Original Article

The relationship between stress during pregnancy with leptin and cortisol blood levels and complications of pregnancy in mother

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Abstract:
Objective: pregnancy is one of the most stressful periods a woman experiences in her life. The present study was an attempt to determine the relationship between maternal stress during pregnancy and cortisol plus maternal serum leptin levels as well as pregnancy outcomes.
Material and Methods: This longitudinal study was conducted on 90 pregnant women in Miandoab city in 2015-2016. The samples were chosen from mothers with a gestational age of 24 to 28 weeks. The participants were asked to fill out Cohen's Perceived Stress Scale (PSS) and Demographic questionnaire and blood samples taken from them. The mothers were then tracked with four-week intervals until the time of delivery and were asked to fill out Cohen's perceived stress scale each time as along with a questionnaire related to maternal outcome. Again, a blood sample was taken at the time of delivery. Data analysis was performed using SPSS 16. Descriptive statistics, Pearson correlation coefficient, and t-test were employed for analysis.
Results: A significant relationship was found between maternal stress and preeclampsia (p=0.008). The relationships between preterm childbirth and maternal cortisol levels in 24-28 weeks (p=0.015), and between preterm child birth and maternal leptin levels at delivery time (p=0.007) were also found to be significant.
Conclusion: Pregnancy and labor, as physically and mentally stressful events, can affect women's physiological and psychological indicators. As a consequence, during pregnancy, the cortisol and leptin index changes in response to the activity of hypothalamic-pituitary axis and autonomic nervous system under stress.
Keywords: Stress, Cortisol, Leptin, Pregnancy Complications

Introduction

Pregnancy is associated with significant physiological and psychological changes which sometimes lead to pathological changes (1). Stressful situations can have adverse effects on women's psychological status. Indeed, stress is the most obvious symptom in pregnant women's behaviors and clinical symptoms (2). For instance, the prevalence of pregnancy stress in England and Sweden was reported as 33-37% and 5-7% respectively (3). Evidence suggests that mothers' reaction to stress changes during pregnancy (4), which is directly correlated with pregnancy outcomes (5, 6). Mothers' stress also predicts delivery problems and complications (7). A pregnant woman's stress affects her and leads to negative perceptions towards delivery and birth, unnecessary fear of childbirth and motherhood, self-medication with alcohol, and activity restriction (8). Studies suggest that preterm birth and gestational hypertension are more likely to occur in mothers with stressful pregnancies (9, 10). Pregnancy stress increases the chance of unplanned cesarean and labor complications (11, 12). To measure the amount of stress during pregnancy, different methods such questionnaires (13) or measurement of biochemical markers, which are indicators of stress (14), are used. Today, cortisol and other hormones produced by the sympathetic nervous system activity, which can be measured from the blood sample, are measured as the indicators of stress (14). Cortisol, which is called the stress hormone, is considered as a decisive indicator in stressful situations (15, 16). Hypothalamic-pituitary-adrenal (HPA) axis is activated in response to stress and releases cortisol in the organism (17). The level of cortisol is a function of actual and perceived stress (18). In a study, Edwards et al. examined the effects of stress during pregnancy on changes in hormonal and behavioral responses affecting serum levels of cortisol and leptin. They found that adipose tissue had an effect on the response to stress with secretion of leptin (19). Glucocorticoids and probably adrenocorticotropic hormone (ACTH) stimulate synthesis and secretion of leptin (20). A number of studies have investigated the relationship between maternal stress and levels of cortisol, and have obtained contradictory results. On the other hand, no studies were found to have only investigated the relationship between perceived maternal stress during

pregnancy plus labor and leptin levels. Accordingly, the present study examines the relationship between stress during pregnancy and leptin plus cortisol levels as well as Complications of pregnancy in pregnant women.

Materials and Methods

The present study is a longitudinal study conducted between November 2015 and the end of April 2016 in Miandoab city. The study population consisted of pregnant women of 24 to 28 weeks. The samples were chosen through available sampling. The sample size was calculated as 90 participants using the correlation coefficient formula a d based on the study conducted by Salari and et al. (21) and considering the correlation coefficient between stress and cortisol level (0.312), confidence level of 5%, and statistical power of 80%. The criterion for inclusion in the study was not having any underlying disease during and before pregnancy. The required information was collected from the reference laboratory where all pregnant women of the city referred to for routine check-ups within weeks 24-28 of pregnancy. The reference laboratory was selected so that the samples would have the same conditions. Since this laboratory was public, it had lower costs and all pregnant women from all over the town (including villages) referred to this center. The objectives and method of study were explained to the participants and their written consents were obtained. Cohen's perceived stress scale (PSS) and a questionnaire developed by the researcher about the demographics of the participants were filled out by the participants. Also, mothers' blood samples taken for routine pregnancy tests in 24-28 weeks of pregnancy were used to test the leptin and cortisol levels of mothers' blood. Subsequent follow-up of the mothers in the study was performed three times with a 4-week interval in 28-32 weeks and 34-36 weeks and at the time of delivery. The first and second follow-ups were performed by telephone with PSS filled out by the participants; on the other hand, for the third follow-up, they were asked to call the researcher, via the number they were provided with, in case they went to the delivery room and had their labor. Also, according to the first day of the last period, the delivery time was estimated and the researcher was present in the delivery room at the time that was determined for the delivery of the participants, even if the participants did not call the researcher. Thus, the third follow-up was performed through presence in the delivery room. At the time of labor, again PSS and a questionnaire related to maternal outcome were filled out. In this stage, the mothers' blood samples that were taken as a routine at the time of reception were used to test the leptin and cortisol levels of mothers' blood. Due to the circadian rhythm of cortisol and in order to minimize the impact of this rhythm on cortisol concentrations, the time of maternal blood sampling in the first stage was within 8-10 A.M, while in the second stage it depended on the time of delivery. The blood samples were centrifuged for 10 minutes at 2500 rpm and their serum samples were separated. Serum samples were placed in closed and coded Eppendorf tubes and were kept in -20℃ in the laboratory. All of the samples were analyzed under the same conditions (environmental, time, place, and analyst). In order to measure leptin and cortisol levels, Bio Vendor kit with sensitivity of 0.2 ng/ml and specificity of 100% for leptin hormone, and DiaMetra kit with sensitivity of 2.44 ng/ml and specificity of 100% for cortisol hormone were used respectively.

Tools

1. Cohen's Perceived Stress Scale (PSS): it includes 14 expressions investigating the participants' feelings and thoughts as well as their general perceived stress during the last month. The 14-item questionnaire including seven negative items and seven positive items was used here. The items were rated on a 5-point Likert scale ranging from "Almost Never" to "Almost Always". Items number 4, 5, 6, 7, 9, 10, and 13 were reverse coded. The lowest and highest scores were zero and 56, respectively (13, 22). Cronbach's alpha for American and Iranian populations was found by Ghorbani et al. as 0.86 and 0.81, respectively (23). The questionnaire's construct validity was established at 0.63 and was significant at $p<0.05$ (24).
2. Demographic questionnaire: this questionnaire was developed by the researcher and had three parts: demographic information (age, address, pregnant women and their husbands' level of education, and job), information about fertility (gravidity, type of delivery, parity, abortion, the pregnancy planning, neonate's gender, desirability of the neonate's gender, mother's interest in pregnancy), and maternal outcomes (preeclampsia, preterm delivery, dystocia, excessive bleeding in childbirth, and spotting during pregnancy).

Data analysis

Data analysis was done using SPSS 16. Further, the relationship between factors ($p<0.05$) was determined using Pearson correlation coefficient and t-test.

Results

In the present study the average age of mothers was 27.48 years and their age ranged from 15 to 40 years. General and reproductive characteristics of the sample are displayed in table 1.

Some of the psychological issues and problems were also investigated in this study: 86.7% had planned pregnancy, the pregnancy in approximately 91.1% of the mothers was intended, 86.7% were happy with their child's gender, and 91.1% of them were interested in their pregnancy.

Maternal perceived stress scores at different time point of pregnancy, as well as cortisol and leptin levels(ng/ml) in mother are shows in table 2.

According to table 3, there was no significant relationship between maternal perceived stress during pregnancy and the time of deliver the two conditions (yes or no) relate to preterm birth, dystocia, spotting during pregnancy

and bleeding at time delivery. Although there was no significant difference between yes or no conditions, the PSS was higher in yes state almost in all cases. there was significant relationship between PSS at the time of delivery and preeclampsia (p=0.028).
Cortisol levels at the delivery were significantly higher with score of perceived stress in 24-28 weeks (p= 0.019, r= 0.246) and total stress score (p=0.046, r=0.211) and there were non –significant correlation between maternal cortisol levels and maternal leptin levels and score of perceived stress in pregnancy and delivery time.
At the time of delivery, the leptin levels showed a significant and negative relationship with preterm childbirth (p= 0.007), i.e. the leptin levels were lower in preterm childbirth. However, the relationship between preterm child birth and cortisol level in weeks 24-28 was significant and positive (p= 0.015). but there weren't significant between maternal leptin and cortisol level and another complications of pregnancy in mother.

Discussion

The findings revealed a significant relationship between perceived maternal stress at the time of delivery and preeclampsia. Shamsi et al. evaluated the risk factors of preeclampsia in Pakistani women (25) and reported a higher level of stress in women with preeclampsia. In another study, it was found that stress had a significant relationship with preeclampsia. Further, stress had a significant relationship with the severity and worsening of preeclampsia (26). This is also confirmed by the findings of Black who suggested that women with sever preeclampsia had higher stress levels compared to those with a mild preeclampsia (27). The findings of the present study in this area agree with those of the previous research.
A significant relationship was found here between cortisol level at the time of delivery and perceived stress score. Similarly, the relationship between perceived stress score and cortisol level in late pregnancy was found to be significant in another study, though no significant relationship was established between cortisol level and early pregnancy (28). On the other hand, the findings revealed no significant relationship between the perceived stress score during pregnancy and plasma cortisol levels (29). A significant relationship was reported between severe stress based on visual analogue scale at the time of labor and salivary cortisol (21). In addition, Plus et al. reported a significant relationship between mothers' state anxiety and salivary cortisol levels in early and late pregnancy (30). The findings of our study are in agreement with most previous studies in this regard. The difference between our findings and some of the other studies can be attributed to the use of different instruments to measure stress levels during pregnancy.
We found a significant relationship between preterm childbirth and maternal plasma cortisol level in weeks 24-28 of pregnancy. Previous studies have suggested that the mean concentrations of maternal plasma cortisol in women with preterm labor are higher compared to their counterparts. It indicates that cortisol plays a significant role in the mechanism of preterm labor in some women (31). In another study, plasma cortisol levels in women giving preterm birth were found higher than in cases of normal delivery, implying the importance of maternal hypercortisolemia in preterm labor (32). According to these findings, the risk of preterm delivery grows by high blood cortisol level (31, 33, 34).
In the present study, a negative significant relationship was observed between preterm childbirth and maternal plasma leptin level at the time of delivery. The mechanism of leptin in preterm birth generally unknown in the literature. Some studies claim that increased level of leptin in preterm delivery is closely linked to antenatal exposure to corticosteroids (35).
The literature suggests that the risk of preterm labor before week-34 of gestation decreases by a higher level of leptin. Wuntakal et al. reported that induced myometrium contraction is determined by availability of leptin and may prove helpful in preventing preterm birth (36). In a study on 1304 pregnant women in weeks 16–27 of gestation, Shroff et al. showed that the level of maternal leptin was higher in women who delivered at term than in those with premature delivery. The difference was still observed after controlling for diabetes, blood pressure disorders, and pre-pregnancy BMI (37). In the same vein, Palchevska et al. studied 110 neonates and found that that leptin levels were higher for term infants (37). This was further confirmed in the study by Laivuori et al. (38).
Limitations: Environmental conditions and circadian rhythm affect cortisol levels. This was controlled, as much as possible, by taking samples in the morning in weeks 24-28 of pregnancy. However, at the time of delivery, due to its unexpected and emergency nature, it was beyond the researcher's controllability to control this condition.
Conclusion: The present study found a significant relationship between preeclampsia and average stress score in pregnancy. In addition, there was a significant relationship between leptin and cortisol levels in maternal serum and preterm childbirth. These findings indicate the negative and undesirable impact of stress on pregnancy outcomes. Other studies can be conducted to discover the possibility of predicting the pregnancy outcomes by measuring the cortisol and leptin levels in blood serums in other stages of pregnancy. Note that in order to draw safer conclusions, there is a need for more longitudinal studies with larger samples.
Ethical Issues: Information about the participants remained confidential thought the study and the results were disseminated collectively.

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Table 1: General and reproductive characteristics of the sample		
Variable		Frequencies(percent)
Locality	City	35(38.9)
	Village	55(61.1)
Mother's education	< High school	37(41.1)
	High school graduate	41(45.6)
	College graduate	12(13.3)
Father's education	< High school	35(38.9)
	High school graduate	46(51.1)
	College graduate	9(10)
Mother's job	Housewife	87(96.7)
	Employed	3(3.3)
Father's job	Unemployed	7(7.8)
	Employed	83(92.2)
Gravida	First	24(26.7)
	Second	45(50)
	Third or more	21(23.4)
Type of delivery	NVD	61(67.8)
	C/S	29(32.2)
Parity	Non	28(31.1)
	1	48(52.3
	2 or more	14(15.5)
Miscarriage	1	13(14.4)
	2 or more	3(3.3)
Sex of the fetus	Boy	54(60)
	Girl	36(40)

Table 2: Maternal perceived stress score and cortisol and leptin levels (ng/ml) of the sample (Mean ± SD)				
Time	**24-28 week Mean ± SD**	**28-32 week Mean ± SD**	**32-36 week Mean ± SD**	**Delivery Time Mean ± SD**
Mean score of perceived stress	25.54±4.33	24.50±4.35	26.01±4.34	31.35±5.04
Level Cortisol (ng/ml)	295.44±9.16	N/A	N/A	298.38±16.62
Level leptin(ng/ml)	34.77±12.24	N/A	N/A	29.94±12.68

Table 3: The Relationship Between Perceived Stress Score's Mother and Complications of Pregnancy (pvalue)				
	24-28	**28-32**	**32-36**	**Delivery time**
Preeclampsia	0.799	0.727	0.722	0.028*
preterm birth	0.165	0.198	0.295	0.249
Dystocia	0.427	0.346	0.525	0.727
spotting during pregnancy	0.358	0.221	0.283	0.968
Bleeding in delivery	0.380	0.271	0.806	0.238
The mean of groups were compared by student *t*-test. Statistically, P<0.05 was significant				

Table 4: The relationship between maternal perceived stress and cortisol and leptin levels of mother					
	24-28	**28-32**	**32-36**	**delivery time**	**Total stress score**
Cortisol (24-28 weeks)	P= 0.619 r= 0.053	P= 0.855 r= 0.020	P= 0.368 r= -0.096	P= 0.623 r= -0.052	P= 0.755 r= -0.033
Leptin(24-28 weeks)	P= 0.229 r= -0.128	P= 0.608 r= -0.055	P= 0.496 r= -0.073	P= 0.899 r= -.014	P=0.314 r= -0.107
Cortisol (delivery time)	P= 0.019* r= 0.246	P= 0.141 r= 0.157	P= 0.073 r= 0.190	P= 0.626 r= -0.052	P= 0.046* r= 0.211
Leptin (delivery time)	P= 0.771 r= -0.031	P= 0.564 r= 0.062	P= 0.477 r= 0.076	P= 0.213 r= -0.133	P= 0.861 r= -0.019
Pearson test was used .Statistically, P<0.05 was significant					

Table 5: The relationship between cortisol and leptin maternal whit Complications of Pregnancy (pvalue)				
	Cortisol(24-28 weeks)	**leptin (24-28 weeks)**	**Cortisol (delivery time)**	**Leptin (delivery time)**
Preeclampsia	0.530	0.056	0.454	0.418
preterm birth	0.015*	0.912	0.676	0.007*
Dystocia	0.737	0.318	0.511	0.873
spotting during pregnancy	0.597	0.614	0.622	0.441
Bleeding in delivery	0.675	0.420	0.209	0.904
The mean of groups were compared by student *t*-test. Statistically, P<0.05 was significant				