Serum concentration of angiogenic (CXCL1, CXCL12) and angiostasis (CXCL9, CXCL10) CXC chemokines are differentially altered in normal and gestational diabetes mellitus associated pregnancies

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Abstract

Background The present study was aimed and designed to determine the serum levels of CXCL1 and CXCL12 as angiogenesis along with CXCL9 and CXCL10 as angiostasis, chemokines in, Gestational diabetes mellitus mothers (GDMM) and normal pregnancy mothers (NPM) and neonates who delivered by them.

Methods We have recruited 63 pregnant GDMM and 63 normal pregnant mothers at the third trimester of pregnancy to this cross-sectional study. Cord blood specimens were obtained from neonates who were delivered from GDMM and NPM. The serum and cord blood levels of chemokines were measured by ELISA in studied groups. Data were analyzed by chi-square and student's *t* test between two groups. The *P*-values less than 0.05 were considered significant.

Results Our results revealed that the serum levels of CXCL1, CXCL9 and CXCL12 were increased in GDMM, while no alteration was found in the serum levels of CXCL10 when compared to NPM. We have observed that in neonates the serum levels of angiogenic chemokines followed an inverse fashion when compared to angiostasis chemokines. Interestingly, CXCL1 and CXCL12 were both increased in neonates who were delivered by GDMM, while, CXCL9 and CXCL10 were decreased in neonates delivered by GDMM.

Keywords CXCL1 · CXCL9 · CXCL10 · CXCL12 · Chemokine · Gestational diabetes mellitus

Abbreviations

APGAR	is a quick test performed on delivered infants at 1 and 5 min after birth
BMI	Body mass index
FBS	Fasting blood sugar
GCT	Glucose challenge test
GDM	Gestational diabetes mellitus
INF	Interferon PAI-1, Plasminogen activator inhibitor-1
TNF- α	Tumor necrosis factor- α

T2DM	Type 2 diabetes mellitus
MetS	Metabolic syndrome
HbA1C	The hemoglobin A1C
ELISA	Enzyme linked immunosorbent assay
SPSS	Statistical package for the Social sciences
GDMM	Gestational diabetes mellitus mothers
NPM	Normal pregnancy mothers

Introduction

The gestational diabetes mellitus (GDM), is defined as one of the most prevalent complications which occurs during pregnancy and is associated with various degrees of impaired glucose intolerance [1, 2]. The clinical state of GDM is most often occurred during the latter half of the pregnancy and is described by carbohydrate intolerance with variable severity [3, 4]. It has also been well evidenced that, the prevalence of GDM is proportional to the frequency of type 2 diabetes mellitus (T2DM) within a community [5, 6]. GDM is amongst the most frequent

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pregnancy associated metabolic disorders, affecting 1–10% of all pregnancies. Most of the pregnant mothers who are suffering from GDM often return to the normal glucose tolerance subsequent to delivery, however, they are still at increased risk of progressing diabetes later on their life. GDM is also considered as a pre-diabetic state, and in fact, the disorder may display many characteristics and abnormalities that are specific for T2DM and commenced during very early stages of the T2DM [6–8]. GDM is associated with inflammation and a pathophysiologic link has been reported between GDM, T2DM and metabolic syndrome (MetS) [9–11]. Sufficient levels of both epidemiological and experimental evidences have provided indications for presence of a remarkable link between markers of subclinical inflammation and cardiovascular diseases, T2DM and MetS [10]. It is now well established that inflammatory markers such as CRP (C-reactive protein), IL-6, PAI-1, and TNF- α are elevated while adiponectin is inversely attenuated in mothers with a previous history of GDM [12, 13]. Chemokines as a subdivision of low molecular weight cytokines play pivotal parts in the regulation of defensive actions and reconstruction of damaged tissues [14]. The chemokines molecular weight is approximately between 8 and 10 kDa and are protein in both nature and structure that serve as pro-migratory factors for immune cells and recruit these cell types toward the damaged and inflamed regions in order to eliminate infectious agents and repair infected tissues [14]. They interact with their specific cognate receptors which are expressed on the cell surfaces and fit in seven transmembrane domain G protein coupled rhodopsin-like bio-structures [15]. Four main subfamilies of chemokines designated as CC, CXC, CX3C and C are described so far. CXCL1, CXCL9 and CXCL10 all fit in pro-inflammatory and inducible, whilst, CXCL12 is functionally a constitutive and homeostatic chemokine [16–18]. CXCL1 and CXCL12 both exhibit angiogenesis, activities whereas, CXCL9 and CXCL10 have anti-angiogenesis properties and display angiostasis activities [19–21]. CXCL9 and CXCL10 exert their functions via binding to CXCR3 (a specific receptor for CXCL9 and CXCL10) [22, 23]. In an almost similar mode of expression as CXCL10, the CXCL9 is also similarly expressed by a broad range of cell types in response to some members of cytokine family including, IFN- α/β and IFN- γ [24]. CXCL9 also attracts T-cells and NK cells to the insulted and infections regions, in addition to, injury and inflammation related pathologies. Furthermore, it has also been reported that CXCL12 and its relevant receptors (CXCR4 and CXCR7) in addition to serving as a particular actor in phenomenon of neovascularization (formation of new blood vessels play paramount roles in regulation of hematopoietic cell trafficking and homing of bone marrow precursors within the bone marrow niches [24, 25]. As stated earlier, CXCL1 and CXCL12 are both functionally angiogenesis and aid forming of new blood vessels while, CXCL9 and CXCL10 exhibit angiostasis properties and thus inhibit phenomenon of

neovascularization and forming of new blood vessels [6, 8, 26]. A body of evidences have addressed the involvement of some other CXC chemokines in pathogenesis of GDM [27–29], however, the role of CXCL1, CXCL9, CXCL10 and CXCL12 in GDM pathogenesis yet to be fully elucidated. Our previous studies on the field of pregnancy [24] encouraged us to extend our investigations towards exploring whether if these multi-functional CXC chemokines are altered in pregnant mothers with GDM and neonates who delivered by these mothers. Regardingly, since the event of GDM is pathophysiologically associated both with angiogenesis (formation of new blood vessels) and inflammation, we have aimed the present study to determine whether if CXCL1 and CXCL12 (as angiogenesis CXC chemokines) levels in GDM mothers and their neonates.

Material and methods

Disease diagnosis and specimen collection

Peripheral blood specimens were collected from 63 pregnant mothers with GDM and 63 pregnant mothers without any specific disorder, including GDM. The demographic information of the participants such as sex, age and duration of diabetes obtained and is presented in Table 1. The state of GDM was diagnosed by expert gynecologists on the basis of WHO (World Health Organization) criteria for GDM. Both clinical and para-clinical findings including, therapeutic protocols such as drug therapy, fasting blood sugar (FBS) and the hemoglobin A1C (HbA1C) status, in addition to other related clinical aspects of the disorder are also recorded in Table 1 [30].

During the clinical conditions all of mothers were routinely monitored by expert gynecologists and within of an hour delivery blood specimens were collected from participants and subjected to serum isolation. All of sampling processes were also performed both under supervision of expert gynecologists as well as assistance of expert midwifery team. All of mothers who had normal delivery the event of labor occurred after 37 weeks of gestation, according to the first day of last catamenia. The GDM sufferers and normal pregnant mothers were matched for age and number of deliveries. The exclusion criteria were, having smoking behavior, infection, allergy, corticosteroids and antibiotics consuming, exhibiting history of hypertension, vaginal bleeding and irregular catamenia. In fact, mothers have been automatically eliminated from the study if were displaying these characteristics.

Only mothers willing to participate in the study have signed a written informed consent form, prior to sample collection. The approval of the Ethical Committee has been granted by the Rafsanjan University of Medical Sciences. All of the names have also been changed to codes.

Table 1 Indicates the demographic characteristics and some paraclinical parameters of mothers

Variable	GDM Mothers		Mothers without GDM	
Age(year) (Mean $\pm$ SEM)	29.6 $\pm$ 1.21		30.52 $\pm$ 4.5	
Duration of diabetes (week)	29		—	
Duration of pregnancy (week)	38.01 $\pm$ 1.12		38.17 $\pm$ 1.82	
Cholesterol(mg/dL)	3.94 $\pm$ 0.94		3.77 $\pm$ 0.92	
Maternal BMI (Mean $\pm$ SEM)	29.7 $\pm$ 1.65*		22.5 $\pm$ 1.5	
FBS mg/dL (Mean $\pm$ SEM)	First FBS	Last FBS	First FBS	Last FBS
	91.7 $\pm$ 2.35**	136.8 $\pm$ 10.7	88.1 $\pm$ 4.5	94 $\pm$ 5.1****
GCT(Mean $\pm$ SEM)	213.56 $\pm$ 3.8*		134 $\pm$ 2.7	
APGAR(Mean $\pm$ SEM)	6 $\pm$ 1*		8 $\pm$ 1	
Delivery status	Caesarean Section	Normal Delivery	Caesarean Section	Normal Delivery
	38(70.8%)	16(29.2%)****	3(5.5%)	51(94.5%) ****
Multiple Pregnancy	1.48 $\pm$ 7		1.46 $\pm$ 7	

FBS, Fasting blood sugar; APGAR, APGAR is a quick test performed on a baby at 1 and 5 min after birth; mg/dL, gram/deciliter; BMI, Body mass index (kg/m^2); GCT, Glucose Challenge Test

* $P < 0.05$; significant difference with NPM (by Mann–Whitney U-test)

** $P < 0.05$; significant difference between first FBS and last FBS in GDMM

*** $P < 0.05$; s significant difference between last FBS in GDMM and NPM

**** $P < 0.05$; significant difference between deliveries with caesarean section and normal delivery in each group

Assessment of chemokines serum level

Further serum isolation, CXCL1, CXCL9, CXCL10 and CXCL12 were assayed by enzyme linked immunosorbent assay (ELISA) by specific CXC chemokine kits [31, 32]. The employed kits were purchased from, R&D systems, UK. Assays were performed in accordance with the manufacturer's guidelines. The sensitivity for kits was 2 pg/mL and inter and intra-assay assessments of reliability of kits were conducted as well [33].

Statistical analysis

All of statistical analysis was performed using the statistical package for the SPSS (Inc., Chicago, IL, USA) version 18. All of experiments were conducted for every separate sample, and all achieved results were reported as the mean values of the three independent experiments. Data were analyzed using chi-squared and students *t*-test between two groups. The normality distributions of data were examined by *Kolmogorov–Smirnov test*. Data were presented as mean $\pm$ SEM. The *P*-values less than 0.05 were considered significant.

Results

Demographic and clinical status of mothers

Analyses of the demographic data have indicated that the mean age for GDM mothers and normal pregnant mothers

was 29.6 $\pm$ 1.21 and 30.52 $\pm$ 524.5 years, respectively. Our finding showed that there exist no significant difference between GDM pregnant mothers and normal pregnant mothers regarding age, cholesterol and multiple pregnancy ($P > 0.05$). The mean duration of pregnancy was 38.01 $\pm$ 1.12 and 38.17 $\pm$ 1.82 weeks in GDM sufferers and NPM, respectively. The maternal BMI in GDMM and NPM was 29.7 $\pm$ 1.65 and 22.5 $\pm$ 1.5, respectively. Approximately, 1.48 $\pm$ 7% and 1.48 $\pm$ 7% of GDMM and WPM exhibited history of multiple pregnancies, respectively. We found that in GDM mothers, the mean first FBS was 91.7 $\pm$ 2.35 mg/dL and the last was 136.8 $\pm$ 10.7 mg/dL, while, it was 88.1 $\pm$ 4.5 mg/dL and the last was 94 $\pm$ 5.1 mg/dL. The GCT level was 213.56 $\pm$ 3.8 mg/dL in GDM and 134 $\pm$ 2.7 mg/dL in normal pregnant mothers (Table 1). The duration of diabetes in GDM mothers was approximately 29 weeks. As we observed, the level of cholesterol in GDM patients was 3.94 $\pm$ 0.94 mg/dL and for control mothers was 3.77 $\pm$ 0.92 mg/dL. The APGAR was 6 $\pm$ 1 number in GDMM while in NPM it was 8 $\pm$ 1 number. Our results demonstrated that 70.8% of GDMM patients underwent caesarean section and only 29.2% of them had normal delivery. We have also observed that only 5.5% of NPM underwent caesarean section and 94.5% had normal delivery (Table 2). We observed a significant difference between caesarean section and normal delivery in studied groups ($P < 0.05$). The APGAR status, BMI and GCT levels were markedly different in GDMM in compare to NPM ($P < 0.05$). There also exists a remarkable difference between the first and last FBS in GDMM whilst a significant difference yet to be observed in NPM in this case ($P < 0.05$).

Table 2 Indicates the demographic characteristics and some paraclinical parameters of neonates

Variable	neonates with GDM mothers		neonates without GDM mothers	
	Caesarean Section	Normal Delivery	Caesarean Section	Normal Delivery
HCC (cm)	34.12 ± 2.2		33.94 ± 1.87	
Fetal birthweight (g)	3345 ± 567		3212 ± 521	
Fetal birth height (cm)	49.53 ± 2.96		50.1 ± 2.82	
Gestational age at labor (weeks)	38.01 ± 1.12		38.17 ± 1.82	
Delivery status	Caesarean Section	Normal Delivery	Caesarean Section	Normal Delivery
Respiratory Distress	yes	no	no	
	17(29.2%)	46 (70.8%)**	63(100%)	
Abnormality	yes	no	yes	no
	4(20%)	20(80%)**	2(3.7%)	62(96.2%)

Data are presented as mean ± SEM

* $P < 0.05$; significant difference between neonates delivered by GDMM and NPM (by Mann–Whitney U-test)

** $P < 0.05$; significant difference between caesarean section and normal delivery in each group

HCC, Head circumference of children; g, gram; cm, centimeters

Demographic and clinical status of neonates who delivered by mothers with and without GDM

Statistical analysis of the demographic factors in neonates indicated that neonatal HCC was 34.12 ± 2.2 cm in neonates who were delivered by GDMM and 33.94 ± 1.87 cm in neonates delivered by NPM. The fetal birth weight was 3345 ± 567 g and 3212 ± 521 g in neonates who were delivered by GDMM and NPM, respectively. The fetal birth height was also 49.53 ± 2.96 cm and 50.1 ± 2.82 cm in neonates who were delivered by GDMM and NPM, respectively. Present results indicated that the gestational age at labor was 38.01 ± 1.12 weeks and 38.17 ± 1.82 weeks in who were delivered by GDMM and NPM, respectively (Table 2). There did not find any significant differences between HCC, fetal birth weight and height and gestational age at labor in neonates who were delivered by GDMM and NPM.

The neonatal respiratory distress in neonates delivered by GDMM underwent caesarean section was 29.2% while it was 70.8% in NPM ($P < 0.05$). The neonatal abnormality in neonates delivered by GDMM underwent caesarean section was also 20% but it was 80% in neonate delivered by NPM ($P < 0.05$) (Table 2).

Serum levels of chemokines in mothers

The serum levels of CXCL1, CXCL9, CXCL10 and CXCL12 were measured in GDMM and NPM in this investigation. Our findings demonstrated that the serum levels of CXCL1 was 298.8 ± 51.8 pg/mL and 201.7 ± 42.1 pg/mL in GDMM and NPM, respectively. We found that the serum levels of CXCL9 was 271.2 ± 9.6 pg/mL and 199.9 ± 7.4 pg/mL in GDMM and NPM, respectively. A The serum level of CXCL12 was $107 \pm$

5.3 pg/mL and 86.1 ± 4.1 pg/mL in GDMM and NPM, respectively. These findings have demonstrated the serum concentrations of CXCL1, CXCL9 and CXCL12 were all increased in GDMM in compare to NPM (Fig. 1a); ($P < 0.05$). In an inverse fashion, the serum levels of CXCL10 has sustrainly remained unchanged. 128.48 ± 8.4 pg/mL and 140.8 ± 5.9 pg/mL in GDMM and NPM, respectively and no significant alteration was observed in CXCL10 levels in GDMM in compare to NPM Fig. 1b).

Serum levels of chemokines in neonates

We have indicated that the serum levels of CXCL1 was 368.2 ± 43.7 pg/mL and 145.2 ± 35.1 pg/mL in neonates who were delivered by GDMM and NPM, respectively. These findings revealed that the serum levels of CXCL12 was 132 ± 7.5 pg/mL and 88 ± 4.5 pg/mL in neonates who were delivered by in GDMM and NPM, respectively. We found that CXCL1 and CXCL12 were considerably elevated in neonates who were delivered by GDMM in compare to who were delivered by NPM (Fig. 2a). Our results showed that the an inverse fashion CXCL9 and CXCL10 neonatal levels were considerably decreased in neonates who were delivered by GDMM, in compare to neonates who were delivered by NPM. The CXCL9 level was 125.5 ± 8.8 pg/mL and 199.9 ± 7.5 pg/mL in neonates who were delivered by GDMM and NPM, respectively. we have also observed that the serum levels of CXCL10 was 79 ± 6.7 pg/mL and 142 ± 5.3 pg/mL in neonates who were delivered by GDMM and NPM, respectively ($P < 0.05$) (Fig. 2b). Overall, these two IFN- γ inducible and angiostasis CXC chemokines (CXCL9 and CXCL10) were differentially expressed in neonates, compared to CXCL1 and CXCL12 (as angiogenesis chemokines).

Fig. 1 Shows serum level of chemokines in GDM and NPM. *: significant difference with NPM (P value<0.05). Data are presented as mean $\pm$ SEM

Discussion

The clinical state of GDM is defined as a disorder in which carbohydrate is not well tolerated with various severities [34,

35]. GDM is also identified as a pre-diabetic clinical condition offering a unique opportunity to study abnormalities that may occur during initial stages of the fetal development [36, 37]. Four main subfamilies of chemokines nominated as CC, CXC,

Fig. 2 Shows serum level of chemokines in neonates delivered by GDM and neonates delivered by NPM normal mothers. *: significant difference with neonates delivered by NPM (P value<0.05). Data are presented as mean $\pm$ SEM

CX3C and C are identified, to date [38–40]. It is now well evidenced that members of cytokine/chemokine network are critically involved in patho-physiology of pregnancy. Therefore, in the current study we sought to determine the circulating levels of CXCL1, CXCL9, CXCL10 and CXCL12 in pregnant mothers who were suffering from GDM and mothers with normal pregnancy along with their neonates. The findings of present investigation demonstrated that CXCL1, CXCL9 and CXCL12 serum levels were increased in GDM in compare to NPM but CXCL10 was remained unchanged significantly. We have observed that serum levels of CXCL1 and CXCL12 were significantly increased in neonates who were delivered from GDM in when compared to neonates who were delivered by NPM, but CXCL9 and CXCL10 followed a reverse fashion. Although, we have previously shown differential expression of CXCL10 and CXCL12 in term and preterm neonates [24]. To best our knowledge, there found no previous study evaluating these specific CXC chemokines in GDM and neonates who were delivered by them, and hence, this is first investigation on serum levels of these chemokines in this clinical state. Altered levels of several cytokines during progression of pregnancy have been reported, but these changes were not associated with GDM [41]. It has also been demonstrated that TNF- α was elevated in circulation of pregnant mothers, however, it not differed in GDM compared to NPM. Although, we examined paired serum levels of these chemokines in samples (mother and neonates) and showed their changes [5, 42]. In line with our study, it has been well identified that pregnancy is most often associated with insulin resistance. In our investigation we showed that both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly higher in GDM. Hypertensive disorders are known to be two to three-folds more frequent in GDM, proposing insulin resistance as an important etiological factor for the disorder [43]. This may possibly reflect several factors such as, advanced maternal age or differences in maternal obesity and therefore increased BMI are within the most important modifiable factors for the presence of GDM [44]. Consistent with this report several other studies also reported that the CXCL12 plasma level was significantly related to insulin resistance in pregnancy [41, 45, 46]. In a study Mohamed H Mahmoud et al., that the offspring of diabetic mice have exhibited several postpartum complications, such as significant aberrant overexpression of activating transcription factor-3 (ATF-3), significant elevation of the plasma levels of pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α) and reactive oxygen species (ROS), marked decreases in the plasma levels of IL-2 and IL-7, significant inhibition of CCL21 and CXCL12 mediated chemotaxis of B and T-lymphocytes, and a markedly decrease in the proliferative capacity of antigen-stimulated B and T-lymphocytes. [47]. Results of our study support previous data indicating increased TNF- α as an upstream target for chemokines expression [48, 49]. As we also previously reported a close relation between TNF- α and pro-inflammatory chemokines [24], a significant correlation was reported between

TNF- α and vascular cell adhesion molecule (VCAM) that propose a role for TNF- α in endothelium damage. Inflammation is also a primitive event in pathogenesis of GDM [50]. Thus, there may exist a relation between TNF- α production as an inflammatory mediator, as well as endothelial damage and hence a link might possibly be speculated between increased levels of angiogenesis chemokines (CXCL1 and CXCL12) and endothelium damage in GDM patients in our study. Therefore, correlation which was found during pregnancy in mothers with GDM between angiogenesis and angiostasis chemokines is maintained later in life, especially in those mothers developing T2DM is an aspect that warrants further study. If shown to be so, inflammation, as well as these chemokines in particular these CXC chemokines, might probably be involved in the GDM complications presented by mothers with glucose intolerance [51]. Accumulating evidences also are in favour of the fact that these chemokines alongside with IL-6 are all elevated in patients with T2DM patients [52], which their elevated levels have been described in GDM as well [53]. This is consistent with elevated CXCL1, CXCL12 levels because consensus elements for regulatory effects of IL-6 have been revealed on the regulatory of the genes of these chemokines [6, 24].

Conclusion

In conclusion, our observation may possibly suggest that these CXC chemokines are involved in pathogenesis of GDM. They either could be considered as useful biomarker for diagnostic purposes or promising target for GDM therapy and also speculation of prognosis to expect the development of further diabetes in mothers or neonates. However, these types of studies are limited to several restrictions, especially specimen collection from both neonates and mothers, but our future focus will be on an in-depth mechanistic research program to look at the pattern of these and other chemokine ligand/ receptor pairs at both mRNA and protein level in GDM.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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