (51.2%)	–	–
Rhesus (n=204)	**Neg**	**Pos**	**Unknown**	–	–
	3 (1.5%)	113 (55.3)	88(43.1%)	–	–
Syphilis (n=204)	Pos	Neg	Unknown	–	–
	10 (4.9%)	114 (55.9%)	80 (39.2%)	–	–
No. of clinic visits(n=204)	**≤ 5**	**≥ 5**	**Unknown**	–	–
	57 (27.9%)	13 (6.36%)	134 (65.7%)	–	–
Gestational age (n=204)	**23-32wks**	**33- 40wks**	**>40wks**	**Unknown**	**Mean (n=?)**
	88 (54.3%)	71(43.8%)	3 (1.85%)	42(20.5%)	31w4d
Maternal comorbidity (n=204)	**HPT**	**Infection**	**GDM**	**Cardiac**	**Thyroid**
	60 (29.4%)	27 (13.2%)	5 (2.45 %)	2 (0.98%)	2 (0,98 %)

Placental lesions

The Amsterdam criteria was used to classify placental findings to identify the placental lesions associated with stillbirths **(**Table 2.2).

Table 2.2: Number of placentas per category (Amsterdam criteria) (n = 204)

Amsterdam	No. of placentas	Percentage	CI
Maternal vascular malperfusion	74	36.3%	29.68% - 43.28%
Fetal vascular malperfusion	21	10.3%	6.49% - 5.30%
Villitis of unknown origin	3	1.47%	0.30% - 4.24%
Chorioamnionitis	50	24.5%	18.77% - 31.00%
Normal	10	4.9%	2.38% - 8.83%
Delayed villous maturation	22	10.8%	6.88% - 15.87%
Other	20	9.80%	6.09% - 14.73%

There were no abnormalities visualised on histology in ten (4.9%) placentas. Out of 204 histology reports, total of seventy-four (74) placentas (36.3%) had lesions fitting the criteria for maternal vascular malperfusion. Placental abnormalities fitting with chorioamnionitis were found in 50 placentas (24.5%). Furthermore 31 (62%) of the 50 placentas had acute chorioamnionitis. One patient had *Treponema pallidum* spirocheates isolated on placental immunohistochemistry.

Twenty-one placentas (10.3%) had lesions of fetal vascular malperfusion. A total of 22 (10.8%) placentas had changes for delayed villous maturation. Placental insufficiency changes occurred in five (5) placentas accounting for 2.45% of cases. Twenty-seven placentas (13.2%) were classified under placental compromise since they have lesions of MVM but did not fulfil the criteria for MVM. There were twenty (7.8%) placentas which showed placental abnormalities not fitting any criteria when using the Amsterdam criteria.

Other Histopathological findings

On histology, varying locations, percentages and extent of placental infarction occurred in 93 (46%) placentas. In 75% of placentas with infarction, the infarction was more than 10%. All infarction less than 5% were from preterm placentas except in 4 placentas which 2 were from term pregnancies. In two placentas the gestational age was unknown. One hundred and fifteen 115 (56.4%) placentas had cord abnormalities observed which included hypercoiling, knots and twists. Retro-placental clot was found in 49 placentas accounting for 24% of placentas. In 22 (n = 49) (44.9%) placentas with an abruptio, the abruptio was associated with maternal vascular malperfusion. Villitis occurred in 3 cases, in which all had chorioamnionitis therefore there were no cases of villitis of unknown aetiology.

HIV lesions

HIV blood results were available in 171 out of 204 mothers accounting for 84% of mothers in this study. In 33 mothers (16.7%) the HIV status was unknown to the researcher. Out of the 33 mothers with unknown HIV status, one was unbooked. A total of (41/171, 24%) were HIV positive whereas (130/171,76%) were negative. Out of the 41 HIV positive mothers, 20 mothers (48.9%) had a viral load result available. Those with viral load results (n=20), ten (50%) of mothers were virally suppressed.

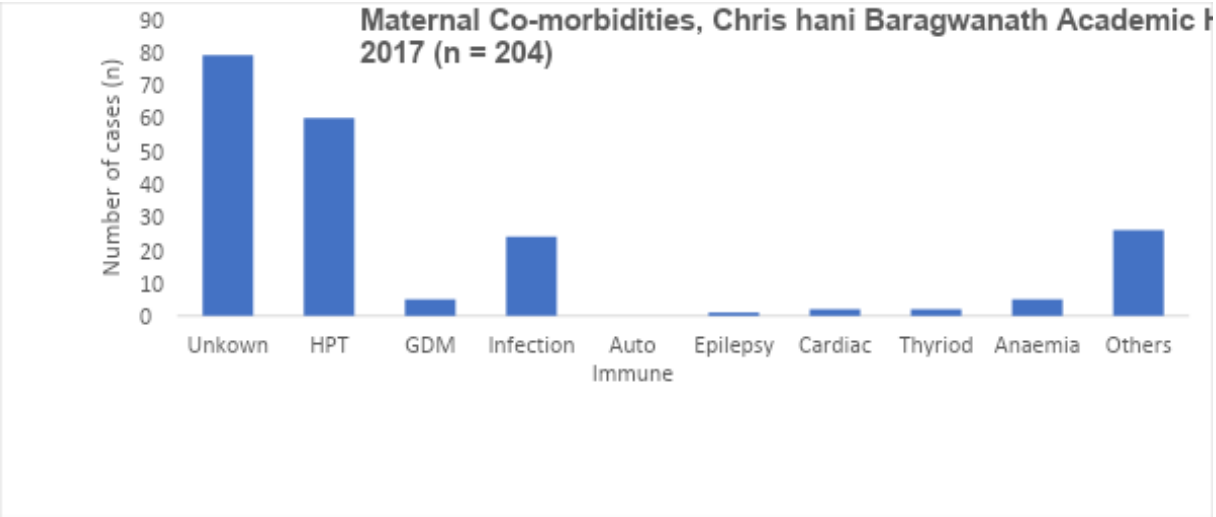

Figure 2.3: Maternal Co-morbidities, Chris Hani Baragwanath Academic Hospital, 2017

Hypertension was found in 60 mothers who had stillbirths. A total of 24 mothers had maternal infection (11.8%) on clinical assessment and laboratory results. A total of 50 mothers (24.5%) had chorioamnionitis on placental histology. Other maternal comorbidities found in the mothers included 5 mothers (2.45%) with diabetes in pregnancy, 2 mothers (0.98%) suffered from a cardiac condition, 2 (0.98%) had thyroid disease in pregnancy, 5 patients (2.45) had anaemia in pregnancy at presentation to antenatal clinic and 1 patient (0.49%) was epileptic.

Maternal syphillis

In the 204 mothers, 124 mothers had syphilis results available. A total of 114 (91.9%) out of 124 mothers were RPR negative. Syphilis serology was positive in 10 patients (8.1%) and one patient had *Treponema Pallidum* spirochetes isolated on placental immunohistochemistry. In 80 mothers (39.2%) the RPR result was unknown to the researcher due to the files not obtained owing to poor filling system and no records of results were found from the NHLS system. One patient with a positive RPR test also had a positive placental immunohistochemistry.

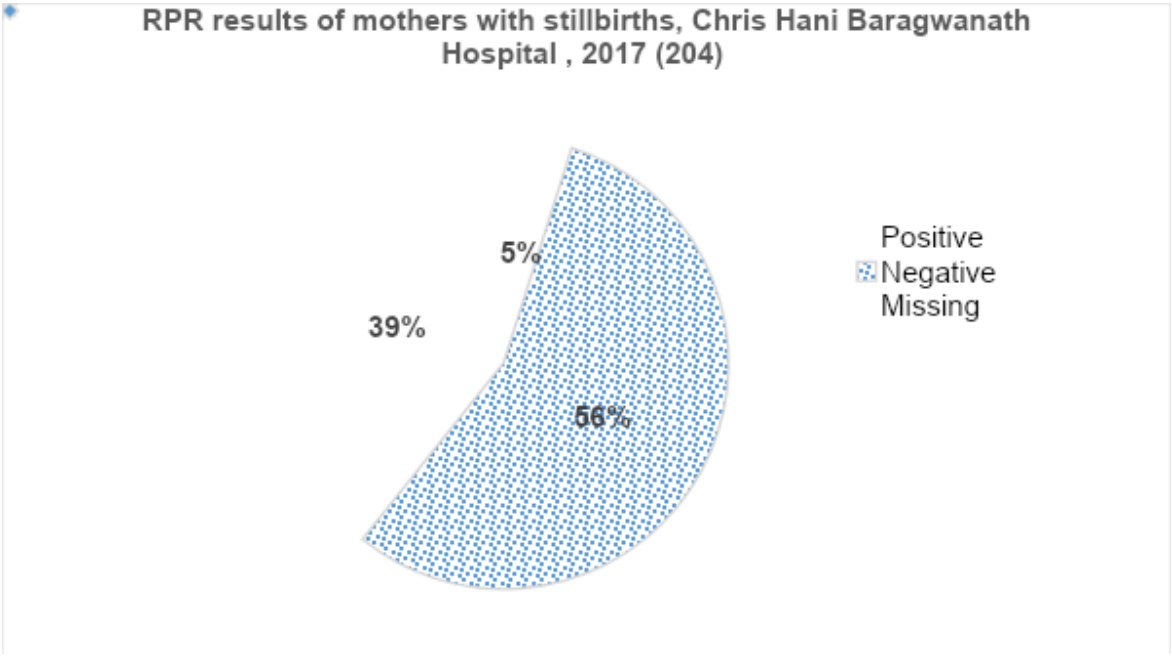

Fig 2.4 RPR results of mothers with stillbirths, Chris Hani Baragwanath Hospital, 2017

A total of 5 patients (50%) with a positive RPR on haematological examination had changes in keeping with chorioamnionitis on placental histology.

2.5 Discussion

Placental histology is an important tool to determine the possible causes of stillbirth. In this study to determine the placental causes of stillbirth and lesions associated with stillbirths, maternal vascular malperfusion was the most common placental lesion affecting a third of women who gave birth to stillbirths. Acute Chorioamnionitis was the second most common finding seen on placental histology. More than half of the placentas had infarction. Infarction has been shown to be present in placentas of healthy live fetuses. The extent and position of infarction on the placenta seems to determine its significance. Placental infarction less than 5% and marginal infarction is normal at term[7,13]. Placental abruptio was commonly found in placentas fitting the criteria for MVM

Hypertension and hypertension related complications in pregnancy were the leading maternal comorbidity found in stillbirths with MVM as the most common placental lesion in these placentas.

Studies done have described a similar association with MVM[10, 11], including the Boston Birth Cohort which showed a strong association between hypertensive disorders of pregnancy with maternal vascular malperfusion in livebirths[9]. They analysed the association of MVM with hypertensive disorders, gestational / pregestational diabetes and obesity. MVM was strongly associated with hypertensive disease in pregnancy and its complications. A prospective cohort study was done in low risk nulliparous pregnant women to estimate the incidence and relative risk of developing MVM[10]. Maternal risk factors, biomarkers, dopplers and placental structure ultrasound were used in isolation and together to estimate the risk of MVM. MVM was seen in 1 in 12 nulliparous low risk patients and these mothers had a more than 4 times higher chance of developing preeclampsia and small for gestational age babies[10]. Complications of preeclampsia, IUGR, abruption and stillbirths was found in 8% of healthy nulliparous women[10]. MVM is found in both placentas of healthy women and high risk patients, but an association between hypertension and possibly stillbirth appears to be stronger[10].

In this study 8.1% of the mothers had syphilis during their pregnancy. The syphilis rate in this study is much higher than the global rates according to the Global health observatory data reported estimates of 1% in pregnancy in the year 2017. Syphilis prevalence was lowest in

Europe (0.12%) and highest in Africa with a rate of 3.04% in 2017[12]. In this study half of the mothers with syphilis had chorioamnionitis.

There was an equal number of stillbirths in both HIV positive women who were virally suppressed and women who were not suppressed, however this finding was statistically not significant because of a small sample size of mothers with viral load available for the study, further studies needs to be done. The viral load and CD4 count is important in understanding the role of anti-retroviral therapy in both the fetus and mother during pregnancy. We further looked at histological changes in placentas of HIV positive mothers. Maternal vascular malperfusion was found in 34% of HIV positive mothers, 22.8% of HIV negative mothers and 8.1% HIV unknown mothers. Studies have been done to find the effect of HIV on the placenta[15]. According to the study done in Brazil which described that HIV positive mothers had an increased number of small for gestational age fetuses, preterm labour and IUGR babies[13].

Acute chorioamnionitis was the second most common finding on placental histology. In a study done in Stellenbosch University in 2015 to describe histopathology of the placenta in HIV positive women, the same findings were observed[14], MVM was the main placental finding in these women followed by ascending infections associated with chorioamnionitis and small placenta.

A total of 34% of the HIV mothers who delivered a stillbirth had hypertension in pregnancy. Some studies have shown an increase risk of hypertension during pregnancy in mothers on active antiretroviral[15, 16]. The one study found an increased risk of hypertension in mothers with an increase in serum lipid profile using first generation protease inhibitors[15]. In 2015, according to a systematic review where they compared findings of different articles looking at the association between antiretrovirals and hypertension[17]. A non-significant association between antiretrovirals and pregnancy induced hypertension[17] was found. This finding was also observed in a Toronto matched cohort study comparing the risk of preeclampsia in HIV pregnant mothers on HAART with HIV negative mothers[18]. There was also no increase in preterm labour, preeclampsia and small for gestational age fetuses but an increase in low birth weight babies was evident[18].

The majority of the stillbirths occurred prematurely with 54% of the stillbirths in the second trimester of pregnancy (early premature) and 46% were in the third trimester of pregnancy. The prematurity rate for stillbirths before 37weeks was 2 per 1000. The overall prematurity rates for livebirths at Chris Hani Baragwanath Academic Hospital for the year 2017 was 151.1 per 1000 births accounting for 15% of live births. This is inkeeping with the national preterm birth rate of 15% in South Africa in the year 2017. The most common cause of preterm delivery being infection and chronic maternal conditions. This is correlating with the findings in this study. The recent prematurity rates at CHBAH are ranging between 21 to 23% which portrays increasing numbers of premature births. Poor follow up to antenatal clinic was associated with an increased risk of stillbirth[19, 20]. This variable could not be assessed in this study due to a high number of missing files and clinical frequency is undefined. In studies that assessed follow up of pregnant women, mothers with infrequent clinic visits had an increased risk of poor fetal and maternal outcome. Mothers with poor antenatal care attendance and late antenatal booking led to late referral to a tertiary hospital if required and affecting pregnancy outcomes. Emphasis on early booking and regular antenatal clinic follow up has shown to improve fetal and maternal outcome. Antenatal care is a public health initiative for early detection and prevention of complications in normal pregnancies and promote good pregnancy outcomes[20]. The basic antenatal care plus in South Africa (BANC plus) recommends eight clinic visits including the booking visit to allow for adequate exposure to health workers to improve the quality of antenatal care[21]. This is supposed to replace the old system where pregnant woman were recommended to have five clinic visits throughout their pregnancy period. This was supported by the cluster randomised trial in 2001 which showed improved maternal and perinatal outcome with BANC plus compared to the old model[22]. BANC plus was introduced to our system in April 2017, which would explain the low numbers of patients meeting the recommended clinic visits.

2.6 The limitations of this study

This was a retrospective study, with limited availability of clinical data. Histology reports did not have a standardised reporting criteria, and available data was extracted to reclassify the findings using the Amsterdam criteria. Essential antenatal blood results were not always available.

2.7 Strengths of the study

This study describes stillbirth rates, and placental lesions in women who had placental histology results reported by a pathologist according to a classification used internationally. This provides more insight into the causes of stillbirth in low and middle income countries where placental histology is not routinely available.

2.8 Implications

Stillbirths can be reduced by firstly understanding the causes of stillbirths. Increasing the availability of histopathology services gives greater insight in to causes of stillbirths and preventable measures.

2.9 Conclusion

MVM is the most predominant finding on placental histology followed by changes of chorioamnionitis. More studies are required to understand the causes of MVM and correlate with stillbirths. The majority of mothers with stillbirths had an underlying placental pathology. The evaluation of placentas in women with stillbirths is essential to understand the causes of unexplained stillbirths and to improve antenatal care.

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Appendix A: Approved Research Protocol

1. Introduction

The WHO definition of a stillbirth is a baby born with no signs of life at or after 28weeks gestation. [1] Increased survival of live infants born between 24-27 weeks led to inclusion of stillbirths in these gestations, therefore altering the WHO definition of perinatal mortality rate and stillbirth. [1] The number of stillbirths is an important reproductive health indicator and public health problem. The South African Saving Babies Report 2012 - 2013 had 1 412 355 births with 32 662 stillbirths and 14 1576 early neonatal deaths from 588 PPIP sites. [2] This represents only 75.6% of all births in institutions using the district health information system[2]. In 2013, 2.8 million newborns died worldwide and stillbirths were estimated at 2.7 million. [3] These numbers indicate a serious health challenge. Lack of information on causes and patterns leading to stillbirth has made it difficult to find strategies to prevent stillbirths.

The causes of stillbirths were investigated, as described in a systematic review, by performing a post-mortem, placental histology, karyotyping and various laboratory investigations. [4] They concluded that placental histological findings were an important part of the evaluation in determining the cause of stillbirths. Post mortem examination, placental histology and karyotype are strongly recommended as part of diagnostic evaluation. [4] A systematic review was done to identify the causes of death that can be diagnosed from placental pathology. [5] The reviewers included studies that classified the causes of death and investigated the significance of specific placental abnormalities in stillbirths. Placental infarction was identified as one of the causes of stillbirths in 53.8% of studies, abruption in 76.9% and chorioamnionitis in 38.4%. The abnormalities that occurred most frequently were infarction, infection and placental failure. [5]

Placental infarction is defined as villous cell death occurring due to local obstruction of maternal utero-placental circulation. [6] Single, marginal infarcts involving less than 5% of the villous tissue are commonly found in placentas of live infants and pose no risk to the fetus. Extensive placental infarction involving more than 30% of villous tissue results in neonatal asphyxia, low birth weight and intrauterine death. [6] Placental infarction is associated with maternal hypertension, pre-eclampsia, Rh incompatibility, connective tissue disorders, retro-placental haematoma and maternal thrombophilia. [6] Infarction has been strongly related with

fetal death, preterm birth and growth retardation in utero and may recur in subsequent pregnancies. [7]

2. Literature review

Placental histology in live births and stillbirths

The Institute of Child Health and Human development performed a study comparing placental histology in stillbirths and live births. [8] The placental disorders were grouped into developmental, inflammatory and circulatory. Frequently observed placental abnormalities included inflammatory, thrombotic and retro placental hematomas. [8]

Chorioamnionitis and inflammatory lesions predominantly caused by infection were common before 24 weeks gestation. Retro placental hematoma had a higher prevalence in stillbirths compared to live births (23.8% vs 4.2%) except in gestational age less than 24 weeks in which case the prevalence was similar at 36%. This association may imply a contribution to preterm births and stillbirths. Parenchymal infarction was more prevalent in stillbirths compared to live births. The occurrence was greater than 35% at 24 to 31 weeks gestation and 32 to 34 weeks gestation compared to 6.4% in live births. Maternal circulation lesions were more frequent in stillbirths than live births. The outcome from this study indicated than inflammation and retroplacental hematoma are predominant in early stillbirths and live births whilst parenchymal infarction and thrombosis were common in later gestation in stillbirths. The power of this study was inclusion of a control group of live births and use of a standard protocol to analyse the placental pathology.

A systematic review by Amina et al. found that maternal disease was predominantly associated with stillbirths.[9] The most common maternal conditions causing stillbirths included hypertension (HPT) in pregnancy, maternal disease conditions, trauma and infection. Improvement of screening and adequate antenatal care of pregnant mothers would markedly lower the stillbirth rate in Low and Middle-income countries.

Prediction and prevention of stillbirths

To identify the process and a capability to foresee and prevent stillbirth, a case control study was done in 2011 in United States of America. [10] A total of 25 placental pathologies were investigated, with 15 of them strongly associated with stillbirth. Out of the 15 placental pathologies ten were also associated with fetal growth abnormalities in stillbirths. The

remaining five pathologies had absence of fetal growth abnormalities. Placental infarction was found in stillbirths and live births with fetal growth anomalies but with a higher percentage in stillbirths compared to live births. Stillbirths had a lower gestational age and birth weight compared to live infants.

These findings suggest that placental action supports fetal growth and development and its impairment results in fetal growth affectation consequently leading to stillbirths. It also shows that placental abnormality can lead to stillbirth without fetal growth affectation. Five relations amongst placental findings, fetal growth anomalies and stillbirth were observed. These included; stillbirth with fetal growth anomalies, stillbirth without fetal growth anomalies, live births with fetal growth anomalies, live birth and stillbirth with fetal growth anomalies in the same manner and lastly, stillbirth and live birth with fetal growth anomalies in a different manner. The birth weight to placental weight ratio could be estimated during the prenatal period using estimated fetal weight to placental volume ratio obtained using ultrasound. According to this study, the indication for delivery would be an abnormal ratio which carries an increased risk for stillbirth.

Association between IUGR fetuses and Stillbirths

Normal growth of the fetus is dependent on adequate supply of oxygen and nutrients from maternal circulation. [7] Adequate and proper functioning of the placenta is of vital importance for a healthy pregnancy. Placental pathology and inadequate oxygenation lead to adaptive changes in the fetus and placenta to compensate for the dysfunction. [11] Failure of adaptive changes ultimately lead to fetal demise.

Causes of IUGR can be classified into placental, fetal or maternal. Most stillbirths amounting to 60% are unexplained. Unexplained stillbirths are stillbirths were the cause of fetal demise cannot be explained by fetal, placental, maternal or obstetric cause.[12, 13]Abnormal placentation is associated with development of preeclampsia, preterm labour, miscarriages, IUGR and intrauterine fetal death. Placental abnormalities can produce contrasting results in different pregnancies, fetal demise in one pregnancy and IUGR in another. [14]

In a study done in 2011 to investigate changes in placental histology in pregnancies of IUGR fetuses and stillbirths of unknown cause. [7] Placentas were collected in pregnancies with IUGR, unexplained stillbirths and live healthy fetuses. Placental infarction was observed in 58% of fetuses with IUGR, 62 % in intrauterine fetal death and 4% in live fetuses.

Intervillous thrombosis was found only in intrauterine fetal deaths and it may be a feature of intrauterine death. No major villous stem occlusions were found in placentas of IUGR fetuses and stillbirths. Chorionic vessel occlusion was found in IUGR and intrauterine fetal death. This outcome was not found in placentas of healthy babies. It was concluded from the study that infarction, chronic villitis, intervillous thrombosis, haemorrhagic endovasculitis, placental intravascular thrombi, perivillous fibrin deposition, fibrinoid necrosis, erythroblastosis and villous edema were found to be the abnormalities causing a normal fetus to become growth restricted and die, but the magnitude of these lesions and clinical effect could not be defined in the study. It was concluded from the study that a relationship exists between changes in the placentas of IUGR fetuses and intrauterine fetal death. [7]

The saving babies 2012-2013 report had estimated and concluded that most unexplained stillbirths are undiagnosed IUGR fetuses and post term pregnancies, congenital infection and congenital abnormalities. [2]

Stillbirths and Preeclampsia

A sub analysis of a population-based case control study of stillbirths was performed to compare placental abnormalities observed between stillbirths in pre eclamptic women and women without pre-eclampsia. [15] A comparison of placental pathology between livebirths and stillbirths in pre-eclamptic women was also conducted. Placental abnormalities were responsible for 23.6% of stillbirths, with obstetric conditions at 29.3%, fetal genetics at 13.7%, infections 12.9%, umbilical cord abnormalities 10.4%, hypertensive disorders 9.2% and other maternal conditions at 7.8%. Parenchymal infarction was the lesion predominantly found in women with pre-eclampsia in preterm deliveries. This group had an increased number of histologic placental abnormalities and more severe abnormalities compared to term pregnancies. Placental lesions observed were predominantly lesions of maternal malperfusion. There was an increased amount of placental hypoplasia and placental vascular lesions in deliveries before 34 weeks gestation compared to term deliveries

IUGR was associated with decreased fetoplacental ratio implying a poorly effective placenta. Preeclampsia was associated with higher fetoplacental ratio when compared with stillbirths unaffected by preeclampsia.[15]This finding is likely to be due to decreased fetal perfusion.

Infections and stillbirths

A study was performed in Alabama 2003 to determine the connection between different perinatal infections and stillbirths. [16] Four mechanisms of infection were recognised. These included direct infection through intact and ruptured membranes, placental injury and severe maternal illness. Organisms found to cause infections included bacteria, viruses and protozoa. Infection was responsible for 10-25% of stillbirths in developed countries. This incidence is much higher in developing countries. It was concluded from this study that reduction of maternal infections may significantly decrease the stillbirth rates in developing countries. Syphilis, Malaria and intrauterine ascending infections (E. coli, Group B Streptococcus and Ureaplasma Urealyticum) were found to be the most frequent causes of stillbirth in developing countries. The two most common viral infections were Parvo virus and Coxsackie virus. In developed countries bacterial ascending infection is the most frequent cause of intrauterine infection causing stillbirths. [16]

Stillbirth, HIV and HAART

Studies from the pre-HAART era showed an increase in stillbirth rates among HIV infected women. [17] A study was done in Gaborone Princess Marina Hospital in November 2010 with the aim of identifying the causes of death in HIV infected women. [17] A comparative group of HIV negative women with stillbirths was used. Specimen taken included the placenta for histology, HIV DNA and PCR for stillbirths and verbal autopsies. To acquire information about the pregnancies, obstetrics records, maternal medical records, history of antiretroviral use and delivery was obtained. Placental insufficiency suggestive of hypertension was found predominantly on stillbirths in both HIV positive and HIV negative women, but with a higher percentage in patients on HAART in the HIV positive group. There was evidence of hypertension in 71% of patients with placental insufficiency before delivery. There was no association found between intrauterine HIV infection and stillbirth. There was no vertical HIV transmission in patients on HAART and very low transmission in HIV infected women not on treatment and the group on AZT (Zidovudine). HIV transmission was found in severely immunocompromised women. In this study HAART appeared to increase the risk of placental insufficiency. This finding was also observed in European studies. [17]

In a study done in Spain, preeclampsia and fetal death were low in HIV infected women pre-HAART and increased markedly during the HAART era. [18]

In a systematic electronic search of articles measuring serum lipid concentration and incidence of dyslipidaemia in HIV infected pregnant women on HAART and the women not

on HAART. [18] There was an increase in serum lipid profile in women using first generation Protease inhibitors as part of their HAART regimen and women treated with HAART at conception. [19]

3. Aim

This study aims to identify histopathological changes in placentas of stillbirths at Chris Hani Baragwanath Academic hospital, and to determine the association between stillbirth and infarction.

4. Objectives of the study

a) To describe histopathological findings on placental assessment of stillbirth at Chris Hani Baragwanath Academic Hospital
b) To describe the association between stillbirth and placental infarction
c) To describe the association between placental infarction in stillbirths and HIV

5. Methodology

The study will be a cross-sectional study and the data will be collected retrospectively. The subjects will include all the stillbirths where the placenta was sent for histology to determine the cause of death. The placental histology reports of stillbirths in the year 2017 period will be retrieved to identify the placental lesions and association with the most likely cause of death. Placental histology results and maternal records from 01/01/2017 to 31/12/2017 will be retrieved and reviewed. Additional information including antenatal records will be obtained from clinical records.

6. Study design

6.1 Study population

The study will be done at Chris Hani Baragwanath Academic Hospital located in Soweto Township in Gauteng. It serves as a referral centre for greater Soweto, Orange farm, Eldorado Park and Lenasia. It is the referral hospital for Bheki Mlangeni District Hospital, Sebokeng Hospital and Thelle Mogoerane. The maternity department delivers more than 20000 babies per year. It is one of the teaching institutions used by the University of the Witwatersrand medical school. Stillbirths were estimated to be ± 625 in 2017 from January to December 2017.This places the stillbirth rate at 32 stillbirths per 1000 deliveries.

6.2 Inclusion criteria

The subjects will include all stillbirths with placenta sent for histology to determine the causes of stillbirths in the community utilising Chris Hani Baragwanath Academic Hospital.

6.3 Exclusion criteria:

- Stillbirth with estimated gestational age at less than 24weeks gestation and fetuses under 500g
- Stillbirths where a termination of pregnancy was done for maternal wellbeing.

6.4 Sampling and data collection

The researcher will access all available placental histology reports in the NHLS system from stillbirths. A period sample with no sampling strategy will be employed from the period of January 2017 to December 2017. The patient records will be obtained to access clinical information.

6.5 Data analysis and interpretation

The study will employ quantitative techniques. Descriptive data analysis will be used for most of the data collected, using means ± standard deviations (for normally distributed continuous data), medians and ranges (for non-normally distributed continuous data), and proportions with percentages (for categorical data). Precision will be managed by using 95% confidence intervals. No specific hypotheses have been set yet, but where necessary, hypothesis testing will employ tests such as Chi-squared, Fisher's exact, Student's T and Mann-Whitney to explore associations between various predictors and outcomes.

7. Ethical considerations

Permission will be sought from the Wits HREC, the CHBAH CEO and the Gauteng province through the National Health Research Database.

8. Project timelines

	Nov 2018	Dec	Jan 2019	Feb	March	April	May	June
Writing of protocol and approval of research committee and Ethics	0	0	0					
Data collection				0				
Data Analysis					0			
Write up						0	0	
Submission								0

9. Appendix B: Data Collection sheet

Age: _____

Parity/Gravidity

_____

Booking bloods:

- RPR_____
- Hb_____
- Rh_____
- HIV_____
- If reactive: vl and cd4 count
- _____

Clinic visits in the present pregnancy:_____

_____

Obstetric history (maternal conditions)

HPT	Yes	No	Epilepsy	Yes	No
GDM	Yes	No	Cardiac disease	Yes	No
Infection	Yes	No	Thyroid disease	Yes	No
Autoimmune disease	Yes	No			
Smoking	Yes	No	Medications	Yes	No

Other Yes No

Histology report

Fetal appearance at birth: Congenital abnormalities: Yes No

Fetal weight: _____

Fetoplacental weight: _____

MSB/FSB: _____

Cord:

No. of cord vessels: _____

Cord abnormalities: Yes No

Membrane abnormalities: Yes No

Placental weight: _____

Placenta maternal surface: Infarction Yes No

Calcification Yes No

Retro placental haematoma Yes No

Placental cut surface: Infarction Yes No

:if yes, percentage of infarction_____

Intervillous thrombus: Yes No

Intervillous Fibrin: Yes No

Villitis: Yes No

Other chorionic plate abnormalities_____

Other decidua plate abnormalities: _____

_____

_____

Post mortem results or any other results done to find cause of death if available:

_____

Appendix C: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr P Maseko
School of Clinical Medicine
Department of Obstetrics and Gynaecology
Charlotte Maxeke Johannesburg Academic Hospital

E-mail: masekopk78@gmail.com

CC: Supervisor: Drs S Maswime and A Wise <Smaswime@gmail.com>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 03/04/2018

REF: *R14/49*

PROTOCOL NO: **M171121** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *A review of placental histological changes in stillbirths at Chris Hani Baragwanath Academic Hospital*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

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18 May 2018

Applicant: Dr Pearl Maseko
Institution: University of the Witwatersrand
Department: Obstetrics and Gynaecology
Email: masekopk78@gmail.com
Cell: 082 776 8197

Re: Approval to access National Health Laboratory Service (NHLS) Data

Your application to undertake a research project "**Review of placental histological changes in stillbirths at Chris Hani Baragwanath Academic Hospital**" using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you ***without patient names*** to conduct the proposed study as outlined in the submitted application

.

Please note that approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

PP. [signature]

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research