

# Placental Changes in Gestational Diabetes Controlled with Insulin versus Diet without Insulin Compared with Normal Uncomplicated Pregnancy: Clinical and Histopathological Study

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**Abstract:** *The fetus, placenta and mother constitute a triad of contributors to pregnancy outcome. When pregnancy is complicated by a medical problem like, diabetes mellitus which affects maternal health, architecture and functions of the placenta may even jeopardize the fetal normalcy. The placenta being the bridge between maternal and fetal activities, considered as a window through which maternal dysfunctions and their impacts on fetal well being can be understood. The aim of this research is studying the pathological changes of placenta of women with gestational diabetes mellitus and to compare the results with normal pregnancies. Methods: It was an observational study conducted in Zagazig University hospitals, 20 placentae from pregnant women clinically diagnosed with gestational diabetes mellitus (GDM) controlled only with diet and life style modifications and another 20 placentae from pregnant women clinically diagnosed with gestational diabetes mellitus (GDM) controlled with insulin and 20 placentae from uncomplicated normal pregnant women were collected from labour room and operation theatre of department of obstetrics and gynaecology. Confirmed gestational diabetic cases were selected purposively from gestational diabetes clinic of endocrinology unit while controls were taken sequentially. Pathologic features of each placenta were recorded in histopathology department. Results: The results showed that placental weight, diameter, surface area and central thickness from diabetic mothers controlled with insulin were significantly more than diabetic mothers controlled only by diet or mothers of normal uncomplicated pregnancies, while no significant differences were observed in shape and site of umbilical cord insertion. The terminal villi in placenta of GDM controlled by insulin showed a significant varying degree of changes like, the increased number of syncytial knots, villous edema and fibrinoid necrosis however, these changes were very minimal in cases of GDM treated only with diet and controlled without need of insulin. Conclusion: The placenta of women with gestational diabetes mellitus treated with insulin even controlled shows significant variation in gross morphology and light microscopy that can be associated with impaired function of placenta, leading to possible adverse perinatal outcome. In the other hand, the placenta of women with gestational diabetes mellitus controlled with diet alone showed minimal changes with no significant differences from normal uncomplicated pregnancy. Control of GDM or better prevention by education, diet, optimum weight control and life style modifications should be strictly encouraged and insulin use should be the last resort.*

**Keywords:** gestational diabetes, pregnancy, insulin, placenta, diet

## 1. Introduction

Since it is one the commonest metabolic problems of pregnancy, an accurate diagnosis of gestational diabetes mellitus (GDM), *i.e.*, high plasma glucose first identified during pregnancy, is critical to the care of pregnant women [1]. Five decades ago, GDM was used to detect pregnant women who were at a higher risk of developing type 2 diabetes mellitus (DM2) after childbirth [1, 2]. Currently, GDM is used to predict morbidity in index pregnancy; many trials have confirmed that it is related to multiple maternal and fetal complications like pre-eclampsia, caesarean sections and birth injuries [2]. 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 increases [3]. About 2% to 5% of the total pregnancies may be affected by diabetes mellitus. Among pregnancies complicated by diabetes mellitus, about 65% cases involve gestational diabetes mellitus whereas 35% cases are associated with pre-existing diabetes mellitus [4]. Placenta is a vital organ for fetal development and is a mirror of maternal and fetal status. It derived from both fetal and maternal tissues, the maternal portion being the decidua

basalis and the fetal portion is chorion frondosum [5]. So, as a mirror, placenta reflects the intra-uterine status of the fetus. Its metabolic functions are complex and it undergoes changes continuously throughout gestation in weight, structure, shape and function in order to support prenatal life [6]. In GDM, when the intra-uterine environment for fetus become hostile, the placenta tries to exert its reserve capacity by changing its morphological structure, as well as some pathological changes occur that are compounded principally of some disturbances in its normal rate of maturation [7]. Some workers have claimed that placentas from diabetic women show no unusual feature while others have observed frequent abnormalities in such placentas but failed to agree on any consistent pathologic pattern. This confusion is partly due to the inclusion, in many studies, of gestational diabetes mellitus (GDM) in whom there was either superadded pre-eclampsia or intrauterine fetal death; as these complications may obscure the significance of diabetic placentas. Also the degree of control and the modality used in control either with insulin or diet alone should be taken in consideration. So it is therefore not surprising that the results obtained have been inconsistent and indeed, often contradictory [8]. In our study, all the patients with GDM were perfectly controlled whatever the modality

of treatment with insulin or without. Also other co-morbid condition such as hypertension, hypothyroidism, anaemia, jaundice, and maternal malnutrition even tobacco abuse, smoking, alcoholism etc. were excluded from study to create a chance of studying the true effect of GDM and modality of treatment on placenta without bias of other pathologic co-morbid conditions or even diabetes control status which, of course well known active player.

## 2. Materials and Methods

It was a prospective comparative study, conducted in Zagazig University hospitals between September 2013 and September 2014. After due approval from institutional ethics committee and informed consent was obtained, 20 placentae from pregnant women clinically diagnosed with gestational diabetes mellitus (GDM) controlled only with diet and life style modifications and another 20 placentae from pregnant women clinically diagnosed with gestational diabetes mellitus (GDM) controlled with insulin and 20 placentae from uncomplicated normal pregnant women were collected from labour room and operation theatre of department of obstetrics and gynaecology. Confirmed gestational diabetic cases were selected purposively from gestational diabetes clinic of endocrinology unit while controls were taken sequentially. Pathologic features of each placenta were recorded in histopathology department. A pre-structured and pre-tested proforma was used to collect the data. Personal details of mothers like name, age and address were recorded. Social history regarding habits of mothers like smoking, tobacco chewing and consumption of alcohol were taken. Detailed obstetric history regarding parity, period of gestation, bad obstetric history in past, type of pregnancy (singleton or multiple), mode of delivery and abruptio placentae was recorded. Medical history regarding anaemia, jaundice, malnutrition, cardiovascular disease, cerebrovascular disease, respiratory disorders, psychiatric illness and any other major illness was taken and recorded. A general physical examination was done for anaemia, jaundice and nutritional status of mothers. Blood pressure, weight and relevant investigations were recorded from bed head tickets. The placentae with attached membranes and umbilical cord were collected soon after delivery and washed in running tap water to clean all blood. Surface dried between blotting papers and examined for morphological characteristics like shape and type of insertion of umbilical cord. The membranes were trimmed and the cord was cut at about 2 centimeters from its insertion. The placenta was then weighed using baby weighing machine. The central thickness was measured by long knitting needle. Two diameters of the placenta were measured with the non stretchable measuring tape and the mean of the two was calculated. The evaluation of macroscopic placental parameters was performed according to protocols published by Benirschke [9].

For light microscopy, two cm. wedge of tissue was taken from the centre of each placenta and fixed in 10% formalin for one week. The tissue was dehydrated and followed by embedding in paraffin and 7 micron serial sections were generated with the help of rotatory microtome. The tissue sections were stained with hematoxyline and eosin.

Histological appearance of the terminal villi of placenta was assessed and following observations were made. **Trophoblast:** The number and position of syncytiotrophoblast, syncytial knots and cytotrophoblast nuclei was assessed. **Villous stroma:** The density of villous stroma was assessed qualitatively by looking for the presence of collagen fibres and density of the background fibrillar material. **Fetal capillary:** Evidence of capillary proliferation and obstruction within the capillary lumen was sought in each case [10, 11].

**Inclusion criteria:** Pregnant women with age between 20-38 years, para 1 to 5, gestational age between 37-42 weeks, deliveries by either vaginal route or caesarean section with singleton pregnancy were included. The control group comprised pregnant women who did not experience complications during pregnancy and who had normal laboratory tests while study group comprised pregnant women with clinically confirmed gestational diabetes mellitus. **Exclusion criteria:** Pregnant women who did experience any complication during pregnancy like hypertension, hypothyroidism, anaemia, abruptio placentae, multiple pregnancies, jaundice, maternal malnutrition, cardiovascular disease, cerebro-vascular disease, psychiatric illness, respiratory disorders, tobacco abuse, smoking, alcoholism etc. were excluded from study.

**Statistical analysis:** The data was entered on Microsoft excel 2010 and analyzed. The results for each parameter (numbers and percentages) for discrete data and average (mean  $\pm$  standard deviation) for continuous data are presented in Tables. Proportions were compared using Chi-square test of significance. The F test was used to determine whether there was a statistical significant difference between control and studied groups. A *P*-value of less than 0.05 was considered to be statistically significant.

## 3. Results

In present study, the mean age  $\pm$  SD (years), weight  $\pm$  SD (kg), height  $\pm$  SD (cm) and the body mass index (BMI)  $\pm$  SD (Kg/m<sup>2</sup>) were studied in all groups of study and summarized in table 1. Generally speaking; age, weight and BMI were more in GDM group controlled with insulin than the other two groups but we cannot consider this note of clinical significance due to limited number of the patients and purposeful mode of patients choice.

**Table 1:** Distribution of characteristics of study subjects

Characteristic	GDM controlled with insulin (Mean $\pm$ SD) n=20	GDM controlled with diet without insulin (Mean $\pm$ SD) n=20	Control (Mean $\pm$ SD) n=20
Age (years)	30.4 $\pm$ 3.5	29.3 $\pm$ 4.3	29.3 $\pm$ 4.3
Weight (kg)	79.7 $\pm$ 8.2	66.2 $\pm$ 6.1	66.2 $\pm$ 6.1
Height (m)	1.57 $\pm$ 0.1	1.56 $\pm$ 0.1	1.56 $\pm$ 0.1
BMI (Kg/m <sup>2</sup> )	32.3 $\pm$ 1.5	27.09 $\pm$ 1.8	27.09 $\pm$ 1.8

In controls majority of cases were in para-2 (40%) followed by para-1 (35%), and in GDM group controlled with insulin majority of cases were in para-1 (45%) followed by para-2

(35%) while in GDM group controlled with diet without insulin cases with the same like the control group.

The mean placental weight± SD (gram), the mean placental diameter± SD(cm), the mean placental central thickness± SD (cm) and the mean placental surface area± SD(cm<sup>2</sup>) were calculated in all groups of study and summarized in table 2. There were highly significant differences in these parameters in GDM group controlled with insulin in comparison with the other two groups (p < 0.01) while no significant differences of these parameters in GDM group controlled with diet without insulin in comparison of control group (p > 0.05).

**Table 2:** Placental morphometry between gestational diabetics and normal pregnancies

Parameters Of placenta	GDM controlled with insulin (Mean ± SD) n=20	GDM controlled with diet without insulin (Mean ± SD) n=20	Control (Mean ± SD) n=20
weight(gram)	437.3 ± 52.1	392.8 ± 44.6	389.4 ± 31.3
diameter(cm)	17.1 ± 1.1	15.6 ± 1.8	15.2 ± 1.3
thickness(cm)	2.3 ± 0.8	2 ± 0.8	1.9 ± 0.4
surface(cm <sup>2</sup> )	218.3 ± 36.9	188.9 ± 34.2	181.6 ± 32.7

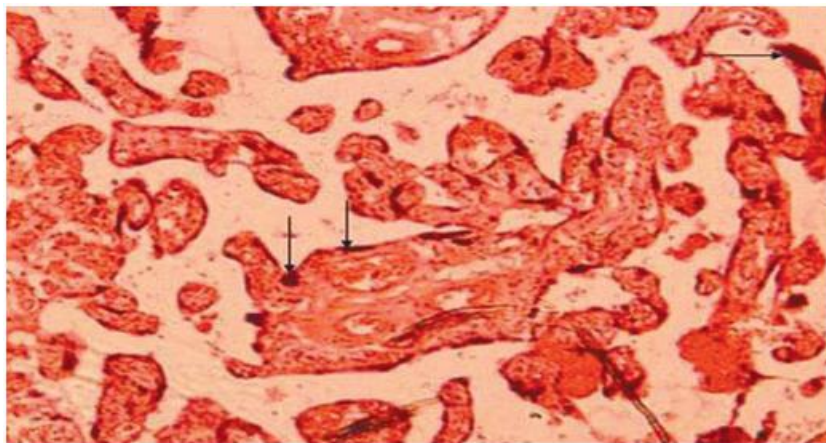
In control group 40% placentae were of round shaped and 60% were of oval shaped, and in GDM group controlled

with insulin 35% of placentae were round shaped and 65% were oval shaped while in GDM group controlled with diet without insulin were same like control group . The difference was not significant (p > 0.05).

In control group umbilical cord insertion was central in 25%, eccentric in 60% and marginal in 15 % of placentae and in GDM group controlled with insulin it was central in 20%, eccentric in 65% and marginal in 15% of placentae while in GDM group controlled with diet without insulin was central in 20%, eccentric in 60% and marginal in 20% of placentae. And again the difference was not significant (p > 0.05).

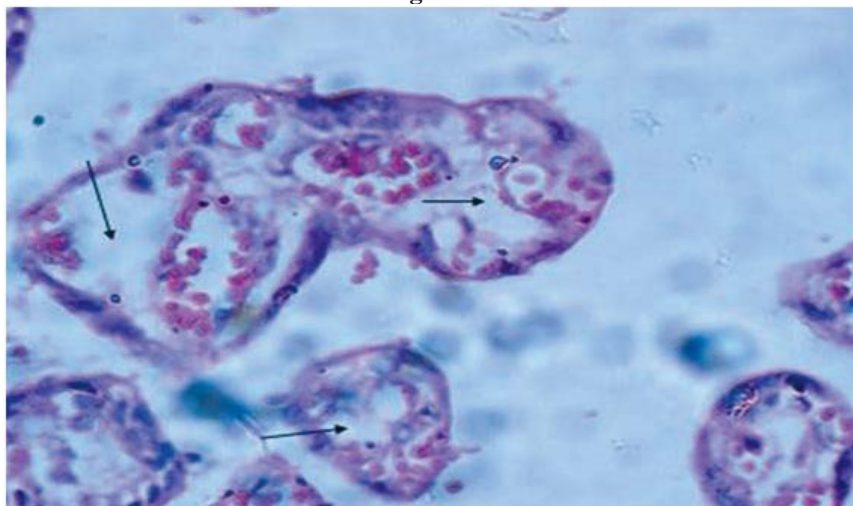
The light microscopic findings.

The terminal villi in placentae of GDM group controlled with insulin showed a varying degree of changes like, the increased number of syncytial knots (Fig.1). The stroma of the villi demonstrated villous edema (Fig. 2) and fibrinoid necrosis (Fig. 3). Villous fibrosis (Fig. 4) and foetal capillary proliferation (Fig. 5) was observed in very limited number. The histopathological changes in GDM group controlled with diet without insulin were minimal changes in the terminal villi and none of them showed villous edema, villous fibrosis or fetal capillary proliferation resemble normal placentae.



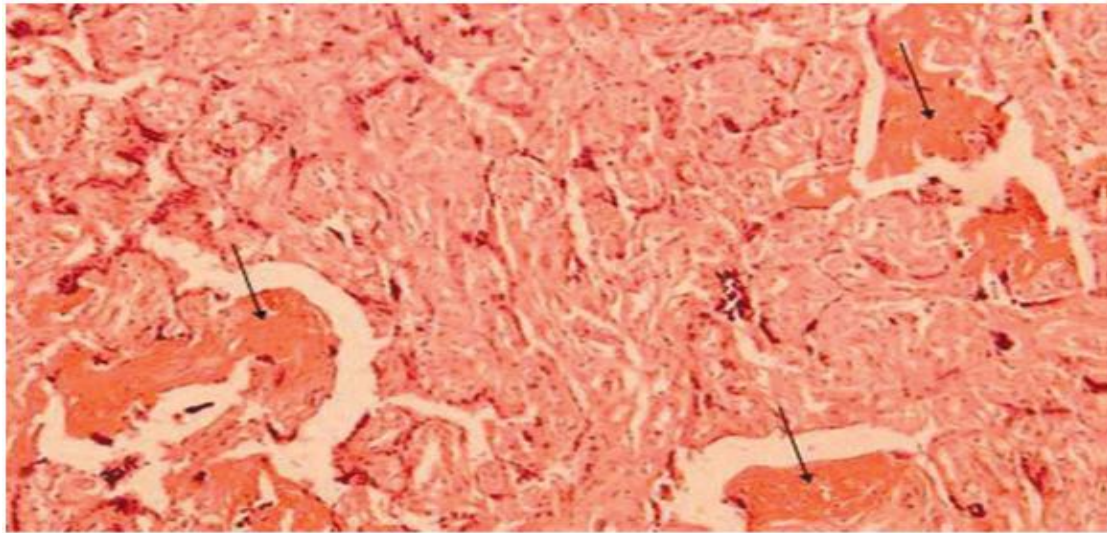
Arrow marked areas of syncytial knot formation. (H & E40X).

**Figure 1**



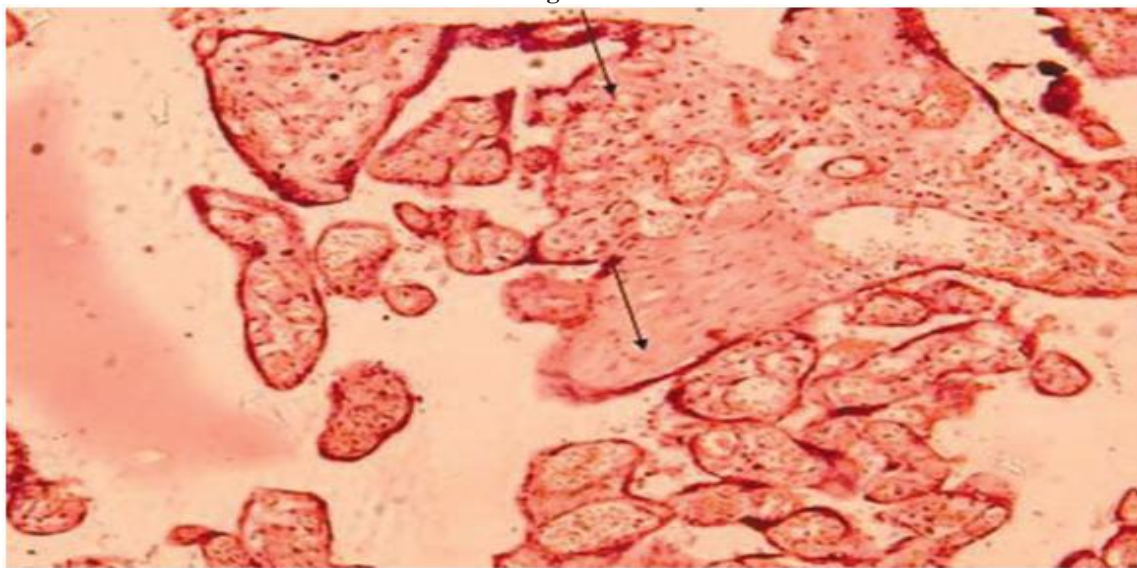
Arrow marked areas of villous edema. (H & E 40X).

**Figure 2**



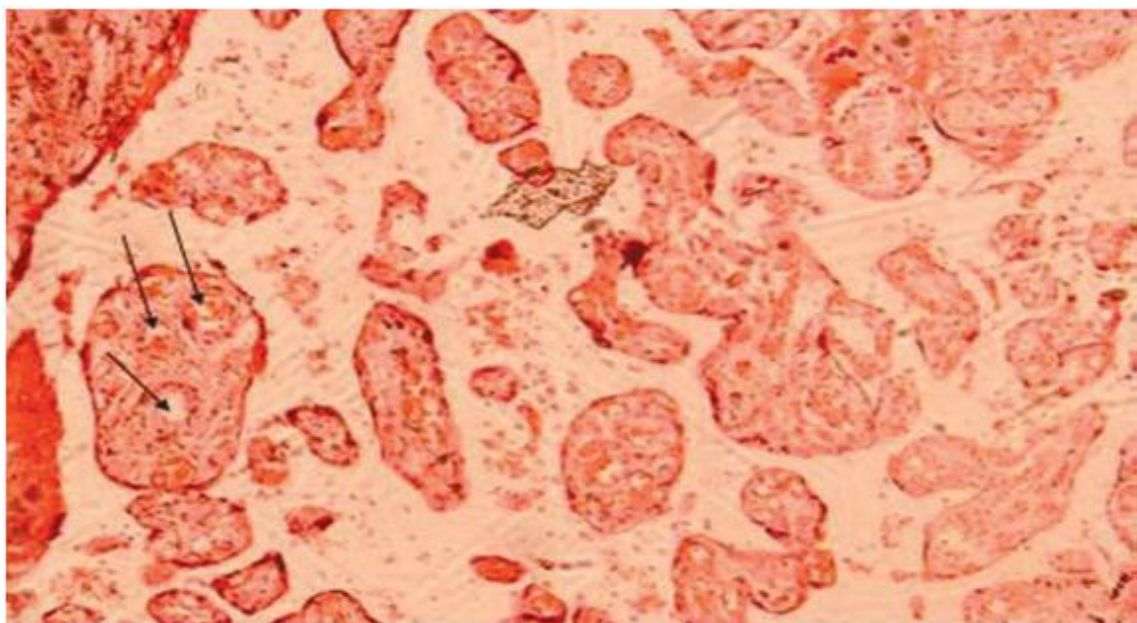
Arrow marked areas of fibrinoid necrosis and congestion of villi. (H & E 40X).

**Figure 3**



Arrow marked areas of villous fibrosis. (H & E 40X)

**Figure 4**



Arrow marked areas of capillary proliferation. (H & E 40X)

**Figure 5**

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#### 4. Discussion

The placenta forms a functional unit between the mother and the fetus that plays pleiotropic role during fetal growth. Therefore, any pathological event that concerns the mother or the fetus will influence the normal function of the placenta, occasionally resulting in morphological and histological change. These abnormalities of the placenta may lead to adverse fetal outcome [12]. Reports on the pathology of placenta in GDM are numerous but often contradictory. The inconsistency may be explained in part, by the fact that the categories of diabetic pregnant women are very heterogeneous [8]. The delineation of placental lesions in maternal diabetes has been also made unduly complex by the superimposed hypertension and other associated complications. In the present study, all the patients with GDM were perfectly controlled whatever the modality of treatment with insulin or without. Also other co-morbid condition such as hypertension, hypothyroidism, anaemia, jaundice, and maternal malnutrition even tobacco abuse, smoking, alcoholism etc. were excluded from study to create a chance of studying the true effect of GDM and modality of treatment on placenta without bias of other pathologic co-morbid conditions or even diabetes control status which, of course well known active player. The weight of placenta is an important and functionally significant parameter as it is related to villous area and fetal metabolism. In the present study, the mean placental weight, the mean placental diameter, the mean placental central thickness and the mean placental surface area in GDM group controlled with insulin was more as compared to control group and this difference was found highly significant ( $p < 0.01$ ). Similar findings were reported in previous studies by Verma et al (2010) [8], Chowdhury et al (2011) [13] and Saha et al (2014) [14]. The weight gain in placentae of diabetic mothers may be attributed to macrosomia and compensatory hyperplasia. Macrosomia affects the fetus and fetal part of placenta. This macrosomia may be attributed to fetal hyperinsulinemia in response to hyperglycaemia in fetuses of diabetic mothers [1, 3, 15]. The interesting point in the present study that, there are no significant differences of these parameters in GDM group controlled by diet without insulin and control group. Similar findings were reported in previous study by Verma et al (2010) [8]. In the same track, several differences were identified in the light microscopy of terminal villi from the placentae of GDM group controlled with insulin; large number of syncytial knots (Increased number of syncytiotrophoblast nuclei which showed chromatin clumping, a feature typical of senescence, and were usually arranged in clusters known as syncytial knots as defined by Jone & Fox, 1977 [11]), fibrinoid necrosis, villous edema, villous fibrosis and proliferation of the capillaries. While placentas of GDM group controlled with diet alone without insulin showed minimally increased number of syncytial knots, fibrinoid necrosis and none of them showed villous edema, villous fibrosis or fetal capillary proliferation resembling more to the control group. More or less similar results were found in previous old studies (16,17) where they found in addition fibrin necrosis and thickening of basal membrane of trophoblast in the placenta of poorly controlled diabetes. In our study, all the patients were perfectly controlled whatever the used

modality with insulin or without. Of course, it would be logical to assume that the less severe and better controlled the patients diabetic state, the less striking would be the placental abnormalities but according to our results we add that, there is something in insulin itself apart from diabetes control had an impact in placental pathology in gestational diabetes. Actually, it is not big surprise as many of diabetes complications occur in controlled diabetic status and usually related to compensatory hyperinsulinemia of insulin resistance or induced by medications. Also we had no data about uncontrolled transitional period of shifting the patients from diet control to insulin yet in my opinion, it had no or minimal effect. Anyhow, large scale study include big number from different races are needed for more clarification.

#### 5. Conclusion

The placentae of women with gestational diabetes mellitus treated with insulin even controlled shows significant variation in gross morphology and light microscopy that can be associated with impaired function of placenta, leading to possible adverse perinatal outcome. In the other hand, the placentae of women with gestational diabetes mellitus controlled with diet alone showed minimal changes with no significant differences from normal uncomplicated pregnancy. Control of GDM or better prevention by education, diet, optimum weight control and life style modifications should be strictly encouraged and insulin use should be the last resort.

#### References

- [1] Mukesh M. et al Gestational diabetes mellitus: An update on the current international diagnostic criteria. *World J Diabetes* 2015 June 25; 6(6): 782-791
- [2] Kumar A, Goel MK, Jain RB, Khanna P, Chaudhary V. India towards diabetes control: Key issues. *Australas Med J*. 2013; 6(10):524-531.
- [3] Pankaj S., Jai P., Anjali J. Gyan C. Effect of gestational diabetes mellitus on gross morphology of placenta: a comparative study. *Int J Anat Res* 2015; Vol 3(1):889-94.
- [4] Kalra P, Kachhwaha CP, Singh HV. Prevalence of gestational diabetes mellitus and its outcome in western Rajasthan. *Indian J Endocr Metab*. 2013; 17(4):677-680.
- [5] Fox H, Neil J, editors. *Pathology of the placenta*. 3<sup>rd</sup> ed. Philadelphia: Elsevier Saunders; 2007.
- [6] Teasdale F. Gestational changes in the functional structure of the human placenta in class relation to fetal growth: a morphometric study. *Am J Obstet Gynecol*. 1980; 137: 560-8.
- [7] Fox H. Pathology of the placenta in maternal diabetes mellitus. *Obstet Gynecol*. 1969; 34:792-8.
- [8] Verma R, Mishra S, Kaul J. Cellular changes in the placenta in pregnancies complicated with diabetes. *Int. J. Morphol.*, 28(1):259-264, 2010.
- [9] Benirschke K. The placenta: How to examine it and what you can learn. *Contemp Obst and Gynaecol*. 1981; 17:117-119.

- [10] Jones, C. J. & Fox, H. Ultrastructure of the normal human placenta. *Electron. Microsc. Rev.*, 4(1):129-78, 1991.
- [11] Jones, C. J. & Fox, H. Syncytial knots and intervillous bridges in the human placenta: an ultrastructural study. *J. Anat.*, 124(Pt 2):275-86, 1977.
- [12] Hargitai B, Marton T, Cox BM. Examination of human placenta. *J clin Pathol.* 2004; 57:785-792.
- [13] Chowdhury AHMMM, Shamim KM, Ferdousi R, Begum JA, Banu LA. A comparative study of effects of different grades of maternal established diabetes mellitus on placental and neonatal weight. *Bangladesh J Anat.* 2011; 9(1):53-58.
- [14] Saha S, Biswas S, Mitra D, Adhikari A, Saha C. Histologic and morphometric study of human placenta in gestational diabetes mellitus. *Ital J Anat Embryol.* 2014; 119(1):1-9.
- [15] Queenan JT. Management of high risk pregnancy. 4th ed. England: Blackwell science; 1999; 261-70.
- [16] Yang, H. X. Placental pathology in gestational diabetes. *Zhonghua Fu Chan Ke Za Zhi*, 28(12):714-6, 1993.
- [17] al-Okail, M. S. & al-Attas, O. S. Histological changes in placental syncytiotrophoblast of poorly controlled gestational diabetic patients. *Endocr. J.*; 41(4):355-60, 1994.