International Journal of General Medicine
and Pharmacy (IJGMP)
ISSN(P): 2319-3999; ISSN(E): 2319-4006
Vol. 5, Issue 1, Dec- Jan 2016, 17-28

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

CORRELATION OF USS PLACENTAL PARAMETERS TO ACTUAL MEASUREMENTS AND ITS RELATIONSHIP WITH FETOMATERNAL HAEMORRHAGE

OLUSEYI OLABOYEDE A. ATANDA[1], ADEWALE SAMSON ADEYEMI[2], ADEMOLA A. AREMU[3], MUSA A. MUHIBI[4] & ADETUNJI OLADENI ADENIJI[5]

[1]Department of Obstetrics & Gynaecology, Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Nigeria

[2,5] Department of Obstetrics & Gynaecology, Ladoke Akintola University of Technology Ogbomoso, Nigeria

[3]Department of Radiology, Ladoke Akintola University of Technology Ogbomoso, Nigeria

[4]Department of Haematology, Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Nigeria

ABSTRACT

PURPOSE: Fetomaternal haemorrhage takes place across the placenta in normal pregnancies without identifiable risk factors and as well as when there are obstetric interventions or trauma related complications of pregnancy. This loss of fetal blood into maternal circulation would stimulate antibody production in the Rh D Negative pregnant woman, who is carrying a Rh D positive baby and can lead to dire consequences in subsequent pregnancies.

This study is to evaluate whether placental parameters (weight, volume, thickness and grade) measurements at term using ultrasonography and direct measurements after delivery, are true predictors of fetomaternal haemorrhage in parturients.

METHODS: In all consenting parturients, baseline bio-data, maternal blood group, Rh D status were recorded. Obstetric ultrasound was done at 36 - 38 weeks for booked parturients and in labour, for unbooked parturients, to assess the placental parameters and grade. Maternal blood sample and cord blood were taken at delivery. Maternal blood sample was tested for fetal blood cells using Kleihauer - Betke test. The FMH was calculated using Mollison's formula. Baby blood group and Rhesus status was determined from the cord blood. The placentae of these parturients were collected and its weight and volume were measured. Data generated were analyzed with Statistical Package for Social Scientists (SPSS) version 17 software. Level of statistical significance was set at $p < 0.05$.

RESULTS: A total of 600 parturients were studied, of which, 208 parturients (34.7%) had demonstrable fetomaternal haemorrhage. Large FMH were noted in 8 (1.4%) out of the 208 parturients with demonstrable FMH. None of the placental parameters [placental weight ($p = 0.893$); placental volume ($p = 0.666$); placental thickness ($p = 0.361$); and placental grade ($p = 0.585$)] showed any significant association with fetomaternal haemorrhage. CONCLUSION: Placental parameters did not prove to be predictive of FMH. There is need for further multi-centre and multi-racial studies with larger sample size to further explore other likely determinants and risk factors of FMH and the role(s) of the placenta in this regard.

KEYWORDS: Fetomaternal Haemorrhage, Kleihauer-Betke Test, Parturients, Placental Parameters

INTRODUCTION

Fetomaternal Haemorrhage (FMH) may be defined as the passage of fetal blood cells into the maternal circulation. It may pass unnoticed, but uncommonly and not rarely, it could be associated with massive volume of FMH leading to dire consequences to the fetus in utero and or the newborn.[1, 2]

The placenta is a fascinating organ, especially when its functions are considered. The placenta does not maintain absolute integrity of the fetal and maternal circulations. This is evidenced by numerous findings of the passage of cells between mother and fetus in both directions.[3, 4]Fetomaternal haemorrhage occurs transplacental; therefore there may be a link between fetomaternal haemorrhage and the placenta. Our literature search did not find any other study directly relating placenta parameters to FMH apart from that recently done in our centre.[5]

This study is designed to address the limitations of the recent study, [5]which studied the postpartum placenta parameters in 300 parturients and USS measurement of the placenta was not done. It is designed to evaluate sonographically placental thickness and pathological changes at term and analyze for correlation with physical measurements of the placental parameters (weight and volume) at delivery, with a view to determining whether placental parameters can truly be a predictor of incidence and severity of fetomaternal haemorrhage in parturients. This may probably reduce the large number of patients with fetomaternal haemorrhage that obstetric risk factors have failed to identify, apart from being a non-invasive screening tool that would have predicted FMH at term prior to delivery.

METHODOLOGY

This hospital based cross-sectional study was conducted at LAUTECH Teaching Hospitals (LTH) at both the Osogbo and Ogbomoso facilities in the Department of Obstetrics and Gynaecology.

This study was conducted between June 2010 and December 2012. Parturients irrespective of booking status and mode of delivery were included in the study after due counseling and consent. Pregnant women who declined their consent to participate were excluded from the study. Exclusion also included patients with Diabetes Mellitus, Haemoglobinopathies, Intrauterine Growth Restriction, Multiple Gestation, Eclampsia and Still births. Participants were recruited at both the antenatal clinics for booked parturients and in the labour ward for the unbooked. Booked parturients were recruited at 36 - 38 weeks, while unbooked parturients were recruited at presentation in the labour ward.

Approval from the ethical committee of LAUTECH Teaching Hospital was obtained for the study.

The sample size for this study was determined using Fischer's formula.[6] The prevalence of 17.63%[5] from the last study done at our hospital, was used in the template to calculate the sample size and was corrected for population less than 10,000.

Baseline maternal Biodata, parity, age, estimated gestational age, blood-group, Rh D status and haemoglobin electrophoresis, route/mode of delivery, obstetric interventions, baby blood group and birth weight were recorded for each patient in data collection forms "A".

All booked parturients recruited for the study had obstetric ultrasound at EGA of 36 - 38 weeks to determine placental thickness and placental grade. Consented unbooked participants had ultrasound in labour to assess same placental parameters. For the purpose of this study, Sonoace X4, a

2-Dimension ultrasound machine manufactured by Medison Co Ltd, Seoul, Korea in 2008 was used. The machine was located in the labour ward unit for convenience.

These findings were also recorded in data collection forms "B". The thickness of the placenta was measured longitudinally at the point of cord insertion.

Two mls of cord blood from a cord vessel of the placenta and two mls of maternal blood were collected into different EDTA bottles at delivery and labeled serially with "M" for maternal sample and 'B" for baby's cord blood sample. Both had the same serial number. Samples were processed at the haematology laboratory immediately, and when taken overnight stored in a refrigerator and processed later in the day. Samples were maintained at room temperature (16 - 22^{o}C) during transit as extremes of temperature compromise the quality of the sample. Samples were processed within 72 hours in order to administer Anti-D immunoglobulin to mothers who required Anti-D prophylaxis.

The maternal whole blood sample was diluted 1:2 with phosphate buffered saline and mixed well.[7] A standard thin blood film was prepared from each maternal blood sample. Each slide was spread evenly and air dried, then examined under the microscope to ensure that the red cells are touching but not overlapping each other. Immediately after air drying, the films were fixed for 5 minutes in 80% ethanol. It was then rinsed rapidly in water and allowed to stand vertically on blotting paper for about 10 minutes to dry.

The prepared slides were processed using the acid elution test of Kleihauer – Betke.[7, 8, 9] This test is dependent on the fact that adult haemoglobin is more readily eluted through the cell membrane in the presence of acid than is fetal haemoglobin which is more resistant to elution at a low pH of 1.5.[7]

The slide was placed for 20 seconds in a jar containing 7.5g/l hematoxylin in 90% ethanol (solution A) mixed with 24g $FeCl_3$ and 2.5 mol/l HCl (solution B). This is the acid elution solution at approximately pH 1.5 which was treated with a New Methylene blue buffer. After staining with the elution solution, the slide was rapidly counterstained in a solution of 2.5g/l eosin, also for 20 seconds. The slide was rinsed in tap water and allowed to air dry. The fetal red blood cells were readily distinguished from the empty maternal cells. The fetal red cells stood out as brightly stained rose pink cells in a field of "ghost" red blood cells. The new methylene blue buffer made reticulocytes stain blue while the fetal cells stained pink, thus allowing the two cells to be easily distinguished.

The slide was viewed using a x10 objective low power field to check for adequate staining and even distribution of fetal cells. The slide was then viewed using the x40 objective. A minimum of 10,000 cells were counted under the microscope and a ratio of fetal (F) to adult cells (A) generated.

On each day of the testing, blood from a newborn and blood from an adult male obtained from the blood bank (without any haemoglobinopathy) were prepared the same way and inspected on the same slide to serve as positive and negative controls respectively.

Calculation of FMH using an acid elution technique was based on the following assumptions: Fetal red cells are approximately 22% larger than maternal cells. Only 92%of fetal red cells stain darkly. Maternal red cell volume is approximately1800mls [7, 10] The fetal bleed was calculated thus:

$$\textit{Uncorrected volume of bleed} = 1800 \times \frac{\textit{fetal cells counted (F)}}{\textit{Adult cells counted (A)}}$$

Corrected for fetal volume (1.22) = (1800 × F / A) ×1.22 = J.

100/92 =1.09. *Corrected for staining efficiency (1.09) = J × 1.09 = Fetal bleed.*

A shortcut method of achieving this same calculation is to multiply the ratio of adult to fetal cells by 2400 as described originally by Mollison (1972)[11] and still in use in current guideline [7]i.e.

2400 × [F/A] = corrected bleed.

Baby blood group and Rhesus status were determined from the cord blood obtained and also recorded into the same data collection form "C" along with number of fetal cell counted and calculated volume of fetal bleed.

Placentae of all consented parturients were collected at delivery. The umbilical cord was clamped at the insertion point immediately after delivery to minimize blood loss from the fetal vasculature. The placenta was washed under running tap water, wiped to drain excessive fluid, mucous and maternal blood. It was trimmed after ligating the umbilical cord at approximately 5mm from the insertion point and removing attached membranes and blood coagula. [12] Placental weights have been reported to vary considerably if the fetal membranes and most of the cord are left attached and the adherent maternal blood clot is not removed; the weight may be greater by nearly fifty percent.[13] The trimmed placental weight was determined using an electronic balance while the volume was estimated by the method of Scherle, which involved submerging the whole placenta in water and measuring the water displaced in millilitres using a measuring jar.[12, 14]These values were recorded in data sheet "B".

All data collection forms were reviewed to eliminate forms with incomplete data entry. The data forms were merged and entered into the computer for data analysis.

Data generated was analyzed with Statistical Package for Social scientists for Windows (Version 17.0, SPSS Inc. Chicago, Illinois, USA). Frequency tables, cross-tabulations and correlations were determined. Pearson's correlation was applied to continuous variables. Level of statistical significance was set at $p < 0.05$.

RESULTS

In this prospective cross sectional study, a total of 600 parturients were included in the final study. Data from 30 parturients (27 parturients due to the exclusion criteria and 3 with incomplete data) were excluded from the analysis.

From the study population, the mean maternal age was 29.01 ± 4.38(Table 1), 77% of the parturients were either primigravidae or secondary gravidae while the others were between para 3 and para 6. A total of 600 babies were also studied with an almost equal sex distribution; female babies were 51% and male babies were 49%. The distribution of birth weight of the delivered babies showed over 90% of babies having a birth weight of between 2500g and 3900g.The mean birth weight was 3200.33g ± 396.65 (Table 1).

Table 1: Descriptive Statistics of Data

Factors	Mean (± Sd)
Age Of Parturients (Years)	29.01 (± 4.38)
Birth Weight (Grammes)	3200.33 (± 396.65)
Uss Placental Thickness (Mm)	21.44 (± 1.47)
Measured Placental Weight (Grammes)	516.89 (± 41.84)
Measured Placental Volume (Mls)	506.51 (± 41.97)

Table 2: Obstetric Factors, Maternal Age and Maternal Blood Group and Rhesus factor with Distribution of FMH

Factors	FMH		p-value	Remarks
	Negative	Positive		
Mean Age of parturients (in years)	29.1 ± 3.9	28.9 ± 5.3	*0.735	NS
Mean parity	2.0 ± 1.	2.0 ± 1.2	*1.000	NS
Mean estimated gestational age	39.0 ± 1.7	38.6 ± 1.5	*0.193	NS
Mean birth weight (in grammes)	3173 ± 394.6	3251 ± 399.1	*9.247	NS
Maternal blood group A+ A- B+ B- O+ O- AB+	 88 (22.4%) 4 (1.0%) 108 (27.6%) 8 (2.0%) 160 (40.8%) 4 (1.0%) 20 (5.1%)	 40 (19.2%) 4 (1.0%) 52 (25.0%) 0 (0.0%) 88 (42.3%) 20 (9.6%) 4 (1.9%)	**0.193	NS
Maternal rhesus factor Positive Negative	 376 (67.1%) 16 (40.0%)	 184 (32.9%) 24 (60.0%)	**0.081	NS

* T-test used ** Chi-square test used NS – Not Significant

The means of the morphology of the 600 placentae studied are as follow; mean placental thickness measured on USS was 21.44mm ± 1.47, mean placental weight was 516.89g ± 41.84 and mean placental volume was 506.51mls ± 41.97 (Table 1). The distribution of placental volume, placental weight, USS measured thickness of placenta and grade of placenta areas shown a taglance fromTable4below. Fetomaternal haemorrhage was positive in 208 (34.7%) parturients of the study population with 8 (1.4%) out of these having large FMH and in 392 (65.3%) parturients, there was no demonstrable FMH. There was no incidence of massive fetomaternal haemorrhage recorded.

In the study population, comparing obstetric factors between the two groups with and without FMH, obstetric factors {parity (p = 1.000); EGA (p = 0.193); birth weight (p = 0.247)}; and maternal age (p = 0.735) showed no significant difference with FMH (Table 2). Also, maternal blood group (p = 0.193) showed no significant difference between the two groups (Table 2). There was also no significant FMH (p = 0.081) when comparing Rh positive parturients against Rh negative parturients(Table2). In this study, there was positive correlation between placental weight and placental volume (0.999) which was significant (p = <0.005), placental weight also had positive correlation with placental thickness (0.634) and significant (p = <0.005). There was positive correlation between placental thickness and baby's birth weight (0.641) which was also significant (p = <0.005) (Table 3). These correlations were significant at the 0.01 level – 99% degree of confidence. This implies that as the placental weight increases, so does the placental volume, as placental weight increases, the placental thickness will also increase and placental thickness increases as baby's birth weight increases. Placental parameters were explored as possible determinants of fetomaternal haemorrhage. Placental weight (p = 0.893) did not show any significant difference between the two groups (Table 4). Volume of placenta (p = 0.666) did not have an influence on FMH as there was no significant difference in the two groups (Table 4). Placental thickness (p = 0.361) was also not significantly different when comparing both groups (Table 4). The observed grade of placenta on USS (p = 0.585) did not influence occurrence of FMH as there was no significant difference in the two groups (Table 4).

Table 3: Pearson's Correlation for Placental Parameters and Baby Birth Weight

Variables	Correlation	Significance
Placental Weight Vs Placental Volume	0.999**	<0.005
Placental Weight Vs Uss Placental Thickness	0.634**	<0.005
Uss Placental Thickness Vs Baby Birth Weight	0.641**	<0.005

Correlation is significant at the 0.01 level (2 tailed)

**= CORRELATION IS POSITIVELY SIGNIFICANT

Table 4: Pattern of Volume of Fmh Compared with Measured Placental Weight, Volume, Uss Thickness And Grade

Factors	FMH (Percent)		Chi-square	p-value
	Negative	Positive		
Grouped placental weight (in grammes) < 450 450 – 550 > 550	 48 (12.2) 240 (61.2) 104 (26.6)	 28 (13.5) 132 (63.4) 48 (23.1)	0.227	0.893 Not Significant
Grouped placental volume (in mls^3) < 450 450 – 550 > 550	 60 (15.3) 248 (63.3) 84 (21.4)	 36 (17.3) 140 (67.3) 32 (15.4)	0.813	0.666 Not significant
Grouped placental thickness (in mm) < 20 ≥ 20	 76 (19.4) 316 (80.6)	 28 (13.5) 180 (86.5)	0.833	0.361 Not significant
Placental grade on USS 0 1 2 3	 4 (10.0) 116 (29.6) 252 (64.3) 20 (5.1)	 0 (0.0) 76 (36.5) 128 (61.5) 4 (1.9)	1.942	0.585 Not significant

DISCUSSIONS

In this hospital based cross sectional study, the incidence of FMH is 34.7%, while that of large FMH is 1.4%. This incidence of significant FMH is much higher than that recorded in earlier studies by Adeniji et al (2008 and 2013)[5, 15]done in the same centre. Though these values are noted to be higher than values quoted in much older literatures.[1]However, there are many other recent studies reporting similar higher incidences. Salim et al (2005)[16]reported an incidence of between 6.4% and 9.6% of large FMH in the different study groups in Israel. A study in Kenya by Kizza A. P. et al (1990) [17] also reported an incidence of 32.2% and went further to suggest that higher values are been reported in blacks than in Caucasian studies.[17] Another study in Mannitoba, Kenya reported an incidence of 64.5% with a much higher incidence of large FMH compared with other studies.[1]In a recent study which looked at FMH in women undergoing caesarean section, an incidence of 28.1% was alsoreported.[18] The incidence of large FMH of 1.4% recorded in this study was consistent with most studies which had reported an incidence of between 0.3- 2.5%.[18]Wiley and D'Alton (2010) did a review of publications of FMH from January 1966 – December 2008 and concluded that there were inconsistencies in incidences of FMH reported.[19]

These inconsistencies may be due to poor reproducibility reported and the fact that FMH studies are highly dependent on technical skills. These reasons may not be solely responsible for the high incidence in this study. It is also likely due to the fact that this study was also conducted among blacks as suggested earlier by Kizza A. P. et al (1990) [17] that

incidences are higher in blacks.

Maternal obstetric risk factors and maternal blood group had been implicated as determinants of FMH.[1, 2]However, individual studies in literature have reported inconsistencies in risk based approaches to FMH.[2, 20] In this study, parity (p = 1.000), EGA (p = 0.193), baby birth weight (p = 0.247), maternal blood group (p = 0.193) and age of parturients (p = 0.735), did not show any significance for FMH (Tables 2). Maternal Rh factor (p = 0.081) did not also pose a significant risk factor for FMH as there was no significant FMH between the Rh positive and Rh negative groups (Table 2). The Rh negative parturient is important because of the possible iso-immunization that could occur when carrying a Rh positive fetus. The 42 years review of FMH by Wiley and D'Alton also confirmed these inconsistencies and maintains that more than 80% of cases of FMH remained unexplained.[19]

Placental parameters studied in this population showed similarity with other studies.[13, 21] The mean of placental parameters reported here (Table1) when compared with those reported in standard texts show slight differences. The mean volume and weight of 506.51mls and 516.89g respectively are slightly higher than the reported means of 497mls and 508g. The mean thickness of 21.44mm reported here is however lower than that of 23mm reported earlier.[13] It should be noted that these means are from Caucasian studies and there is paucity of research work in Africans to compare with. A wide variation has been reported in literature for these individual placenta parameters [13, 22, 23, 24]and when the means are considered as a range as reported in some literature, [13]these results are also within the range. No study was cited in Nigeria reporting placental parameters.

The positive correlation between USS thickness and baby birth weight (0.641), placental weight and placental volume (0.999) and placental weight with USS thickness (0.643) (Table 3) is in accordance with all other studies comparing placental parameters,[21, 25, 26, 27]except the previous placenta study[5]done in our centre which did not show a positive correlation between weight and diameter of placenta. In this study, there were linear relationships between all these placental parameters; placentae with reduced thickness on USS were associated with a low birth weight baby and reduced placental weight and volume at birth. Also, in those with increased placental thickness, there was increase in placental weight and placental volume. It can be inferred that one placental parameter is predictive of the other and eventual birth weight of the baby.

In this study, none of these morphological parameters of the placenta including grade of placenta proved significant statistically for FMH (Tables 4). This study aimed at assessing whether placental anatomy (weight, volume and thickness) influences the occurrence and severity of FMH in normal pregnancies. Assuming these placental parameters in this study showed a significant difference between the two groups, the placenta could then have been suggestive of FMH. Considering that we already know that a positive correlation exists between these placental parameters, if obstetric USS was done at 36 weeks and the placental thickness was suggestive of FMH, this would lead to increased surveillance for FMH in Rh D Negative pregnant women. In such women the placental weight and volume would be measured at birth to further predict severity of FMH and identify those Rh D Negative pregnant women who would require Kleihauer-Betke test to determine volume of FMH and dose of anti- D immunoglobulin to be administered. The findings in this study did not show that placental parameters could be used as determinants of FMH which is in contrast to the previous study[5]done in the same centre with a smaller sample size. These two results from our centre appear as isolated conflicting reports as no other study was cited in literature comparing placental morphology with FMH.

Sivarao, S. (2002) [14] and Mayhew, T. M. (2005)[28] had suggested that placental morphology and parameters can

provide valuable information on pregnancy outcomes. Some studies have implicated the placenta in some case reports of FMH. Diseases of inflammation of the placenta such as preeclampsia and chorioamnionitis were reported to be associated with FMH.[29] Some other studies have also reported an association between the placenta and low birth weight babies, maternal diabetes mellitus, intra uterine growth restriction and fetal hydrops. Some of these changes include placentomegaly while some have small placenta with reduced thickness.[25, 30, 31] In a review by Wiley and D'Alton, (2010), [19] it was suggested that certain placental lesions such as intraplacental haemorrhage, intervillous thrombosis, placental infarcts and retroplacental haematoma were more frequently observed in pregnancies with evidence of FMH.[19] However, the morphology of these placentae were not mentioned. Another study by Al-Mufti et al, (2003), [32] which compared FMH and its severity with number of fetuses, reported that there was increased severity with higher order pregnancies. Also, Adeniji et al, (2008), [15] suggested similar relationships. Al-Mufti went further to suggest that the increase in fetomaternal cell trafficking was possibly related to increased placental surface area and vasculature. In another study by Wegrzyn, P. et al, (2005), [33] which looked at placental parameters at 11-14 weeks and prediction of chromosomal defects, the study did not prove significant except for trisomies 13 and 18 which were associated with early onset fetal growth restriction. In all these highlighted pathological situations, the placenta is either smaller than the average or there is placentomegaly, but, in none of them was the placenta morphology directly examined as a contributort o FMH.

The placental grade in this study did not also influence occurrence of FMH (Table 4). FMH occurred in the different grades of placenta and there was no consistency. No study was cited relating placental grade with occurrence of FMH. Earlier studies had only implicated the grade of placentain the prediction of fetal pulmonary maturity.[30]

The difference between this study and earlier studies mentioned may probably be due to the fact that pathological conditions were not present in our study group. One may then ponder to ask whether it is the pathological conditions of the placenta without the influence of morphology that contributes to FMH. Also, our findings contradicts the previous study[5] done in our centre which assessed some placental parameters and FMH; demonstrable and large FMH occurred only in placentae weighing more than 500g, whereas in this study it occurred in placenta weighing less than and greater than 500g This study therefore suggests that there was no critical weight to predict occurrenceof FMH.

The limitation of this study may be the fact that placental parameters such as volume and surface area were not measured ultraso no graphically.

There have been cases of unexpected massive fetomaternal haemorrhage without antecedent identifiable risk factors; such findings have stressed the need to find better ways of predicting FMH in the absence of clinical suspicion. However, this study assessing placental parameters as determinants of fetomaternal haemorrhage did not provide solution to this intriguing concept. Also, as there have been conflicts in various reports looking at obstetric risk factors as determinants of FMH, the Rh-D negative woman with a Rh-D positive baby should have Kleihauer-Betke test to determine volume of FMH and appropriate dose of anti-D immunoglobulin instead of the standard dose given to all.

From the foregoing, it is hereby recommended that globally, more studies, with larger sample sizes preferably multicentre and multiracial are required to establish determinants and risk factors for FMH, and these could possibly give more consistent results.

In Africa, there is a need to investigate FMH further and confirm whether truly there is a higher racial difference in the incidence of FMH.

For now, the understanding of FMH remains far from complete.

ACKNOWLEDGEMENTS

We are grateful to all our patients who consented to participate in this study and all our resident doctors that assisted in the conduct of the study.

DISCLOSURE

The authors report no conflicts of interest in this work.

REFERENCES

1. de Almeida V, Bowman J M. Massive fetomaternal haemorrhage. Manitoba Experience. Obstet Gynecol 1994; 83: 323-8.
2. Giacoia G P. Severe fetomaternal hemorrhage: a review. Obstet Gynecol Surv 1997; 52: 372-80.
3. Gary Cunningham F, Norman Gant F, Kenneth J, et al. The Placenta and Fetal Membranes, Chapter 5, in Williams Obstetrics, 21st ed. New York: The McGraw –Hill Companies, Inc. 2001
4. Benirschke K. Timing fetal injury: the role of the placenta. OBG Management 2000(March): 72-87.
5. Adetunji O. Adeniji, Oluseyi A. Atanda, Musa A. Muhibi, Adewale S. Adeyemi. Role of Placenta Parameters in Predicting Significant Feto-Maternal Haemorrhage. ijcm 2013; 4: 133-136. doi:10.4236/ijcm.2013.43024
6. James Bartlett E, Joe Kortlik W, Chadwick Higgins C. Organizational Research: Determining Appropriate Sample Size in Survey Research. Information Technology, Learning and Performance Journal 2001; Vol 19, No. 1: 43-50.
7. Austin E, Bates S, de Silva M, Howarth D, Lubenko A et al. Guidelines for the Estimation of Fetomaternal Haemorrhage. BCSH FMH 2009.
8. BCSH. Blood transfusion and Haematology task forces. The estimation of fetomaternal haemorrhage. Transfusion Medicine 1999; 9: 87-92.
9. Raafat A, Fraser N, Main R, Urbaniak S J. A quality assurance scheme for the Kleihauer test: The Scottish experience 1988-1996. Transfusion Medicine 1997; 7; 3: 221-6.
10. Howarth D J, Robinson F M, Williams M, Norfolk D R. A modified Kleihauer technique for the quantification of fetomaternal haemorrhage. Transfusion Medicine 2002; 12; 6: 373-78.
11. Mollison P L. Quantification of transplacental haemorrhage. BMJ 1972; 3; 818: 115.
12. Claire Langston, Cynthia Kaplan, Trevor Macpherson, Elizabeth Manci et al. Practice Guideline for Examination of the Placenta. Arch Pathol Lab Med 1997; 121: 449-476.
13. Gary Cunningham F, John Hauth C, Keneth J, et al. Implantation, Embryogenesis and Placental Development, Chapter 3, in Williams Obstetrics. 22nd ed. New York: The McGraw-Hill Companies, Inc. 2005.
14. Sivarao S, Vidyadaran M K, Jammal A B E, Zainab S, Goh Y M, Ramesh K N. Weight, Volume and Surface

Area of Placenta of Normal Pregnant Women and their Relation to Maternal and Neonatal parameters in Malay, Chinese and Indian Ethnic Groups. Placenta 2002; 23: 691-696.

15. Adeniji A O, Mabayoje V O, Raji A A, Muhibi M A, Tijani A M, Adeyemi A S. Feto-maternal haemorrhage in parturients: Incidence and its determinants. J Obstet Gynecol 2008; 28; 1: 60-3.
16. Salim R, Ben Schlomo, Nachum Z, Mader R, Shaler E. The incidence of large fetomaternal haemorrhage and the Kleihauer-Betke test. Obstet Gynecol 2005; 105: 1039-1044.
17. Kizza A P, Rogo K O. Fetomaternal haemorrhage in Kenya. East Afr Med J 1990; 67(11): 801-7.
18. Denise M. Pelikan, Humphrey H. Kanhai, Go delieve M. de Goot-Swiggs, Hans J. Tanke, Sicco A. Schergon. Fetomaternal haemorrhage in women undergoing caesarean section. [https://openaccess.leidenuniv.nl/bitstream/handle/1887/4347/05.
19. pdf?sequence=10] (Submitted for publication and accessed online via internet Google search on 30-03-2013).
20. Blaire J. Wiley, Mary E. D'Alton. Fetomaternal Hemorrhage. Obstet Gynecol 2010; 115: 1039-51.
21. Lau M S K, Tan J V K, Tan T Y T, Gomez J M, Yeo G S H. Case of FMH Resulting in Hydrops. Ann Acad Med Singapore 2003; ss32: 642-4.
22. Gabbe S G, Niebyl J R, Simpson J L. Obstetric Ultrasound: Assessment of Fetal Growth and Anatomy, Chapter 10, in Obstetrics - Normal and Problem Pregnancies. 4th ed. Philadelphia: Churchill Livingstone, Inc. 2002.
23. Gabbe S G, Niebyl J R, Simpson J L. Placental and Fetal Physiology, Chapter 2, in Obstetrics - Normal and Problem Pregnancies. 4th ed. Philadelphia: Churchill Livingstone, Inc. 2002.
24. Burton G J, Kaufman P, Huppertz B. Anatomy and genesis of the Placenta, in Physiology of Reproduction. 3rd ed. New York: Elsevier, 2006.
25. Berthold Huppertz, John Kingdom C P. The Placenta and Fetal Membranes, Chapter 3, in Dewhurst's Textbook of Obstetrics and Gynaecology. 7th ed. Oxford: Blackwell Publishing, 2007.
26. Mayhew T M, Ohadike C, Baker P N, Crocker I P, Mitchell C, Ong S S. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. Placenta 2003; 24: 219-226.
27. Virupaxi R D, Potturi B R, Schirol V S, Desai S P, Hukkeri V B. Morphology of the placenta and its relation with small for date babies in 950 live births. Rec-Res Sci Tech 2011; 3(2): 123-126.
28. Fawzia A. Habib. Prediction of Low Birth Weight Infants From Ultrasound Measurement Of Placental Diameter and Placenta thickness. Ann Saudi Med 2002; 22(5-6): 312-314.
29. Mayhew T M. The Human Placenta And The Search For Structural Correlates Of Fetal Well-Being. In Proceedings of a workshop: Comparative Placentology. Havemeyer Foundation Monograph Series No. 17, 2005.*(www.havemeyerfoundation.org/PDFfiles/Monograph17.pdf*) accessed online 29-03-2013.
30. Scholz C, Herman C, Kachler A, Kainer F, Friese K, Makrigiannakis A, Jeschke U. Association of placental inflammation with fetomaternal haemorrhage and loss of placental mucin-1.Arch Gynecol Obstet 2012

Mar;285(3):605-12.

31. Benirschke K, Kaufman P. Pathology of the Human Placenta. 4th ed. New York: Springer. 2000.
32. Benirschke K, K P. Architecture of Normal Villous Trees, Chapter 7, in Pathology of the Human Placenta. New York: Springer Verlag. 2002. p116-154.
33. Al- Mufti R, Hambley H, Farzaneh F, Nicolaides K H. Distribution of fetal erythroblasts enriched from maternal blood in multifetal pregnancies. Human Reprod 2003; 18; 9: 1933-6.
34. Wegryzyn P, Faro C, Falcon O, Peralta C F A, Nicolaides K H. Placental volume measured by three-dimensional ultrasound at 11 to 13+6 weeks of gestation: relation to chromosomal defects. Ultrasound Obstet Gynecol 2005; 26: 28-32.