Original Research Article

Effects of Metformin and diet on Placental Morphology in Women with Gestational Diabetes Mellitus

ABSTRACT

Objective: The purpose of this study was to see how food organization and Metformin affected placental morphology in women with GDM.

Methods: 66 GDMs were registered after providing informed consent permission. Against who standards, 35 patients of GDM through blood sugar levels 140 mg/dl remained allocated Set B (2500-3000Kcal/day and 30-minute walk three times a week). They remained reserved on diet control, while 34 patients of GDM through blood sugar levels >140 mg/dl have been delegated Set C and remained reserved on diet with tablet Metformin (550mg TDS). Lastly, 28 healthy pregnant women remained retained in Set A as controls. Placentas were stored and analyzed for morphology after delivery.

Results: Heavy placentae thru extensive villous immature, charangoists, and syncytial knots were observed in set B, while fibrinoid necrosis and calcification were observed in set C. Placental and cord width were significant in Set B against A, but only cord width was relevant in Set C against A in gross morphology. In light microscopy, charangoists, infarction, and syncytial loops showed detected in sample 2 against with a villous maturity; moreover, charangoists and syncytial knots have been found in appendix B versus C placental width, but C versus A results were negligible.

Conclusion: In comparison to the diet group, metformin exhibited beneficial benefits on placental morphology that were equivalent to normal controls.

Keywords: Pharmaceutical Effects, Placental Morphology, Gestational Diabetes Mellitus

INTRODUCTION

GDM is defined as glucose intolerance discovered during second trimester of pregnancy. This is generated primarily by diabetogenic impact of placental hormones and is related by considerable embryonic and motherly significances. The global occurrence of GDM is 4-10%, but the incidence in our community is 4-4.48%, but the results are substantially worse owing to an absence of education and medical treatment facilities.

The placenta has a role in dietary intake of the developing baby. That is discoid-formed assembly coated in membranes and consists of several villi carrying minute blood arteries and mesenchymal supporting tissue under microscope. Modifications in parental milieu have an impact on the structural and functional of this key organ. In type 2 diabetes mellitus, the placenta undergoes several gross and microscopic changes, including

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Comment [U2]: What is the basis of this cut off value. WHAT IS THIS VALUE… RBS OR FPG?

Comment [U3]: WHAT DOES THIS MEAN

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Comment [U5]: You mean in group C

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a rise in proliferative degree of trophoblasts, stromal cells, and villous capillaries. This is primarily owing to increasing possessions of endogenous fetal insulin. Some other major factor is hypoxia, particularly happens in placental matter when fetal development and requirements increase. As a result, the volume, length, and depth of the placenta grow. Extra sugar in the maternal blood is also retained in placental tissue. Pathophysiology can be connected to changes in placental epidermal growth hormone, diabetes, and other tissue regeneration that govern placental formation and growth. This apparently causes the placenta to grow larger and thickness. Moreover, all hypoxia indicators are enhanced in GDM individuals' placentas when examined under a microscope. Elevated serum glucose levels require pharmaceutical therapy in addition to diet and exercise. Despite the fact that insulin is the global standard medication, it has been shown that though with insulin treatment, there had been a risk of maternal weight increase, macrosomia newborns, and unexpected term fatalities. This eventually results in a~~n~~ rise in the size in addition heaviness of the placenta. Additionally, all hypoxia parameters are significantly enhanced in GDM patients' placentas on microscopic examination. Elevated serum glucose levels require pharmaceutical therapy in addition to diet and exercise. Although insulin is a gold mainstay of treatment, this remained shown that also through insulin treatment, here would be a risk of maternal weight increase, macrosomia infants, unexpected term death of fetuses, and stillbirths. The use of insulin causes well-recognized hypoxic alterations in the placenta. In contrast to past, whenever oral anti-diabetic medications remained deemed to remain teratogenic, Metformin is now considered to remain harmless as a set B medication. This causes euglycemia by increasing insulin confrontation, increasing glucose absorption, and decreasing intracellular glucose synthesis. Moreover, this enhances capillary function, lowers hyperglycemia, and slows incidence and intensity of micro and macro vascular problems in DM type-2.

METHODS

This study was carried out at Services Hospital in Lahore. Fasting and random blood sugar levels of prenatal clinic participants were evaluated for this ongoing clinical investigation. GDM was diagnosed using a glucometer and rechecked with lab data using WHO standards (FBS > 110mg/dl and RBS > 130mg/dl). In dangerous/critical individuals, just like those with a history of GDM or a poor obstetric history, a 55 G oral glucose challenge test (value RBS 150mg/dl) was used to make the diagnosis, and an OGTT was used to verify it. Nonprobability survey method was used, and 29 normal girls with no co-morbidities (Group A) and 65 GDM women were recruited in the research after providing written informed permission. They have been encouraged to consume only 2800 calories per day, through food charts provided and 32 minutes of walking three times each week. In Group C, 32 GDM patients by RBS more than 140 mg/dl were given Metformin 500mg tablets coupled through stringent food management treatment (2600 calories per day and 35 minutes of walking three times per week). Metformin was begun at 500 mg and gradually raised to 1600 mg based on the patient's tolerance and glycemic levels. Those individuals remained followed in the prenatal hospitals on a regular basis until they reached term.

Comment [U7]: A GRAMMAR MISTAKES

Comment [U8]: NOT WHO CRITERIA FOR DIAGNOSIS OF GDM

Comment [U9]: BETTER DEFINATION OF CONTROL GROUP NEEDED

Comment [U10]: IN ABSTRACT ITS 66

Comment [U11]: WHY ONLY THIS AMOUNT AND WHICH GROUP IS THIS. SIGNIFICANCE OF THIE EXERCISE

Comment [U12]: WHY USE THE RBS VALUE INSTEAD OF THE PREFERED FPG VALUE

Comment [U13]: HOW OFTEN WAS THIS MONITORED AND THERE IS NEED EXPLAIN EXACTY WHAT IS MEAN BY PATIENT'S TOLERANCE AND GYCAEMIC CONTROL

Table-I: Gross Morphology within and between groups N=75 (n=25).

S. No.	*Groups Characteristics*	*Placental size1(cM)*	*Placental size2 (cm)*	*Placental width (cm)*	*Placental weight (gm)*	*Cord length (cm)*	*Cord width (cm)*	*Cord vessels (n)*
1	Group A	16.32±2.34	14.00±1.91	2.12±0.58	567.6±138.9	41.98±7.88	1.34±0.45	3±0
2	Group B	15.06±2.41	12.88±2.92	2.84±0.62	590±147.9	42.96±7.4	1.84±0.34	3±0
3	Group C	15.88±2.58	13.92±2.72	2.20±0.5	626.4±122.6	45.54±7.37	1.68±0.45	3±0
				P-value				
1	Group B v/s A	0.06	0.12	0.00*	0.58	0.65	0.00*	NA

2	Group C v/s A	0.5	0.9	0.6	0.119	0.1	0.01*	NA
3	Group B v/s C	0.25	0.12	0.001*	0.34	0.22	0.16	NA

Key: Group A: Normal control group, Group B: GDM females on diet control, Group C: Metformin treated group, *Statistically significant, NA: Independent t test not applicable.

On every visit of their treatment, their therapy, any adverse effects, and blood sugar level were all examined at each appointment, and adequate management was reaffirmed. Individuals (6 in group B and 7 in group C) were omitted from the trial because they gave birth elsewhere or were given insulin as part of their Metformin therapy. Placentas were obtained from these individuals within 32-44 minutes after birth and kept in 12 percent formalin in labelled containers. Following the dissection, the placenta was grossly morphologically evaluated (Tables I and II), and tissues were collected from the 7 o'clock, 12 o'clock, besides central locations for slide preparation. The portions subsequently treated with alcohol and xylene before being cut into blocks with paraffin wax. Sections were cut onto 4mm thin films using a manual microtome for slide processing, and slides were made. Following drying, the slides remained discolored with hematoxylin and eosin, PAS, and trichome stains and examined under a light microscope. Microscopically, hypoxic values in the placenta were identified. (Table-III).

SPSS version 16 was used to tabulate and analyses the data. The results were found to be statistically significant if the P value was less than 0.06. The mean of quantitative variables was evaluated using the Dependent t test, while percentages of categorical variables were evaluated using chi square tests. Due of low cell counts, the Fisher exact test was utilized instead of the chi square test.

Comment [U14]: Why a P value of 0.06? What was your P value for significance? IT'S NOT CLEAR WHETHER IT'S 0.05 or 0.001. It is all mixed up

RESULTS

Substantial changes in FBS, RBS, and HbAIC were identified between diabetics and non-diabetics. HbAIC was measured at 36 weeks of pregnancy and found to be significantly lower in group C significantly higher in group B. (Table-IV).

Comment [U15]: HbAIc only appearing in results section and was not mentioned in the merhodology.

For placental breadth and cord width, the differences between groups B and A were statistically expressive (p value of 0.001). The only significant difference between groups C and A was in cord width (p=0.01), whereas all other characteristics were comparable. Whenever group B and C were evaluated, placental width was significant statistically (p= 0.001), but all other measures were non-significant, indicating that diet-controlled GDM placentas were thicker than Metformin-treated placentas.

The results between all the three groups (normal control, GDM on diet therapy, GDM on metformin therapy) were statistically non-significant. (Table-II) On light microscopy hypoxic placental feature between group B and A were statistically significant for villous immaturity, charangoists,

Table-III: Microscopic morphology within and between groups N=75 (n=25).

S. No.	Groups	Characteristics											
		Villous immaturity		Chorangiosis		Infarction		Villous fibroid necrosis		Calcification		Syncytial Knots	
		P	A	P	A	P	A	P	A	P	A	P	A
1	Group A	4(16%)	21(64%)	6(24%)	19(76%)	7(28%)	18(72%)	18(72%)	7(28%)	11(44%)	14(56%)	4(16%)	21(84%)
2	Group B	10(40%)	15(60%)	13(52%)	12(48%)	14(56%)	11(44%)	19(76%)	6(24%)	10(40%)	15(60%)	14(56%)	11(44%)
3	Group C	5(20%)	20(805)	5(20%)	20(80%)	9(36%)	16(64%)	22(88%)	3(12%)	15(60%)	10(40%)	7(28%)	18(72%)
		P-value											
4	Group B v/s A	0.05*		0.04*		0.04*		0.74		0.77	0.25	0.003*	
5	Group C v/s A	>0.9^		0.73		0.54		0.15		0.15		0.3	
6	Group B v/s C	0.12		0.01*		0.15		0.24^				0.04*	

Key: Group A: Normal control group, Group B: GDM females on diet cont Group C: Metformin treated group,
*Statistically significant, P: Present, A: Absent, ; ct test applied due to decrease cell count.

Overall, 3 groups' findings (normal control, GDM on diet therapy, and GDM on metformin therapy) remained statistically meaningless. (Table-III) On light microscopy , the changes in hypoxic placental characteristics between groups B and A for villous immature, charangoists, and Key: Set A is a normal control group, while Section B is a group of ladies with GDM who are on a diet.

*Statistical significance for P: Present, A: Absent, infarction, and syncytial knots (p=0.04, 0.05, 0.06, and 0.04, correspondingly). (Fig.1). All of the microscopic metrics for groups C and A were statistical non-significant, indicating that metformin-treated placentas remained equivalent to normal controls placentas. (Fig.1). For microscopic characteristics, the findings for groups B and C were substantial for charangoists and syncytial knot development (p= 0.02 & 0.05 respectively), indicating that way of eating placentae exhibited charangoists and syncytial knots when compared to Metformin-treated placentae.

DISCUSSION

GDM is linked to structural and functional changes in the placenta, which can result in fetal hypoxia, illness, and stillbirth. These might be seen as visible and microscopic changes in the morphology of placentae, the primary communication tissue seen amongst mother and the growing kid. Changes in placental morphology caused by hypoxia in GDM include villous maturity, ischemia, and villous fibrotic and necrotic areas. Charangoists, calcification, syncytial knots formation from usual placentas in rapports of villous childishness, infarction, chorangiosis, and making of syncytial knots. Verma et al. (2018) found that in GDM preserved just through diet, placentae displayed fibrosis and ischemia alterations, increased syncytial knots, moderate edoema, and fibrinoid necrosis, that remains consistent with the current findings. Metformin-treated placentas exhibited non-significant results in gross placental morphology when compared to normal controls, with the exception of cord width. Altogether residual gross and microscopic hypoxia indicators remained not statistically significantly different across groups, suggesting that it was near to control. According to light microscopic data, metformin-treated placentas exhibited considerably reduced thickness, charangoists, and syncytial knot development than way of eating placentas. The remainder microscopic hypoxia indicators were statistically lesser in metformin set than in diet control set.

Campbell described a specific instance in 2012 wherein the GDM individual having preeclampsia on Metformin died intrauterine and placental morphology exhibited severe abnormalities such like villous dysmaturity, thorianites, and villi fibrosis. Though, this was uncertain yet if the placental variations are induced by only gestational DM or by a grouping of GDM and hypertension.

The majority of in-vivo and in-vitro investigations had demonstrated that Metformin reduces gluconeogenesis predominantly via inhibiting lactate absorption in adipocytes. Some other main element that has been observed is a decrease in Actin polymerization in hepatocytes, that leads to a reduction in hepatocyte glucose production from glycogen. This also interferes with oxidation of the breathing

Comment [U16]: Discussion section is inadequate. There is need to discuss the findings in comparison to other researchers' findings on similar work. Discussion on blood results missing. Effect of the drug on blood glucose and HbAic need to be discussed.

chain in the mitochondria of liver cell at the cellular level. Metformin also dramatically reduces HbAIC. Metformin-treated placentae, as seen in Fig.1C, may contribute to the drug's favorable benefits in GDM sufferers. Metformin's various functions in diabetic cells may explain why it has a positive effect on placenta associated to diet alone.

Table-IV: Maternal characteristics

s v/s A) (C v/s A) & (B v/s C) N=75.

S. No.	*Maternal Characteristics*	*AGE (years)*	*WEIGHT (kg)*	*FBS (mg/dl)*	*RBS (mg/dl)*	*HbA_1C 1*	*HbA_1C 2*
		Mean ± S.D	*Mean ± S.D*	*Mean ± S.D*	*Mean ± S.D*	*%*	*%*
1	Group A	29.0±4.37	73.84±9.97	72.24±9.34	126.8±35.8	4.84	4.97
2	Group B	30.08±3.16	78.54±6.93	90.9±16.8	148.72±38.9	5.34	5.74
3	Group C	29.76±3.41	77.9±7.6	104.4±13.12	171±37.44	5.28	5.42
				P-value			
1	Group B v/s A	0.32	0.059	0.00*	0.03*	0.00*	0.00*
2	Group C v/s A	0.49	0.11	0.00*	0.00*	0.001*	0.00*
3	Group B v/s C	0.75	0.75	0.00*	0.04*	0.693	0.01*

Group A: Normal control group, Group B: GDM females on diet control, Group C: Metformin treated group, *Statistically significant, FBS: fasting blood sugar at the time of enrollment; RBS: Random blood sugar at the time of enrollment, HbA_1C 1: at the time of patient enrollment, HbA_1C 2: at 36 weeks of gestation.

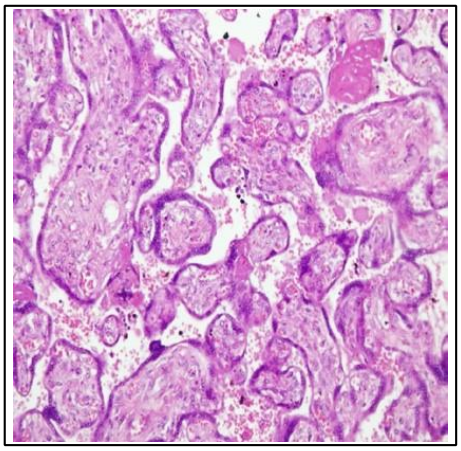


Fig.1A: Histology of normal healthy placenta.

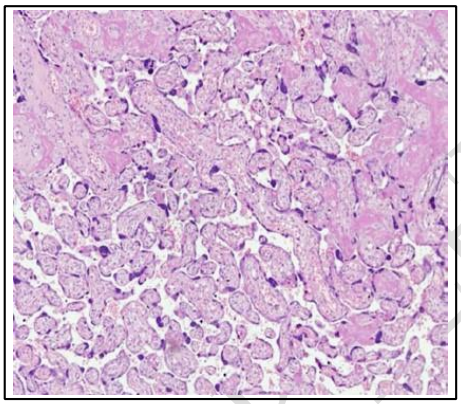
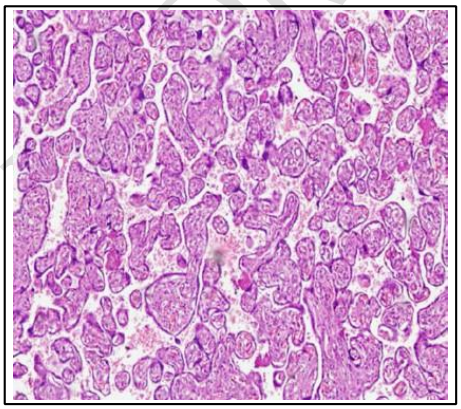


Fig.1B: Diet control placenta.



CONCLUSION:

In summary, once way of eating and Aminoglycoside placentas were compared to normal control placentas, Metformin-treated placentas had morphology comparable to traditional control placentas, but diet-controlled placentas exhibited substantial gross and histological alterations. Large-scale educations of Methimazole placentas using electron microscopy and immunohistochemistry appear to be a potential topic for the new researchers.

Comment [U17]: NOT AN APPROPRIATE CONCLUSION OF THE FINDINGS

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