ORIGINAL ARTICLE

MORPHOMETRICAL ANALYSIS OF INTERVILLOUS SPACE AND VILLOUS MEMBRANE THICKNESS IN MATERNAL ANAEMIA

Nazma Kiran, Alia Zubair, Henna Khalid, Ambreen Zafar

Department of Pathology, Army Medical College, Rawalpindi, Pakistan

Background: Anaemia in pregnancy is linked with an increased risk of preterm delivery, low birth weight, perinatal and maternal mortality and it is related with variable histo-morphological changes in placenta which show a reflection for the poor foetal outcome. The objective of this study is to assess the micro-morphometery of intervillous space and villous membrane of placenta in anaemic mothers. **Methods:** This case control study was carried out at Army Medical College, National University of Sciences and Technology Islamabad in collaboration with Department of Obstetrics and Gynaecology, Military Hospital, Rawalpindi, Pakistan, from December 2011 to November 2012. A total of 75 placentas were included, that were divided into study and control group. In control group (n=15) placentas were taken from mothers having normal haemoglobin levels and study group (n=60) included placentas from anaemic mothers having haemoglobin less than 11g/dl. Study group was subdivided into three groups, i.e., mildly (10.0-10.9g/dl), moderately (7.0-9.9g/dl) and severely (<7g/dl) anaemic group. Three representative sections were taken from placenta, i.e., one close to umbilical cord (A), one from periphery (C) and one midway between A and C (B).Ocular micro-meter was used to measure intervillous space and villous membrane thickness. **Results:** Intervillous space was prominent in study groups (41.26±16.33ìm) as compared to control group (15.98±3.81ìm) (p<0.05). Thickness of villous membrane was significantly less in study group as compared to control group (2.97±0.70) (p<0.05). **Conclusion:** The present study showed wide intervillous space and thin villous membrane in study group as compared to control group reflecting adaptive changes in response to hypoxiain maternal anaemia.

Keywords: Intervillous space, villous membrane, maternal anaemia, Placenta

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INTRODUCTION

Anaemia in pregnancy is a public health issue, mainly in developing and underdeveloped countries.[1] In pregnant women this condition is linked with an increased risk of preterm delivery, low birth weight, perinatal and maternal mortality.[2] According to WHO standards, maternal anaemia is present when the haemoglobin level in blood is less than 11gm/dl.[3] Global prevalence of maternal anaemia is 41.8%, in South-east Asia it is 48.2% and in Pakistan 39.1% pregnant women are anemic.[4] In many developing countries, prevalence rates of up to 75% are reported.[5] In Pakistan, anaemia during pregnancy is supposed to be more common and according to local studies it is approximately 90%, varying slightly geographically.

Infants of anaemic women are born with reduced iron stores and are at risk of anaemia during infancy and increased risk of infant morbidity and mortality.[3]Normal foetal development depends on the appropriate function and growth of the placenta.[6]Severe maternal anaemia may impair the oxygen delivery to the foetus and results in intrauterine growth retardation, still birth, low birth weight and neonatal deaths.[3] If after child birth, the placenta is studied thoroughly, it provides great knowledge about the prenatal wellbeing of mother and baby.[7]

The placental tissue can be described as a sum of parenchymal, non-parenchymal and pathological components. The parenchyma comprises intervillous space and villi along with blood vessels; the non-parenchymal component comprises decidual and chorionic plates, fetal blood vessels and maternal intercotyledonary septa.[8]

Anaemia in pregnancy is related with variable histomorphological changes in placenta, including widening of intervillous space and decrease in thickness of villous membrane, which show a clear reflection for the poor foetal outcome.[9]

In maternal anaemia the placenta adjusts by thinning of villous membrane and increase in the volume of intervillous space so that diffusion capability is retained at normal levels. As described in previous data that demonstrates a negative correlation between levels of maternal haemoglobin and volume of intervillous space.[10] Morphometric analysis of villous membrane thickness, carried out in Ukraine, revealed that mean thickness of villous membrane is considerably reduced in case of anaemia due to an increase in the volume fraction of foetal capillaries. Therefore, morphometric diffusing capability of villous membrane is retained.[11]

There is a need to explore the extent of above mentioned structural changes, because severity of these histomorphological parameters is correlated with the efficiency of placenta to support the growth of a foetus, and this condition is likely to be related to insufficient function of the placenta.[12] The present study was aimed to evaluate the histopathological parameters of the placenta in maternal anaemia in quantitative manner, to assess the effects of anaemia on placenta, in our setup.

MATERIAL AND METHODS

This case control study was carried out in Pathology Department of Army Medical College, Rawalpindi, National University of Sciences and Technology (NUST), Islamabad in collaboration with Military Hospital, Rawalpindi from December 2011 to November 2012. A total of 75 placentas, after informed consent, were included in the study, that were divided into control (Group A, n: 15) and study group (Group B, n: 60). Cases included in control group had normal hemoglobin levels, i.e., ≥11g/dl and the study group comprised placentas from anaemic mothers (haemoglobin <11g/dl). Study group was subdivided into three groups according to severity of anaemia, i.e., mildly (10.0–10.9g/dl), moderately (7.0–9.9g/dl) and severely (<7g/dl) anemic group.[13] The placentas of the mothers having systemic disorders, i.e., diabetes mellitus, hypertension, asthma and antepartum haemorrhage were not included in the study.[14]

Three representative sections were taken from each placenta, i.e., one close to umbilical cord (A), one from periphery (C) and one midway between A and C (B). Additional sections were taken from pathological area, if any.[15,16] The tissues were processed and routine staining was done with H & E stain. The slides were then examined under light microscope for intervillous space and villous membrane thickness.

For morphometric analysis, width of intervillous space was measured in 5 random fields per each slide under 100X objective with ocular micrometer and mean was calculated. Measurement of villous membrane was done in the region of syncytiotrophoblast and capillary endothelium with minimal stroma and without intervening nucleus. Thickness of villous membrane was measured with ocular micrometer. Five random terminal villi per each slide were selected under 100x objective and mean was calculated.[17]

Data was analysed using SPSS-17. Mean and standard deviation were calculated for quantitative variables. Percentage was calculated for qualitative variables. Quantitative variables were compared through analysis of variance (ANOVA) while qualitative variables were compared through chi-square test. Pearson correlation test was used for calculating relationship between variables. *p*-value less than 0.05 was considered as significant ($p<0.05$).

RESULTS

The mean haemoglobin of control group was 11.83g/dl and of study group was 8.62g/dl with significant *p* value ($p<0.05$).Among the study group, mild anaemia was found in 19 cases (25.3%) with mean haemoglobin level of 10.4g/dl. Maximum number of cases (n: 26) revealed moderate anaemia (mean 8.6g/dl).Severe anaemia (mean 6.4g/dl) was found in 15 cases.

Analysis of intervillous space and villous membrane thickness is demonstrated in (Table-1). Intervillous space can be seen in mild anaemic group (Figure-1) and Figure-2 is showing thin villous membrane in severe anaemia. The difference in intervillous space of the placenta was statistically significant ($p<0.05$) between Group-A & B1, A & B2, A & B3, B1 & B2 and B1 & B3 (Table-2). Intervillous space increased with severity of anaemia and a strong, linear and negative correlation (Pearson's correlation coefficient: -0.707) was found between intervillous space and mother's haemoglobin level (Figure-3).

According to the data presented in Table-2, cases with mild anaemia (Group-B1) did not reveal much difference in villous membrane thickness when compared with non-anaemic placenta (control group) (Figure-4). Villous membrane thickness was markedly reduced in placentas with severe anaemia (1.78±0.37µm) (Figure-5).

The quantitative difference between mean thickness of villous membrane was statistically significant ($p<0.05$)between group A and B2, A and B3, B1 and B2 and B1 and B3 (Table-3).It was found that thickness of villous membrane was significantly less in study group as compared to control group. Positive, linear and strong correlation (Pearson's correlation coefficient: 0.658) was found between villous membrane and mother's haemoglobin level (Figure-6).

Table-1: Width of intervillous space and villous membrane thickness in control and study groups

Groups		Number of cases (n)	Intervillous space (µm) Mean±SD	Villous membrane Mean±SD
Control group (Group A)		15	15.98±3.82	2.97±0.70
Study group (Group B)	Mildly anaemic group (Group B1)	19	28.29±11.07	2.85±0.72
	Moderately anaemic group (Group B2)	26	45.71±16.84	2.06±0.39
	Severely anaemic group (Group B3)	15	49.96±10.70	1.78±0.37

Table-2: Comparison of width of intervillous space and villous membrane thickness in control and study groups

Groups	Intervillous space	Villous membrane
Control (Group A) vs. mildly anaemic (Group B1)	$p<0.05$*	$p>0.05$
Control (Group A) vs. moderately anaemic (Group B2)	$p<0.05$*	$p<0.05$*
Control (Group A) vs. severely anaemic (Group B3)	$p<0.05$*	$p<0.05$*
Mildly (Group B1) vs. moderately anaemic (GroupB2)	$p<0.05$*	$p<0.05$*
Mildly (Group B1) vs. severely anaemic (Group B3)	$p<0.05$*	$p<0.05$*
Moderately (Group B2) vs. severely anaemic (Group B3)	$p>0.05$	$p>0.05$

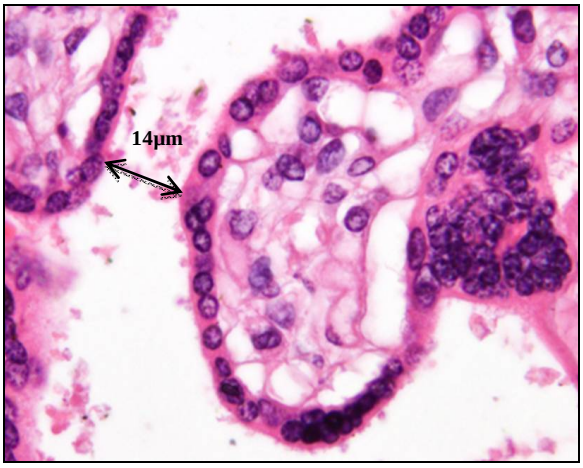

Figure-1: Photomicrograph of placental tissue of mildly anaemic group (B) exhibiting tertiary villi and intervillous space (H&E stain x 1000)

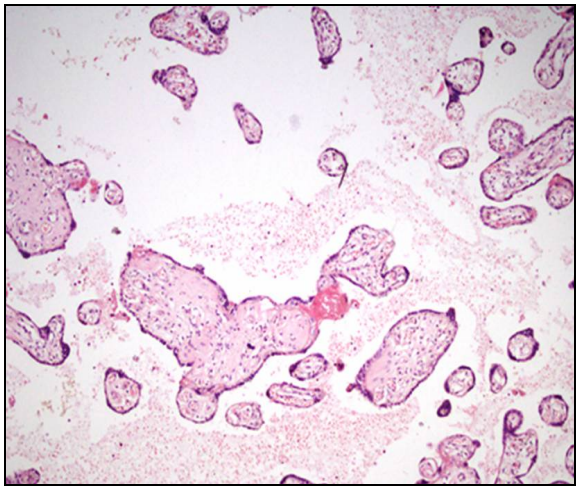

Figure-2: Photomicrograph of placental tissue of severely anaemic group (B3) revealing chorionic villi with prominent intervillous space (H&E stain x 100)

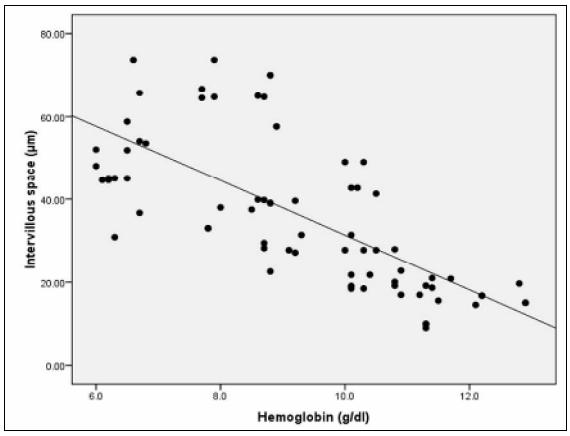

Figure-3: Correlation between intervillous space and mother's haemoglobin level

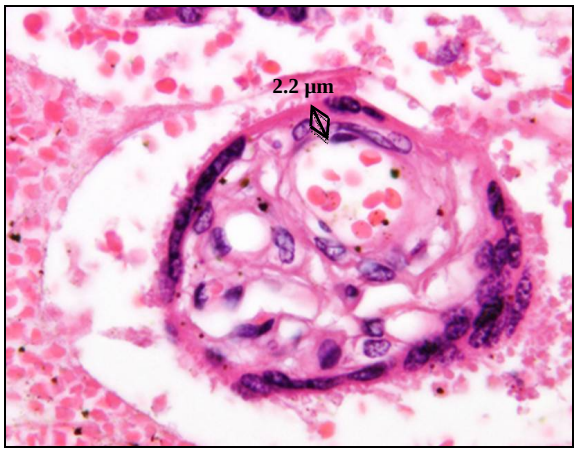

Figure-4: Photomicrograph of placental tissue of mildly anaemic group (B1) revealing tertiary villous with thick villous membrane (H&E stain x 1000)

Figure-5: Photomicrograph of placental tissue of severely anaemic group (B3) indicating tertiary villous with thin villous membrane (H&E stain x 1000)

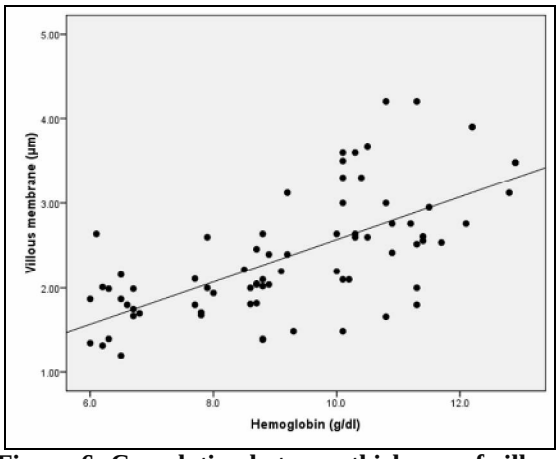

Figure-6: Correlation between thickness of villous membrane and mother's haemoglobin level

DISCUSSION

Anaemia is potentially a harmful hematological disorder that may occur in pregnancy and is known to have effects on many important organs of body. Severe anaemia in pregnancy is considered as a cause of pre-placental hypoxia which may give rise to fetal hypoxia and its complications.

The placenta is a functional component among foetus and mother. Therefore, any pathological incident related to mother or foetus, will affect the function of placenta, causing histopathological changes. With this background, the present study was undertaken to evaluate the spectrum of histopathological changes in placenta during pregnancy.

Histological appearance of intervillous space revealed some differences in two groups. It was prominent in study groups as compared to control group ($p<0.05$). Huang *et al* also made similar observation i.e., the intervillous space is found to be increased in the anaemic group and there is a significant negative relationship among total volume of intervillous space and mother's haemoglobin level.[18] When pregnancy is associated with maternal anaemia, villous mass is decreased due to decreased number of villi and their size, with resultant increase in intervillous space.[10,19]

Sections of placenta revealed that the villous membrane separated the maternal and foetal blood between intervillous space and villous capillaries. Thickness of villous membrane was measured and was found significantly less in study group ($p<0.05$). Several studies support this result. In maternal anaemia mean thickness of the villous membrane is considerably decreased because the placenta adjusts by thinning of villous membrane so that diffusion and exchange is maintained at normal levels.[13] It is obvious that the effect of anaemia on placental growth is due to hypoxia. This result could be compensatory response including thinning of villous membrane.[11]

Severity of anaemia may also have different impacts on thickness of villous membrane in placenta. Although the studied population revealed decrease in villous membrane thickness, however, different findings are also available in previously published studies. One study documented thickening of villous membrane with increase in the severity of anaemia and it was observed that there is also cytotrophoblastic proliferation with thickening of villous membrane as a villous response to decreased uteroplacental blood flow showing that the placenta is hardly insufficient.[9]

Analysis of villous membrane thickness was the second component of the present study. Actual assessment of thickness is difficult because of the problem of asymmetrical appearance of the membrane arising from two sources, firstly natural disparity in real thickness and secondly due to sectioning artifacts. To avoid bias in assessment, measurement was done in area of minimum thickness.[20]

CONCLUSION

The present study showed wide intervillous space and thinning of villous membrane in study group as compared to control group. Thus our study specified some of the adaptive changes which placenta has to adapt to overwhelm the effects of anaemia. Quantitative analysis of placental changes is crucial in study of placenta as normal pregnancies can also show substantial placental changes.

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Address for Correspondence:

Dr. Nazma Kiran, Department of Pathology, Army Medical College, Rawalpindi, Pakistan. **Cell**: +92-0334-5351936
Email: nazmakiran@hotmail.com