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MORPHOLOGICAL AND HISTOLOGICAL STUDY OF PLACENTA IN ANAEMIC MOTHER OF GWALIOR REGION

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ABSTRACT

Introduction: Placenta is provide nutrition and exchange of gases in foetus. so it is a vital organ .it also help in synthesis of oestrogen and progesterone. Placental abnormalities can be early warning system for foetal problems. Anaemia is commonest haematological disorder that occurs in pregnancy. In present study we find out morphological and histological changes in placenta of anaemic mothers and its effects on foetal outcome. Aim of this study is to find out effect of anaemic mother over foetal outcome, gestational age and baby birth. **Material and methods:** The present study was carried out on 60 placentas from the Department of Obstetrics and Gynaecology, Gajra Raja medical college Gwalior. All the placenta were collected immediately after delivery. The placenta were preserved in 10% saline for microscopic examination. This study was divided into 2 Groups-group 1 control group. it comprised 30 placentas from mothers having no sign and symptoms of anaemia and haemoglobin level more than 11 gm%. Group 2 study group-placenta divided into 3 groups depending on the severity of anaemia. Group2a-mild anaemia, haemoglobin level 11gm%- 10gm%.Group 2b- moderate anaemia, haemoglobin level 10gm%-7gm%. Group 2c – severe anaemia, haemoglobin level less than 7gm%. **Results:** It was found that the period of gestation decreased with severity of anaemia. Number of cotylendons decreases with severity of anaemia. Umbilical cord insertion was more towards margin. Incidence of fibrosis increase with increase in severity of anaemia. In present study microscopic feature observed that capillaries per villous increase in number and are dilated with increasing grade of anaemia. Cytotrophoblastic proliferation increase with increase in severity of anaemia. **Discussion:** It was found that all the cases of study groups that with the high parity (67% of cases multigravida) severity of anaemia increases. The present study also

correlates findings of high incidence of premature (40%) low birth weight 40% and foetal loss (52% with the increase in severity of anaemia. We found that weight of placenta increase with mild anaemia but decrease in weight of the placenta with the severity of anaemia (average weight 443.33 gms of the study group and 480 gms of control group).

INTRODUCTION

Placenta is a fountainhead of human existence because it is a means for provide nutrition and exchange of gases (O_2 and CO_2) in foetus. So, it is a vital organ which is absolutely essential for survival, growth and development of foetus.[1]

The term placenta was introduced by Realddus Columbus who used this Latin word for a 'circular cake' (Plakous = Placenta = cake). In the old testament. The study of placenta is by necessity, retrospective in nature. Yet it provides reflection of the hazards, the foetus has been subjected during its growth and development. The placenta is the most accurate record of infant prenatal experience.

Placenta is a focus of increasing interest in modern obstetrics because significant pathology afflict the placenta, often before affecting the foetus.[2]

Placenta abnormalities therefore can be an 'early warning system' for foetal problem.

The physiological anaemia starting from 6^{th} week onwards but manifest by 8^{th} week of pregnancy, and progresses till 34^{th} week and is aggravated if undernutrition taken by mother.[3,4]

In the present study we find out morphological and histological changes in placenta of anaemic mothers and its effects on foetal outcome.[5]

As most of the perinatal foetus deaths, were related to the insufficient O_2 supply in utero, placenta plays a pivotal role in the transport of O_2 to the foetus.

It was suggested that a test of placental efficiency is urgently required and could be fundamental importance in progress of foetus.[6]

Though the placenta is a organ of very limited life span, yet our information on the factors limiting the span are imprecise, it performs number of function which is other organs of body involve highly specialized tissues including transport of metabolites in two directions both to

and from the foetus and synthesis of important hormones and proteins.[7-8]

High mortality and morbidity was always associated with low birth weight babies in which the role of placenta become pivotal.[9]

This study aims to find out morphological and histological changes in placenta of anaemic mothers and effect of anaemia (in mothers) over fetal outcome, gestational age and baby birth. To know, it is how much important to correct anemia in antenatal period of prevent worst fetal outcome.

MATERIAL AND METHODS

60 placentas, neonates and mothers constituted the material for present study. The study was done in Department of Anatomy in association with Department of Pathology and Department of Obstetrics and Gynaecology, Gwalior.

Group I: Control Group

It comprised of 30 placentae from mothers having no signs and symptoms of anaemia and their hemoglobin level were recorded to be more than 11 gm%.

Group II: Study Group

It comprised of 30 placentae obtained from anaemic mothers whose hemoglobin levels were less than 11 gm%. According to the WHO report (1989)[10] placenta divided into 3 groups depending on the severity of anemia.

Group IIa- Mild anaemia (Haemoglobin level 11 gm/dl – 10 gm/dl) Group IIb – Moderate anaemia (Hb level 10-7 gm/dl)

Group II c – Sever anemia (Hb level < 7 gm/dl) Each group II contain 10 placentae.

Criteria for selection of cases

1. All cases belonging to age group 20-35
2. Gestational age ranging from 28-42 weeks
3. We have taken primi as well as multi gravida
4. They had no racial, cultural or environmental differences
5. The mothers was not have any systemic disease (antepartum haemorrhage, toxaemia of pregnancy, blood group incompatibility and any systemic disease).

Gross examination of placenta

1. Examine as soon as possible after delivery in the fresh state.
2. Note the amount of blood and clots in the container and search for separate pieces of membranes, cord and placenta.
3. Examine after delivery in the following order membranes, cord and placenta.
4. The distance from the placental margin to the nearest point of rapture was measured.
5. Membranes were examined from completeness, colour number of cotylendons, calcification insertion, decidual necrosis, retromembraneous haemorrhage, meconium staining, colour and transparency.
6. Attachment of umbilical cord to placenta is noted whether central, eccentric or velamentous, number of umbilical vessels.
7. Umbilical cord was cut one centimeter from the placenta surface and membranes were trimmed off.
8. Any abnormality in placenta such as accessory lobe or bilobed placenta also recorded.
9. The fetal surface was examined for colour, opacity, squamous metaplasia, thrombosis of fetal surface vessels.
10. The maternal surface was examined for completeness, normal fissures, laceration, depressed areas, retroplacental hemorrhage.
11. The maximum diameter, thickness in the centre, weigh and shape was noted.

Section for histology

The placenta was held gently with one hand, maternal side up on flat surface and parallel sections with a large sharp knife were cut.

Three 2 cm piece were removed from both group of placenta (control and anaemic group) that included both the foetal surface and the maternal surface, one piece taken from periphery of placenta, one pieces taken so that the fetal surface vessels are cut at right angles to their long axis, fixed for 24 hours, 3 mm section were taken (through and through) and further used for processing and staining.

Fixative used – 10% formalin

Method of processing

1. **Dehydration:** The preserve tissues were thoroughly washed in running tap water for 4-6 hours. They were then passed through upgraded alcohol as follows:

   50% alcohol – 1 hour

70% alcohol – 1 hour

90% alcohol – 1 hour

Absolute alcohol I – 1 hour

Absolute alcohol II – 1 hour

2. **Clearing:** Clearing of tissues was done in xylene.
3. **Embedding:** The cleared tissue was put in xylene wax (50% v/v) for one hour and then in molten paraffin wax for 12 hours (Melting point – 56°-58°C) in the oven. The paraffin block of tissues were made with the help of Lochart's metallic blocks.
4. **Sectioning:** The serial paraffin section of 5 micron thickness were cut by rotatory microtome and floated in water bath, having temperature 5°-10°C. The sections were then spread on the slide smeared with adhesive solution (Mixture of equal amount of glycerol and egg albumin). The slide were dried on hot plate having temperature 5-10°C.

Deparaffinisation of sections: The slides were put in xyelene, two changes each for 5-10 minutes in order to remove the extra cellular and intracellular wax.

After blotting the slides, these were put in descending grades of alcohol i.e. absolute 90%, 70% and 50% for 2 minutes each. The slides were then washed in running tap water for 2 minute and then taken for routine H & E staining.

Histological staining procedure

Hematoxylin and cosin staining: Haemotoxylin and cosin stain is most widely used histological stain. The haematoxyline component stains the cell nuclei blue/black, with good intranuclear detail while the cosin stains cytoplasm and most connective tissue fibers in varying shades and intensities of pink, orange and red.

Method of staining

1. Dewax section, hydrate through graded alcohols to water.
2. Stain and Harris haematoxylin (5-15 min)
3. Wash in running tap water until sections become blue (for 5 min/less)
4. Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 5-10 sec.
5. Wash well in tap water until sections are again become blue (for 10-15 min).
6. Blue by dipping in an alkalin solution followed by 5 min tap water wash.
7. Stain in 1% Eosin for 10 min.
8. Wash in running tap water (1-5 min)

9. Dehydrate through alcohols, clean and mount.

RESULTS

Present study was divided in two groups

Group I – Control group (30 cases) Group II – Study group (30 cases)

The study group was further divided according to severity of anemia. Group IIa - Cases of mild anaemia – 10 cases

Group IIb - Cases of moderate anaemia – 10 cases

Group IIc - Cases of severe anaemia – 10 cases

Table 1: Incidence of cases in various groups.

Groups	No. of cases	Percentage of cases
I	30	50
IIa	10	16.66
IIb	10	16.66
IIc	10	16.66

Parity

In control group 67% were primi gravida and 33% were multi gravida, whereas in anemic group 33% were found to be primi gravida and 67% were multi gravida.

Table 2: Incidence of cases of primi gravida and multi gravida in both groups.

Group	Primi Gravida		Multi Gravida	
	No.	%	No.	%
II	20	67%	10	33%
	10	33%	20	67%

Gestational age

The gestational age of mother were calculated from last menstrual period (LMP) and by doing per abdominal examinations in both groups.

It was observed that in the control group 87% were delivered in the gestational age of 37 to 40 weeks. Rest of the cases were found to be either less than 37 weeks of gestation or more than 40 weeks of gestation, whereas in the study group higher incidence was in the gestational age of less than 37 weeks considering all the anemic group.

Table 3: Incidence of cases according to gestational weeks in various groups.

Groups	< 37 weeks		37-40 weeks		40-42 weeks	
	No.	%	No.	%	No.	%
I	2	6.5	26	87	2	6.5
IIa	2	20	6	60	2	20
IIb	4	40	4	40	2	20
IIIc	5	50	4	40	1	10

Placenta

Table 4: Mean placental weight and diameter in various groups.

Groups	Mean placental weight (in gm)	Mean placental diameter (in cc)
I	480	16
IIa	500	17
IIb	480	16
IIc	350	15

In Control Group – Mean Placenta weight – 480 gm

In Study Group – Decrease in Placental weight with severity of anemia.

Table 5: Incidence (in %) of various types of shapes in both groups.

Groups	Discoidal	Circular	Oval	Bilobed
I	77	23	0	0
II	63.5	32	3.23	1.27

In Control Group – Most common shape – Discoidal

In Study Group – Most common shape – Discoidal (63.5%) but circular shape is more than study group because with severity of anemia surface area of placenta was reduced making the shape circular.

Fibrosis (Fibrin deposition)

White patchy area of fibrosis were found in both groups. It was present in 6% cases of control, 20% cases in group IIa, 25% cases in group IIb, and 65% cases in group IIc.

Umbilical cord insertion

Table 6: Incidence of various types of umbilical cord insertion in both groups.

Types of U.C. Insertion	Control Group (% of Cases)	Study Group (% of Cases)
Central	67	40
Eccentric	33	58
Velamentous	0	2

In Control group – Most common type of umbilical cord insertion – Central

In Study group – Most common type of umbilical cord insertion – Eccentric

Microscopic examination

Observations of microscopic examination were done under following headings:

1. Capillaries Per villous: In control group each villous had minimal number of small capillaries but in study group capillaries per villi were seen to be increased in number and dilated with severity of anemia.
2. Cytotrophoblastic proliferation: (More than one layer of cytotrophoblast in a villi)
   In control group we did not observe any case of cytotrophoblastic proliferation, whereas in study group cytotrophoblastic proliferation increases with severity of anemia.
3. Thickening of Basement membrane of trophoblast: In control group basement membrane was observed to be normal, whereas thickening of it increased with severity of anemia.
4. Thickening of basement membrane of villous capillaries: In control group basement membrane of villous capillaries was observed to be normal where as it is increased in thickness with severity of anemia.
5. Vasculosyncytial membrane: Cases of vasculosyncytial membrane were not observed in cases of control group whereas in study group this was observed in cases of moderate anemia as well as severe anemia.
6. Fibrosis: In control group scattered and minimal amount of fibrosis seen whereas in study group amount of fibrosis tend to increase in villi and in intervillous spaces with increasing grades of severity of anemia.
7. Syncytial knots: Syncytial knots occasionally seen in control group, whereas in study group number of syncytial knots kept increasing with increase in severity of anemia.

Table 7: Incidence of cases of foetal outcome in study group.

Groups	I.U.D. No. of Cases	Premature body No. of cases	L.B.W. Baby No. of cases	F.T.N.D. No. of cases
I	0	0	0	30
IIa	0	2	4	4
IIb	1	3	3	3
IIc	2	4	4	0

It was observed that in control group, no case was found of I.U.D., prematurity, L.B.W. baby. 100% cases were delivered as full term baby. While in study group cases of I.U.D., cases of prematurity, and cases of L.B.W. baby increase with severity of anaemia and cases

delivered as full term baby decrease in comparison to control group.

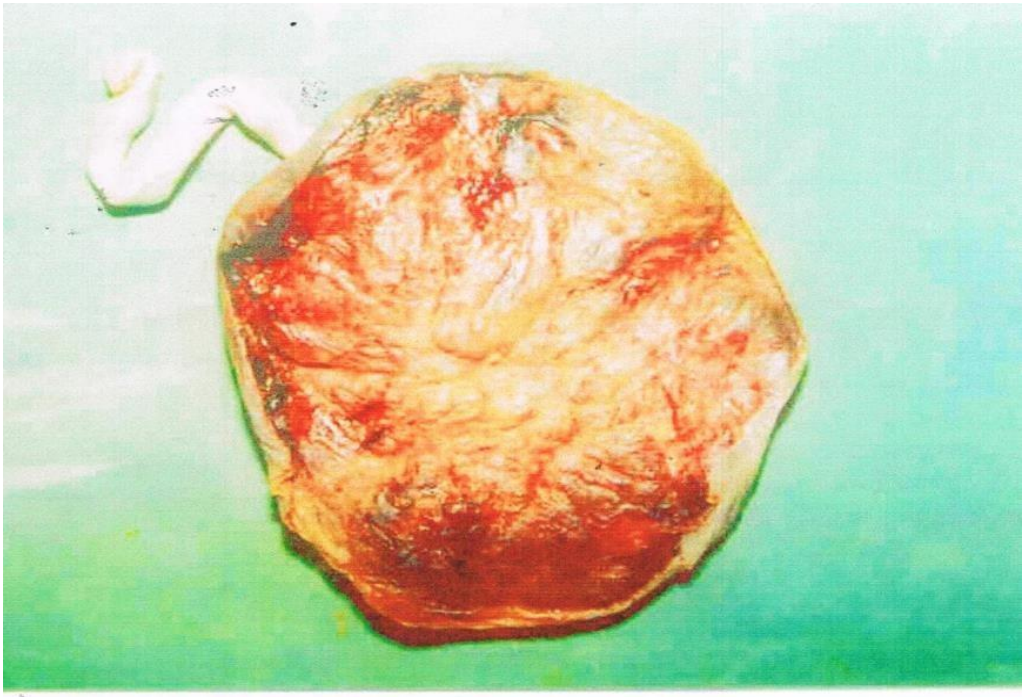

Figure 1: Gross morphology of placenta of control group showing circular shape.

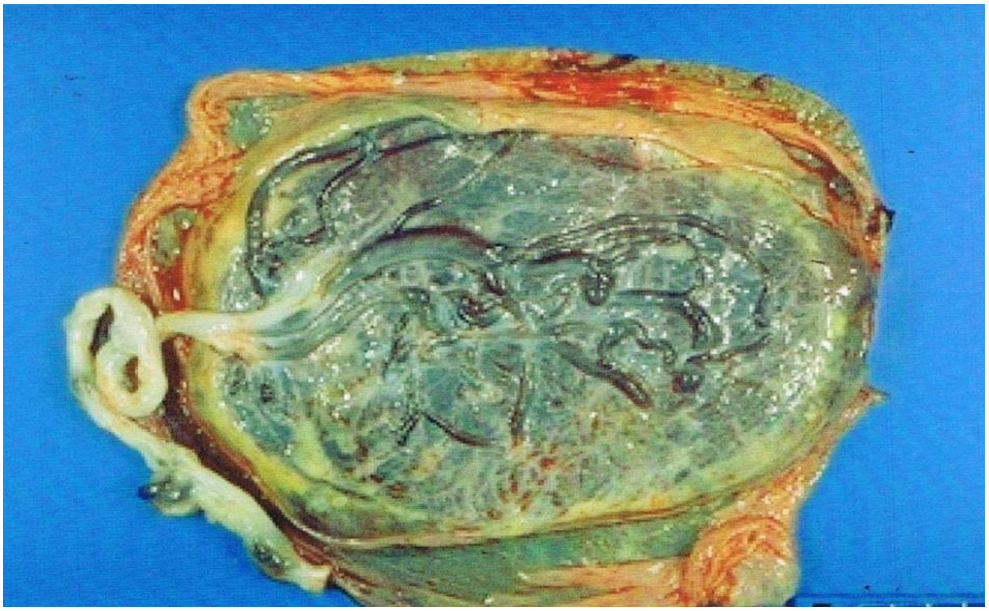

Figure 2: Gross morphology of placenta of study group showing discoidal shape with eccentric attachment of umbilical cord.

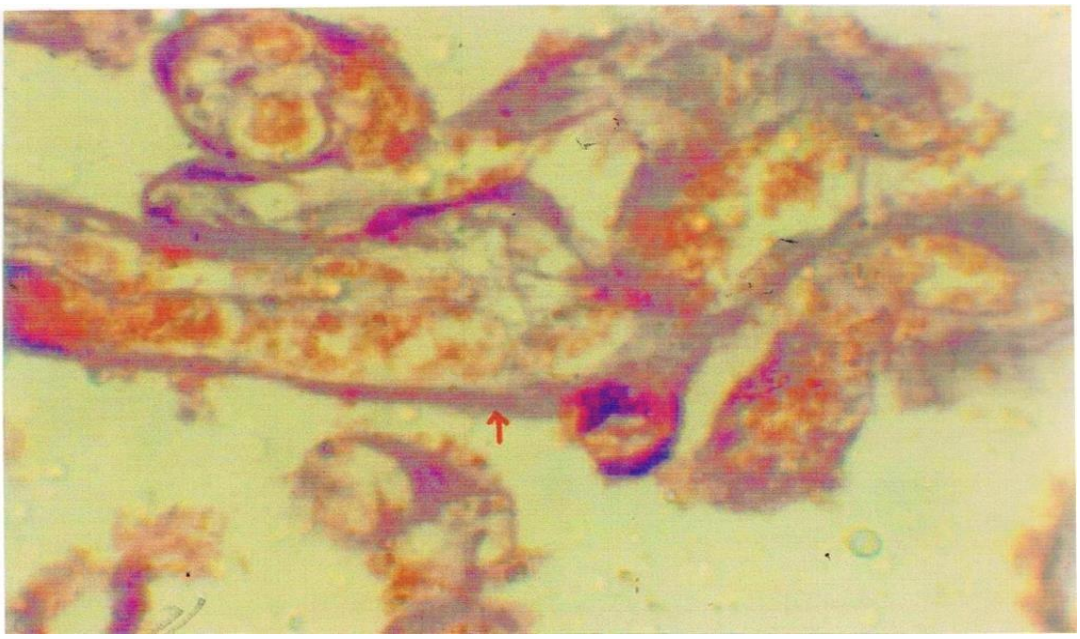

Figure 3: Photo micrograph of placental villi with inter villous space (moderate anemia) Arrow showing thickened basement membrane of cytotrophoblast.

High magnification

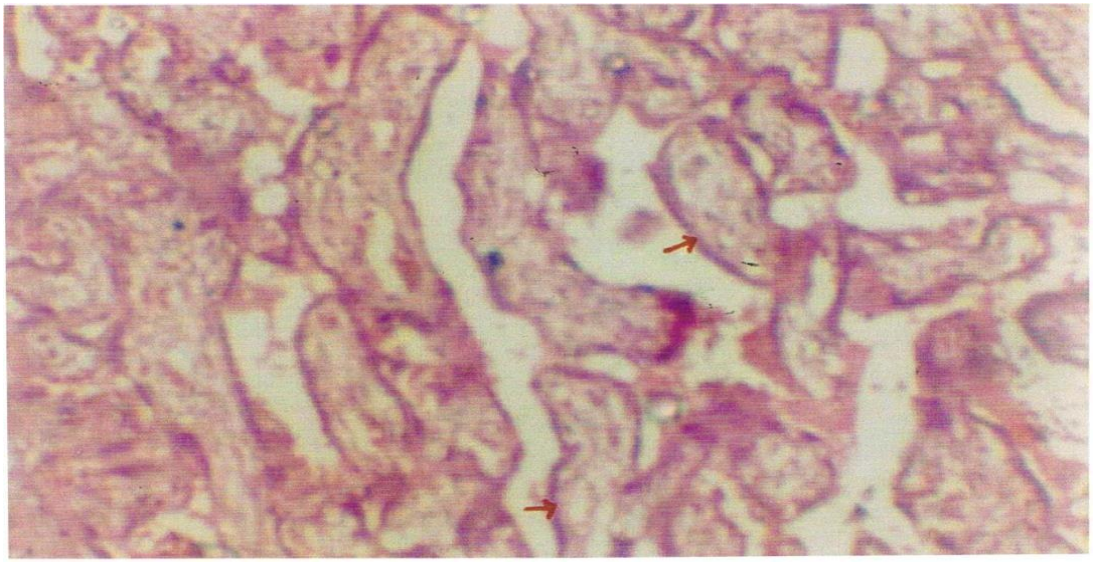

Figure 4: Photo micrograph of placental villi with inter villous space (Severe anemia) Arrow showing high thickened basement membrane of trophoblast. Low magnification.

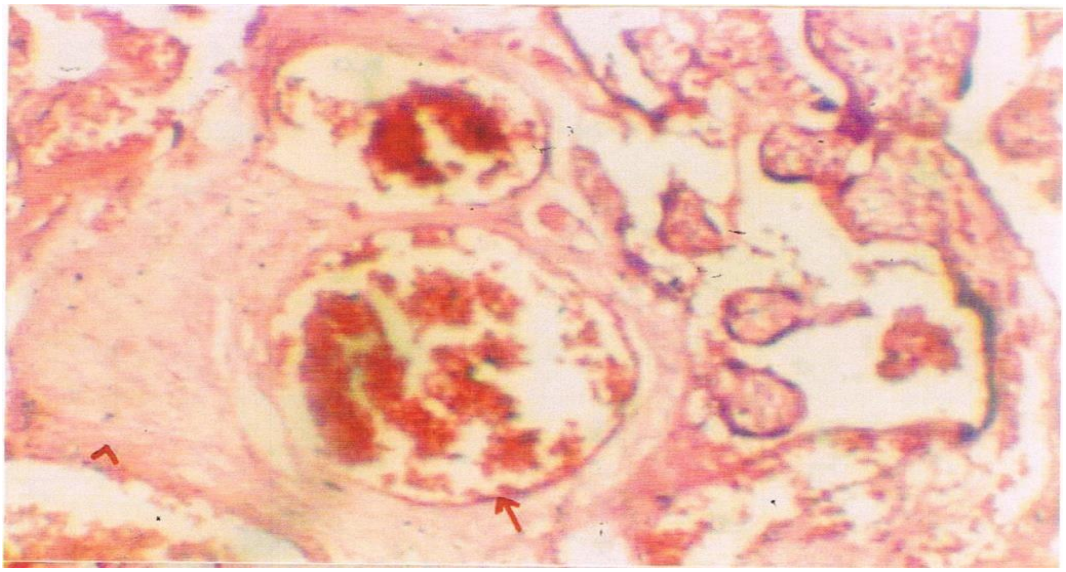

Figure 5: Photo micrograph of placental villi with inter villous space (Severe anemia) Arrow showing markedly dilated capillaries. Arrow head showing villous fibrosis. Low magnification.

DISCUSSION

It was found in all the cases of study group that with the high parity (67% of cases multigravida) severity of anemia increase. This was also observed by other authors where the increase in parity leads to high grade of anemia. It happens due to repeated blood in subsequent pregnancies.[11,12]

It was found in all cases of study group that total period of gestational decreases with the severity of anemia. In our study 33.33% cases were noted at less than 37 weeks. Vijaylaxmi et al[13] also observed in their study of 1040 cases that mother delivered at < 37 weeks of pregnancy were 3.9% in non anemic cases and 26% in anemic cases.

The present study also correlates findings of high incidence of premature (40%) low birth weight 40% and foetal loss (20%) with the increase in severity of anemia. It was observed by Kelly[14] that woman with mild anemia had a 30-40% increased risk and those with moderate to severe anemia had about 70% increased risk of preterm birth.

In the present study we found that weight of placenta increase with mild anemia but decrease in weight of the placenta with the severity of anemia (average weight 443.33 gms of study group and 480 gms of control group). Small placental weight associated with anemic mothers was also reported by Wong et al.[15]

Purnima[16] explained that explained that small placenta of severely anemic mother is due to retarded growth of placenta with decrease in total placental DNA suggesting decrease in cell number and thus reduction in size, where there is early stop of cell division in anemia (normally cell division stops after 36 weeks of gestation).

So conclusion is that due to mild to moderate anemia in mother because of hypoxia compensatory placental hypertrophy occur. But when anemia is severe, placenta affected so much that it can not go under compensatory hypertrophy.

CONCLUSION

Anemia is most common nutritional disorder in world. Nutritional anemia is most common among pregnant women. Severity of anemia among pregnancy was judged by criteria suggested by W.H.O.

It was found that period of gestation decreased with severity of anaemia. Various workers have attributed this to either degenerative changes or increased intervillus fibroid deposition causing premature delivery where placenta could not further compensate to the insult caused by hypoxia.

Placenta from anemic mother have comparatively low eight.

In the present study number of cotylendons decreases with severity of anemia (average no. of cotylendons in control group 17, in study group 11).

Present study also showed that the umbilical cord insertion was more towards margin. (In study group 58% of cases were eccentric whereas in control group it was 33% with increase in severity of anemia).

In present study, we found discoidal shape placenta (77% in control group and 63.5% in study group) circular shape (23% in control group and 32% in study group) and oval 3.23% and bilobed or with accessory lobe 1.27% (both found in study group only).

Incidence of fibrosis increase with increase in severity of anemia.

In the present study, it was observed that the capillaries per villous increase in number and are dilated with increasing grade of anemia.

It was found that cytotrophoblastic proliferation increase with increase in severity of anemia. Basement membrane thickening is a consequential by product of cytotrophoblastic proliferation which is also observed in our study and inferred that there is basement membrane thickening with increasing severity of anemia.

Sinusoidal dilatation of terminal villus capillaries forming vasculosyncytial membrane increase with severity of anemia.

We observed that stromal fibrosis increase with severity of anemia.

The foetal vessels of these villi undergo complete sclerosis and hence thickening of capillary wall.

The study concludes that the placental examination offers a lot of information of prognostic significance for the new born. The placenta plays a major role in the survival and growth of foetus. Case of anemia in pregnancy, examination of placenta give indication of time frame characteristics of insult by morphological and histological examination of placental tissue.

For microscopic examination of placenta 3 cm × 3 cm size tissue was taken from placenta. Sections was taken and mounted on slides. Sections were taken and mounted on slides.

Microscopic study of placenta of anemic mothers showed markedly increased syncytial knots, and increased cytotrophoblastic proliferation, thickening of cytotrophoblastic and villous capillary basement membrane which increase thickness of placental barrier and this adversely affects foetal well being.

Increased villous capillarization of terminal villi in placenta of anaemic mothers is considered as a adaptation in hypoxic environment.

Extensive stromal fibrosis in placenta of anaemic mothers is due to normal aging process and reduced uteroplacental blood flow.

There is a threshold for level of haemoglobin and consequently for oxygen transport. Below which placental function is affected. This explain the pathogenesis of incrased frequency of premature birth, foetal death and perinatal mortality in foetus of pregnant mother with anemia.

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