Original Investigations

Comparison of glucose degradation product and receptor levels in dıabetic and normal pregnancy

Şimşek Tanin et al. Glycation products and diabetes

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Abstract

Objectives: The aim of our study was to asses the diagnostic values of new biochemical markers which can be alternative to OGTT and determine the differences between our groups.
Materials and Methods: A total 180 women were included into this prospective randomized study. The patients were divided into three groups. All the women were screened with 50 gr and 100 gr GDM screening tests. All three groups were evaluated in terms of biochemical parameters such as serum human advanced glycation end products (AGEs) levels, carboxymethyl lysine (CML) levels and receptor for advanced glycation end product levels (RAGE/AGER), body mass index (BMI), age, fasting glucose levels, obstetrics' parameters and gestational age.
Results: Age found as statistically insignificant in the control group, impaired glucose tolerance group and gestational diabetes group. Whereas fasting glucose levels and BMI in 3 groups found as statistically significant. AGEs, CML, RAGE/AGER levels found as statistically significant (p=0,000).
Conclusion: In this study AGEs, CML, RAGE/AGER levels usage for diagnosis and screening of gestational diabetes investigated. In this study, we appointed that advanced glycation products significantly higher in impaired glucose tolerance and gestational diabetes cases.
Keywords: Gestational diabetes mellitus, RAG /AGER, CML, Impaired glucose intolerance

Introduction

There is not a complete consensus on diagnosis, screening, follow-up and treatment of gestational diabetes mellitus (GDM). While GDM is the leading cause of maternal and fetal morbidity and perinatal mortality, the rates of fetal loss and illness in GDM pregnancies increased about four times compared to normal pregnancies (1). The world's most common screening test is 50-gram (1 hour) glucose test and its sensitivity is between 60 % and 80 %. 100 g oral glucose tolerance test (OGTT) is used as a diagnostic test. Definition of impaired glucose tolerance (IGT) has been shown to be an inter-metabolic disorder between normal glucose tolerance and diabetes (prediabetes). IGT is a major risk factor for diabetes mellitus. Within 10 years, progression to the diabetes is between 20-50% ratio (2).

Regulation of blood sugar levels following gestational diabetes mellitus diagnosis during pregnancy prevents fetal macrosomia which is associated with shoulder dystocia, birth trauma, increased rate of cesarean section. In addition, metabolic complications such as hypoglycemia, polycythemia, hypocalcemia and hyperbilirubinemia, which occur in neonatal period, can be prevented to a great extent and maternal and fetal morbidity and mortality can be reduced (3).

Although there are many discussions on the definition of GDM, there is a close relationship between GDM and perinatal morbidity and mortality. Therefore, accurate and timely diagnosis and treatment have gained importance. OGTT has been used for diagnosis of diabetes for years and OGTT criteria are frequently updated by organizations such as the American Diabetes Association (ADA) and the World Health Organization (WHO) (4, 5). The development of quick, effortless, cheap and reliable methods has become essential in diagnosis. Scientists are searching for new parameters that will contribute to the screening of diabetes. For this purpose, studies on parameters such as ketonic bodies, glycosylated hemoglobin, fructosamine, microalbumin and advanced glycation products are ongoing. Protein glycation and advanced glycation products play an important role in the development of diabetic complications (retinopathy, nephropathy, neuropathy, rheumatoid arthritis, cardiomyopathy, osteoporosis). The accumulation of glycation end products on proteins and free amino acids has been associated with diabetic vascular, renal, retinal, and neural complications (6). At first, this nonenzymatic process was reversible, but, later, it becomes irreversible. The increase in circulating glycation end products lead to renal insufficiency, and excretion of the metabolites in the urine. If the glycation end products accumulated in the tissue, renal and microvascular complications could occur due to crosslinking with collagen. There is a need of studies for clinicians to routinely use these new diagnostic parameters as an alternative to OGTT in the near future. For this purpose; we investigated whether or not the advanced glycation products have a role in IGT or GDM.

Materials and Methods

Department of Obstetrics and Gynecology Clinic of a tertiary hospital, followed-up pregnants between 24-28 weeks taken to Clinical Biochemistry Laboratory for GDM screening as in 2003 ADA recommendation (7). 50 g glucose was given orally at any time of day without consideration of fasting. One hour after glucose was administered, plasma glucose was measured by glucose oxidase method. In this study, 140 mg / dl value used as a threshold value of blood sugar in the plasma as a suggestion of ADA and ACOG (5, 7). Following the administration of 50 g of oral glucose, if blood glucose level at the first hour was above 140 mg / dL mmol / L), 100 g OGTT was also requested for definitive diagnosis.

Pregnant women who had type 1 and type 2 diabetes, multiple pregnancies, diagnosed endocrinopathies, kidney and liver disease, under 18 year old pregnant women and who used drugs that could affect insulin secretion or susceptibility were excluded from the study group .

After 100 g OGTT, 95 mg / dL (5.3 mmol / L) of fasting glucose, 180 mg / dL (10 mmol / L) of the first hour, 155 mg / dL (8.6 mmol / L) in the second hour and 140 mg / dL (7.8 mmol / L) in the third hour were used as the diagnostic criteria of Carpenter and Coustan and GDM was diagnosed in the presence of any two values exceeding the threshold value (8, 9). At any value above the threshold value impaired glucose intolerance was diagnosed.
First of all, three groups were formed among 180 pregnant women who were accepted to investigate. GDM group with GDM diagnosis (n = 59), IGT group with impaired glucose intolerance (n = 50) and control group with below threshold values (n = 71) were formed.
Then fasting serum human advanced glycation products (AGEs), carboxy methyl lysine (CML) and advanced glycation product receptor (RAGE / AGER) levels were determined by enzyme-linked immunosorbent assay (ELISA) method based on the sandwich model. All cases were informed and confirmed for study. The study protocol was in compliance with the Declaration of Helsinki and approved by Local Ethic Committee. An informed consent was yielded from the subjects. This study was supported by the Scientific Research Projects' Unit with the number of 2015TF/T-211.

Statistical Analysis
SPSS 15.0 program was used for statistical analysis. After the distribution of data was evaluated by Kolmogorov-Smirnov method, One-Way ANOVA test was used for the comparison of normal distribution parameters and Kruskal Wallis test was used for the comparison of non-normal distribution parameters. Data were given as mean ± standard deviation. Chi-square test was used for comparison of qualitative data. The results were evaluated as 95% confidence interval and significance was evaluated as $p < 0.05$.

Results
180 pregnant women were included in the study. Three groups were formed among these women. A group of 59 patients diagnosed with gestational diabetes mellitus (GDM) was included in our study as a group of GDM, a group of 50 patients diagnosed with impaired glucose intolerance (IGT) and a group of 71 women who were below the threshold values as a control group. The results of our study in which we evaluated AGE, RAGE, CML levels and evaluated the relation of fasting blood glucose, OGTT, systole / diastole ratios, and BMI in groups were as follows:
There were no statistically significant difference in age, gestational week, gravida, parity, living and abortus numbers between the control group, IGT and GDM groups. There was no statistically significant difference in diastolic blood pressure ($p = 0,178$) while systolic blood pressure was statistically significant ($p = 0,002$) between the groups (Table 1).
Fasting blood glucose values, BMI values, GDM in previous pregnancy and family history of DM were statistically significantly higher in the GDM group ($p = 0.001$). RAGE / AGER levels between the groups were statistically significant ($p = 0,000$). Comparisons of the RAGE / AGER values between each one of 3 groups were evaluated significantly among themselves. CML and AGE levels between the control group, IGT and GDM groups were statistically significant ($p = 0,000$) (Table 2).

Discussion
In this study, we compared 50 g OGTT and 100 g OGTT performed between January 2015 and January 2016 in our hospital and examined the glucose degradation products and receptors in pregnant women with GDM and impaired glucose tolerance by comparison with the control group. Our aim was to investigate whether glucose degradation products are involved in the early diagnosis of IGT or GDM. There was a significant difference in the levels of the markers between the groups like as our hypothesis of 'We can find a statistically

significant difference between the control group and the IGT and GDM groups in terms of AGE, RAGE, CML'. In the literature, there are no other studies investigating the relationship between glucose degradation products and impaired glucose tolerance and GDM.
The risk of diabetes in women at age 30 is 4.4% whereas at age 35 it is 6.5%. In our study, mean age of diabetic group was 32,34 ± 5,43 and it was slightly higher than control group (30,93 ± 3,43) which was not statistically significant (p> 0,05). In Dornhorst and Rossi's 1998 study, age was accepted as one of the unchangeable risk factors (10). In our study, when we compared age groups, we found that patients with negative screening test were statistically younger than those with GDM and impaired glucose tolerance group (p <0.0001). There was no statistical difference between the other groups. This finding confirms that older age is a risk factor for GDM (11).
As the number of pregnancies increased, there was no significant difference in diagnosis of gestational diabetes mellitus by 100 g OGTT. As mentioned earlier, we know from literature that the number of pregnancies is not among the risk factors for gestational diabetes (International Diabetes Study Group) (12). 51.1% of those questioned about diabetes in family history had a positive diabetes story. In the absence of DM family history, the risk of diabetes is 4.4%, while in cases of positive DM family history the risk increases to 8.8% (13). There is a risk of recurrence of GDM in subsequent pregnancies. In a prospective study, GDM repeated in 47 (52%) of 90 pregnancies with GDM (14, 15). In our study, 16.2% of the cases had GDM history in previous pregnancies.
The risk is 3.6% when BMI is 22 while it increases to 8.6% when BMI is 25. The risk also increases in positive family history with the weight gain of the person. In our study, 100 g OGTT was worse in the women with high BMI. Dudhbhai and colleagues also found that BMI was more common in pregnancies with bad 100 g OGTT (16). In our study, we found that the fasting blood sugar of the first application was higher in 100 g OGTT defects and this value was statistically significant (p <0.05), Perruchini also reported that the initial fasting blood glucose (FBG) was high in bad OGTT retrospectively (17).
The presence of chronic hyperglycemia in diabetes leads to the formation of heterogeneous compounds termed advanced glycation end products (AGEs) as a result of nonenzymatic glycation of macromolecules such as amino group containing amino acids, proteins, peptides, phospholipids and nucleic acids. Diabetes-associated AGEs accumulate in tissues and lead to micro- and macro-complications of diabetes. Currently the structure of many AGE species has been clarified and it has become possible to identify them by using various properties (18-23). HbA1c is one of the endogenously formed glycation products and indicates the glycation of the hemoglobin molecule. HbA1c is not only a test used to diagnose diabetes but it is also used for follow-up in diabetic individuals and indirectly informs about the last 8-10 weeks blood glucose level. Without doubt AGEs are more suitable for the evaluation of total glycation products. Because the AGE levels measure both glycation products absorbed from the outside and endogenously glycated products of all proteins and nucleic acids. The AGE test also reveals glycation products formed by fructose and galactose (24, 25).
The groups were compared in terms of RAGE / AGER, CML and AGE values. The mean of RAGE / AGER measurements of GDM diagnosed pregnants was 954.29 ± 216.24 whereas the mean of RAGE / AGER measurements of detected impaired glucose intolerance was 840.35 ± 182.85. The difference in RAGE / AGER levels between the groups was statistically significant (p = 0,000).
The CML levels of the study groups were 433.01 ± 57.49 in the control group. The mean of CML measurements of GDM diagnosed pregnancies was 865,60 ± 174,70 whereas the mean of CML measurements of detected impaired glucose tolerance was 530,14 ± 100,74. The difference in CML levels between the groups was statistically significant (p = 0,000). The mean of AGE measurements of GDM diagnosed pregnancies was 93.99 ± 16.70, while the

mean of AGE measurements of detected impaired glucose intolerance was 82.78 ± 12.46. The difference in AGE levels between the groups was statistically significant (p = 0,000). The high CML and AGE levels in the GDM-diagnosed pregnancies supports similar results in similar studies (26-29).

AGE formation and its effects play an important role in the development of chronic complications of diabetes mellitus. A clear understanding of the structure of these heterogeneous compounds, standardization of measurement methods, a clear understanding of the pathophysiology of long-term complications play a significant step in determining treatment options, and have a role as a new tool in monitoring the development of late complications from glycation in diabetic patients.

Conclusion

Currently, there are still debates about gestational diabetes screening, diagnosis, and follow-up methods and through significant detection of biochemical markers in our study; in the future it has been hoped that these markers will be further standardized with further and broader studies and used in routine screening, diagnosis or follow-up of the diabetes. Our results suggest that AGE, RAGE and CML values may contribute to early GDM diagnosis, but there is a need for extensive studies to determine sensitivity, specificity and suitability based on cost analysis in order to use these parameters in screening and diagnosis.

Ethics

Ethics committee approval: The study protocol was in compliance with the Declaration of Helsinki and approved by Local Ethic Committee.

Informed Consent: An informed consent was yielded from the subjects.

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Table 1. Clinical and anthropometric characteristics of the study groups

Parameters	Control n=(71)	IGT (n=50)	GDM (n=59)	p
Age	30,93±3,43	32,92±5,46	32,34±5,43	0,059
BMI (kg/m²)	27,58 ± 4,64	30,98 ± 4,63	33,43 ± 5,96	0,000
Gravida	2 [1-6]	3 [1-7]	2 [1-5]	NS
Family history of DM	2,8%	20,9%	28,8%	0,000
History of GDM (%)	2,8%	20,9%	28,8%	0,000
Gestational age (week)	26,5±1,3	27,2±1,8	27,4±1,7	0,689
Systole (mmHg)	112,01±13,71	116,96±16,71	121,36±15,02	0,002
Diastole (mmHg)	70,75±14,30	73,40±10,61	74,58±9,88	0,178

Data presented as mean ± SD, median [IQR] or proportions.
Differences evaluated by nonparametric chi-square test, respectively

Table 2. Laboratory characteristics of the study groups

Parameters	Control (n=71)	IGT (n=50)	GDM (n=59)	p
Fasting blood glucose (mg/dl)	86,79 ±14,43	99,76±30,28	104,58 ± 38,36	0,001
RAGE/AGER (pg/ml)	676,19±97,51	840,35±182,85	954,29± 216,24	0,000
CML (pg/ml)	433,01±57,49	530,14±100,74	865,60±174,70	0,000
AGE (pg/ml)	69,91±8,84	82,78±12,46	93,99±16,70	0,000
50 g OGTT	118,17±25,82	157,10±11,63	173,20±20,60	0,000
100 g OGTT 0.h (mg/dl)	-	94,74±8,46	104,50±12,48	0,000
100 g OGTT 1.h (mg/dl)	-	171,20±11,20	189,67±21,62	0,000
100 g OGTT 2.h (mg/dl)	-	147,54±11,82	168,91±21,93	0,000
100 g OGTT 3.h (mg/dl)	-	118,06±17,85	140,53±21,39	0,000

Data presented as mean ± SD