Hs-CRP and TNF-α effects on postnatal umbilical coiling: impact assessment of the gestational diabetes mellitus in a prospective cohort study

Hamideh Akbari  
Sayad Shirazi hospital, Golestan University of Medical Sciences

Fateme Monemi  
Sayad Shirazi hospital, Golestan University of Medical Sciences

Atefe Notej  
Sayad Shirazi hospital, Golestan University of Medical Sciences

Alireza Khajavi  
Shahid Beheshti University of Medical Sciences

Omolbanin Asadi Ghadikolaei  
Iran University of Medical Sciences

Fereshte Abdolmaleki  
Iran University of Medical Sciences

Laily Najafi (✉ lailynajafi@yahoo.com)  
Iran University of Medical Sciences

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Abstract

Background

Hence, no study has been conducted to demonstrate specifically the relationship between gestational diabetes mellitus (GDM) status, inflammatory factors, and postnatal umbilical coiling index (pUCI), as understanding this relation could help to select the best appropriate interventions to save the fetus.

Method

To assess the impact of high sensitivity C-reactive protein (hs-CRP) and Tumor necrosis factor-Alpha (TNF-α) (in maternal venous and umbilical cord (UC) blood) on pUCI, comparing GDM and non-GDM groups. This prospective observational study contained 40 parturients in each of GDM and non-GDM groups, matched for maternal age, ethnicity, and parity. GDM diagnosis was confirmed by 24–28 weeks of gestation (WOG) and two-step strategy. The covariates of interest were maternal hs-CRP and TNF-α, measured at 37-41th WOG, and their UC analogous, measured during delivery. The gross morphologies were assessed immediately after delivery. The UC coiling was quantitatively assessed by the postnatal umbilical coiling index (pUCI).

Results

No significant difference of hs-CRP and TNF-α, in maternal venous blood or UC blood, was found between GDM/non-GDM groups. The mean (SD) of pUCI in GDM and non-GDM groups were 0.28 (0.15) and 0.24 (0.21) (p-value = 0.441), respectively. In GDM group, none of the four covariates of interest had significant effects on pUCI. Among the non-GDM patients, merely the UC hs-CRP had a direct association with pUCI, a Pearson correlation of r = 0.54 (p-value < .01).

Conclusions

In GDM group, no apparent relationship was observed between inflammatory factors and pUCI, although a direct association was detected between UC hs-CRP and pUCI in the non-GDM.

Tweetable abstract

The umbilical cord high sensitivity C-reactive protein is directly correlated with postnatal umbilical coiling.

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance presenting in variable severity of hyperglycemia, firstly diagnosed during pregnancy, and is associated with an increased risk of fetal morbidity and mortality(1). The metabolic impairments in GDM status create an abnormal environment in peripheral blood, and subsequently vascular structure alterations may occur.
which affect the function and development of placenta (2). GDM persuades excessive chronic hypoxia stress and inflammatory response in placenta(2), and this inflammatory cascade and vascular endothelial dysfunction plays an important role in the progression and pathogenesis of GDM (3).

Conversely, mild but significant inflammatory activity is involved in the development of normal pregnancy, which might have important physiological roles(4), and pregnancy is a pro-inflammatory and anti-inflammatory condition, depending upon the stage of gestation (5).

Accordingly, genetics, maternal characteristics, placental and umbilical cord structure and functions are the most important health factors that influence the fetus. In the umbilical cord as the vigorous lifeline of the fetus (6), we could watch coiling, as a unique feature of the human umbilical cord and can be evaluated by the umbilical coiling index (UCI) (7). UCI is quantified macroscopically after delivery by dividing the total number of complete vascular coils by the umbilical cord length in centimeters (postnatal UCI (pUCI)) (7, 8), and the changes of UCI is individualized (9).

The mechanisms underlying the changes in cord coiling are not fully understood (10). The importance of UCI abnormalities could be due to adverse prenatal outcomes caused by abnormal flow, constriction or thrombosis in the umbilical cord (10). Adverse perinatal outcomes in UCI abnormalities especially represent in pregnancies complicated by diabetes (7, 10–13).

GDM is one of the most important risk factors for abnormal coiling(14) which has an injurious effect on umbilical vessels and the connective tissue(15). The augmented perinatal fatality and morbidity associated with GDM may be the result of a vascular etiology (15).

Vascular endothelial dysfunction and elevated serum levels of inflammatory and endothelial markers have also been observed in women with GDM (3).

Simple differences in mechanical properties of the cord, growth response of the cord to metabolic and pharmacological abnormalities as well as intrinsic vascular structural changes and variations in placental morphology affecting placental blood flow and maternal-fetal gas exchange are the reasons for the correlation between GDM status, coiling abnormalities and adverse perinatal outcomes (16, 17).

Thus far, no study has been conducted to demonstrate specifically the relationship between GDM status, inflammatory factors, and pUCI, as understanding this relation could help to select the best appropriate interventions to save the fetus. Therefore, the inflammatory biomarkers were assessed as predictors of coiling abnormalities. This seems to be beneficial to select pregnancies for intensified fetal monitoring.

We hypothesized that GDM generates inflammatory changes in maternal and umbilical cord (UC) samples, which could affect the pUCI value. The aim of the current study was to assess the effect of high sensitivity C-reactive protein (hs-CRP) and Tumor necrosis factor- Alpha (TNF-α) (maternal venous blood and UC blood) on postnatal umbilical coiling in GDM and non-GDM.
Methods

Subjects

This prospective observational study recruited 80 singleton parturients (mother-infant pairs), during April 2018 until September 2019. This study was performed in the Sayad Shirazi hospital (tertiary perinatal center), located in Gorgan, Golestan, Iran.

The parturients were recruited at the 37th week of gestation (WOG) (determined by the first day of the last menstrual period or 1st trimester ultrasound scan using crown-rump length) and followed until delivery and postpartum period. The sample size was calculated by G power software (version 3.1), power= 90%, α =5%, d= 0.15, prevalence macrosomia =7%, 10% probable drop rate (6).

The outcomes and covariates of interest were fully measured for these parturients, who comprised of two groups of GDM and non-GDM (40 parturients in each group), and matched for maternal age, ethnicity, and parity.

The exclusion criteria included unavailable demographic, maternal, laboratory, neonatal and gross morphological data, or being afflicted by overt diabetes, presence of gross fetal anomalies, history of chronic hypertension, smoking or substance abuse, systemic disease, use of medications other than routine pregnancy supplements, single-artery UC, chorioamnionitis, placenta previa, placenta abruption, fever, and multifetal pregnancy.

Face to face interviews were performed in the first and the following prenatal visits by a single trained physician. Maternal and fetal detailed history, anthropometric variables, obstetrical, physical examination, and paraclinical parameters were collected from the patient’s files.

GDM was diagnosed according to the American Diabetes Association criteria at 24 to 28 weeks of gestation (WOG) using two-step strategy (100 grams Oral Glucose Tolerance Test (100-g OGTT)) (18-20). The non-GDM group included the parturients who were not complicated by GDM.

The medical nutrition therapy was prescribed for all GDM patients, and after 2 weeks, insulin therapies were assigned, due to ethical considerations. The appropriate insulin therapy was selected for the parturients, due to their self-monitoring blood glucose, and their socioeconomic status.

Blood pressure (BP) was measured in a standard condition (sitting position, after 5 minutes of resting, and ceasing smoking, drinking tea or coffee, and eating food for at least half an hour).

Blood glucose was measured by the Enzymatic Calorimeter method using a standard kit (EliTech kit) supplied by EliTech Group (France).

The maternal serum samples were collected and evaluated according to the hs-CRP and TNF-α during 37-41th WOG in the aforementioned hospital’s lab and immediately after delivery the same tests were
performed for the UC’s blood samples of 80 neonates. The hs-CRP and TNF-α were analyzed by ELISA method, using Monobind kit (Germany) (ng/ml) and Manual kit (America) (ng/L), respectively.

Then infantile evaluation was performed and entered into the checklist. All of the gross morphologic assessments were performed for all of the parturients immediately after delivery. To avoid diagnostic error, all of the evaluations were performed in the same physical conditions, and by same instruments and procedures.

**Definition of terms**

The umbilical cord coiling is quantitatively assessed by the UCI, the pUCI was determined by dividing the total number of complete UC twists by the total UC length in centimeters after delivery, resulting in range variability (0-1) (21, 22). One coil was defined as complete 360 degrees of umbilical artery around umbilical vein (7, 10, 14, 23, 24).

**Gross morphology assessment**

The following gross morphologies were assessed in this study: presence of UC coiling, existing complete vascular coils by 5 centimeters cord length (UCI), UC length, UC diameter, and number of vascular coils.

Macroscopic examination of placenta and UC was carried out in accordance with previously published protocols (25). A true pUCI was grossly evaluated within 24 hours after delivery and cord clamping in the fresh state, and the specimen was handled with great care, avoiding lacerations by a trained single physician who was blinded to the parturients’ clinical characteristics, laboratory data and pregnancy outcomes. Immediately after birth both placental and fetal ends of the cord was clamped and cut straight at five cm from the fetal insertion taking care not to milk and stretch the cord (which may affect the UCI) (26). Five centimeters was added to the length of each cord, to account for the portion of the cord that remained attached to the fetal umbilicus (27). The length and diameter of the UC were measured against a non-elastic tape graduated in centimeters, from its insertion into the placenta up to neonatal clamp. The placenta was allowed to separate spontaneously. Immediately after delivery, the placenta and the UC were preserved in a labeled, clean and dry plastic container full of normal saline (28) with an airtight lid. They were kept in a dry, clean laboratory with constant temperature maintained at 5°C and were washed clean of blood before examination.

**Intrapartum and neonatal outcomes**

Intrapartum outcomes included gestational age (GA) at delivery, emergency cesarean delivery, preeclampsia, premature rupture of membranes (PROM), polyhydramnios, and meconium stained amniotic fluid. Neonatal outcomes were inspected by a blinded pediatrician after delivery. Neonatal outcomes included neonatal sex, height, weight, and head circumference, first and fifth minute Apgar score, respiratory distress syndrome, cardiopulmonary resuscitation (CPR) and O2 consumption after CPR, neonatal intensive care unit admission, duration of hospital admission, fetal complications, large for GA (LGA), small for GA (SGA) and preterm delivery.
Statistical analysis

The independent sample t-test was used to compare the GDM and non-GDM groups, in terms of the continuous variables with normal distributions, and for the non-normal ones, non-parametric Mann-Whitney U test was utilized. Moreover, for comparing the discrete variables between two groups, chi-squared test was the tool.

To assess the effect of each one of the covariates of interest; maternal hs-CRP, UC hs-CRP, maternal TNF-α, and UC TNF-α on the pUCI, multivariate regression models containing the assessed covariate plus systolic BP, body mass index (BMI), Family history of diabetes mellitus (DM), and a binary variable comparing GDM/non-GDM patients were fitted. Since the response variable (pUCI) was a rate, the Poisson regression model was fitted, which provided the incidence-rate ratios, as the inferential tool.

Statistical analysis was performed using SPSS (version 16, SPSS Inc., Chicago, IL, USA).

Results

In this study, the average of GA at delivery time was 37.8 (1.1) vs. 37.8 (1.4) weeks (p-value = 0.09), for GDM and non-GDM groups, respectively. Demographic and reproductive characteristics of parturients are summarized in Table 1.
Table 1
Demographic and reproductive characteristics of parturients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>GDM (N = 40)</th>
<th>Non-GDM (N = 40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (yrs., mean(SD))</td>
<td>29.9 (5.5)</td>
<td>28.7 (5.6)</td>
<td>0.327</td>
</tr>
<tr>
<td>Relativity (n (%))</td>
<td>6 (15.0)</td>
<td>6 (15.4)</td>
<td>0.962</td>
</tr>
<tr>
<td>Gravidity (median (IQ))</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
<td>0.694</td>
</tr>
<tr>
<td>Parity (median (IQ))</td>
<td>0 (0–1)</td>
<td>1 (0–2)</td>
<td>0.080</td>
</tr>
<tr>
<td>History of Abortion (n (%))</td>
<td>2 (1–3)</td>
<td>1 (0–2)</td>
<td>0.041</td>
</tr>
<tr>
<td>History of stillbirth (n (%))</td>
<td>1 (2.5)</td>
<td>3 (7.5)</td>
<td>0.615</td>
</tr>
<tr>
<td>History of macrosomia (n (%))</td>
<td>0 (0.0)</td>
<td>1 (2.5)</td>
<td>0.506</td>
</tr>
<tr>
<td>History of GDM (n (%))</td>
<td>5 (12.5)</td>
<td>1 (2.5)</td>
<td>0.201</td>
</tr>
<tr>
<td>Family history of DM (n (%))</td>
<td>17 (42.5)</td>
<td>3 (7.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (mean(SD))</td>
<td>29.5 (5.1)</td>
<td>25.6 (4.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Maternal systolic BP (mean(SD))</td>
<td>117.9 (11.7)</td>
<td>109.5 (10.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Maternal diastolic BP (mean(SD))</td>
<td>74.9 (6.8)</td>
<td>71.4 (9.5)</td>
<td>0.066</td>
</tr>
</tbody>
</table>


The mean (SD) of GA of GDM diagnosis was 22.3(9.3) and the mean (SD) of GDM duration was 15.4(9.3) weeks, all of the GDM group received insulin therapy, and the type of insulin therapy was combination of short and long acting insulin in 52.5% of cases, 25% long acting, and 22.5% short acting insulin. The mean (SD) of insulin duration was 13.3(8.9) weeks.

The laboratory findings are compared between GDM and non-GDM groups, in Table 2, implying no significant differences in hs-CRP and TNF-α (maternal or UC), between two groups.
Table 2
Laboratory findings of two GDM and non-GDM groups.

<table>
<thead>
<tr>
<th></th>
<th>GDM (^\dagger) ((N = 40))</th>
<th>Non-GDM ((N = 40))</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Hs-CRP ((median \ (IQR)))</td>
<td>4633.5 (2277.6-8519.3)</td>
<td>6579.2 (2409.1-13235.1)</td>
<td>0.40</td>
</tr>
<tr>
<td>UC Hs-CRP ((median \ (IQR)))</td>
<td>27.1 (7.3–55.2)</td>
<td>13.5 (0-89.6)</td>
<td>0.50</td>
</tr>
<tr>
<td>Maternal TNF-(\alpha) ((median \ (IQR)))</td>
<td>105.3 (85.1-277.9)</td>
<td>130.4 (93.9-226.3)</td>
<td>0.31</td>
</tr>
<tr>
<td>UC TNF-(\alpha) ((median \ (IQR)))</td>
<td>163.3 (140.7-297.9)</td>
<td>186.1 (158.3-373.9)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

GDM: Gestational diabetes mellitus. Hs-CRP: high-sensitivity C-reactive protein.
UC: Umbilical Cord. TNF-\(\alpha\): tumor necrosis factor alpha.

All neonates were alive; and delivered by cesarean section in 85% of GDM and 39.5% of non-GDM parturients \((p-value = < 0.001)\). Comparing the intrapartum and neonatal outcomes, no significant statistical difference was detected. No PROM, SGA and preterm delivery was reported. Although polyhydramnios and neonatal intestinal atresia was detected in one of the GDM parturients, and in another a LGA neonate was distinguished.

The gross morphology findings were compared between GDM and non-GDM groups, implying no significant difference regarding the presence of UC coiling, number of vascular coils, UC length and diameter. The mean (SD) of pUCI in GDM and non-GDM groups was 0.28 (0.15) and 0.24 (0.21) \((p-value = 0.441)\), respectively.

According to Fig. 1, among the GDM parturients, none of the four covariates of interest, i.e. hs-CRP and TNF-\(\alpha\) in maternal venous blood or UC blood, showed an apparent relationship with the pUCI, and among the non-GDM parturients, merely the UC hs-CRP had a direct association with pUCI. The Pearson correlation analysis also confirmed it, where the only significant correlation was found between UC hs-CRP and pUCI \((r = 0.54, p-value < .01)\). Although no statistically significant correlations were detected, there seemed to be reverse relations between (maternal and UC) TNF-\(\alpha\) and pUCI, in both GDM and non-GDM groups.

The multivariate regression models were fitted taking the pUCI as dependent variable, using the covariates of interest hs-CRP and TNF-\(\alpha\) in both forms of maternal venous blood and umbilical cord blood, and adjusted for the confounders systolic BP, BMI, family history of diabetes and GDM/non-GDM status. The findings are reported in Table 3. None of the four assessed covariates showed any significant effect on pUCI.
Table 3
The multivariate Poisson regression models evaluating the effects of the covariates hs-CRP and TNF-α (maternal and UC forms) on pUCI, adjusted for confounders.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>IRR</th>
<th>p-value</th>
<th>Confidence interval (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal hs-CRP</td>
<td>1.000001</td>
<td>0.98</td>
<td>0.99992 to 1.00007</td>
</tr>
<tr>
<td>UC hs-CRP</td>
<td>1.0001</td>
<td>0.18</td>
<td>0.99993 to 1.0003</td>
</tr>
<tr>
<td>Maternal TNF-α</td>
<td>0.9998</td>
<td>0.63</td>
<td>0.9989 to 1.0006</td>
</tr>
<tr>
<td>UC TNF-α</td>
<td>0.9998</td>
<td>0.64</td>
<td>0.9989 to 1.0006</td>
</tr>
</tbody>
</table>

**IRR**: Incidence-rate ratio, **pUCI**: Postnatal umbilical coiling index

**Hs-CRP**: high-sensitivity C-reactive protein. **UC**: Umbilical Cord.

**TNF-α**: tumor necrosis factor alpha.

**Adjusted for the confounders; systolic blood pressure, BMI, family history of diabetes, and GDM/non-GDM status.**

**Discussion**

This prospective observational study investigated the effect of hs-CRP and TNF-α (maternal and UC) on postnatal umbilical coiling in GDM and non-GDM pregnancy.

The present study’s investigators have not found any previous publications correlating the GDM status, postnatal umbilical coiling and inflammatory factors.

We found no significant difference of hs-CRP and TNF-α, in maternal venous blood or UC blood, between two groups. The same result was detected by Gomes et al. for TNF-α, between GDM and non-GDM groups (29). The same non-significant difference in UC hs-CRP was demonstrated between GDM and non-GDM groups by other studies (30, 31). In addition, no significant difference of pUCI in both groups were detected, the same as other studies (6, 21, 32).

In GDM group, the maternal and UC hs-CRP had no apparent effects on pUCI, although no significant association was observed, there seemed to be direct relations. Among the non-GDM parturients, merely the UC hs-CRP had a direct and significant association with pUCI, in contrast with maternal hs-CRP.

No statistically significant correlations were detected between maternal/UC TNF-α and pUCI in both groups, though there seemed to be reverse relations.

In the multivariate regression models, adjusted for the confounders systolic BP, BMI and family history of diabetes, none of the four assessed covariates showed any significant effect on pUCI.

For normal pregnancy development, significant inflammatory activity is needed which might have important physiological roles (4).
In the prepartum period; the metabolic and immunological disorders besides the inflammatory status alterations, could affect the fetal developing environment (4). The inflammation is an important determinant of adverse fetal and perinatal outcomes (33). The fetal response to inflammation is considered by increased levels of pro-inflammatory cytokines in the amniotic fluid and UC blood (33, 34); which induces inflammation of chorionic plate, placental and UC vessels (fetal vasculitis) (35). However, the association between perinatal inflammation and biochemical inflammatory indicators in UC blood and maternal circulation are not completely understood. TNF-α and CRP have been mentioned for their role in the inflammatory process and are most commonly assessed in relation to insulin resistance and obesity (36, 37). TNF-α have been recognized as being responsible for hepatic production of the acute phase reactants such as cytokines and CRP (38). Hs-CRP is an acute phase reactant biomarker and is produced by liver in response to pro-inflammatory cytokines, which may be used as adjunctive test in the diagnosis of inflammation (33). The plasma levels of hs-CRP will be increased within a day after the onset of inflammation and tissue injury, when diagnostic clinical signs and other laboratory tests may be non-specific, and remains elevated until after stimulus resolves (39). Elevation of serum and UC hs-CRP in the neonate is due to endogenous hepatic synthesis, since very low quantities of hs-CRP cross the placenta (33, 40). Perinatal inflammation is associated with increased UC inflammatory cytokines including hs-CRP (41), but the impact on inflammatory markers in the early neonatal period is less well-described (33).

TNF-α is mainly produced by activated macrophages and monocytes (42), it affects insulin secretion and sensitivity through influencing B-cell function and insulin signaling pathways, which possibly induces GDM (43). It's level is controversial in this way, no changes was observed during pregnancy vs. postpartum (4) in contrast with Germain's (44) study which demonstrated more in normal pregnancy, vice versa Graham presented that most pro-inflammatory cytokine expression, including TNF-α was reduced during normal pregnancy(45).

The prepartum pro-inflammatory and anti-inflammatory condition is depended to the severity of comorbidities, the trimesters that were evaluated (5), and also pregnancy duration (46) and the mode of delivery may affect the concentrations (47).

Hs-CRP, TNF-α and other adipokines were found to be highly correlated with obesity which contributes to insulin resistance and this correlation leads to many diseases, notably GDM and preeclampsia during pregnancy (48). TNF-α and hs-CRP are mostly raised at the end of pregnancy (37).

GDM diagnosis was performed during second trimester, which probably exposed the infant to intrauterine metabolic changes, inflammatory process and epigenetic programming for nearly 24 to 28 WOG, which predispose infants to long-term abnormality (49). The hyperglycemia is contributed to produce reactive oxygen species which concluded oxidative tresses, and so pro-inflammatory cytokines and TNF-α is produced (50). So, according to elevation of serum inflammatory biomarkers and endothelial markers in GDM, the vascular endothelial dysfunction and placental angiogenesis is observed (3, 50). In another view, the metabolic impairments in GDM status cause vascular structure alterations, so consequently
affect the morphology, function and development of placenta and UC (2, 50). GDM induces chronic hypoxia and inflammatory response in placental and UC vascular endothelial cells (2). A hypoxic placenta may release cytokines and inflammatory factors (TNF-α and CRP) which could induce vascular endothelial dysfunction (51, 52). This inflammatory cascade and vascular endothelial dysfunction plays an important role in the progression and pathogenesis of GDM (3).

Cvitic et al. and the other studies presented more expression of TNF-α in placenta in GDM parturients (53–55), in contrast with UC that low TNF-α level was detected (50, 55, 56). Increment of TNF-α levels in GDM may according to increased oxidative stress and imbalance in the expression of pro-inflammatory and anti-inflammatory cytokines which may contribute to impaired glucose metabolism (55, 57).

In prepartum period, hs-CRP is correlated with maternal serum glucose of GDM parturients, when measured at the 3rd trimester as a standard screening time (58, 59), which is similar with the present study. According to the adverse perinatal outcomes in GDM parturients, a vascular etiology is seems as an important cause (15, 60), and also metabolic and pharmacological abnormalities are the other causes. Additionally; the mentioned adverse fetal morbidities in UCI abnormalities, especially represent in pregnancies complicated by GDM (7, 10–13). GDM as one of the most important etiologies for coiling abnormalities(14) has an deleterious effect on umbilical vessels and the connective tissue(15).

The influence of UCI on perfusion pressure is unknown, it is hypothesized that the pressure reduction caused as a weaker stimulus to angiogenesis with failure to form vasculo-syncytial membranes (13). Consequently, a raised pressure in the coiling abnormalities also seen more frequently detected in diabetes, which leads to terminal villi congestion and consequently angiogenesis increment (13). Besides the effect of insulin on placental vascularity, the other factors such as angiogenic factors and inflammatory/oxidative stress factors also induce angiogenesis (61, 62). Adverse fetal outcomes were attributed to abnormal UCI (63), which predisposed the vessels of the umbilical to stenosis, thrombosis, constriction, occlusion, torsion of umbilical vessels, cord entanglement (10). These abnormal mechanisms lead to compress the fetoplacental blood flow, maternal-fetal gas exchange abnormality and fetal hypoxia (7, 16, 17), which may strengthen and exaggerated in GDM parturients because of the reduction of the Wharton's jelly content, decrement of mucopolysaccharide synthesis and the collagen molecules changes(15). The fetal hypoxia leads to more oxidative and nitrative stresses (64).

Conclusively, all these conditions induced placental blood supply decrement(16, 17); which may affect the number of coils was an important etiology to all mentioned conditions (14).

All the above-mentioned changes were noticed in nearly one third of diabetic parturients and it seems that the poor glycemic control is significantly related to these changes, these changes are prominently described in pre-gestational diabetes not in GDM and occult form(13).

In similar way, Mayhew et al. defined normal vascular function in GDM parturients with good glycemic control (65) in contrast with Leach, which described vascular insufficiency with appropriate glycemic control (66). The mentioned inflammatory activity may affect from intra-partum GDM treatment (by life style changes, medical nutrition therapy, glycemic control) according to ethical consideration, decrement
of GDM severity, on-time diagnosis and appropriate follow-ups, in GDM group. We suggested the aforementioned considerations; as confounding factors that affect our results.

In the literature review, no study has been conducted to demonstrate specifically the relationship of biochemical inflammatory biomarkers, and pUCI in parturients complicated by GDM.

The strengths of this study were that all evaluations were demonstrated in a referral obstetric hospital (tertiary perinatal center). In this prospective observational study, the authors could follow the late prenatal, perinatal and postnatal period. The study population comprised GDM and normal pregnancy, providing valid comparison between groups. Additionally, for UCI calculation, the total length of the cord was considered and rapid bilateral clamping of the cord was performed to avoid urgent blood drainage. Using merely “two-step strategy” (100-g OGTT) for GDM diagnosis of all cases to prevent over diagnosis by “one-step strategy”.

**Conclusions**

These are the first data to show that in GDM group, no apparent relationship was detected between inflammatory biomarkers with pUCI although a direct association was detected between UC hs-CRP and pUCI in non-GDM.

The limitation of the present study could be mentioned as the small sample size, according to that the impact assessment of some variables could not be achieved. Consequently; further investigations in larger populations with serial and early evaluation of inflammatory biomarkers to understand the correlations between the aforementioned and other inflammatory indicators with UCI; as screening tests for detection of coiling abnormalities and prediction of pUCI in different comorbidities such as GDM, preeclampsia and so on.

**Abbreviations**
Declarations

Ethics approval and consent to participate

The ethics committee of Golestan University of medical sciences approved the study protocol (IR.GOUMS.REC.1397.273, IR.GOUMS.REC.1397.272) and all participants signed the written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent to participate was obtained by all subjects. We confirm that all methods were performed in accordance with the relevant guidelines and regulations

Consent for publication

Not applicable.

Availability of data and material
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interest**

The authors declare that they have no competing interests.

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**Authors’ contributions**

HA and LN designed the study. LN and AK contributed to the analysis and interpretation of data. LN and AK drafted the manuscript. FM, AN, and FA critically revised the manuscript. All authors approved the final draft.

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**Author details**

[1] Clinical Research Development Center, Sayad Shirazi hospital, Golestan University of Medical Sciences, Gorgan, Iran.

[2] Student Research Committee, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. alireza.khajavi.student@gmail.com

[3] Endocrine Research Center, Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Tehran, Iran.

**Disclosure of interests**

The authors declare that there are no financial and non-financial conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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    40.
Figures

Figure 1

The correlation between high sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor-Alfa (TNF-α) (in maternal venous blood and umbilical cord blood) and pUCI, in GDM and non-GDM groups.