

HISTOPATHOLOGICAL STUDY OF PLACENTA IN ANAEMIA OF PREGNANCY IN RURAL PUDUCHERRY

Rajesh Kumar M¹, Rathna S², Sowmya S³, Gandhi N⁴, Venkatraman J⁵

¹Postgraduate Student, Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

²Associate Professor, Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

³Professor and HOD, Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

⁴Professor, Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

⁵Assistant Professor, Department of Pathology, Mahatma Gandhi Medical College and Research Institute, Puducherry.

ABSTRACT**BACKGROUND**

Placenta is a mirror which reflects the intrauterine status of the foetus. It is a vital organ for maintaining and promoting normal foetal development. It undergoes different changes in weight, volume, structure, shape and function continuously throughout the gestation to support the prenatal life. Anaemia during pregnancy is one of the common and serious haematological disorder which causes changes in the structure of placenta, profound changes on the maternal circulatory system and it has serious effects both on mother and foetus. The examination of placenta in-utero as well as postpartum provides insight into prenatal health of baby and mother.

The study was undertaken to identify the histomorphological findings of all placentas and to correlate normal and abnormal placental findings in relation to anaemia of pregnancy.

MATERIALS AND METHODS

Fifty placental specimens from mothers who had anaemia of pregnancy and fifty placental specimens from mothers who had normal antenatal period were taken up for the study. The specimens were received in the Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital for a period of 2 years and were studied. The clinical data related to mother and foetus were recorded. The macroscopic appearance of the placenta was documented. Representative bits were given for routine processing and stained with Haematoxylin and Eosin stain for detailed microscopic study.

RESULTS

Placental weight, umbilical cord length and birth weight were reduced in placenta of anaemia group in comparison with control group. Increased incidence of increase in number of cotyledons, calcification and infarction was noted in placenta of anaemia group as compared to control group. The microscopic parameters like syncytial knots, calcification, infarction, fibrinoid necrosis and fibrotic villi were increased with decreased villous vascularity in anaemia group in comparison with control group. In all these variables, p value was found to be statistically significant.

CONCLUSION

Anaemia in pregnancy alters the placental morphology. Early recognition of anaemia and proper management may therefore be crucial for normal placental function. Thus, we conclude that histopathological examination of placenta is indispensable and has to be carried out as a routine protocol.

KEYWORDS

Placenta, Anaemia, Macroscopy, Microscopy.

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BACKGROUND

Placenta is a mirror which reflects the intrauterine status of the foetus. It is a vital organ for maintaining and promoting normal foetal development. It connects the developing foetus to the uterine wall thereby allowing nutrient uptake, waste elimination and gas exchange via mother's blood supply.¹ It is an important organ for the continuation of pregnancy and

foetal nutrition and has evoked great interest among pathologist and obstetrician to understand the unique biological status of this complex organ.² The examination of placenta in-utero as well as postpartum provides much insight into prenatal health of baby and mother.³

Anaemia during pregnancy is one of the common and serious haematological disorders, which is associated with late abortions, prematurity, low birth weight and stillbirths.⁴ Severity of anaemia among pregnant women is assessed based on haemoglobin percentage suggested by WHO. A level of haemoglobin below 11 g/dL during pregnancy is considered anaemia.⁵ The commonest aetiological cause for anaemia during pregnancy is iron deficiency which is mainly due to nutritional deficiency or increased demand in pregnancy. Anaemia leads to hypoxia and causes changes in structure of placenta, profound changes on the maternal circulatory system and has serious effects both on mother and foetus.⁵ Since placenta can show various macroscopic and microscopic

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Corresponding Author:

Dr. Venkatraman J,

Assistant Professor,

Department of Pathology,

Mahatma Gandhi Medical College and

Research Institute, Puducherry.

E-mail: drvenkatraman1983@gmail.com

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changes in maternal and foetal disease process, the current study was conducted in relation to the aetiology.

MATERIALS AND METHODS

It is a descriptive study on placental specimens which were received in the Pathology Department of Sri Manakula Vinayagar Medical College and Hospital from October 2014–May 2016. Fifty placental specimens from mothers who had anaemia of pregnancy and fifty placental specimens from mothers who had normal antenatal period were included for the study after obtaining Ethical Committee Clearance.

The following were included in Control Group

- Singleton uncomplicated term pregnancy.
- Baby weight >2.5 Kg.
- Gestational period >37-41 weeks.
- Blood pressure <140/90 mm of Hg throughout pregnancy.
- Age matched non-anaemic mothers whose haemoglobin value was 11 g/dL and above.

The following were included in Anaemia Group

- Singleton pregnancy.
- Gestational period >37-41 weeks.
- Blood pressure <140/90 mm of Hg throughout pregnancy.
- Anaemic patients whose haemoglobin value below 11 g/dL were included in the study.

Conditions such as associated obstetric complications of pregnancy, associated medical disorders of pregnancy and multiple pregnancies (e.g. twins, etc.) were excluded from the study.

The clinical data related to mother, foetus and the mode of delivery was recorded. The placentas from both control and anaemia groups were collected immediately following expulsion, and washed in the running tap water to remove the blood clots. The shape (round or oval) of the placenta was assessed by stretching it flat on the cutting board. The weight of the placenta was measured on a standard weighing scale. Placental diameter was measured using a thread and the thickness was measured at the centre of placenta after giving serial cuts. The foetal surface was examined for colour, transparency, subchorionic thrombus, insertion of umbilical cord and attachment of foetal membranes. The extra placental membranes were inspected for completeness, adherent blood clot, colour and transparency. The umbilical cord length was measured and it was examined for the number of vessels, true knots, false knots and insertion (central, eccentric or velamentous). The membranes were trimmed and a strip was taken as "Swiss roll". The cord was cut at about 4 cm from its insertion. The cut end of the cord was examined for the number of blood vessels. Portions of the cord from the proximal and distal regions were taken for paraffin embedding. The maternal surface of the placenta was inspected for completeness, adherent blood clots, number of cotyledons, calcification and infarction. The whole placenta was left for fixation in 10% formal saline for 24-48 hours. After fixation, the placenta was cut into vertical strips (bread loaf manner) of 0.5 cm thickness each and the macroscopic lesions were examined. The villous parenchyma was examined for calcification, infarction and intervillous thrombosis. The tissue

bits for microscopic examination were taken from cut end of umbilical cord, at placental junction, strip of membrane roll, margins of placenta, central area of placenta, any fibrotic area, any calcified area and any infarcted area. The tissue bits were then processed through series of dehydration steps and embedded in wax blocks. Tissue sections of 5 µm thicknesses were cut from paraffin embedded blocks and stained by conventional Haematoxylin and Eosin (H & E) stain.

Different histological features were quantified. One hundred terminal villi were counted in the maternal subdivision of each of the sections. Thus, in each placenta four hundred villi were counted and the incidences of various histological features were determined. The mean numbers of villi showing syncytial knots, vasculosyncytial membrane, fibrinoid necrosis, infarction, calcification and villous stromal fibrosis were determined.

Evaluation of Macroscopic Lesions

Infarction

Infarction was noted and confirmed on microscopic examination. Placentas were classified according to the presence or absence of infarction and expressed as percentage.

Calcification

Calcification was noted and confirmed on microscopic examination. Placentas were classified according to the presence or absence of calcification.

Evaluation of Histological Features

Villous Vascularity

The number of villi showing normal vascularity, increased vascularity or decreased vascularity was recorded and the results were expressed as a percentage of the total villi.

Syncytial Knots

The number of villi with syncytial knots was recorded and the results were expressed as a percentage. A true syncytial knot was only accepted as such if it was visible on low power magnification and the counts were carried out using high power magnification.

Fibrinoid Necrosis

The number of villi showing fibrinoid necrosis was noted and the total of such villi was then expressed in percentage.

Fibrosis of Villi

The number of fibrotic villi was expressed as a percentage. An individual villus was classed as "normal" or "fibrotic".

The different histological findings were observed against the background of normal standards of histology of placenta as described by Stacey et al⁶ and Fox H.⁷

Statistical Analysis

All the gross and histological features were compared between the placenta of anaemia group and control group. The data was arranged in excel workbook under suitable headings. The mean, standard deviation and standard error was calculated for different parameters. They were statistically analysed using Chi square test for qualitative variables and Student 't' test for quantitative variables. This was done using EPI info version 3.5.3. In all the tests, 'p' value less than 0.05 was taken to be statistically significant.

RESULTS

During the study period, fifty placental specimens from mothers who had anaemia of pregnancy were studied. The macroscopic and microscopic features of these specimens were compared with fifty placental specimens from the control group. Of the 50 anaemia cases, 21 (42%) were primigravida and 29 (58%) were multigravida suggesting that anaemia is more common in multigravida. Mean birth weight between anaemia group (2375.0±329.08) was less when compared to control group (2932.0±209.17). Mean placental weight in anaemia group (438.0±40.56) was less when compared to control group (476.8±33.16).

The correlation between birth weight and placental weight was calculated using Pearson correlation coefficient. Among anaemic mothers, the correlation coefficient was 0.62, positive which indicates as placental weight increases birth weight also increases. Among control group, the correlation coefficient was 0.55, positive and statistically significant which indicates as placental weight increases birth weight also increases. Placental coefficient is defined as the ratio of placental weight to foetal weight. Normally it is 0.10 to 0.18. Mean placental coefficient between anaemia groups (0.18±0.02) was more when compared to control group (0.17±0.01) (Table 1).

The mean length of the umbilical cord in anaemia group was 49.72±5.29 cm and that in control group was 54.46±3.12 cm. Hence, in the current study, umbilical cord length was significantly less in anaemia group. Calcification was seen in 12 (24%) placentas of anaemia group and 4 (8%) placentas of control group. Infarction was seen in 28 (56%) placentas of anaemia group and 9 (18%) of control group (Table 2).

The mean number of cotyledons seen in placenta of the anaemia group was 18.9±3.02 and that in control group was 17.46 ±1.58. Syncytial knots were present on more than 30% of the villi in 32 (64%) placentas of the anaemia group and 7 (14%) placentas of the control group. Hence, increased proportion of placenta in anaemia group showed syncytial knots on more number of villi when compared to control group (Table 3).

40% of villous vascularity in anaemic patients was normal compared to 70% in control group. 10% of villous vascularity in anaemic patients was increased compared to 18% in control group. 50% of villous vascularity in anaemic patients was decreased compared to 12% in control group (Table 4).

Fibrinoid necrosis was seen in more than 3% of the villi in 29 (58%) placentas of the anaemia group and 4 (8%) placentas of the control group (Table 5). Fibrosis of villi was seen in more than 3% of the villi in 29 (58%) placentas of the anaemia group and 4 (8%) placentas of the control group (Table 6).

Parameters	Anaemia (n=50)	Control (n=50)	p value
Birth Weight (g)	2375.0±329.08	2932.0±209.17	<0.001*
Placental Weight (g)	438.0±40.56	476.8±33.16	<0.001*
Placental Coefficient	0.18±0.02	0.17±0.01	<0.001*

Table 1. Association of Mean Birth Weight, Placental Weight and Placental Coefficient in Anaemia Group and Control Group

Sl. No.	Macroscopic Lesions	Anaemia Group (n=50)		Control Group (n=50)		Chi-Square Value	p value
		Number of Placenta	%	Number of Placenta	%		
1	Calcification						
	Absent	38	76	46	92	4.76	0.029*
Present	12	24	4	8			
2	Infarction						
	Absent	22	44	41	82	15.49	<0.001*
Present	28	56	9	18			

p value based on Chi square, *statistically significant p value (<0.05)

Table 2. Association of Infarction and Calcification between Anaemia and Control Group

Group	Percentage of Villi with Syncytial Knots	Number of Placenta	%	Chi-Square Value	p value
Anaemia	≤30%	18	36	26.27	<0.001*
	>30%	32	64		
Control	≤30%	43	86		
	>30%	7	14		

p value based on Chi square, *statistically significant p value (<0.05)

Table 3. Association of Syncytial Knots between Anaemia Group and Control Group

Group	Particulars of Villous Vascularity	Number of Placenta	%	Chi-square Value	p value
Anaemia	Normal	20	40	16.88	<0.002*
	Increased	5	10		
	Decreased	25	50		
Control	Normal	35	70		
	Increased	9	18		
	Decreased	6	12		

p value based on Chi square, *statistically significant p value (<0.05)

Table 4. Association of Villous Vascularity between Anaemia Group and Control Group

Group	Percentage of Villi Showing Fibrinoid Necrosis	Number of Placenta	%	Chi-Square Value	p value
Anaemia	≤3	21	42	28.27	<0.001*
	>3	29	58		
Control	≤3	46	92		
	>3	4	8		

p value based on Chi square, *statistically significant p value (<0.05)

Table 5. Association of Fibrinoid Necrosis between Anaemia Group and Control Group

Groups	Percentage of Fibrotic Villi	Number of Placenta	%	Chi-Square Value	P value
Anaemia	≤3	21	42	28.27	<0.001*
	>3	29	58		
Control	≤3	46	92		
	>3	4	8		

Table 6: Association of Fibrotic Villi between Anaemia Group and Control Group

DISCUSSIONS

In the present study, total of 100 placentas were studied which included 50 from mothers with uncomplicated pregnancies, considered as control group and 50 from mothers with anaemia, considered as anaemia group. The macroscopic and microscopic lesions were quantified and compared between the two groups. The incidence of anaemia in multigravida (58%) is more when compared to primigravida (42%). The mean weight of the placenta in anaemia group (438.0±40.56 g) was less compared to control group (476.8±33.16 g). Mongia et al⁸ in his study observed that decreased placental weight in low haemoglobin concentration, similar to the present study. But Beischer et al⁹ and Lao et al¹⁰ in their study observed that the maternal anaemia was associated with placental hypertrophy.

In the present study, correlation between the placental weight and birth weight indicated a positive correlation in both anaemia and control groups. This indicates that if the placental weight decreases the birth weight of the infant also decreases. Ren et al¹¹ in their study observed that in maternal anaemia, the foetal weight decreases as the placental weight decreases. The results were similar in the current study. Placental coefficient is defined as the ratio of placental weight to foetal weight. Normally it is 0.10 to 0.18. The placental coefficient decreases as the placental weight increases and high placental co-efficient is seen if the placental weight decreases.¹²

In the present study, placental coefficient in anaemia group (0.18±0.02) is more when compared to control group (0.17±0.01). Similar results were seen in the study conducted by Rangnekar AG et al¹³ The mean length of the umbilical cord in anaemia group was less (49.72±5.29 cm) when compared to control group (54.46±3.12 cm). These results were similar to the study conducted by Adil et al.¹⁴

Calcification is common in human placenta and it is a normal feature of maturation and senescence of this organ. Increased calcification of placenta has complications like foetal growth restriction and foetal distress. Tindall et al¹⁵ in their study reported that placental calcification was low in anaemia. But in the current study, placental calcification was seen more in anaemia group (24%) when compared to control group (8%). In current study, placental infarcts were found to be significantly more in anaemia group (56%) when compared to control group (18%). Begum et al¹⁶ and Huang et al¹⁷ observations were similar to the present study. In the current study, the mean number of placental cotyledons were significantly increased in anaemia group (18.9±3.02) when compared to control group (17.46±1.58). The results were similar to the study conducted by Mongia et al.⁸ Knots in more than 30% of the villi are considered excessive. In the present study, syncytial knot counts were more in anaemia group (64%) when compared to control group (14%). Mongia et al⁸

and Kristina et al¹⁸ studied histological features of placenta in anaemia which showed an increase in the number of syncytial knots.

The villi of the mature placenta usually contains between 2-6 capillary vessels which are sinusoidally dilated. In the present study, there was significant decrease in villous vascularity in anaemia group (50%) as compared to control group (12%). Joshi et al¹⁹ in their study observed that villous hypovascularity was seen in anaemic mothers. Placenta in which fibrinoid necrosis involving up to 3 percent of placental villi is considered as normal and placenta in which the percentage of villi showing fibrinoid necrosis of more than three percent is considered as abnormal.²⁰ In the present study, fibrinoid necrosis was more in anaemia group (58%) when compared to control group (8%). Mehrota et al²¹ and Joshi et al²⁰ in their study observed increased intravillous and perivillous fibrin deposition. Both of their results were in concurrence to the present study. In term placenta, less than 3 percent of villi show fibrosis, and if more than 3 percent of villi in a placenta show fibrosis it is considered as abnormal.²² In the present study, fibrotic villi were more in anaemia group (58%) when compared to control group (8%). Usha Rusia et al²³ and Mehrota et al²² observed that villous fibrosis increases in amount with increasing grade of anaemia. Both of their results were similar to the present study.

CONCLUSIONS

Placenta depicts the most accurate record of prenatal experience of an infant and it undergoes different changes in weight, volume, structure, shape and function continuously throughout the gestation to support the prenatal life. Placental morphology plays a vital role in both maternal and foetal well-being during pregnancy. Macroscopic examination of placenta in anaemia showed decreased mean foetal birth weight, placental weight and cord length. There was increased incidence of increased number of cotyledons, calcification and infarction. Microscopic examination of placenta in anaemia showed excessive syncytial knots, increased fibrinoid necrosis, increased fibrotic villi, and decreased villous vascularity. Anaemia in pregnancy alters the placental morphology. Early recognition of anaemia and proper management may therefore be crucial for normal placental function. Thus, we conclude that histopathological examination of placenta is indispensable and has to be carried out as a routine protocol.

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