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RESEARCH ARTICLE

STUDY OF HISTOPATHOLOGICAL CHANGES IN PLACENTA IN ANAEMIA DURING PREGNANCY

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ABSTRACT

Anaemia is a potential hazardous hematological disorder that may occur in pregnancy. It is important risk factor for uteroplacental insufficiency and is associated with late abortions, prematurity, low birth weight and stillbirths. The present study aimed to assess the histological changes of placentas associated with maternal anaemia (mothers with Hb level < 11 g/dl) were fifty (50) and those collected from control mothers were fifty (50). All the deliveries were at full term (37-41 weeks) periods and without any obstetric complications or diseases. Microscopic analyses of the placentas were done and these findings were compared. Statistical analysis was performed by using t-test for comparing the mean values of fetal weights, placental weights and placental indices of the maternal anaemia group with those of control group. It was observed that the mean fetal weight of pregnancy with anaemia group was less than those of the control group. The mean placental weight in pregnancy with the anaemia group was more than the control group. Also, the mean placental index of the maternal anaemia group was higher than that of the control group. Histopathology study revealed decreased villous vascularity, excessive syncytial knots, increased fibrinoid necrosis of the villi, increase in villous stromal fibrosis in placentas of anaemic mothers as compared to those of the controls. Incidence and severity of the above lesions increases with increase in severity of anaemia. We concluded that maternal anaemia resulted into bigger, heavier placentas and smaller fetuses, whereas placental morphological changes showed signs of chronic hypoxia and placental insufficiency.

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INTRODUCTION

The placenta is the most remarkable organ which aims preparing the fetus for extrauterine life. It contributes much for the well being of the fetus in utero by its protective, nutritional and respiratory function though it does not become part of body of neonate (Cunningham, 1993). Despite its important role in human fetal development, study of the placenta has lagged behind that of fetus might be due to clinical indication for placental examinations have no gold standards. A number of anatomists and embryologists worked through the 1990s to provide some basic knowledge. Later enlightenment has transposed through the efforts of placental pathologists such as Benirschke, Driscoll, Fox and Naeye. Their work as well as their colleagues shows that careful examination of placenta can shed light on the etiopathogenesis of a number of maternal and fetal disorder (Cunningham, 1993; Hargitai, 2004). Anaemia is said to have occurred in pregnant women when the hemoglobin level is less than 11 g/dl (WHO, 2008). It occurs in 40 to 80 % of the pregnant women due to various causes such as Iron and folic acid deficiencies, intestinal parasitic infections malaria and hemoglobinopathies (Meda *et al*., 1999).

Initial observations on placenta were based on macroscopic examination but currently attention is focused on microscopic abnormalities as they provide crucial information.

MATERIALS AND METHODS

The present prospective study was carried out in R.C.S.M Govt. Medical College, Kolhapur, India from November 2014 to May 2016. After obtaining clearance from ethical committee of the institute and written informed consents, subjects were enrolled in the study. Fifty (50) placentas were collected from cases of maternal anaemia (Hb < 11 g/dl) and Fifty (50) placentae were collected from mothers without anaemia (Hb >11 g/dl) and taken as controls. All the deliveries, both control group and anaemic group, were at full term (37-42 weeks) and the antenatal periods were without any complications or diseases like pre-eclampsia or diabetes. The placenta from both control and anemic group was collected immediately following expulsion and washed in tap water to remove the blood clots. Birth weights of fetuses were noted from the Clinical sheets.

Then the following were recorded: Weights of placentas were measured by means of an electronic weighing scale.
Placental index was calculated by dividing placental weight from fetal weight. From the placentas pieces of tissue were obtained: each piece included the entire thickness of the placenta, from maternal to fetal surfaces. The tissues were then fixed in 10% formalin solution, processed for wax-embedding and section cutting (Carleton's Histological Technique, 1980). Five-micrometer sections were cut and stained with Harris' Haematoxylin and Eosin stain. At least 4 sections were made from each placenta. Statistical analysis was performed by using t-test for comparing the mean values of fetal weights, placental weights and placental indices of maternal anemia group with those of control group.

RESULTS

We observed that in placentas of the maternal anaemia group birth weights of fetuses ranged from 2100-2800 gm (2514 ± 179.86gm). Weights of placentas in this group ranged from 440-510 gm (484 ± 13.88 gm). Mean placental index was calculated 0.193 ± 0.018 (range 0.11-0.26). In the control group of our study, the birth weights of fetuses ranged from 2400-3100 gm (2818 ± 198.91 gm). Weights of placentas ranged from 440-520 gm (475 ± 22.51). Mean placental index was 0.168 ± 0.007 (range: 0.08-0.2). In the present study we observed that mean fetal weight in maternal anaemia group was less than that of control group (significantly less, p value - 0.0001). The mean placental weight of the maternal anaemia group was more than that of control group, though it was not statistically significant. We calculated that the mean placental coefficient index of the maternal anaemia group was significantly more than that of the control group (Table 1). We also observed that placental coefficient index in severe anaemic group was more that of mild and moderate anaemic groups. It also more in moderate anaemic group than mild anaemic groups. It suggests that placental weight increases with increase in severity of anaemia (Table 2). Villous vascularity was decreased in 2/50 placentae of control group and 14/50 of study group. The difference of proportion of placentae showing decrease villous vascularity between two groups was statistically significant ($p<0.05$). Syncytial knots were present on >30 percent of the villi in 15/50 (30%) placentae of the study group and 06/50 (12%) placentae of the control group. The difference of proportion of placentae showing high villous syncytial knot counts (>30% of the villi) between the two groups was statistically significant ($p<0.001$) (Table 3). Fibrinoid necrosis was seen in >3 percent of the villi in 6/50 (12%) placentae of the control group and 20/50(40%) placentae of study group. The difference of proportion of placentae showing fibrinoid necrosis in >3 percent of the villi between the two groups was statistically very significant ($p<0.001$). (Table 4) Increase in villous stromal fibrosis was seen in 4/50 (08%) placentae of the control group and 29/50(58%) placentae of the study group. The difference of proportion of placentae showing increase in stromal fibrosis in >3 percent of the villi between the two groups was statistically very significant ($p<0.001$). (Table 5) Above findings show that higher proportion of placentae in study group show syncytial knots on more number of villi, more fibrinoid necrosis and increase in villous stromal fibrosis . Vasculo-syncytial membranes were present in >20 percent of the villi in 06/50 (07%) placentae of the control group and 06/50(12%) placentae of the study group.

The difference of proportion of placentae showing vasculo-syncytial membranes in >20 percent of the villi between the two group was statistically not significant ($p>0.05$) and none of the placentae in both the groups showed deficiency of membranes (i.e. <5% of the villi) or had high vasculo-syncytial membrane count (i.e. >30% of the villi) (Table 6).

DISCUSSION

Severe anaemia during pregnancy has a significant impact on mother and fetus. It is a cause of pre-placental hypoxia, which may give origin to fetal hypoxia resulting in serious problems for the developing fetus. In the present study we observed that the mean placental weight and placental index of the maternal anaemia group was more than that of control group. The mean fetal weight in the maternal anaemia group was less than that of control group. Our observation was similar to other studies (Beischer *et al*., 1970; Barker *et al*., 1990; Godfrey *et al*. 1991; Biswas, 2014). Villous vascularity was significant decrease in study group (14/50) as compared to control group (2/50). (Fig-1A & B) Khanna *et al*. (1979) Sabharwal *et al*. (1987) and Gyatri *et al*. (1983) reported similar findings in their studies. According to Fox, (1965) diminished villous vascularity is an effect of increase in stromal fibrosis. Syncytial knots are composed of aggregates of small, closely packed densely staining nuclei protruding from the villous surface into the intervillous space. (Fig-2 A&B) In the literature, it is hypothesized that villous hypovascularity leads to formation of syncytial knots, indicating sequestration of aged nuclei is being accelerated or augmented so as to use optimally the amount of trophoblast available for transfer purpose (Fox, 1965). Increased syncytial knots in placentae in anaemia suggested that an attempt was being made to form new villi so as to increase an effective surface area for exchange (Dhall, 1994). Syncytial knots in more than 30% of the villi are considered excessive (Fox, 1965). In the present study, high villous syncytial count (i.e. over 30% of the villi showing syncytial knots) were seen in 15/50(30%) placentae of anaemia group as compared to 06/50(12%) placentae of control group. Rohini *et al*. (2013) Soni *et al*. (2013) and Biswas *et al*. (2014) reported increase in syncytial knots in case of anemia. Fibrinoid necrosis is seen as a nodular mass of homogeneous acidophilic material in the villi.

Placentae in which fibrinoid necrosis involving upto three percent of placental villi is considered as normal (Fox, 1968). Fibrinoid necrosis may be a degenerative change in villous trophoblast (Fox, 1968). Recent literature suggests it to be an immunological reaction within the trophoblastic tissue (Fox, 1965). (Fig-3) In the present study, 20/50 (40%) placentae of anaemia group showed more villi with fibrinoid necrosis count as compared to 6/50 (12%) placentae of control group. Rohini M *et al*. (2013) and Biswas S *et al*[7] reported increase in fibrinoid necrosis in case of anemia. In term placenta normally 3 percent of villi may show increase in stromal fibrosis (Soni, 2013) (Fig- 4A & B). Increase villous stromal fibrosis seen in the anaemic placentae may be the result of relative hypoxia in the peripheral part of the placental lobule. Increase in villous stromal fibrosis in the anaemia cases was reported in all the previous studies. In the present study, 29/50 (58%) placentae of anaemia group revealed increase in the incidence of villi with stromal fibrosis as compared to 4/50 (8%) placentae of control group. The difference between the two groups was statistically very significant ($p<.001$).

Table. 01 Mean weight, Placental weight and Placental coefficient in both the groups

Parameters	Control Group (n-50)	Study Group (n-50)	P value
Birth Weight (gm%)	2818 ± 198.91	2514 ± 179.86	0.0001
Placental Weight (gm%)	475 ± 22.51	484 ± 13.88	0.0191
Placental Coefficient	0.168 ± 0.007	0.193 ± 0.018	0.0001

Table No. 02 Mean weight, Placental weight and Placental coefficient in Mild, Moderate and Severe Anaemic group

Parameters	Group A	Group B	P	Group B	Group C	p	Group A	Group C	P
Birth Wt. (gm%)	2661±94.4	2522±116.38	0.001	2522±116.38	2307±146.8	0.001	2661±94.4	2307±146.8	0.001
Placental Wt. (gm%)	480±12.7	482±14.36	0.67	482±14.36	494±9.7	0.011	480±12.7	494±9.7	0.002
Placental Coefficient	0.18±0.01	0.19±0.012	0.01	0.19±0.012	0.213±0.02	0.002	0.18±0.01	0.213±0.02	0.001

Table 03: Incidence of increased syncytial knots

Group	Percentage of villi with syncytial knots	Number of placentae	Percentage	z value	p value
Control group (n-50)	≤ 30%	44	88		
	>30%	06	12	2.2	<0.05
Study group (n-50)	≤ 30%	35	70		
	> 30%	15	30		

Table 04: Incidence of Fibrinoid necrosis

Group	Percentage of villi showing fibrinoid necroses	Number of placentae	Percentage	z value	p value
Control group (n-50)	≤ 3	44	88		
	>3	06	12	3.2	<0.05
Study group (n-50)	≤3	30	60		
	>3	20	40		

Table 05. Incidence of increased fibrotic villi (Increased villous stromal fibrosis):

Group	Percentage of villi showing fibrinoid necroses	Number of placentae	Percentage	z value	p value
Control group (n-50)	≤ 3	46	92		
	>3	04	08	5.3	<0.001
Study group (n-50)	≤3	21	42		
	>3	29	58		

Table 06. Incidence of Vasculo-syncytial membranes

Group	Percentage of villi with vasculo syncytial membrane	Number of placentae	Percentage	z value	p value
Control group (n-50)	≤ 20	43	86		
	>20	07	14	0.3	0.766
Study group (n-50)	≤20	44	88		
	>20	06	12		

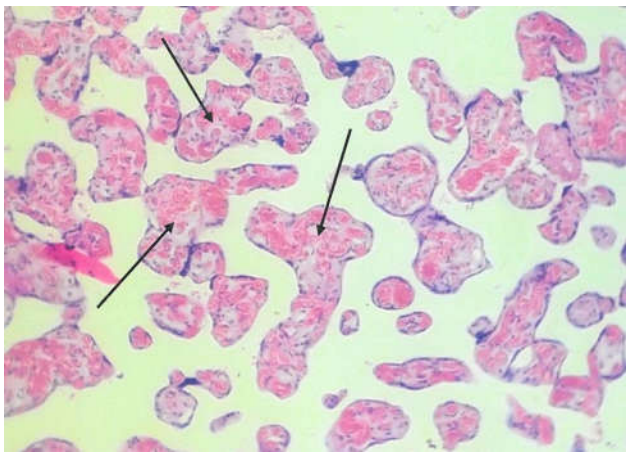

Fig. 1 (A). Photomicrograph showing increased villous vascularity (H & E, x100)

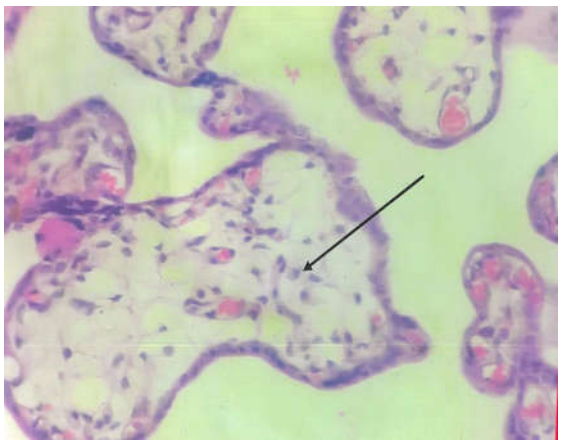

Fig. 1(B). Photomicrograph showing decreased villous vascularity (H & E, x400)

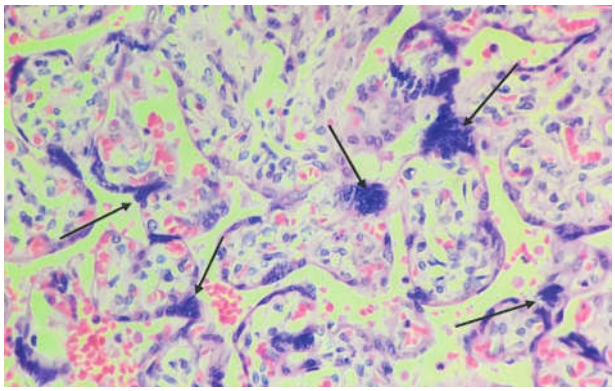

Fig. 2(A). Photomicrograph showing villi richly endowed with syncytial knots (H & E, x100)

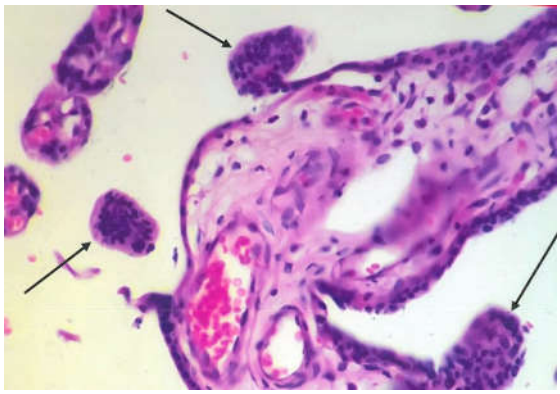

Fig. 2(B). Photomicrograph showing villous with syncytial sprouts (H & E, x400)

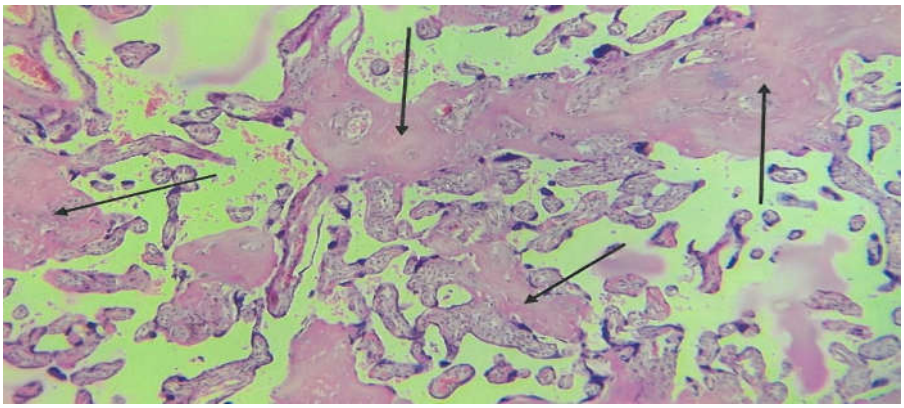

Fig. 3. Photomicrograph showing Fibrinoid necrosis (H & E, x100)

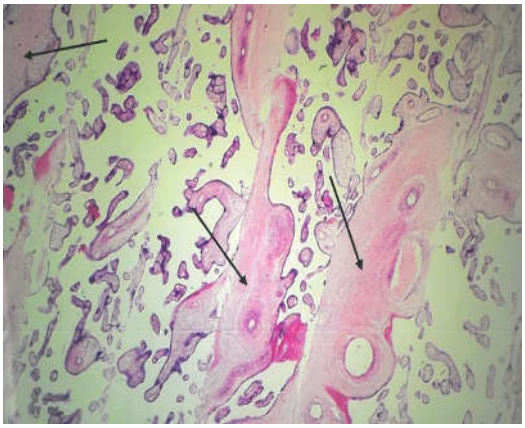

Fig. 4(A). Photomicrograph showing villous stromal fibrosis (H & E, x100)

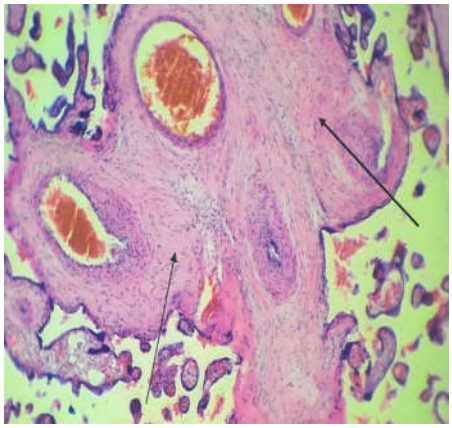

Fig. 4(B). Photomicrograph showing villous stromal fibrosis (H & E, x400)

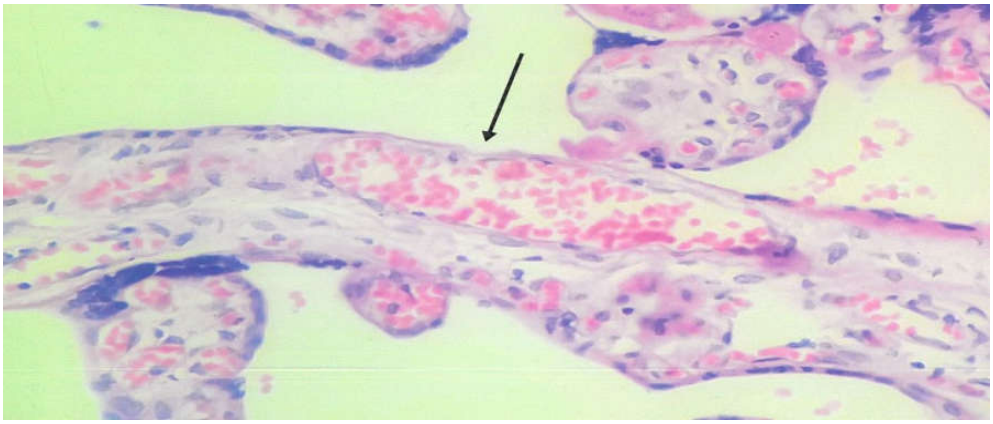

Fig. 5. Photomicrograph showing vasculo syncytial membrane (H & E, x100)

Vasculo-syncytial membranes are focally differentiated areas of syncytiotrophoblast which are specifically concerned with materno-fetal transfer. It consists of attenuated anuclear syncytiotrophoblast stretched over, and in close apposition to, a sinusoidally dilated vessel (Fox, 1965). It gives a good indication of the ability of the placenta to supply oxygen to the foetus (Fox, 1965). Placentae in which between 6 and 30% of the villi show vasculo-syncytial membrane are said to have a normal count (Fig-5). In the present study all placentae in both groups had vasculo- syncytial membrane count within normal range. None of the placentae showed deficiency of membrane nor had high vasculo-syncytial membrane count.

Conclusion

Histopathological examination of placenta in anaemia shows decreased villous vascularity, excessive syncytial knots, increased fibrinoid necrosis of the villi, increase in villous stromal fibrosis. Incidence and severity of the above lesions increases with increase in severity of anaemia. Quantitative determination of placental changes is essential in study of placenta as normal pregnancies can also show significant placental changes.

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