Research Paper

CLINICAL INVESTIGATION

The relation of oxidative stress biomarkers with proinflammatory cytokines in gestational diabetes

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Abstract

Background: It has been shown that the synthesis and secretion of cytokines is influenced by the imbalance in oxidant/antioxidant status. In this study, the relation between the circulating levels of oxidative stress biomarkers and proinflammatory cytokines was searched in gestational diabetes mellitus (GDM) patients in order to evaluate the possible role of oxidative stress in ongoing proinflammatory condition and impaired glucose homeostasis.

Methods and findings: 33 pregnant women with GDM and 20 pregnant free of any maternal or fetal disorders were included in the study. Visfatin, TNF-α, IL-1β, IL-6, IL-10 and anti-ox-LDL levels were determined by ELISA. Malondialdehyde (MDA) levels and total antioxidant status (TOS) were measured spectrophotometrically. In the GDM group, MDA levels were significantly elevated (p=0.001), while the increment in TOS showed a borderline significance (p=0.05) as compared to the control group. Anti-ox-LDL of both groups remained unchanged. Serum IL-6 and IL-1β were significantly high (p=0.039, and p=0.04) in the GDM group, visfatin and IL-10 were similar in both groups. Slight but no significant increases in TNF-α levels were observed. TNF-α was associated with anti-ox-LDL (r=0.307, p=0.038) and HbA1c (r=0.352, p=0.05), MDA were correlated with TNF-α (r=0.420, p=0.004), TNF-α /IL-1β ratio (r=0.421, p=0.018), and with HbA1c (r=442, p=0.013). Anti-ox-LDL was correlated with TNF-α /IL-10 ratio (r=-0.361, p=0.046).

Conclusion: Our results indicated that MDA and TOS levels are elevated in close association with hyperglycemia and some proinflammatory cytokines in GDM patients. According to our study, reestablishment of the oxidant/antioxidant balance should be considered as one of the main therapeutic targets in order to establish normoglycemia as well as to outweigh the inflammatory state in GDM.

Keywords: Gestational diabetes ▪ proinflammatory cytokines ▪ Malondialdehyde (MDA) ▪ Total oxidant status (TOS) ▪ ox-LDL antibody

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Introduction

Gestational diabetes (GDM) is a status of carbohydrate intolerance in varying degree of severity which occurs during pregnancy. Insulin resistance and disturbances in carbohydrate metabolism may develop in pregnancy because of the necessity to supply appropriate nutrients for fetal growth and placental metabolism [1]. GDM has been shown to associate closely with increased synthesis of proinflammatory cytokines, the molecules that is known to involve in developing insulin resistance and GDM [2,3]. Lipid accumulation in adipocytes due

to maternal weight gain causes the marked production of proinflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, and visfatin [2,3]. There are studies to demonstrate the importance of these cytokines in insulin resistance and GDM [1]. Visfatin is an adipocyte-derived protein, and its secretion is mediated by plasma levels of glucose and insulin. Although plasma visfatin level has been found increased in some cases with type 2 diabetes, metabolic syndrome and obesity [4,5], controversial findings have also been reported [6]. TNF-α participates in the development of insulin resistance [7,8]. Its effect on insulin secretion may be either via inhibiting insulin signaling or reducing expression of regulatory molecules [9,10]. IL-6 and IL-1 β are other cytokines that participate in the inflammatory process [11], whereas IL-10 has an anti-inflammatory property by specifically counteracting with the responses caused through TNF-α, and helps to normalize the glycemic status [12,13]. Low concentrations of IL-10 in plasma have been reported in patients with gestational diabetes [8,14]. The intensity of hyperglycemia has been shown to alter the synthesis of TNF-α and IL-10, resulting in the elevation of TNF-α/ IL-10 ratio [15].

During pregnancy, maternal oxygen consumption is increased. Synthesis of reactive oxygen species (ROS) is increased owing to the mitochondria-rich placenta and increased amount of transitional metal ions such as iron. Consequently, the prooxidant-antioxidant balance is relatively disturbed, and total oxidant levels are increased inthe circulation [16,17]. Bukhari et al. have observed an elevation in serum total oxidant status (TOS), malondialdehyde (MDA) and homocysteine levels, and decreased antioxidant molecules during pregnancy [18]. In cases with GDM, high glucose level is an additional factor to increase oxidative stress due to auto-oxidation of glucose [19]. Enhanced superoxide generation from mitochondrial electron-transport chain and non-enzymatic protein deterioration are among consequences of hyperglycemia [20,21].

Lipids are the most sensitive biomolecules in oxidative stress conditions. MDA is a biochemical marker of lipid peroxidation that has mutagenic and atherogenic potency by interacting with proteins and DNA [22]. The levels of oxidized low density lipoprotein (ox-LDL) in plasma are increased mostly due to the oxidative stress. Modified lipoprotein profile and elevated ox-LDL levels have been demonstrated both in patients with diabetes mellitus and pregnancies with GDM [23,24]. Oxidative changes in LDL molecule cause chemotaxis of monocytes and T lymphocytes by inducing B-cell activation, and lead to antibody secretion against ox-LDL [25], which has protective effect on atherosclerosis and metabolic disorders [26].

In women with GDM, variations in plasma cytokine levels and oxidative stress biomarkers have been reported [7,11,14,27]. However, the association of cytokines with oxidative indices such as MDA, total oxidant status (TOS) and anti-ox-LDL have not yet been defined. In the present study, we aimed to measure the levels of visfatin, TNF-α, IL-1β, IL-6, and IL-10 in plasma of women with GDM and to evaluate their association with oxidative stress parameters.

Methods

Subjects

The study group consisted of 33 pregnant women with gestational diabetes (median age 32, range 20-47), and pregnant women without any maternal and fetal disorders (n=20, median age 28, range 18-37) served as controls. Median gestational age was 36 weeks. Gestational diabetes was diagnosed between 24th and 26th weeks of gestational age and diagnosis was achieved following a two-step GDM screening according to American Diabetes Association (ADA) [28]. The glycemic control of GDM subjects was solely provided by diet and exercise. The patients with renal failure (creatinine >1.5 mg/dL or glomerular filtration rate <70 mL/min), hepatic insufficiency, severe anemia and requiring insulin therapy were excluded from the study. All women delivered a live birth.

Venous blood samples were collected in vacutainer serum separator tubes (Becton Dickinson, Plymouth, UK), centrifuged at 4°C for 15 minutes, at 1000 xg, and serum aliquots were stored at -80° C for the measurements of TNF-α, IL-10, IL-1β, IL-6, MDA and TOS. For HbA1c, blood samples were collected in vacutainer tubes containing K2-EDTA and HbA1c levels were measured within four hours.

The study was approved by the Ethical Committee of Istanbul University (project no: 2014/399). An informed consent was taken from each patient.

Methods

HbA1c levels were determined using cation-exchange high performance liquid chromatography [HPLC] system with Bio-Rad Turbo II (Bio-Rad, Richmond, California, USA).

Serum visfatin, IL-6, TNF-α, IL-10, IL-1β concentrations were determined by the enzyme-linked immunosorbent method (ELISA, Assaypro, St Charles, USA) with intra-assay coefficient of variation (CV) being <5%, and interassay CV <8%. Serum visfatin level was measured using ELISA kit (Sunred

Biological Technology, Shanghai, China) with intra- and inter-assay CVs being 9.0% and 11%, respectively. Anti-ox-LDL was measured by the sandwich enzyme immunoassay technique (Immundiagnostik AG, Bensheim, Germany) with intra-assay CV below 6%, and interassay CV below 12%.

MDA levels were measured using thiobarbituric acid as described previously [29]. Results were calculated by using the molar extinction coefficient of the product (1.56×10^{-5} $M^{-1}cm^{-1}$). TOS concentration of the samples was determined spectrophotometrically using the commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey). In this method, the oxidants present in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ions and the color intensity at 530 nm is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with H2O2, and the results are expressed in μmol H_2O_2 Eq/L. The TOS assay had an intra-assay CV 3.0% with a two level-control.

Demographic parameters (weight, height and waist/hip circumferences) were measured, and BMI of the subjects was calculated as the ratio between weight and height squared (kg/m^2).

Statistical analysis

The data were analyzed using SPSS 15 (SPSS, Chicago, IL, USA). The results were expressed as mean ± SD or median (range). Mann–Whitney U test was performed to compare the data between the groups. Correlation analyses were carried out by the Pearson test. Statistical significance was defined as $p<0.05$.

Results

Baseline demographic characteristics of the subjects are shown in Table 1. There was no significant difference between the groups with respect to age, weeks of gestation, BMI, average weight gain during pregnancy, parity, or family history of type 2 diabetes.

Data obtained from the women with GDM were compared with those in the control group (Table 2). Serum IL-6 and IL-1β levels were found significantly high in the former group (p=0.039, and p=0.04, respectively). An increase in TNF-α level was by approximately 33%, but the difference did not give a statistical significance. Visfatin levels in GDM group showed a slight decrease, while IL-10 levels were similar in both groups.

There was a significant elevation of MDA levels in the GDM group compared to the control group (p=0.001). The increase in TOS level was 19% with a borderline significance (p=0.05). Anti-ox-LDL levels of both groups remained unchanged.

Significant correlations between oxidative markers and cytokines were obtained in the GDM group. MDA levels were positively correlated TNF-α (r=0.420, p=0.004), TNF-α /IL-1β ratio (r=0.421, p=0.018 respectively), and with HbA1c levels (r=442, p=0.013). TNF-α was also associated with anti-ox-LDL (r=0.307, p=0.038) and HbA1c (r=0.352, p=0.050). Anti-ox-

Table 1. Demographic characteristics and birth outcomes of the study groups. Median (range).

Demographic characteristics	GDM (n=33)	Normal pregnancy (n=20)
Age [years]	31 [20-47]	28 [18-37]
Parity	3 [1.0-6.0]	1.0 [1.0-3.0]
BMI [kg/m^2]	27 [17-44]	24 [21-26]
Gestational weeks at delivery	36 [35-38]	35 [34-38]
Birth weight [g]	3133 [2390-3800]	3177 [2150-3500]
Family history of DM [n]	21	8

Table 2. The levels of cytokines and oxidative stress biomarkers in women with gestational diabetes and controls [means ± SD].

	GDM	Normal pregnancy	p
Visfatin (ng/mL)	3.8 ± 2.9	4.97 ± 5.47	0.426
IL-6 (pg/mL)	3.1 ± 0.9	2.7 ± 0.7	0.04*
TNF-α (pg/mL)	6.61 ± 7.53	4.41 ± 3.03	0.59
IL-1β (pg/mL)	1.62 ± 0.4	1.39 ± 0.2	0.04*
IL-10 (pg/mL)	88.3 ± 66.5	71.8 ± 13.8	0.73
TNF-α/ IL-10	0.08 ± 0.1	0.06 ± 0.05	0.56
HbA1c % (mmol/mol)	5.1 ± 0.3 (32 ± 4)	4.9 ± 0.17 (30 ± 2)	0.11
MDA (nmol/mL)	22.9 ± 5.5	16.3 ± 2.5	0.001*
TOS (μmol H_2O_2 Eq/L)	4.3 ± 1.5	3.6 ± 1.1	0.05*
ox- LDL antibody (U/mL)	79365.5 ± 23461	80796.6 ± 15680	0.65

LDL was correlated only with TNF-α /IL-10 ratio (r=-0.361, p=0.046).

Significant correlations were also obtained between proinflammatory cytokines in the GDM group. Visfatin levels were associated with IL-10 (r=0.565, p=0.001) and IL-6 (r=0.511, p=0.003), TNF-α was associated with IL-6 (r=-0.318, p=0.032) and IL-10 (r=0.588, p=0.000). Only IL-1 β was associated with the birth-weight of the newborn (r=0.385, p=0.05). HbA1c was correlated with maternal BMI (r=0.597, p=0.001).

Discussion

Gestational diabetes is a chronic inflammatory state of insulin-sensitive adipose tissue and liver. Lipid accumulation in adipocytes increases ROS generation through activation of NADPH oxidase and the synthesis of proinflammatory cytokines, including TNF-α, IL-6, and IL-1 [30]. These proinflammatory mediators and cellular stress have been shown to activate JNK and NF-κB pathways [31,32], thus causing insulin resistance in the target tissues.

In this study, MDA levels were higher in patients with GDM than that in glucose-tolerant pregnant women. This finding was in good agreement with previous reports [33,34]. Additionally, a significant association was seen between MDA levels and HbA1c, indicating a relation between enhanced lipid peroxidation and poor glycemic control [35]. The increase in TOS levels also indicated an imbalance between oxidant/antioxidant parameters. Increased MDA generation has previously been shown in the maternal and cord plasma [36,37]. In experimental studies, aberrant ROS generation in type 1 and 2 diabetes mellitus have been observed [38,39]. It has been suggested that induction of oxidative stress in placenta and adipose tissue might disrupt the balance between oxidant agents and antioxidant defense system [40,41]. Diminished activity of scavenging enzymes and low levels of glutathione that have been detected in several studies supported this hypothesis [27,33,35].

When the proinflammatory cytokines were examined in GDM patients, IL-6 and IL-1β levels were found elevated, while visfatin and IL-10 levels were unaffected with regard to normal pregnancies. TNF-α levels showed an increment, but the difference between groups did not reach a significant level, possibly due to the scattering values of TNF-α. However, we obtained the significant associations of TNF-α level with MDA and HbA1c. These findings suggested that increased oxidative stress and hyperglycemia might lead to increased TNF-α synthesis or vice versa [30,32]. A slight increment in TNF-α/IL-10 ratio was seen. The changes in immunoregulatory cytokine profile might indicate an ongoing inflammatory response at the maternal-fetal interface. Moreli et al have reported a profoundly high TNF-α / IL-10 ratio in hyperglycemic pregnancies and concluded that the increase might indicate an altered synthesis of proinflammatory cytokines that results in impaired glucose homeostasis [15]. There are several other studies supporting their findings [2,8,41].

On the other hand, the reports with regard to IL-6, visfatin and IL-1β levels are controversial. While some authors have observed high values of these cytokines [4,5,42-44], the others revealed contrary results [14,35,45,46]. Despite increased synthesis, plasma cytokine levels may not be influenced due to the presence of various factors that prevent the transition of cytokines into the circulation. Among these factors, hypoxic environment due to hyperglycemia and pre-existing inflammatory conditions should be encountered [47]. According to our study, TNF-α appears to be associated more closely than other cytokines with poor glycemic control and oxidative stress parameters.

The ox-LDL antibodies have been suggested to remove ox-LDL from the circulation and artery wall [48]. These antibodies have been demonstrated to prevent ox-LDL binding to its receptors present on macrophages. It is known that the ox-LDL antibodies decrease the proinflammatory cytokine levels and also have beneficial effects on insulin-resistance. The macrophages that synthesize proinflammatory cytokines are down-regulated with the action of the ox-LDL antibodies resulting in reduced inflammation and insulin resistance [49]. In our study, mean levels of anti-ox-LDL in the patients with GDM were found similar to those from the control group. However, the observed significant correlations between anti-ox-LDL concentrations and TNF-α as well as TNF-α /IL-10 ratio might be indicative of inducing effect of TNF-α on antibody synthesis of ox-LDL. Although several studies have established an inverse relation between anti-ox-LDL and HbA1c levels in type 1 diabetic patients [50,51], we could not find such an association in our GDM patients. This finding might be explained by the fact that blood glucose levels of our study group were kept in normal range solely by diet and exercise, and none of the GDM patients required insulin treatment.

In conclusion, our results indicated that levels of MDA are markedly elevated in close association with hyperglycemia together with proinflammatory cytokines TNF-α and IL-6 due to the result of impaired balance between prooxidant and oxidant system in patients with GDM.

According to the results of our study, the maintenance of the oxidant/antioxidant balance

should be one of the therapeutic aspects in order to establish normoglycemia as well as to outweigh the inflammatory state in GDM.

Conflicts of Interest Statement

The authors declare that they have no conflicts of interest.

Executive summary

Background: It has been shown that the synthesis and secretion of cytokines is influenced by the imbalance in oxidant/antioxidant status. In this study, the relation between the circulating levels of oxidative stress biomarkers and proinflammatory cytokines was searched in gestational diabetes mellitus (GDM) patients in order to evaluate the possible role of oxidative stress in ongoing proinflammatory condition and impaired glucose homeostasis.

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Conclusion: Our results indicated that MDA and TOS levels are elevated in close association with hyperglycemia and some proinflammatory cytokines in GDM patients. According to our study, reestablishment of the oxidant/antioxidant balance should be considered as one of the main therapeutic targets in order to establish normoglycemia as well as to outweigh the inflammatory state in GDM.

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