Morphological and histological placental characteristics in relation to pregnancy outcome in an unselected population.

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ABSTRACT

Macroscopic and histological characteristics of the placenta and umbilical cord are thought to be associated with the perinatal outcome. However, a lack of appropriate quantitative data on placental morphology makes it difficult to study its effects on the pregnancy and neonatal outcome. The umbilical cord coiling, its insertion onto the chorionic plate and the placental shape varies from one pregnancy to another. There has been very little attempt at quantifying placental and umbilical cord morphology.

Widespread use of digital measurement techniques in biomedical science has superseded manual measurements. Though this technique has been applied to the placental measurements, no standardized methodology has been used. This study formally compares manually and digitally obtained placental measurements using proprietary software.

This study derives indices describing the cord coiling (cord coiling index), the relationship of cord insertion to the placental centre (cord centrality index) and the shape of the placenta (eccentricity) in placentas from unselected pregnancies and common obstetric outcome groups. This study establishes a quantitative relationship of birth weight to the placental weight and circumference. It demonstrates that these indices in pregnancies are affected by common obstetric outcome groups such as; pre-eclampsia (PET), pregnancy induced hypertension (PIH), gestational diabetes mellitus (GDM) and delivery of a small for gestational age (SGA) baby.

This study defines the various histological abnormalities of the placenta using strict criteria. It further investigates the incidence of these predefined histological abnormalities of the placenta in an unselected population and common obstetric outcome groups.

The guidelines published on placental examination recommend a detailed histopathological examination of the placenta in cases of adverse perinatal outcome. However, there seems to be some degree of uncertainty whether any association exists between histopathological findings and perinatal outcome. This study examines the morphological indices and histology of the placenta in infants admitted to the Neonatal Unit.

This study attempts to identify the relationship between the pregnancy outcome, neonatal characteristics and placental pathology by examining the pregnancy and neonatal outcome in all cases with abnormal placental pathology.

This study shows significant correlation between manual and digital measurements of the placenta. Digital measurement is a reproducible and relatively simple method and can be of potential importance in the future studies in this field. The quantitative analysis performed shows that the cord insertion is most commonly “off centre” and the shape of the placenta is “elliptical”, this finding is contrary to the traditional belief that the cord insertion is central and the placental shape is round. The study demonstrates that the morphological indices in common obstetric outcome groups are not different from the non-affected cases. It also reveals a significant positive correlation between birth weight to placental weight and circumference.

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CONTRIBUTION OF THE CANDIDATE

The current study was conducted at Addenbrookes Hospital, Departments of Fetal Medicine and Pathology. The candidate prepared the protocol and submitted the study to the ethical committee. The candidate prepared the study posters and designed the pathology form defining the study inclusions.

She displayed the study posters in the Department and informed all the midwives in the delivery unit. The candidate prepared clear instructions and a flow chart for the delivery unit staff for this study.

The candidate was responsible for the recruitment of the patients and counselling them for participation in the study. The candidate made her available to all participating patients to discuss any queries of the study.

The candidate learnt and personally performed most macroscopic examination and imaging of the placenta. All histological examinations were performed by pathologists; Dr Flora Jessop, Addenbrookes Hospital, Cambridge and Prof. Neil Sebire, Great Ormond Street Hospital, London. The candidate played an active role in liaising with the Department of Fetal Medicine and Pathology at Addenbrookes' and Great Ormond Street Hospital, London.

The candidate prepared the obstetrics and neonatal part of the study database and established it on the perinatal server of the Addenbrookes Hospital. She was responsible for entries of all participating patients in the database. She collected all the data, mostly from the obstetric and neonatal database and remaining from the patients' case notes.

The candidate performed all statistical analyses of the study. She prepared the manuscripts and submitted the papers to scientific journals under the auspices of her supervisors.

PUBLICATIONS

Published in peer-reviewed journals

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Pathak S, Hook E**,** Hackett G**,** Murdoch E**,** Sebire NJ**,** Jessop F**,** Lees C**.** *Cord coiling, umbilical cord insertion and placental shape in an unselected cohort delivering at term: Relationship with common obstetric outcomes.* **Placenta.** 2010; 31: 963-8.

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** *Relationship between placental morphology and histological findings in an unselected population near term***-** accepted **Virchows Archiv**, March 2011

Submitted Papers

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** Macroscopic and Histological Features of Placentas at 34-43 weeks' Gestation and their association with infant admission to the Neonatal Unit- short communication, **BJOG** April 2011

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** Placental histology at or near term in relationship to obstetric outcome- **Virchows Archiv** April 2011

Papers in preparation

Placental morphological and histological features: relevance to fetal distress.

Specific placental histology in relation to the obstetrics and Neonatal outcome.

PRESENTATIONS

Oral Presentation

20.03.2009	Digitalising the placenta East Anglian Obstetric and Gynaecological Society (EAOGS), Cambridge.

Oral Poster

17.09.2009	The relationship between cord centrality index, eccentricity and cord coiling in the term placenta. 19th World Congress on Ultrasound in Obstetrics and Gynaecology (ISUOG), Hamburg, Germany, 13-17th September, 2009.
17.09.2009	The Relationship between placental circumference, placental weight and birth weight at term. 19th World Congress on Ultrasound in Obstetrics and Gynaecology, Hamburg, Germany, 13-17th September, 2009.

12.10.2010	Placental weight, cord insertion, shape and cord coiling in hypertensive disorders of pregnancy. 20th World Congress of International Society of Ultrasound in Obstetrics and Gynaecology, 10-14th October, 2010. Prague, Czech Republic.
12.10.2010	Placental weight, cord insertion, shape and cord coiling in pregnancies with gestational diabetes mellitus (GDM). 20th World Congress of International Society of Ultrasound in Obstetrics and Gynaecology, 10-14th October, 2010. Prague, Czech Republic.
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ABBREVIATIONS

AC	Abdominal circumference
aCL	Anti cardiolipin antibodies
AFI	Amniotic fluid index
aPL	Anti phospholipid antibodies
APS	Anti phospholipid syndrome
BPD	Bi parietal diameter
CCI	Cord centrality index
COREC	Central office of research and ethics committee
CLD	Chronic lung disease
CRL	Crown rump length
CRP	C-reactive protein
CTG	Cardiotocography
DCDA	Dichorionic diamniotic
DLC	Differential leucocyte count
FL	Femur length
GDM	Gestational diabetes
HC	Head circumference
ITP	Idiopathic thrombocytopenic purpura
IUD	Intra uterine death

IUGR	Intra uterine growth restriction
IVT	Intervillous thrombus
LA	Lupus anticoagulant antibodies
MCA	Middle cerebral artery
MCDA	Monochorionic diamniotic
MPFD	Massive perivillous fibrin deposition
NEC	Necrotizing enterocolitis
NGA	Normal for gestational age
NICU	Neonatal intensive care unit
OGTT	Oral glucose tolerance test
PDA	Patent ductus arteriosus
PET	Pre-eclampsia
PI	Pulsatility index
PIH	Pregnancy induced hypertension
PMNs	Polymorphonuclear leucocytes
PPROM	Preterm premature rupture of membranes
PROM	Premature rupture of membranes
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
SCBU	Special care baby unit
SGA	Small for gestational age

SLE	Systemic lupus erythematosus
SUA	Single umbilical artery
TLC	Total leucocyte count
TORCH	Toxoplasma, Rubella, Cytomegalovirus and Herpes
UCI	Umbilical coiling index
VUE	Villitis of unknown etiology

DEDICATION

I dedicate this work to Rahul, Akanksha, Akshita and my parents who have been a source of inspiration in everything I do

CHAPTER 1 : INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The placenta is a neglected human organ. Pathological assessment of the placenta may provide considerable information which may have significant clinical impact on mother and the neonate. According to the guidelines of the Royal College of Pathologists, any sample of diagnostic value removed from the human body should be histologically examined, with only a few exceptions.[1] One of the exceptions is the healthy human placenta, but even with valid indications the human placenta is one of the most under-examined specimens.[2] There are no gold standards for placental examination for clinical indications. There is also evidence that the quality of reports on the investigation of the placenta is very variable.[3] There is no consistency in the gross and histological reporting of the placenta. There is also a considerable discrepancy rate in the diagnosis of placental disease, and it is common for general surgical pathologists not to recognise placental lesions that may have clinical relevance.[4] It has been suggested that the standards of placental surgical reporting can be improved, possibly by the use of templates and checklists for reporting of placentas.[3]

The placental and umbilical cord measurements vary greatly from one pregnancy to another. There has been an increasingly growing interest in the morphometry of the placenta and the umbilical cord. We do not know if umbilical cord coiling, abnormal insertion of umbilical cord onto the chorionic plate and chorionic plate shape are linked with perinatal outcome. As per normal practice, morphology of the placenta and umbilical cord are defined qualitatively. Traditionally coiling of the cord, cord insertion and the placenta shape has been described qualitatively by almost all studies. These indices may have clinical associations with pregnancy outcome though there is a need to derive quantitative morphological indices, describing the above parameters in order to improve the accuracy of diagnosis. In recent years many researchers have emphasised an importance of hyper or hypo coiling of the cord.[5-8] These studies have suggested adverse perinatal outcome with abnormal (hyper/hypo) insertion. However, there is still uncertainty with regards to the exact association of abnormal coiling to

the perinatal morbidity and mortality. It is believed that peripheral insertions of the umbilical cord are linked to poor pregnancy and neonatal outcome. There is confusion with the paracentral (eccentric) cord insertion type, as most studies conducted in the past have explained the paracentral insertion qualitatively. Central and paracentral insertions have no clinical significance; however, an extreme eccentric insertion may be associated with poor pregnancy and neonatal outcome.[9] Shapes of the placenta have been described as round, oval, irregular, star shaped, multi-lobate.[10] Irregular chorionic plate shape may have an association with altered placental function resulting into adverse outcomes such as low birth weight and placenta weight ratio.[10]

To avoid the subjective bias in placental morphological analysis, we derived quantitative indices describing umbilical cord insertion, as "Cord Centrality Index" and chorionic plate shape as "Eccentricity". The coiling of the umbilical cord has been defined by an already established method as "Cord Coiling Index".[11]

A common practice of examination of the placenta, following an adverse perinatal outcome, such as pre term labour, severe intra uterine growth restriction (IUGR), still birth or poor neonatal outcome is to perform a manual measurement of the placenta and the umbilical cord. Digital imaging is being used in many sub disciplines of medicine including digital measurements of the placenta.[12-13] In the current study, digital measurements of the placenta were performed using an image processing programme, Image J. This java image processing software has been used in various studies and compared with other advanced routinely used investigative techniques such as CT and MRI.[14]

There are certain potentially treatable maternal conditions, recurrent placental or inherited fetal conditions that can be identified by placental examination. Therefore, there is a need to standardise the method and reporting of placental examination. In order to advance and promote better understanding of placental pathology, the College of American Pathologists have encouraged clinicians to perform studies on the placenta and umbilical cord.

The American college of Pathologists published guidelines in 1991 to encourage properly designed placenta studies with appropriate outcome parameters necessary to draw conclusions.[15]

Tragedies such as perinatal death or severe neurological impairment are increasingly recognised as being associated with pathological conditions of the placenta and the umbilical cord. These frequently develop long before labour and delivery and cannot be prevented by even the most attentive obstetric care. Under these circumstances, examination of placenta and umbilical cord in the delivery room as well as in the pathology laboratory can make a crucial contribution to the investigation of instances of pregnancy failure.

The placenta is a functional unit between the mother and the fetus. Therefore, any pathological event concerning the mother or the fetus may influence the normal function of the placenta, occasionally resulting in morphological changes. Severe abnormalities of the placenta may lead to adverse fetal outcome. However, placental lesions are not necessarily the cause of unfavourable obstetric outcome, and some structural changes may be the consequences of poor fetal condition.[16]

Placental examination can be helpful in identifying the etiology of stillbirth, preterm delivery, intrauterine growth restriction, and neurodevelopment impairment. It may be possible to determine whether the pathological condition that endangered the well being of the fetus was an acute or a chronic process.[17-18] Conditions with the risk of recurrence can be recognised, resulting in adequate treatment and preventive measures during subsequent pregnancies. There has been study performed to determine whether placental investigations assist in determining the cause of still birth and found some placental lesions were associated with clinical causes of still birth such as placental infarction, leukocyte infiltration and chorioamnionitis. Assessment of placenta can aid classification of still birth, even when full infant post-mortem is declined.[19]

Placental examination may have medico legal implications for example, concerning the aetiogy of long term neurodevelopment sequelae or the approximate timing of an intrauterine death.[20-21]

The placenta is one of the important means of establishing that the fetal damage may cause adverse pregnancy outcome independent of clinical care.

The entire literature is dominated by small case series of placental abnormalities rather than defining incidence of histological pathologies of the placenta in a general population. Previous studies on placental pathology have been largely confined to the association of particular outcomes in relation to specific abnormalities, usually in a small number of selected cases. The majority of studies are retrospective and notable also is the minimal consideration in published studies given to the combined effects of multiple placental lesions.

Another major concern is that most studies of the placenta are based on abnormal placentas, which makes it difficult to differentiate between the "pathologically abnormal" versus "normal variant". There are few studies that analyse normal placentas statistically and identify the normal variants of histological lesions during the course of pregnancy.

Recent advances such as more advanced ultrasound techniques, MRI in fetal medicine and placental pathology are now such that the opportunity exists to establish clearly which placental lesions represent the greatest threat to a healthy pregnancy and to a positive neonatal outcome. It is believed from that certain placental lesions are associated with poor neonatal outcome; conversely, there are relatively recently described placental lesions such as abnormal cord coiling, Villitis of unknown aetiology and thrombotic vasculopathy where the precise clinical implications are still not well understood.

This study will unite antenatal observations and neonatal outcomes with macroscopic and microscopic examination of a cohort of unselected pregnancies delivered in a single unit. We expect through this research to propose quantitative indices and establish a rationale for identifying both normal and pathological appearances of placental histology, by linking the findings with pregnancy and neonatal outcome.

1.2 PLACENTA

1.2.1 Overview of placental anatomy

The placenta is the most important and the only organ between mother and fetus, serving multiple functions. It acts as an endocrine organ producing several types of hormones e.g. lactogen, chorionic gonadotrophins etc. It allows the exchange of oxygen and CO_2, whereby transfer of oxygen takes place from maternal blood to the fetus, and carbon dioxide goes out from fetus to mother. It allows the transfer of carbohydrates, protein, amino acid, polypeptides, lipids, vitamins, water, electrolytes and pharmacological agents from the mother to the fetus.

A placenta has a maternal surface (basal plate) and a fetal surface (chorionic plate). The basal plate and chorionic plate meets at the placental margin and form the smooth fetal membranes. The space between chorionic and basal plate is filled with the intervillous lakes of maternal blood.

Basal plate

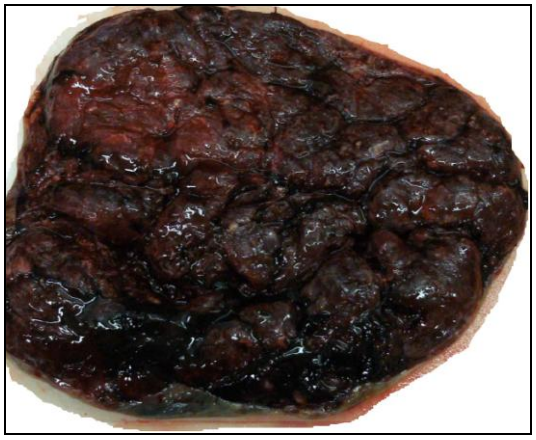


Figure1.1 Maternal surface of a normal term placenta with cotyledons (study photograph)

The basal plate is the placental surface, attached to the uterine wall during pregnancy, once separated called basal plate or maternal surface. Therefore the basal plate would have both fetal as well as maternal component to it, seen microscopically. The maternal surface is opaque, dark red in colour, consists of protuberant growths called cotyledons, usually 10-40 in a healthy normal term placenta, these cotyledons are separated by grooves, occupied by placental septa in situ (fig 1.1). These septas can extend even up to the fetal surface.

Microscopic view

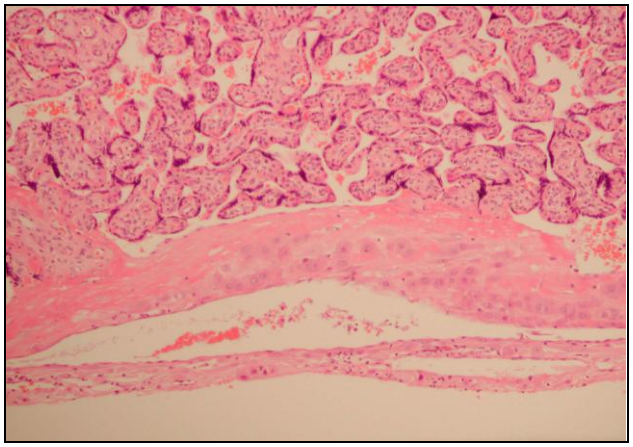


Figure 1.2 Microscopic view of basal plate of the placenta (study photograph)

Figure 1.2 shows the microscopic view of the basal plate. The basal plate has fetal extravillous trophoblasts and all types of maternal cells from uterine deciduas and deciduas basalis. The villi are surrounded by the maternal endometrium. The trophoblast cells, lining the endometrial side of the villi, synthesize extracellular matrix, fibronectin. In microscopic examination of the basal plate, dilated maternal spiral arteries lined by fibrinoid matrix together with endovascular trophoblasts can be seen.

Chorionic plate

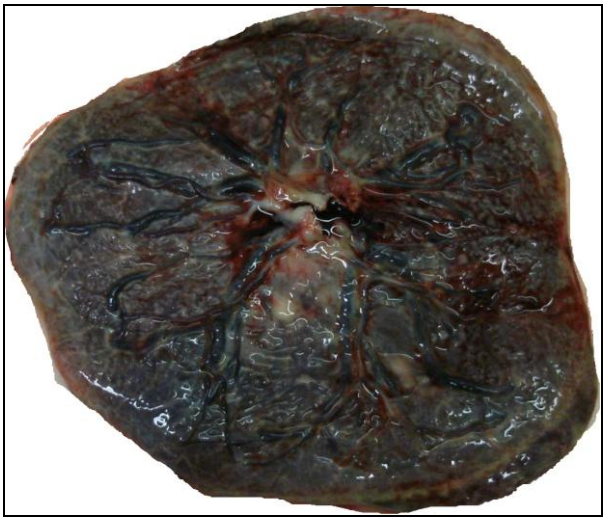


Figure 1.3 Macroscopic view of chorionic plate of the placenta (study photograph)

This is the fetal surface of the placenta; a variable amount of whitish-yellow subchorionic fibrin can be seen under the chorion (fig 1.3). There is no clinical importance of subchorionic fibrin unless it is massive and extensive. Amnion is composed of epithelium and amniotic mesenchyme. Amniotic mesenchyme is attached to the chorionic mesenchyme loosely, which can be very easily separated. The umbilical cord is attached onto the fetal surface. Chorionic vessels run in the chorionic mesenchyme, which are then continuous with the vessels of the umbilical cord that eventually supplies the villous tree. The chorionic veins give rise to one single umbilical vein. Umbilical vessels give rise to multiple branches, which spread all over the fetal surface, up to the peripheral margin of the placental disc.

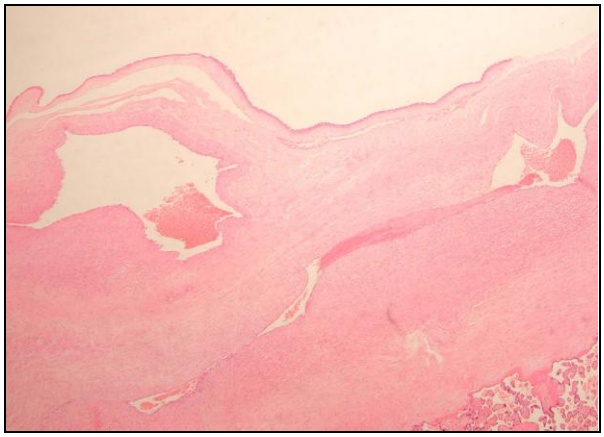


Figure 1.4 Microscopic view of the chorionic plate of the placenta (study photograph)

Stem villi are composed of dense connective tissue, which are surrounded by arteries and vein. The arteries are thick walled compared to the veins. Peripheral extension of the stem villi gives rise to immature intermediate villi, which implant in the basal plate and mature intermediate villi. Immature intermediate villi are predominant form of villous tree in immature placentas, between 8-20 weeks of pregnancy.[22] Mature intermediate villi develop early in third trimester and this type makes up to quarter of villi in a mature placenta.

The lateral expansion of the chorionic plate of the placenta plateaus by the middle of third trimester.[23] At 30-32 weeks gestation, growth of the placenta is mainly by arborisation of the villous tree, resulting into the growth of its thickness.[24]

1.2.2 Placental Shape

Normal development of a placenta is the one of the important requirements for a healthy pregnancy, regulating fetal growth and fetal health. The placental shape is evidence of a normal development of the placenta. The placenta is normally considered to be a round discoid shape; however the shape has been described in various ways such as round, oval, irregular, star shaped, bi-lobate, multi-lobate, circumvallate, circummarginate and many more (figure 1.5). The placenta is the primary source of nutrients and oxygen to the fetus. A variable maternal utero placental environment affects macroscopic placental structure as a change in shape.[10] Therefore, the shape of the chorionic plate is a reflection of the function of the placenta and an abnormal placental shape could indicate abnormalities in utero-placental environment.

The clinical significance of abnormal placental shape has led to mixed opinions. One view is that irregular shapes are associated with lower birth weight for placental weight, suggesting variable shaped placentas have altered function.[10] Some believe that abnormal shapes (circumvallate and circummarginate) are simply variants of normal shape and has no clinical significance[25] while one of the large studies of 7666 cases has shown association of circumvallate placenta to the higher incidence of preterm labour, placental abruption and intra uterine fetal death.[26] Another study showed higher odds of intra uterine fetal death (OR 4.7; 95% CI 1.4-15.1), preterm delivery at < 32 weeks (OR 4.7; 95% CI 1.6-14.1) and intrauterine growth restriction (OR 4.7; 95% CI 1.4-15.1) with abnormal shape of placenta than did the women with a normal placental shape.[25 27]

A placenta with accessory lobes (succenturiate lobes) can potentially be detected antenatally by ultrasound; most have no clinical significance but may be associated with retained accessory lobe after delivery leading to either post partum haemorrhage or uterine sub involution. Multilobate placenta may be associated with velamentous insertion or blood vessels traversing in between the lobes, covering cervix, causing vasa previa.

Traditionally placental shape is described qualitatively but recently a study emerged with quantitative analysis of the shape of the placenta.[10] The same group related the variability in the chorionic plate shape to the structure of the underlying vascular tree. Figure 1.5 shows the different variants of placental shapes. First column shows the normal round to oval shape, while 2nd column with star shaped placenta and 3rd column has placentas with accessory lobes.

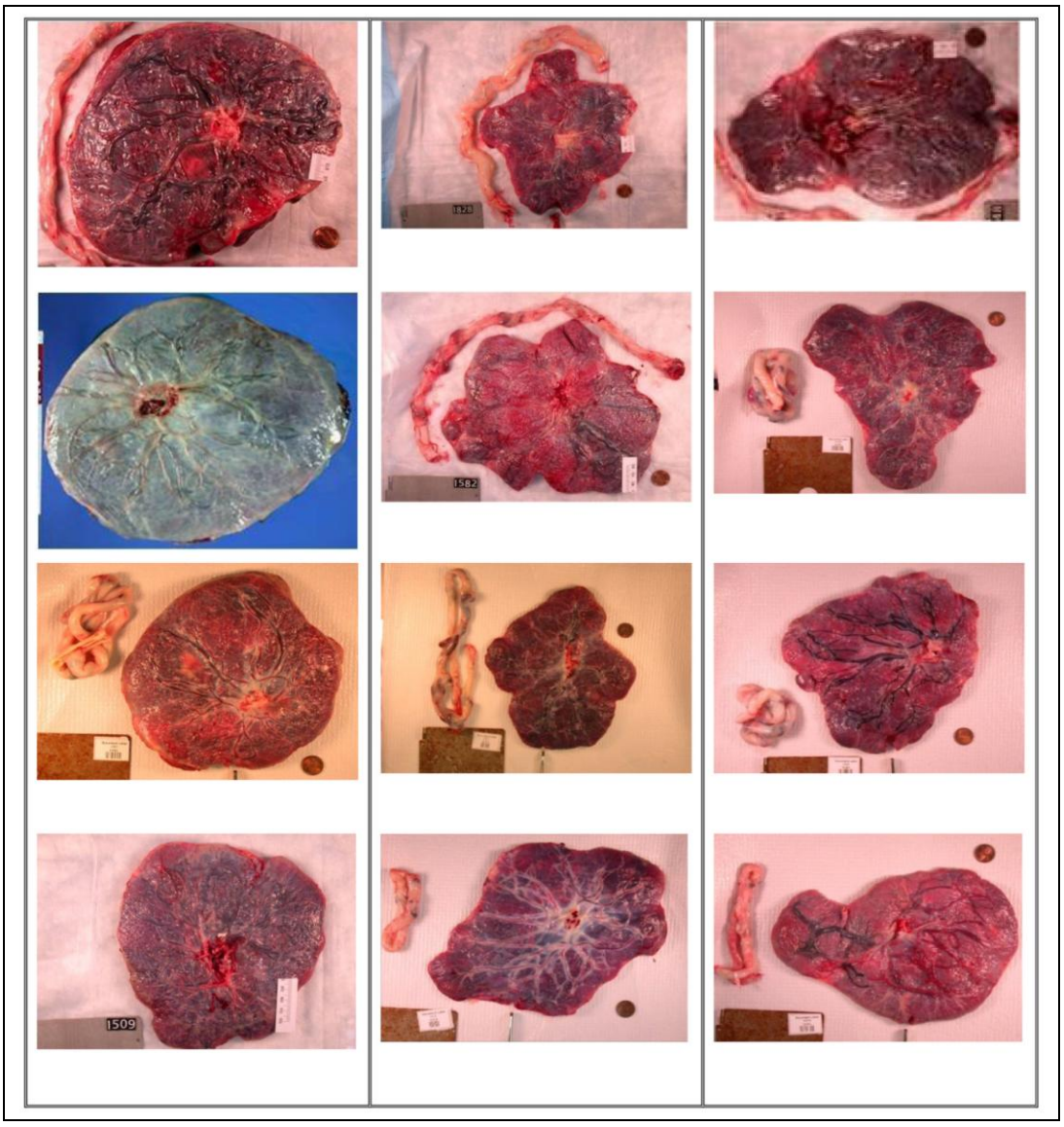


Figure1.5 Shape variants of the placenta

(Source- Yampolsky M, Salafia CM, Shlakhter O, Haas D, Eucker B, Thorp J. Modeling the variability of shapes of a human placenta. *Placenta* 2008;29(9):790-7).[10]

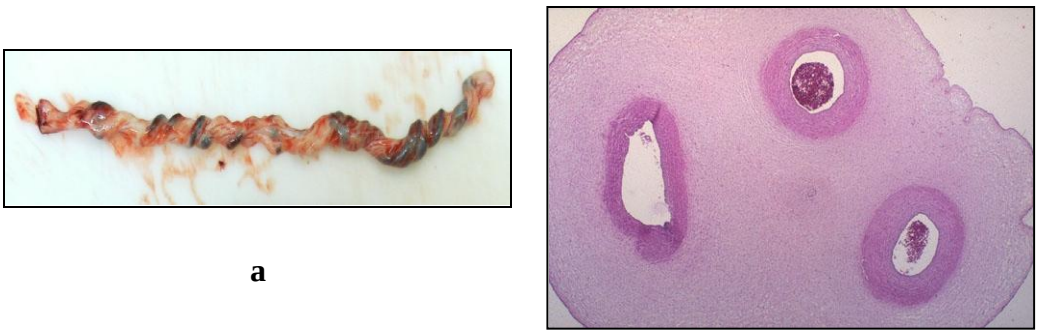
1.3 UMBILICAL CORD

1.3.1 Embryology & Development of the Umbilical Cord

The primitive umbilical ring is the junction between amnion and embryonic ectoderm. At the fifth week of development, structures passing through this ring are the connecting stalk containing allantois and umbilical vessels, yolk sac (vitelline duct) with vitelline vessels and the canal connecting intra and extra embryonic cavities. Extra-embryonic mesoderm grows towards the centre to form the chorionic cavity, a place which is occupied by the proper yolk sac. During the development, the yolk sac rotates towards the implantation site. The embryo then folds into the amniotic cavity. Subsequent expansion of the amniotic cavity occurs at the expense of chorionic cavity. Later amnion envelops the connecting stalk and yolk sac stalk together and that forms the primitive umbilical cord. During early development, 13-40 days post conception umbilical cord forms at the site of the connecting stalk, which joins the extra embryonic mesoderm to the embryonic disc. Proximally the primitive umbilical cord also contains some intestinal loops. By the end of the third month amnion has expanded in such a way that it comes in contact with chorion, obliterating chorionic cavity. The yolk sac shrinks and gets obliterated. As the development progresses, the connecting stalk containing allantois, vitelline duct and the umbilical vessels gets smaller in diameter and increases in length. Later allantois and vitelline duct are obliterated. Umbilical vessels remain at the end of the development, which is surrounded by the Wharton's jelly. There are two umbilical arteries, formed from two embryonic allantoic arteries. Initially there are two allantoic veins but as the development progresses, within first two months, the right allantoic vein disappears and only left forms the umbilical vein.

1.3.2 Overview of Umbilical cord anatomy

In a typical three vessel umbilical cord there are two umbilical arteries and one umbilical vein, suspended in a mesodermal mucoid matrix stroma, called Wharton's jelly. There are no nerves or lymphatic vessels in the umbilical cord. Wharton's jelly consists of myofibroblasts and ground substance.[28-29] This combination of loose gel and contractile cells gives the umbilical cord tensile strength and umbilical vessels are protected against any pressure or compression. The umbilical arteries do not have an internal elastic lamina and the media of the artery is composed of peripherally arranged spiral muscles. The umbilical vein does have an elastic lamina and the smooth muscle layer is thinner than the umbilical artery. Therefore, during the antenatal period, the protective elements for any umbilical cord are amniotic fluid, Wharton's jelly and the helical coiling of the umbilical vessels.



a **b**

Figure 1.6 Macroscopic and histological view (H & E stain) of a normal 3 vessel view of the umbilical cord (a)study photograph (b) Websource-http://library.med.utah.edu/WebPath/PLACHTML/PLACIDX.html#5)

Umbilical cord length

It is still not well understood, what factors exactly decide the length of the umbilical cord. However, the evidence so far available suggests fetal movement producing the tensile force on the umbilical cord and genetic factors play an important role in deciding the length of cord. Most studies on the placenta and umbilical cord to date have measured cord which is attached with the placenta. Ideally the measured length of the cord should also include the measurement of the cord attached to the baby. As that length can be variable depending on the condition of the baby on delivery, its length taken only on the part attached to the placenta will not be a true measurement of the cord.

It is believed that the tensile strength produced from the fetal movements is an important deciding factor for the length of the cord; conditions which affect the fetal movements' in-utero will affect the length of the cord too. Conditions restricting fetal movements such as skeletal dysplasia, amniotic bands, oligohydramnios, multiple pregnancies, uterine malformation usually have a short cord.[30-32]

Boyd and Hamilton (1970)[33] reported on lengths at various gestation as outlined below in the table. This table reflects the increasing length with increasing gestation.

Gestation (month)	Length of fetus (mm)	Average cord length (cm)	Range (cm)
Third	31-60	6	2.3 – 10.5
Fourth	61-100	15	5 – 25
Fifth	101-150	25	14.5 – 45
Sixth	151-200	28	21 – 48
Seventh to term	200-340	35	22 – 48 (or more)

Table 1.1 Umbilical cord length as per gestation (Source-Boyd and Hamilton-1970)[33]

Though the umbilical cord is extremely variable in length, an average normal length of umbilical cord is 55-60 cm.[9] The minimum cord length which allows the normal vertex delivery is 32cm.[34]. Since this study, most authors have accepted this length as a minimum length, anything less than this is considered to be a short cord. Considering this definition of short cord, the incidence of short cord has been reported as 0.4-0.9% [35-37], while in one study incidence of cord less than 35 cm was 2%.[38]

A short cord has been associated with intrauterine fetal distress and neonatal asphyxia.[39] It is believed that a possible cause of fetal hypoxia with short cord is excessive traction on the cord during descent of the fetus, which results into occlusion of the cord vessels. However, this remains speculative as the pH and base deficit of the umbilical blood were the same for neonates with normal umbilical cord length.[38]

There is no agreed definition, as to what should be termed as excessive long cord. Some studies have defined cord length more than 80 cm as long cords[38], while others have taken 100 cm as the upper limit of the cord length.[36] These studies have defined incidence of long cords as 3.7%[12] and 0.5%[10] respectively. Long cords have been associated with increased knotting (true knots), torsion and cord prolapsed.[40]

Umbilical cord diameter

The umbilical cord diameter depends upon the number of vessels present, size of the umbilical vein and the fluid content of Wharton's jelly. By what, factors determining the amount of water content in Wharton's jelly are not clearly understood. The normal cord diameter is 1-2 cm and the cord can be oedematous in clinical situations such as maternal diabetes mellitus. Fetal outcomes are better with increased jelly in the cord, while cords with reduced Wharton's jelly are more prone to compression and abnormal fetal heart rate pattern, an absence of Wharton's jelly around umbilical vessels have been reported to be associated with perinatal death.[41]

Umbilical cord coiling

The umbilical cord is protected by Wharton's jelly, coiling of the umbilical vessels and the amniotic fluid. The umbilical cord is a coiled, helical structure but the origin of coiling remains unknown. Possible explanations of the origin of coiling have been given as torsion by active or passive movements, causing the embryo to rotate around its umbilical cord axis[42], presence of Roach muscle, which is a small bundle of muscles lying just beside the umbilical artery, contributing to the coiling[43], and hemodynamic forces of the fetus.[44]

Umbilical cord coiling is well established by 9 weeks of gestation. It is unknown whether there are genetic factors involved in coiling or if it is only acquired but the degree, tensile stress and the number of coils seems to be affected by in-utero fetal movements.[45] Considering the fetal movements as a theory for coiling of the umbilical cord, hypocoiling is seen in cases of fetal anomalies affecting fetal movement such as amniotic bands, oligohydramnios, uterine malformations, twins and pregnancy affected by chromosomal anomalies[46] whereas hypercoiling is more common in parous women, male fetus (presumed to be more active) have longer cord than female fetus, maternal cocaine use and longer umbilical cords.[47] The hypocoiled cord is more prone to kinking, compression and stasis.

Frequency of noncoiled cord is reported as 4.3-4.9%[5 48] while hypercoiling has been reported as high as 20% in unselected placentas.[6]

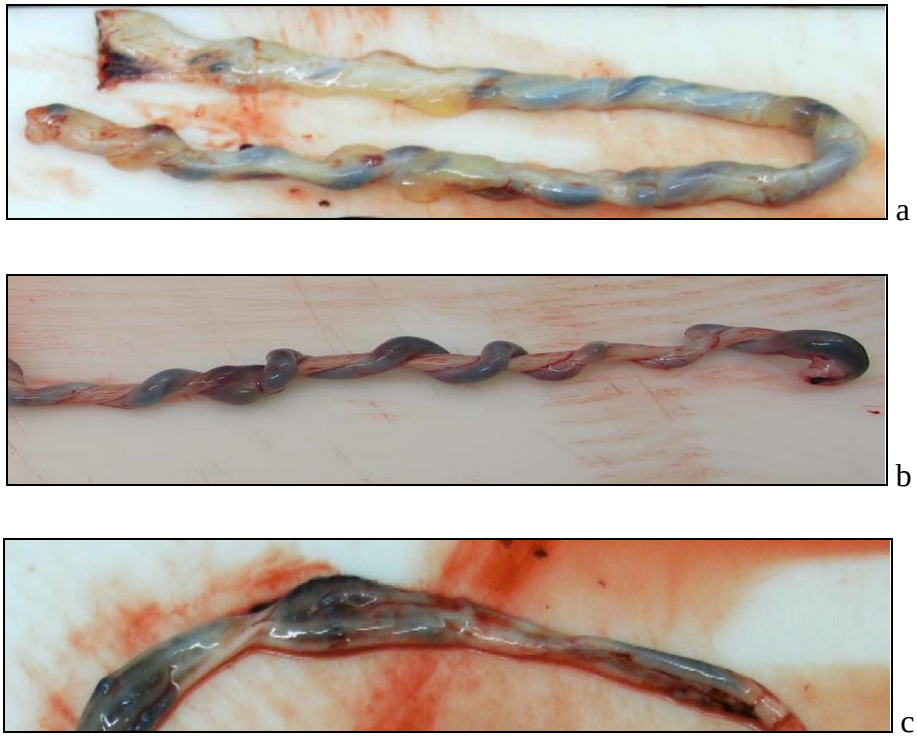


Figure 1.7 Coiling of the umbilical cord (a) Normal coiling (b) Hypercoiling (c) Hypocoiling (study photographs)

Coiling is either anti-clockwise (left) or clockwise (right). Anti clockwise coiling is more common. To define the direction of coils, the umbilical cord is placed vertically and vessels on the anterior surface of the cord are noted; if the direction is towards the left hand of the observer, it is noted as left sided coiling and right sided if it directed towards observer's right hand. Studies have observed the direction of coiling either from placental end[49] or from the fetal end [50], however the direction of the coiling would not in fact alter whether viewed from the placental or the fetal end.[51] The prevalence of anti-clockwise coiling varies from 65 to 76%.[8 49] It is not very well understood why anti-clockwise pattern is more common, few researchers have proposed a possible explanation to this, as cranial arteries also have a spiral form, there may be a relationship between the direction of umbilical arteries spirals and left or right sided cerebral dominance.

1.3.3 Umbilical Cord Coiling Index

Umbilical cord coiling was first described in terms of the number of coils in 1954 [42] as "The index of Twist", which was calculated by dividing the total number of coils by the umbilical cord length in centimetres. The coiling pattern was described as negative for anti-clockwise and positive for clockwise coiling. Later studies[11] defined coiling as the term "The Umbilical coiling Index" (UCI). UCI was determined by so called Strong's formula by dividing the total number of complete umbilical vascular coils by the umbilical cord length (in centimetres), UCI was reported as 0.21 ± 0.07 coils/cm, there being at least one coil in every 5 centimetres of the cord. Therefore hypocoiling of the umbilical cord will be defined for UCI smaller than 10th percentile (i.e. less than or equal to 0.1), while hypercoiling as UCI above 90th percentile (i.e. equal to or more than 0.3).

Antenatal Assessment of Cord Coiling Index

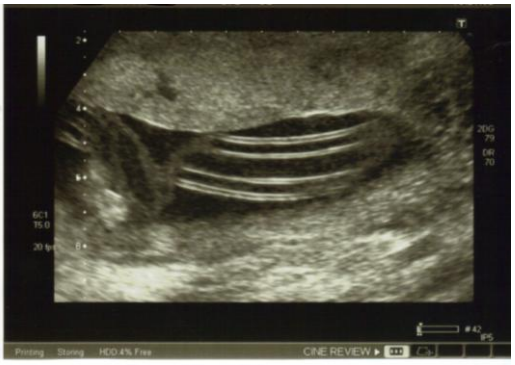
The umbilical cord can be studied sonographically for various prenatal abnormalities or possible pathologies. Apart from traditional antenatal assessment of the umbilical cord, which includes only the number of blood vessels in the umbilical cord and Doppler assessment of the umbilical arteries, further detailed assessment can be done prenatally, such as the amount of Wharton's jelly, diameter of umbilical vessels[7], coiling pattern and coiling index.[49 52]

Though the reproducibility of these measurements is not yet at a level where they can be put to widespread use.

UCI can be calculated for the cord either at fetal end, placental end or the central part. There is more coiling towards the fetal end as compared to the placental end[53], therefore UCI will be increasing as we move from the placental end to the fetal end. In studies the UCI has been measured at the placental end at the umbilical cord insertion, or the fetal end near the umbilical cord entrance into the fetal abdomen and in the middle of these two[49] to obtain the true UCI.

To assess the UCI prenatally, in a longitudinal image of the umbilical cord, a pair of coils is identified and the distance between the coils is measured and UCI is calculated. However it is not clear as to which section of the umbilical cord should be chosen and how many intercoil distances should be measured to obtain the true UCI (postnatal UCI).

Coiling of the umbilical cord is fully developed by end of the first trimester and it does not change after this except that the length of the cord keeps getting bigger in between the coils.[42] [54] In the second trimester, the entire umbilical cord can be visualised better in its entirety as compared to the examination in third trimester, where with relatively reduced liquor volume and the growing fetus, obtaining full view of the longitudinal section of the umbilical cord can be challenging especially of the fetal end of the umbilical cord. Therefore the most appropriate time to assess prenatal UCI should be second trimester with relatively greater amount of the amniotic fluid as compared to the fetal size, which allows the visualization of the most part of the cord. Studies have shown that sonographic evaluation of umbilical cord coiling in the second trimester correlates with the true UCI at birth.[55]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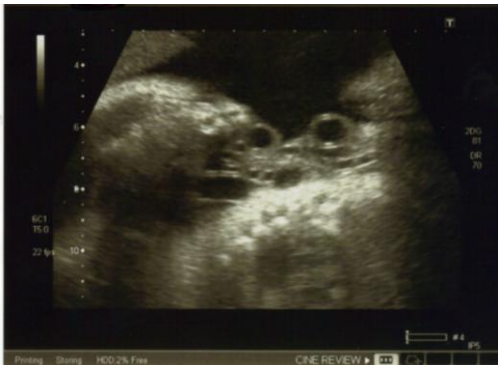
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Figure 1.8 Antenatal ultrasound of cord coiling in (a) 2nd and (b) 3rd trimester of the same patient

Postnatal Assessment of Cord Coiling Index

The true UCI is the one which is assessed after birth. At delivery, the direction and the number of coiling are noted and umbilical cord coiling index is calculated by the standard Strong's formula that is defined as dividing the total number of completed coils by the length of the umbilical cord.

Antenatal versus Postnatal Assessment of Cord Coiling Index

The sensitivity of second trimester sonographic measurement for predicting hypocoiling or hypercoiling ranged from 9-40%[49 52] as compared to the postnatal measurement of UCI. The distension of the umbilical vessels with the fetal blood resulting into tighter coiling of the vessels and therefore increased UCI could be the possible explanation for this low sensitivity[56], or more coiling being towards the fetal end as compared to the placental end, or limited visualisation of the umbilical cord after the second trimester due to the relative fetal size and the amount of liquor volume, making it difficult to measure UCI.

Most studies are not able to examine the umbilical cord which remains attached to the baby and the cord attached to the neonate could be of any length. Whether that makes any difference in the calculation of post-partum UCI, considering that umbilical cord is more coiled on the fetal end, is not clear.

1.3.4 Clinical significance of abnormal cord coiling

Maternal risk factors for abnormal vascular coiling have been proposed as extremes of maternal ages, obesity, gestational diabetes mellitus and pre-eclampsia[57], while others did not find any association between UCI and maternal age.[58-59]

Hypocoiling and hypercoiling of the cord whether detected prenatally or postnatally, have been correlated with adverse perinatal outcome. Researchers have related different variables to the abnormal coiling of the umbilical cord. Hypocoiling has been associated with trisomies, preterm delivery, fetal death, increased intrapartum complications and interventional deliveries for fetal distress, Apgar score less than 7 at 5 minutes, velamentous cord insertion and single umbilical artery, while hypercoiling of the umbilical cord has been linked with trisomies, small for gestation age, fetal asphyxia and single umbilical artery.[5 8] Abnormal cord coiling is also associated with the thrombosis of the chorionic plate vessels, umbilical venous thrombosis and umbilical cord stenosis.[6]

There is still controversy regarding the association of hypocoiling and adverse pregnancy outcome. A possible explanation for hypocoiling and adverse perinatal outcome has been attempted by showing a significant correlation between umbilical coiling index and umbilical venous blood flow[7 60], which further suggest that quantitative analysis of umbilical venous blood flow may be a useful tool in conditions such as intra uterine growth restrictions.[60-61] Umbilical cord is considered as a pumping system, the coiling of the cord is believed to contribute to the fetal venous return by creating a umbilical venous pressure.[62] Increased coiling would perhaps make this pumping system more efficient and therefore increase umbilical blood flow while the hypocoiling of the cord would lead to reduced blood flow and eventually affects the growth of the fetus causing intrauterine growth restriction.[62] It is also thought that overcoiling makes a cord less flexible and undercoiling makes a cord floppy, making an undercoiled cord more prone to entanglement.[63]

Complicated pregnancies and UCI

Studies have investigated those placentas and umbilical cord, which needed a histological examination of the placenta for various indications such as fetal demise, intrauterine growth restriction, preterm delivery, pre-eclampsia, diabetes mellitus, macroscopic abnormalities of placenta, fetal asphyxia, and intra uterine infection. Fetal death, preterm delivery, umbilical artery pH<7.05, fetal structural and chromosomal anomalies, fetal placental vessel thrombosis, fetal hypoxia and low birth weight were associated with hypercoiling while chorioamnionitis, lower Apgar score at 5 minutes were associated with hypocoiling. Fetal demise and structural or chromosomal fetal congenital anomaly cases were associated with hyper and hypocoiling of the cord.[63]

As the umbilical cord coiling index can be assessed prenatally, the association of abnormal cord coiling index with perinatal outcome can be a predictive measure of adverse antenatal and perinatal outcomes. However, considering the low sensitivity of prenatal detection, larger studies are needed to assess the prenatal ultrasound findings to the perinatal outcome.

In summary there is still controversy and the significance of abnormal UCI (hypercoiling and hypocoiling), its association with adverse perinatal outcome remains unclear. However, it is a very simple but significant examination in assessing cases particularly with unexplained adverse outcome such as fetal death.

1.3.5 Umbilical cord insertion

Different ways in which the umbilical cord can be attached onto the fetal surface i.e. chorionic plate of the placenta have been defined qualitatively as central, eccentric, marginal (Battledore) and velamentous (membranous).

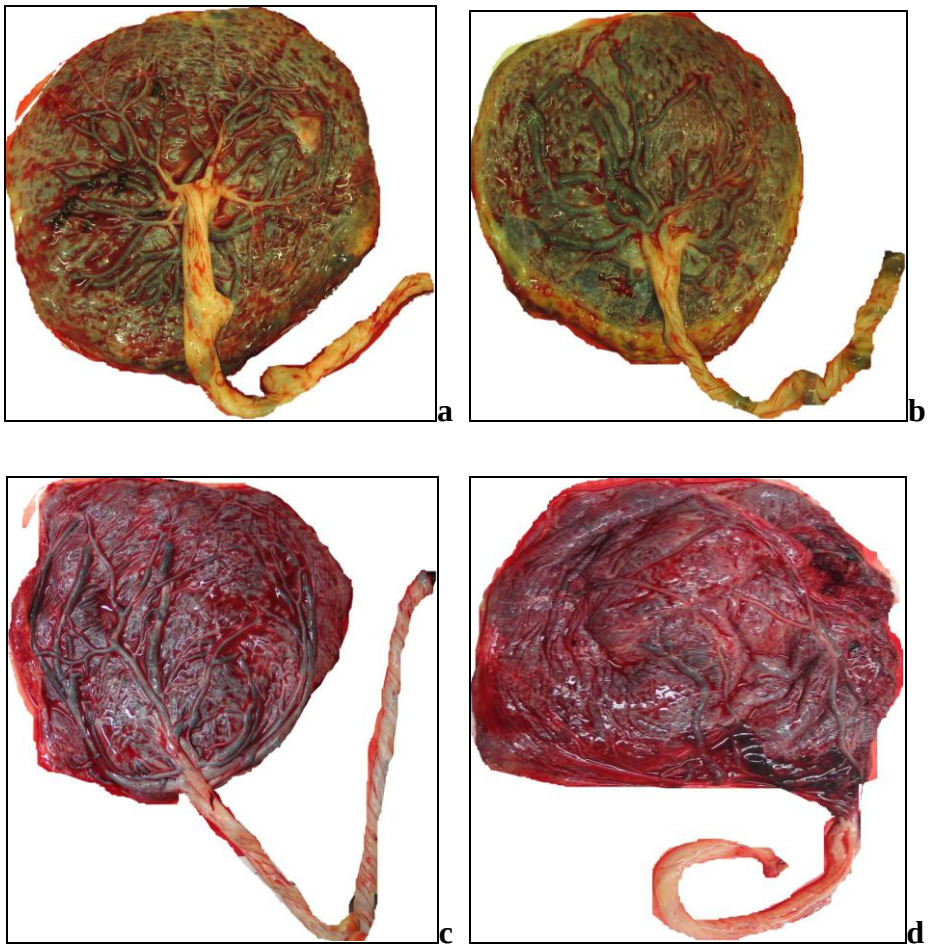


Figure 1.9 Qualitatively described cord insertions (a) Central, (b) Eccentric, (c) Marginal, (d) Velamentous (study photographs)

Central and eccentric insertions account for more than 90% of cord insertions and have no clinical importance, while marginal insertion may be more susceptible to vessel rupture and has been associated with intra uterine growth restriction, still birth and neonatal death.[9]

The incidence of velamentous insertion increases with maternal smoking, advanced age or diabetes mellitus and among multiple births, congenital malformation and in vitro fertilisation pregnancies.[9]

Central insertion is defined as where the umbilical cord is inserted onto the centre of chorionic plate. The cord is inserted at the margin of the chorionic plate in marginal insertion and membranous insertion where the umbilical cord is not inserted into the placenta, but into the membranes. However, eccentric (also called paracentral) cord insertion has not been clearly defined. Though it is understood that the insertion anywhere between central and marginal insertion is eccentric insertion, eccentric insertion can be near to the centre or it can be near to the chorionic plate margin. It depends on the precision of the observer as well, an insertion exactly at the centre is called central, but slightly off the centre, is defined as central or eccentric. It is not clear whether an extreme eccentric insertion (eccentric insertions near the chorionic plate margin) should be defined as an eccentric insertion or marginal. Although the difference between extreme eccentric umbilical cord insertion and marginal cord insertion may be small, this differentiation is important as eccentric umbilical cord insertion is the most common type of umbilical cord insertion in pregnancies with normal outcome while some studies have shown an increased incidence of marginal umbilical cord insertion in pregnancies with adverse outcomes such as miscarriage, fetal congenital anomalies, preterm labour and intrauterine growth restriction.[64-66]

It has been suggested that pathological variations (marginal and velamentous) result from disturbance of implantation or faulty implantation of fertilised ovum, which eventually prevent central insertion of umbilical cord from taking place.[66-67]

The incidence of marginal insertion varies widely possibly due to the different interpretation of "extremely eccentric" and marginal insertion. Different studies have quoted the incidence between 2-9%.[36 68-70] Researchers have different views on the outcomes related to marginal umbilical cord insertion.

Studies have shown an association between marginal cord insertion and increased incidence of congenital malformation[66], neonatal asphyxia[71], and preterm labour[64], while others did not find an increased incidence of above complications with marginal cord insertion.[72]

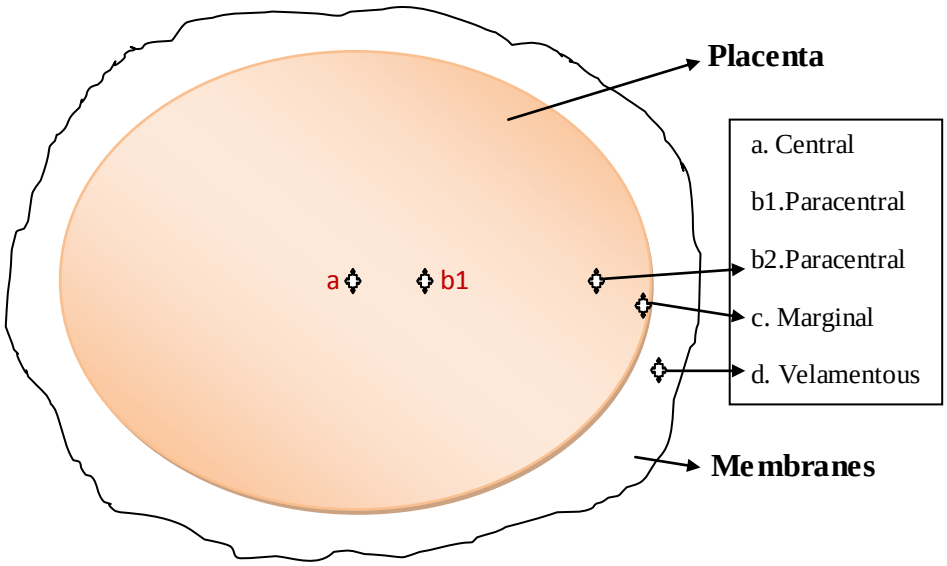


Figure 1.10 Illustration of qualitatively described cord insertions onto the chorionic plate

Velamentous insertion

Velamentous insertion is where the umbilical cord is inserted in between the fetal membranes, amnion and chorion and runs in the membranes unsupported by the placenta before the cord becomes inserted onto the placental fetal surface. Therefore the unprotected umbilical vessels will travel a certain distance before passing onto the placental surface. These membranous vessels are prone to compression, thrombosis, rupture, and haemorrhage especially if vessels are traversing across the cervical os and are ruptured with membranes during delivery, resulting into fetal morbidity and mortality (vasa praevia). The incidence of velamentous insertion is generally agreed as around 1%, with a range of 0.5-1.6%.[68 73-74]

The incidence of velamentous insertion is higher in multiple pregnancies[68], in cases with single umbilical artery[75], congenital fetal malformation and miscarriage[66], while other researchers[69] did not find the association between velamentous insertion and malformation. One of the largest and most detailed studies revealed higher association of velamentous insertion with intrauterine growth restriction, preterm delivery, abnormalities in the fetal heart rate patterns during labour and low Apgar score at birth.[74]

The pathogenesis and etiology of velamentous insertion is not yet clear.[66] [68] One theory is that the yolk sac adheres to the chorion far from where it should have been and this causes abnormal implantation of the umbilical vessels on to the membranes. Another theory suggests that the body stalk does not migrate towards usual decidua basalis, rather umbilical cord arises from chorion which is most vascularised. With the progress of pregnancy vascularity of deciduas capsularis reduces while it increases for deciduas basalis. Therefore part of chorion closer to deciduas basalis, the one which is the site for the definite placenta becomes the most vascularised. That results into cord inserted into the membranes. Another view of velamentous insertion was due to the abnormal implantation. Blastocyst implanting obliquely (rather than facing) in the endometrium, will result into abnormal implantation of the cord. Some favour the theory of "trophotropism", explaining that initially umbilical cord is inserted as normal but later with the growth of placental tissue more laterally leaving central part atrophic and that process leaves the umbilical cord in the membranes.

Prenatal diagnosis of velamentous cord insertion has been successfully made using conventional ultrasound and more so by using colour Doppler ultrasound.[74] Velamentous cord insertion has also been associated with raised maternal serum hCG levels during the second trimester[76] though there is no explanation for this association.

Despite all the possible complications associated with velamentous cord insertion, most pregnancies with velamentous cord insertion pass through antenatal period as well as intrapartum phase without having any complications.

1.4 AIMS AND OBJECTIVES OF THE STUDY

- To compare manual and digital measurements of different axes of the placenta in unselected pregnancies.

- To derive indices for placental morphology from digitally derived measurements.

- To establish criteria and normal ranges for objective determination of macroscopic placental features, including shape and umbilical cord insertion site.

- To establish the quantitative relationships of birth weight to the placental weight and circumference.

- To define the incidence of predefined placental histopathological abnormalities in an unselected cohort of 1159 women with singleton pregnancy delivering in a single unit in relation to macroscopic features.

- To determine the relationship between the predefined placental morphology and predefined abnormalities in relation to specific obstetric and neonatal outcomes of pregnancy.

CHAPTER 2 : METHODS

2.1 DESIGN

This was a prospective study of unselected mixed risk women with singleton pregnancy booking for delivery at the Rosie Hospital. This unit delivers both high and low risk care to women in Cambridge and the Eastern Region of England, and has approximately 6000 deliveries per annum. The study received ethical approval from The Peterborough and Fenland Research Ethics Committee, Cambridgeshire (LREC Ref.no:07/Q0106/51). Recruitment was achieved over 13 months in 2007-2008. Written maternal consent was obtained for placental examination. The study was undertaken in the Department of Fetal Medicine, Obstetrics and Gynaecology, Rosie Hospital and the Department of Pathology, Addenbrookes Hospital, Cambridge.

2.2 MATERIALS AND METHODS

2.2.1 Recruitment of patients

A total of 1159 women with a singleton pregnancy, booking at Rosie were recruited for the study. Study posters, explaining the study were produced to make pregnant women and staff involved aware of the study. Posters were displayed in the antenatal clinics, ultrasound Department, antenatal wards and various places in the delivery unit. The study aims/objectives and methodology were also discussed in Departmental meetings. Subjects were recruited prospectively on an ad-hoc, case-by-case basis. All women presenting to the Rosie Hospital with a viable singleton intra-uterine pregnancy, and booking for delivery at the Rosie during the recruitment period, were invited to join the study. Subjects excluded from the study were those who were unable to give adequate informed consent to participate in the study (for example language difficulty, learning disability), subject with twins or higher order multiple pregnancies and subjects who had a non-viable fetus on booking.

The gestational age at which patients delivered is shown in appendix 1.

The patient information sheet and consent form were designed according to the guidance on COREC (Central Office of Research Ethics Committee). Patient information sheets included information explaining in a non-scientific manner, the purpose of the study, consenting for the study, advantages and disadvantages of taking part in the study, issues related to confidentiality of data, and the people involved in the research. (Appendix 2)

Generally speaking, there was a separation in time between the patient information sheets being given to patients and consent being sought (often at the time of admission to the delivery unit in labour), as per the instructions of the Research Ethics Committee. This meant that consent was a 'two stage' process; not all women given patient information sheets subsequently consented. This tended to bias recruitment away from severe preterm deliveries as women admitted to the delivery unit in this situation, and those looking after them, were less willing to consider discussing and consenting to this study.

Antenatal clinic

Antenatal clinics in the Rosie Hospital, Addenbrookes run from Monday to Thursday every afternoon. All women in the antenatal clinics were approached individually. All women had gestational age assigned from a first trimester ultrasound at 10-14 weeks using either crown rump length (CRL) or bi-parietal diameter (BPD) measurement. No selection was made based on any maternal characteristics. The study was explained to them and an information sheet about the study was given to them. They were asked to read the information sheet, encouraged to ask questions if unsure about anything and then to sign the written informed consent form, if they agreed to take part in the study. The research participants were only required to sign the consent form. There were no extra treatments or procedures during the participant's pregnancy because of this study. Women received ante-natal care as normal from the obstetric and community teams. This study did not affect the care they received, their appointments or delivery. Research participants were not contacted after the study and all records were anonymised.

A consent form was attached to the antenatal hand held notes (normally carried by the pregnant woman each time she attends antenatal clinic at the Hospital or to her midwife), to make it easier for the midwives at the delivery unit to confirm that woman had consented for the study.

Delivery Unit

Midwives and other delivery unit staff were informed about the study. Study posters were displayed. Flow charts of how to collect the placentas after the delivery were also displayed for the midwives. Midwives were reminded of the study especially at the hand over times so that it was made sure that each and every midwife in the delivery unit was aware of the study and the placentas were kept for the study. A special folder was made for the placental study which had study's information sheets, consent forms, flow charts of placental collection methods and the lab forms for the study. Every delivering woman was asked again if she agreed to participate in the study. Midwife delivering the women were asked to confirm the written consent of the women for the study. Placenta was examined grossly by the midwife as per routine and a microbiological swab was taken if required clinically. Placenta was placed in a double bag with the woman's addressograph on it, which was kept in a dry clean 1-2 litre bucket with a wide top air tight lid within 20 minutes of delivery. The placental bucket was kept in the fridge with the temperature maintained at 4-6°C. 2 fridges were procured in the delivery unit to keep the placentas before placentas were collected to the pathology Department. Midwives and other staff were regularly updated about the progress of study by regular presentations in the Departmental meetings.

2.2.2 Transportation of placenta

A medical laboratory assistant was employed solely for the study who collected placentas from the delivery unit each morning Monday to Friday to the dedicated research section in the Department of pathology.

Each placenta was accompanied by the dedicated pathology form with the brief information of the patient on it. (Pink form in appendix 6) Each placenta was registered on the Hospital server and assigned a pathology number, for example PF07 or PF08...Once the database was complete, all identifiable information of the patients was deleted for example name of the patient, Hospital number and the pathology number. A study number was given to each placenta included in the study. All macroscopic examinations findings were recorded on the back of the pathology form in a predefined section.

2.2.3 Examination of Placenta

Manual Measurements

Placentas were examined at a dedicated perinatal lab in the pathology Department. Each day placentas were examined grossly, photographed and sections cut for microscopic examination.

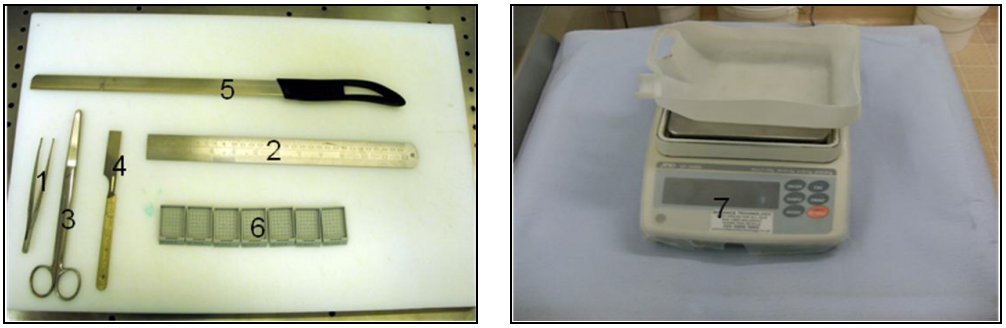


Figure 2.1 Instruments used for gross examination of placenta and cutting sections.

Instruments used were (fig 2.1)

1. Toothed holding forceps.

2. Fixed measuring scale (cm)

3. Heavy cutting Mayo's scissors

4. Swan Morton surgical steel sterile skin graft blade.

5. 12 inch (300mm), disposable macro knife with a non slip integral handle with the double honed edge.

6. Cassettes with covers

7. A & D GF-3000 top-loader digital fixed weighing scale (grams)

8. Camera- Canon Power shot G5, 5mega pixel camera. (picture not shown)

For each placenta the identity was confirmed and a study number was given and it was anonymised so that once cut it can only be identified with the study number. A Pathology form was designed specifically for the study where gross and microscopic findings of each placenta were reported. Fresh placentas placed in labelled clean plastic buckets with a labelled request form were received in the Histopathology Department, Addenbrookes Hospital.

The placenta was first examined grossly for the presence of any macroscopic lesions such as thrombosis, fibrin, infarcts and haematoma.

The placentas were then photographed. Photographs were taken of maternal surface with trimmed membranes and trimmed cord, fetal surface and cross sections of the placenta.

The point of insertion of the umbilical cord into the chorionic plate was noted and recorded as central, paracentral, marginal or velamentous. For the qualitative comparisons, we used the most frequently used terminology for cord insertion.[33]

Central insertion is defined as any part of the umbilical cord inserting within 2cm of the centre of the chorionic plate; paracentral greater than 2 cm from the centre and within the margin of the chorionic plate; marginal at the placental margin and velamentous insertion as being outside the chorionic margin into the membranes.

The cord was then excised from the point of insertion into the chorionic plate; blood was drained from placenta as much as possible, adherent blood clots from the maternal surface of placenta were removed. All membranes were trimmed off and the placenta was then weighed on a fixed scale. Weight was noted in grams. The placentas were weighed in over 90% of cases within 24 hours but in all cases within 72 hours in accordance with published guidance for handling the placenta.[17 68]

The Umbilical cord length was measured in centimetres using an accurate, non stretchable fixed measuring scale. The diameter of the umbilical cord was noted in centimetres using a fixed scale from the fetal end of the cord.

The direction and the number of coiling of the umbilical cord were also noted. Direction of the cord was noted as left, if the direction of coils directed towards the examiners left hand, right sided coiling if directed towards right hand.

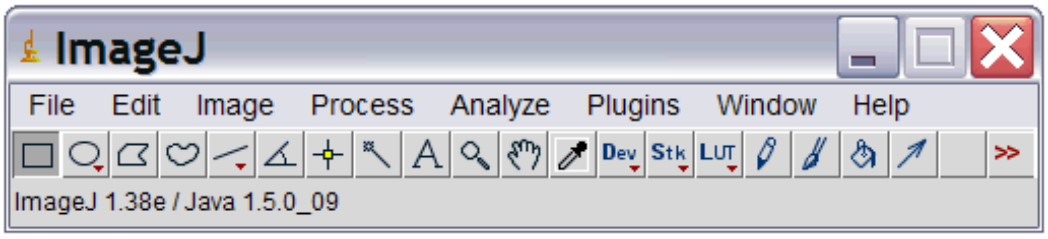
Cord Coiling Index: The cord coiling index was calculated using an established method by dividing the number of coils by the length of the umbilical cord in centimetre.[11] Therefore the coiling index is the total number of completed vascular coils per centimetre length of the cord. A complete vascular coil is defined as a 360^0 round coiling.

Placentas were cut and sampled for histological examination. 3 standard blocks were taken from the cord which included maternal end, fetal end and one from the central area. 3 full thickness central parenchyma blocks and one full thickness peripheral block were taken. One block from the maternal surface was taken. Additional blocks were taken from the macroscopic lesion areas.

Small samples from few unselected placentas were also archived in "RNA later" for possible future molecular analysis.

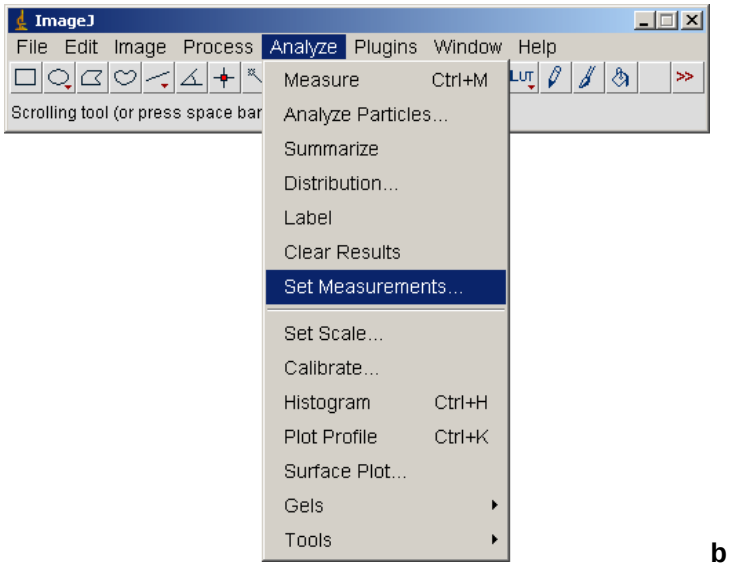
Digital Measurements

The placentas were photographed using Canon Power shot G5, 5-mega pixel camera. Though the tripod was not used, but a uniform vertical distance and angle was kept for each photograph taken. Each fresh placenta was placed with the fetal surface uppermost, membranes were trimmed and all blood clots were removed from the fetal surface. A 15-centimetre ruler was placed in every placental picture for calibration purposes. A line was drawn between two points of known distance on the ruler in every picture with the placenta. The length of the line was calculated automatically in pixels by the software and that pixel was converted into centimetre by setting the scale on the software. This scale was adjusted for each picture separately. Each picture was uploaded on the Hospital server and identified by a study number; the details were entered into a secure database. Images were analysed using Image J software. Image J is a freely available public domain Java based image processing program developed by the National Institute of Health. In the current study, digital analysis of the placenta was performed using version 1.38e which was downloaded from website (http://rsb.info.nih.gov/ij) and the dimensions measured and shown in Figure below.



a

Figure 2.2 (a) Image J tool bar for placental measurements.



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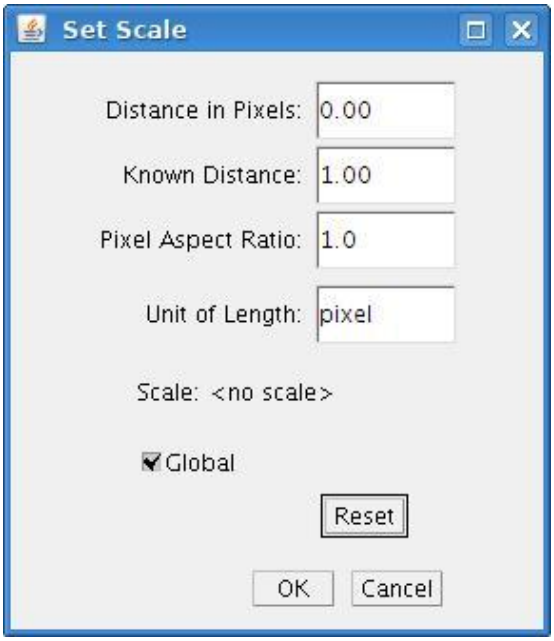


c

Figure 2.2 (b-c) Image J scale setting for placental measurements.

The point of umbilical cord insertion was noted on image.

The widest measurement of the placenta on an X-axis (A) and Y-axis (B); both measurements being perpendicular to each other.

Measurements on X-axis(C) and Y-axis (D); perpendicular to each other passing through the umbilical cord insertion.

Shortest distance of umbilical cord insertion to the chorionic plate margin on X-axis (e) and Y-axis (d)

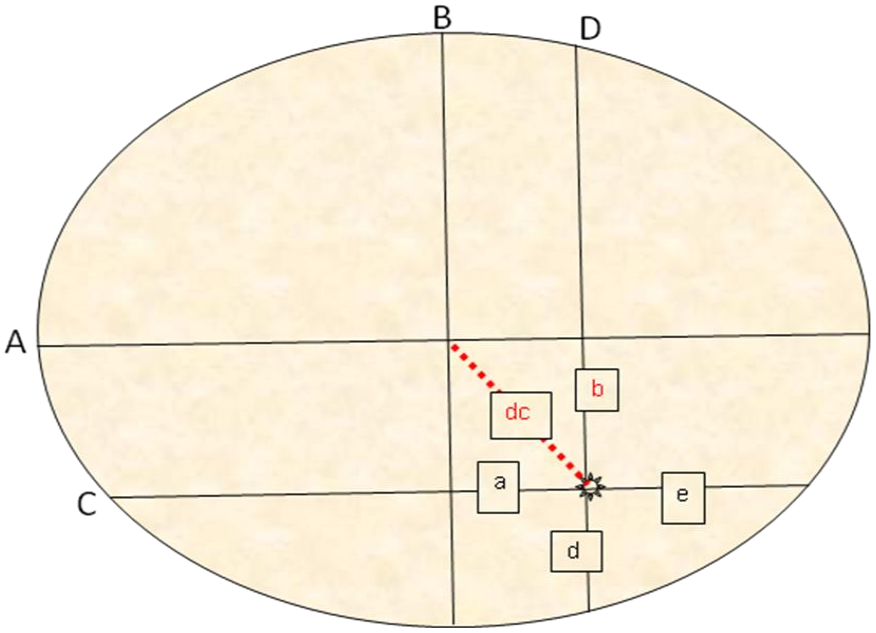


Figure 2.3 Depiction of the placental measurements.

a = (½ C) – e

b = (½ D) – d

dc = Distance of cord insertion from centre

Derivation of digital parameters

Distance of umbilical cord insertion from placental centre was calculated mathematically according to the Pythagorean Theory. 'a' was calculated by subtracting 'e' (shortest distance of umbilical cord insertion to the chorionic plate margin on X-axis) from the half of 'C' (X-axis passing through the insertion of umbilical cord). 'b' was calculated by subtracting 'd' (shortest distance of umbilical cord insertion to the chorionic plate margin on Y-axis) from half of 'D' (Y-axis passing through the insertion of umbilical cord). Since $dc^2=a^2 + b^2$, where dc is the distance of umbilical cord insertion from the centre. The formula for calculating the distance of the cord insertion from the centre is:

$$dc = \sqrt{a^2 + b^2}$$

$$dc = \sqrt{(1/2C - e)^2 + (1/2\mathrm{D} - \mathrm{d})^2}$$

Cord Centrality Index (CI) – a ratio that describes the distance of the umbilical cord insertion from the chorionic plate margin. It will range between 0 and 1 (except in cases of velamentous cord insertion, where the value may be greater than 1 as the insertion may be further from the centre than half the longest diameter).The smaller the CI, the closer the umbilical cord insertion to the placental centre; the greater the CI, the further away the cord insertion:

$$CI = \frac{\text{Distance of umb cord insertion from centre}}{\text{Half of the longest diameter of the placenta}}$$

Placental shape; eccentricity- Eccentricity is derived from the mathematical formula describing eccentricity for an ellipse/oval. This is the ratio of the distance between the foci to the length of the major axis [77]. In this present study, this term; "eccentricity" therefore has been referred in a manner consistent with its common and established

usage in applied mathematics, engineering and astronomy, to describe the features of an ellipse. The term is therefore used here to describe the shape of placenta in relation to how circular or ovoid it is. The value of an eccentricity should fall between 0 and 1, 0 indicates that the shape of placenta is circular while values towards 1 indicate an elliptical shape of the placenta.

$$\text{Eccentricity} = \sqrt{1 - \left(\frac{\text{minor axis}}{\text{major axis}}\right)^2}$$

Circumference of the placenta was calculated by the following formula, assuming the placenta is an ellipse;

$$\text{Circumference} = \pi\,(\text{half of major axis} + \text{half of minor axis})$$

Circumference was calculated for both digital as well as manual images. The mean of both circumferences (manual and digital) was calculated. The mean percentage difference was also calculated between the two measurements.

Intra-observer digital measurements

To assess the reliability and accuracy of the digital measurements, one observer (SP) re-measured the randomly selected 20 placentas and compared the results with the measurements of the same placentas performed previously by same observer.

Inter-observer digital measurements

Two observers (SP, CCL) performed the digital measurements on 20 randomly selected placentas simultaneously and the two observer's measurements were compared.

Fixation of placental tissues

To preserve placental tissues, all blocks were placed in the 10% neutral buffered formalin solution for overnight. Tissues are fixed by cross-linkages formed in between the proteins, by which the antigenicity of the tissue is maintained. Buffered part of formalin prevents acidity that would promote autolysis. Formalin fixes the tissues slowly but penetrates well into the tissues.

Tissue Processing

Placental tissue processing involves the process by which tissues can be made into thin microscopic sections. Placental tissues samples taken in our study were processed in the histology laboratory in the Department of Histopathology at Addenbrookes Hospital, by the dedicated and trained medical laboratory assistant for the study, who then prepared the slides for the examination by the pathologists' member of the team.

Tissue processing involved embedding of the tissues in the paraffin first followed by cutting the thin sections of the tissues with the help of microtome shown in figures 2.4 (a-d).

Once prepared in thin sections, tissues were stained with Haematoxylin and Eosin.

The stained tissue section was covered with the cover slip and ready to be examined by the pathologist team members.

Figure 2.4 Machines used in tissue preparation.

The placental pathology findings were linked to the pregnancy and neonatal outcome. The histological, obstetric and neonatal variables were recorded on a password secured Access database using Microsoft access version 2003; the entry fields are outlined below. Gestation age at delivery was calculated by early dating ultrasound for each patient. Term pregnancy defined by completed 37 weeks of gestation and preterm is delivery before 37 completed weeks.

2.3 DEFINITIONS OF CLINICAL GROUPS

Pre-eclampsia (PET)

PET was defined after 24 weeks as blood pressure ≥ 140/90 on 2 or more occasions, at least 6 hours apart with significant proteinuria of either more than 300 mg/l in 24 hours collection in a previously normotensive woman.[78-79] Women with ≥ 2+ in a urine sample in absence of a demonstrable urinary tract infection were also included in this group.

Pregnancy induced hypertension (PIH)

PIH was defined as blood pressure ≥ 140/90 on 2 or more occasions, at least 6 hours apart in pregnancy after 20 weeks without any evidence of proteinuria.

Gestational Diabetes Mellitus (GDM)

According to WHO criteria, gestational diabetes is carbohydrate intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy. We defined the **gestational diabetes** (GDM) in our study according to the WHO criteria: fasting blood glucose level ≥ 7 m.mol/L or 2 hours post 75mg of oral glucose load ≥ 7.8 m.mol/L. [80]

Small for Gestational Age (SGA)

Birth weight percentile less than the 10th for gestational age defined the **SGA** group. To calculate these we used the standard UK 4-in-1 charts (girls and boys), which are the growth charts designed principally for Hospital use for preterm and term babies and their follow up.[81]

2.4 OBSTETRIC FIELDS - recorded for each participant of the study are shown in table 2.1.

General	Pregnancy	Ultrasound details	Delivery/Neonatal outcome	Admitted to Neonatal Unit
Maternal age	Diabetes	GA when uterine artery Doppler performed	Live birth/still birth/neonatal death	Duration of admission in Neonatal Unit
Parity	Pre-eclampsia	Right/left uterine PI	Birth weight/centile	RDS
Pregnancy loss	PIH	Right/left uterine notch	Neonatal gender	CLD
USS EDD	Essential hypertension	GA at scan prior to delivery	Neonatal resuscitation	Ventilation/CPAP/ respiratory support (days)
Smoking status	Thrombophelia	Umbilical artery PI	Neonatal outcome	Post natal steroids
Drug abuse	APS	Ductus venosus PI	NICU admission	USS
Medications	SGA≤ 10 centile	MCA PI		Seizures
Ethnicity	Clinical chorioamnionitis	AFI		ROP
	Abruption	CTG-STV		NEC
	GA at delivery			Inotrops
	Onset of labour			PDA
	Mode of delivery			Thrombocytopenia
				Anaemia
				Coagulopathy
				Proven infection
				Renal impairment
				Survival to discharge
				Age at death

Table 2.1 Obstetric variables of the study.

2.5 PROTOCOL FOR EXAMINATION OF PLACENTA

2.5.1 Macroscopic examination: A specifically designed form for the study which included gross and microscopic findings was used.

Umbilical cord examination	Placental examination	Standard blocks taken
Length (cm) Diameter (cm) Coiling index (turns/length) Direction of cord spiral Cord insertion site True knots	Trimmed weight (g) Measurements in 3 dimensions (cm) Present/absence of any macroscopic lesions including infarct (s), haematoma, fibrin, maternal surface thrombus	Cord x 3 (maternal, fetal end and central) Membranes Central parenchyma blocks (full-thickness) x 3 Peripheral parenchyma block (full-thickness) x 1 Maternal surface x 1 block

Table 2.2 Protocols for examination of the placenta and umbilical cord.

2.5.2 Microscopic examination: Checklist for reporting cord and membranes are described in table 2.3.

Umbilical cord and membranes	Placental parenchyma
Funisitis	Chorionic plate-inflammation, infection, FTV
Chorioamnionitis	Stem villi-inflammation, infection, FTV, mesenchymal dysplasia, storage disease
Maternal vascular disease in any adherent deciduas	Distal villi-hypoplasia, Chorangiosis, villitis, infection, storage disease
Meconium staining/necrosis	Intervillous space-inflammation, infection, haemorrhage, fibrin (massive)
Amnion nodosum	Maternal vessels-mural fibrin deposition, atherosis

Table 2.3 Checklist for histological examination of the placenta and umbilical cord.

2.6 **DATA ANALYSIS**

The database of the study initially was divided into two parts and was set up on two different servers of the Hospital, for the sake of convenience and ease for the research team. One database included Obstetrics and Neonatal outcome information, set up on the perinatal server.

The second part of the database was set up onto the histology server, which included all macroscopic and microscopic findings of histopathology of placenta.

Both databases were held on a secure server with password access to members of the research team only within Addenbrookes Hospital. Every placenta recruited for the study was given a pathology number. The pathology database had all the macroscopic and microscopic features of each placenta by its pathology number and Hospital number.

Once all the information, Obstetrics, neonatal outcome, macroscopic and microscopic examinations findings of each placenta were entered onto the databases, the two database were merged matching with the Hospital number of the patients. Once merged, a study number (1, 2, 3.....) was given to each placenta. Hospital numbers and pathology numbers were deleted to make the database completely anonymised. Once anonymised, it was not possible to link the outcome data to any patient's identifiable information.

Multivariate analyses of a range of clinical and histological parameters were done. Correlation of pregnancy and neonatal outcome to placental and umbilical pathology was performed with statistical advice from the research team with, where appropriate, specialist advice from the University of Cambridge and University of East Anglia.

The data analysis was performed using SPSS (Statistical Package for Social Science) software, window version 15and 18; SPSS, Chicago IL, USA.

Descriptive statistics for nominal data were expressed in absolute numbers and percentages. Mean value and SD are presented for normally distributed continuous variables. For non-normally distributed variables, median and ranges are presented.

A test of normality was performed by using frequency histograms, Q-Q plots and Kolmogorov-Smirnov test. Frequency histograms and Q-Q plots the observed data against expected data. Kolmogorov-Smirnov test quantifies the discrepancy between observed and expected distribution.

To explore the association between the variables, Pearson correlation coefficient (r) was analysed. The value of "r" varies between -1 to +1. The sign of –ve and +ve shows whether there is negative or positive correlation between the variables and the number value signifies the strength of correlation; 1 being the strongest and 0 being no correlation between the variable. A linear relationship between the variables was plotted on a scatter plot. Coefficient of determination was calculated as r^2. This enables us to know how much variance is shared by two variables.

T-test was used to compare means of continuous data, if normally distributed. If data did not follow a normal distribution, not even after logarithmic transformation, in those data to compare means, Mann-Whitney-U test was performed. Non parametric test was also performed if the sample size was small. A p-value of ≤ 0.05 was considered statistically significant.

Z- Score

Z-score or the standard score allows us to calculate the probability of a score occurring within the normal distribution and also helps to compare two scores of different normal distributions.

In the current study, to establish the relationship between different continuous variables (birth weight, placenta weight, placenta circumference), z scores for the variables were calculated to normalise the data for the gestational age within the gestation range 33-43 weeks, using the means and SD derived from this population.

Bland Altman Plot[82-83]

Bland Altman is a statistical method of data plotting used in analysing the agreement between two different methods. This plot analyses the correlation between two methods that are designed to measure the same variable or parameter. Bland Altman plot can also be used to compare a new measurements technique against an available standard method.

The mean of the two measurements is plotted on the x-axis and y-axis contains the difference between the two values.

The limits of agreement (95% confidence interval of the difference between the two methods, specified as average difference +/- 1.96 SD of the difference) were also computed on the Bland Altman Plot.

In this study, Bland-Altman Plot was used to analyse the agreement between manual and digital measurements of the placenta.

CHAPTER 3 : A COMPARISON OF MANUAL AND DIGITAL PLACENTAL MEASUREMENTS

Summary Points:

- This study compared manual and digital placental dimensions in 888 placentas.
- The key findings were significant positive correlation between manual and digital measurements.
- This chapter concludes that the digital measurement of the placenta is a simple and reproducible method of measurement compared to the traditional methods.

This chapter is based on:

Pathak S, Jessop F, Hook L, Sebire NJ, and Lees C. *Placental weight, digitally derived placental dimensions at term and their relationship to birth weight.* **J Maternal Fetal Neonatal Med.** 2010 Oct; 23(10):1176-82

3.1 INTRODUCTION

Placental measurements have traditionally been performed manually following delivery with a ruler and little is known about the correlation between the manual and digitally derived placental measurements following the widespread introduction of digital photography for recording of pathological specimens. [12 84]

Digital imaging and subsequently derived measurements are now commonplace in many other areas of medicine, for example in MRI organ volume estimation[85] and for 2D distance measurements in biology, astronomy, nuclear medicine, laboratory medicine, immune-histochemistry and Histopathology and physics, image analysis using Image J software (a semi-automated web based freeware image analysis programme) has been widely applied. Similar imaging software has been used for quantification of bone metastases in prostatic cancer in which results were closely correlated with manual counting of lesions[86] and for quantifying erythema and pigmentation in skin diseases.[87] In hepatic CT volumetry, volumes of virtual liver resection specimens measured with Image J were compared with specimen weights and calculated volumes during pathology examination after resection; the two methods of measurement were closely correlated ($r^2 = 0.98$ $p<0.0001$).[88]

Image J has also been compared with computed tomography (CT) imaging software to quantify soft tissue cross-sectional areas obtained from abdominal and mid thigh areas with correlation coefficient (r^2) of 0.99 and the two methods were comparable (95% confidence interval +2.5cm^2 or +1.4cm^2) using the Bland-Altman method.[14]

Correlation coefficients have been used to compare manual and digital measurements, whereas a more appropriate approach would be a Bland-Altman plot to analyse agreement between two methods which are designed to measure the same parameter[82] and shows the relationship between the two methods of measurements. This information is important to establish validity of digital measurement for future studies where image analysis based measurements will be increasingly used.

The aims of the study are to compare manually and digitally obtained long and short axes placental measurements and the derived placental circumference using both correlation coefficients and the Bland-Altman plot.

3.2 METHODS

Detail of methodology is described in chapter 2. Briefly, for the purposes of this chapter, 888 placentas were analysed, where the digital measurements were available.

3.3 STATISTICAL ANALYSIS

Details of the statistical methods are described in chapter 2. The limits of agreement (95% confidence interval of the difference between the two methods, specified as average difference +/- 1.96 SD of the difference) were also computed on the Bland Altman Plot. The Bland-Altman Plot was used to analyse the agreement between manual and digital measurements of the placenta for:

-maximum diameter (long axis of the placenta)

-minimum diameter (short axis of the placenta)

-circumference.

Pearson correlation coefficients (r) were derived using bivariate relationships for both manual and digital measurements; and r^2 was calculated. One observer (SP) performed all the measurements.

In order to determine the reliability of digital measurements, 20 randomly selected placentas were re-measured by same observer (SP) after 6 months. Another randomly selected 20 placentas were measured by 2 observers (SP, CCL) simultaneously.

3.4 RESULTS

Analysis of this study includes 888 placentas. In all placentas, all manual and digital measurements were obtained. Table 3.1 shows the demographics of the population studied.

n=888	
Maternal age ± SD (years)	30.8 ± 5.6
% Caucasian (n)	89.8 (797)
% nulliparity (n)	48.2 (428)
% Smokers >5 cigarettes per day (n)	9.6 (85)
Birth weight ± SD (gms)	3470 ± 496
Mean gestation at delivery ± SD (weeks)	39.4 ± 1.4
Mean Placenta weight ± SD (gms)	478.44 ± 96.7

Table 3.1 Baseline demographics of the population studied.

Table 3.2 details the comparison of the manual and digital measurements.

	Minimum	**Maximum**	**Mean**	**SD**
Long axis of placenta (cm)				
Manual	14.00	36.00	20.77	2.66
Digital	12.70	30.70	18.96	2.47
Short axis of placenta (cm)				
Manual	7.00	24.00	17.83	2.07
Digital	8.90	22.00	15.91	1.87
Circumference (cm)				
Manual	36.11	89.49	60.60	6.24
Digital	38.31	77.56	54.76	5.71

Table 3.2 Manual and digital measurements of the placenta.

Intra-observer variability

In 20 randomly selected placentas; one observer (SP) re-measured the same placentas digitally at an interval of 6 months. The intra-observer correlation coefficient and coefficient of variation were 0.99 and 1.8% respectively for long axis, 0.98 and 2.1% for short axis measurements.

Inter-observer variability

These measurements are based on 20 randomly selected placentas, measured by two observers (CCL, SP) simultaneously. The inter-observer correlation coefficient and coefficient of variation were 0.98 and 3.3% respectively for long axis, 0.97 and 2.6% for short axis measurements.

Outliers

The outliers were noted while comparing long, short axes and the circumference of the placenta. These numbers were re-checked on the manual as well as digital measurements. However these numbers of outliers were very small to have made any significant effect on the result.

Comparison of manual versus digital measurements:

Long axis of placenta**:**

Manual versus digital measurements showed a Pearson correlation coefficient (r) of 0.74 ($p<0.0001$); linear correlation r^2 0.54 and coefficient of variation 9.2%. The Bland–Altman plot mean difference between manual and digital measurements was 1.80 cm with 95% CI (+/-1.96SD) -1.86 and 5.46 cm.

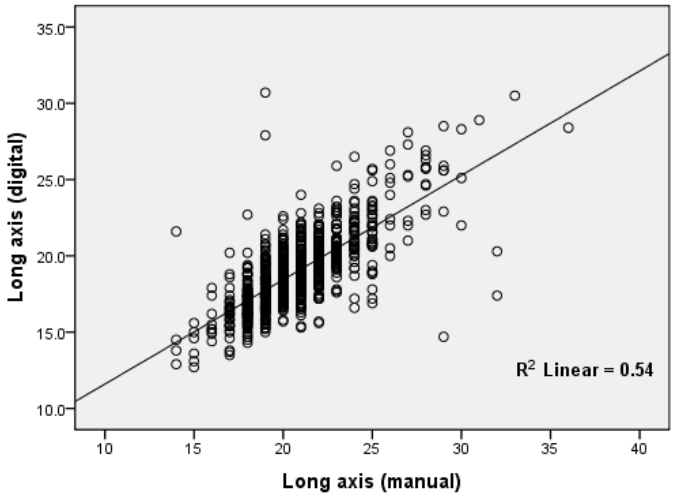


Figure 3.1 Scatter plot showing correlation coefficient for manual versus digital measurements of long axis of the placenta

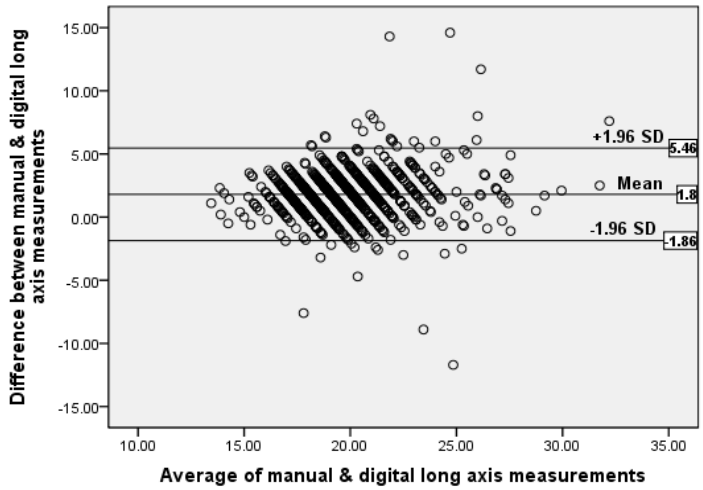


Figure 3.2 Bland Altman plot for long axis of the placenta: manual versus digital measurements.

Short axis of placenta:

The Pearson correlation coefficient (r) was 0.70 ($p<0.0001$); r^2 0.49 with coefficient of variation of 10.3%. The Bland–Altman plot mean difference between the long axes measurements taken manually and digitally was 1.91 cm with 95%CI (+/- 1.96SD) -1.1 and 4.92 cm.

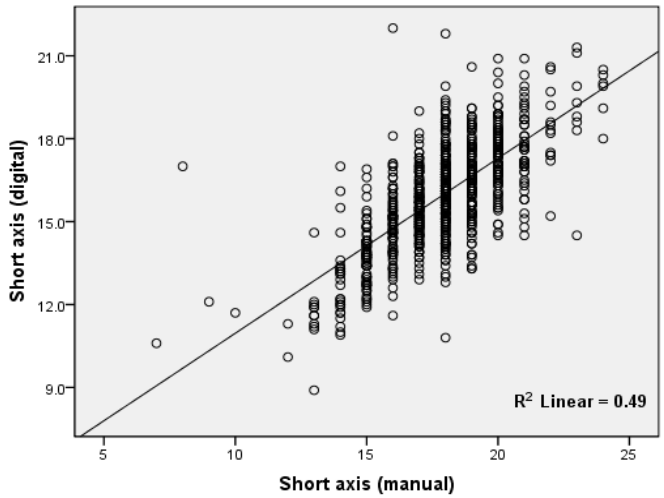


Figure 3.3 Scatter plot showing correlation coefficient for manual versus digital measurements of short axis of the placenta.

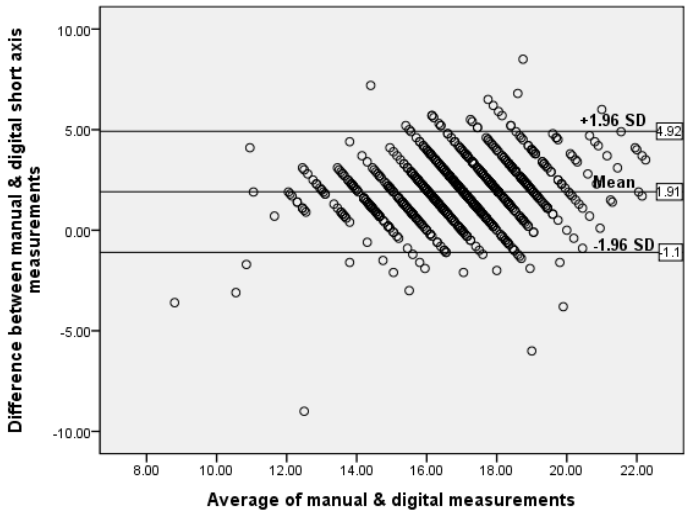


Figure 3.4 Bland Altman plot for short axis of the placenta: manual versus digital measurements.

Circumference of placenta:

The Pearson correlation coefficient (r) was 0.77 ($p<0.0001$), r^2 0.59 with coefficient of variation 7%. The Bland–Altman plot mean difference between the measurements taken manually and digitally was 5.83 cm with 95% CI -2.19 and 13.85 cm.

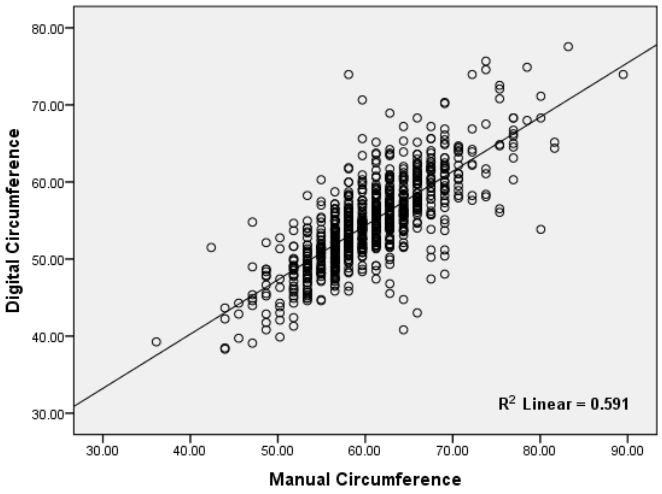


Figure 3.5 Scatter plot showing correlation coefficient for manual versus digital measurements of circumference of the placenta.

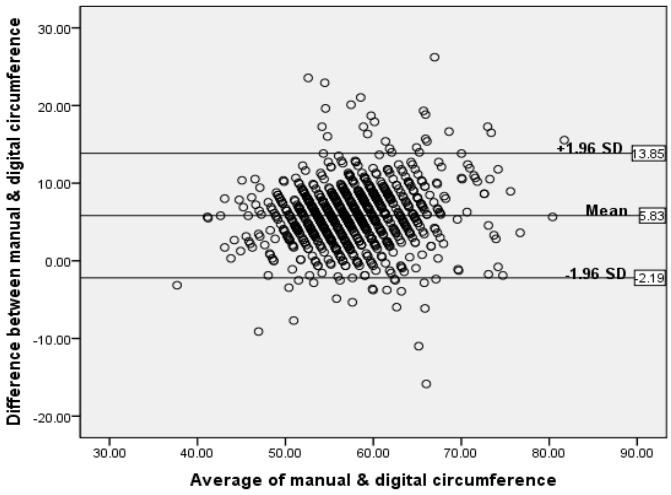


Figure 3.6 Bland Altman plot for circumference of the placenta: manual versus digital measurements.

3.5 DISCUSSION

We report manually and digitally derived measurements following delivery at gestation from 33-43 weeks. This data shows significant positive correlation between manual and digital measurements.

Previous descriptions of manual versus digital measurements including those on the placenta[84] have reported good correlation between the two measurements. However, the most appropriate method for comparing two different methods of deriving the same measurement is not a correlation coefficient, since one measurement technique may lead to systematically larger or smaller measurements than the other but still have a correlation coefficient close to 1. Moreover the correlation coefficient also depends on the way the sample is chosen, and it has meaning only for the population from which the study subjects can be regarded as a random sample.[83] Comparison of digital measurements with those from previous studies using manual measurements should therefore be approached with caution. The problem of interpretation and comparison is to a large extent overcome by use of the Bland-Altman plot,[82] which was used in this study. This plot compares two methods of measurement. For example, in the current study, digital measurements appear systematically shorter for both long and short axes of the placenta, as evidenced by the crude comparisons (Table 3.2) and Bland-Altman plots; the 95% limits agreements of the mean difference between the manually and digitally derived placental circumferences were -2.19 and 13.85 cm though the inter and intra observer coefficients of variation are <5% (Figure 3.6).

The reason for this difference in measurement is not clear, and as comparative data has not been presented in this format before, it is not clear if the finding is unique to this study or others. Other studies reporting digital placental measurements did not use a Bland-Altman analysis for the two types of measurement, so although the correlation between the two types of measurement is high, it is not possible to ascertain whether there was a systematic over or under measurement from digital recordings.[12 84] This finding does however sound a potential note of caution that deserves to be investigated in future studies.

The digital measurements of placenta of the current study are however comparable to those reported in two recent studies (Table 3.3). It must be noted that one included placentas from women delivering after 24 weeks; however 86% were from deliveries after 37 weeks.[12] So with this proviso, the results can be compared accepting that the minimum range might be lower in that study as a result of some placentas being from earlier gestation deliveries.

	Present Study			Coall et al [13]			Salafia et al [12]		
Measurements (cm)	**N**	**Mean**	**Range**	n	Mean	Range	n	Mean	Range
Long axis of Placenta	**888**	**18.9**	**12.7-30.7**	482	22	14-35	628	21	12-43
Short axis of Placenta	**888**	**15.9**	**8.9-22.0**	482	18	8-26	628	18	9-29
Circumference of placenta	**888**	**54.7**	**38.3-77.5**	482	71.8	49-111	-	-	-
Distance-centre to cord insertion	**888**	**3.3**	**0.1-10.0**	482	4.0	0.2-11.5	628	3.6	0.1-23.7

Table 3.3 Digital placental measurements reported by two recent comparable studies.

In summary, digital measurement of the placenta is reproducible and a relatively simple of method measurement compared to traditional manual measurement as it is very easy to perform, does not require much training and digital method also allows the measurements to be done at a later stage. Image J software is also freely available at no cost making it accessible to researchers in countries where financial resources may be limited. The measurements derived are comparable to the other studies though the digital measurements in our study appear to be systematically smaller than those derived by conventional manual measurements. Whether this is a feature of this study-for example Image J software- or is more likely to occur with digital measurements in general is not clear, neither are data available that would resolve this from otherwise comprehensively reported studies of this type.[12 84]

CHAPTER 4 : MORPHOLOGY: UMBILICAL CORD COILING, UMBILICAL CORD INSERTION AND PLACENTAL SHAPE IN UNSELECTED PREGNANCIES

Summary Points:

- This study derived the morphological indices for coiling of the cord (cord coiling index), insertion of the cord (cord centrality index), and shape of the placenta (eccentricity) in unselected 888 pregnancies at 33-43 weeks.
- The key findings were suggestive that cord is inserted off the centre and shape of the placenta is oval or elliptical, not round.

This chapter is based on

Pathak S, Hook E**,** Hackett G**,** Murdoch E**,** Sebire NJ**,** Jessop F**,** Lees C**.** *Cord coiling, umbilical cord insertion and placental shape in an unselected cohort delivering at term: Relationship with common obstetric outcomes.* **Placenta.** 2010; 31: 963-8.

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** Morphological features of placenta in relation to common obstetric outcomes: Paper in progress.

4.1 INTRODUCTION

It has been known for many centuries that the placental shape, size, cord insertion and pattern of cord coiling varies widely between pregnancies. In current obstetric and perinatal pathology practice, qualitative terms are generally used to describe the placenta, for example, "velamentous", "central" and "marginal" cord insertions, "round" or "oval" placental shape, among many others.

There has been little attempt at quantifying or describing the relationship between measurements, including the size of the placenta, the shape of the chorionic plate (fetal surface), distance of the umbilical cord insertion from the centre of the placenta and the deviation in placental shape from the suggested normal circular appearance. Little is known about these variables and their association with pregnancy and neonatal outcome. Though several studies on umbilical cord coiling index have emerged in the last few years [5-8] [52], there remains uncertainty in relation to the significance of abnormal umbilical cord coiling detected ante or postnatally.

Umbilical cord coiling is either anti-clockwise (left) or clockwise (right). Anti clockwise coiling is more common. The prevalence of anti-clockwise coiling varies from 65-76%. [8] [49] It is not well-understood why the anti-clockwise pattern is more common. The umbilical cord coiling index has been defined as the number of completed coils per centimetre of the umbilical cord; a cord coiling index mean value of 0.21 (+/-0.07) is defined as normal in the delivered placenta [11]. Though some studies have defined 'hypocoiling' if the cord coiling index is less than 10th centile and 'hypercoiling' if more than 90th centile for gestation, the frequency of non-coiled cord is reported as 4.3-4.9% [5] [48] while hypercoiling has been reported as high as 20% in unselected placentas in other studies.[6]

Different types of cord insertions have been explained qualitatively in most of the studies in the past. An insertion anywhere between central and marginal insertion is generally considered to be eccentric, but none of these terms have been quantitatively described, making comparative studies of outcome difficult.

The type of cord insertion may also be described as how far the insertion point is located from the centre of the placenta, or alternatively, how close the umbilical cord insertion is to the chorionic plate margin. The distance of the umbilical cord insertion from the placental centre has been proposed as a clinically useful marker of placental insufficiency.[89-90] Rath and colleagues [91] calculated a measure of relative insertion eccentricity by dividing the minimum distance from the umbilical cord insertion to the margin of the placenta by the radius of the placenta; however this is a poor measure of eccentricity in irregularly shaped placentas. Others have defined the relative insertion eccentricity by dividing the minimum distance between the centre of the placenta and the insertion of the umbilical cord by the average radius of the placenta.[13]

The placenta is normally described as either round or oval; however it can be described as irregular, bilobate or multilobate, among others. Irregular placental shapes have been associated with lower birth weight: placenta weight ratio, suggesting they may be associated with altered placental function.[10]

The aim of this study is to derive ratios for the shape of the placenta, relationship of cord insertion to its centre and cord coiling in singleton pregnancy placentas from an unselected reference population of gestation age ranging from 33-43 weeks.

For placental shape and cord insertion we utilized reproducible, rapidly obtained digital measurements in placentas to morphometrically define the cord insertion and placental shape[10] using Image J, a freely available public domain Java based image processing program developed at National Institute of Health. Details have been explained in chapter 2 (section 2.2.3).

Cord coiling was measured manually in the same placentas.

4.2 METHODS

In this chapter, analysis is based on digital measurements of 888 placentas at 33-43 weeks gestation without making any attempt at selection by any maternal characteristic.

Details of manual, digital measurements and derivation of the morphological indices are described in chapter 2. Briefly cord coiling index was derived using established method by dividing the number of coils by the length of the umbilical cord.[11] Digital analysis was performed using image J software and analysed the morphological indices; cord centrality index and eccentricity.

4.3 STATISTICAL ANALYSIS

Categorical data are presented as the number and the percentage. Continuous data is presented as mean with the SD. Descriptive statistics was analysed for manual and digital placenta and umbilical cord measurements used in this study.

Approximate normality was checked for cord coiling Index, centrality index, and eccentricity index for the population, using the frequency histograms for each category. Q-Q plots for the same variables were also plotted between observed and expected distribution.

4.4 RESULTS

888 placentas from singleton pregnancies, 33 to 43 weeks were examined; in all placentas, digital measurements were obtained. Demographics for this chapter are outlined in chapter 3 (Table 3.1)

Table 3.2 outlines the manual and/or digital measurements used in the study.

4.4.1 Umbilical cord coiling and coiling index

For umbilical cord coiling, in 704 out of 888 cases (79.3%), the cord was left coiled and 145 out of 888 cases (16.3%) right coiled. There was no coiling in 19 umbilical cords (2.1%). Direction of coiling was bi directional in 5 (0.6%) cases. In these cases the number of the completed coiling were counted as explained in methods. In the remaining 02 cords, cord was too short to calculate the coiling index. Data were not available for 13 cases as the cords had been removed before the placenta reached the research team. Cord coiling index ranged from a minimum value of 0.00 to a maximum of 1.0 with a mean value of 0.20 ± 0.09. Frequency histograms and Q-Q plot revealed an approximate normal distribution for coiling index. Figures below shows the frequency histogram and Q-Q plot of coiling index in reference population.

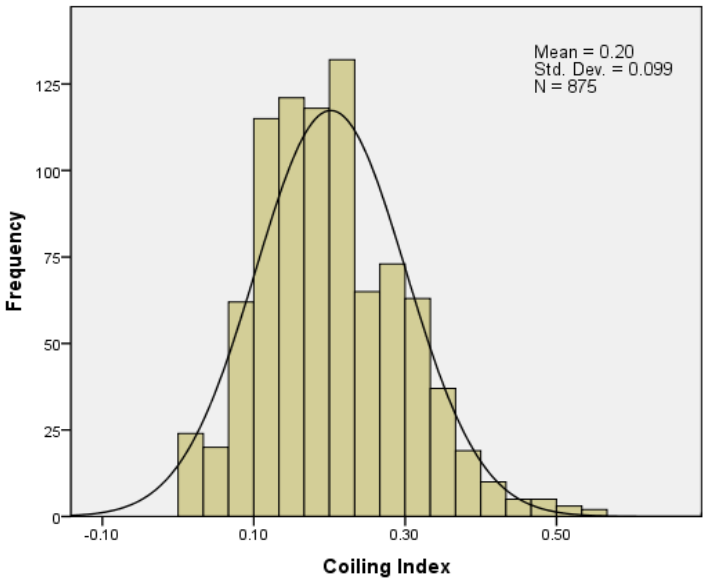


Figure 4.1 Frequency histogram of Coiling Index at 33-43 weeks.

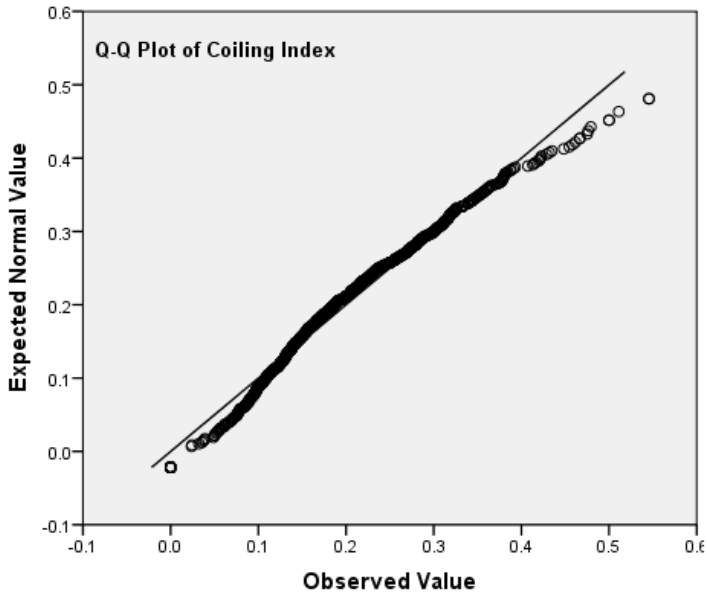


Figure 4.2 Q-Q plot for Coiling Index at gestation 33-43 weeks.

4.4.2 Umbilical cord insertion

Qualitative description of the type of umbilical cord insertion in relation to previous standards showed that the frequency of umbilical cord insertion was 'central' in 245/888 (27.6%), 'paracentral' in 567/888(63.9%), 'marginal' in 74/888(8.3%) and 'velamentous' in 2/888(0.2%).Table below shows the different types of umbilical cord insertion documented in previous studies and in the current study.

Author (Year)	Krone (1961)	Gerlach (1962)	Krone (1965)	Dorste (1971)	Agboola (1978)	**Present Study (2010) (n)**
Central %	25	28	14.9	22.8	26.5	27.6 (245/888)
Paracentral %	64	62.5	65.4	62.2	62.5	63.9 (567/888)
Marginal %	10	8	7.9	14.7	9.2	8.3 (74/888)
Velamentous %	1	1.5	1.8	-	1.8	0.2 (2/888)

Table 4.1 Qualitative description of cord insertion in different studies.

4.4.3 Cord centrality index

The minimum distance of the cord insertion from the centre of the placenta was 0.15 cm and maximum distance 10.05 cm with the mean of 3.35 cm ± 1.9, irrespective of the direction of umbilical cord coiling.

The Cord centrality index ranged from 0.02 to 1.0 with the mean of 0.36 ± 0.21. Frequency histograms and Q-Q plot of cord centrality index indicated an approximate normal distribution. Figure 4.3 shows the frequency histograms of cord centrality index in unselected population and fig 4.4 is an explanatory picture of placenta denoting mean centrality index as 0.36. Fig 4.5 is Q-Q plot for the cord centrality index in reference population.

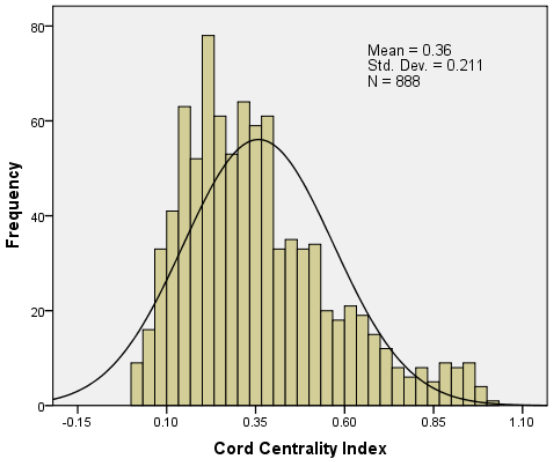


Figure 4.3 Frequency histogram of Cord Centrality Index at 33-43 weeks.

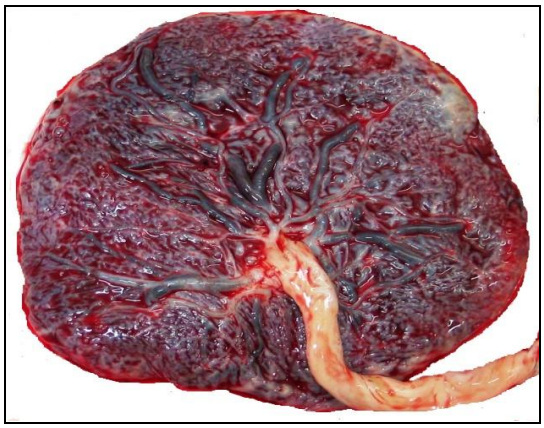


Figure 4.4 Explanatory photograph of a placenta showing Cord Centrality Index of 0.36.

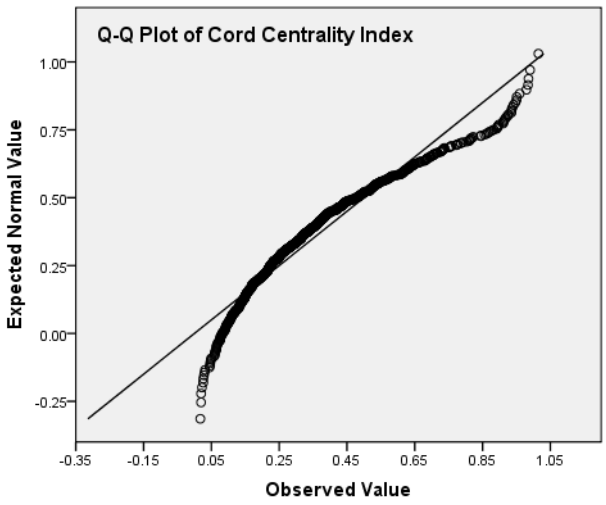


Figure 4.5 Q-Q plot for Cord Centrality Index.

4.4.4 Eccentricity

The placental eccentricity index ranged from 0.00 (circular shape of placenta) to 0.91(highly elliptical shape) with a mean of 0.49 ± 0.18. The frequency histograms and Q-Q plot indicated a normal distribution (Fig 4.6, 4.8). Figure 4.7 shows the explanatory placental picture with mean eccentricity of 0.49.

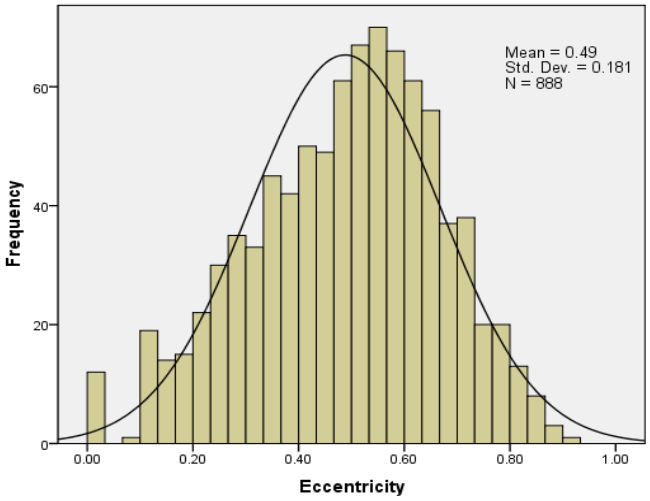


Figure 4.6 Frequency histogram of placental Eccentricity at 33-43 wks.

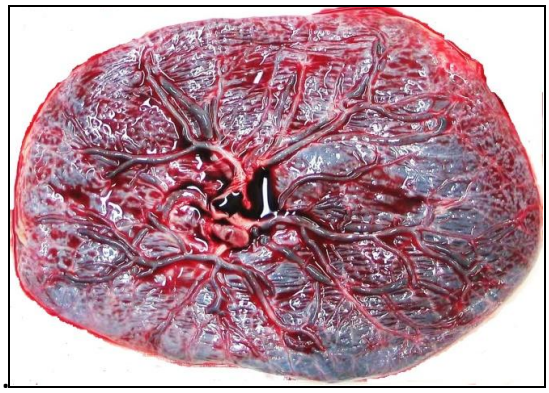


Figure 4.7 Explanatory photograph of a placenta showing Eccentricity of 0.49.

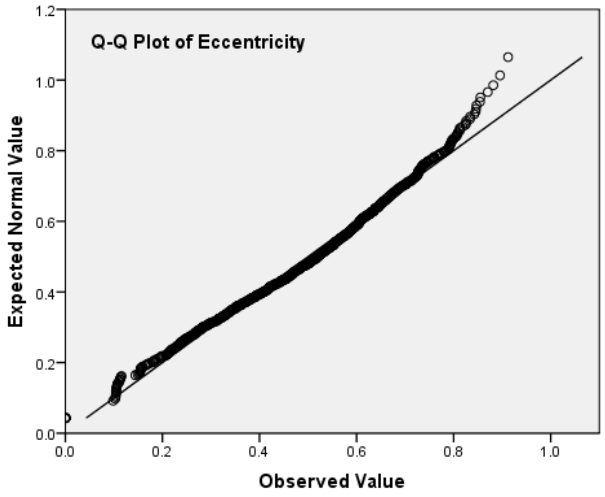


Figure 4.8 Q-Q plot for Eccentricity at 33-43 weeks.

Table 4.4 shows the 5th, 50th and 95th percentile ranges for cord centrality, eccentricity and cord coiling index for placentas at 33-43 weeks. Within this gestation range, there was no effect of gestation on any of these ratios.

	5th	50th	95th
Eccentricity	0.16	0.49	0.76
Cord centrality index	0.08	0.36	0.79
Cord coiling index	0.06	0.20	0.37

Table 4.2 Percentile ranges of morphological indices at 33-43 weeks.

4.5 **DISCUSSION**

Most studies done on placenta and umbilical cord are based either in high risk population or histology in adverse perinatal outcome. Very few studies have focused on the morphology of placenta in unselected reference population. Out of those few studies only handful studies have quantified the morphology of placenta.[12-13] Therefore there is no established baseline quantified morphological characteristics of placenta in an unselected population.

Although not clearly understood, abnormal coiling of the cord (Hypo/hypercoiling) has been shown association with perinatal morbidity such as trisomies, preterm delivery, fetal death, increased intrapartum complications and interventional deliveries for fetal distress, low Apgar score, velamentous cord insertion and single umbilical artery, small for gestation age, fetal asphyxia and single umbilical artery.[5 8 56] Abnormal cord coiling has also been found with thrombosis of the chorionic plate vessels, umbilical venous thrombosis and umbilical cord stenosis.[6]

The umbilical cord coiling index in this study was 0.20 coils/cm ± 0.09 with a preponderance of left sided coiling (79.3%). The cord coiling index in this study is in accordance with the published data.[11] and so does the left sided preponderance of the coiling.[8 45 49]

Quantitatively degree of deviations from central insertion into the chorionic plate has been defined as cord centrality index. In this study the data from unselected population of pregnancies delivering at gestation of 33-43 weeks, shows the mean value of cord centrality as 0.36, which signifies a markedly ‘off centre’ insertion (Figure 4.4).

In recent years shape of the chorionic plate has been the area of interest. Studies have linked the irregular and abnormal shape to the perinatal morbidities such as low birth weight, suggesting variably shaped placentas have altered function.[10] The mean eccentricity index in this study is 0.49 indicating the shape of the chorionic disc more ellipse than round (Figure 4.7).

Though subjective and qualitative description of the placenta has a role predominantly in clinical reporting, deriving these morphological indices will allow entirely objective analysis of the particular index and other continuous variables in relation to outcomes and other placental findings.

In summary, this study showed in the unselected reference population, mean of the coiling index (0.20) was similar to that shown by previous studies, centrality index as 0.36 i.e. cord insertion off the centre and eccentricity as 0.49 i.e. chorionic disc is elliptical or oval, not round.

These data are sufficiently robust to enable them to be used as a basis for future studies both descriptive and of outcome in pregnancy. It is, therefore, appropriate to dispel the widely held beliefs that the placenta is, in its natural and normal state, circular and that the cord normally inserts into its centre.

CHAPTER 5 : MORPHOLOGY: UMBILICAL CORD COILING, UMBILICAL CORD INSERTION AND PLACENTAL SHAPE IN PREGNANCIES WITH COMMON OBSTETRIC OUTCOME.

Summary Points:

- This study derived the morphological indices for the placenta and the umbilical cord in the pregnancies with common obstetric outcomes; PET, PIH, GDM and SGA and compared the indices with reference population.
- Key findings: The morphological indices; cord coiling index, cord centrality index and eccentricity were not different in pregnancies with outcome groups.

This chapter is based on:

Pathak S, Hook E**,** Hackett G**,** Murdoch E**,** Sebire NJ**,** Jessop F**,** Lees C**.** *Cord coiling, umbilical cord insertion and placental shape in an unselected cohort delivering at term: Relationship with common obstetric outcomes.* **Placenta.** 2010; 31: 963-8.

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** Morphological features of placenta in relation to common obstetric outcomes: Paper in progress.

5.1 INTRODUCTION

There have been recent studies on placental shape, cord coiling and umbilical cord insertion.[10] [92] Studies have made attempts to correlate perinatal outcome with abnormal placenta shape, coiling and umbilical cord insertion. Various studies have attempted to explain possible causes and features in respective common obstetric outcome group pregnancies however these have almost exclusively defined placental and umbilical cord features qualitatively by using descriptive terms, making direct comparison between groups problematic.

Attempts at quantitative investigation have involved calculating placental shape from using a 3D model of placental vascular growth. This suggested that an irregular placental outline is an indication of sub-optimal branching structure of the vascular tree, accounting for the lower birth weight observed in non round/oval placentas.[10]

There is also a renewed interest in the placental shape and developmental programming. Analysis of the relationship between placental surface area and hypertension in later life has been described in subgroups defined by the mother's height and socio-economic status; the risk of developing hypertension was associated with reduced placental weight and area.[93] Furthermore, pregnancies complicated by pre-eclampsia had reduced placental surface area and more oval placentas; the short diameter strongly associated with the severity of the pre-eclampsia.[94]

Chapter 4 defines the cord coiling, insertion and shape (eccentricity) in unselected reference population. In this chapter, same placental indices have been investigated in pregnancies affected by pre-eclampsia (PET), pregnancy induced hypertension (PIH), gestational diabetes mellitus (GDM) and delivery of a small for gestational age (SGA) baby.

The aim of this study is to derive indices describing the cord coiling, relationship of cord insertion to its centre and the shape of placenta, at gestational age 33-43 weeks placentas from women who developed pre-eclampsia, pregnancy induced hypertension, gestational diabetes or small for gestational age with birth weight less

than 10th centile. This study also aims to compare these morphological indices in women with and without pre-eclampsia, PIH, GDM or delivered SGA babies.

Cord coiling was measured manually in the placentas. For cord insertion and placental shape, we utilized digital measurements in placentas to morphometrically define the cord insertion and placental shape using image J.

5.2 METHODS

All measurements of the placenta were derived from image J software following standard photography using a common protocol and scale for calibration.[95]

This chapter includes the digital measurements in 888 women delivered at 33-43 weeks. Macroscopic indices derived were coiling index, cord centrality index, eccentricity and the placental circumference. Frequency of these indices was noted in common obstetric outcome groups of PET, PIH, GDM and SGA.

Circumference was calculated both for manual as well as digital measurements. For this chapter, all analysis on the circumference is taken for digital measurements only.

Definitions of the common obstetric outcome categories are described in chapter 2.

We compared data in a specific outcome group to the reference group excluding those particular cases with that outcome. We did not seek to compare data for common obstetric outcome groups with a normal population.

5.3 STATISTICAL ANALYSIS

Descriptive statistics of manual and digital measurements were analysed. A test of normality (the Kolmogorov-Smirnov test) was performed on the cord coiling Index, cord centrality index, and eccentricity index. Kolmogorov-Smirnov tests the normality of the data distribution, and quantifies the discrepancy between observed and expected distribution. A p value greater than 0.05 indicates normality of the distribution; however considering the small numbers of common obstetric outcome groups, the frequency histograms and Q-Q plots were also plotted for each variable to test the normality of the distribution.

Considering the small number for common obstetric outcome group cases, as a precaution, the non parametric Mann-Whitney U test was performed to test the significant difference of coiling Index, cord centrality index, and eccentricity in the specific common obstetric outcome groups and non affected population.

A Mann-Whitney U test 2 tailed significant values (p) was significant if value was ≤ 0.05.

5.4 RESULTS

888 placentas from singleton pregnancies, 33 to 43 weeks were examined; in all placentas, digital measurements were obtained. Common obstetric outcome groups were; Pre-eclampsia (PET=2.3%), Pregnancy induced hypertension (PIH=2.9%), Gestational diabetes (GDM=4.3%) and small for gestational age of $\leq 10^{th}$ percentile (SGA=9.2%). Table 5.1 describes the baseline maternal demographics of the study population including the common obstetric outcome groups in the population.

N=888	Mean ± SD
Maternal age ± SD	30.8 ± 5.6
% Caucasian (n)	89.8 (797)
% nulliparity (n)	48.2 (428)
% Smokers >5 cigarettes per day (n)	9.6 (85)
% Gestational Diabetes (n)	4.4 (39)
% Pre-eclampsia (n)	2.3 (20)
% Pregnancy induced hypertension (n)	2.9 (26)
% SGA $\leq 10^{th}$ birth weight percentile (n)	9.2 (82)
Birth weight ± SD	3470 ± 496
Mean gestation at delivery ± SD	39.4 ± 1.4

Table 5.1 Baseline demographics of the reference population with common obstetric outcome groups.

Table 5.2 outlines the specific manual and digital measurements used in this chapter for reference population, PET, PIH, GDM, and SGA.

	*Reference Population (n=888)	*PET (n=20)	*PIH (n=26)	*GDM (n=39)	*SGA (n=82)
Long axis	18.9(12.7-30.7) ±2.4	18(15-23.2) ± 2.2	18.9(12.9-25.6) ± 2.7	19.1(12.7-25.8) ± 2.5	17.7(12.7-25.9) ± 2.5
Short axis	15.9(8.9-22) ±1.8	15.2(8.9-18.9) ± 2.3	15.9(12.1-21.3) ±2.1	15.9(10.6-20.4) ±2.4	14.4(8.9-18.2) ±1.8
Distance of cord insertion	3.3(0.2-10.1) ±1.9	3.6 (1.1-7.5) ±1.9	3.6(0.4-9.6) ±2.4	3.0(.2-7.7) ±1.7	3.5(0.2-8.3) ±1.9
Cord length	41.5 (0-104) ± 14.1	43.5 (26-71) ±14.3	43.5(21-103) ±16.9	37.7(14-77) ± 14.2	35.7(0-64) ±12.4
Cord coils	8.4 (0-34) ± 4.9	8.4 (2-16) ±3.9	9.0(0-20) ±5.8	7.2(0-20) ±4	7.4(0-19) ±4.1

*All values are mean (range) ± SD
Table 5.2 Placental measurements in reference and outcome groups.

5.4.1 Umbilical cord coiling and coiling index

Table 5.3 is the comparative table describing the directions of cord coiling in reference and common obstetric outcome groups. In all groups left sided coiling was in preponderance ranging from 70-92%. There was no coiling in 3.8% in PIH and 1.7% in SGA. There was no non- coiling observed in the cords in the GDM and SGA groups.

Table 5.4 describes the statistics of cord coiling index in all common obstetric outcome groups. The mean coiling index ranged from 0.19-0.22.

Kolmogorov-Smirnov test of normality revealed a p value of 0.82, 0.71, 0.80 and 0.18 for PET, PIH, GDM and SGA respectively indicating a normal distribution of data in all common obstetric outcome cases, however considering the small number of cases, frequency histograms Figures 5.1 (a-d) and Q-Q plots were also plotted, which showed normal distribution.

Direction of cord coiling (%)	Left	Right	Bi-directional	No coiling	Missing data or cord too short to measure
Reference	79.3	16.3	0.6	2.1	1.7
PET	70	25	-	-	5
PIH	73.1	23.1	-	3.8	-
GDM	92.1	5.3	-	-	2.6
SGA	78	18.4	-	2.4	1.2

Table 5.3 Direction of cord coiling in reference and outcome groups.

Coiling Index	N	Mean (range)±SD
Reference	875	0.20 (0-1) ± 0.09
PET	19	0.20 (0.06-0.5) ± 0.10
PIH	26	0.20 (0-0.38) ± 0.09
GDM	39	0.20 (0-0.43) ± 0.09
SGA	81	0.21(0-0.55) ± 0.11

Table 5.4 Description of Cord Coiling Index in reference and outcome groups.

Mann-Whitney U test was performed to test the difference in coiling index in common obstetric outcome groups and it was compared to the non-affected cases.

A Mann-Whitney U test revealed no significant difference in the coiling index for pre-eclampsia; (n=19), U=7891, z=-0.22, p=0.82, affected cases (mean=0.20, median=0.15) versus non affected cases (mean=0.20, median=0.19). Results for PIH; (n=26), U=10778, z=-0.20, p=0.83, affected (mean=0.20, median=0.18) versus non affected group (mean=0.20, median=0.19). Cases of GDM (n=39), U=15475, z=-0.49, p=0.63, affected cases (mean=0.20, median=0.18) versus non affected cases (mean=0.20, median=0.19). Non parametric test showed a non significant difference (p=0.54) in SGA (n=81), U=30814, z=-0.62, (mean=0.21, median=0.20) versus non affected cases (mean=0.20, median=0.19).

In each category above, p value was ≥ 0.05, no significant difference in the coiling index of the groups with or without the conditions defined.

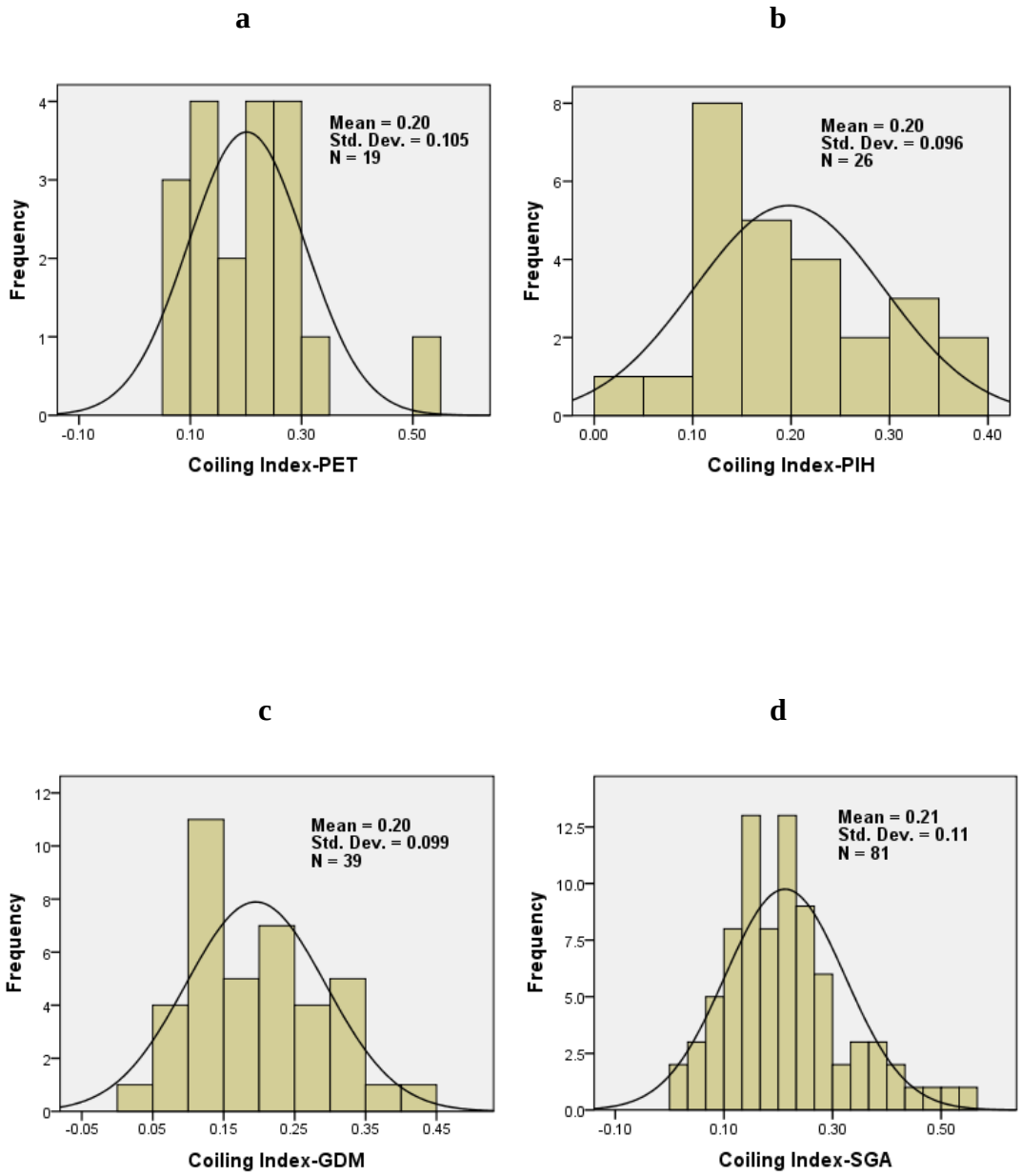


Figure 5.1(a-d) Frequency histograms of coiling index of obstetric outcome groups at 33-43 weeks.

5.4.2 Umbilical cord insertion

Qualitative description of the type of umbilical cord insertion in relation to previous standards showed that the frequency of central and paracentral umbilical cord insertion remains highest even in common obstetric outcome groups.

Table 5.5 shows the different types of umbilical cord insertion in reference and outcome groups. Comparing within the groups, incidence of central and paracentral insertion was similar to the unselected reference population. Though the incidence of marginal and velamentous appeared higher in pregnancy induced hypertension; 19.3% and 3.8% respectively, but statistically it was a non significant difference ($p \geq 0.05$).

Cord Insertion	Central (%)	Paracentral (%)	Marginal (%)	Velamentous (%)	Not known (%)
Reference	27.6	63.9	8.3	0.2	-
PET	25	65	5	-	5
PIH	23.1	53.8	19.3	3.8	-
GDM	34.2	57.9	7.9	-	-
SGA	25.6	61	12.2	-	1.2

Table 5.5 Qualitative description of cord insertion in reference and common obstetric outcome groups.

5.4.3 Cord centrality index

The mean distance of cord insertion from the centre ranged from 2.99 -3.63 cm in all common obstetric outcome groups, irrespective of the direction of umbilical cord coiling.

Table 5.6 describes the statistics of Centrality index in all common obstetric outcome groups. The mean centrality index ranged from 0.31-0.40. Kolmogorov-Smirnov test of normality revealed a 2 tailed p value of 0.97, 0.52, 0.78 and 0.06 for PET, PIH, GDM and SGA respectively indicating a normal distribution of data in all common obstetric outcome cases, however considering the small number of cases, frequency histograms Figures 5.2 (a-d) and Q-Q plots were also calculated, showing normal distribution.

Centrality Index	N	Mean (range) ± SD
Reference	888	0.35 (0.02-1.01) ± 0.21
PET	20	0.40 (0.12-0.89) ± 0.20
PIH	26	0.39 (0.04-0.99) ± 0.27
GDM	39	0.31 (0.02-0.93) ± 0.18
SGA	82	0.40 (0.02-0.99) ± 0.24

Table 5.6 Description of Centrality index in reference and outcome groups.

Mann-Whitney U test revealed no significant difference in the centrality index of PET (n=20), U=7370, z=-1.15, p=0.24; affected (mean=0.41, median=0.38) versus non affected (mean=0.36, median=0.32); PIH (n=26), U=10742, z=-0.36, p=0.71, affected (mean=0.40, median=0.31) versus non affected (mean=0.36, median=0.32); GDM (n=39), U=14804, z=-1.07, p=0.28; affected (mean=0.32, median=0.31) versus non affected (mean=0.36, median=032); SGA (n=82), U=29627, z=-1.55, p=0.12 affected (mean=0.40, median=0.35) versus non affected (mean=0.35, median=0.32).

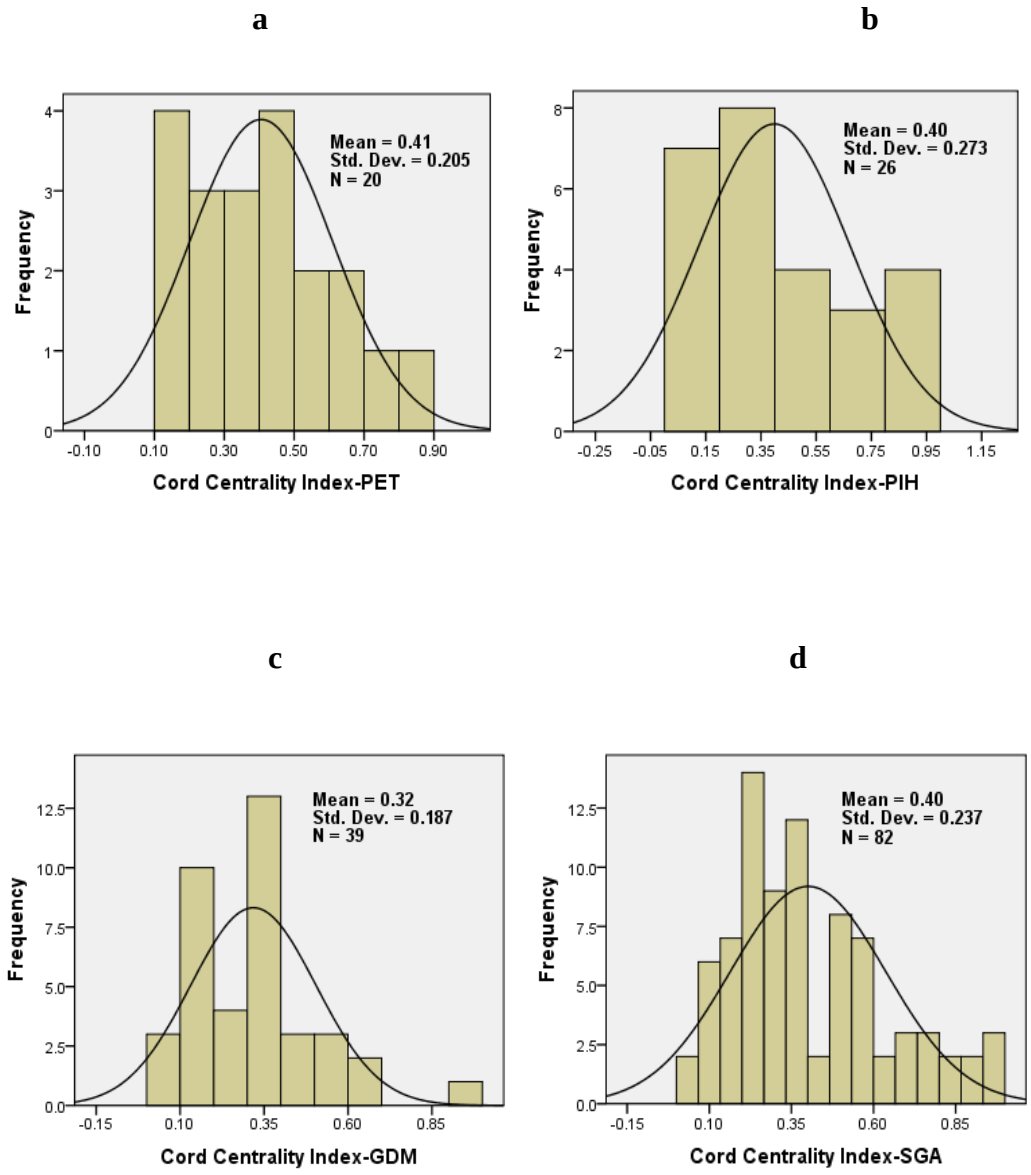


Figure 5.2 (a-d) Frequency histograms of Cord Centrality Index in outcome groups at 33-43 weeks.

5.4.4 Eccentricity

Table 5.7 describes the eccentricity in all common obstetric outcome groups. The mean eccentricity ranged from 0.47-0.52 in all common obstetric outcome groups. Kolmogorov-Smirnov test of normality revealed a 2 tailed p value of 0.98, 0.73, 0.43 and 0.99 for PET, PIH, GDM and SGA respectively indicating a normal distribution of data in all common obstetric outcome cases, however considering the small number of cases, frequency histograms Figures 5.3 (a-d) and Q-Q plots were also displayed, showing a normal distribution.

Eccentricity	**N**	**Mean (range)** ±SD
Reference	888	0.49 (0-0.91) ± 0.18
PET	20	0.47 (0.11-0.91) ± 0.19
PIH	26	0.50 (0.25-0.79) ± 0.14
GDM	39	0.49 (0-0.85) ± 0.20
SGA	82	0.52 (0.20-0.91) ± 0.18

Table 5.7 Description of Eccentricity in reference and outcome groups.

Mann-Whitney U test revealed no significant difference in the eccentricity of PET (n=20), U=8095, z=-0.51, p=0.60; affected (mean=0.47 , median=0.50) versus non affected (mean=0.49, median=0.51); PIH (n=26), U=109812, z=-0.17, p=0.86, affected (mean=0.50 , median=0.52)versus non affected (mean=0.49, median=0.51); GDM (n=39), U=15718, z=-0.49, p=0.63; affected (mean=0.50 , median=0.50) versus non affected (mean=0.49, median=0.51); SGA (n=82), U=29920, z=-1.41, p=0.16, affected (mean=0.52, median=0.52) versus non affected (mean=0.48, median=0.51).

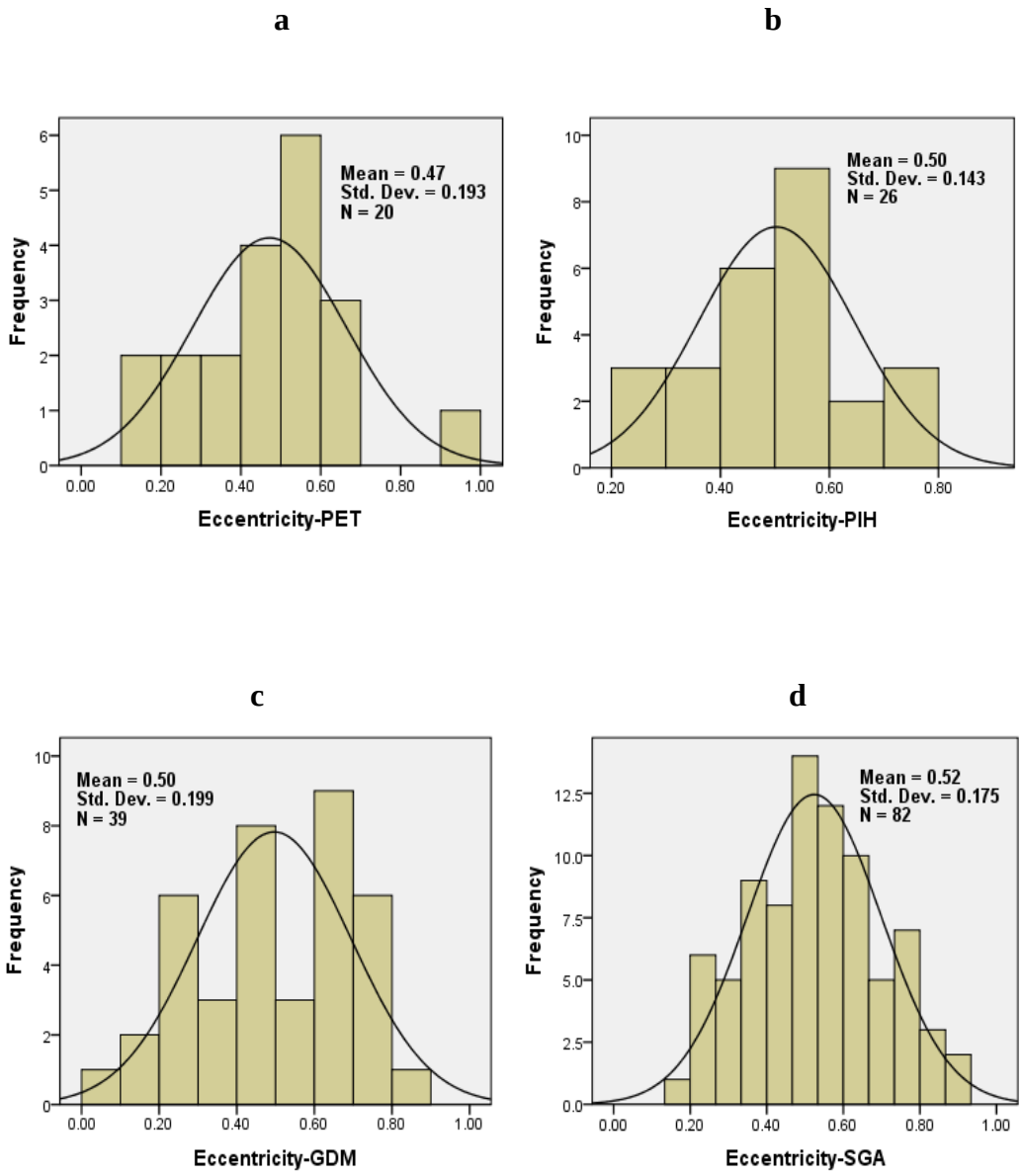


Figure 5.3 (a-d) Frequency histograms of placental Eccentricity in obstetric outcome groups at 33-43 weeks.

5.5 DISCUSSION

This data highlight the morphological indices of the placenta and umbilical cord in common obstetric outcome pregnancies and compare these indices in the non-affected cases.

The coiling index is one of the most studied umbilical cord related parameters in high risk pregnancies. Pre-eclampsia and gestational diabetes have been linked to the abnormal coiling, both non coiling as well as hyper coiling.[57 96] Hypercoiling has been associated with SGA (OR 2.10).[8] This study however showed no significant difference in umbilical cord coiling index with pre-eclampsia, PIH, GDM or SGA compared to that of population without these common obstetric outcome groups.

Qualitatively marginal cord insertion are associated with intra uterine growth restriction, stillbirth and neonatal death[9], and velamentous insertion with low birth weight, IUGR, diabetes mellitus.[9 74] In the current study, there was no significant difference in the incidence of central, paracentral, marginal or velamentous cord insertions in common obstetric outcome groups as compared to the non-affected groups.

There are no studies quantitatively comparing the difference between abnormal insertion of the umbilical cord in common obstetric outcome groups and a reference population. The distance of umbilical cord insertion from the centre (or from the chorionic plate margin) has been considered a marker of placental insufficiency.[90 97] Despite there being association of peripheral cord insertion with common obstetric outcome groups, shown in the various above studies, quantitatively defined, in the current study there was no significant difference in cord centrality index for pre-eclampsia, PIH, GDM and SGA groups compared to the reference population.

There are no studies describing the shape of placenta quantitatively in the common obstetric outcome groups of hypertensive disorders of pregnancy, GDM or SGA. Further, none have compared the shapes of the placenta quantitatively between common obstetric outcome groups and a reference population, though a recent study has shown a correlation between the short axis of the placenta and the severity of the pre-eclampsia.[94] This study did not find any statistically significant difference in eccentricity between the populations affected with the common obstetric outcome groups to that of un-affected rest of population.

To summarise, coiling index, cord centrality index and eccentricity in this study was not different in the adverse pregnancy outcomes that has been defined previously, compared to the data without the disease from unselected reference population.

CHAPTER 6 : MORPHOLOGY: PLACENTAL WEIGHT AND CIRCUMFERENCE-SIGNIFICANCE AND CORRELATION WITH BIRTH WEIGHT IN UNSELECTED PREGNANCIES AND COMMON OBSTETRIC OUTCOME GROUPS.

Summary points:

- This study described the baseline data for the ratio of birth weight to the placental weight and placental circumference in the reference population of 1159 at 33-43 weeks of gestation.
- Key findings: Study analysed the relationship of birth weight to the placental weight and circumference and showed a strong positive correlation of birth weight to the placental weight and circumference.
- This means that bigger the placenta in weight and size, heavier is the baby and vice versa, which may sound obvious, but has not been shown quantitatively before.
- In cases with PET, these ratios were significantly smaller compared to cases not affected by PET.

This chapter is based on:

Pathak S, Jessop F, Hook L, Sebire NJ, and Lees C. *Placental weight, digitally derived placental dimensions at term and their relationship to birth weight.* **J Maternal Fetal Neonatal Med.** 2010 Oct; 23(10):1176-82

6.1 INTRODUCTION

The weight of the placental is an important reflection of the placental function. The placenta weight is one of the important characteristics of the placental size. Two important constituents of the placenta size are chorionic plate surface area, and the disc thickness. The area of the uterus which is covered by the placenta is the surface area of the chorionic plate [98], on the other hand thickness of the chorionic disc shows the extent of the arborisation of the villous.[33] The placenta weight can be a 'sentinel' indicator of nutritional and/or environmental problems. However, it is not clear, whether weighing the placenta reflects the "true" weight as it is extremely difficult to evaluate the exact amount of fetal and maternal blood present in the placenta at the time of weighing? It is difficult to assess the amount of blood remained in the intervillous space. The amount of fetal blood trapped in the placenta will depend to some degree upon the timing of cord clamping after delivery of the baby.

Moreover there are no standard techniques as to how a placenta should be weighed, some weigh the placenta with attached membranes and the cord and also without removing the adherent maternal clots, whilst other will weigh placenta with membranes trimmed off and with or without cutting the cord and with or without removing any adherent maternal clots.

More importantly, one has to consider the fact that a placenta loses a variable but progressive weight while kept in the refrigerator, if not weighed soon after delivery. The placenta stored in the lab to be weighed later, loses weight over a period of time, one study showed that stored placenta loses 4% of its weight in 12 hours, 6% in 24 hours and 10% in 48 hours.[99] This problem perhaps could be avoided by keeping the placenta in the low temperature or fix in formalin; however it is not advisable to freeze or fix the placenta before examination as both these procedures can obliterate the most useful microscopic characteristics and make even the macroscopic examination more difficult. Fixation of the placenta also produces variable changes in the weight which then further makes difficult to weigh the placenta accurately.

There are different factors which may affect the placental weight for that given pregnancy. Placental weight varies with ethnicity [100], gestation age, maternal socio-economic status, smoking, maternal age, sex of the infant and birth weight.

Feto-placental weight ratio represents the balance between fetal and placenta growth. Any alteration in any of them will suggest disturbance in the intrauterine status and would warrant further investigation. Birth weights, placental weights, and birth weight /placental weight ratios have been correlated with perinatal morbidity and mortality. Less than 10th centile of the placenta weight has been associated with Intrauterine growth restriction, pre-eclampsia, increased intervillous fibrin deposition, villitis of unknown origin and trisomy whilst high placental weight has been linked with maternal diseases such as diabetes mellitus, anaemia, and fetal disorders such as fetal anaemia, hydrops, congenital syphilis and nephritic syndrome.[101-102] As few studies have shown the importance of the weight of the placenta, it has encouraged the researchers to explore more about the clinical significance of placental weight. The placental weight percentile curves according to the gestation age and gender for that population have been described.[103] These percentile curves are specific for that population but if one consider the ratio of birth weight to the placenta weight, probably that should not vary much between the different sets of populations and it has long been argued if this ratio is likely to yield useful information rather than the weight of the placenta alone.

A positive correlation has been seen between the placental weight and the infant weight at birth. Birth weight of the infant is said to be directly proportional to the placental weight.[104-105] Studies have even suggested that small for gestational age infants have smaller placentas than the placentas for appropriate weight for gestation infants, suggesting that fetal growth depends on the actual weight of the placenta.

The placenta weight/ fetal birth weight ratio is 1:2.9 at 24 weeks which increases to 1:6.8 at 40 weeks[106], any change in this ratio will simply reflect the altered relationship of fetal-placental unit. In the recent literature it has been emphasised more onto the placental and fetal weight ratio, which was said to be more accurate rather than taking the weight of the placenta alone and correlating it to the neonatal outcome.

Higher placental/fetal weight ratio has been correlated with high perinatal morbidity and mortality.[107] Even in these cases one could argue, placenta gets bigger as a compensatory mechanism in high risk pregnancies such as pre-eclampsia, severe anaemia, heavy smoking in pregnancy and it won't be surprising to get the higher placental /fetal weight ratio associated with higher perinatal morbidity and mortality. Studies are reported showing that anaemia during pregnancy is associated with large placental weight and a high ratio of placental weight to birth weight.[108 109]

Placenta weight/fetal weight ratio has been well known as an predictor of developing disease later in adult life as intrauterine environment has an important effect on blood pressure and hypertension in adults. Large British cohort studies have suggested that having a discordance of larger placenta with a small baby may lead to circulatory adaptation in the fetus, altered arterial structure in the child, and hypertension in the adult.[110] This may have important implications for the prevention of adult hypertension, which appears to have its origin in fetal life. Higher placenta weight in the most recent pregnancy has also been correlated significantly to the reduced survival with the breast cancer, if diagnosed during pregnancy or within 2 years thereafter.[111]

However placenta weight only, per se should have no clinical importance unless considered along with gestational age and clinical picture.

Further, a few recent studies establish the nature of the relationship between birth weight and placental weight and circumference; positive correlations between placental and infant weight are reported[105 112-113], but these data do not refer to an unselected population. Moreover, the available data on the birth weight/placenta circumference is qualitative. There is no available data on quantitative analysis on placental circumference and its correlation with birth weight.

The aims of this chapter:-

Analysis of mean placenta weight, placenta circumference and birth weight in an unselected reference population.

Analysis of mean placenta weight, placenta circumference and birth weight in common obstetrics outcome groups e.g. Pre-eclampsia (PET), pregnancy induced hypertension (PIH), gestational diabetes (GDM) and small for gestational age (SGA).

Defining the relationship between placental weight and placental circumference (manual) to birth weight in the common obstetric outcome population and compare the ratios to the similar ratios derived for non affected cases in unselected reference population.

6.2 METHODS

Details of the methods are described in chapter 2.

1159 placentas from singleton pregnancies, 33 to 43 weeks were examined. Placental circumference taken in this chapter is manual circumference. Definitions of the common obstetric outcome pregnancies have been defined in chapter 2.

6.3 STATISTICAL ANALYSIS

Descriptive analysis of birth weight, placenta weight, and placenta circumference was performed in reference population, pre-eclampsia (PET), pregnancy induced hypertension (PIH), Gestational diabetes (GDM) and Small for gestational age at birth (SGA).

Normality was checked for birth weight, placenta weight and placenta circumference for reference population, using the frequency histograms and Q-Q plots.

Descriptive analysis was performed for ratio of birth weight to placenta weight and placental circumference in reference population, PET, PIH, GDM and SGA.

To establish the relationship of birth weight to the placental weight and circumference, Z score of all the three was calculated to normalise the data for the gestational age within the gestation range 33-43 weeks, using the means and SDs derived from this population. A linear relationship was analysed on a scattered plot for birth weight versus placenta weight and birth weight versus placenta circumference. Pearson correlation coefficient (r) was derived and r^2 was calculated for both comparisons. As multiple comparisons were made, p value of ≤ 0.01 was considered significant.

T test was performed to test if there is any significant difference of ratio of birth weight to placenta weight and placenta circumference in the non-affected group compared to the group affected with PET, PIH, GDM or SGA. A T test 2 tailed significant values (p) was significant if value was ≤ 0.05.

6.4 RESULTS

In the study data of 1159 placentas, specific common obstetric outcome pregnancy categories were chosen; Pre-eclampsia, Pregnancy induced hypertension, Gestational diabetes and small for gestational age of $\leq 10^{th}$ percentile. Table 6.1 describes the baseline maternal demographics of the study population including the common obstetric outcome groups in the population.

N	1159
Maternal Age ± SD	30.9 ± 5.73
% Nulliparity (n)	47.6 (552)
% Smokers (n)	9.3 (108)
% Caucasians (n)	90.7 (1051)
% PET (n)	2.5 (29)
% PIH (n)	2.6 (30)
% GDM (n)	4 (46)
% SGA <10 Centile (n)	9.4 (109)
Mean gestation age at delivery ± SD	39.5 ± 1.49
% Female (n)	46.7 (541)
% Male (n)	53.3 (618)

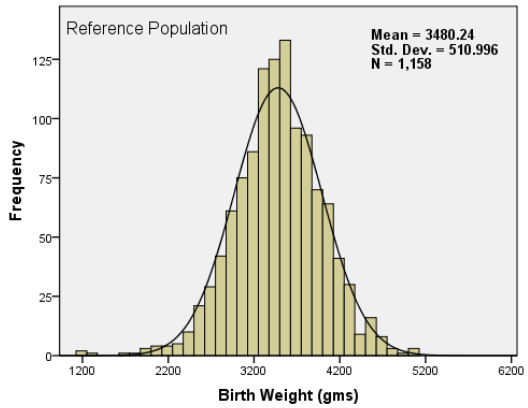
Table 6.1 Baseline demographics of the reference population.

Table 6.2 describes the statistics of mean ± SD of birth weight, placenta weight and placenta circumference (manual) in reference population and all common obstetric outcome groups. Table shows the crude comparison of the means of birth weight, placenta weight and placenta circumference in different groups.

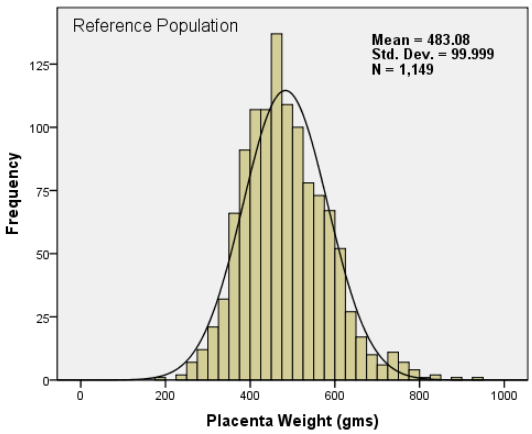
Mean ± SD	**Gestation age range (Weeks)**	**Birth weight (gms)**	**Placenta weight (gms)**	**Placenta circumference (cm)**
Reference	33-43	3480 ± 511	483 ± 100	60.30 ± 6.28
PET	33-41	3076 ± 823	495 ± 117	59.42 ± 7.27
PIH	36-42	3451 ± 510	464 ± 102	61.81 ± 8.75
GDM	37-41	3511 ± 462	506 ± 92	61.26 ± 7.55
SGA	33-42	2671 ± 341	377 ± 68	55.95 ± 5.49

Table 6.2 Birth weight, placental weight and circumference in reference and obstetric outcome groups.

Figure 6.1(a-c) represent the frequency histograms of birth weight, placenta weight and circumference in reference unselected population. There was data missing for 1 birth weight, 10 placental weights and 9 placental circumferences in the reference population.



a



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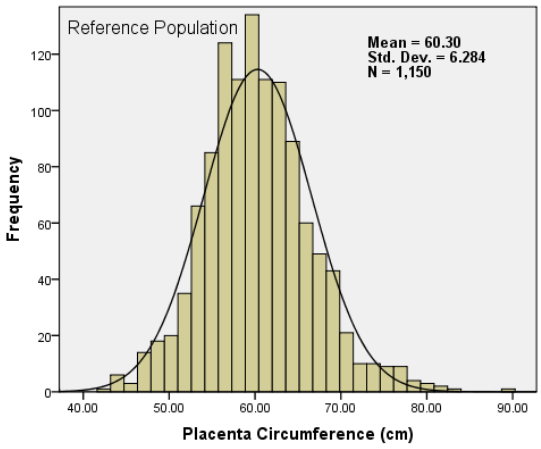


c

Figure 6.1(a-c) Frequency histograms of birth weight, placenta weight and circumference in reference population.

Table 6.3 and 6.4 describes the statistics for the ratio of birth weight to placenta weight and placenta circumference.

Birth Wt / Placenta Wt				Coefficient of Variation	
	Mean	Median	SD	Mean centred	Median centred
Reference	7.38	7.32	1.20	16.2%	16.4%
PET	6.60	6.49	1.03	15.6%	15.9%
PIH	7.69	7.63	1.67	21.5%	21.7%
GDM	7.07	6.94	1.08	15.3%	15.7%
SGA	7.27	7.29	1.30	17.2%	17.2%

Table 6.3 Birth weight and placenta weight ratio in reference and common obstetric outcome groups.

Birth Wt/placenta circumference				Coefficient of Variation	
	Mean	Median	SD	Mean centred	Median
Reference	58.05	58.12	8.07	13.9%	13.9%
PET	54.14	53.42	10.00	18.5%	18.8%
PIH	56.47	55.47	8.60	15.2%	15.6%
GDM	57.70	57.71	7.39	12.8%	12.8%
SGA	48.27	48.66	6.14	12.7%	12.6%

Table 6.4 Birth weight and placenta circumference ratio in Reference and common obstetric outcome groups.

Tables 6.5 (a-d) illustrates the direct comparisons of birth weight and placenta weight in cases with common obstetric outcome versus cases not affected by the outcome groups.

a

Birth Wt / Placenta Wt	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
No PET	7.40	7.34	1.19	16.1%	16.3%
Present PET	6.60	6.49	1.03	15.6%	15.9%
Overall	7.38	7.32	1.20	16.2%	16.4%

b

Birth Wt / Placenta Wt	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
No PIH	7.38	7.31	1.18	16.0%	16.2%
Present PIH	7.68	7.63	1.66	21.5%	21.7%
Overall	7.38	7.32	1.20	16.2%	16.4%

c

Birth Wt / Placenta Wt	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
No Diabetes	7.40	7.34	1.20	16.2%	16.4%
GDM	7.07	6.94	1.08	15.3%	15.7%
Overall	7.38	7.32	1.20	16.2%	16.4%

d

Birth Wt / Placenta Wt	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
NGA	7.39	7.32	1.19	16.1%	16.3%
SGA	7.27	7.29	1.30	17.2%	17.2%
Overall	7.38	7.32	1.20	16.2%	16.4%

Table 6.5 (a-d) Birth weight and placental weight ratios in common obstetric outcome groups

Tables 6.6 (a-d) illustrates the direct comparison of birth weight and placental circumference ratio in cases with and without obstetric outcome groups.

a

Birth Wt / Placental Circumference	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
No PET	58.14	58.16	8.00	13.8%	13.8%
Present PET	54.14	53.42	10.00	18.5%	18.8%
Overall	58.05	58.12	8.07	13.9%	13.9%

b

Birth Wt / Placental Circumference	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
No PIH	58.09	58.14	8.06	13.9%	13.9%
Present PIH	56.47	55.47	8.60	15.2%	15.6%
Overall	58.05	58.12	8.07	13.9%	13.9%

c

Birth Wt/ Placental Circumference	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
No Diabetes	58.08	58.16	8.10	14.0%	13.9%
GDM	57.70	57.71	7.39	12.8%	12.8%
Overall	58.05	58.12	8.07	13.9%	13.9%

d

Birth Wt / Placental Circumference	Mean	Median	SD	Coefficient of Variation	
				Mean	Median
NGA	59.05	58.86	7.56	12.8%	12.8%
SGA	48.27	48.66	6.14	12.7%	12.6%
Overall	58.05	58.12	8.07	13.9%	13.9%

Table 6.6 (a-d) Birth weight and placental circumference ratios in common obstetric outcome groups

T test was conducted to compare the birth weight ratios to placenta weight and circumference for all common obstetric outcome groups and the reference population.

Results of test in the birth weight: placenta weight is as follows: - (affected versus non-affected cases)

PET (n=26), Mean=6.60 ± 1.03, p=0.001 (two tailed). The mean difference of the two means was 0.79 with 95% confidence interval of 0.34 to 1.27.

PIH (n=30), Mean=7.68 ± 1.66, p= 0.16, GDM (n=46), Mean = 7.07 ± 1.08, p= 0.07, SGA (n=107), Mean=7.28 ± 1.25, p= 0.32, and unselected reference population, Mean = 7.39 ± 1.20.

T test results for birth weight: placenta circumference is as follows: - (affected versus non-affected cases)

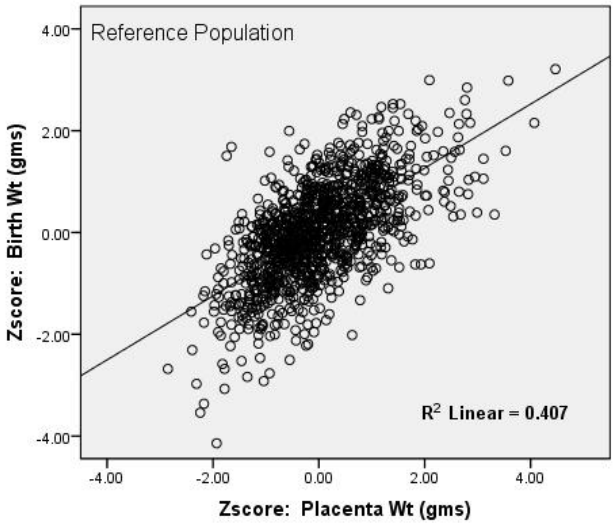
PET (n=26), Mean=54.14 ±10, p=0.01 (two tailed), the mean difference of the means was 3.99 with 95% Confidence interval of 0.86 to 7.13.

PIH (n=30), Mean =56.47 ± 8.60, p= 0.28, GDM (n=46), Mean=57.70 ± 7.39, p=0.76. In cases with SGA-(n=108) Mean=48.27± 6.14, p=0.00. The mean difference of the means was 10.79 with 95% CI of 9.53 to 12.04. Unselected reference population had mean value of 58.05 ± 8.07.

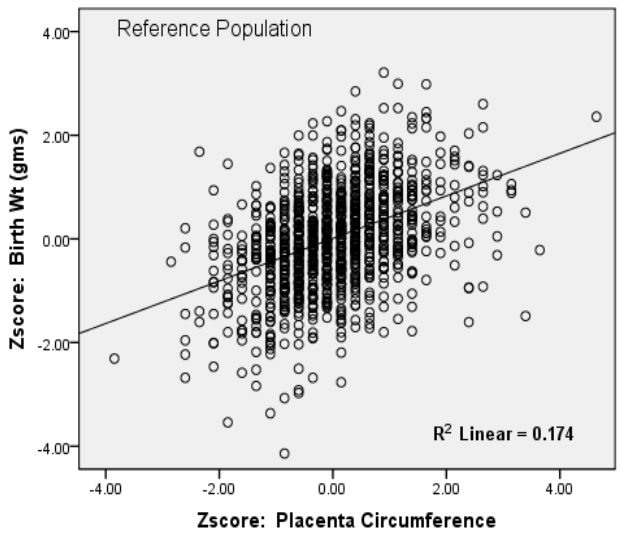
Figures 6.2 (a-b) shows the scatter plots and the linear relationship with significant Pearson's correlation coefficient for z: birth weight to z: placenta weight and z: birth weight to z: placenta circumference for reference population, r as 0.638 and 0.417 respectively.

For common obstetric outcome groups, z- score of birth weight and placental weight (figures 6.3-6.6), the Pearson's correlation coefficient (r) was 0.78, 0.49, 0.59, 0.45 with p values 0.001, 0.006, 0.001 and 0.001 for PET, PIH, GDM and SGA respectively, showing high positive correlation.

Similarly significant results were obtained for z-score of birth weight and placenta circumference are shown in figures 6.7- 6.10, with Pearson's correlation coefficient (r) as 0.54, 0.39, 0.50 and 0.35 with p values 0.004, 0.034, 0.001 and 0.001 for PET, PIH, GDM and SGA respectively. These figures also compare the relationship in the ratios with and without the disease.



a



b

Figure 6.2 (a-b) z score of birth weight versus placental weight and circumference in reference population.

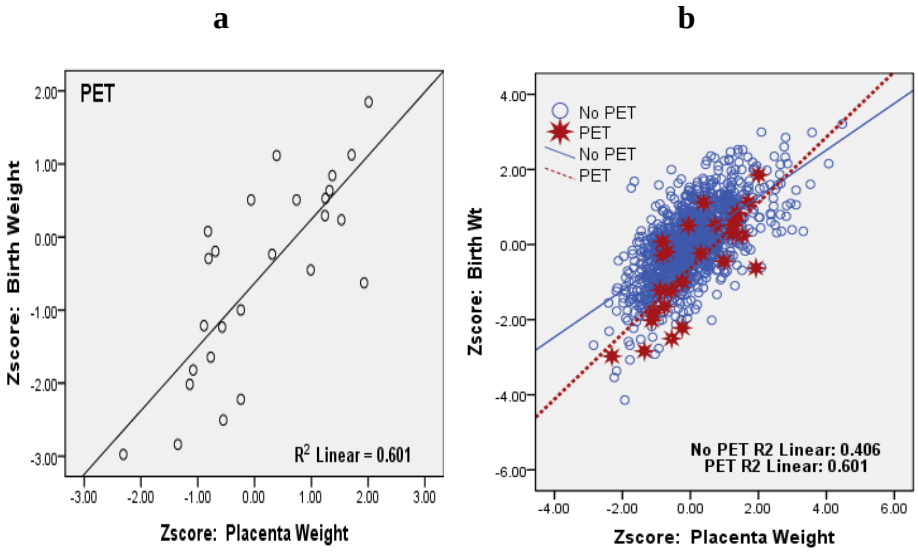


Figure 6.3 (a-b) z score of birth weight versus placental weight in PET.

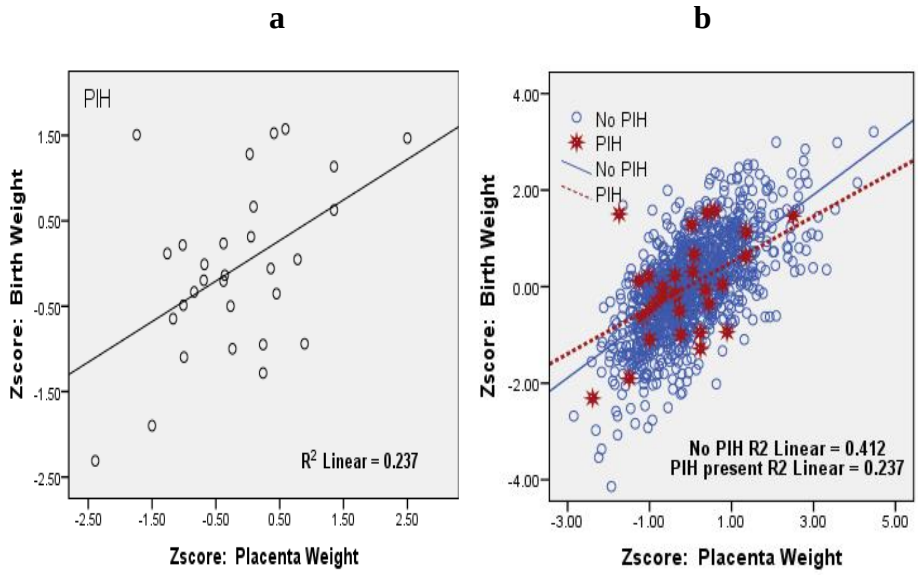


Figure 6.4 (a-b) z score of birth weight versus placental weight in PIH.

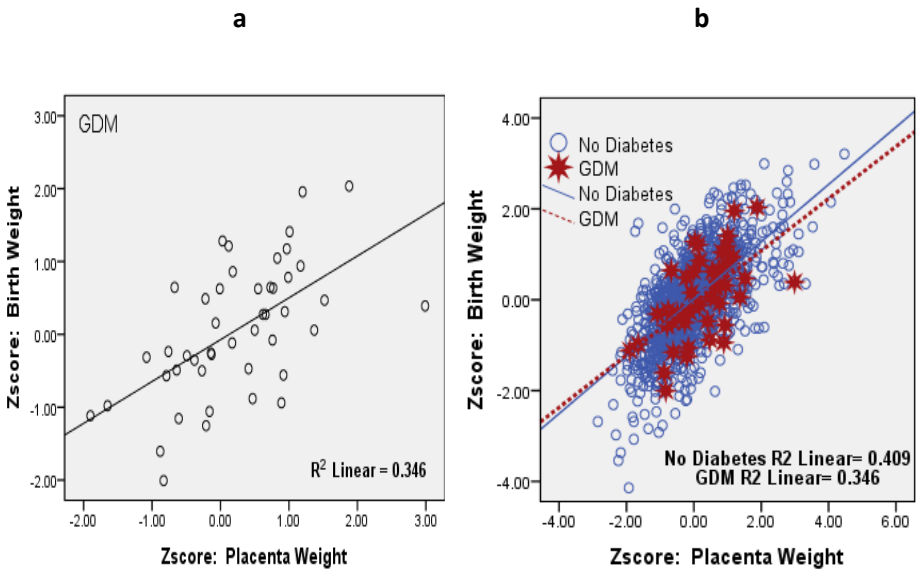


Figure 6.5 (a-b) z score of birth weight versus placental weight in GDM.

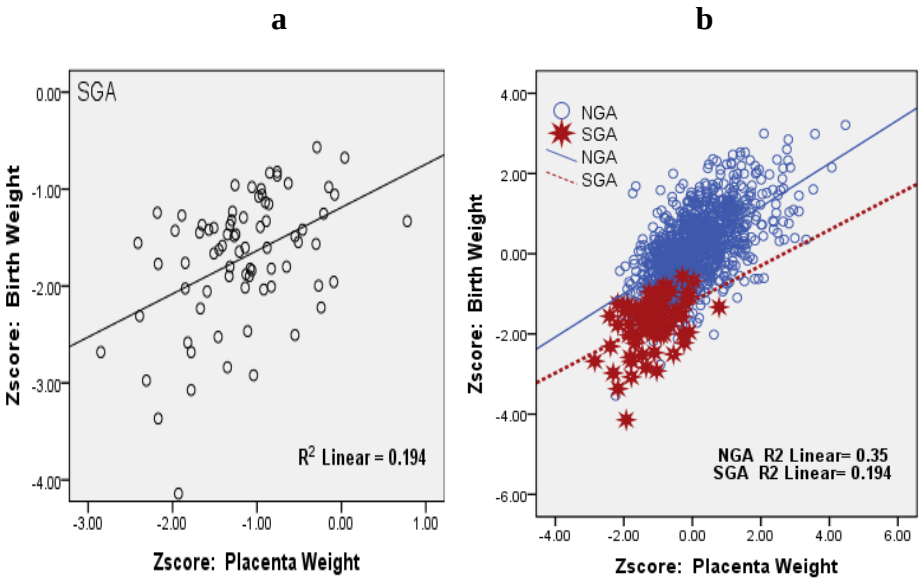


Figure 6.6 (a-b) z score of birth weight versus placental weight in SGA.

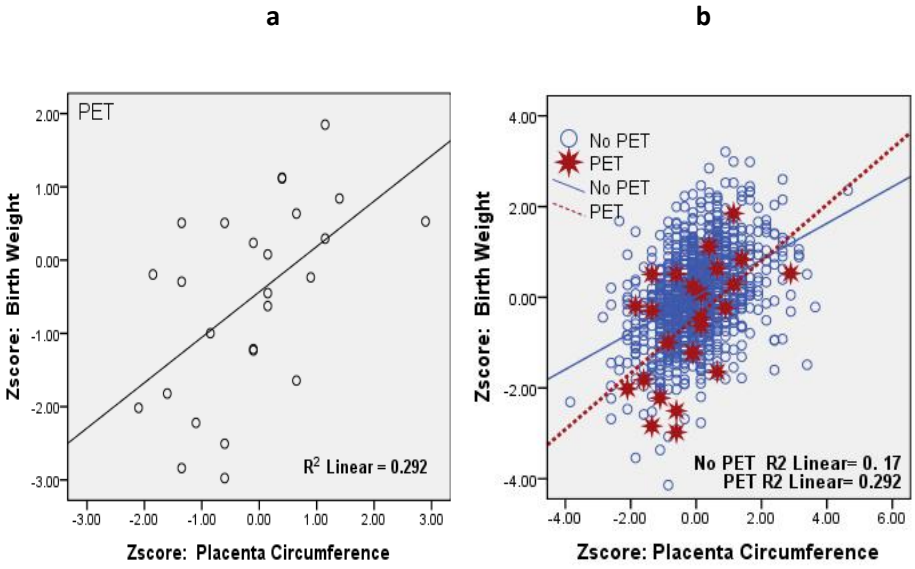


Figure 6.7 (a-b) z score of birth weight versus placental circumference in PET.

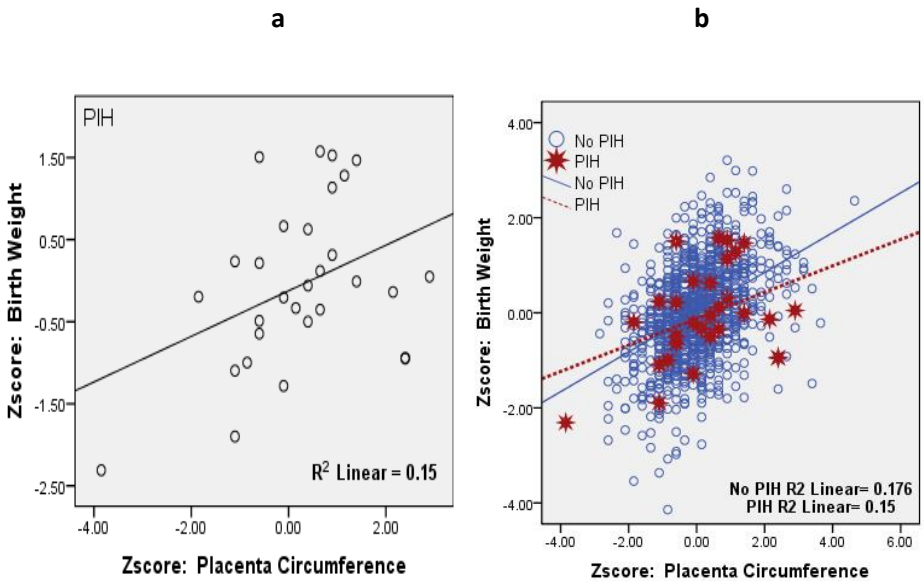


Figure 6.8 (a-b) z score of birth weight versus placental circumference in PIH.

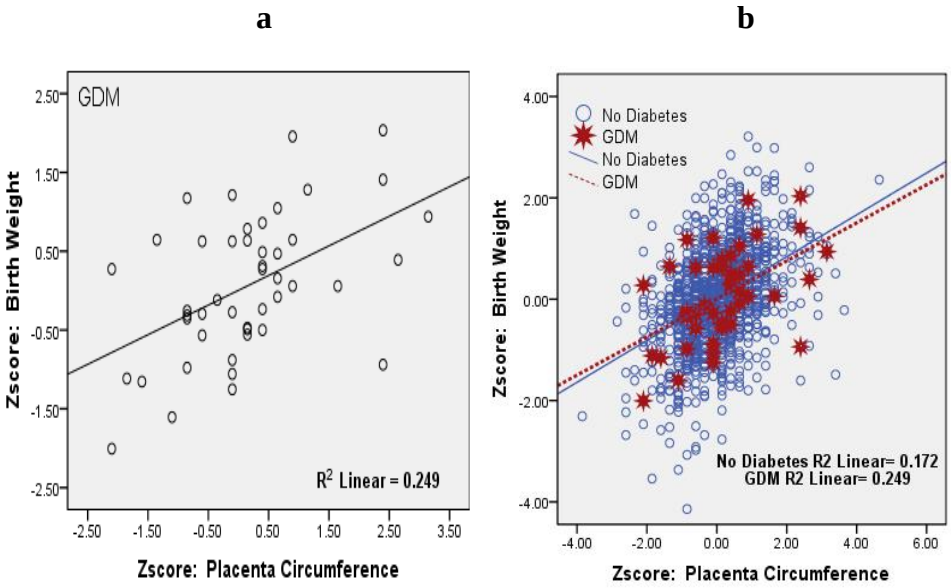


Figure 6.9 (a-b) z score of birth weight versus placental circumference in GDM.

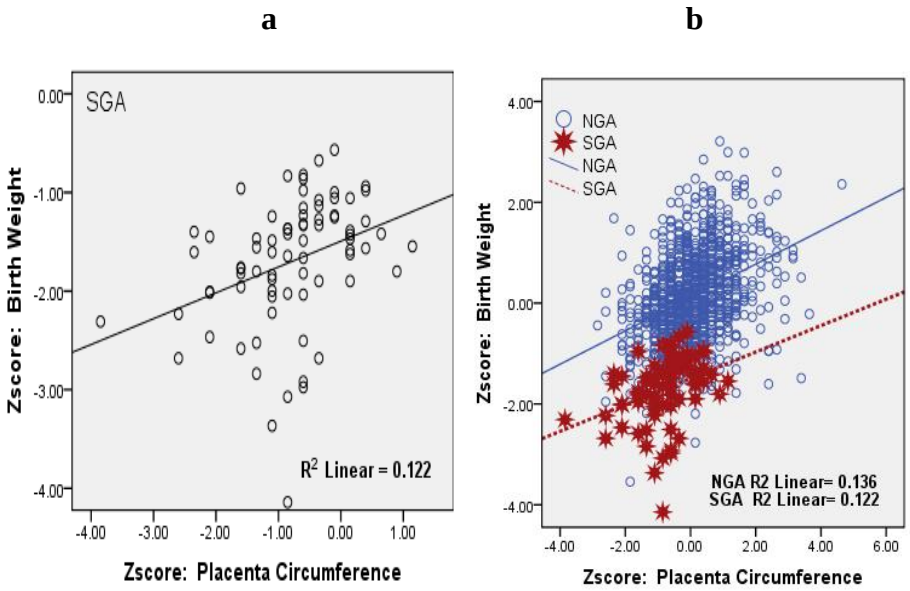


Figure 6.10 (a-b) z score of birth weight versus placental circumference in SGA.

6.5 DISCUSSION

The normal birth: placental weight ratio has been shown to range between 6.5 to 7.1 at term 37-42 weeks gestation[114-115] Another study showed the birth weight: placental weight ratio ranging from 6.3 to 8.46 at 34-43 weeks study.[116] The birth weight: placental weight ratio of 7.39 ± 1.20 in reference population from 33-43 weeks that was derived, is therefore within the limits of these ranges. This ratio does have relevance as the ratio of birth weight: placental weight has been considered to be more important than placental weight alone when considering adverse perinatal outcome such as fetal and neonatal demise.[107 117]

In the common obstetric outcome population, birth weight: placental weight has been compared with non affected population. In cases with PET, this ratio has been found significantly (p=0.001) smaller as compared to non PET cases (6.60 versus 7.40). Mean difference between the groups was 0.79 with 95% confidence interval of 0.34-1.27, Studies have shown that often infants are small for gestational age for a small placenta in pre-eclampsia and large for large for gestational age.[118] In the current study, cases with PIH, GDM and SGA did not show this ratio any significantly different from that of non-affected groups.

The other ratio, this study has looked at is birth weight: placenta circumference, which has not been shown before in previous studies. Whether it is a useful ratio or not, it is not clear yet but this can be used as a baseline for the future studies on the same area.

This study showed significant difference in birth weight/placental circumference ratio in cases with PET and SGA. In cases of PET, mean difference of this ratio was 3.9 from non-affected population with confidence interval of 0.86-7.13 and p value was 0.01. For SGA, mean difference was 10.79 (48.27 versus 59.05) with 95% CI as 9.53-12.04

with p value of <0.01. Though it would seem obvious that for a low birth weight, size or circumference of placenta should also be smaller, but the current study did not notice this ratio any different in cases with PIH and GDM as compared to the non-affected population values.

This study also showed strong correlation between placental weight and circumference to the birth weight in all common obstetric outcome groups and reference group (scatter plots with positive correlation with significant Pearson's correlation coefficient's value; figures 6.2-6.10), which also confers that higher the birth weight, higher is the placental weight and the circumference and vice versa. Although this may sound obvious but these relationships have not been shown before in this manner, quantitatively.

The values of placental weight and birth weight/placental weight ratio in reference population will provide a baseline for future studies based on the similar subjects.

CHAPTER 7 : HISTOLOGY AND ITS RELATIONSHIP WITH PLACENTAL MORPHOLOGY

Summary points:

- This study has described the baseline data of predefined histological lesions of the placenta in common obstetric outcome groups of PET, PIH, GDM and SGA and in reference population and its relationship with its morphology.

  Key points: are as follows-

- There was no significant difference in the incidence of predefined histological lesions in the pregnancy outcome groups.
- Most placentas from an unselected population (72%) or common pregnancy outcome groups (66-80%) are histologically normal.
- Placental morphological indices were not different in predefined histological lesions.
- Macroscopic abnormalities of the placental shape and cord insertion cannot predict presence or absence of histological features and are of limited clinical significance.

This chapter is based on:

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** *Relationship between placental morphology and histological findings in an unselected population near term***-** accepted **Virchows Archiv**, March 2011

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** Placental histology at or near term in relationship to obstetric outcome- **Virchows Archiv.** April 2011

7.1 INTRODUCTION

Though there have been some agreed standards for the placental histological examinations[16-17], still there are no uniformly defined standards and guidelines for placental examinations. Not only that, there are not even agreed definitions for most histological pathologies. Very few Obstetricians truly understand these pathological lesions and their implications for the past and the future pregnancy. Frequently, pathologists report the placenta examination as either "NAD" or small area of marginal infarction, which may not have any bearing onto the clinical outcome while Obstetricians "blame" the only available pathology of placenta, as the possible cause of adverse perinatal outcome.

Another major concern is that most of the placenta literature is based on abnormal placentas, which makes it difficult to differentiate for many features between the "pathologically abnormal" versus "normal variant". There is a definite need for studies to analyse normal placentas statistically and to identify the normal variants of histological lesions during the course of pregnancy. In best of my knowledge the pattern of histopathological abnormalities of the placenta from unselected population has not been described before.

Complications such as pre-eclampsia, pregnancy induced hypertension, gestational diabetes, and small for gestational age are associated with higher perinatal morbidity and mortality. Since the placenta and umbilical cord is the functional unit between the mother and the fetus, the placenta may be expected to reflect in some way the health of both the baby and the mother. One might therefore hypothesize that certain obstetric outcomes might exhibit specific effects or histological lesions on the placenta. Numerous studies have been performed on placentas in these obstetric outcomes, in an attempt to explain the possible effects of these on the pregnancy and histology of placenta. However none of the studies highlights any of the lesions as characteristic of any one particular obstetric outcome category, which then makes the whole picture very confusing. Moreover, incidence of these histological lesions present in obstetric outcomes has not been studied in unselected cohort.

PET/PIH

Due to the incomplete invasion of trophoblast, spiral arteries remains less elastic leading to maternal vasospasm, this eventually causes high resistance to blood flow passing through these vessels and reduced utero-placental blood flow. The placenta in cases of pre-eclampsia and PIH may exhibit many pathological features suggestive of the disease but there may be completely normal placental findings macroscopically as well as microscopically even in cases of severe disease. Therefore the absence of these features should not exclude the pathology, as these pathological features may be characteristic but not the universal finding.

Pathological findings of the placenta in PET and PIH depend largely on the severity and the duration of the disease. Pathological features described in association with pre-eclampsia and pregnancy induced hypertension are villous maturation, decidua arthropathy, retroplacental haematomas, intervillous thrombosis, fetal artery thrombosis, varying degree of perivillous fibrin deposition and chorioamnionitis.

GDM

According to the UK diabetes statistics, about 1.8 million people are diagnosed with the diabetes by 2004 and by 2010, this will increase to 3 million. With the increasing incidence of diabetes, the incidence of gestational diabetes is growing. 2-5 % of women delivering in England and Wales per year have diabetes during pregnancy. Approximately 87.5% of these have gestational diabetes.

There are many structural and functional changes in placenta in diabetes depending upon the gestational period affected of the pregnancy. Microscopic features reported in diabetes are so varied that at times they seem to contradict each other. These differences could either be due to the different methodologies or may be due to the difference in degree of severity of diabetes and difference in gestational age at delivery. Histological lesions documented in diabetes are villous immaturity, oedema, Chorangiosis and proliferative endarteritis.

Small for Gestational Age

Small for gestational age (SGA) infant has been defined in this study as the birth weight less than 10th percentile of the gestational age. SGA infants may or may not be growth restricted (IUGR). IUGR is associated with 6-10 times higher perinatal morbidity and mortality compared to normal weight neonates.
Microscopic examination of the placenta in small for gestational age infants varies from case to case. Some placentas will exhibit entirely normal histology while others may show various histological abnormalities. Some of the common histological features which can be found in SGA are hypovascularіy of terminal villi, abnormal maternal spiral arterioles, increased fibrin deposition, villitis of unknown aetiology.

Further, there is no data whether there is any relationship of histological lesions with placental morphological characteristics such as cord coiling, umbilical cord insertion site and placental shape.

The aim of this chapter is to establish the incidence of predefined placental pathological abnormalities in the common obstetric outcome pregnancy outcomes groups of pre-eclampsia, pregnancy induced hypertension, gestational diabetes and small for gestational age infants compared to non-affected pregnancies and the rest of the unselected reference population. This chapter also aim to describe the relationship between morphological parameters of the placenta, as previously defined in chapter 4 and the common categories of histological lesions.

7.2 METHODS

Details of the methods are described elsewhere (chapter 2). Briefly; analysis of this chapter is based on all cases with available histology analysis report. The population of the study was an unselected population irrespective of the risk status of the pregnancy.

Histological findings of the placenta from the unselected population were compared with the common obstetric outcome group. Common outcome groups considered were pre-eclampsia (PET), pregnancy induced hypertension (PIH), gestational diabetes (GDM) and small for gestational age (SGA).

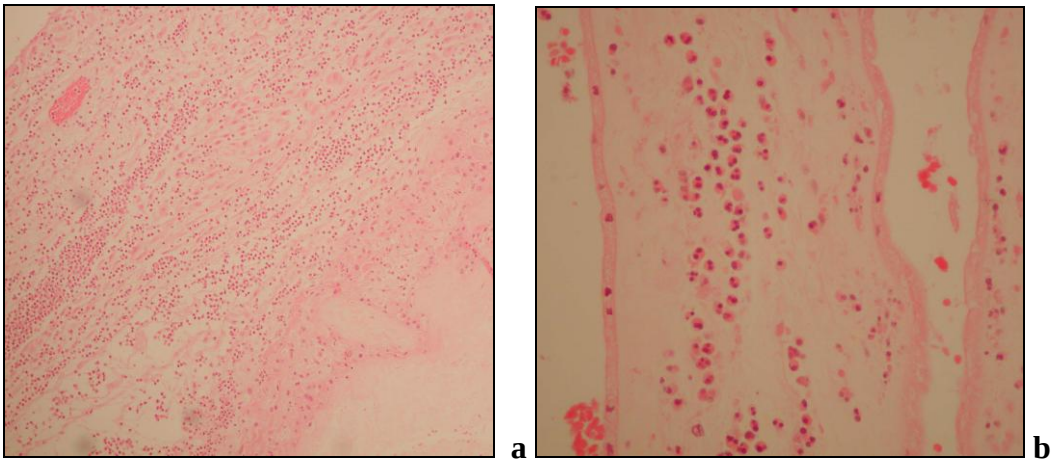
Definitions of the common obstetric outcome groups of PET, PIH, GDM and SGA are described in Chapter 2.

There were certain placentas where more than one histological lesion was present. Each placental lesion has been counted separately. Similarly, in the clinical groups, certain cases had more than one clinical obstetric outcome group and each group has been counted separately.

Digital measurements (Cord centrality and eccentricity indices) were available on 888 placentas. Manual measurements (Coiling index) were performed on 1141 cases, as in 18 cases either the umbilical cord was missing or was too short to measure.

MAJOR PATHOLOGICAL GROUPS

Chorioamnionitis



Figures 7.1 Microscopic view of chorioamnionitis (a) low power (original magnification x 40) (b) high power (original magnification x 100) (study photograph).

Histologic chorioamnionitis is inflammatory changes (not infective process) in placenta, umbilical cord, decidua, chorion and amnion. Chorioamnionitis is most of the time is an acute process but cases of chronic chorioamnionitis has also been described in literature, where placental membranes are infiltrated with chronic inflammatory cells.[119] In one large study of chronic chorioamnionitis, it was noted to have lymphocytic inflammation of the fetal membranes. Villitis of unknown aetiology was also noted in 70% of these cases examined, though the severity of villitis did not seem to have any correlation with the severity of lymphocytic infiltration of the membranes.[120]

Traditionally acute inflammation of chorion and amnion membranes and amniotic fluid infection has been taken together.[121-122] Studies done in 1980s have shown the recovery of the organisms in acute inflammatory lesions of the placenta from subchorionic plate[123-124] and chorioamniotic space.[121]

Clinical chorioamnionitis is suspected when there is presence of fever, uterine tenderness, and foul smelling vaginal discharge, maternal and or fetal tachycardia, leucocytosis, however none of these symptoms per se are not specific for chorioamnionitis and therefore clinical diagnosis is unreliable for the diagnosis histologically.

There is a poor correlation between clinical and histological chorioamnionitis. However there are many important questions to consider before judging the relationship between clinical and histologic chorioamnionitis; such as diagnostic criteria for clinical chorioamnionitis in the given set up; bacterial culture of placenta/amniotic fluid in support of diagnosis of chorioamnionitis; and if the diagnoses of clinical chorioamnionitis have features suggestive of histologic chorioamnionitis?

Most studies suggest that there is a poor correlation between clinical and histologic chorioamnionitis. In cases with confirmed histological chorioamnionitis, there may be no signs or symptoms suggestive of clinical chorioamnionitis at all.[125]

Studies have examined cases diagnosed with clinical chorioamnionitis to confirm the diagnosis histologically as well and also examined the effect of chorioamnionitis on neonates, neonatal sepsis at less than 7 days and found poor correlation between clinical and histologic chorioamnionitis and neonatal sepsis.[126] Another large study involving 6,294 singleton deliveries, there was no histological evidence of chorioamnionitis in 38% cases diagnosed clinically as chorioamnionitis (3%). Neonatal sepsis was also found only in 36% of neonates without histologic evidence of chorioamnionitis as compared to the 60% of neonates with confirmed histologic chorioamnionitis.[127]

Studies have also reported positive placental bacterial culture in absence of histologic chorioamnionitis[128] while other studies could only isolate bacteria in 70% cases of histologic chorioamnionitis.[129]

Therefore histologic chorioamnionitis is considered more sensitive and specific and is a gold standard for the diagnosis of chorioamnionitis as compared to the clinical diagnosis.[122 130 131]

For this study, following have been grouped together as "**Ascending genital tract infection**".

-Neutrophils in the fetal membranes- Chorioamnionitis

-Neutrophils at the choriodecidual junction- Choriodecidual junction syndrome

-Inflammation of umbilical cord- Funisitis

-Neutrophils in subchorion fibrin

- Subacute or chronic chorioamnionitis

Chronic placental underperfusion

We defined as villous changes small, hypovascular chorionic villi with increased syncytial knots, placental infarction >1cm (non-peripheral) and/or atherosis. Presences of any or all of the features were classified in this category.

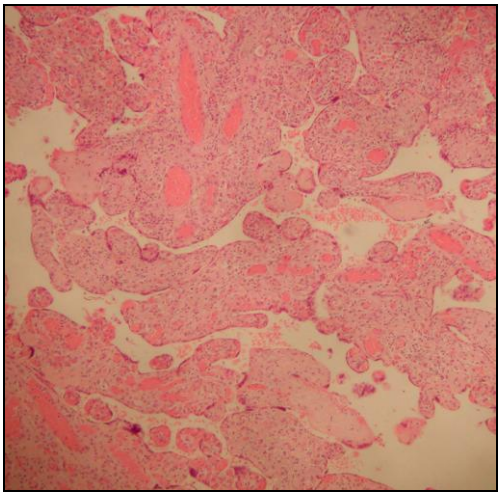
Intervillous Thrombus (IVT)

These are localised, nodular and circumscribed, old or recent thrombi within the intervillous space. Some authors have described these lesions as intraplacental haematomas as well[114] but others view was to consider these as thrombi only as the lesions are entirely within the part of vascular system, intervillous space.[117] Incidence of IVT varies from 3-50% in full term uncomplicated pregnancies.[132, 133] Macroscopically, intervillous thrombus can look dark red or brown, greyish or whitish depending upon the age of the lesion, usually round or oval lesion.

Thrombus could be single or multiple thrombi anywhere within placenta, varying in size, from few mms up to many centimetres. While histologically, erythrocytes can be seen with fibrin strands in fresh thrombus representing mainly red blood cells, and as the thrombus degenerate, depending on the size, it can displace the intervillous space. Infarction of the surrounding villi can also be seen.

IVT can also be revealed on antenatal ultrasound as echo-poor placental "cavities".[134] Small thrombi are common and have no clinical significance on the placental function. Presence of fetal cells in the intervillous space and therefore in the maternal circulation is common especially in third trimester; it is estimated around 15-30% of women will have some degree of slow leakage of fetal cells into the maternal circulation.[135] It becomes significant in cases with either extremely big or multiple intervillous thrombi causing significant fetomaternal haemorrhage. Significant fetomaternal haemorrhage will have its consequences such as fetal anaemia, hydrops, end organ damage and fetal death.

Villitis of unknown aetiology (VUE)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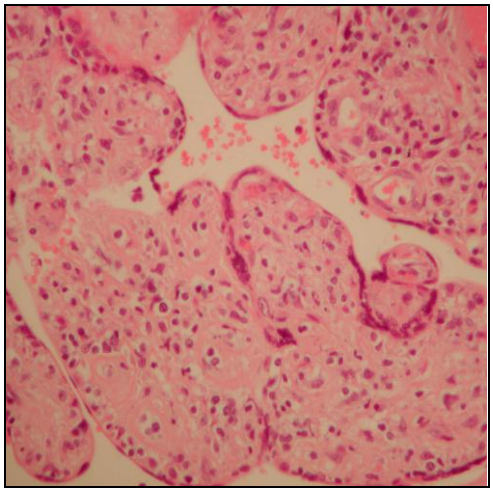
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Figures 7.2 Microscopic view of VUE (a) low power (original magnification x 40) (b) high power (original magnification x 100) (study photographs).

Presence of morphologically increased chronic inflammatory mononuclear cells and fibrinoid necrosis affecting groups of chorionic villi in absence of known clinical or histological aetiological factor/organisms (such as TORCH group, enterovirus, varicella, syphilis, syphilitic spirochetosis, and other bacterial and protozoal infections) is called as villitis of unknown etiology.

Incidence of chronic Villitis varies in different studies; probably due to the different population studied and/or different histological criteria in different units. Ethnic, environmental and socio-economic factors play an important role in the incidence of Villitis of unknown etiology. Incidence reported in United Kingdom is 13.6%[136], while it is 7% in United States[137], 14.2% in New Zealand[138] and 33.8% in Argentina.[139]

VUE could be focal or diffuse or low grade (involving less than 10 villi) or high grade (involvement of more than 10 villi). High and low grade classification is clinically helpful as it correlates more specifically to the clinical complications e.g.

High grade villitis is associated with adverse neurologic outcome.[140] Macroscopically there may be no obvious features.

In cases of severe villitis, placenta is often pale in colour and also has an irregular consolidation of the villous parenchyma.

Histologically features of VUE vary widely. There could be focal or multiple lesions. Focal or low grade patterns are usually seen in clinically asymptomatic cases while microscopic findings suggestive of severe villitis usually represents clinically significant; e.g. IUGR, fetal demise and other clinical complications. Some pathologists suggest that diagnosis of chronic villitis should not be made based on a single focus of VUE involving less than 5 villi.[141] Other histological features associated with villitis of unknown aetiology can be fetal villous thrombosis, haemorrhagic endovasculopathy, villous stromal sclerosis, Chorangiosis, decidual plasma cells, chronic chorioamnionitis, perivillous fibrin deposition and occasional presence of granulomatous focus with giant cells.

VUE may not have any clinical effects, but if severe, increase the risk of recurrent reproductive failure[142], IUGR, [138] [143-144] perinatal asphyxia[145] and adverse fetal neurological outcomes.[140]

Fetal thrombotic vasculopathy

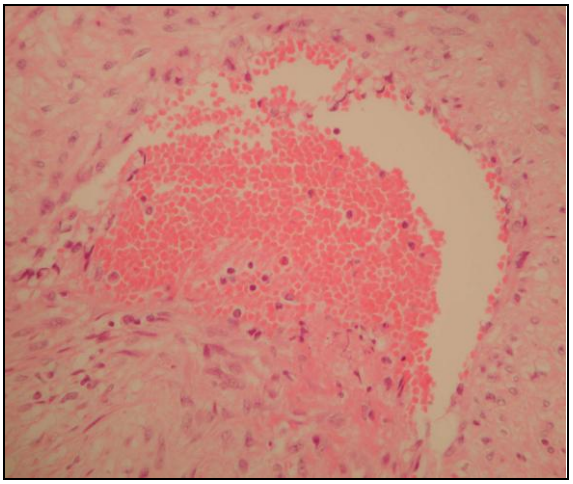


Figure 7.3 Microscopic view of fetal thrombotic vasculopathy (original magnification x 40) (study photograph).

Fetal thrombotic vasculopathy is a placental lesion due to the disturbances of fetal blood flow where there is occlusion of the chorionic or major fetal villous stem artery by a thrombus, which cuts the vascular supply of the area, causing avascular villi and resulting into the macroscopically detectable lesions.

Macroscopic appearance depends upon the age of the lesion. New lesions will be evident in the chorionic plate vessels. Vessels will be engorged; at times even the thrombi can be seen in the large vessels of chorionic plate. Once organised, lesion will be firm to palpate and later gets calcified and hard to touch. Lesions in placental parenchyma are discrete firm and pale patchy regions.

It is very easy to confuse these lesions with infarction. These pale areas are easier to identify on a fixed specimen as compared to a fresh placenta.

Microscopically appearance will depend upon the age of the lesion; early lesion will be represented by presence of thrombus in the chorionic vessels, reactive endothelial swelling in parenchyma followed by endothelial disruption with erythrocyte extravasations. Older lesion will be evident as intramural fibrin thrombus, fibroblast growth, erythrocyte fragmentation and later calcification in chorionic vessels and parenchyma.

Therefore fetal thrombotic vasculopathy is a thrombotic lesion in the fetal circulation; associated with hypercoagulable states and if extensive, can lead to high perinatal morbidity such as perinatal liver disease[146-147], Neonatal thrombosis and neonatal neurological abnormalities leading to neonatal stroke, seizures, chronic neurodevelopment deficits[148-150], IUGR, oligohydramnios, perinatal asphyxia, hydrops fetalis as well as neonatal mortality.

In this study diagnosis of this histopathological condition is made with the presence of –Downstream changes and no thrombus

Thrombus but no downstream effects

Abnormal vessel architecture with fibrin, recanalisation with multiple channels.

Massive Perivillous Fibrin Deposition

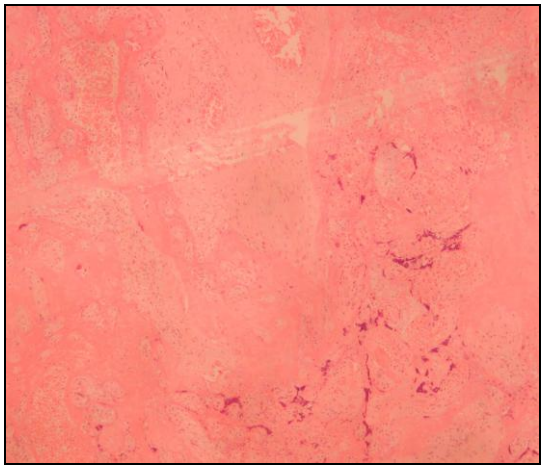


Figure 7.4 Microscopic view of massive perivillous fibrin deposition (original magnification x 40) (study photograph).

Massive perivillous fibrin deposition is an important clinical entity, which is poorly understood but has clinically significant impact on the current pregnancy as well as effects onto the future pregnancy, particularly with its recurrence tendency.

Massive perivillous fibrin deposition (MPFD) is significant deposition of fibrin material in the villous parenchyma, which is present as macroscopically extensive lesion, extending from basal to chorionic plate with trophoblast proliferation or involving more than 30% of the villous parenchyma.[117] Some degree of fibrin in the intervillous space is seen normally in nearly all term placentas as a result of turbulent intervillous blood flow.[117] This will not have any clinical significance and therefore are not pathological lesions. Authors have described it as significant if; its macroscopically large lesion and obviously extensive, extending from basal to chorionic plate with trophoblast proliferation or involving more than 30% of the villous parenchyma.[117] Others have defined it as MPFD only if the full thickness of the placenta is involved with the fibrin deposition with at least 50% villi involved as seen at least on one slide.[151]Another study has defined massive perivillous fibrin deposition in their own way that they considered significant perivillous fibrin only

where at least 20-30% of villi in the central basal part of placental parenchyma were entrapped in fibrin.[143]

It is believed that fibrin deposition entrapping up to the 30% of the villi will have no detrimental effects onto the overall function of the placenta i.e. fetus growth and its oxygenation, therefore anything affecting over 30% of villi, theoretically should be called massive or extensive, which will have clinical effects and that is the amount Pathologists and Obstetricians should take as significant and should be called as "massive" fibrin deposition.

In current study, we defined MPFD as perivillous fibrin extending from basal to chorionic plate with trophoblast proliferation. Placental pictures of these cases were then analysed macroscopically to ensure that at least 30% of total volume was involved in order to make the diagnosis.

On gross examination massive perivillous fibrin deposition are visible as irregular strands and areas of pale, greyish/yellowish coloured indurations on cut sections and clearly demarcated by the surrounding normal placental tissue. Mostly fibrin deposition is seen in the peripheral areas of the placenta. However fibrin deposition can be centrally located in the placenta. If the fibrin is mainly involving the maternal floor as in maternal floor infarct, basal plate is thick, pale and fibrin deposition will be localised to basal plate.

On histology, villi are entrapped within the fibrin material with eosinophilic infiltration and obliterate the intervillous space. Depending upon the severity and the duration of the fibrin deposition, syncytiotrophoblast and capillary endothelium of the affected villi undergo necrosis and completely disappear. Trophoblastic basement membrane is thickened. With progressive lesion, fibrosis of the stroma is seen and ultimately obliteration of the villous vessel is seen. In the process syncytiotrophoblast disappears but cytotrophoblastic cells proliferates and grow into the surrounding fibrin and such cells sometimes detach from the parent villus and appear as isolated cell mass in the fibrin.

Clinical implications of massive MPFD has been observed in cases of miscarriages[152], recurrent miscarriages[153], pre term labour, IUGR[151 154-156], IUFD.[154-156]

Chorangiosis

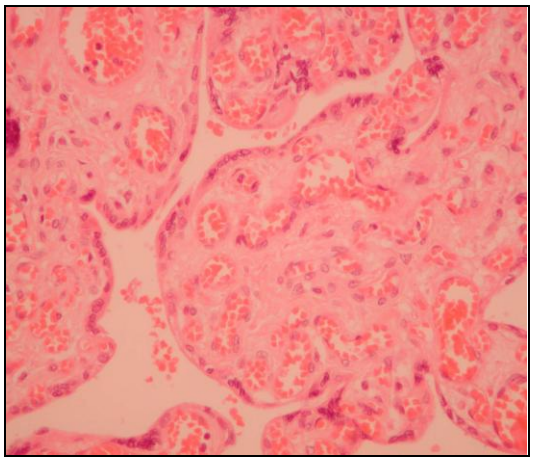


Figure 7.5 Microscopic view of Chorangiosis (original magnification x 100) (study photograph).

Defined as a vascular lesion where there is vascular hyperplasia of the chorionic villi, defined as the occurrence of 10 or more villi with 10 or more capillaries in 10 or lower power microscopic fields.

Chorangioma

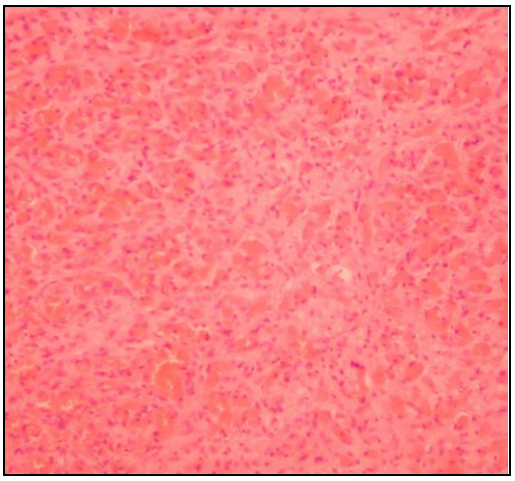


Figure 7.6 Microscopic view of chorangioma (original magnification x 40) (study photograph).

Chorangioma is defined as well circumscribed, nodular lesion composed of vascular channels, stromal cells and surrounded by trophoblasts.

Features of maternal pre-eclampsia

Included cases with fully developed atherosis only.

Chronic histiocytic intervillousitis

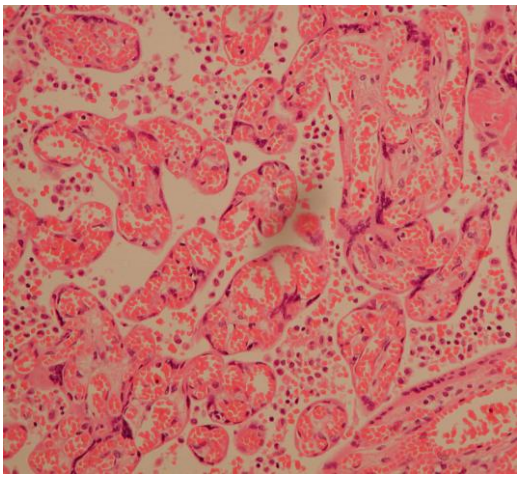


Figure 7.7 Microscopic view of chronic histiocytic intervillousitis (original magnification x 100) (study photograph).

Marked mononuclear inflammatory cell infiltrate with associated fibrin deposition in the intervillous space without associated villitis or chorioamnionitis.[117]

Features of acute abruption

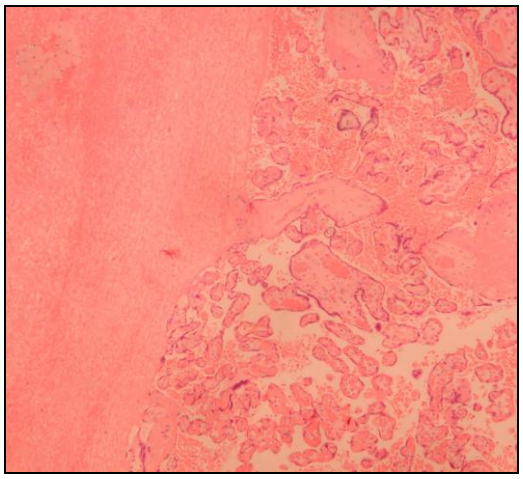


Figure 7.8 Microscopic view of retroplacental haemorrhage (original magnification x 40) (study photograph).

Features of acute abruption include the presence of significant retroplacental haematoma or haemorrhage or clot with signs of compression of the basal plate and congestion.

Table 7.1 summarises the diagnostic criteria for all predefined histological categories in the study.

Diagnosis	**Diagnostic criteria**
Ascending genital tract infection	Any combination of: neutrophils in the fetal membranes, accumulating at the choriodecidual junction or umbilical cord.
Chronic placental underperfusion	Small, hypovascular chorionic villi with increased syncytial knots, placental infarction >1cm (non-peripheral) and/or atherosis.
Intervillous Thrombus	Localised, nodular and circumscribed old or recent thrombi within the intervillous space.
Villitis of unknown aetiology	Morphologically increased chronic inflammatory cells affecting groups of chorionic villi in the absence of known clinical or histological aetiological factor/organisms (such as TORCH group, enterovirus, varicella, syphilis, syphilitic spirochetosis, and other bacterial and protozoal infections)
Fetal thrombotic vasculopathy	Lesions of the placenta due to occlusion of the chorionic or major fetal villous stem artery by a thrombus, causing avascular distal villi
Chorangiosis	10 or more villi with 10 or more capillaries / villous cross section in 10 or lower power microscopic fields.
Maternal Pre-eclampsia	Included cases with fully developed atherosis only
Massive perivillous fibrin deposition	Significant deposition of perivillous fibrin involving more than 50% of the villous parenchyma
Chorangioma	A well circumscribed, nodular lesion composed of vascular channels, stromal cells and surrounded by trophoblasts.
Chronic histiocytic intervillositis	Marked mononuclear histiocytic inflammatory cell infiltrate with associated fibrin deposition in the intervillous space without associated villitis or chorioamnionitis
Acute abruption	The presence of significant retroplacental haematoma or haemorrhage or clot with features of compression of the overlying parenchyma.

Table 7.1 summary of the diagnostic criteria for predefined histological categories.

7.3 STATISTICAL ANALYSIS

Prevalence of the predefined histological abnormalities was noted in the unselected population. Comparison of incidence of common histological groups was performed between the groups non-affected and affected with PET, PIH, GDM and SGA, using chi square test. A p value of ≤ 0.05 was considered significant.

Normality of distribution was checked for cord coiling index, centrality index and eccentricity index for the reference population, ascending genital tract infection, chronic placental underperfusion, intervillous thrombus and villitis of unknown aetiology groups, by analysing the spread of the distribution in each frequency histogram. As the variables coiling index, cord centrality index and eccentricity showed an approximate normal distribution, a parametric test (T-test) was performed to compare the means of affected with non-affected cases. A p value of <0.05 was considered significant.

7.4 RESULTS

Table 7.2 describes the baseline maternal demographics of the study population including the common obstetric outcome groups in the population.

N	1159
Maternal Age ± SD	30.9 ± 5.73
% Nulliparity (n)	47.6 (552)
% Smokers (n)	9.3 (108)
% Caucasians (n)	90.7 (1051)
% PET (n)	2.5 (29)
% PIH (n)	2.6 (30)
% Essential Hypertension (n)	0.4 (5)
% GDM (n)	4 (46)
% IDDM (n)	0.3 (3)
% SGA <10 Centile (n)	9.4 (109)
Mean gestation age at delivery ± SD	39.5 ± 1.49
% Female (n)	46.7 (541)
% Male (n)	53.3 (618)

Table 7.2 Demographics of the reference population.

The placenta and umbilical cord morphology in unselected population is described in chapter 4. Briefly; table 7.3 describes the statistics of the major macroscopic indices analysed in the study. Manual measurements were available for 1141 cases and digital for 888 cases.

	N	**Mean (range) ± SD**
Coiling Index	1141	0.20 (0-1) ± 0.10
Cord Centrality Index	888	0.36 (0.02-1.01) ± 0.21
Eccentricity	888	0.49 (0-0.91) ± 0.18

Table 7.3 Major morphological indices in the reference population.

Histology analysis was performed in 1125 cases, as in 34 placentas of the study, histology results were not available. Table 7.4 outlines the incidence of all histological lesions in different clinical outcome groups as well as in all unselected reference population. The majority of placentas (72.4%), as expected, did not show any histological abnormality in reference population. However; almost 30% placental histology revealed histological abnormalities.

Predefined histological features	Reference Population % (n=1125)	PET % (n=24)	PIH % (n=27)	GDM % (n=45)	SGA % (n=107)
Normal histology	72.4(814)	79.1(19)	66.6(18)	80(36)	74.8(80)
Ascending genital tract infection	10.9(123)	8.3(2)	11.1(3)	4.4(2)	10.3(11)
Chronic placental underperfusion	7.2(81)	4.2(1)	14.8(4)	2.2(1)	9.3(9)
Intervillous thrombus	4.7(53)	8.3(2)	3.7(1)	6.8(3)	-
Villitis of unknown aetiology	3.7(42)	-	11.1(3)	-	3.7(4)
Fetal Thrombotic Vasculopathy	1.0(11)	-	-	2.2(1)	0.9(1)
Chorangiosis	0.8(9)	-	-	2.2(1)	-
Maternal Pre-eclampsia	0.6(7)	-	-	2.2(1)	0.9(1)
Massive perivillous fibrin deposition	0.4(5)	4.2(1)	-	-	1.9(2)
Chorangioma	0.4(4)	4.2(1)	-	-	0.9(1)
Chronic Histiocytic Intervillousitis	0.3(3)	-	-	-	0.9(1)
Acute Abruption	0.1(1)	-	-	-	-

*****Total % is >100%, as some placentas had more than 1 histological lesion and each histological lesion has been counted separately. P >0.05 in all the groups

Table 7.4 Incidence of predefined histological lesions in reference population and common obstetric outcome groups.

Amongst common obstetric outcome groups of PET, PIH, GDM and SGA, the normal histology was revealed in 79.1%, 66.6%, 80% and 74.8% respectively.

Coiling index of normal histology cases (n=806) was statistically significantly lower compared to cases with abnormal histology (n=308, p=0.02). Coiling index of those with ascending genital tract infection and chronic placental underperfusion were not significantly different whilst cases with intervillous thrombus and villitis of unknown aetiology, showed more coiling compared to non-affected cases (p=0.024 and 0.009 respectively).

Cord centrality index-There was no significant difference noted in case of centrality index between any of the groups

Eccentricity- There was no difference noted in the values of eccentricity between any groups.

Table 7.5 describes the comparative morphological indices in the reference population and in the most common histological lesions of the study versus non-affected cases in each category.

	Coiling Index (SD)	p value (comparison with non-affected cases)	Centrality Index (SD)	p value (comparison with non-affected cases)	Eccentricity (SD)	p value (comparison with non-affected cases)
Reference population	0.20±0.10 (n=1141)	NA	0.36±0.21 (n=888)	NA	0.49±0.18 (n=888)	NA
Normal histology	0.20±0.10 (n=806)	NA	0.36±0.21 (n=615)	NA	0.48±0.18 (n=615)	NA
Ascending genital tract infection	0.20±0.09 (n=122)	NS	0.37±0.23 (n=98)	NS	0.52±0.17 (n=98)	0.07
Chronic placental underperfusion	0.21±0.11 (n=80)	NS	0.32±0.21 (n=68)	NS	0.48±0.19 (n=68))	NS
IVT	0.23±0.10 (n=53)	0.02	0.34±0.20 (n=33)	NS	0.54± 0.16 (n=33)	0.08
VUE	0.24±0.10 (n=39)	0.009	0.35±0.23 (n=33)	NS	0.47±0.15 (n=33)	NS

Table 7.5 Comparative analysis of the morphological indices of the placenta in reference population and abnormal histology groups.

NA-Not applicable NS-Non significant.

7.5 DISCUSSION

This chapter has described the pattern of histology in common obstetric outcome groups and unselected population. Strict criteria were followed for the diagnosis of histological abnormalities. Incidence of various histological pathologies varies widely in literature; one of the reasons being lack of standard definitions for these lesions and every researcher has defined the lesions with their definitions and therefore literature lacks uniformity on the subject.

There have always been a debate whether the detailed examination of every placenta by the pathologists should be a routine component of obstetric-neonatal care; group in favour of this option believe that this helps identifying placental pathology especially in cases of adverse perinatal outcome, which further helps in the management of the pregnancy and more so for the future pregnancy [18], however, the histological examination and perinatal outcome in most pregnancies is normal, for example in the current study, the frequency of histologically normal examination was observed in over 70% of cases. Therefore this decision should be left on to the individual unit to decide their own protocol of routine examination of each placenta by the pathologists, based on their manpower and financial resources.

For the diagnosis of chorioamnionitis, clinical picture is not a reliable tool and it has been proven by many studies that histological diagnosis of chorioamnionitis is a gold standard. In the current study interestingly there were no cases of clinically diagnosed chorioamnionitis or any other antenatal sepsis and yet there were significant number (123-10.9%) of cases of ascending genital tract infection. That further enforces that there is very poor correlation between clinical and histological infection. However, the limitation of this study is that the information for clinical suspension of chorioamnionitis was collected only from the protos database of the department, not a note trawl. We don't have separate data on clinical markers such as WCC, CRP, prolonged rupture of membranes, use of antibiotics in labour.

Incidence of Inter villous thrombus in this study was 4.7% in unselected pregnancies, while literature has reported incidence varying from 3-50% in full term uncomplicated pregnancies.[132-133] This wide variation in the incidence may well again be due to the lack of standard definition and protocols as well as the population studied.

Villitis of unknown etiology is another major histopathological entity with high degree of inter-observer variation.[157] As there is no standard criteria for its diagnosis, which makes it even more difficult to understand. It is important to understand VUE in a better way, as it has been associated with IUGR and with its tendency of recurrence in the following pregnancy and that too with increasing severity. Incidence of VUE in this study was much lower (3.7%), this is even lower than the incidence noted in one of the historic study with British population (13.6%)[136]

The incidence of fetal thrombotic vasculopathy in the current study was only 1% of all the histology cases, which was significantly lower than reported in other series.[150] [158]

Incidence of massive perivillous fibrin deposition is relatively low, quoted as low as 0.028%[155] to 0.09%.[154] Incidence of MPFD was very low to draw any meaningful conclusion. However it is important to understand as it has important clinical effects and also has a recurrence rate of 10-50% in subsequent pregnancies.[151] [155]

It has not been clear if the histological lesions present in the placentas from obstetric outcome groups are any different from the rest of the unselected population. Many histological lesions are documented in pregnancies with these obstetric outcome groups but most, if not all of them, are not specific to any of the outcome group of the pregnancy.

This chapter aimed to identify if the frequency and type of predefined histological lesions observed in placentas from the common obstetric outcome group are any different from unselected reference population.

Pre-eclampsia/Pregnancy induced hypertension

The macroscopic and histological changes in the placenta from cases with PET and PIH may be characteristic but may not be there even in severely affected cases. Frequency of ascending genital tract infection features in PIH, were similar to that found in a recent study[159], however the frequency of IVT (3.7% versus 18.9%) and Chorangiosis (0% versus 10.9%) was much less than observed in the similar study. A slightly higher proportion of IVT cases were observed in pre-eclampsia (8.3%). Features suggestive of chronic placental underperfusion were also important feature noted in cases of both pre-eclampsia (4.2%) as well as PIH (14.8%).

Villitis of unknown aetiology is one of the most common histological lesions identified in term placentas and is associated with adverse perinatal outcome especially with IUGR[143-144]. The incidence of VUE has been reported in up to 17% in term cases of pre-eclampsia[160], though we did not observe any cases of VUE in pre-eclampsia subset, however VUE was noted in 11.1% cases of pregnancy induced hypertension. Whether it has anything to do with the pathophysiology in PIH or not, probably needs further exploration. This difference could either be due to the high degree of inter-observer variation or due to the population studied. Most studies have been based on the adverse outcome of the pregnancy.

Retroplacental haematoma can be seen in up to 12-15% cases of pre-eclampsia but we did not notice any case in our series. Pre-eclampsia is a hypercoagulable state, where there is intravascular coagulation and micro thrombosis affecting multiple organs. Utero-placental impairment occurs in cases of pre-eclampsia. There is increased antifibrinolytic activity in hypertensive disorders of pregnancy. Total effect of increased hypercoagulation and antifibrinolytic activity is believed to contribute toward the increased deposition of fibrin. Therefore one might expect to see significant perivillous fibrin deposition with all the cases of severe pre-eclampsia but that doesn't seems to be the case. Only a few studies have shown this association[161] while most authors did not find this finding any different from normal population.

In the current series, only one case of MPFD (4.2%) was found in PET but none in PIH. There were no cases of fetal thrombotic vasculopathy in either pre-eclampsia or PIH, although some studies have documented its incidence in term pre-eclampsia as 5%.[160]

Gestational Diabetes Mellitus

It is thought that the histological features in gestational diabetes are similar to those of pre existing diabetes, however less pronounced and reduced in frequency in cases of GDM as compared to that of overt diabetes.[162 163] The pathological findings of the placenta do not predict the severity of the disease, neither relate to the duration of the disease especially with pre existing diabetes.[163] Placentas from diabetic pregnancies show increased incidence of vascular pathological changes. Chorangiosis is an example of such findings, reported to be 40% in GDM.[164] This study examined the histological lesions specifically in placentas from known diabetic group while our study has quoted the incidence in an unselected population. The incidence of fetal thrombotic vasculopathy was noted in GDM subset as 2.2%, again indicating the hypercoagulable state in diabetes.

Small for Gestational Age

Histological lesions found in SGA were features of ascending genital tract infection (10.3%), chronic placental underperfusion (9.3%), VUE (3.7%), features of maternal pre-eclampsia (0.9%), MPFD (1.9%), Chorangioma (0.9%), fetal thrombotic vasculopathy (0.9%) and chronic histiocytic intervillousitis (0.9%). These are the expected histological features secondary to the maternal vascular compromise leading to placental ischemia, which one would expect in cases of IUGR. A recent study found the most common histological lesions in IUGR (birth weight<10th percentile clinically) was Chorangiosis (39%)[160], however this study did not show any case of Chorangiosis in SGA group.

Comparing the histological lesions in the above obstetric outcome groups to that of the unselected reference group, results were as follows-

Most placentas, as expected, showed normal histology in PET (79.1%), PIH (66.6%), GDM (80%) and SGA (74.8 %). These results were not different from the non-affected cases and unselected reference population (72.4%). There was no difference in the frequency of ascending genital tract infection in the common obstetric outcome groups as compared to the unselected population.

In the current study there were no IVT observed in SGA, while in cases with PET, PIH and GDM, difference was not significant.

Reports on the frequency of villitis of unknown aetiology in cases of pre-eclampsia and other hypertensive disorders of pregnancy compared to the normotensive pregnancies have been mixed; some studies found the incidence higher [165-166], while others did not find any difference.[167] Frequency of VUE was higher (11.1%) in cases with PIH but interestingly there were no cases observed in PET.

Small for gestational age group placentas also showed similar frequency of VUE (3.7 %) to that of unselected reference population (3.7%).

The other abnormal histology cases were observed in very small number to perform any meaningful comparison to the reference population

In summary most cases of placentas from unselected population and even common obstetric outcome groups are histologically normal. There was no significant difference in the frequency of the predefined histological lesions in obstetric outcome groups as compared to that of the reference population. In the common obstetric outcome groups, histological examination may be normal even in the presence of a severe disease.

Further, this chapter also reports a statistically significant, but small, difference in the cord coiling index in cases with normal compared to abnormal histology (p=0.02), in particular, cases with IVT and VUE show relative hypercoiling. The clinical significance of this finding remains uncertain and it is difficult to postulate a plausible mechanism whereby both of these unrelated histological features are related to cord coiling.

There was no difference in cord centrality index and eccentricity between the common histological groups. We therefore propose that cord centrality and placental shape have no significant effect on these histological lesions.

Certain common histological lesions are possibly associated with increased umbilical cord coiling but placental macroscopic features such as shape, and cord insertion have no association with histological abnormalities.

Macroscopic abnormalities of placental shape and cord insertion cannot predict the presence or absence of histological features and are of limited clinical significance.

Most histological abnormalities are either studied in uncomplicated pregnancies or in complicated pregnancies but in best of my knowledge, no one has presented the baseline histologic profile in unselected reference population. This reference range of histological conditions can be used as a baseline for future studies based on the same principals.

CHAPTER 8 : MORPHOLOGY AND HISTOLOGY OF THE PLACENTA IN INFANTS ADMITTED TO THE NEONATAL UNIT COMPARED TO UNSELECTED PREGNANCIES.

Summary Points:

- This study described the placental morphological indices and incidence of predefined histological lesions in cases where infants were admitted to the Neonatal Unit.
- Morphological indices were not different in the placentas from infants admitted to the Neonatal Unit.
- Histological features of ascending genital tract infection were more frequent in the placentas from infants admitted to the Neonatal Unit.
- There was no difference in the incidence of all other predefined histological lesions of the placenta in infants admitted to the Neonatal Unit.

This chapter is based on:

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** Macroscopic and Histological Features of Placentas at 34-43 weeks' Gestation and their association with infant admission to the Neonatal Unit- submitted as short communication, **BJOG**. April 2011.

Pathak S, Sebire NJ**,** Jessop F**,** Hackett G**,** Murdoch E**,** Hook E**,** Lees C**.** Placental morphological and histological features: relevance to fetal distress. Paper in preparation.

8.1 INTRODUCTON

Examination of the placenta may be useful investigation to the clinician to determine the underlying mechanism which has possibly resulted in perinatal morbidity and even mortality. If any placental or umbilical cord lesion is found, it could guide the clinicians for the future pregnancy in terms of further investigations required, pre-pregnancy counselling and care for the future pregnancy.[168-169]

Despite growing interest in the morphology and histology of placentas, no large studies exist, which have specifically investigated placental features in infants particularly those admitted to the Neonatal Unit. Therefore it is not clear if the placenta from infants who were admitted to the Neonatal Unit is any different from that of the reference population.

Guidelines suggest that placentas from pre tem deliveries and adverse perinatal outcome should be sent for the detailed histological examination.[16-17] However, it is not clear whether these placental examinations can predict specific neonatal complications, or provides information useful for the management of the admitted neonates.

This chapter aimed to define the macroscopic indices and frequency of histological lesions in the admitted cohort and compare those to that of the non admitted group. Therefore we aimed to address the question as to whether it is important to perform detailed macroscopic examination and histology of the placentas for all neonates admitted to the Neonatal intensive care unit.

8.2 METHODS

For this study, cohorts of neonates admitted in the Neonatal Unit from the prospectively recruited cohort of 1159 women (chapter 2) have been studied. Neonates were admitted either directly from delivery unit or from the post natal wards, while the women were still admitted in the unit after delivery. 71 neonates were admitted to the Neonatal Unit in Rosie Hospital, Cambridge during the study period 2007-2008.

Placentas from those neonates admitted to the Neonatal Unit (includes NICU+SCBU) is referred to as "admitted cohort", while the remainder of the population is referred to as "non admitted cohort". The reference or unselected population included both admitted as well as non admitted groups.

Manual Measurements

Recorded variables from umbilical cord examinations included Umbilical cord length, number of coiling, direction of coiling, number of vessels and the insertion of umbilical cord onto the chorionic plate.

Manual measurements and the indices derived from them were placental weight, length of axes of placenta, cord coiling index, placental circumference.

Manual measurements were available in 68 cases of infants admitted to the Neonatal Unit.

Digital measurements

Digital analysis of the placenta is described in chapter 2. Measurements and indices derived digitally were length of axes of placenta, distance of cord insertion from the centre, cord centrality index, eccentricity, and placental circumference.

Digital measurements were available in 51 cases of neonates admitted to the Neonatal Unit.

Main macroscopic indices studied in admitted cohort and compared to that of reference population are **coiling index, cord centrality index and eccentricity**. Derivation of these indices is described in chapter 2.

Histology of the placenta

Predefined histological lesions were examined; the criteria and definitions of these histological lesions are detailed in chapter 7. Briefly; the histological categories were, no significant histological abnormality, features of ascending genital tract infection, features of chronic placental underperfusion, intervillous thrombus, villitis of unknown aetiology, fetal thrombotic vasculopathy, Chorangiosis, features of maternal pre-eclampsia, massive perivillous fibrin deposition, chorangioma, chronic histiocytic intervillousitis, and features of acute abruption.

Out of 71 placentas from infants admitted to the Neonatal Unit, 4 histological examinations findings were not available, therefore histology for 67 placentas were analysed and compared to the 1058 placentas from non admitted cohort. The frequency of each lesion was individually assessed in the admitted cohort and compared to the same lesion present in non admitted group.

8.3 STATISTICAL ANALYSIS

Descriptive statistics of manual and digital measurements of the umbilical cord and placenta was performed. Frequency histograms were plotted for coiling index, cord centrality index and eccentricity, showing normal distribution. Normality was also confirmed with Q-Q plots to see the difference between observed and expected values of each variable.

Though the macroscopic variables showed normal distribution, still due to the smaller sample size, we used a non parametric test; the Mann Whitney U test to compare the difference of means (medians) of coiling index, centrality index and eccentricity to that of rest of the non admitted population. A p value of ≤ 0.05 was considered significant.

Histological lesions were defined in cases, where infants were admitted to the Neonatal Unit and the frequency of each histological lesion was compared to that of the non admitted cohort.

8.4 RESULTS

Table 8.1 describes maternal and fetal characteristics for those babies admitted to the Neonatal Unit. The obstetric outcome groups considered were pre-eclampsia, pregnancy induced hypertension, gestational diabetes and small for gestational age.

Mean (range) ± SD or % (n)	Neonates admitted to Neonatal Unit (n=71)	Non admitted (n=1088)
Maternal age	31 ± 5.64	30.8 ± 5.74
% Caucasian (n)	91.4 (64)	90.7 (987)
% nulliparity (n)	54.9 (39)	47.2 (513)
% Smokers >5 cigarettes per day (n)	12.7 (9)	9.1 (99)
% Pre-eclampsia (n)	8.5 (6)	2.1 (23)
% Pregnancy induced hypertension (n)	1.4 (1)	2.7 (29)
% GDM (n)	5.6 (4)	3.9 (42)
% SGA (n)	19.7 (14)	8.7 (95)
% Induced onset of labour (n)	26.8 (19)	19 (207)
% Emergency Caesarean Section (n)	21.1 (15)	10.9 (119)
Mean gestation at delivery (Weeks)	38.3 ± 2.78	39.5 ± 1.33
Birth weight	3192 ± 812	3498 ± 479
% Males (n)	47.9 (34)	53.7 (584)
% Females (n)	52.1 (37)	46.3 (504)
% Neonates requiring immediate resuscitation (n)	31 (22)	1.5 (16)

Table 8.1 Demographic population of infants admitted to the Neonatal Unit.

22 neonates (31%) required resuscitation immediately after they were born. 4 (5.6%) of the neonates were resuscitated with facial oxygen and stimulus only, while 13 (18.3%) required face mask ventilation, 4 neonates (5.6%) were intubated and 1 needed cardiac massage. Table 9.2 describes the measurements of placenta and umbilical cord taken manually and digitally and the indices derived from these.

	Manual (n=68) Mean ± SD		**Digital (n=51) Mean ± SD**
Cord length	40.40 ± 14.19		
Cord coils	8.09 ± .59	**Distance of cord insertion from centre**	3.24 ± 1.87
Coiling index	0.20 ± 0.09	**Cord centrality index**	0.35 ± 0.21
Placenta weight	478 ± 116	**Eccentricity**	0.50 ± 0.16
Long axis	20.37 ± 2.70	**Long axis**	18.91 ± 2.46
Short axis	17.22 ± 2.63	**Short axis**	15.88 ±1.74
Circumference	59.01 ± 7.38	**Circumference**	54.61 ± 5.84

Table 8.2 Manual and digital measurements of placenta and cord and in infants admitted to the Neonatal Unit.

Table 8.3 describes qualitatively different types of umbilical cord insertion onto the chorionic plate. Frequency of each type of insertion was compared to that of the non admitted neonatal and unselected population. Of the clinical importance: marginal and velamentous cord insertion were not any different in admitted cohort compared to that of non admitted cohort (Marginal-7 versus 6.3%, velamentous-1 versus 0.6%).

	Admitted group% (n=71)	Non admitted% (n=1088)	Ref Population% (n=1159)
Central	23.9	22.6	22.7
Paracentral	56.3	61	60.4
Marginal	9.9	6.3	7.0
Velamentous	1.4	0.6	0.6
Missing	8.5	9.4	9.3

Table 8.3 Qualitatively described cord insertions in infants admitted, non admitted to the Neonatal Unit and reference population.

Table 8.4 compares the macroscopic indices in the Neonatal Unit admitted cohort versus non admitted group.

	Admitted to Neonatal Unit	n	Mean ± SD	p value
Coiling Index	Yes	68	0.20 ± .09	NS
	No	1073	0.20 ± .10	
Cord Centrality Index	Yes	51	0.35 ± .21	NS
	No	837	0.36 ± .21	
Eccentricity	Yes	51	0.50 ± .16	NS
	No	837	0.49 ± .18	

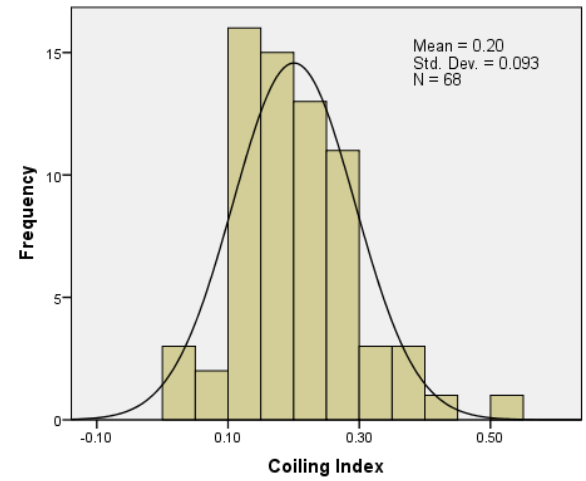
*NS-Non Significant

Table 8.4 Morphological indices in the infants admitted versus not admitted to the Neonatal Unit.

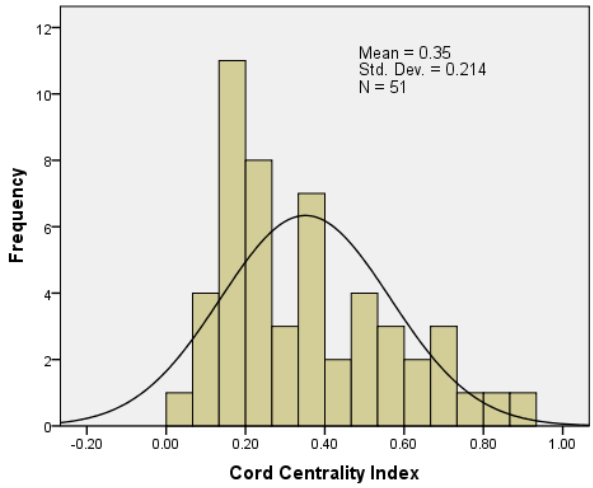
78.9% of cords showed left coiling, similar to the reference population (77.9%). Cord coiling index ranged from a minimum value of 0.00 to a maximum of 0.50 with a mean value of 0.20 ± 0.09. Frequency histograms and Q-Q plot revealed an approximate normal distribution for coiling index. Figure 8.1 shows the frequency histogram of coiling index in admitted cohort. A Mann-Whitney U test revealed no significant difference in the coiling index for admitted (n=68, mean=0.20, median=0.18) versus non admitted group (n=1073, mean=0.20, median=0.19) with 2 tailed p value of 0.82.

The Cord centrality index ranged from 0.05 to 0.9 with the mean of 0.35 ± 0.21. Frequency histograms and Q-Q plot of cord centrality index indicated an approximate normal distribution. Figure 8.1 shows the frequency histograms of cord centrality index in the admitted group. A Mann-Whitney U test revealed no significant difference in the cord centrality index for admitted cohort (n= 51, mean=0.35, median=0.31) versus non admitted cohort (n=837, mean=0.36, median=0.32) with 2 tailed p value of 0.67.

The placental eccentricity index ranged from 0.00 (circular shape) to 0.79 (Highly elliptical shape) with a mean value of 0.50 ± 0.16. The frequency histograms and Q-Q plot indicated a normal distribution. Figure 8.1 shows the frequency histograms of eccentricity in admitted group. A Mann-Whitney U test revealed no significant difference in the eccentricity of admitted cohort (n=51, mean=0.50, median=0.51) versus non admitted group (n=837, mean=0.49, median=0.51) with p value of 0.61.



a



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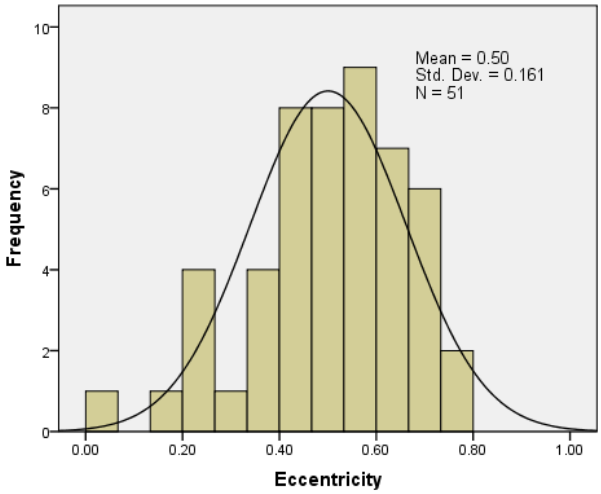

c

Figure 8.1 (a-c) Frequency histograms of morphological indices in infants admitted to the Neonatal Unit.

Table 8.5 illustrates the frequency of predefined abnormal histology in placentas of admitted infants and compared to the non admitted population. 65.7% cases of placentas from admitted cohort showed normal histology of placenta, which was slightly less than that of non admitted population (72.8%). Features of ascending genital tract infection were more frequent in admitted infants (17.9%) as compared to the non admitted group (17.9% versus 10.5%, p=0.04) but the frequency of other lesions was not significantly different between the groups. There was no significant difference in the incidence of chronic placental underperfusion (6 vs 7.3%), intervillous thrombus (6% versus 4.6%), fetal thrombotic vasculopathy (1.5 versus 0.9%) and VUE (4.5% vs 3.7%). Chorangiosis was noted only in 1 case of admitted group. There were no cases of MPFD, chorangioma, chronic histiocytic intervillousitis, and acute abruption observed in the admitted neonate's placentas.

Histological features	%Infants admitted to NICU (n=67)*	%Non-admitted group (n=1058)*	%Reference population (n=1125)*
Normal histology	65.7 (44)	72.8 (770)	72.4 (814)
Ascending genital tract infection	17.9 (12)	10.5 (111)	10.9 (123)
Chronic placental underperfusion	6 (4)	7.3 (77)	7.2 (81)
Intervillous thrombus	6 (4)	4.6 (49)	4.7 (53)
Villitis of unknown aetiology	4.5 (3)	3.7 (39)	3.7 (42)
Fetal thrombotic vasculopathy	1.5 (1)	0.9 (10)	1.0 (11)
Chorangiosis	1.5 (1)	0.8 (8)	0.8 (9)
Maternal Pre-eclampsia	-	0.7 (7)	0.6 (7)
Massive perivillous fibrin deposition	-	0.5 (5)	0.4 (5)
Chorangioma	-	0.4 (4)	0.4 (4)
Chronic histiocytic intervillousitis	-	0.3 (3)	0.3 (3)
Acute abruption	-	0.1 (1)	0.1 (1)

*Total number is > n (>100%), as some placentas had more than 1 histological lesion and each histological lesion has been counted separately.

Table 8.5 Incidence of predefined histological categories in admitted, non-admitted and reference population.

8.5 DISCUSSSION

The studies detailed in this chapter to evaluate whether there was any difference in placental morphologic and histologic characteristics in neonates admitted to the Neonatal Unit as compared to those where neonates were not admitted to the Neonatal Unit.

Several studies have emerged in recent years on umbilical cord coiling index in normal as well as in complicated pregnancies and these studies have made an attempt to link cord coiling to the pregnancy outcome.[5-8 52] However, there still remains the uncertainty as regards the significance of abnormal cord coiling. In this study umbilical cord coiling and the coiling index did not show any significant difference in the admitted group as opposed to that of the non-admitted group (0.20 versus 0.20, p=0.82).

Abnormal cord insertion (in particular marginal and velamentous) has been linked to the adverse perinatal outcome. To avoid subjective bias, results of this study were based on quantitative analysis of cord insertion, using the index; cord centrality index, indicating how far the cord insertion was from the centre or the chorionic plate margin. In this study there was no significant different between the cord centrality index of admitted versus non admitted group (0.35 versus 0.36, p= 0.67)

The placental surface area has been linked to the development of hypertension in later life.[93] Few studies have even analysed the chorionic plate shape quantitatively.[10] In the current study, there was no difference in the quantitatively defined placental shape, eccentricity index in the admitted group to that of non admitted group (0.50 versus 0.49, p=0.61)

Therefore all macroscopic indices, coiling index, centrality and eccentricity index showed no difference in two groups. 34.3% of newborns admitted to the Neonatal Unit showed abnormal histology of the placentas compared to 27.6% of those not admitted.

Amongst the histological categories, the incidence of ascending genital tract infection was significantly greater in admitted group as compared to the non admitted group (17.9% versus 10.5%, p=0.04). Apart from an excess of histological evidence of ascending genital tract infection, there were also no significant differences in other placental histological findings between these groups. This is not entirely surprising; half of the infants admitted to the Neonatal Unit were for indications such as grunting/mild respiratory distress, hypoglycaemia and poor feeding. These clinical observations are almost always unrelated to fetal pathology, hence would not be expected to be associated with placental abnormalities.

To summarise, this study did not find any major morphological or histological differences in the examination of the placentas of Neonates admitted to the Neonatal Unit. Therefore we would not support the recommendation that every placenta from the admitted cases should have a detailed pathological examination in term or near term cases, however medico-legal aspect of these cases cannot be ignored where it may well be one of the legal requirements to have thorough examination of the placenta.

CHAPTER 9 : CLINICAL OUTCOMES IN RELATION TO PLACENTAL HISTOLOGY

Summary Points:

- This study describes pregnancy and neonatal outcome in cases with abnormal placental histology.
- Specific clinical outcomes are compared.
- The key findings were that there was no difference in the clinical outcome between the histological groups.

9.1 INTRODUCTION

In the previous chapter (chapter 8), gross and histological characteristics of placenta in infants admitted to the Neonatal unit were compared to those placenta where infants were not admitted to the Neonatal unit. In this chapter a different relationship is explored i.e. whether abnormal histology of the placenta has any bearing on the clinical and neonatal outcome? This area is dominated with many controversies, partly due to the relatively deficit in communication between the pathologist and obstetricians and also due to high degree of inter-observer variability among pathologists in defining histological groups.

The placental pathology has been associated with adverse neonatal outcome especially neurodevelopment outcome, even in normally grown term infants.[140 170] For example; chorioamnionitis has been linked to the cerebral palsy as its one of the major predisposing factor, only about 10% cases of cerebral palsy have been attributed to intra partum events, the majority of the cases thought to be linked to events earlier on in the pregnancy.

Placental injury or abnormal histology may be present in the form of intra uterine growth restriction, abnormal fetal weight, and low birth weight placenta weight ratio during antenatal and neonatal period.

The aim of the studies in this chapter was to identify specific pregnancy and neonatal outcome in the most common placental histological categories observed in the study). This compared the pregnancy and neonatal outcome in all abnormal histology cases to that of normal histology cases. Clinical outcome were examined in most common placenta pathology categories of: ascending genital tract infection, chronic placental underperfusion, intervillous thrombus, villitis of unknown aetiology and fetal thrombotic vasculopathy.

9.2 METHODS

As previously described (chapter 2 &7), of 1159 cases, histology was available in 1125 cases. Abnormal placenta histology was noted in 313 cases while remaining 812 cases showed normal placenta histology. Individual placenta pathology was noted in 313 pathology cases. How these pathological categories are defined, is explained in chapter 7.

For this chapter specifically following information were observed.

Maternal factors recorded were maternal age, parity, gestational age, incidence of common obstetric outcome pregnancies, onset of labour, CTG abnormalities, mode of delivery especially incidence of emergency caesarean sections.

Neonatal factors recorded were neonatal outcome, birth weight, gender of the neonate, number of admissions to the neonatal unit, Apgar score and cord blood pH (arterial and venous with base excess). Neonates requiring resuscitation was also observed. Neonates, admitted to the neonatal unit, were observed for number of admission days in the neonatal and especial care unit, findings of cranial ultrasound, presence of hydrocephalus, seizures, retinopathy of prematurity, necrotising enterocolitis, patent ductus arteriosus, thrombocytopenia, anaemia, infection proven by laboratory findings, any evidence of renal impairment and finally if the baby was discharged home or transferred out to another unit for longer stay in the Hospital.

9.3 STATISTICS

Data from all abnormal placental histology cases was compared to the data from normal histology. Individual placental pathology cases were also compared to that of the normal histology. To explore the relationship between two categorical variables, Chi-square test for independence was used. Means were compared of the continuous variables using t-test. 2 tailed significance value ≤ 0.05 was used as statistically significant. Numerical variables were expressed as mean ± SD.

9.4 RESULTS

Out of total 1159 histology cases, data was available for 1125 cases. Normal histology was observed in a majority of cases 812cases (72.2%), while 313 cases (27.8%) showed abnormal placenta histology.

Table 9.1 represents the predefined histologic categories in cases with abnormal placenta histology.

Abnormal histology	% (n-313)*
Ascending genital tract infection	39.3 (123)
Chronic placental underperfusion	25.9 (81)
IVT	16.9 (53)
VUE	13.4 (42)
Fetal thrombotic vasculopathy	3.5 (11)
Chorangiosis	2.9 (9)
Maternal PET	2.2 (7)
MPFD	1.6 (5)
Chorangioma	1.3 (4)
Chronic histiocytic intervillousitis	0.9 (3)
Acute abruption	0.3 (1)

*Total number is > 313 (>100%), as some placentas had more than 1 histological lesion and each histological lesion has been counted separately.

Table 9.1 Frequency of predefined histological categories in abnormal placental histology.

Table 9.2 presents the maternal and neonatal characteristic data in cases with placental pathology versus placentas with normal histological findings.

Total n=1125	Abnormal histology(n=313)	Normal histology(n=812)
Maternal age years ± SD	30.5 ± 5.6	31 ± 5.8
Mean Gestational age ± SD	39.8 ± 1.3	39.4 ± 1.5
% Nulliparity (n)	57.8 (181)	44.3 (359)
% Smoker (n)	10.5 (33)	8.9 (72)
% Maternal PET (n)	1.6 (5)	2.3 (19)
% Maternal PIH (n)	2.9 (9)	2.2 (18)
% Maternal GDM (n)	2.9 (9)	4.4 (36)
% SGA≤10th percentile (n)	8.6 (27)	9.8 (80)
% Induced labour (n)	17.3 (54)	20.1 (164)
%Emergency caesarean delivery (n)	12.5 (39)	10.9 (89)
Mean Birth Weight ± SD	3523 ± 497	3466 ± 504
%Males (n)	53.7 (168)	52.9 (430)
% admitted to Neonatal unit (n)	7.3 (23)	5.4 (44)
Apgar score (at 5 min) ± SD	9.85 ± 0.4	9.83 ± SD 0.6
Cord Blood pH (Arterial) ± SD	7.25 ± 0.07	7.25 ± 0.08
BE (Arterial) ± SD	-4.50 ± 2.6	-4.35 ± 2.7
Cord Blood pH (Venous) ± SD	7.31 ± 0.07	7.31 ± 0.07
BE (Venous) ± SD	-4.22 ± 2.19	-4.17 ± 2.51

Table 9.2 Maternal and neonatal characteristics in cases with normal and abnormal placental histology.

Table 9.3 compares the different resuscitation measures required immediately after delivery in the group with and without placental pathology. Majority of the cases, over 90% in both groups did not require any resuscitation. None of the neonates required intubation or cardiac massage in the placenta pathology group.

	Abnormal histology % (n=313)	**Normal histology % (n=812)**
None	95.8 (300)	97.1 (788)
Facial oxygen + Stimulus	2.3 (7)	1.5 (12)
Face mask ventilation	1.9 (6)	1.1 (9)
Intubation	-	0.2 (2)
Intubation + Cardiac massage	-	0.1 (1)

Table 9.3 Neonatal resuscitation in abnormal versus normal placental histology groups.

Table 9.4 compares the neonatal outcome in the admitted neonates in abnormal versus normal histology group. The incidence of respiratory distress syndrome (RDS) was higher in the placental pathology group (26% versus 15.9%), however, this difference was not statistically significant ($p>0.05$). There was no statistically significant difference in the mean ventilation, CPAP, total respiratory support days in the group with and without placental pathology. There was no significant difference in the admission days to NICU, SCBU or total admission days in the unit in either of the group.

	Abnormal histology	Normal histology	P value
No. of neonates admitted	N=23	N=44	
% RDS (n)	26 (6)	15.9 (7)	NS
% CLD	0	0	
Ventilation days (Mean ± SD)	0.09 ± 0.4	0.10 ± 0.37	NS
CPAP days (Mean ± SD)	0	0.30 ± 1.17	NS
Mean total days respiratory support ± SD	0.13 ± 0.46	0.33 ± 1.14	NS
Hydrocephalus (n)	0	0	
Seizures (n)	0	0	
ROP (n)	0	0	
Feed Intolerance (n)	2	9	NS
PDA (n)	0	1	
Thrombocytopenia n)	0	0	
Anaemia (n)	0	0	
Coagulopathy (n)	0	0	
Proven infection (n)	0	0	
NEC (n)	0	0	
Renal Impairment (n)	0	1	
NICU admission days (Mean ± SD)	0.17 ± 0.7	1.86 ± 4.8	NS
SCBU admission days (Mean ± SD)	5.65 ± 1.7	6.53 ± 3.5	NS
Total Hospital stay days (Mean ± SD)	5.83 ± 1.9	8.21 ± 6.2	0.07
Survival to discharge (n)	23	43	NS
Discharged home from Rosie Hospital (n)	23	40	NS
Transferred out to different unit (n)	0	3	

NS-Non significant

Table 9.4 Neonatal outcome in abnormal versus normal placental histology.

Tables 9.5 (a-b) shows the pregnancy and neonatal outcomes in placentas with abnormal histology groups and the comparison to the normal histology data.

All values in % (n) or Mean ± SD	Ascending genital tract infection (n=123)	Chronic placental underperfusion (n=81)	IVT (n=53)	VUE (n=42)	Fetal thrombotic vasculopathy (n=11)	Normal histology (n=812)
Induced labour	18.7 (23)	12.3 (10)	15.1(8)	16.7(7)	27.3(3)	20.1(163)
Emergency c/section	14.6(18)	11.1(9)	17(9)	0	9.1(1)	10.9(89)
Males	56.1(69)	58(47)	60.4(32)	31(13)	9.1(1)	52.9(429)
Maternal PET	1.6(2)	1.2(1)	3.8(2)	0	0	2.3(19)
Maternal PIH	2.4(3)	4.9(4)	1.9(1)	7.1(3)	0	2.2(18)
Maternal GDM	1.6(2)	1.2(1)	5.7(3)	0	9.1(1)	4.4(36)
SGA≤10th percentile	8.9(11)	12.3(9)	0	9.5(4)	9.1(1)	9.8(80)
Admitted to neonatal unit	9.8(12)	4.9(4)	7.5(4)	7.1(3)	9.1(1)	5.4(44)
Apgar score at 5 min	9.80 ± 0.4	9.83 ± 0.4	9.92 ± 0.27	9.95 ± 0.22	9.91 ± 0.30	9.85 ± 0.66
Arterial cord Blood Ph	7.24 ± 0.06 (n=47)	7.26 ± 0.07 (n=23)	7.25 ± 0.07 (n=14)	7.23 ± 0.07 (n=14)	7.32 ± 0.07 (n=5)	7.25 ± 0.08 (n=254)
BE-Arterial	-5.0 ± 2.40 (n=47)	-4.5 ± 2.96 (n=23)	-3.99 ± 2.26 (n=14)	-5.00 ± 2.30 (n=14)	-2.34 ± 2.13 (n=5)	-4.35 ± 2.69 (n=254)
Venous cord blood Ph	7.30 ± 0.07 (n=47)	7.32 ± 0.06 (n=22)	7.31 ± 0.06 (n=14)	7.30 ± 0.04 (n=14)	7.38 ± 0.10 (n=6)	7.31 ± 0.07 (n=260)
BE-Venous	-4.63 ± 2.10 (n=47)	-4.02 ± 2.52 (n=22)	-3.99 ± 1.85 (n=14)	-4.51 ± 2.24 (n=14)	-3.15 ± 1.93 (n=6)	-4.17 ± 2.51 (n=260)
Resuscitation required	9.8(12)	1.2(1)	0	0	0	3(24)
RDS	1.6(2)	3.7(3)	1.9(1)	0	9.1(1)	15.9(129)

Table 9.5 (a) Pregnancy and neonatal outcomes in cases with abnormal and normal histology.

	Ascending genital tract infection (n=123)	Chronic placental underperfusion (n=81)	IVT (n=53)	VUE (n=42)	FTV (n=11)	Normal histology (n=812)
Mat Age	29.98 ± 5.70	30.73 ± 5.17	30.32±5.39	31.81±5.64	28.36 ± 6.28	31.00±5.78
GA at del(wks)	40.11 ± 1.30	39.70 ± 1.21	39.74±1.00	39.74±1.15	38.82 ± 1.60	39.37±1.49
Birth Wt (gms)	3584 ± 476	3390 ± 524	3660±426	3434±523	3382 ± 512	3466±504
NICU admission (days)	0	.25 ± 0.50	.75±1.50	0	0	1.86±4.84
SCBU admission (days)	5.58 ± 1.08	3.75 ± 2.22	4.75±2.63	7.00±2.00	8.00 ± 2.00	6.53±3.50
Total Hospital admission (days)	5.58 ± 1.08	4.00 ± 2.16	5.50±3.70	7.00±2.00	8.00 ± 2.00	8.21±6.19
Ventilation days	0	0	.50±1	0	0	0.10±0.37
CPAP days	0	0	0	0	0	0.30±1.17
Total days resp support	0	.25 ± 0.50	.50±1	0	0	0.33±1.14

Table 9.5 (b) Pregnancy and neonatal outcomes in cases with abnormal and normal histology.

9.5 DISCUSSION

Should all placentas be sent for detailed examination of placenta if there is adverse perinatal outcome; it is still not clear whether the pregnancy and neonatal outcomes are any different in abnormal placental histology group as compared to that of normal histology.

In this study, there were no differences observed in gestational age at delivery, prevalence of PET, PIH, GDM and SGA, rate of induction of labour or emergence Caesarean section in either of the group. Even there was no difference in the rate of admission in neonatal unit in placenta pathology versus normal cases (7.3% versus 5.4% with p=0.26). There was no significant difference in the mean admission days to NICU (p=0.10), SCBU (p=0.26) or in the total spent in the neonatal unit (p=0.07).

Cord blood gases (arterial and venous) were not any different in abnormal histology versus normal histology groups (Art-7.25 versus 7.25, Ven-7.31 versus 7.31).

There was no difference observed in the incidence of respiratory distress syndrome in the placentas with normal versus abnormal histology, with no difference in mean ventilation days (p=0.92), days on CPAP (p=0.22) or in total days on respiratory support (p=0.42).

None of the individual histological categories showed any increased prevalence of the PET, PIH, GDM or SGA.

39.3% of all placental histology cases were of ascending genital tract infection; however none of these cases had any clinical signs or symptoms suggestive of chorioamnionitis or any other intrauterine sepsis. The result of this study is in accordance with the other studies showing poor correlation between clinic and histologic chorioamnionitis.[126-127] Though the histological diagnosis of the chorioamnionitis is considered a gold standard, the clinical significance of this is not entirely clear. In this study, there were no significant differences observed in pregnancy and neonatal outcome of normal versus abnormal histology groups.

In term pregnancies, chorioamnionitis occurs in labour and clinically remains largely irrelevant, while in preterm deliveries, this will be of much greater clinical relevance. A recommendation therefore from this analysis is that every placenta does not need detailed pathological examination in deliveries at or close to term. However this data can only be represented for the population at or near term but cannot be extrapolated to pre term deliveries.

CHAPTER 10 : DISCUSSION

Key Findings:

- Close correlation between manual and digital placental measurements using software image J has been defined.
- Morphological indices of the placenta and the umbilical cord defined for more objective analysis.
- These morphological indices were not different in common obstetric outcome groups as compared to those of the reference population.
- This study has shown quantitatively, strong positive correlation of birth weight to the placental weight and circumference.
- For the first time, this study has described the baseline data of predefined histological lesions in the reference population and the pregnancies with common obstetric outcome.
- Cases of IVT and VUE showed relative hypercoiling of the umbilical cord. Most placentas from unselected population (72%) as well as common obstetric outcome groups (66-80%) were histologically normal.
- Morphological indices and predefined histological abnormalities were not any different in the placentas from infants admitted to the Neonatal Unit.
- There were no differences in the pregnancy and Neonatal outcome in cases with abnormal predefined histological lesions.

10.1 OVERVIEW OF THESIS

This study was a prospective study of an unselected reference population of women with singleton pregnancies, delivering in a single unit in Cambridge, Eastern region of England. The study received ethical approval from Cambridgeshire 3 Research Ethics Committee. 1159 patients were recruited for the study over a period of 13 months in year 2007-2008. This study largely included term cases (33-43 weeks).

The study predominantly defines the morphological and histological characteristics of the placenta to the pregnancy and neonatal outcomes. This study has surprisingly never been done before. The study derived the placental morphological indices quantitatively using the image analysis, and showed a strong positive correlation between manual and digital measurements of the placenta. Based on the derived indices; cord coiling, insertion and the shape of the placenta has been described quantitatively, avoiding the bias of qualitative method. The study has also derived the ratio of birth weight to placental weight and the circumference in the reference population. These morphological indices and the ratios will form the bases of future studies.

No baseline quantitative data for placental characteristics exists. Previous studies have either been performed on high risk population and/or analysed placental characteristics qualitatively, which makes it difficult to compare the data.

This study has defined the criteria for diagnosing the histological lesions of the placenta and the incidence of these predefined histological lesions in the unselected population.

As most cases in any unselected population are “normal” and have a normal outcome, they therefore do not require further investigations of the placenta. This study has shown this in a more objective manner and we cannot recommend that there is need for routine detailed placental examination in an unselected term population.

This study was not easy to do, and many problems were encountered. It required involvement of great number of personnel such as dedicated laboratory assistant for daily transportation of placenta from delivery unit to pathology lab. Extra biomedical scientists were required for daily tissue sectioning and processing for further analysis. Procuring a fridge with greater capacity near labour ward was difficult. One of the most challenging tasks of this study was to keep the midwifery staff motivated and encouraged throughout the recruitment period so that all the placentas from participants were kept for the study from the patients recruited. Since a detailed and thorough macroscopic examination of the placenta was not possible in the delivery unit, a dedicated study area was required in the pathology lab. The study required trained perinatal pathologists, as the general pathologist may not be trained for the detailed placental histological examination, required by the study.

10.2 MANUAL VERSUS DIGITAL MEASUREMENTS

This study compared the manual measurements of placenta to the digital measurements of image J software. The data showed a strong positive correlation between manual and digital measurements using correlation coefficient and a more precise method of comparison of the two measurements; Bland Altman analysis. The results of digital measurements of the study were comparable to other recent studies (Table 3.3).

The findings are of potential importance for future studies in this area, since with the widespread introduction of relatively cheap and accurate digital imaging technologies and low cost image analysis software, imaging based measurements are likely to become increasingly commonly used. The advantages of such image based studies includes the ability to perform measurements in a batch at a subsequent time, rather than delaying placental or tissue clinical examination, and the storage of the raw data images which can be revisited at a later date for review or further measurements. For most such studies examining measurements in relation to outcome, the precise measurements are less important since provided the same methodology is used for all group valid comparisons can be made.

There was a close correlation in manual measurements of long axis, short axis and circumference of the placenta to that of digital measurements, though the digital measurements appeared to be systematically smaller than the traditional manual measurements. The reason for this difference was not clear. As the comparative data have not been presented in this format before, it is difficult to know if this finding was unique to this study or whether there are other reasons.

Digital measurement of the placenta is reproducible and a relatively simple method of measurement compared to traditional manual measurements. Image J software is also freely available making it accessible to researchers in countries where financial resources may be limited.

10.3 PLACENTAL MORPHOLOGICAL INDICES

Studies on the placenta and umbilical cord have generally investigated adverse outcomes where there is an indication for histological examination of the placenta, such as fetal demise, intrauterine growth restriction, preterm delivery, pre-eclampsia, diabetes mellitus, macroscopic abnormalities of placenta, fetal asphyxia, and intra uterine infection. Few have attempted to establish baseline characteristics in an unselected population delivering at term. This population studied approximates in maternal demographics, the incidence of pregnancy conditions and birth weight/gestation outcomes to a typical UK/European booking population.

Hypocoiling and hypercoiling of the cord (whether detected in antenatal or postnatal period) have been reported to be associated with adverse perinatal outcome, although causality remains uncertain. Hypocoiling has been associated with trisomies, preterm delivery, fetal death, increased intrapartum complications and interventional deliveries for fetal distress, Apgar score less than 7 at 5 minutes; velamentous cord insertion and single umbilical artery. Hypercoiling has also been linked with trisomies, but also small for gestation age, fetal asphyxia and single umbilical artery [5 8 56].

Abnormal cord coiling has also been found with thrombosis of the chorionic plate vessels, umbilical venous thrombosis and umbilical cord stenosis [6].

The coiling index is probably one of the most frequently reported of umbilical cord related parameters in common outcome groups. Pre-eclampsia and gestational diabetes have been suggested as maternal risk factors for abnormal coiling [57]. In gestational diabetes both non coiling and hyper coiling were significantly more frequent than in normal pregnancies[96]. In addition, hypercoiling has been associated with SGA (OR 2.10) [8]. The umbilical cord coiling index in this study was 0.20 coils/cm with a preponderance of left sided coiling (79%), according with published data which suggest a range of 65% to 87.5% [8 45 49].[11]. Our data however showed no significant difference in umbilical cord coiling index with pre-eclampsia, PIH, GDM or SGA compared to the reference population.

Whilst central and paracentral cord insertions presumably represent variants of normal and have no clinical importance, marginal insertion has been suggested as being more susceptible to vessel rupture and associated with intra uterine growth restriction, stillbirth and neonatal death [9]. The incidence of velamentous insertion increases with maternal smoking, advanced age or diabetes mellitus and among multiple births, congenital malformation and in vitro fertilisation pregnancies [9]. However, in our study we did not observe any cases of velamentous insertion of the cord in GDM. Although the difference between extreme paracentral umbilical cord insertion and marginal cord insertion may be small, this differentiation may be important as paracentral umbilical cord insertion is the most common type of umbilical cord insertion in pregnancies with normal outcome while some studies have shown an increased incidence of marginal umbilical cord insertion in pregnancies with adverse outcomes such as miscarriage, fetal congenital anomalies, preterm labour and intrauterine growth restriction [64-66].

The morphological index for the degree of deviation from central insertion into the chorionic plate is expressed in this study as the cord centrality index (CI). The data of this study indicated that in unselected population of pregnancies delivering at term, the 'normal' cord insertion is not in fact central. In the current study mean cord centrality index was 0.36, which signifies a markedly 'off centre' insertion (Figure 4.4).

Transposing these results to the traditional descriptive categories, the results concur with historical as well as newer studies (Table 4.1). Though subjective and qualitative description of the placenta has a role predominantly in clinical reporting, deriving this index allows entirely objective analysis of this and other continuous variables in relation to outcomes and other placental findings.

There are no studies comparing quantitatively the difference between abnormal insertion of the umbilical cord in common obstetric outcome groups and a reference population. The distance of umbilical cord insertion from the centre (or from the chorionic plate margin) has been considered a marker of placental insufficiency [90 97]. Peripheral cord insertion is also strongly related to abnormal placental shape in severe IUGR with abnormal umbilical artery Doppler.[171] The association of velamentous insertion with IUGR, low birth weight infants and pregnancies affected with diabetes mellitus has been reported [9]. One of the largest studies of its type on 12,750 patients, found an increased risk of low birth weight (OR 2.32) and small for gestational age infants (OR 1.54) with velamentous insertion[74], and another an increased incidence of velamentous cord insertion in low birth weight than controls [73]. Historic studies have reported an increased incidence of marginal cord insertion in pregnancies with IUGR [64-66]. In the current study there was no significant difference in cord centrality index for pre-eclampsia, PIH, GDM and SGA groups compared to the reference population.

The shape of the placenta has been thought to closely mirror the pattern of chorionic vascular growth; studies modelling the placental vascular tree have suggested that irregular shapes of placenta are common and associated with low birth weight, suggesting variably shaped placentas have altered function [10]. Abnormal shape has been associated with increased intra uterine fetal death, extreme preterm delivery and fetal growth restriction [172]. The data of the present study indicated that the 'normal' term placenta was not round. The eccentricity index in this study varied from 0 to 1 with mean eccentricity of the chorionic disc 0.49, in other words more elliptical than round (fig 4.7). There are no studies describing the shape of placenta quantitatively in the common obstetric outcome groups of hypertensive disorders of pregnancy, GDM or SGA. Further few have compared the shapes of the placenta quantitatively

between obstetric outcome groups and a reference population, though a recent study has shown a correlation between the short axis of the placenta and the severity of the pre-eclampsia [94]. The current study did not find the statistically significant difference in shape (eccentricity) of the placenta between the reference population and any of the common obstetric outcome groups.

It is, therefore, appropriate to dispel the widely held beliefs that the placenta is, in its natural and normal state, circular and that the cord normally inserts into its centre. Further, coiling index, cord centrality index and eccentricity in this study was not any different in the pregnancy outcome groups compared to the data from pregnancies which were not affected by those outcomes.

10.4 BIRTH WEIGHT/ PLACENTAL WEIGHT AND CIRCUMFERENCE

The normal birth weight: placental weight ratio has been shown to range between 6.5 to 7.1 at term 37-42 weeks gestation[114-115], and 6.3 to 8.46 at 34-43 weeks.[116] The birth weight: placental weight ratio of 7.39 ± 1.20 in the reference population from 33-43 weeks that we derive is therefore within the limits of these gestational age ranges. This ratio may have relevance as the ratio of birth weight: placental weight has been considered to be more important than placental weight alone when considering adverse perinatal outcome such as fetal and neonatal demise.[107 117]

This relationship between birth weight and placental weight is also important as a larger placental weight: birth weight ratio has been associated with chronic hypertension and other adverse cardiovascular outcomes in later life.[110 173]

Confusingly, the relationship of birth weight and placental weight has been described differently in other studies, and gestational age strongly affects the ratio with the ratio being lower at earlier gestations: 2.9 at 24 weeks and 6.8 at 40 weeks.[115] Some studies describe the feto-placental weight ratio[116], others as its inverse-placental weight: birth weight ratio.[113] One UK based study[174]found that the placental: birth weight ratio at term (39-41 weeks) ranged from 19.5 to 20.4%, which would be roughly be equal to birth: placental weight ratio of about 5; another measured the

placenta: birth weight ratio at term (>37 weeks) in a large cohort as 0.177 i.e. birth: placenta weight ratio as 5.6.[113] These ratios are smaller than those observed in the current study.

Ethnicity does not seem to affect this ratio, as smaller births in Asian pregnancies are associated with smaller placentas and greater birth weight with higher placental weight; therefore the ratio is thought to remain essentially the same.[174] The population in this current study is mainly Caucasians as has been mainly the case in European based studies.

Birth weight/placenta weight of the reference population has been compared with the common obstetrics outcomes in this study. This ratio is significantly smaller ($p=0.001$) in cases with PET (6.60 ± 1.03) than the cases not affected by PET (7.40 ± 1.19). The mean difference between the two groups was 0.79. This study did not show any difference in this ratio in cases with SGA, PIH and GDM compared to the non-affected population.

Another outcome analysed in this study was the ratio of birth weight and placental circumference. This ratio has not been presented previously. We described it as we wished to establish whether a relationship existed between the birth weight and the placental dimensions rather than with weight or volume. Whether or not this ratio is a clinically useful index, describing it and deriving a relatively robust range and ratio at gestation between 33-43 weeks allows other researchers to compare their findings to it. In this study, a significant difference was observed in birth weight/placenta circumference ratio in cases with PET and SGA. In cases of PET and SGA, the mean difference of this ratio was significantly less than non-affected population. The study did not disclose this ratio any different in cases with PIH and GDM as compared to the non-affected population values.

The birth weight: placental weight ratio, derived in this study was consistent with the data from studies. The data of the birth weight ratio to the placental weight and circumference as robust as they were from an unselected population of women

delivering at gestational age of 33-43 weeks whose gestations have in all cases been calculated from a first trimester scan.

There was a strong correlation between placenta weight and circumference to the birth weight in all common obstetric outcome groups and reference groups. (scatter plots with significant Pearson's correlation coefficient's values-figures 6.4-6.10), which also confers that higher the birth weight, the higher is the placenta weight and the circumference and vice versa.

10.5 PREDEFINED HISTOLOGICAL LESIONS

These data describe the pattern of histology in unselected population irrespective of the risk status of the pregnancy. To add this, the study follows strict criteria for the diagnosis of histological abnormalities. The incidence of various histological pathologies varies widely in literature; one of the reasons being lack of standard definitions for these lesions. Every research group has defined the lesions according to their definitions and therefore the literature lacks uniformity on the subject.

Guidelines suggest that all placentas should be examined by the trained perinatal pathologists: Placental pathology should be a routine component of obstetric-neonatal care, as it would provide detailed information, helpful in the post natal management of adverse pregnancy outcome.[18] However the histological examination and perinatal outcome in most pregnancies is normal, for example in the current study, the frequency of histologically normal examination was observed in over 70% of cases, even in the subset of common obstetric outcome pregnancies more than 66% placentas did not show any histological lesions. Therefore the data from this study do not support the recommendations for the routine examination of placenta by the pathologist.

The diagnosis of chorioamnionitis clinically is unreliable as the clinical signs and symptoms are non specific. These symptoms may be suggestive of some other pathology, not necessarily chorioamnionitis.

Above all most cases of chorioamnionitis (confirmed histologically) do not even have the clinical features of infection per se. Therefore only a histologically made diagnosis of chorioamnionitis should be taken as a reliable diagnosis. Interestingly, in the current study we had no cases of clinically diagnosed chorioamnionitis and yet we had significant number (123; 10.9%) of cases of histological ascending genital tract infection. This enforces the fact that there is a very poor correlation between a clinical diagnosis and histological infection. There was no difference in the frequency of ascending genital tract infection in the common obstetric outcome groups as compared to the unselected population.

The reported incidence of IVT varies from 3-50% in full term uncomplicated pregnancies. [132 133] This study reported the frequency of IVT in unselected cohort of 4.7%; comparing the incidence of IVT in uncomplicated pregnancies to small for gestational age (<10 centile) has shown no difference in the incidence of the lesion.[133] In current study there were no differences in IVT observed in SGA, PET, PIH and GDM, compared with reference population.

Villitis of unknown etiology is one of the most commonly found placental lesions in the 3rd trimester. This entity is still unknown to many clinicians and pathologists are still over or under diagnosing the condition. VUE is primarily seen in the term placenta; more than 80% cases occur after 37 weeks and all after 32 weeks of gestation.[114] Histology findings suggestive of chronic villitis at less than 32 weeks should lead to a high suspicion of infectious pathology rather than making a diagnosis of villitis of unknown etiology.

The diagnosis of VUE can be difficult specially when there is minor degree of inflammatory change. A high degree of inter-observer variation can be seen in the diagnosis of villitis of unknown etiology.[157] There also exists different histological criteria for the diagnosis of villitis by different authors. Perhaps if agreed standard criteria for the diagnosis of VUE; this condition then can be better understood by all.

Villitis of unknown etiology has been described to have a recurrent tendency in successive pregnancies and recurrent villitis tends to be more extensive and has even

stronger association with intra uterine growth restriction.[175] [176] The incidence of VUE in current study what we find was lower (3.7%) than the incidence noted in one of the study of the UK population (13.6%).[136] The frequency of VUE was higher (11.1%) in cases with PIH but interestingly there were no cases observed in PET.

We also report relatively infrequent histological lesion: fetal thrombotic vasculopathy, Chorangiosis, massive perivillous fibrin deposition (MPFD) in the study.

In summary: these histological abnormalities are either studied in uncomplicated pregnancies or in complicated pregnancies but to the best of our knowledge, no study has presented the baseline histologic profile in unselected population. We described an unselected population, the prevalence of the histological abnormalities is low and therefore the power of the study to determine clinical significance is low. However the range and incidence of histological abnormalities can be used as baseline data for future studies.

This study also investigated the relationship between the macroscopic placental indices to the placental histology. The effect of and/or association between histological lesions and the morphological characteristics of the placenta have not been previously comprehensively described in an quantitative manner, using objective measurements and strict histological criteria combined with completely blinded histological review.

The study did not find an association between placental macroscopic features such as the shape (Eccentricity), and cord insertion (Cord centrality index) with the histological abnormalities. Placental morphology can therefore not predict the presence or absence of histological abnormality.

10.6 PLACENTA IN NEONATES ADMITTED TO THE NEONATAL UNIT CLINICAL AND OUTCOMES IN ABNORMAL HISTOLOGY GROUP

Macroscopically; the cord coiling index, cord centrality index and placental eccentricity in infants admitted to the Neonatal Unit did not show any significant difference as compared to that of macroscopic indices of the cases where infants were not admitted. The cord coiling index was no different in infants admitted to the Neonatal Unit. The cord centrality index showed no difference in Neonatal Unit cases, neither was eccentricity. However microscopically; 34.3% of infants admitted to the Neonatal Unit had histological abnormalities according to our pre defined histological groups.

Clinical chorioamnionitis is associated with adverse perinatal outcomes, including cerebral palsy, abnormal fetal heart rate pattern, fetal hypoxia, chronic lung disease, broncho-pulmonary dysplasia, IUGR, fetal sepsis, fetal and neonatal death. These will obviously increase the rate of neonatal admission. However, it is difficult to determine whether the increased incidence of ascending genital tract infection is a true reflection of the Neonatal Unit admitted cohort or is due to any particular clinical outcome group.

The incidence of chronic placental underperfusion, IVT, VUE, fetal thrombotic vasculopathy and Chorangiosis was very small number to draw any meaningful conclusions. No cases of MPFD, chorangioma, chronic histiocytic intervillousitis, and acute abruption were observed in the placentas where infants were admitted to the Neonatal Unit.

Comparing all cases of abnormal placental histology to normal histology, there was no significant difference in maternal age, gestational age, prevalence of PET, PIH, GDM and SGA, rate of induction of labour or emergency caesarean section. There was no difference in the rate of admission to the Neonatal Unit based on the histology of the placenta. There was no difference in cord blood gases (arterial and venous) in both groups. In both groups over 95% of neonates did not require any resuscitation.

The incidence of respiratory distress syndrome was not different in the two groups. Similarly, there was no significant difference in mean ventilation days, days on CPAP, total days on respiratory support, mean admission days to NICU, SCBU or in the total spent in the Neonatal Unit.

Individually comparing with the most common histological categories, there was no significant difference between the cases with abnormal and normal histology and the incidence of maternal pre-eclampsia, PIH, GDA and SGA was not increased in the abnormal histology group.

Out of 313 cases with abnormal placenta histology, the most common histological category was ascending genital tract infection, which constituted 39.3% of all placental pathology cases. In this study there were neither cases of clinical suspected chorioamnionitis nor any obvious cases of antenatal sepsis. Clinical signs and symptoms which may be suggestive of chorioamnionitis are non specific; there is a poor correlation between clinical and histologic chorioamnionitis.[126-127] We included funisitis (umbilical cord inflammation) and the inflammation at the choriodecidual junction within the ascending genital tract infection group. Intrauterine infection due to any cause is well known to be linked with preterm deliveries.[177-180] Intrauterine infection and subsequent resulting fetal infection has been implicated in neonatal and fetal injury[181-182], cerebral palsy[183] and chronic lung disease.[184] The end state is infection is fetal infection and increased neonatal mortality due to neonatal sepsis; Chorioamnionitis results in increased perinatal morbidity and mortality.

Diagnosis of chorioamnionitis clinically is an unreliable tool as the clinical signs and symptoms suggestive of chorioamnionitis are non specific. However its clinical significance is not clear as most term cases would have no clinical concerns.

In summary, this study showed no obvious utility to examining the placenta routinely or in the case of neonatal and fetal pathology. However in such cases, the significance of detailed placental examination by the pathologist would still remain from medico-legal purposes.

We recommend that the strict criteria for the definitions for all common histological categories and standard protocols should be developed for better understanding of the disease.

10.7 REFLECTIONS ON PROBLEMS IN THESIS

Ethics committee/ R&D /Consent

Patients were provided the consent form with the study information in the antenatal clinics. They were asked to sign the consent forms if agreed to participate in the study. Consent forms were kept in the patients notes. At the time of delivery, the evidence of consent was confirmed by the delivery unit staff and placenta was sent to the study attached with a study pathology form with a confirmation of the consent. During the study, there were problems with regard to the consent for the placental study. The research team identified some shortcomings in filing the written consent form, in the patients' notes, as per the study protocol. In the interest of research governance, the team agreed to review all case notes when a concern was raised.

Where there was no evidence of signed consent form in the patients' notes, the cases were not included in the study and the patient information was removed from the study database.

There were some cases where there was no evidence of the written consent form in the case notes, but the completed and signed study pathology form by the attending doctor or midwife was present with the placenta stating explicitly that the consent has been taken. These cases also have not been included in the current study, as they are pending ethics committee decision.

We very much regret that we do not include these cases but feel we have no alternative in absence of any response from the ethical committee. (Reference letter in appendix)

10.8 LIMITATIONS OF STUDY

All pregnant women with singleton pregnancy were approached for the study irrespective of their gestational age at the time of recruitment. Therefore we perhaps did not recruit patients who either had pre term delivery or developed for example early onset pregnancy complications for example early onset pre eclampsia, IUGR etc. Because of the laborious consenting process, women in whom there were clinical difficulties at preterm gestations are less likely to have consented to take part in this study. However the incidence of term complications of pre eclampsia, PIH, GDM and SGA are broadly similar to those reported in other studies from unselected population. We do plan to extend this study including specific cases of early onset PET, IUGR, clinical abruption cases, diabetes and IUFD.

We also could not recruit those placentas where placenta those were delivered manually, making it difficult to perform measurements.

We did not have control on the length of the umbilical cord left on the neonates. We only relied on the cord which was received with the placenta. This problem is encountered in most studies performed on the umbilical cord. Therefore fetal end of the cord is not truly "fetal" end. The method we have used to derive the placental circumference worked well for regularly shaped placentas, however was more approximate where a placenta was a very abnormal shape. There is no geometrical way of reliably defining the axes of the placenta and its centre where a placenta has a grossly irregular shape. This is an invariable limitation of this type of study, however we did not exclude any placentas on the basis of very irregular shape. Furthermore for consistency rather than deriving placental circumference through direct tracing, we derived this in all cases from the major and minor axes which form the basis of the eccentricity and the cord centrality indices.

In the reference population, 123 cases (out of 1125) showed signs suggestive of histological ascending genital tract infection; none of these cases had a record made of clinical chorioamnionitis. However we relied on the obstetric database "protos" for the clinical diagnosis. We did not consider individual clinical markers such as

WCC, CRP, history of rupture of membranes or use of antibiotics in labour as diagnostic in themselves as clinical chorioamnionitis.

10.9 FUTURE WORK

Following demonstration of quantitative morphology of the placenta in unselected population, this work now allows the future studies to perform further analysis based on the morphology of the placenta.

This data will serve as baseline dataset for all future studies on placental morphology in unselected as well as common obstetric outcome pregnancies.

During the period of the study, placental tissues in some cases were stored in RNA later for possible future molecular based studies.

Future work which could follow this study are:

Study on increased number of abnormal cases now that we have a large reference population to compare with nested cases control studies.

Analysis on cases of preterm pre-eclampsia, SGA, placental abruption, IUD, diabetes including IDDM and clinical chorioamnionitis.

To define incidence of rare histological findings in abnormal outcome groups.

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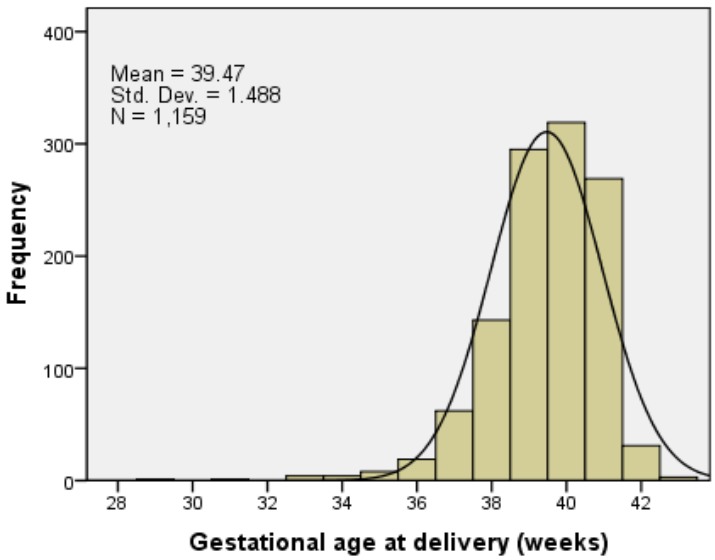
APPENDICES

APPENDIX 1: GESTATIONAL AGE AT DELIVERY

Gestational age (Weeks)	n (%)
24-28	0
29	1 (0.1)
30	0
31	1 (0.1)
32	0
33	4 (0.3)
34	4 (0.3)
35	8 (0.7)
36	19 (1.6)
37	62 (5.3)
38	143 (12.3)
39	295 (25.5)
40	319 (27.5)
41	269 (23.2)
42	31 (2.7)
43	3 (0.3)



APPENDIX 2: ETHICS APPROVAL LETTER

Cambridgeshire 3 Research Ethics Committee
(formerly Peterborough & Fenland Research Ethics Committee)
Victoria House
Capital Park
FULBOURN
Cambridge
CB21 5XB

Telephone: 01223 597597
Facsimile: 01223 597645

05 June 2007

Dr Flora Jessop
Consultant Paediatric/Perinatal Pathologist
Department of Pathology, Box 235
Addenbrooke's Hospital
Hills Road
Cambridge
CB2 0QQ

Dear Dr Jessop

Full title of study: **Outcomes in pregnancy and the neonatal period: correlation with placental examinations**
REC reference number: **07/Q0106/51**

Thank you for your letter of 08 May 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 04 June 2007. A list of the members who were present at the meeting is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised. The favourable ethical opinion is conditional upon the revised Consent Form being given an updated version number and/or date. Please send the Committee a copy of the revised Consent Form as soon as possible. Please note that the approved Patient Information Sheet is the revised sheet of 080507.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA. There is no requirement for [other] Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application (Lock code: AB/106222/1)		19 March 2007
Investigator CV: Dr Flora Jessop		15 March 2007
Protocol	1.1	19 March 2007
Covering Letter: Re initial application		19 March 2007
Letter from Sponsor: Priya Shimoga, Addenbrooke's R&D		22 March 2007
Patient Information Sheet		08 May 2007
Participant Consent Form		
Participant Consent Form		
Response to Request for Further Information: Letter from Dr Flora Jessop		08 May 2007
Letter from funder: Keith Day		30 October 2006
Applicant's checklist		19 March 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Feedback on the application process

Now that you have completed the application process you are invited to give your view of the service you received from the National Research Ethics Service. If you wish to make your views known please use the feedback form available on the NRES website at:

https://www.nresform.org.uk/AppForm/Modules/Feedback/EthicalReview.aspx

We value your views and comments and will use them to inform the operational process and further improve our service.

07/Q0106/51	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Mr Stuart Kent
Vice-Chair

Email: lynda.mccormack@eoe.nhs.uk

APPENDIX 3: STUDY INFORMATION FORM

Addenbrooke's Hospital NHS

Cambridge University Hospitals NHS Foundation Trust

Studies on your Placenta

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others if you wish. This leaflet:

- Outlines the aim of the study and what will happen to you if you take part.
- Outlines more detailed information about the conduct of the study.

Please do ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

2. **What is the purpose of the study?**

This study focuses on the placental examination and is being carried out jointly between the Rosie Hospital, Addenbrooke's Histopathology Department & Great Ormond Street Hospital for Sick Children. We plan to examine placentas from women delivering their babies at the Rosie over a two year period, and will correlate the findings to pregnancy outcome and establish baseline features of the placenta.

3. Why have I been chosen?

We are asking all mothers who are expecting one baby (i.e. not twins) booking at the Rosie.

4. **Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. Once you have signed the consent form, we will send your placenta for pathological examination and there is nothing further you would need to do.

5. What will happen to me if I take part?

We are asking only one thing: to be able to examine your placenta (afterbirth) once baby is born. It is normally disposed of, however we will examine it in the laboratory and link the details of your pregnancy outcome anonymously with your maternity and baby's records. This study will not in any way affect the care you receive, your appointments or delivery. You will not be contacted after the study is over.

6. What do I have to do?

You will be provided the relevant information of the study and then asked if you wish to take part in the study. You then need do is to sign the consent form (part 2) attached to this information sheet.

7. What are the possible benefits of taking part?

Normally, placentas are not sent for pathology examination. There is no direct benefit to you from taking part in the study, however as you are participating in this study, we'll examine the afterbirth and a report would be available for, if requested by your doctor in the hospital looking after you during the pregnancy.

8. **What happens to my placenta?**

Studies have linked the pregnancy and neonatal outcomes with the changes in the placenta. We'll examine the placentas and link the examination findings to the pregnancy and neonatal outcome. The placenta (afterbirth) will be examined in the laboratory and some small pieces of tissue will be taken and examined under the microscope. The tissue will then be held in long term (minimum 25 years) secure storage at Addenbrooke's and Great Ormond Street Hospitals. These tissues from the placenta will be used for the further studies which will be subjected to ethical approval.

9. Will my taking part in the study be kept confidential?
We will follow ethical and legal practice and all information about you will be handled in confidence. Information will be collected by one of the research team members. The anonymised database linking placental pathology with all outcome data will be kept on a restricted research database at Addenbrooke's.

10. What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (S Pathak, ext: 58137). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

11. What will happen to the result of the study?
We intend to use the result of this study in future pregnancies management. We intend to publish the results and you will not be identified in any report/publication.

12. Who has reviewed the study?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by Peterborough Research Ethics Committee.

13. Who will be doing the research?
The research group is: Mr C Lees (Consultant in Obstetrics and Fetal Medicine), Mr G Hackett (Consultant Obstetrician and Gynaecologist), Dr E Murdoch (Consultant Neonatologist), Dr F Jessop (Consultant Paediatric Pathologist), Dr N Sebire (Consultant Pathologist), Dr L Hook (Pathology Subspeciality Fellow) and Dr S Pathak (Research Fellow in Fetal Medicine). The study funded jointly by Addenbrooke's Charities (Charity number: 1048868), Cambridge Fetal Care, and Great Ormond Street Hospital.

14. Contact Details:
The research fellow conducting this study is: **Dr Sangeeta Pathak** If you have any queries about this study, observations or complaints, then please contact her by telephone (extension 58137) or e-mail: sangeeta.pathak@addenbrookes.nhs.uk

APPENDIX 4: CONSENT FORM

Addenbrooke's Hospital NHS
Cambridge University Hospitals NHS Foundation Trust

Patient Identification Number for this trial:

CONSENT FORM

Title of Project: Placental Correlates of Pregnancy and Neonatal Outcomes.

Name of Researcher: Dr Sangeeta Pathak

1. I confirm that I have read and understand the information sheet dated 08.05.2007 (Version 1.4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of any of my medical notes, my baby's medical notes and data collected during the study, may be looked at by responsible individuals from the research team of Cambridge University Hospitals NHS Trust and Great Ormond Street Hospital where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I consent for my placental tissues to be stored at Addenbrookes Hospital and Great Ormond Street Hospital for further studies. ☐
5. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of Person Taking consent	Date	Signature

When completed, 1for patient; 1 for researcher site file; 1(original) to be kept in medical notes.

Chairman: Dr Mary Archer Chief Executive: Dr Gareth Goodier

APPENDIX 5: STUDY POSTER

PLACENTAL STUDY

(LREC Reference no. 07/Q0106/51)

RECRUITMENT NOW OPEN

PLEASE CONSIDER DONATING YOUR PLACENTA FOR OUR RESEARCH STUDY

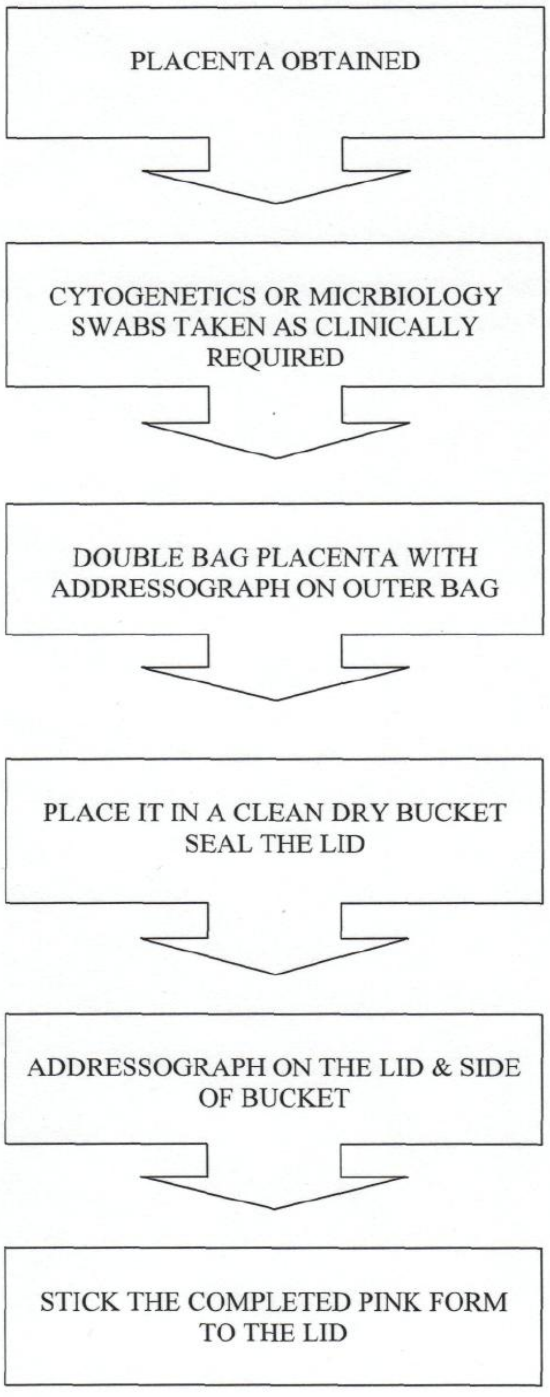
Patient information forms in clinic, ultrasound and on the delivery unit

Contact: Dr Sangeeta Pathak Ext: 58137

APPENDIX 6: INSTRUCTIONS TO MIDWIVES

PLACENTAL PROJECT- "STUDIES ON YOUR PLACENTA"

ENSURE CONSENT FORM FOR "PLACENTAL PROJECT" IS SIGNED AND FILED IN THE NOTES.



APPENDIX 7: PATHOLOGY FORM DEFINING STUDY INCLUSIONS

Addenbrooke's Hospital NHS
Cambridge University Hospitals NHS Foundation Trust

East Anglia Perinatal and Paediatric Histopathology Services and Fetal Medicine Department.

REQUEST FORM

(Study contact: Dr Sangeeta Pathak ext 3660.)

Request for Examination of Placenta for the **Studies on Your Placenta Project** LREC07/Q0106/51 (A completed consent form must be filed in the notes plus a copy for the researcher).

Section 1

Addressograph

Gestation:

Delivery date:

Ensure that, if clinically required, samples for Cytogenetics + Microbiology have been taken.

If placental histopathology is required, complete green form as normal and enclose with this form.

Section 2:

Name of person completing this form:

Name of person taking consent:

Section 3

FOR RESEARCH USE ONLY

RNA later sample taken: Y N

(Page 2: for laboratory use only)

Page 1

Page 2: please note, this page for laboratory use only

Department of Histopathology, Addenbrooke's Hospital Cambridge

PLACENTA WEIGHT SHEET

Number		**Name**	
Hospital number		**Date**	
Technician		**Path**	

MACRO

Trimmed	
Dimensions	
Cord length	
Cord diameter	
Number of vessels	
Insertion	
Direction of coil	
Coiling	

1		A	
2		B	
3		C	
4		D	
5		E	
6		F	
7		G	
8		H	
9		J	
10		K	
11		L	
12		M	
13		N	

MICRO

Page 2

APPENDIX 8: COMMUNICATION LETTERS TO LREC

Ref: KDD/9740

30th October 2006

Dr FA Jessop
Department of Histopathology
Box 235
Addenbrookes Hospital
Cambridge

Dear Dr Jessop

Outcomes in pregnancy and the neonatal period: correlation with placental examination
Grants Committee Meeting 18th October 2006
Minute No.31/06(E)

I am pleased to confirm that your application for funding from the General Medical Research Fund for the above project was granted at the recent Grants Committee Meeting. The amount of the grant is £15,300 for one year only to be allocated as follows;
£13,300 for Histopathology MLA for one year including on-costs
2,000 for consumables – wax, chemicals, cassettes etc.

In no circumstances should total expenditure exceed this allocation or be used for any purpose other than that described in your application of 29th August 2006. Any proposed significant variation between the categories of expenditure must be referred to the Chairman of the Research Advisory Committee for prior approval. It is expected that the grant will be spent within 18 months. If you envisage going over this period, you should let me know. In most cases a re-application to the Research Advisory Committee will be required if any element of the grant remains unspent at the end of this period. It is not acceptable for approved projects to be transferred to another investigator without seeking Chairman's Action or attending the Committee for approval.

It is a condition of the grant that a report is made by you to the Research Advisory Committee, and I should be glad if you would submit a short report (not exceeding 300 words) 12 months from the date of this letter and a final report at the end of the project.

If the grant includes a salary, you must contact Medical Staffing, or Personnel if appropriate, to make sure contracts of employment are in order. The cost centre **9740** has been given to your grant and this reference should be quoted on all correspondence. If you have any query please do not hesitate to contact me or **Carol Tabor, Charities Section, on extension 2184 / Box 130** in the first instance

Yours sincerely

Keith Day
Secretary to the Trustees
cc: Dr John Bradley, Dr CE Hook
Mr CC Lees, Mr GA Hackett, Dr E Murdoch, Dr N Sebire

FAJ/LREC/jw

Monday 12th October 2009

Department Histopathology
Box 235
Hills Road
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Switchboard: 01223 245151
Direct Dial: 01223 274642
flora.jessop@addenbrookes.nhs.uk
www.addenbrookes.org.uk

Dr R Griffiths
Chair
Cambridgeshire 3 Research Ethics Service
Victoria House
Capital Park
FULBOURN
Cambridge
CB21 5XB

Dear Dr Griffiths

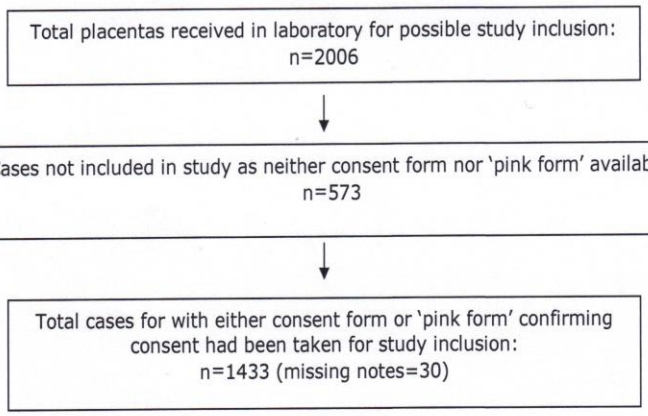
Re: Outcomes in pregnancy and the neonatal period: correlation with placental examinations, reference number 07/Q0106/51

I am writing to update you about the progress of this study. It has recently come to the attention of the investigators involved in this study that there has been some difficulty in filing, in the patients' notes, the completed consent forms for entry into the study, as per the study protocol.

We concluded that the most appropriate course was to review all case notes (numbering 2006) for women we believed had given informed consent to be included in the study.

By way of background, our protocol provided for two separate documents to be completed relating to consent:

1. a signed consent form to be archived in the clinical notes
2. a pink paper request form (the 'pink form') confirming that a patient had consented, to accompany the placenta to histopathology.



Cont.../

In 250 of 1433 cases, we have a 'pink form' completed by the attending doctor or midwife stating explicitly that consent has been taken, but we are not able to trace the consent form itself in the notes. This 'pink form' is signed by the person taking consent. We believe that this form indicates that consent for inclusion in the study was obtained from the patient, and so would also like to include these women in our study.

We would be very grateful for your guidance in this matter, and would be glad to meet with you or attend the committee in person if you require any more information.

Yours sincerely

Dr Flora Jessop
Consultant Paediatric Pathologist

On behalf of Mr G Hackett, Mr C Lees, Dr E Murdoch and Prof N Sebire

2

Cambridge University Hospitals NHS
NHS Foundation Trust

FAJ/LREC/jw

Tuesday, 5th January 2010

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Dr R Griffiths
Chair
Cambridgeshire 3 Research Ethics Service
Victoria House
Capital Park
FULBOURN
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CB21 5XB

Dear Dr Griffiths

Re Outcomes in pregnancy and the neonatal period: correlation with placental examinations; LREC ref 07/Q0106/51.

I wrote to you in October 2009 to make you aware of some issues with consent which have arisen in this study, and am contacting you to determine whether you are now able to issue further guidance.

I would be very grateful if you were able to advise on the inclusion of those placentae where a consent form had not been archived in the clinical notes, but which were submitted to histology with a request form counter-signed to confirm that consent for inclusion in the study had been obtained. These cases are currently not included in the study pending LREC advice, but it would be very helpful to the progress of the project if you were able to provide guidance on this point.

I do understand that you may be waiting to review this matter with the wider committee, in which case it would be very valuable to the project team to know when you may be able to consider it. Please do let me know if you require any further information.

With best wishes

Yours sincerely

Dr Flora Jessop
Consultant Paediatric Pathologist

Copy to: Mr G Hackett, Mr C Lees, Dr E Murdoch, Dr S Pathak and Prof N Sebire

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