Original Article

Association of HbA1c and Fructosamine with insulin resistance indices in gestational diabetes

Fazloddin Fahimi Moghaddam [1], Jamshid Mehrzad [2*], Jafar Saeidi [3], Ahmad Ghasemi [4]

[1] PHD Candidate of Biochemistry, Department of Biochemistry, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran. [2] PHD of Biochemistry, Department of Biochemistry, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran. [3] PHD of Physiology, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran. [4] PHD of Clinical Biochemistry, Department of Basic Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran.

Abstract

Objectives: The levels of HbA1c and Fructosamine (FRA) in pregnant women with GDM (gestational diabetes mellitus) compared to non-GDM ones have been evaluated, then the association between the above two compounds with insulin resistance indices has been investigated. Methods: Between September 2018 and March 2019, this study was performed on 400 pregnant women (50 with GDM and 350 without diabetes) referred to Kashmar Health Center Laboratory. Glycemic markers and insulin resistance indices were compared between women with GDM compared to Non-GDM ones by Mann-Whitney U and Independent sample t-tests. Linear regression test was used to investigate the relationship between insulin resistance indices with HbA1c and FRA in the two above groups. Results: Significant differences were observed in the levels of HbA1c (P <0.001) and FRA (P <0.001) between women with GDM and Non-GDM group. Results of linear regression considering age and group effects resulted in no significant correlations between HbA1c and IR indices (QUICKI (β= -1.98, P =0.22), HOMA-IR (β = 0.03, P=0.39), HOMA-B (β = 0.00005, P=0.41), 1/Insulin (β = -0.85, P=0.32), G/I (β = -0.005, P=0.62)). Likewise, no significant correlations between FRA and IR indices observed (QUICKI (β= 9.79, P =0.64), HOMA-IR (β = 0.07, P=0.88), HOMA-B (β = 0, P=0.67), 1/Insulin (β = -2.52, P=0.82), G/I (β = 0.02, P=0.86)). Conclusion: HbA1c and FRA were not directly affected by insulin resistance, and it can be concluded that any increase in insulin resistance may not be an exclusive factor for the elevation of HbA1c and FRA levels.

Keywords: Gestational diabetes mellitus, OGTT, Fructosamine; HbA1c, insulin resistance indices

Introduction

GDM is commonly referred to any degree of glucose resistance that is detectable at the beginning or during pregnancy [1].

Euglycemic status (keeping blood glucose in the normal range) is maintained by an increase in insulin secretion in pregnant women, and GDM occurs in women who are unable to raise insulin sufficiently [2].

Glycated Hemoglobin (HbA1c) is formed by hemoglobin glycation in a non-enzymatic pathway that is mainly used to measure moderate blood sugar levels over the past 2-3 months since that the erythrocytes life span is 120 days. In diabetes, higher levels of HbA1c indicate poorer control of blood glucose [3].

Fructosamine (FRA) is formed by an irreversible enzymatic reaction between serum proteins and glucose, especially albumin. Given the short half-life of albumin in the blood, FRA shows an average blood glucose level over a period shorter than HbA1c, approximately 4 weeks [4].

Insulin resistance indices (fasting indices: 1/Insulin, Glucose/Insulin, HOMA-IR, HOMA-B, QUICKI) were used to evaluate insulin resistance [5, 6].

Our hypothesis in this study is whether insulin resistance can, over time, increase HbA1c and FRA, without a marked and persistent increase in fasting or random blood glucose levels or not. So we investigated the insulin resistance indices, as well as the levels of HbA1c and FRA in GDM and non-GDM pregnant women (NGT or normal glucose tolerance). Then

Address for correspondence: Jamshid Mehrzad, PHD of Biochemistry, Department of Biochemistry, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran.
E-mail: mehrzadjam@yahoo.com

How to cite this article: Fahimi Moghaddam, F., Mehrzad, J., Saeidi, J., Ghasemi, A. Association of HbA1c and Fructosamine with insulin resistance indices in gestational diabetes. Arch Pharma Pract 2020;11(S1):102-6.

we looked at the relationship between the amount of HbA1c and FRA with insulin resistance in the two above groups. As far as we know, no study has been done so far.

Material and Methods

Subjects

This study is based on case-control analytical studies and the exposure and outcome of this study are pregnancy, and some laboratory parameters, respectively.

Nonprobability sampling method and convenience model (Accidental sampling) were used, so the study was performed on all pregnant women who referred to the Central Laboratory of Kashmar (Iran; Khorasan Razavi province) during a 6-month period (between September 2018 and March 2019) and were screened at 24 to 28 weeks of gestation for GDM using a universal one-step GDM screening program. Of all these pregnant women, 350 non-diabetic pregnant women and 50 pregnant women with GDM were identified and studied during this period, according to the sampling model used. A questionnaire was prepared for each of these women and written informed consent was obtained from all participants.

Measurements and assessments

For all subjects, oral glucose tolerance test (OGTT), HbA1c, FRA and fasting insulin were evaluated. OGTT was performed according to the criteria outlined by the "International Association of the Diabetes and Pregnancy Study Group" (IADPSG). IADPSG criteria, have determined the diagnosis of GDM using 75 grams of oral glucose in the OGTT test in 2013, and even one blood glucose level more than the threshold below is sufficient to diagnose GDM: Fasting blood sugar (FBS): 92 mg/dl; Blood glucose one hour after glucose intake: 180 mg/dl; Blood glucose two hours after glucose intake: 153 mg/dl [7].

The normal range of FRA in human serum is between 29 to 48.3 mg/dL. The normal level of HbA1c in this method for pre-diabetic patients is between 5.7% and 6.4%, greater than or equal to 6.5% for diabetics, and therapeutic target in diabetic patients is less than 7%.

Blood samples were drawn after an 8 to 10-hour overnight fast. A glucose oxidase method (Selectra XL, Vital Instrument Co. Netherlands; Bionik kit, Iran) was used to determine serum glucose levels, and an enzyme immunoassay (ELISA) kit (Autobio Plate Reader, China; DiaMetra kit, Italy) was used for insulin measurement. To measure Fructosamine, an assay based on the reaction of FRA with NBT (nitrobutetrazolium) was used. Formazan dye compound that came from resuscitation of NBT by FRA was measured at a wavelength of 550 nm (Autobio Plate Reader, China; Abcam Fructosamine Assay Kit, USA).

The whole blood sample obtained on the anticoagulant $EDTAK_2$ from the subjects was used for evaluating HbA1c. The method of HbA1c measurement in this study is based on the reaction of the Cis-diol form of HbA1c with boronate and deposition of this compound on the cartridge filter and then measurement of this deposition by laser reading. The CERA-STAT 2000 branded HbA1c kits and Reader instrument were used (HbA1c Reader; South Korea).

Several indices were calculated to assessment of insulin resistance (HOMA-IR, QUICKI, HOMA-B, 1/Insulin, G/I).

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using this formula [8, 9].

$$\text{HOMA-IR} = (\text{Fasting Glucose, mmol/l} \times \text{Fasting Insulin, } \mu\text{IU/ml})/22.5$$

Calculation of Quantitative Insulin Sensitivity Check Index (QUICKI) was done using the mathematical formula as follows [10]:

$$\text{QUICKI} = 1/[\log(\text{Fasting insulin, } \mu\text{U/ml}) + \log(\text{Fasting glucose, mg/dl})]$$

Homeostasis model assessment for estimation of index β-cell secretion (HOMA-B), the index of insulin secretion capacity, was calculated as follows[11]:

$$\text{HOMA-B} = (360 \times \text{insulin, micro-unit/lit})/\text{Glucose, mg/dl} - 63)$$

Two other indices, 1/Insulin (1/fasting insulin) and G/I (fasting glucose/fasting insulin) were measured too.

The association between insulin resistance (IR) and increased levels of HbA1c and FRA in GDM and healthy non-GDM patients was assessed with statistical analysis.

Statistical analysis

Data were expressed as medians and interquartile ranges. To search for a normal distribution of data, the Kolmogorov-Smirnov test was used. Chi-square test and Fisher's exact test were used to examine Non-numeric variables in both groups. Then, to study for our hypotheses, the Mann-Whitney U test and independent sample t-test were used, according to the data distribution. Finally, for investigating the relationship between insulin resistance indices with HbA1c and FRA variables linear regression test was used. A p-value < 0.05 was considered to indicate statistical significance. Data were analyzed using the SPSS version 16.0 for Windows software. Based on the Kolmogorov-Smirnov test, none of the measured and calculated parameters in this study (except age, BMI and weight gain) had a normal distribution. As a result, nonparametric tests were used for statistical analysis. For age, BMI and weight gain parameters, Independent sample t was used.

Results

Metabolic parameters

A summary of the results of the experiments and some anthropometric indices in this study are presented in Table 1. The fasting insulin levels were significantly higher in the NGT group than in the GDM group.

The IR indices values were significantly worse in the GDM group than in the NGT group. In the study of insulin resistance indices, the median of the 1/insulin index was a statistically significant decrease in the GDM group compared to NGT group. FBS/fasting insulin (G/I) was significantly higher in diabetic mothers than NGT group ($p < 0.001$). The difference in HOMA-IR between diabetic and non-diabetic groups was statistically significant ($p<0.001$). QUICKI had a statistically significant difference in the two groups ($p < 0.001$). HOMA-B was not significantly different between the two groups.

The difference of HbA1c in the diabetic and healthy group was statistically significant ($P < 0.001$). The median of the FRA level in diabetic and non-diabetic women showed a statistically significant difference ($p < 0.001$).

The result of studying the relationship between IR indices and two other variables (HbA1c and serum FRA), in diabetic and NGT groups showed no significant relationship between them (Tables 2,3), taking into account two age and group factors (healthy women and GDM groups).

Discussion

As far as we know, this is the first study about the relationship between IR Indices with FRA and HbA1c in pregnant women with GDM.

FRA and HbA1c are both used to monitor diabetes. Each of them provides information for a specific period of time about the measured analyte. Since the half-life of hemoglobin(Hb) in red blood cells (RBCs) is approximately 6-8 weeks, HbA1c measurements show an average glucose concentration over this period of the time [12]. FRA, unlike what was said about HbA1c, explains the short-term control of diabetes. Higher blood glucose levels and more severe increases in blood glucose levels after eating sweet meals in women with GDM than non-GDM ones can increase the level of HbA1c in the GDM group, during the RBC life span.

Low levels of HbA1c have been observed in pregnant women to non-pregnant women, possibly due to lower fasting blood glucose as well as a shorter life span of red blood cells in these individuals [13].

Radder et al. [14] reported in their studies that the level of HbA1c in healthy pregnant women varied between 5% and 6%; although this result is different from the results in our study that the median HbA1c in healthy mothers was 4.09%, but this result shows a good agreement with what Balaji V et al. [15] calculated. In their study, the mean of HbA1c in women with NGT and GDM was calculated by 5.3% and 6% respectively.

In a study by Agarwal et al. In the UAE, it was found that the HbA1c value less than 5.5% for rejecting GDM has a sensitivity of 82.1% and the value higher than 7.5% for HbA1c to prove GDM in one is 95.8% [16].

The results of our study showed a statistically significant difference in HbA1c and FRA values between the two groups (P-value<0.001). However, the distinct differences between the results of HbA1c values in different studies are often due to the method of testing HbA1c in each study. It seems that FRA testing along with other laboratory tests can be used to diagnose GDM and increase the accuracy and precision of screening in pregnant women.

In 2006, Li and Young concluded that FRA can be evaluated for monitoring GDM but cannot be used to predict GDM in early pregnancy. [17].

Study of Delgado et al., showed that FRA can be more appropriate for evaluation of glycemic control in patients with GDM as it allows for short-term monitoring [18]. The results of Bostani Fergush (2014) showed that despite all the effects of pregnancy on hemoglobin level, HbA1c is still more reliable than other tests for monitoring the status of diabetic patients.

Insulin resistance indices

Insulin helps maintain glucose homeostasis by stimulating glucose uptake in muscle and adipose tissue and inhibiting gluconeogenesis in the liver. The insulin concentration needed to produce half the maximum effect is called "insulin sensitivity" (IS). Decreased responsiveness to insulin's metabolic effects, including stimulation of glucose consumption or inhibition of hepatic glucose production (HGP), is called "insulin resistance" (IR). IR plays an important pathophysiological role in metabolic syndrome [19].

Various techniques such as “Euglycemic Clamp” and “Insulin Suppression Test” have been used to IR evaluating. These methods are very laborious and time-consuming and lasts several hours and are not very practical in medical diagnostic laboratories [20]. Conversely, there are simpler methods that use fasting glucose and insulin to measure IR, the same ones we used in this study.

It has been shown that IR occurs earlier in the individual than diabetes and is involved in the pathological stages leading to diabetes. Many studies have shown that IR screening can predict the incidence of diabetes between 10-20 years before it occurs [21].

Indices such as the homeostasis model evaluation (HOMA-IR) that is based on fasting glucose and fasting insulin levels are also widely used to express IR in diverse populations [22, 23].

We showed that difference of HOMA-IR in the diabetic and healthy mothers is statistically significant (P-value <0.001) and it seems that HOMA-IR can be a valuable indicator of determining IR in pregnant women even several weeks before the usual GDM screening week (24 to 28). Since the HOMA-B variable in our study was not significantly different between GDM and NGT groups (P-value <0.001), it seems that HOMA-B cannot be considered a reliable indicator for the diagnosis and screening of GDM.

The quantitative evaluation index of IS or QUICKI is useful for diabetic and non-diabetic people. Katz et al. examined the association between different glucose clamp studies and found that there was a significant (r=0.78) relationship between QUICKI and the IS clamp (r=0.78) [10]. Our present study obtained QUICKI was statistically significantly different between GDM and NGT groups (P-value <0.001). This index, like HOMA-IR, can be used to assess IR in pregnant women. Unlike HOMA-IR, the higher value of this index shows a lower risk of GDM. Good correlation with results of standard euglycemic clamp method and its possibility to be used in early screening of GDM diagnosis for all pregnant women can make these indices as a valuable criterion for IS and IR assessment.

Kwak reported a mean HOMA-IR of 2.2 to 3.2 for Korean women with a history of GDM [24]. These values are not significantly different from the values obtained in the Sae Jeong Yang study (mean HOMA-IR of 2.3 in GDM subjects) [25].

Although FBS/fasting insulin (G/I) and 1/insulin have been used only in theoretical and less operational terms in different studies, however, in the present study, these two indices in the diabetic mothers were significantly lower than the healthy mothers (P-value <0.001).

Therefore, quantitative evaluation of IR may be useful for detecting the presence and severity of diabetes, especially in those who have not yet developed abnormal glucose tolerance or diabetes. Although the existence of IR can be deduced from clinical findings, there is currently no common way to quantify it in the clinic, and its quantification is largely limited to research studies [6].

Kirwan et al. Investigated IS using the OGTT as well as the calculation of fasting glucose/insulin levels in an attempt to predict IS in women before and during pregnancy. They found that the estimation of insulin sensitivity in pregnant women by the OGTT test was significantly better than that of fasting insulin and glucose and G/I [26].

In the present study, we found no significant relationship between IR indices with HbA1c and FRA. This could mean, it is not expected that the presence of insulin resistance in a person can certainly increase HbA1c or FRA in him. This result also suggests that using FRA and HbA1c tests are not reliable tests for determining IR in a person and they cannot be used in place of insulin resistance indices. Therefore, FRA and HbA1c are directly affected by plasma glucose levels, and the mere presence of insulin resistance cannot be a reason for their rise in the blood.

Conclusion

Although HbA1c and FRA measurement can be useful in the diagnosis of GDM, along with other diagnostic tests, they cannot replace glucose tolerance testing. As a conclusion of this study, these tests can be helpful in GDM diagnosis. The results of this study showed that IR could not be a factor in increasing HbA1c and FRA. Overall, it can be concluded that IR may increase FRA and HbA1c if it raises blood sugar, and the presence of IR may not be sufficient reason for this increase, lonely. Finally, based on the results of this study, it can be stated that the measurement of HbA1c and FRA cannot be used to determine IR and its severity in a person.

Acknowledgments

The present study was supported by a grant of Islamic Azad University (Neyshabour branch)

Conflicts of interest

The authors have no conflicts of interest.

References

1. Metzger, B.E., D.R. Coustan, and O. Committee, Summary and recommendations of the fourth international workshop-conference on gestational diabetes mellitus. Diabetes care, 1998. 21: p. B161.
2. Poirier, C., et al., MicroRNAs in pregnancy and gestational diabetes mellitus: emerging role in maternal metabolic regulation. Current diabetes reports, 2017. 17(5): p. 35.
3. Miedema, K., Standardization of HbA1c and optimal range of monitoring. Scandinavian Journal of Clinical and Laboratory Investigation, 2005. 65(sup240): p. 61-72.
4. Smit, F.C., The development of a new reference range for fructosamine for the pathcare pathology group, Somerset West, South Africa. 2010, Cape Peninsula University of Technology.
5. Dina, R.C., et al., Clinical and Biological Markers of Insulin Resistance. Romanian Journal of Diabetes Nutrition and Metabolic Diseases, 2010. 17(3): p. 177-185.
6. Borai, A., et al., Selection of the appropriate method for the assessment of insulin resistance. BMC medical research methodology, 2011. 11(1): p. 158.
7. Coustan, D., et al., International Association of Diabetes and Pregnancy Study Groups. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study: paving the way for new diagnostic criteria for gestational diabetes mellitus. Am J Obstet Gynecol, 2010. 202(6): p. 654.
8. Bonora, E., et al., Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes care, 2000. 23(1): p. 57-63.
9. Matthews, D., et al., Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and

insulin concentrations in man. Diabetologia, 1985. 28(7): p. 412-419.
10. Katz, A., et al., Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. The Journal of Clinical Endocrinology & Metabolism, 2000. 85(7): p. 2402-2410.
11. Wallace, T.M., J.C. Levy, and D.R. Matthews, Use and abuse of HOMA modeling. Diabetes care, 2004. 27(6): p. 1487-1495.
12. American Diabetes Association, A.D., Gestational diabetes mellitus. Diabetes care, 2004. 27(suppl 1): p. s88-s90.
13. Makris, K. and L. Spanou, Is there a relationship between mean blood glucose and glycated hemoglobin? Journal of diabetes science and technology, 2011. 5(6): p. 1572-1583.
14. Radder, J. and J. Van Roosmalen, HbA1c in healthy, pregnant women. Neth J Med, 2005. 63(7): p. 256-259.
15. Balaji, V., et al., A1C in gestational diabetes mellitus in Asian Indian women. Diabetes care, 2007. 30(7): p. 1865-1867.
16. Agarwal, M.M., et al., Gestational diabetes: a reappraisal of HBA1c as a screening test. Acta obstetricia et gynecologica Scandinavica, 2005. 84(12): p. 1159-1163.
17. Kui, L. and H.-x. Yang, Value of fructosamine measurement in pregnant women with abnormal glucose tolerance. Chinese medical journal, 2006. 119(22): p. 1861-1865.
18. Delgado, R., et al., Plasma fructosamine to evaluate metabolic control among women with gestational diabetes. Revista medica de Chile, 2011. 139(11): p. 1444-1450.
19. Wilcox, G., Insulin and insulin resistance. Clinical biochemist reviews, 2005. 26(2): p. 19.
20. Yeckel, C.W., et al., Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. The Journal of Clinical Endocrinology & Metabolism, 2004. 89(3): p. 1096-1101.
21. Lillioja, S., et al., Impaired glucose tolerance as a disorder of insulin action. New England Journal of Medicine, 1988. 318(19): p. 1217-1225.
22. Wallace, T. and D. Matthews, The assessment of insulin resistance in man. Diabetic Medicine, 2002. 19(7): p. 527-534.
23. Kim, J., et al., Insulin secretion and sensitivity during oral glucose tolerance test in Korean lean elderly women. Journal of Korean medical science, 2001. 16(5): p. 592.
24. Kwak, S.H., et al., Subsequent pregnancy after gestational diabetes mellitus: frequency and risk factors for recurrence in Korean women. Diabetes Care, 2008. 31(9): p. 1867-1871.
25. Yang, S.J., et al., Insulin secretion and insulin resistance in Korean women with gestational diabetes mellitus and impaired glucose tolerance. The Korean journal of internal medicine, 2013. 28(3): p. 306.
26. Kirwan, J.P., et al., Clinically useful estimates of insulin sensitivity during pregnancy: validation studies in women with normal glucose tolerance and gestational diabetes mellitus. Diabetes care, 2001. 24(9): p. 1602-1607.