GESTATIONAL DIABETES MELLITUS SEEMS TO BE ASSOCIATED WITH INFLAMMATION

Ayla Aktulay, Yaprak Engin-Ustun, Mehmet Sahin Ozkan, Salim Erkaya, Mustafa Kara, Oktay Kaymak and Nuri Danisman

Department of Obstetrics and Gynecology, Zekai Tahir Burak Women Health Training and Research Hospital, Ankara, Turkey

SUMMARY – The aim of this study was to investigate whether gestational diabetes mellitus (GDM) is associated with inflammation by comparing serum levels of human chitinase-3-like protein 1 (YKL-40), neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR). This case control study included 29 pregnant women with GDM and 29 pregnant women with normal glucose tolerance matched for age (±2 years) and pre-pregnancy body mass index (±2 kg/m^2). The YKL-40/CHI3L1 levels were measured, and NLR and PLR investigated. There were no statistically significant differences in maternal age, gestational age, gravidity and parity. Higher YKL-40 levels were recorded in pregnant women with GDM compared to control subjects (203 (65-300) ng/mL *vs*. 159.2 (14-290) ng/mL, p=0.007). NLR and PLR were significantly higher in GDM compared with control group. In conclusion, GDM is associated with high levels of YKL-40, NLR and PLR, which indicate inflammatory status.

Key words: *CHI3L1 protein, human; Diabetes, gestational; Leukocyte count; Neutrophils – cytology; Lymphocytes – cytology; Blood platelets – cytology*

Introduction

Gestational diabetes mellitus (GDM), developing in 6%-7% of pregnant women, is defined as carbohydrate intolerance that begins or is first recognized during pregnancy[1]. Even though the association between inflammation and insulin resistance is well known, the data related to the role of inflammation in GDM are conflicting. In a recent study, Ozyer *et al.* revealed that maternal serum levels of inflammatory mediators were not related to GDM at the time of the glucose challenge test in the late second or early third trimester[2].

A new biomarker, human chitinase-3-like protein 1 (YKL-40), has been found to be elevated in cancer and inflammatory diseases[3-5]. Patients with type 2 diabetes had elevated YKL-40 and high sensitivity C-reactive protein (hsCRP) levels, and it was related to insulin resistance[4]. According to the study by Kyrgios *et al.*, serum levels of YKL-40 were elevated in obese youth and represented a marker of insulin resistance even in childhood[5]. Schaller *et al.*, on the other hand, disclosed that YKL-40 levels were not different between GDM and control patients either during or after pregnancy. The role of YKL-40 in GDM seems to require further evaluation[6].

The neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) have been considered as systemic inflammatory response markers. Sefil *et al.* suggested that increased NLR might be associated with increased HbA1c in patients with type 2 diabetes mellitus[7].

This study aimed to evaluate whether GDM is associated with inflammation in terms of serum levels

Correspondence to: *Ayla Aktulay, MD*, 435. Cadde Zirvekent Zambak Sitesi 72/49 Çankaya, 06550 Ankara, Turkey
E-mail: aaktulay@yahoo.com

Received October 13, 2014, accepted November 4, 2015

of YKL-40 and NLR. To our knowledge, this is the first study investigating the relation of YKL-40, NLR and GDM.

Materials and Methods

This case control study was conducted in a tertiary referral center, with approval of the Local Ethics Committee. Twenty-nine healthy pregnant women and 29 women with GDM having singleton pregnancies, gestational age of 34 to 40 weeks, were included in the study. A history or new diagnosis of corticosteroid-treated asthma, liver disease, renal disease, Crohn's disease, ulcerative colitis, celiac disease, and history of type 1 or type 2 diabetes were determined as the exclusion criteria. Twenty-nine pregnant women with normal glucose tolerance and 29 women with GDM were included in the control and case groups, respectively. Screening for GDM was conducted with the 50-g glucose loading test at 24th-28th week of gestation. The threshold value of plasma glucose level to define an abnormal glucose challenge test result was 140 mg/dL. The patients with positive screening results underwent a 100-g oral glucose tolerance test (OGTT). GDM diagnosis was based on two or more values from a 3-hour 100-g OGTT meeting or exceeding the criteria developed by Carpenter and Coustan[8]: fasting >5.3 mmol/L; 1 hour >180 mg/dL; 2 hours >155 mg/dL; 3 hours >140 mg/dL. Body mass index (BMI, kg/m^2) was calculated in study subjects. The case and control groups were matched for age (±2 years) and pre-pregnancy BMI (±2 kg/m^2). Age, gestational age, gravidity, and parity were recorded.

The YKL-40/CHI3L1 levels were measured with the Human Chitinase-3-like Protein 1 (YKL-40/CHI3L1) ELISA Kit, Catalog No. CSB-E13608h (96T) kit. Fibrinogen levels were evaluated by the HemosIL®, Fibrinogen-C XL (Catalog No. 0020003910) kit. Complete blood count analysis was performed at hematology laboratory within 2 hours of collection of blood samples on a Beckman Coulter Gen-S autoanalyzer (High Wycombe, UK). NLR was calculated by dividing absolute neutrophil count by absolute lymphocyte count and PLR was calculated by dividing platelet count by absolute lymphocyte count.

Statistical analyses were performed by the SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) for Windows 15.0 program. The normality of data distribution was determined by Kolmogorov-Smirnov test. Continuous variables were presented as mean ± standard deviation (SD) and compared by using the t-test for independent samples when showing normal distribution. Mann-Whitney U test was used for results not displaying normal distribution or for comparison of nonparametric data. The strength of association between the variables was calculated using Pearson's correlation test for parametric variables and Spearman Rho correlation test for nonparametric variables. The level of significance was set at $p<0.05$.

Results

Table 1. Demographic and clinical characteristics of the two groups

	GDM group (n=29)	Control group (n=29)	p
Age (years)*	29.3±5.2	27.0±6.1	0.154
Body mass index (kg/m^2)*	29.1±5.2	27.1±3.3	0.06
Gravidity**	3 (1-8)	2 (1-5)	0.086
Parity**	1 (0-5)	0 (0-4)	0.249
Gestational age (days)*	259±21	256±19	0.197
Neutrophil/lymphocyte ratio*	5.1±1.9	3.3±0.9	0.0001
Platelet/lymphocyte ratio*	144.7±48.3	114.4±40.4	0.018
YKL-40 (ng/mL)**	203 (65-300)	159.2 (14-290)	0.007
Fibrinogen (mg/dL)*	442.9±83.6	412.5±69.3	0.209

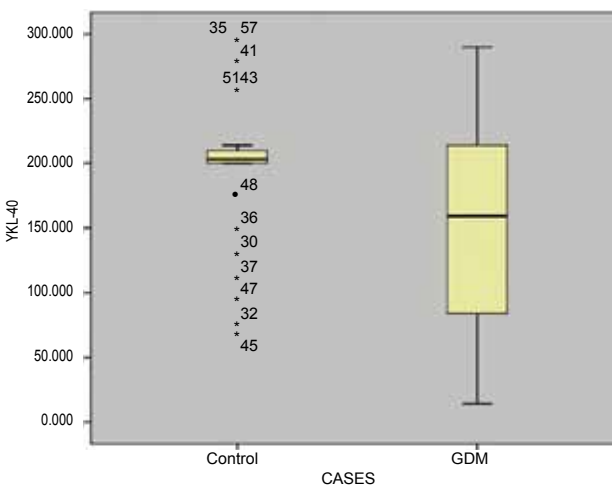
GDM = gestational diabetes mellitus; *values are mean ± standard deviation; **values are median (minimum-maximum); YKL-40 = human chitinase-3-like protein 1; $p<0.05$ = level of statistical significance

There were no statistically significant between-group differences between the group according to maternal age, gestational age, gravidity, parity and BMI (Table 1). Higher YKL-40 levels (203 (65-300) ng/mL *vs.* 159.2 (14-290) ng/mL, p=0.007) were recorded in pregnant women with GDM as compared to control group (Fig. 1). NLR and PLR were significantly higher in GDM compared with control group (Table 1).

No correlation was found between YKL-40 (r=-0.172, p=0.210) and age, YKL-40 and BMI (r=-0.059, p=0.662), and YKL-40 and glucose levels (r=0.039, p=0.787).

Discussion

Gestational diabetes mellitus is associated with serious maternal and fetal complications, among which unexplained stillbirth, an increased frequency of hypertension, and the need for cesarean delivery can be included. These complications have a substantial economic impact, including costs of monitoring and treating sick mothers and neonates[9,10]. The pathophysiology of GDM deserves further evaluation. Segovia *et al.* have reported that inflammatory mediators might influence fetal adipose tissue, liver and skeletal muscle, and lead to insulin resistance and GDM[11]. Ategbo *et al.* investigated circulating levels of cytokines and adipokines in 59 women with GDM, compared to 60 age-matched controls[12]. In this study, adiponectin, interleukin (IL)-2 and interferon-γ were found to be decreased. Cytokines such as tumor necrosis factor-α and IL-6 were elevated in patients with GDM[13].



GDM = gestational diabetes mellitus; *values are mean ± standard deviation; **values are median (minimum-maximum); YKL-40 = human chitinase-3-like protein 1; p<0.05 = level of statistical significance

Fig. 1. Human chitinase-3-like protein 1 (YKL-40) levels in the two groups.

Information on the physiological function and the mechanisms by which YKL-40 mediates its effects is still insufficient. In patients with type 2 diabetes, YKL-40 and hsCRP levels were found to be elevated, and this was associated with insulin resistance[4]. Hansen *et al.* report on patients with pancreatitis and secondary diabetes mellitus to have elevated levels of YKL-40 and IL-6 compared to healthy subjects, suggesting that YKL-40 is not a primary mediator of diabetes mellitus but a consequence of the diabetic state[3]. According to Celik *et al.*, plasma YKL-40 levels are increased in patients with polycystic ovary syndrome compared to healthy subjects[14].

Schaller *et al.*, contrary to our results, revealed that YKL-40 was not different between GDM and controls either during or after pregnancy[6]. They suggest that the absence of difference in YKL-40 levels between the GDM and control groups might be due to a similar inflammatory status at the time of measurements and explain this finding by the short duration of metabolic changes during GDM, which was in contrast to the results in type 2 diabetes. However, the present study revealed that there was an association between elevated YKL-40 levels and GDM. All studies investigating YKL-40 in diabetic patients point to the role of YKL-40 in the progression/prognosis of patients with diabetes[3-5].

We also found significantly higher NLR and PLR in GDM compared with the control group. To our knowledge, there is only one study investigating NLR in GDM[15]. Similar to our results, Yilmaz *et al.* showed increased NLR level to be a powerful and independent predictor of GDM[15].

In conclusion, GDM is associated with high levels of YKL-40, NLR and PLR, which indicate inflammatory status. As the number of studies on GDM increases, its association with inflammation will become clearer.

References

1. Moyer VA; U.S. Preventive Services Task Force. Screening for gestational diabetes mellitus: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2014;160:414-20.

2. Ozyer S, Engin-Üstün Y, Uzunlar O, Katar C, Danışman N. Inflammation and glycemic tolerance status in pregnancy: the role of maternal adiposity. Gynecol Obstet Invest. 2014;78:53-8.
3. Hansen M, Nielsen AR, Vilsboll T, Lund A, Krarup T, Knop FK, *et al*. Increased levels of YKL-40 and interleukin 6 in patients with chronic pancreatitis and secondary diabetes. Pancreas. 2012;41:1316-8.
4. Rathcke CN, Johansen JS, Vestergaard H. YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistance. Inflamm Res. 2006;55:53-9.
5. Kyrgios I, Galli-Tsinopoulou A, Stylianou C, Papakonstantinou E, Arvanitidou M, Haidich AB. Elevated circulating levels of the serum acute-phase protein YKL-40 (chitinase 3-like protein 1) are a marker of obesity and insulin resistance in prepubertal children. Metabolism. 2012;61:562-8.
6. Schaller G, Brix JM, Placher-Sorko G, Höllerl F, Schernthaner GH, Schernthaner G. YKL-40 concentrations are not elevated in gestational diabetes. Eur J Clin Invest. 2010;40:339-43.
7. Sefil F, Ulutas KT, Dokuyucu R, Sumbul AT, Yengil E and Yagiz AE, *et al*. Investigation of neutrophil lymphocyte ratio and blood glucose regulation in patients with type 2 diabetes mellitus. J Int Med Res. 2014;42:581-8.
8. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes mellitus. Am J Obstet Gynecol.1982;144:768-73.
9. Johnstone FD, Nasrat AA, Prescott RJ. The effect of established and gestational diabetes on pregnancy outcome. Br J Obstet Gynecol. 1990;97:1009-15.
10. Deveer R, Deveer M, Akbaba E, Engin-Ustun Y, Aydogan P, Celikkaya H, *et al*. The effect of diet on pregnancy outcomes among pregnant women with abnormal glucose challenge test. Eur Rev Med Pharmacol Sci. 2013;17:1258-61.
11. Segovia SA, Vickers MH, Gray C, Reynolds CM. Maternal obesity, inflammation, and developmental programming. Biomed Res Int. 2014;2014:1-14.
12. Ategbo JM, Grissa O, Yessoufou A, Hichami A, Dramane KL and Moutairou K, *et al*. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. J Clin Endocrinol Metab. 2006;91:4137-43.
13. Kirwan JP, Hauguel-De Mouzon S, Lepercq J, Challier JC, Huston-Presley L, Friedman JE, *et al*. TNF-alpha is a predictor of insulin resistance in human pregnancy. Diabetes. 2002;51:2207-13.
14. Celik C, Abali R, Guzel S, Bastu E, Kucukyalcin V, Yilmaz M. Elevated circulating levels of YKL-40 are a marker of abnormal glucose tolerance in women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 2012;77:893-7.
15. Yilmaz H, Celik HT, Namuslu M, Inan O, Onaran Y, Karakurt F, *et al*. Benefits of the neutrophil-to-lymphocyte ratio for the prediction of gestational diabetes mellitus in pregnant women. Exp Clin Endocrinol Diabetes. 2014;122:39-43.

Sažetak

MOGUĆA POVEZANOST GESTACIJSKOG DIJABETESA S UPALOM

A. Aktulay, Y. Engin-Ustun, M. Sahin Ozkan, S. Erkaya, M. Kara, O. Kaymak i *N. Danisman*

Cilj ovoga istraživanja bio je procijeniti je li gestacijski dijabetes melitus (GDM) udružen s upalom i to usporedbom serumskih razina humanog hitinazi-3-sličnog proteina 1 (YKL-40) te omjera neutrofila/limfocita (NLR) i omjera trombocita/limfocita (PLR). U ovo istraživanje parova bilo je uključeno 29 trudnica s GDM i 29 trudnica s normalnom tolerancijom glukoze. Dob (± 2 godine) i indeks tjelesne mase prije trudnoće bili su podjednaki u obje skupine. Mjerene su razine humanog hitinazi-3-sličnog proteina 1 (YKL-40/CHI3L1) te ispitani omjeri NLR i PLR. Nije bilo statistički značajnih razlika u dobi, gestacijskoj dobi i gravidnosti. Zabilježene su više razine YKL-40 u trudnica s GDM u usporedbi s kontrolnim trudnicama (203 (65-300) ng/mL prema 159,2 (14-290) ng/mL, p=0,007). NLR i PLR bili su značajno viši u skupini s GDM nego u kontrolnoj skupini. U zaključku, GDM je udružen s visokim razinama YKL-40, NLR i PLR koji ukazuju na upalno stanje.

Ključne riječi: *CHI3L1 protein, humani; Dijabetes, gestacijski; Leukociti, broj; Neutrofili – citologija; Limfociti – citologija; Trombociti – citologija*