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RESEARCH ARTICLE

Advanced Glycation End Products (AGEs) level and insulin resistance in women with gestational diabetes

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Abstract

Objective: to study the level of the oxidative stress marker, Advanced Glycation End products (AGE)s in women with gestational diabetes, and to assess its usage as a biomarker in addition to the insulin resistance indexes in the diagnosis of gestational diabetes.

Methods: A case control study was carried out from January to June/2014. Sixty pregnant women at third trimester between (37-41) weeks of gestation and average age between (18-45) years old were enrolled in this study. They were admitted to different hospitals in Baghdad. All cases selected for this study underwent elective caesarian section. Thirty healthy pregnant women at the same average of age involved as control group. Patients and controls were comparable in age.

Results: Patient's AGEs, FSG, HbA1c, C-peptide concentration and HOMA-IR were found significantly increased when compared vs. the control (p-value <0.0001). While patient's insulin level showed no significant difference (p-value 0.062) when compared with control.

Conclusion: Depending on the ROC analysis, AGEs was found more accurate than HOMA-IR. So, it may be consider as a good biomarker in addition to the HOMA-IR in diagnosis women with gestational diabetes.

INTRODUCTION

Gestational Diabetes Mellitus (GDM) is a condition in which women without previous history of diabetes exhibit high blood glucose levels during pregnancy, especially during second to third trimester. [(1)] It is usually effect (3-10) % of pregnancies and develops in one of 25 pregnancies worldwide. GDM usually disappears after pregnancy, but women with GDM and their children are at an increased risk of developing type 2 diabetes later in life. [(2)] A study done by O'Reilly et al. 2011 concluded that gestational insulin use, non-European ethnicity, a family history of type 2 diabetes mellitus, and an elevated body mass index (BMI) were factors associated with persistent dysglycemia in women who have had gestational diabetes mellitus. [(3)] Glycated hemoglobin (HbA1c) identifies average plasma glucose concentration. It develops when haemoglobin, a protein within red blood cells that carries oxygen throughout the body joins with glucose in the blood, becoming 'glycated'. It is an overall picture of what the average blood sugar levels have been over a period of weeks/months (over the past 8-12 weeks). [(4)] In 2009, the American Diabetic Association added that HbA1c $\geq$ 6.5% is another criterion for the diagnosis of diabetes. Therefore, it is highly recommended to measure HbA1c during pregnancy, as additional diagnostic criteria and to anticipate the maternal and fetal complications if it is abnormally elevated. [(5)] Insulin is a 51-amino acid polypeptide (small protein) hormone, it is produced and stored in beta cells of the pancreas and degraded within about one hour after its initial release into circulation, it is central to regulating carbohydrate and fat metabolism in the body. [(6, 7)] A decrease in insulin sensitivity (i.e. an increase in insulin resistance) is normally seen during pregnancy to spare the glucose for the fetus. This is attributed to the effects of placental hormones. Pregnancy is associated with increase in the beta-cell mass and increase in insulin level throughout pregnancy but certain pregnant women are unable to up-

regulate insulin production relative to the degree of insulin resistance, and consequently become hyperglycemic, developing gestational diabetes. [5]

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Oxidative tissue and organ damage play roles in DM and its complications. Hyperglycemia can cause oxidative stress particularly by evidence that several biochemical pathways activated during hyperglycemia can increase the production of free radicals. Oxidative stress is a pathophysiological process leading to multiple outcomes in diabetic pregnancies. [8] Pregnancy is susceptible to oxidative stress and antioxidant defenses that can be altered in response to elevated levels of oxidative stress. Beyond hyperglycemia, the increased local oxidative stress, frequently associated with diabetes, directly promote the formation of Advanced Glycation End-products (AGEs). [9] Advanced Glycation End-products (AGEs) are a class of complex often unstable-reactive compounds formed in excess during aging and diabetes mellitus. AGEs formed as the end-stage products of Glycation reactions also known as the Maillard reactions [non-enzymatic glycosylation processes, in which a sugar molecule bonds to either an amino group of long-lived proteins or a lipid molecule to produce non-enzymatic cross-links, these cross-links known as advanced glycation end products (AGEs)]. [10,11] Prevention of AGE-mediated cell toxicity has been proposed as a key strategy in preventing the onset of diabetic complications and some age-related pathology. [12] Yet, beyond hyperglycemia, the dyslipidemia and the increased local oxidative stress, frequently associated with diabetes, all directly promote the formation of AGEs. The rate of increase AGEs in serum is higher in diabetic patients compared to that of non-diabetics. [13] This study was designed to determine the level of AGEs in women with GDM under treatment and to assess the usage of AGEs as an additional biomarker in diagnosis women with GDM.

Methods

A case control study was carried out from January to June/2014. Sixty pregnant women at third trimester between (37-41) weeks of gestation and average age between (18-45) years old were enrolled in this study. They were admitted to different hospitals in Baghdad/Iraq. They were diagnosed by their gynecologists after proper physical, analytical and gynecological examinations and confirmed by ultrasound. All cased selected for this study underwent elective caesarian section.

Patients group consist of thirty pregnant women who proved to have Gestational Diabetes (GDM) depending on OGTT and on (Soluble Insulin or Metformin) as a medication of use. All patients with family history of diabetes, hypertension, thyroid disease, renal diseases, liver diseases, cardiac problems, smoking were excluded from this study. Thirty healthy pregnant women at the same average of age with the same exclusion criteria were involved as control group.

About six milliliters of fasting venous blood sample was aspirated from all pregnant women prior to caesarian section (C/S). One milliliter was transferred to a heparinized (EDTA = Ethylene Diamine Tetra Acetic Acid) tube for glycosylated hemoglobin determination (HbA1c %) using the enzymatic colorimetric method, and the rest of the blood sample was transferred into a plain tube, left for 5 minutes at room temperature, and centrifugation at 3000 rpm for 5 minutes in order to collect serum.

Serum was used to determine, Fasting serum glucose (FSG), insulin concentrations, c-peptide and lipid profile including [Total Cholesterol, Triglyceride (TG), Very Low Density Lipoprotien Concentration (VLDL-C), High Density Lipoprotein Concentration (HDL-C) using the enzymatic colorimetric method. The Oxidized Low Density Lipoprotien Concentration (LDL-C)] was measured using Fredrickson equation. [14] Body mass index (BMI) was calculated by dividing each pregnant women weight (Kg) on their height (m^2). Advanced Glycation End-products (AGEs) concentration was measured using an Enzyme Linked Immunosorbant Assay technique (ELISA); Human AGEs kit (Catalogue No. CSB-E09412h, CUSABIO BIOTECH CO., LTD., China).
Ethical approval and patient permission was obtained from all pregnant women involved to conduct this study.

Statistical analysis

Statistical analysis was performed using SPSS-21 (Statistical Packages for Social Sciences- version 21) and Microsoft Office Excel (Microsoft Office Excel for windows; 2010). Unpaired t *-test* was used to assess significant difference among means. Correlations between parameters were assessed using bivariate correlations. $P < 0.05$ was considered statistically significant.

Results

Sixty pregnant women at third trimester between (37-41) weeks of gestation were divided into two groups:
Group (A): This includes thirty pregnant women with gestational diabetes (Patients group).
Group (B): This contains thirty healthy pregnant women (Control group).

This study was design to determine the level of different biochemical parameters in patients' sera and compare it with healthy pregnant women at the same trimester.

According to data analysis, no significance difference was found between the study groups regarding to their Age, GA (Gestational Age), Parity and BMI. {table(1)}

Table (1): Differences between the Anthropometric Characteristics of Study subjects using student t- test.

Parameter	Patients (n=30)	Controls (n=30)	P value
	Mean±SE	Mean±SE	
Age (year)	31.30±1.09	30.43±0.95	NS
GA (week)	39.33±0.16	38.56±0.17	NS
Parity	2.43±0.16	2.23±0.15	NS
BMI(kg/m²)	28.80±0.27	27.77±0.40	NS

SE= Standard error, GA= gestational age, BMI= body mass index, GDM= gestational diabetes mellitus, NS= not significant and n= number.

In table (2), no significant differences was found regarding to patients' and controls' age percentage when subdivided into (< 20 and ≥ 20) years old. The highest percentage in women with GDM was found between (25-29) years, while the lower percentage was found with those aged (≥ 35) years old.

Table (2): Distribution and Percentage of Study Subjects According to Subdivided Ranges of Age using student t- test.

Age (year)	Patients (n=30)		Control (n=30)		P value
	No.	%	No.	%	
< 20	4	13.3	5	16.6	NS
≥ 20	26	86.6	25	83.3	NS
Mean±SE	31.30±1.09		30.43±0.95		

SE= Standard error, NS= not significant and n= number.

Table (3): Distribution and Percentage of Study Subjects According to Subdivided Ranges of BMI.

BMI (Kg/m²)	Patients (n=30)		Control (n=30)		P value
	No.	%	No.	%	
> 25	11	36.7	8	26.7	NS
25 – 29	16	53.3	18	60.0	NS
≥ 30	3	10	4	13.3	NS
Mean±SE	28.80±0.27		27.77±0.40		

BMI= body mass index, SE= Standard error, NS= not significant and n= number.

Table (3), shows the distribution and the percentage of patients & control groups according to their BMI. The highest percentage was found among GDM women with overweight-ranged (25-29) Kg/m² (53.3%) vs. (60%) at the same overweight BMI in healthy pregnant women.

Table(4):Comparison between the Biochemical Parameters in Patients and Control groups using student t-*test.*

Biochemical Parameter	Patients (n=30)	Controls (n=30)	P value
	Mean±SE	Mean± SE	
Serum Lipid profile (mg/dl):			
S.T. Cholesterol	239.8±3.6	215.1±6.2	0.001
S. Triglyceride	225.2±8.8	180.3±1.3	<0.0001
S. HDL-C	65.3±0.7	63.3±0.9	NS
S. LDL-C	129.4±4.4	115.7±6.5	NS
S. VLDL-C	45.03±1.7	36.1±0.3	<0.0001
AI=(LDL-C/HDL-C)ratio	1.99±0.08	1.85±0.1	NS
FSG (mg/dl)	104.9±2.8	84.2±1.2	<0.0001
HbA1c%	6.51±0.33	5.07±0.08	<0.0001
S. Insulin (µIU/ml)	7.53±0.25	6.85±0.2	NS
S. C-peptide (ng/ml)	1.08±0.07	0.69±0.03	<0.0001
S. AGEs (µg/ml)	5.29±0.28	2.76±0.33	<0.0001
HOMA-IR	1.93±0.06	1.43±0.06	<0.0001

S=serum, T=total, TG= triglyceride, HDL-C= high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, VLDL-C=very low-density lipoprotein cholesterol, AI= atherogenic index, FSG=fasting serum glucose, AGEs=advanced glycation end-products and HOMA-IR= homeostatic assessment model for insulin resistance.

In table (4), serum (T. Cholesterol, Triglyceride and VLDL-C) of GDM women were found increased significantly when compared with healthy pregnant women (0.001, <0.0001 and <0.0001) respectively. While serum (HDL-C, LDL-C) and AI of GDM women were not significantly different (p>0.05) compared with healthy pregnant women.

Regarding serum (FSG, HbA1c and S. C-peptide) concentrations, a highly significant increase was found in women with GDM compared with healthy pregnant women (p<0.0001). No significant difference was found between patients and control serum insulin concentration (p>0.05).

Regarding AGEs level and the HOMA-IR index, a highly significant increased was found among GDM women vs. healthy pregnant women with (p-value <0.0001).

As shown in table (5), AGEs was found with a high sensitivity when compared with HOMA-IR (96.3 % vs. 76.0%), while HOMA-IR found with a high specificity when compared with AGEs (83.3% vs. 70.0%).

Table (5): Specificity, sensitivity and cut off values for Comparison between HOMA-IR and AGEs ROC curves.

Parameter	Sensitivity	Specificity	AUC	SE	95% CI	Cut off
HOMA-IR	76.0%	83.3%	0.856	0.0498	0.741 to 0.933	≤1.71
AGEs(µg/ml)	93.3%	70.0%	0.849	0.0507	0.734 to 0.928	>2.85

AUC: Area under curve

Table (6): Comparison of ROC curves between AGEs and HOMA-IR:

AGEs ~ HOMA_IR	
Difference between areas (AUC)	0.00611
Standard Error	0.066
95% Confidence Interval	-0.123 to 0.136
Significance level P (Area=0.5)	P = 0.926

AUC: Area under curve

Comparison for the ROC curves between AGEs and HOMA-IR revealed that the differences between the two markers were not significant. The non-significant differences between the two markers despite the differences in sensitivity and specificity could be attributed to the overlap between the ROC curves of HOMA-IR and AGEs.

Discussion:

The no significance differences between patients and control anthropometric parameters in this study were agreed with a study done by Vijayam et al. 2007. They reported that there was no statistically significant difference among age, BMI, and gestational weeks of the women in the normal glucose tolerant and GDM groups ($P > 0.05$). [15] Also Guosheng et al., 2009 showed that there were no significant differences in age, gestational weeks and parity between the GDM and normal groups ($P>0.05$). [16] Karthiga Prabhu et al., 2011 have studied 60 pregnant women; 20 with GDM at their third trimester and 40 women as control (with OGTT >140mg/dl). They found no statistically significant difference between their age and parity. [17]

Regarding lipid profile in table (4), GDM women showed significant differences in their Lipid profile: highly significant differences in (S.T. Cholesterol, S. Triglyceride and S.VLDL-C) when compared with healthy pregnant women. While both S. HDL-C and S. LDL-C levels showed no significant differences when compared with healthy pregnant women. A study done by Tarim et al., 2006 resulted that VLDL-C levels in the GDM group were significantly higher than in unaffected (healthy) pregnant women. [18] Also Agata et al., 2007 demonstrated that obesity is characterized by increased production of TG in the liver leading to changes in the composition of VLDL-C and LDL-C particles, which by way of exchange with HDL-C particles, also change their composition leading to increased catabolism of changed HDL-C particles and reduced levels of HDL-C particles in the serum. While in healthy women, increasing obesity is paralleled by increased TG, reduced HDL-C and slightly elevated or unchanged TC. [19] This may explain the elevated levels of TG and VLDL-C, and reduced levels of HDL-C and LDL-C in studied subjects because all of the pregnant women were within the overweight range of BMI, table (3). Also, Karthiga Prabhu et al., 2011 revealed that serum triglyceride were significantly higher in women with GDM as compared with controls. In contrast, total cholesterol, HDL-C, LDL-C did not differ between GDM and control pregnancies. VLDL-C was also significantly more elevated in GDM patients when compared with controls. [17] Results of Karthiga Prabhu et al., 2011 about total cholesterol is unlike this study in which highly significant differences was found between GDM when compared with healthy pregnant women. Pregnancy is a hyperlipidemic state in which the placenta and fetal adrenal cortex cooperatively produce cholesterol vital for fetal neuronal and membrane development. [20]

Khan et al., 2012 confirmed that fasting blood glucose level and HbAlc of GDM was significantly higher than healthy pregnant women (table(4)).[21] Djelmis J. and Ivanisevic M., 2012 reported that women with GDM had significantly higher values of HbA1c, blood glucose and C-peptide than healthy pregnant women.[22] A study done by Prager et al., 1997 demonstrated that the fasting concentrations of C-peptide were higher in GDM as compared with pregnant non-diabetics. They found that after delivery, women with GDM gave a significant reduction of C-peptide levels and basal insulin secretion. They reported that this might indicate that impaired insulin secretion is the predominant defect in GDM. [23]

Homko et al., 2001 have investigated prehepatic insulin secretion in women with GDM and in non-diabetic pregnant controls first during late gestation and then again postpartum; they found that there were no significant differences in serum insulin levels comparing GDM and controls during either late gestation or postpartum. [24] There is a physiological increase in insulin resistance that occurs in all women during the second half of pregnancy, because of increased blood levels of different hormones. Charlotta Nilsson, 2013 showed that women with GDM are more insulin resistant than women without diabetes, which could be due to defective insulin secretion as well as defective insulin action. [25] Amara et al., 2011 also showed that, women with gestational diabetes mellitus feature more pronounced insulin resistance during pregnancy than pregnant subjects with normal glucose tolerance. This insulin resistance state seems to improve after delivery. [26] Typical GDM develops in the second half of pregnancy as a result of gradually increasing insulin resistance. Kereneyi et al., 2002 considered it an early marker of metabolic syndrome. [27]

In this study, in table (4), regarding to HOMA-IR, there was a significant increase in HOMA-IR in women with GDM vs. healthy pregnant women. The results of Hongxiu Zhang et al., 2013 indicated a tendency for progressive increased HOMA-IR values from the normal glucose tolerance to the GDM group. [28] Petrova et al., 2011 have found that the pregnant women with GDM had significantly higher HOMA-IR values compared to pregnant with normal glucose tolerance. They concluded that, pathological IR, common for GDM, is a manifestation of a substantial loss of insulin sensitivity with constant character and does not disappear completely after birth. [29]

Also similar to those reported by Das et al., 2009, have demonstrated that the subset of pregnant women who presented with GDM had significantly higher HOMA-IR values compared to pregnant women with normal glucose tolerance. [30]

This study shows a highly significant increase in AGEs level in GDM women sera compared with healthy pregnant women {table (4)}. Guosheng et al., 2009 studied the relationship between serum AGE levels in diabetic mothers with adverse fetal outcome. They found that women with GDM, whether at mid or late gestation, had significantly higher AGE levels than those in the normal group, with no statistically significant difference between the two groups. After treatment, blood glucose levels in mid-GDMs fell to normal levels by late gestation but, despite this, AGE levels at these gestations were elevated. [16] During a study of Harsem et al., 2008 about Advanced glycation end products in pregnancies complicated with diabetes mellitus or preeclampsia, they resulted that serum level of AGEs was elevated both in patients with type I DM and GDM, but not in preeclampsia, compared with controls. [31] Buongiorno AM. et al. 1997 studied the levels of advanced glycosylation end products (AGEs) in sera of pregnant diabetic women and make a comparison between type I, type II and gestational diabetes mellitus. They found that the levels of AGE in serum of type I and type II diabetes mellitus were not different from those of non-diabetic subjects. The only group that showed raised AGEs levels was GDM. They explain that it is not surprising, since hyperglycemia in those patients may be present without particular signs throughout pregnancy and only be detected when an OGTT is performed during the third trimester. By contrast, type I and type II pregnancies are programmed far in advance and not permitted before a good metabolic control is reached. [32]

Vlassara H. et al. 2003 confirmed that even in the absence of free glucose, once protein had been modified by AGEs; this process can self-perpetuate and may damage tissue through this self-amplification process. This persistently raised AGEs level throughout pregnancy could result in poor fetal - maternal outcome. [33] Buhimschi CS. et al. 2007 detected that high levels of AGEs in mid and late gestation GDM not only directly affect the pregnant mothers but also enter the fetal circulation through the placenta, with high fetal AGEs resulting in various fetal diseases and abnormal prenatal outcome. [34]

Receiver operating characteristic (ROC) analysis is commonly used in clinical research to express the diagnostic accuracy according to area under curve (AUC). At the cut-off score for AGEs, the sensitivity, specificity and area under the ROC curve (AUC) were 93.33%, 70.00% and 0.849 respectively that could make a conclusion regarding ROC analysis, which is, AGEs as an oxidative marker could be consider as a good additional biomarker or indicator for diagnosis of pregnant women with GDM.

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