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Original Research Article

Microscopic Features of Human Placentae in Normal and Gestational Diabetes Mellitus

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Abstract:

Background: Placenta is a multifunctional feto-maternal organ that plays an important role during pregnancy. Gestational Diabetes Mellitus (GDM) is reflected on placenta both macroscopically and microscopically such as enlargement of placenta, abnormalities in villi and degenerative changes. GDM is associated with increased rates of maternal and perinatal morbidity.

Materials and Methods: This is a cross sectional study conducted among two groups, normal and GDM groups to compare the microscopic features of GDM placentae in the Department of Obstetrics & Gynaecology and Department of Anatomy, Govt. Medical College, Thiruvananthapuram.

Results: This study was conducted on 65normal and 65 GDM placentae. Light microscopic features such as syncytial knots, villous stromal fibrosis, fibrinoid necrosis, chorangiosis, were significantly increased in GDM placentae.

Conclusion: Placental examination can shed light upon factors pertaining to the current pregnancy and its outcome, guide postpartum management and helps to predict and prevent the adverse effects in successive pregnancies. Hence, the present work would provide vital information to both Obstetricians and neonatologists.

Keywords: Placenta; Gestational Diabetes Mellitus; Histology of placenta.

Introduction

Placenta is a feto-maternal organ which connects the developing fetus to the uterine wall of the mother. It is responsible for the exchange of gaseous and metabolic products between fetal and maternal circulations and synthesis of hormone. It is essential for maintenance of pregnancy and for promoting normal growth and development of fetus. It begins to meet the demand of the embryo as early as third week of intrauterine life.

The placenta was recognized for the first time as an endocrine organ in the beginning of 20th century and the new focus became the transfer of solutes across the placenta. Despite this journey of evolving understanding of the complexities of the placenta, significant knowledge gaps remain in understanding placental functions. The Human Placental Project sponsored by National Institute of Health (NIH) described eloquently the need for continuing research in this field. The placenta is the least understood human organ and arguably one of the most important for the health of a woman and her fetus during pregnancy, also for the lifelong health of both [1]. 'Placenta' is a Latin word, the Greek equivalent word plakuos means a flat cake [2]. The term placenta was coined by an Italian Surgeon and Anatomist, Realdo Colombo. It is a discoid mass, having maternal and fetal surfaces and a peripheral margin. Maternal surface is finely granular and fetal surface is smooth, covered by amnion with the umbilical cord attached to it. The placenta continuously undergoes changes in shape, weight, structure and functions throughout gestation. It is considered as a window through which maternal dysfunction and their impacts on fetal well-being can be understood. Pregnancy is a diabetogenic state by virtue of various physiological changes which cause insulin resistance.

In normal pregnancy, glucose tolerance decreases by third trimester, though plasma levels of insulin increase. Gestational diabetes mellitus (GDM) is described as glucose intolerance of varying severity with the onset of first recognition during pregnancy and disappears with delivery [3]. About 2 -5% of the total pregnancies may be affected by diabetes mellitus. Among pregnancies complicated by diabetes mellitus, about 65% cases involve gestational diabetes mellitus [4]. India has been

called 'the diabetic capital of the world'. In India the prevalence of GDM is 4-11.6% in urban population and 3% in rural population, varies according to geographical areas and diagnostic methods employed [5].

The placenta of diabetic women has attracted much interest because diabetic pregnancy is characterized by numerous disturbances in fetal growth and development [6]. GDM in pregnancy is reflected on placenta both macroscopically and microscopically such as enlargement of placenta, abnormalities in villi and degenerative changes. This results in reduced blood flow and utero-placental insufficiency which may lead to fetal hypoxia, congenital fetal malformations and unexplained intrauterine death. The extent of these changes depends on a number of factors particularly the quality of glycemic control achieved during the critical periods in placental development [7].

The examination of placenta is of critical value as it can be used in gathering knowledge about identification of pathological process contributing to the adverse outcome and management conducted during pregnancy. By increasing our understanding of the placenta, it may be possible to prevent and treat placental abnormalities, thus ensuring lifelong health of the child and the mother. Hence, the present work would provide vital information to both obstetricians and neonatologists.

Materials and Methods

A cross sectional study with a comparison group was carried out between 09/02/2017 to 08/08/2018 to explore the microscopic features of placentae from normal pregnancies and pregnancies complicated with GDM. The study was conducted after approval by the Human Ethics Committee, Govt. Medical College, Thiruvananthapuram.

After obtaining informed consent from the mother, microscopic features of placenta were studied.

Sample Size: Sample size for the current study was calculated using the information provided by ALPANA HATIBARUAH in the article "A study on macroscopic anatomy of human placenta"

In this study 62.16% of normal placentae at term found to weigh between 400-499 grams.

Sample size, $N=4PQ/ d^2$

P=62(62.16)

Q=100-62=38

d, precision=20% of p =12.4

$N=4X62x38/ (12.4x12.4)^2$

=61.29

Sample size was taken as 65.

Equal number of placentae from pregnancies complicated with GDM was taken and both gross and microscopic features were compared with that of normal pregnancies. Purposive type of non- probability sampling method was used.

Inclusion Criteria

- Placentae from normal pregnancies with gestational age >24 weeks.
- Placentae from GDM complicated pregnancies with gestational age >24 weeks.

Exclusion Criteria

- Multiple pregnancies, Pregnancies with pre-existing diabetes mellitus.
- Pregnancies complicated with pregnancy induced hypertension, hypothyroidism, anaemia, abruptioplacenta, jaundice and malnutrition.

Study Variables

- Syncytial knots
- Villous stromal fibrosis
- Fibrinoid necrosis
- chorangiosis

2cm of tissue was taken from the center of each placenta and immediately transferred to a bottle containing 10% formalin. After a week of fixation, tissue was subjected to histological processing that included dehydration, clearing, embedding, deparaffinisation of sections. Then processed tissue was sectioned by rotary microtome to 5microns thickness and stained with Eosin and Hematoxylin. Section of one normal and one GDM placentae were stained with Van Gieson for better visualization of collagen fibers. The mounted sections were observed under light microscope. 100 adjacent villi were studied for each category of stain. Microscopic features such as Syncytial knots, Villous Stromal Fibrosis, Fibrinoid necrosis, and Chorangiosis were studied from H& E stained slides. Photomicrographs of the stained slides were taken using photomicroscope.

Syncytial Knots: These are aggregations of syncytiotrophoblast nuclei in clusters. For studying syncytial knots, hundred adjacent villi in H&E sections of both normal and GDM placentae were selected. Then the number of villi having syncytial knots was counted and considered the mean values.

Villous Stromal Fibrosis: It is a normal phenomenon and considered abnormal when it is not restricted to the stem villi. The number of villi having villous stromal fibrosis in H&E sections of GDM placentae were calculated from hundred adjacent villi and mean value was compared with that of normal.

Fibrinoid Necrosis: It is a non-cellular, eosinophilic, homogeneous material. Number of villi having fibrinoid necrosis was calculated from hundred adjacent villi in H & E sections of both groups and compared the mean values.

Chorangiosis: Extreme villous hypervascularity is known as chorangiosis, the normal values ranging from 2 to 6 per terminal villus. In this study, minimum 10 vascular profiles per chorionic villus in at least 10 chorionic villi were considered as chorangiosis.

Van Gieson's staining for Collagen Fibers [8]

Preparation of Weighert's Iron Hematoxylin

- Solution A: 1gram of Hematoxylin was added to 100 ml of 95% alcohol.
- Solution B: 4ml of 29% aqueous ferric chloride was mixed with 95 ml of distilled water and 1 ml of concentrated hydrochloric acid.
- Weighert's Iron Hematoxylin was prepared by mixing of solution A and Solution B.

Van Gieson's solution was prepared by adding 4 drops of acid fuchsin to 1ml of picric acid.

Staining procedure:

- Sections were deparaffinized in xylene for 10 minutes.
- Hydrated in graded alcohol and water for 2 minutes each.
- Stained in Weighert's Iron Hematoxylin for 3 minutes.
- Washed in running water for 5minutes.
- Stained in Van Gieson's solution for 5 seconds.
- Rapidly dehydrated in 95% alcohol and absolute alcohol.
- Air dried.
- Cleared in 2 changes of xylene for 3minutes each.
- Mounted in DPX.

Results:

- Collagen fibers – Red.
- Nuclei – Blue / black.
- Other tissue components like muscle, RBC – Yellow.

Statistical analysis: Data was entered in Microsoft excel sheet and statistical analysis was performed using SPSS version 16. Quantitative variables were described by mean and standard deviation. Statistical test of significance for quantitative variables was Students t test. Categorical variables were analyzed by proportion. Statistical test of significance for categorical variables was Chi-square test. A 'p value' less than 0.05 were considered to be statistically significant.

Syncytial Knot: The mean number of villi having syncytial knot formation in normal group was 16.23±5.72, while in GDM group it was 32.02±8.

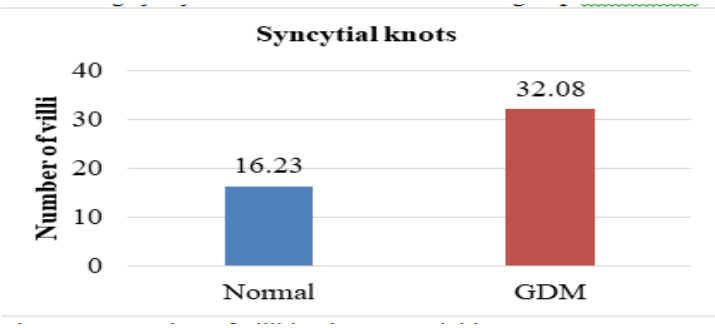
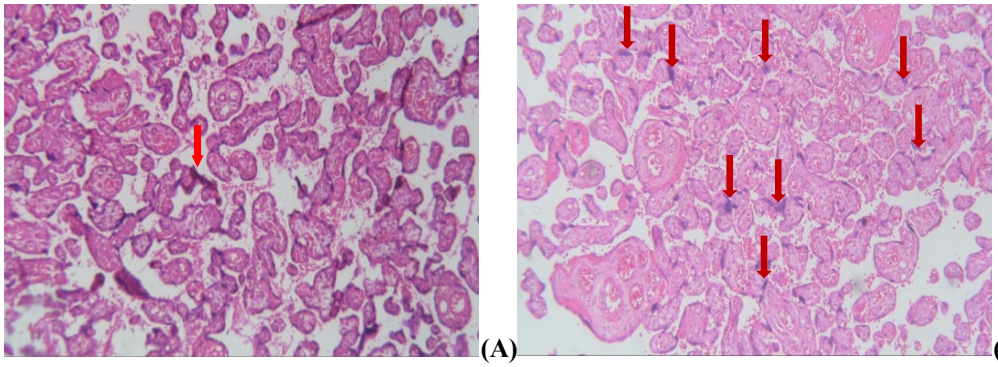


Figure 1: Bar chart showing means number of villi having syncytial knots



(A) **(B)**

Figure 2: Section of a GDM placenta (B) showing increased number of syncytial knots. Compare with the normal placenta (A) (H&E) 100x.

Villous Stromal Fibrosis: The mean number of villi having villous stromal fibrosis in normal group was 1.69±1.413, while in GDM group, it was 5.69±3.640. Villous stromal fibrosis in GDM group was increased which was statistically highly significant.

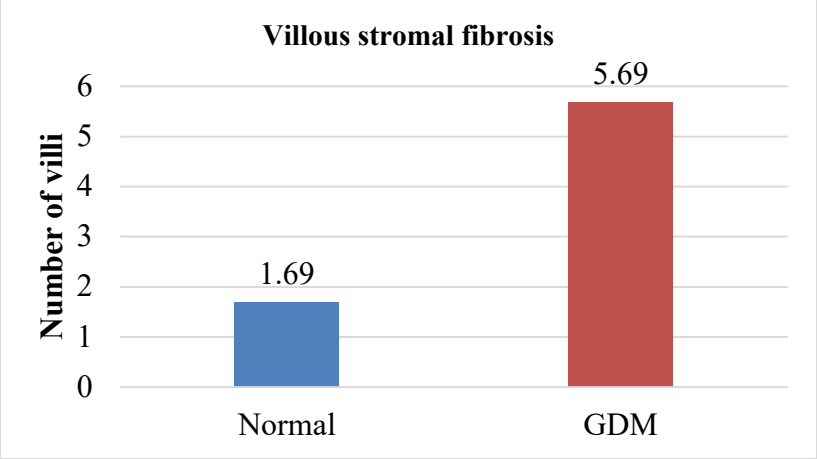
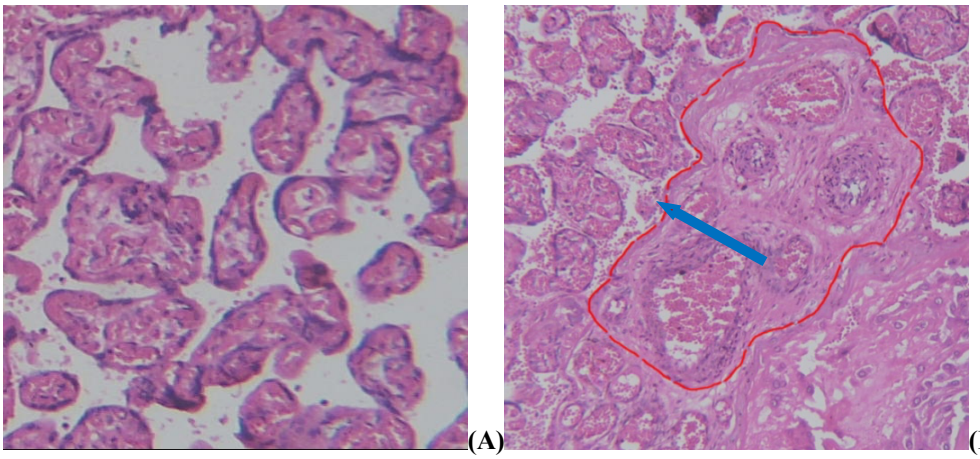


Figure 3: Bar chart showing means number of villi having villous stromal fibrosis



(A) (B)

Figure 4: Section of a GDM Placenta (B) showing villous stromal fibrosis (blue arrows). Dotted red line denotes a villus. Compare with the normal (A) (H&E) 100x.

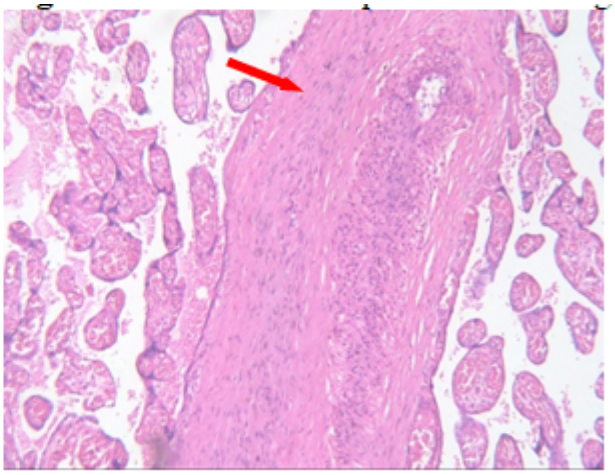


Figure 5: Section of GDM placenta showing extensive villous stromal fibrosis (H & E) 100x.

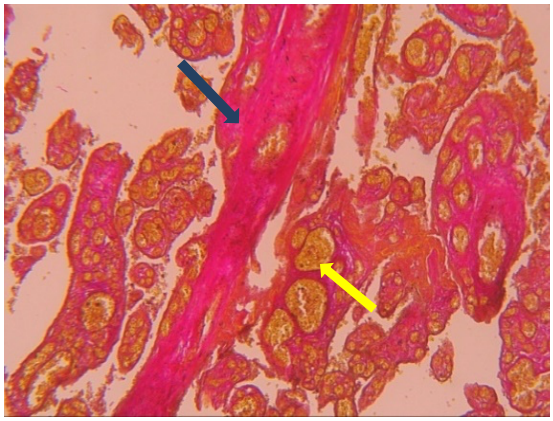


Figure 6: Section of a GDM placenta (Van Gieson stain) 100x. Collagen fibres – reddish pink, RBC – yellow (yellow arrow). Note the extensive fibrosis (blue arrow).

Fibrinoid Necrosis: Mean number of villi having Fibrinoid necrosis in normal group was 2.54±1.846, while in GDM group it was 7.57±3.473.There was a statistically highly significant increase in fibrinoid necrosis in GDM group.

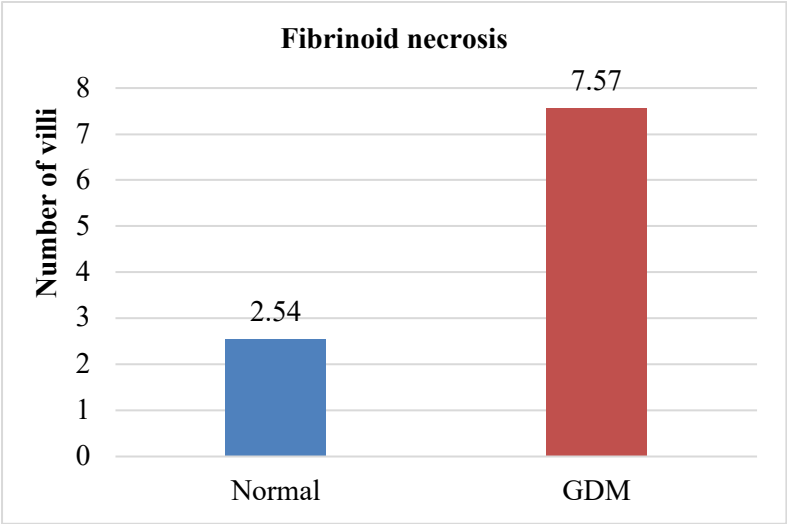


Figure 7: Bar chart showing means number of villi having fibrinoid necrosis

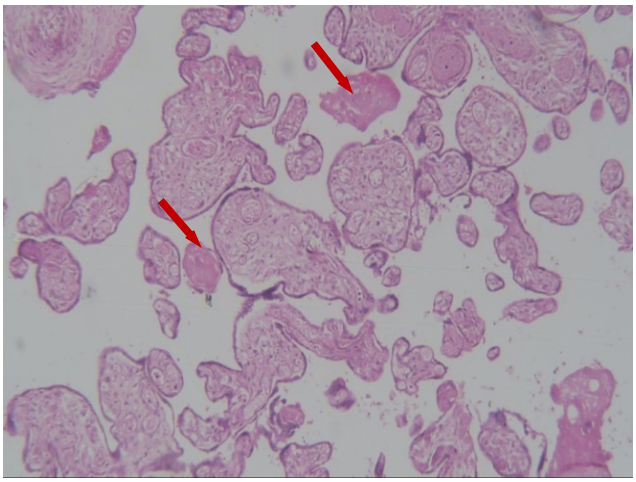


Figure 8: Section of a GDM placenta showing extra villous fibrinoid necrosis (H & E) 100x.

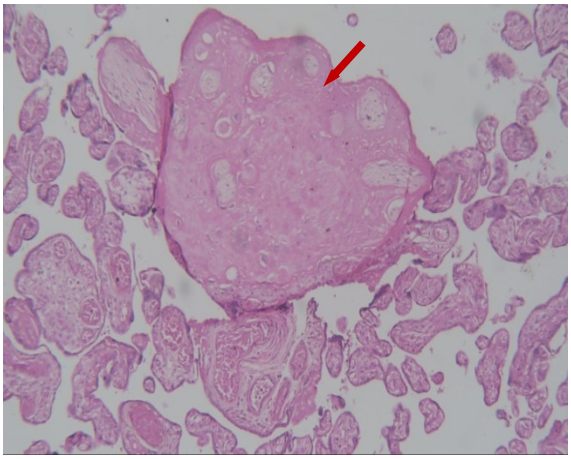


Figure 9: Section of a GDM placenta showing intravillous fibrinoid necrosis (H & E) 100x.

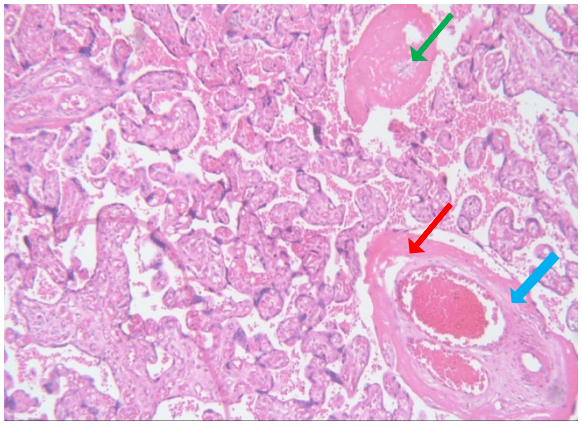


Figure 10: Section of a GDM placenta (H&E stain) 100x, showing fibrinoid necrosis (green arrow), dilated fetal capillary with pericapillary fibrosis (blue arrow), perivillous fibrin deposition (red arrow) and increased syncytial knots.

Chorangiosis: Chorangiosis was seen in 12(18.5%) placentae from GDM group, but none of the normal placentae had this feature on histological examination. Chorangiosis in GDM group was statistically highly significant.

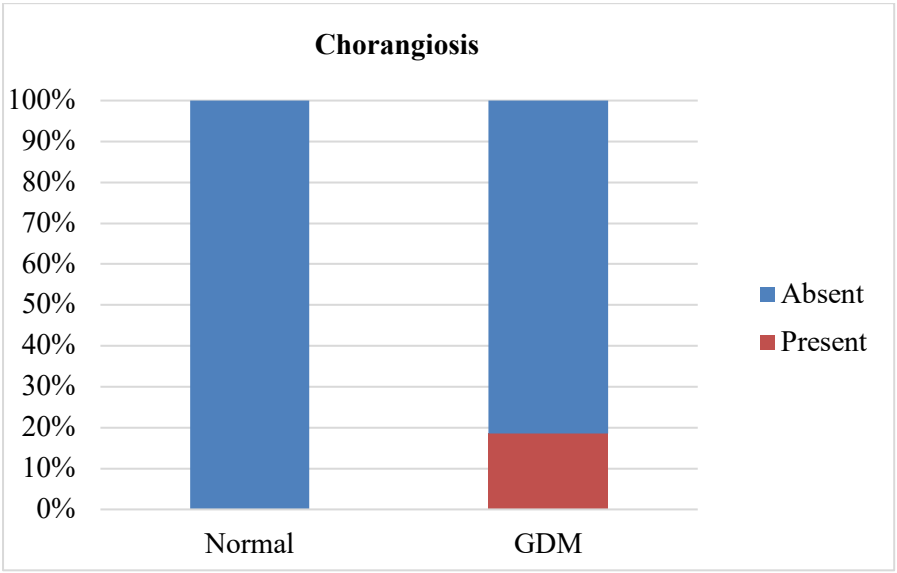


Figure 11: Segmented bar chart showing frequency distribution of chorangiosis.

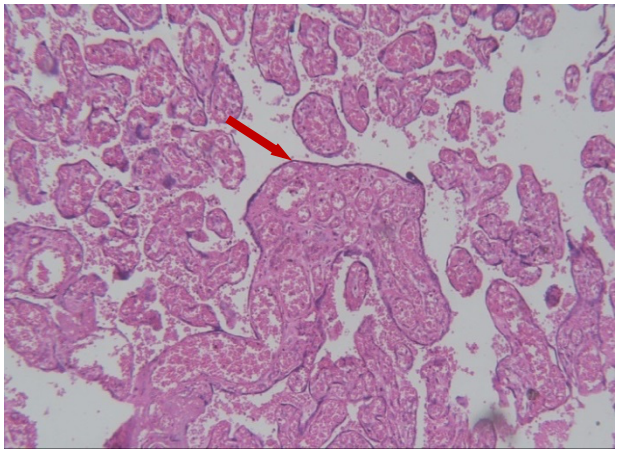


Figure 12: Section of a GDM placenta showing chorangiosis (H&E stain) 100x

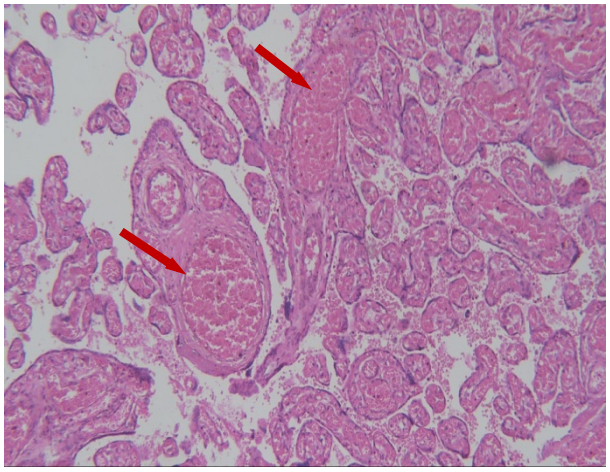


Figure 13: Section of GDM placenta showing dilated and congested fetal capillaries (H & E stain) 100x.

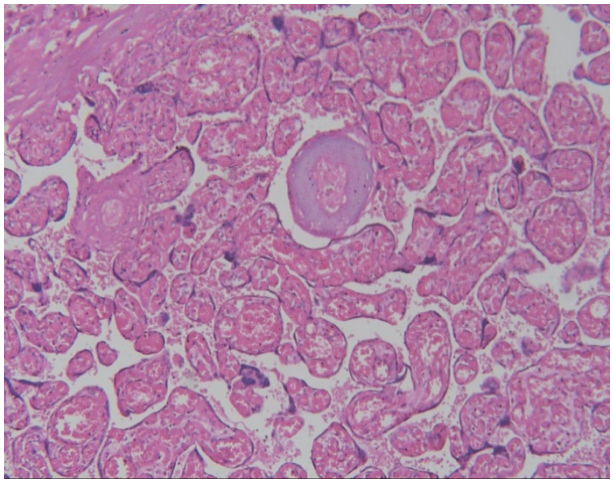


Figure 14: Section of a GDM placenta showing hyaline degeneration as an occasional finding (H&E stain) 100x.

Discussion

The objective of this study was to compare the microscopic features of GDM placentae with that of normal.

Gross variables such as weight, shape, type of attachment of umbilical cord, diameter, number of maternal cotyledons, colour of the fetal membrane of placentae were noted. Microscopic variables such as syncytial knots, villous stromal fibrosis, fibrinoid necrosis, chorangiosis, were studied.

Syncytial Knots: The syncytio-trophoblast layer appeared as strong-basophilic cells lacking intercellular boundaries with darkly-stained and irregularly dispersed nuclei often aggregated or clustered at the villous surface to form syncytial

clumps or knots. Syncytial basophilia could be attributed to the abundance of free ribosomes, RER, lysosomes, phagosomes and secretory vesicles or granules of hormones formed by syncytial cells.

The syncytial knots or clumps appeared as localized clusters with close aggregation of nuclei that undergo marked degenerative changes probably to remove senescent nuclear materials away from the adjacent functional and metabolically active syncytial areas. If these senescent cells were not pushed aside and rather kept in place, the villous surface would be covered by a very thick layer of these aged cells and blood feto-maternal exchange function would be progressively impeded [9].

In the present study, the syncytiotrophoblast and syncytial knots were best examined by H&E stain and we observed significantly increased incidence of syncytial knot formation in placentae of GDM group. Ahmed TM Elshennawy et al. (2016) opined that increased number and frequency of syncytial clumps or knots centrally more than peripherally could be attributed to the more increase of villi themselves due to hypoxia caused by diabetic insult to placenta [10].

A significant increase in syncytial knots formation was noted in villi of diabetic placentae (85%) as compared to placentae of control group (15%) in the study conducted by Mishra P et al. (2017) [11]. Nidhi Mishra et al. (2017) found an increased incidence of synctial knots in placentas of diabetic patients as compared to normal group and it was statistically significant [12].

In the study, mean number of syncytial knots in GDM group was 32.08 as compared to16.23 in normal group which correlated well with the previous studies.

Villous Stromal Fibrosis: Fibrosis of the stem villi is a normal phenomenon in the placenta and it is a good indicator of placental maturation. Fibrosis usually starts at about 15th week post menstruation, usually around the stem vessels and completes a few weeks before term. Stromal fibrosis is considered abnormal when it is not restricted to the stem villi. One theory holds that, collagen production may be stimulated by the increased partial pressure of intra villous oxygen. As diffusion of oxygen from maternal space into the stroma, in the face of inadequate uptake by fetal capillaries, due to poor oxygen perfusion of the villous tree, might result in an increase in the oxygen content in the stroma and stimulates collagen synthesis [13].

In our study, mean number of villi having villous stromal fibrosis was significantly increased in GDM placentae (5.69±3.640) as compared to normal placentae (1.69±1.413).

Soad A Treesh et al. (2015) [15] and Nidhi Mishra et al. (2017) [12] demonstrated increased stromal villous fibrosis in diabetic placentae.

Ahmed TM Elshennawy et al. (2016) documented that the diabetic placentae showed a marked increase in villous stroma with increased fibrous content of collagen and reticular fibers [10].

Mishra P et al (2017) noted an increased villous stromal fibrosis in diabetic placentae (45%) as compared to normal placentae (15%) [11].

Our findings agreed with the previous observations.

Fibrinoid Necrosis: Fibrinoid is a non- cellular, eosinophilic, homogeneous material that can be identified in the placenta. Extra villous fibrinoid has a lamellar structure and, in terms of immunohistochemistry, its superficial layer is a fibrinous layer (blood origin). This type of fibrinoid either fills gaps in the trophoblastic layer, or includes the all chorionic villi or groups of villi. Intra villous fibrinoid was described as a distinct fibrinoid material appearing in the sub trophoblastic space that finally occupies the whole villous stroma. Intra villous fibrinoid deposits are increased in pathological conditions including diabetes [10].

In our study, mean number of villi having fibrinoid necrosis in normal group was 2.54±1.846, while in GDM group it was 7.57±3.473. The difference between two groups was statistically highly significant.

Soad A. Treesh et al. (2015) observed an increase in both extravillous and intravillous fibrinoid necrosis (14).

Pankaj Saini et al. found that fibrinoid necrosis was significantly higher in the diabetic group (6.70 ± 2.14) as compared to a mean value in the controls (2.25 ± 1.66) [15]. Mishra P et al. (2017) reported an increased incidence of fibrinoid necrosis in GDM placenta (87.5%) as compared to normal (12.5%) [11].

In the study conducted by Nidhi Mishra et al. (2017), there was significantly increased incidence of both intra villous and perivillous fibrinoid necrosis in placentas of diabetic patients as compared to normal group [12].

Our findings were in accordance with the previous studies.

Chorangiosis: One of the morphologic features of chorionic villi is their vascularity, the normal values ranging from 2 to 6 per terminal villus and larger numbers defined as hypervascularity. Extreme villous hypervascularity is known as

chorangiosis, In 1958 Hormann, coined the term "chorangiosis". Altshuler (1984) did a detailed study on this entity and defined as at least 10 vascular profiles per terminal/intermediate chorionic villus in 10 chorionic villi per 10 objective microscopic field in at least 10 areas of 3 or more random cotyledons. The increased chorionic villi vasculature by angiogenesis in diabetic placentas represented an adaptive response in a trial to increase the placental respiratory area through increased villous capillary number, surface area, diameter and length but without remodelling [16].

In our study, Chorangiosis was present in 18.5% of GDM placentae, whereas none of the placentae in normal group had this feature on histological examination. In the study done by Kalla Ravi teja (2017), it was observed that control group was having 50% of chorangiosis whereas increased chorangiosis (76.7%) was seen in diabetic group [16]. According to Mishra P et al. (2017), chorangiosis was found in 27.5% of diabetic placentae, whereas it was totally absent in placentae of normal mothers [11]. Nidhi Mishra et al. (2017) found that chorangiosis was present in 15% of diabetic placentae and it was absent in normal placentae [12].

The result of the present study endorsed the results of the above works.

Summary and Conclusion

Placenta is a multifunctional feto-maternal organ, plays an important role during pregnancy. Gestational Diabetes Mellitus is reflected on placenta both macroscopically and microscopically such as enlargement of placenta, abnormalities invilli and degenerative changes. Microscopic features such as syncytial knots, Villous stromal fibrosis, Villous edema, Fibrinoid necrosis, Crowding of villi, dilated blood vessels and chorangiosis were significantly increased in GDM placentae. Placental examination can shed light upon factors pertaining to the current pregnancy and its outcome, guide postpartum management and helps to predict and prevent the adverse effects in successive pregnancies. Hence, the present work would provide vital information to both obstetricians and neonatologists.

References

1. Mir IN, Chalak L. Placenta 'The Least Understood Human Organ'- From Animistic Origins to Human Placental Project. Ann Reprod Med Treat. 2017; 2(2): 1013.
2. Hamilton, Boyd, Mossman's human embryology 4th edition. The Macmillan Press Ltd 1976; chapter 5:129.
3. Metzger B.E., Coustan D.R., Eds. Proceedings of the Fourth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care. 1998; 21 (Suppl. 2): B1–B167.
4. Saxena R. Bedside obstetrics and gynaecology. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; Chapter 13, Gestational Diabetes. 2010; 234-255.
5. Kumar A, Goel MK, Jain RB, Khanna P, Chaudhary V. India towards diabetes control: Key issues. Australas Med J. 2013; 6(10):524-531.
6. Coustan D R. Gestational diabetes In: Management of high risk pregnancy. Blackwell Science England. 1999; 4: 261-9.
7. Desoye G, Shafrir E. The human placenta in diabetic pregnancy. Diabetes Rev. 1996; 4:70-89.
8. McManus J, Mowry R. Staining methods: histologic and histochemical. Hoeber Medical Division, Harper & Row; 1963.
9. Standring S. Gray's Anatomy: The Anatomical Basis of Clinical Practice, 41st ed. Elsevier Limited, London. 2015.
10. Elshennawy TMA. Effect of Gestational Diabetes on Gross Morphology, Histology and Histochemistry of Human Placenta. Endocrinol Metab Syndr. 2016; 5: 227.
11. Mishra DN, Jamila DA, Devi DNS. Pathological Changes in Placentas of Diabetic Mothers & Its Association with Fetal Outcome: 7.
12. Nidhi Mishra, A Jameela, Nalli Sumitra Devi. "Pathological Changes in Placentas of Diabetic Mothers & Its Association with Fetal Outcome." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS). 2017; 16(8): 93-99.
13. Debra S, Heller, Vijay V Joshi. Handbook of placental pathology: 2005; 85-90.
14. Treesh SA. Histological Changes of the Human Placenta in Pregnancies Complicated with Diabetes. J Cytol Histol. 2015; 06(02).
15. Saini P, Pankaj, Jain A. Effect of Gestational Diabetes Mellitus on Gross Morphology of Placenta: A Comparative Study. Int J Anat Res. 2015 Mar 31; 3 (1):889–94.
16. Kalla Ravi Teja, Govindaraj T, Arun kumar SP, Hiremath. Histopathological Study of Placenta in Gestational Diabetes in a Tertiary Care Hospital Sch. J. App. Med. Sci., Nov 2017; 5(11C):4500-4506.