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Study of DNA Damage in Diabetic Mothers and Their Newborn

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ABSTRACT

Background: Previous research declares that gestational diabetes mellitus (GDM) affects both mother and infant during pregnancy and in the long term.

Objective: To assess the pattern of DNA damage in mothers with gestational diabetes during pregnancy and their possible impact on their offspring.

Patients and Methods: A case-control study was designed and conducted on 120 pregnant women and their infants; divided into two groups: 1-Patients group (60 patients had gestational diabetes mellitus during the third trimester of pregnancy and their infants) 2-Control group (60 apparently healthy pregnant women and their infants). **Results:** DNA damage of mother and their infants was significantly increased among the cases group than controls (p=0.0001). There was a statistically significant increase among infants who had DNA damage group than who hadn't regarding HBA1C, random blood sugar (RBS) 3 hr and RBS 6 hr (p<0.05). Also, infants who had DNA damage had statistically significant decrease regarding Ca, RBS 24 hr, RBS 36 hr and RBS 48 hr (p<0.05). While there was no statistically significant difference between the studied groups regarding Hb, hematocrit, RBS, fasting BS, AST, Hemoglobin (Hb) at 1 hr, hematocrit at 1 hr, Hb 24 hr, hematocrit 24 hr, RBS 1hr, RBS 2 hr and RBS 12 hr (p>0.05). C**onclusions:** Hyperglycemia affects maternal and fetal DNA integrity and DNA damage response differently, gestational and mild gestational hyperglycemia, were all related to increased oxidative DNA damage and DNA repair may be thus considered an important mechanism to prevent the deleterious effects of hyperglycemia in the genetic material.

Keywords: DNA damage, Gestational diabetes mellitus, Hyperglycemia, Newborn, Pregnant.

INTRODUCTION

Gestational diabetes is any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies whether insulin or only diet modification is used for treatment and whether or not the condition persists after pregnancy. Studies have reported that women are more than seven times as likely to develop diabetes after GDM, and that approximately 50% of mothers with GDM will develop diabetes within 10 years, making GDM one of the strongest predictors of type 2 diabetes [(1)].

The International Association of Diabetes and Pregnancy Study Groups (IADPSG) showed new criteria for the diagnosis of GDM, based on the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) Study. This criteria use a 75-g oral glucose tolerance test (OGTT) and diagnose GDM when the fasting glucose is ≥5.1 mmol/L (90 mg/dL) and/or when the 1 h post load glucose is ≥10.0 mmol/L (180 mg/dL) and/or when the 2-h post load glucose is ≥8.5 mmol/L (150 mg/dL) [(2)].

It is well known that normal pregnancy is accompanied by a marked increase in insulin resistance, which may be the result of both increased maternal adiposity and the insulin-antagonizing effects of several placental hormones. Therefore, maternal pancreatic beta cell compensation is important for overcoming the insulin resistance provoked by pregnancy and for maintaining the metabolic balance during pregnancy. There are some risk factors that predispose the development of GDM as pre-pregnancy, overweight, obesity, family history of diabetes, advanced maternal age, poor diet, low physical activity before or during pregnancy, history of subfertility or infertility, polycystic ovarian syndrome as well as genetic factors [(3)].

PATIENTS AND METHODS

All subjects included in this study were divided into two groups as: Group I (cases): Included 60 pregnant mothers with gestational diabetes mellitus; their ages ranged between (25 to 38 years) with mean age (31± 3.5) and their infants and **Group II (controls):** Included 60 pregnant mothers, their ages ranged from (21-36 years) with mean age (26 ± 5.5) years and their infants.

Inclusion criteria:

All pregnant mothers with gestational diabetes mellitus (type 1, type 2 and gestational) and their infants.

Exclusion criteria:

Mothers with chronic diseases as chronic renal diseases, chronic liver diseases and chronic heart diseases.

Gestational diabetes mellitus was diagnosed at any time during pregnancy based on any one of the following values: (1) Fasting plasma glucose = 5.1-6.9

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mmol/L (92-125 mg/dL), (2) 1 h post 75 g oral glucose load ≥ 10.0 mmol/L (180 mg/dL) (there are no established criteria for the diagnosis of diabetes based on the 1 h post-load value), (3) 2 h post 75 g oral glucose load between 8.5-11.0 mmol/L (153-199 mg/dL) [4].

Ethical considerations:

The study protocol was approved by the Ethical Committee of Faculty of Medicine, Menoufia University. Informed consent was obtained from mothers and they were informed about the objective of the study, its benefits and the absence of any risk associated with the participation of their children.

All participants were subjected to the following: Detailed history taking included age, address, consanguinity, family history of diabetes, present history (type of diabetes, medication before and during pregnancy, complication of diabetes during pregnancy) and obstetric history (parity, full term or preterm and previous abortion), **Complete general examination of all body systems** and **Laboratory investigations** included complete blood count (CBC), Hb (gm/dl**),** fasting plasma glucose level (mg/dl)**,** post-prandial plasma glucose level after 2 hr (mg/dl)**,** HBA1C (%)**,** liver function (AST(U/L),ALT(U/L))**,** renal function (urea (mg/dL), creatinine (mg/dL))**.**

From infants of these diabetic pregnant mothers: Detailed history taking: personal history (name, age, sex), antenatal (fever, hemorrhage, premature rupture of membranes (PROM) or others), natal (mode of delivery CS or normal vaginal delivery, (full term, preterm or post term), sedation (general or spinal) and use of forceps, post-natal (Apgar and Ballard scores), **Complete general examination of all body systems, Laboratory investigations:** Complete blood count (CBC) Hb (gm/dl), C-reactive protein (CRP) (mg/dl), Random blood sugar (RBS) (mg/dl)**,** Calcium level (gm/dl)**,** magnesium level (gm/dl), total and direct bilirubin level (mg/dl), **Radiological investigation:** including chest x- ray, Echo-cardiology and abdominal ultrasound if needed and **Special investigations, which included:** Estimation of DNA damage in peripheral leucocytes by DNA extraction; done by the use of Gene Jet Whole Blood Genomic DNA Purification Mini Kit and then Gel electrophoresis [5]**.**

Principle:

Samples were digested with proteinase K in the supplied lysis solution. The lysate was then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane. Impurities were effectively removed by washing the column with the prepared wash buffers. Genomic DNA was then eluted under low ionic strength conditions with the elution buffer. Additional materials and equipment required.???

Statistical Analysis

Results were statistically analyzed using a personal computer using SPSS v. 21 (SPSS Inc., Chicago, IL, USA), using descriptive: e.g. percentage (%), mean and standard deviation, median, and range. Analytical: that included: Chi-Square (X^2) and Fishers exact test for qualitative data and Mann-Whitney test and unpaired t test for quantitative data and Pearson's correlation coefficient (r). A value of P less than 0.05 was considered statistically significant.

RESULTS

In our study, there was a statistically significant increased among the cases group than controls regarding age. While there was a statistically significant decreased regarding family history, complication, hyperglycemia, PROM, hypoglycemia, hypertension, parity and gestational age. Also, there was a statistically highly significantly decrease among the cases group regarding mother and infant DNA damage than controls. Also, there was a statistically significant difference between the infants of both groups regarding gestational age, ABGAR score at 1 minute, weight and respiratory rate (Table 1).

Table (1): Data of mothers and their infants

	Variables	Control (N=60)		Cases (N=60)		Test	P-value
Mothers	**Age (years)** - mean ± SD - median (range)	27.00 ± 5.56 26.00 (21-36)		30.85 ± 3.56 31.00 (25-38)		U=4.08	**<0.001***
	Weight (Kg)	87.05 ± 7.441		86.00 ± 4.981		U=0.577	0.564
	Family History of diabetic mothers	No	%	No	%		
	Positive **Negative**	0 60	0.0% 100%	21 39	35% 65%	X^2=57.7	**<0.0001***
	Parity p 1 **p 2** **p 3** **p 4** **p 5**	12 (20.0%) 12 (20.0%) 21 (35.0%) 15 (25.0%) 0 (0.0%)		0 (0.0%) 15 (25.0%) 24 (40.0%) 12 (20.0%) 9 (15.0%)		X^2=21.8	**<0.0001***
	Delivery **1-normal vaginal** **2-caesarian section**	33 (55.0%) 27 (45.0%)		30 (50.0%) 30 (50.0%)		X^2=0.30	0.533
	DNA damage **1) yes** **2) no**	60 (100.0%) 0 (0.0%)		39 (65.0%) 21 (35.0%)		FE= 25.45	**0.0001***
Infants	**Gestational age (weeks)** - Mean ± SD - Median	37.7 ± 0.96 38.00		36.70 ± 1.28 37.00		t=4.341	**<0.0001***
	APGR at 1 minutes	8.60±0.807 9.00 (7-9)		7.25±0.950 7.00 (5-9)		U=6.9	**<0.0001***
	Sex **1- Male** **2- Female**	8.60±0.807 9.00(7-9)		30 (50.0%) 30 (50.0%)		X^2=1.21	0.271
	Weight	36 (60.0%) 24 (40.0%)		3.430±0.5776 3.500 (2.3-4.5)		U=5.017	**<0.0001***
	Respiratory rate	41.25±3.917 40.00 (38-55)		46.65±7.859 46.00 (35-60)		U=3.77	**<0.001***
	Heart rate	138.00±9.735 140.00 (123-152)		138.45±9.537 140.00 (122-155)		U=0.383	0.7
	DNA damage: **1) Yes** **2) No**	57 (95.0%) 3 (5.0%)		36 (60.0%) 24 (40.0%)		X^2=21.07	**<0.0001***

SD: standard deviation t: student t test U: Mann Whitney U test
FE: Fishers exact test X^2: Chi-Square test *: significant

In our study, mothers who had DNA damage had statistically significant increase regarding HBA1C, RBS, Hb at 1st hour, HB at 24 hours and serum calcium (Table 2).

Table (2): Comparison between mother who had or hadn't DNA damage regarding lab investigation and random blood sugar

Cases	Mother DNA damage		U	p-value
	No N=39	Yes N=21		
Hb			0.913	0.36
Mean ± SD	11.09±0.7304	11.25±0.91		
Median	11.20	11.50		
HBA1C			2.2	0.025*
Mean ± SD	7.22± 0.94	7.87± 1.42		
Median	7.00	7.50		
Random B.S			2.66	0.008*
Mean ± SD	170.77± 9.96	237.86± 8.61		
Median	170.00	180.00		
Hb at 1hr			2.79	0.005*
Mean ± SD	17.77±1.61	16.14±2.58		
Median	17.90	15.20		
HB 24hr			3.77	0.0001*
Mean ± SD	16.385±1.37	14.56±2.11		
Median	16.30	13.50		
Ca			3.07	0.002*
Mean ± SD	4.26±0.71	4.71±0.47		
Median	4.10	4.60		
RBS at 1hr			0.42	0.675
Mean ± SD	55.31±12.301	55.29±2.89		
Median	55.00	53.00		
RBS at 2hr			1.6	0.108
Mean ± SD	66.92±6.371	74.43±3.85		
Median	63.00	72.00		
RBS at 3hr			0.49	0.624
Mean ± SD	84.54±15.768	84.00±14.61		
Median	84.00	86.00		

SD: standard Deviation HB**:** Hemoglobin Ca: calcium U: Mann Whitney U test *: significant RBS: random blood sugar

There was a statistically significant increase among infants who had DNA damage group than who hadn't regarding urea, creatinine, ALT, serum Ca, RBS at 6 hr and RBS at 24 hr, and RBS 36 hr (Table 3).

Table (3): Comparison between infant who had or hadn't DNA damage regarding lab investigation and random blood sugar

Cases	Infant DNA damage		U	p-value
	No N=36	Yes N=24		
Hb			0.547	0.584
Mean ± SD	11.05±0.82	11.29±0.76		
Median	11.35	11.1		
Hematocrit			0.887	0.375
Mean ± SD	34.36±3.54	34.54±1.46		
Median	35.03	34.15		
Urea			2.113	0.035*
Mean ± SD	21.58±1.47	27.38±4.89		
Median	22.00	26.50		
Creatinine			2.46	0.014*
Mean ± SD	0.68±0.11	0.76±0.13		
Median	0.65	0.75		
Alt			2.25	0.024*
Mean ± SD	20.92±4.26	25.38±4.08		
Median	18.00	24.50		
AST			1.227	0.22
Mean ± SD	24.50± 4.23	28.25± 3.67		
Median	24.00	28.50		
Ca			2.65	0.008*
Mean ± SD	4.61±0.57	4.14±0.73		
Median	4.50	4.050		
RBS 1hr			0.146	0.89
Mean ± SD	55.50±5.47	55.00±8.47		
Median	54.00	52.50		
RBS 6hr			2.388	0.017*
Mean ± SD	78.67±9.58	79.00±3.32		
Median	80.50	87.00		
RBS 24hr			4.28	0.0001*
Mean ± SD	106.67±12.18	90.87±14.49		
Median	109.50	93.00		
RBS 36hr			4.40	0.0001*
Mean ± SD	102.67±11.57	88.25±9.12		
Median	101.50	89.00		

SD: standard Deviation Hb**:** Hemoglobin Ca: calcium. U: Mann Whitney U test *: significant

There was a significant positive correlation between HbA1C, RBS of mother, FBS of mother and mother DNA damage. There was a significant positive correlation between HbA1C of mother, fasting blood sugar of GDM and DNA damage in infants. There was a significant negative correlation between hemoglobin at 1 hour in infants and mother DNA damage (Table 4).

Table (4): Spearman correlation of data of infants of diabetic mothers

			Mother DNA damage N=60	Baby DNA damage N=60
Mother	Hb a1c	r	0.292*	0.675**
		p-value	0.024	<0.001
	Random B.S	r	0.347*	0.133
		p-value	0.007	0.310
	Fasting B.S	r	0.482*	-0.009
		p-value	<0.001	0.946
Infants	APGR at 1 minutes	r	0.167	-0.181
		p-value	0.203	0.165
	R R	r	0.219	-0.205
		p-value	0.092	0.117
	heart rate	r	0.083	-0.045
		p-value	0.528	0.733
	HBat 1 hr	r	-0.364*	-0.053
		p-value	0.004	0.687
	RBS 1 hr	r	-0.055	0.018
		p-value	0.679	0.893

*: significant (-) negative correlation.

DISCUSSION

This study showed that, there was a statistically significant decrease among the cases group than controls regarding mother and infant DNA damage. Our results correspond to **Moreli *et al.* [6] who** demonstrated that the relationship between type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), and DNA damage is well established. Also, to **Gelaleti *et al.* [7]** who revealed regarding DNA damage, the comet assay was used to evaluate basal DNA damage levels in pregnant women with either diabetes or mild hyperglycemia. The DNA damage levels were lower in the diabetic group compared to the non-diabetic group. In addition**, Gelaleti *et al.* [7]** in their literature reported that diabetes hyperglycemia is related to increased DNA damage **[8]**. In fact, diabetes and hyperglycemia can generate reactive oxygen species (ROS) that may induce genetic damage. Some studies showed increased DNA damage in leukocytes of type 1 and type 2 diabetes mellitus individuals. **Lima *et al.* [9] and Pitozzi *et al.* [10]** previously described that patients with DM2 have a higher frequency of cells with DNA damage than those with DM1. Recently, **Zengi *et al.* [11]** also showed increased oxidative DNA damage in lean normoglycemic offspring of DM2 patients.

While, little studies are known about DNA damage in pregnancy especially in pregnancy complicated by pre-gestational (T1DM or T2DM) or gestational diabetes mellitus (GDM) **[12-14]**, this study showed that there was a statistically significant increase among mothers who had DNA damage group regarding hematocrit (Hct), HBA1C, rRandom B.S, fasting B.S, Ca and RBS 24 hr than who hadn't. Also, there was a statistically significant decrease among mothers who had DNA damage regarding urea, Alt, AST, Hb at 1hr, HB 24 hr and HCT 24 hr. While there was no statistically significant difference between the studied groups regarding Hb, Creatinine and Hct at 1hr and RBS from 1hr-12 hrs, 36 hrs and 48 hrs. Other experimental studies conducted by **Moreli *et al.* [6]** with streptozotocin-induced diabetic rats showed that the levels of basal DNA damage in leukocyte of mothers with severe diabetes (blood glucose ≥ 300 mg/dL) and their respective fetus was higher when compared with the control group **[12,15].** Subsequently, **Lima *et al.* [9]** demonstrated that rats with severe diabetes and their offspring showed higher oxidatively generated DNA damage in leukocyte detected by FPG (form amido pyrimidine-DNA glycosylase) and endonuclease III-sensitive sites when compared to mild diabetes group (blood glucose levels between 120 and 299 mg/dL). Taken together, these experimental results suggest that the intensity of diabetes is related to the levels of oxidative DNA damage. Thus, hyperglycemia may have repercussions at the DNA level that go beyond the pregnant mother.

Another study by **Gelaleti *et al.* [7]** did not detect a significant difference in the urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentration among the three groups (Diabetic, mild gestational hyperglycemia (MGH) and Controls). However, the values increased according to the concentration of glucose, suggesting a relationship between blood glucose and oxidative

stress. Recently, therefore, longitudinal studies, with serial measurements of urinary 8-OHdG concentrations along with indices of insulin sensitivity and secretion across gestation, are needed to elucidate the mechanisms and pathophysiological consequences of maternal oxidative stress during pregnancy. This study showed that, there was a statistically significant increase among infants who had DNA damage group regarding +ve family history, presence of hyperglycemia and hypoglycemia than who hadn't. 87.5% of infants who had DNA damage had +ve family history and hyperglycemia, while none of them had hypoglycemia. Whereas, there was no statistically significant difference between the studied groups regarding age, complications, PROM, hypertension, parity, delivery, gestational age, weight, APGR at 1 minutes, maternal height, maternal weight and systolic BP, diastolic BP, respiratory rate and Heart rate.

This was consistent with **Gelaleti *et al.*** [16]**.** They demonstrated that, the correlation between maternal glycemia and offspring DNA damage levels indicated that increased maternal blood glucose levels aggravate DNA damage in offspring. In that study, correlation analysis within each group revealed a significant positive correlation between maternal glucose levels and offspring DNA damage levels in both the nondiabetic and the diabetic groups. Although all patients in these groups were obese, correlation analysis took into account the glycemic variations in these women and the presence of DNA damage in their infants. The glycemic values found in the groups were within the normal range but, in general, increasing maternal blood glucose levels were associated with increased offspring DNA damage. In the MGH group, no correlation between maternal glucose levels and offspring DNA damage levels was observed, but other factors related to diabetes, such as obesity, hypertension, and insulin resistance were present. These factors might also contribute to DNA damage levels [17]**.** In addition, **Bukhari *et al.*** [18] found that, in obese individuals, DNA damage levels are strongly correlated with triglycerides, low-density lipoprotein-cholesterol, systolic blood pressure, cholesterol, malondialdehyde, and total oxygen stress. **Yıldız *et al.*** [19] demonstrated that patients with hypertension are more commonly affected by ROS-induced DNA damage, and **Song *et al.*** [20] showed that oxidative stress leads to high levels of insulin resistance. This study showed that, there was a statistically significant increase among infants who had DNA damage group regarding HBA1C, urea, creatinine, ALT, Mg, RBS 3 hr and RBS 6 hr than who hadn't. Also, there was a statistically significant decrease among infants who had DNA damage group regarding Ca, RBS from 24 hr-48 hr. While there was no statistically significant difference between the studied groups regarding HB, Hct, Random BS, Fasting BS, AST, HB at 1hr, Hct at 1hr, HB 24hr, HCT 24hr, RBS from 1hr-12hr.

Consistent with our findings, **Mahat *et al.*** [21] found increased levels of glucose (both fasting and 2 h plasma glucose after giving 75 gm of glucose) in prediabetic subjects as compared to control subjects. In addition to this, they also found an increased level of HBA1C in pre-diabetic subjects as compared to controls. Also, with **Mohieldein *et al.*** [22] reported a significant increase in the levels of glucose and HBA1C in pre-diabetic subjects as compared to normoglycemic subjects**. Pereira *et al.*** [23] also revealed that hyperglycemia results in auto-oxidation of glucose, which contributes to the non-enzymatic glycation of proteins and stimulation of polyol pathway. These metabolic changes may lead to an increase in ROS generation, which causes damage to the DNA, including oxidized bases and DNA strand breaks with an increase in 8-OHdG and destruction of endothelial function resulting in atherosclerosis [24]**. Al-Aubaidy *et al.*** [24,25] showed a statistically significant increase in the level of 8-OHdG in prediabetic subjects as compared to control subjects, which is in line with previous studies.

The result of **Al-Aubaidy *et al.*** [24] study indicates cellular damage occurs even before the onset of full-blown diabetes, which is the stage of prediabetes. This may be due to an increased level of glucose in prediabetic subjects, which causes increased formation of ROS that leads to oxidative DNA damage**.** The increased level of HBA1C in prediabetes may also have a purported impact on DNA damage. Hydrogen peroxide, which is generated during hyperglycemic condition, induces the release of iron from hemoglobin and induces more release from glycated hemoglobin than from its non-glycated analog. Free iron participates in Fenton reaction, producing ROS, creating the environment of oxidative stress, and acting as one of the key DNA damaging agents.

CONCLUSION

Hyperglycemia affects maternal and fetal DNA integrity and DNA damage response differently. Gestational and mild gestational hyperglycemia, were all related to increased oxidative DNA damage and DNA repair may be thus considered an important mechanism to prevent the deleterious effects of hyperglycemia in the genetic material. The reflection of oxidative stress pattern warranted the importance of care to these vulnerable groups of mothers and their infants.

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