Received: 06 Dec 2023 | **Accepted:** 28 Jan 2024 **Genel Tıp Derg. Volume** 34/**Issue** 2 (April), 251-257
DOI: 10.54005/geneltip.1400341

ORIGINAL ARTICLE

Maternal and Umbilical Cord Heat-Shock Protein 70 Levels in Patients with Gestational Diabetes Mellitus

Gestasyonel Diyabetes Mellituslu Gebe Hastalarda Maternal ve Umblikal Kordon Heat-Shock Protein 70 Seviyeleri

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How to cite ?

Bertizlioğlu M, Yılmaz S, Tazegul A, Kebapçılar A, Seçilmiş Ö, Gençoğlu Bakbak BB, Celik C, Aktaş G, Unlu A, Abuşoğlu S, Doğan NU. Maternal and Umbilical Cord Heat-Shock Protein 70 Levels in Patients with Gestational Diabetes Mellitus. Genel Tıp Derg. 34(2): 251-257.

ABSTRACT

Objectives: The objective of this investigation is to ascertain whether there is an increase in the levels of heat shock protein 70 (HSP70), a signal for cellular stress, in the maternal bloodstream and umbilical cord of pregnancies affected by gestational diabetes mellitus (GDM). Additionally, the aim is to explore whether variations in the concentrations of Hsp70 in umbilical cord serum can be indicative of the likelihood of early term delivery (between 37 0/7 and 38 6/7 weeks of gestation) in women diagnosed with GDM compared to the control group.
Methods: In this case-control study, 62 individuals diagnosed with gestational diabetes mellitus (GDM) comprised the GDM group while 22 non-diabetic, healthy women scheduled for cesarean section formed the control group. Our analysis encompassed the examination of Hsp70 serum levels in both maternal pregnancies and umbilical cord sera, alongside an evaluation of various biochemical and anthropometric markers. Additionally, the study explored the occurrence of early term delivery among all subjects.
Results: The concentration of Hsp70 in the maternal serum exhibited a notable increase among individuals diagnosed with gestational diabetes mellitus (GDM) compared to healthy pregnant women. Similarly, the levels of Hsp70 in the umbilical cord were elevated in GDM patients although the difference did not reach the commonly accepted significance threshold. Notably, cord Hsp70 levels displayed a statistically significant negative correlation with the time of delivery in women with GDM. This inverse relationship with the time of delivery was also observed in the overall study group, indicating a potential association between cord Hsp70 levels and the timing of delivery.
Conclusion: Maternal Hsp70 was significantly higher in patients with GDM. The obtained results seem to indicate that elevated umbilical cord Hsp70 values may potentially be used as indicators of risk factor for preterm delivery in pregnancies.

Keywords: Gestational Diabetes, Heat-Shock Protein 70, Preterm Birth

ÖZ

Amaç: Bu çalışmanın amacı, hücresel stresin bir belirleyicisi olan Heat shock protein 70 (Hsp-70)'in, gestasyonel diyabet mellitus (GDM) gebeliklerde maternal serum ve umbilikal kordda yüksek olup olmadığını belirlemek ve farklı serum Hsp-70 konsantrasyonlarının umbilikal kordda, GDM'li kadınlarda ve kontrol grubundaki kadınlarda erken doğumun bir göstergesi olarak ilişkilendirilip ilişkilendirilmediğini belirlemektir (37 0/7-38 6/7 hafta gebelik).
Yöntemler: Çalışma, bu durum kontrol çalışmasında sezaryen doğum olan GDM'li 62 hasta (GDM grubu) ve 22 diyabetik olmayan, sağlıklı kadını içermektedir. Hamileliklerde ve umbilikal kord serumlarında Hsp-70 düzeylerini ve tüm konulardaki erken doğumu içeren diğer biyokimyasal ve antropometrik belirteçleri analiz ettik.
Bulgular: GDM'li hastalarda maternal serum Hsp-70 düzeyleri, sağlıklı hamile kadınlardan önemli ölçüde yüksekti. GDM hastalarının umbilikal kord Hsp-70 düzeyleri de sağlıklı hamile kadınlara göre artmıştı ancak genellikle kabul edilebilir düzeyde anlam bulunamadı. Kord Hsp-70 düzeyleri, GDM'li kadınlarda doğum zamanı ile negatif anlamlı bir korelasyon gösterdi. Kord Hsp-70 düzeyleri, aynı zamanda tüm grup içinde doğum zamanı ile negatif anlamlı bir korelasyon gösterdi.
Sonuçlar: Maternal Hsp-70, GDM'li hastalarda önemli ölçüde yüksekti. Elde edilen sonuçlar, yüksek umbilikal kord Hsp-70 değerlerinin, gebeliklerde erken doğum için bir risk faktörü göstergesi olarak potansiyel olarak kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Heat-shock protein 70, Gestasyonel Diyabetes Mellitus, Erken Doğum

Introduction

Gestational diabetes mellitus (GDM) is characterized by varying degrees of glucose intolerance that emerges or is first recognized during pregnancy. This condition is linked to serious adverse perinatal outcomes, including abnormal intrauterine growth and an elevated incidence of both spontaneous and induced preterm births (1). Despite the numerous complications associated with hyperglycemia, the underlying mechanisms contributing to the physiopathology of fetal abnormalities in GDM have yet to be fully comprehended (2). Heat-shock proteins (HSPs), also known as stress proteins, serve as molecular chaperones, playing a vital role in maintaining protein homeostasis (3) and shielding cells from various forms of stress (4). Typically, under normal conditions, HSPs are regarded as intracellular proteins with anti-inflammatory effects (5). However, in situations of cellular stress, these proteins can also be expressed on the cell surface (6).

Peer-Review: Double anonymized - Two External
Plagiarism Checks: Yes - intihal.net
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Selcuk University Press
Genel Tıp Dergisi | e-ISSN: 2602-3741
https://dergipark.org.tr/tr/pub/geneltip
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Crucially, heat shock proteins are instrumental in the development of insulin resistance, contributing to the manifestation of hyperglycemia. This involvement underscores their significance in the physiological response to stress and the regulation of cellular processes associated with metabolic function. Heat Shock Protein 70 is a noteworthy protein in this context, acting as a cytoprotective chaperone with roles in both protein folding and degradation. The induction, transcription, and translation of Hsp70 have associations with a reduction in insulin resistance-related metabolic disorders. This impact extends to factors such as inflammation, mitochondrial function and endoplasmic reticulum (ER) stress.

Elevated levels of extracellular Hsp70 in the plasma are correlated with conditions like obesity and diabetes, both recognized as pro-inflammatory states as discussed in the current review. Conversely, a decrease in the concentration of Hsp70 may impede inflammation and mitochondrial fatty acid oxidation while concurrently enhancing the activation of SREBP-1c, a gene transcription factor intricately involved in ER stress. This intricate interplay underscores the multifaceted role of Hsp70 in regulating various cellular processes associated with metabolic health.

Moreover, increased expression of Hsp70 in brain cells holds the potential to augment insulin sensitivity and restore blood glucose levels to a normal range. This highlights the multifaceted involvement of Hsp70 in regulating diverse pathways associated with insulin resistance and other related metabolic disorders (2). The production of elevated levels of Hsp70 can be initiated by exposure to various stressors such as hyperthermia, ischemia, inflammation, and oxidative stress (1). Recent research findings have indicated that circulating Hsp70 levels were notably elevated in individuals diagnosed with type 2 diabetes mellitus when compared to those without diabetes (3). Diabetes mellitus exerts an impact on the duration of gestation and the likelihood of spontaneous preterm birth (1). The connection between preterm delivery and heat-shock protein (Hsp70) has been a subject of investigation (1, 3-7). However, findings regarding the association between Hsp70 and both preterm and term deliveries have been inconsistent. While some studies report an increased risk of early term birth (1, 4-6), international literature lacks a consensus on whether Hsp serves as an independent risk factor for spontaneous early term or preterm birth (1, 3-7). The existing body of research presents conflicting perspectives on the role of Hsp70 in influencing the timing of delivery. Nevertheless, it is crucial to acknowledge that these studies come with inherent limitations, including a retrospective design in some cases (2), the absence of umbilical cord sera in others (1, 5-7), reliance on serum from high-risk patients for preterm delivery in specific studies (5), and the lack of consideration for gestational diabetes mellitus (GDM) in certain investigations (1, 3). To the best of our knowledge, there is currently no available data regarding the changes in Hsp70 levels in both maternal and cord serum among women with GDM when compared to a carefully matched nondiabetic control group.

In the current study, we sought to address these gaps by conducting a comprehensive assessment of early term delivery. Our focus was on determining whether the levels of serum Hsp70 in umbilical cord samples could potentially serve as a reliable indicator of early term delivery in women with GDM, comparing these findings with a control group of non-diabetic individuals. This investigation aims to contribute valuable insights into the relationship between Hsp70, GDM, and early term delivery, providing a more nuanced understanding of the complex interplay involved.

Material and Methods

The current study received approval from the Ethical Committee and Institutional Review Board of Selcuk University Faculty of Medicine, where the research was conducted. Prior to participation, written informed consents were obtained from all individuals included in the study. The study comprised 62 pregnant women diagnosed with gestational diabetes mellitus (GDM) and a control group consisting of 22 individuals matched for age and body mass index (BMI), all with uncomplicated singleton pregnancies. Pregnant women were recruited from the antenatal clinic of our obstetrics department, with a history of prior cesarean section deliveries and a plan for cesarean section delivery. Exclusion criteria for all participants included the use of medication, smoking, high blood pressure, any acute or chronic diseases, fetal anomalies, and multiple gestation. Detailed clinical histories of all participants were taken and physical examinations were performed. Screening for GDM was performed using a 50 g glucose challenge test (GCT) at the 24th gestational week. This comprehensive approach to participant selection and screening aimed to ensure a detailed and representative study population, taking into account various factors that could potentially influence the study outcomes. The diagnosis of gestational diabetes mellitus (GDM) in our study followed the two-step approach proposed by Carpenter and Coustan. The 50 g glucose screening test was conducted irrespective of the time of day or preceding meals. Subsequently, an oral glucose tolerance test was recommended for all patients whose 1-hour test result equaled or exceeded 140 mg/dl. The criteria for diagnosing GDM included two or more values surpassing the established cut-off levels (95/180/155/140 mg/dl) (8). As for the age and BMI-matched control group, all individuals had negative results in the oral glucose tolerance test. This rigorous diagnostic approach ensured a clear and standardized identification of GDM cases and the selection of suitable controls for comparison. 62 patients diagnosed with gestational diabetes mellitus (GDM) underwent various treatments aimed at maintaining blood glucose levels within target ranges. The defined targets were set to be 105 mg/dl or lower before meals and 120 mg/dl or lower 2 hours after meals, with the goal of managing elevated fasting plasma glucose values (>95 mg/dl) and/or elevated

postprandial values (above 120 mg/dl for 2 hours or 140 mg/dl for 1 hour) (9, 10). If blood glucose levels remained above the specified targets despite dietary intervention, insulin therapy was implemented. The patients were categorized into three groups: Group 1 comprised 22 non-diabetic pregnancies serving as the control group, Group 2 included 28 pregnant women with GDM who underwent controlled dietary intervention, and Group 3 consisted of 34 pregnant women with GDM who received insulin therapy. In the context of this case-control study, a total of 84 pregnant women were included, with 62 in the GDM group and 22 healthy, non-diabetic women serving as controls. This comprehensive approach allowed for a thorough investigation into the impact of different treatments on blood glucose levels and their implications for both GDM and non-diabetic pregnancies. For all participants, detailed information including age, gravidity, and parity was recorded. Additionally, body mass index (BMI) was calculated before delivery as the ratio of weight divided by height squared (kg/m2). Exclusion criteria for both groups comprised multiple pregnancies, infections, chronic illnesses, smoking habits, women younger than 18 or older than 40 years, known clotting disorders, uterine or cervical abnormalities, placenta previa and placental abruption, patients with a history of previous premature delivery, and those with severe maternal diseases such as HELLP syndrome, preeclampsia, and severe chronic diseases. The inclusion of these criteria aimed to eliminate potential confounding factors and ensure a more homogeneous study population, given that these conditions are recognized risk factors in themselves for early term births (between 37 0/7 and 38 6/7 weeks of gestation). Maternal venous blood samples were obtained in the fasting state before Caesarean section, with all subjects receiving lumbar epidural analgesia for pain relief during the procedure. Simultaneously, umbilical cord blood samples were collected at the time of the Caesarean section. These blood samples were drawn into tubes and promptly centrifuged after clotting. The resulting supernatant serum was frozen at -80°C until the time of assay. The analysis of serum Hsp70 was conducted using a commercial ELISA kit from Eastbiopharm, China, with measurements performed by an ELISA reader from Rayto, India. To ensure the reliability of the results, the intra- and inter-assay coefficients of variations for Hsp70 were maintained at levels below 10% and 15%, respectively. The detection range for Hsp70 in the kit spanned from 2 to 600 ng/mL, providing a robust framework for the quantitative analysis of Hsp70 levels in the collected serum samples.

Statistical Analysis

Data analysis was conducted using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). The normality of continuous variable distributions was assessed using the Kolmogorov-Smirnov test, and the homogeneity of variances was evaluated using the Levene test. Descriptive statistics were presented as mean ± SD or median (min-max), as appropriate. For comparisons between the groups (GDM and non-diabetic group), the Independent Samples t-test and Mann–Whitney U test were employed for parametric and non-parametric numeric data, respectively. To assess mean differences among the groups (Group 1, 2, 3), One-Way ANOVA was utilized for parametric numeric data while the Kruskal-Wallis test was applied for non-parametric numeric data. In instances where the p value from the Kruskal-Wallis test statistics was statistically significant, Conover's non-parametric multiple comparison test was used to determine specific group differences. The association between continuous variables was evaluated using Spearman's Rank Correlation analyses. Nominal data were analyzed using Pearson's chi-square, Fisher's exact, or Likelihood Ratio tests, as appropriate. A p value less than 0.05 was considered statistically significant.

Results

The study encompassed 62 patients diagnosed with gestational diabetes mellitus (GDM) (GDM group), alongside 22 non-diabetic individuals. Within the GDM group, 28 received treatment with diet alone (Group 2), while 34 underwent insulin therapy (Group 3). The non-diabetic control group (Group 1) comprised 22 pregnancies. The demographic and clinical characteristics of women in the GDM and control groups are presented in Table I.

The mean maternal serum Hsp70 level in the GDM group (Group 2 + Group 3) was significantly higher than that of the non-diabetic control group, with values of 220.5 ± 96.9 ng/ml versus 166.1 ± 36.2 ng/ml, respectively (p<0.001). In contrast, the cord Hsp70 level in the GDM group (Group 2 + Group 3) was slightly elevated compared to the non-diabetic control group, but this difference did not reach statistical significance (123.2 ± 113.5 ng/ml versus 106.6 ± 71.7 ng/ml, p=0.4) (Figure 1). No significant differences were observed between the groups in terms of age, time of delivery, BMI, birth weight, maternal height, and weight (p>0.05). However, a noteworthy finding was the significantly higher incidence of a family history of gestational diabetes among women in the GDM group compared to the control group (p<0.001).

Table 1. Demographic, clinical characteristics of women with GDM and controls according to groups

	Group I (n=22) Controls	Group II (n=28) Diet	Group III (n=34) İnsulin	p-values
Age (year)	31.4±4.9	34.1±6.1	33.3±6.0	0.265
Maternal height(cm)	159.9±4.9	162.6±8.2	159.8±4.5	0.154
Maternal weight (kg)	76.3±8.8	81.6±10.5	81.7±11.1	0.121
BMI (kg/m^2)	29.9±3.5	31.0±4.2	32.0±4.2	0.172
Gestational age at birth (week)	38.6 (38.0-39.3)	38.7 (38.0-39.5)	38.4 (38.0-39.5)	0.707
Family history GDM	3 (%13.6)[a,b]	16 (%57.1)[a]	22 (%64.7)[b]	<0.001
Birth weight (g)	3237.5 (2825-3950)	3140 (2700-5100)	3150 (2700-4380)	0.785

BMI, body mass index.

[a]Significantly different from the Group 1 and Group 2 (p<0,05)

[b]Significantly different from the Group 1 and Group 3 (p<0,01)

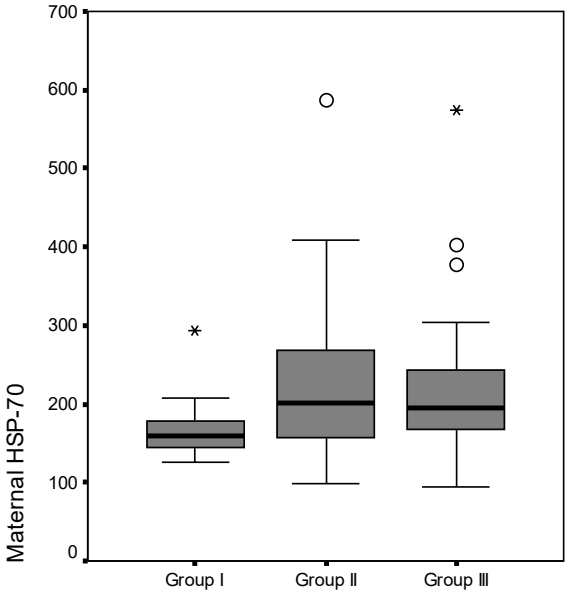

Figure 1. Maternal Hsp70 levels in women with GDM and controls according to groups.

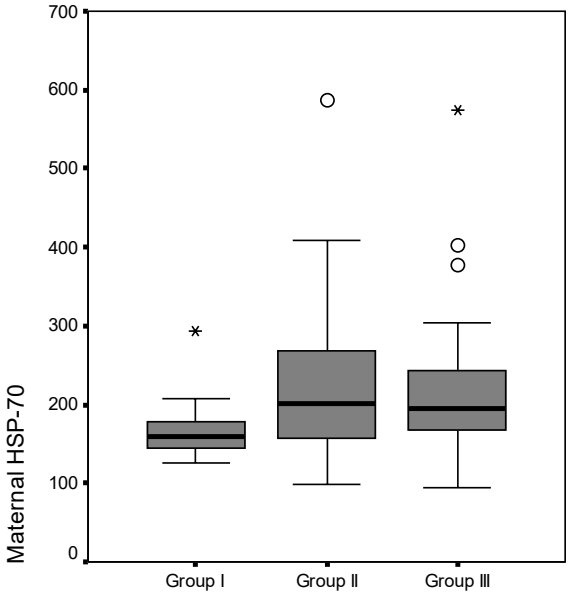

Figure 2. Maternal Hsp70 levels in women with GDM (diet and insulin group) and controls according to groups

Analyzing maternal Hsp70 levels across groups, the graphical representation uses box plots. The horizontal line within each box denotes the median, with the upper and lower boundaries indicating the 25th and 75th percentiles, respectively. The whiskers extending above and below the box represent the maximum and minimum Hsp70 levels. Open circles are used to denote outliers while asterisks specifically highlight extreme cases. This visual representation offers a clear and concise overview of the distribution and central tendency of maternal Hsp70 levels within and across the examined groups.

Maternal serum Hsp70 levels were significantly higher in group 2 than group 1 controls (201.5 [99-587] ng/ml versus 159 [126-293] ng/ml, (p=0.002). Serum Hsp70 levels were also found significantly higher in group 3 than group 1 controls (195.5 [(94-574)] ng/ml versus 159 [126-293] ng/ml, (p=0.002) (Figure 2). No significant differences were found between group 2 and group 3 in Hsp70 levels (p=0.9). Hsp70 levels in cord were not significantly different among groups. Table 2 shows maternal and cord Hsp70 levels of women with GDM (group 2, group 3) and controls according to groups.

The comparison of maternal Hsp70 levels among groups is depicted using box plots. Within each box, the horizontal line represents the median, while the upper and lower borders indicate the 25th and 75th percentiles, respectively. The whiskers extending above and below the box mark the maximum and minimum Hsp70 levels. Open circles on the plot are used to identify outliers, and asterisks highlight extreme cases. This graphical representation provides a visual summary of the central tendency, spread and presence of extreme values in maternal Hsp70 levels across the examined groups.

Table 2. Maternal and cord Hsp70 levels of women with GDM (diet and insulin group) and controls according to groups

	Group I Controls	Group II Diet	Group III İnsulin	p-value
Serum Hsp70 (ng/ml)	159 (126-293) [a,b]	201.5 (99-587)[a]	195.5 (94-574)[b]	0.008
Cord Hsp70 (ng/ml)	107.5 (5-234)	111 (5-541)	121.5 (2-389)	0.557

[a]Significantly different from the Group 1 and Group 2 (p=0,002)
[b]Significantly different from the Group 1 and Group 3 (p=0,002)

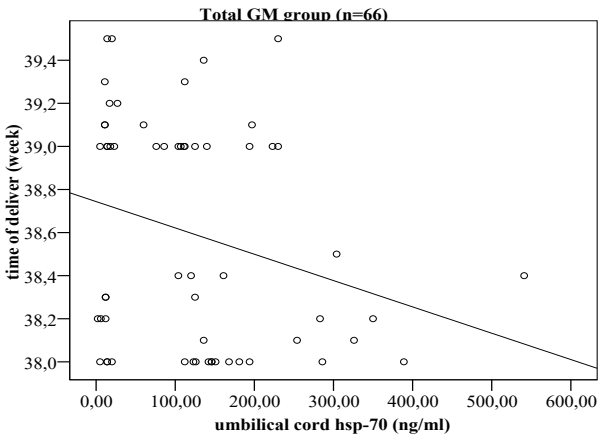

Figure 3. Cord Hsp70 levels showed a negatively significant correlation with time of delivery, (r: - 0.266, p=0.04) in women with total GDM group (diet with insulin; n=66).

Cord Hsp70 levels showed a negatively significant correlation with time of delivery, (Figure 3; r: - 0.266, p=0.04) in women with GDM group (group 2 + group 3). Cord Hsp70 levels also showed a negatively significant correlation with time of delivery, (Figure 4; r: - 0.25, p=0.02) in whole group. No correlations were found between serum and cord Hsp70 levels with age, BMI, birth weight (Table 3).

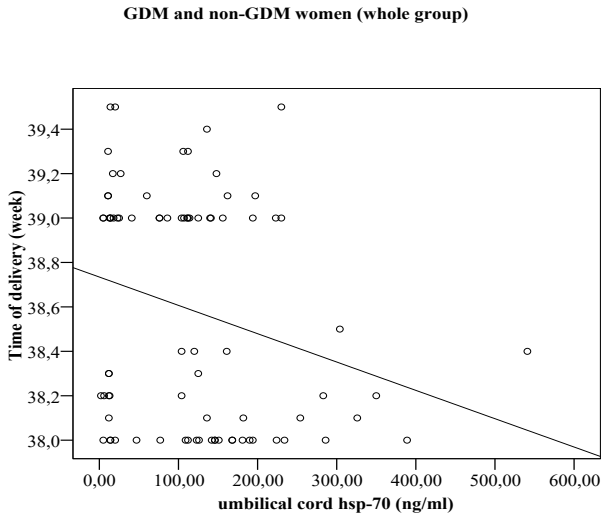

Figure 4. Cord Hsp70 levels showed a negatively significant correlation with time of delivery, (r: - 0.25, p=0.02) in whole group (n=84).

Table 3. Correlations between maternal and cord HSP70 levels and all the other parameters in whole groups

	Serum HSP70		Cord HSP70	
	r	p-values	r	p-values
Age (year)	-0.046	0.676	0.019	0.867
BMI (kg/m²)	0.120	0.278	0.158	0.160
Gestational age at birth	0.047	0.674	-0.226	0.042
Birth weight (g)	0.037	0.738	0.017	0.879
Fasting glucose (mg/dl)	0.066	0.653	0.026	0.861

Discussion

This study represents a pioneering effort, being the first to concurrently assess Hsp70 levels in both maternal and fetal compartments in the context of gestational diabetes mellitus (GDM). Our results, obtained through a meticulously matched case–control design, reveal that individuals with GDM exhibit elevated serum Hsp70 levels in comparison to those without GDM. Although umbilical cord Hsp70 levels were also higher in the GDM group, the difference did not reach statistical significance. Notably, a significant correlation was identified between umbilical cord Hsp70 levels and the time of delivery, both in women with GDM and in the entire study group. These findings shed light on the potential role of Hsp70 in GDM and its association with the timing of delivery, providing valuable insights for further exploration and understanding of the underlying mechanisms.

The molecular mechanisms implicated in the effects of hyperglycemia on inflammation and vascular complications are believed to involve the action of reactive oxygen species within the cell nucleus (11). Our study, focusing on the increase in circulating Hsp concentrations in gestational diabetes mellitus (GDM), was driven by the hypothesis that GDM might trigger a heat shock response. Our results find support from existing studies on type 2 diabetes. For instance, a previous investigation revealed higher serum Hsp70 levels in non-insulin-treated type 2 diabetes subjects compared to their insulin-treated counterparts (12). Another cross-sectional study demonstrated increased levels of Hsp70 in mononuclear cells of type 2 diabetic patients in comparison to normal subjects (13). Reports have also highlighted elevated serum Hsp70 levels in type-1 diabetic patients (14). While previous studies on pregnant patients yielded similar results, our larger-scale research further supports these findings with a more comprehensive examination involving a larger number of participants (4).

Contrastingly, some studies have reported reduced systemic Hsp70 expression in association with elevated glucose levels in non-human primates (5). In our study, participants were categorized into two groups based on treatment models. Among women with GDM (n=62), 28 were treated with diet alone (Group 2), and 34 underwent insulin therapy (Group 3). Interestingly, we observed no significant differences between Group 2 and Group 3 in Hsp70 levels. We hypothesize that the duration of glucose excursion may hold greater significance in influencing Hsp70 levels than the specific treatment model involving insulin or diet. This apparent discrepancy warrants further investigation for a comprehensive understanding of the relationship between treatment modalities, glucose levels and Hsp70 expression.

Studies focused on type 2 diabetes mellitus (DM) have played a crucial role in unveiling the functions of Hsp70 in diabetes. However, there is a notable scarcity of data concerning gestational diabetes mellitus (GDM). In a study by Katarzyna et al., it was reported that levels of Hsp70 in women with pre-pregnancy diabetes were significantly higher than those in women with GDM. These findings suggest a cellular adaptive response to oxidative stress associated with hyperglycemia, potentially linked to the elevated serum Hsp70 levels observed in non-pregnant individuals with diabetes. This strongly implies that chronic hyperglycemia over an extended period may contribute to the observed increase in serum Hsp70 levels in GDM. This underscores the importance of considering the duration and intensity of hyperglycemia in understanding the dynamics of Hsp70 levels in different forms of diabetes. (15).

Increased levels of Hsp70 expression have been detected in the placenta of women underwent conditions such as pre-eclampsia, placental vascular diseases, and other pathological pregnancies. Similarly, heightened expression of cellular stress markers has been noted in the placenta of women

with cardiovascular diseases (CVD). Earlier research has acknowledged Hsp70 as a notable angiogenic factor within the orchestrated response mechanisms in maternofetal tissues. Consequently, there appears to be a reported elevation in both angiogenesis and lymphangiogenesis in the placenta of women with CVD. These findings suggest a potential role for Hsp70 and cellular stress markers in the pathophysiology of pregnancy-related complications and cardiovascular diseases, emphasizing the intricate interplay between stress responses and vascular health during pregnancy. (16). Also, our data presented in this study demonstrate that there is a differential release of Hsp70 from umbilical cord and maternal serum obtained from normal pregnant women and women with GDM. Although no difference in Hsp70 release was observed between GDM group and non-diabetic control group in umbilical cord, it was higher in GDM group. Why Hsp70 is not increased significantly in umbilical cord is exactly remains unknown. The primary rationale for the homogeneity of our sample size can be attributed to the stringent inclusion criteria applied, coupled with the lack of significant differences in obstetric history and smoking habits among the participants. The exclusion of severe maternal diseases, such as preeclampsia and placenta previa, which are recognized risk factors for early term birth, further contributed to the uniformity of the sample. This methodological approach aimed to minimize potential confounding variables, allowing for a more focused examination of the specific factors under investigation and enhancing the internal validity of the study. Secondary explanation may be that prolonged exposure to high glucose levels may be necessary to impair Hsp responses in fetus as mentioned previous studies (17). Nakhjavani M et al. (5) showed that the acute response of serum Hsp70 levels to hyperglycemia was the indicator of average long-term serum glucose level. Therefore, it may be explained by this hypothesis that, GDM patients were much more meet with high glucose levels so that serum levels of Hsp70 were significantly higher in GDM patients than in healthy pregnant women.

Our findings of a negative association between umbilical cord Hsp70 concentrations and early term birth in pregnancies corroborate with previous studies. Increased concentrations of Hsp70 in mononuclear cells of peripheral blood obtained from women in early pregnancy were associated with subsequent miscarriages, stillbirths, and preterm births (18). The work by Ziegert M et al. has outlined the prognostic and diagnostic significance associated with the presence of Hsp60 and Hsp70 antibody complexes in the placenta, as well as the detection of antibodies to Hsp60 and Hsp70 in the blood. However, it's important to note that findings across studies are not entirely consistent. Some investigations exploring the connection between early term delivery and Hsp expression have reported no significant difference in the levels of Hsp in the placenta between full-term delivery and preterm delivery. The variability in results underscores the complexity of the relationship between heat shock proteins and the timing of delivery, indicating the need for further research to better understand these associations (19-21).

As previously noted, a strong correlation has been established between umbilical cord Hsp70 levels and early term delivery. However, it is crucial to emphasize that, as of now, maternal Hsp70 alone is not adequate for predicting early term delivery. Consequently, our ongoing efforts involve the evaluation of the potential to enhance predictive accuracy for early term delivery or assess therapeutic effects by incorporating Hsp70 with other indicators. This comprehensive approach aims to explore whether a combination of factors can provide a more precise understanding of early term delivery risks and treatment outcomes, reflecting the multifaceted nature of the underlying physiological processes. There are several limitations to our study that should be acknowledged. Firstly, the absence of data in large groups of diabetes patients limits the generalizability of our findings. This study can be considered a reasonable starting point for exploring the role of Hsp70 in gestational diabetes mellitus (GDM). Additionally, the lack of assessments such as malondialdehyde (MDA) or cytosolic reactive oxygen species (ROS) production in participants is a notable limitation, as correlating these markers with Hsp70 in women with GDM could provide valuable insights into the underlying mechanisms. This represents one of the most significant limitations of the study. Moreover, our patient cohort was drawn from a representative sample of individuals with GDM in our clinic, which may impact the generalizability of our results. Despite these limitations, our study serves as an initial exploration, and future research with larger and more diverse participant groups is warranted to further elucidate the role of Hsp70 in GDM.

The data presented in this study indicate a distinct variance in Hsp70 levels between normal pregnant women and those with gestational diabetes mellitus (GDM). Although umbilical cord Hsp70 levels were higher in the GDM group, this difference did not reach statistical significance. However, a significant correlation was identified between umbilical cord Hsp70 levels and the time of delivery in women with GDM and the overall study group.

Conclusion

Considering that many causes of early term birth remain unexplained and unknown, future studies are essential for elucidating the roles of both Hsp70 and oxidative stress in the risk of early term delivery in pregnant women. The findings from this study provide a foundation for further exploration and emphasize the need for comprehensive investigations into the complex interplay of various factors influencing the timing of delivery in the context of GDM.

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