

Original Article

# A Comparative Study of Stress Levels, Serum Cortisol, and Thyroid Hormones in Pregnant vs. Non-Pregnant Women

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Stress Levels,  
Serum  
Cortisol, and  
Thyroid  
Hormones in  
Pregnant vs.  
Non-  
Pregnant

## ABSTRACT

**Objective:** To compare the levels of perceived stress, serum cortisol, and thyroid hormones (TSH, T3, and T4) in pregnant and non-pregnant women in a Pakistani population.

**Study Design:** A case control comparative study

**Place and Duration of Study:** This study was conducted at the Department of Physiology, Khyber Medical University, Peshawar from February to July 2024.

**Methods:** The Perceived Stress Scale (PSS) was used to measure stress levels. Blood samples were collected to analyze cortisol, TSH, T3, and T4 levels. Independent t-tests were used to compare the groups. Independent samples t-tests were performed to compare the mean differences in hormonal levels and PSS scores between the two groups. A p-value of <0.05 was considered statistically significant.

**Results:** Pregnant women exhibited significantly elevated cortisol levels ( $p = 0.001$ ) and higher PSS scores ( $p = 0.007$ ) compared to non-pregnant women. No significant differences were observed in TSH levels ( $p = 0.2$ ), while T4 levels were significantly higher in pregnant women ( $p = 0.002$ ). T3 levels showed no significant differences ( $p = 0.144$ ).

**Conclusion:** In pregnant women experience higher perceived stress and elevated cortisol levels. The increased T4 levels in pregnant women point out physiological changes related to pregnancy. These findings emphasized the need for stress management and hormonal monitoring in prenatal care.

**Key Words:** Stress Levels, Serum Cortisol, Thyroid Hormones, Pregnant vs. Non-Pregnant Women

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## INTRODUCTION

Pregnancy in woman's life, related with various physiological, psychological, and hormonal changes<sup>(1)</sup>. These changes have significant impact for maternal well-being and fetal development<sup>(2)</sup>. Particularly, stress during pregnancy can aggravate the hormonal fluctuations, effecting the health of mother and the unborn child<sup>(3)</sup>.

Globally, including in Pakistan, maternal stress has been identified as a important factor in adverse

pregnancy outcomes, preterm labor, miscarriages, underweight child, and developmental delays in infants<sup>(4)</sup>. A study Pakistani study shown that high perceived stress during pregnancy was related with an higher risk of adverse perinatal outcomes, underscores the importance of stress management during pregnancy in Pakistani women.<sup>(5)</sup>

Cortisol known as a stress hormone, had important role in the body's response to stress and is regulated by the hypothalamic-pituitary-adrenal axis<sup>(6)</sup>. Elevated cortisol levels, during pregnancy have been linked with gestational diabetes, hypertension, and preterm birth<sup>(7)</sup>. Evidence about pregnant women suffering elevated levels of stress had significantly higher cortisol levels, that were correlate with hypertensive disorders of pregnancy was reported<sup>(8)</sup>. A study reported the importance of understanding hormonal regulation in pregnant women in Pakistan, where stress levels was effected by sociocultural and economic factors<sup>(9)</sup>.

Although cortisol, thyroid hormones play important role in pregnancy, regulating metabolism, energy production, and fetal development stages<sup>(10)</sup>. During pregnancy the thyroid dysfunction is related with miscarriage, preeclampsia, and preterm birth. Studies in Pakistan, reported thyroid dysfunction in pregnant women was growing concern, with subclinical

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hypothyroidism being prevalent<sup>(11)</sup>. A study reported 12% of pregnant women had abnormal thyroid function, linked with poor pregnancy outcomes<sup>(12)</sup>. This emphasizes the necessity to monitor thyroid hormone levels along stress-related hormones, cortisol in pregnant women.

Numerous studies have discovered the impact of stress on pregnancy outcomes, there is limited literature precisely comparing perceived stress, cortisol, and thyroid hormones between pregnant and non-pregnant women in Pakistan. Stress not only activates the release of cortisol but may also affect thyroid function, leading to hormonal imbalances that can have long-term 'effects on maternal and fetal health'. Considering relationship of both hormones and stress in Pakistani women can support in developing targeted interventions to improve pregnancy.

The objective was to compare the levels of perceived stress, serum cortisol, and thyroid hormones (TSH, T3, and T4) between pregnant and non-pregnant women. This aims that maternal stress being forecaster of adverse outcomes, and 'the prevalence of thyroid dysfunction among pregnant women, it is important to understand how these factors interlinked'. Our study findings could contribute to the expansion of interventions that address stress management and hormonal monitoring in pregnancy, ultimately leading to improved maternal and fetal health outcomes. This study reports the gap in understanding how perceived stress influences hormonal changes during pregnancy in Pakistani women.

## METHODS

The study was conducted Khyber Medical University, Peshawar over the six months from February to July 2024, the study design was case control comparative analysis. To compare stress levels, cortisol, and thyroid hormone between pregnant and non-pregnant women. Ethical approval was obtained before the commencement of the study, and all participants provided written informed consent. The study included a total of 60 participants, that were divided into 30 pregnant women in their 2<sup>nd</sup> trimester and 30 non-pregnant women as controls. A sample technique convenient was used. The sample size calculation of 60 was based on a power analysis, which point out that 30 participants per group would be sufficient to detect significant differences in cortisol and thyroid hormone levels with 80% power and a 5% level of significance. The inclusion criteria for both groups include all participants were aged between 18 to 45 years. Pregnant women were recruited from the obstetrics department in their 2<sup>nd</sup> trimester, while non-pregnant women were selected from the outpatient department, ensuring they had not been pregnant in the past year. Women with chronic conditions, diabetes, thyroid disease, or those on medications affecting cortisol or

thyroid function were excluded to avoid confounding variables.

After obtaining informed consent, demographic data, including age, height, weight, and BMI, were collected. Blood pressure and pulse rates were recorded using standard clinical procedures. Blood samples were drawn from all participants to measure serum cortisol, TSH, T3, and T4 levels. Hormonal analysis was conducted in a certified laboratory using enzyme-linked immunosorbent assays (ELISA). Stress levels were assessed using the Perceived Stress Scale (PSS), a validated questionnaire designed to measure the degree of perceived stress.

Statistical analyses were performed using SPSS version 22.0. Descriptive statistics were calculated for all demographic and clinical variables and chi square test was applied. Independent 't-tests were used' to compare mean values of hormonal levels and PSS scores between pregnant and non-pregnant women. A p-value of < 0.05 was considered statistically significant. Levene's test was used to check for homogeneity of variances.

## RESULTS

The demographic characteristics of the participants were summarized in Table 1. No significant differences were observed in the age distribution between pregnant and non-pregnant women ( $p = 0.343$ ). However, height showed a significant difference ( $p = 0.007$ ), with a greater proportion of non-pregnant women being taller than 170 cm. This demographic comparison indicates that the two groups were generally well-matched in terms of age, but height varied significantly. although there was no significant difference in BMI between pregnant and non-pregnant women ( $p = 0.2$ ), a larger proportion of pregnant women had a normal BMI compared to non-pregnant women. This suggests that pregnant women may have been more closely monitored for healthy weight management, though the difference was not statistically significant.

**Table No. 1: Demographic Characteristics of Pregnant and Non-Pregnant Females**

Demographic Variable	Pregnant Females (n=30)	Non-Pregnant Females (n=30)	p-value
Age (years)			
18-24	16	14	0.343
25-34	11	11	
35-44	2	3	
45 and above	3	0	
Height (cm)			
Short < 160 cm	14	3	0.007
Average 160-170 cm	11	18	
Tall > 170 cm	5	9	
BMI (kg/m <sup>2</sup> )			
18.5-24.9 (Normal)	19	15	0.2
25-29.9 (Overweight)	11	15	

**Table No. 2: Clinical and Hormonal Variables of Pregnant and Non-Pregnant Females**

Variable	Pregnant Females (n=30)	Non-Pregnant Females (n=30)	p-value
Pulse (beats/min)			0.001
Normal 60-100 bpm	3	15	
Elevated > 100 bpm	27	15	
Systolic BP (mmHg)			0.014
Normal < 120	1	0	
Elevated 120-129	12	24	
Hypertensive stage 1 (130-139 mmHg)	11	3	
Hypertensive stage 2 ( $\geq 140$ mmHg)	6	3	
Diastolic BP (mmHg)			0.8
Normal < 80	9	7	
Elevated 80-90	16	15	
Hypertension Stage 1 (90-99 mmHg)	7	6	
Cortisol Level ( $\mu\text{g/dL}$ )			0.009
Normal (5-25 $\mu\text{g/dL}$ )	8	18	
Elevated (> 25 $\mu\text{g/dL}$ )	22	12	
TSH (mIU/L)			0.5
Normal (0.4-4.0 mIU/L)	22	24	
Elevated (> 4.0 mIU/L)	8	6	
T3 (ng/dL) and T4 ( $\mu\text{g/dL}$ )			0.053
Normal	21	27	
Abnormal	9	3	
PSS Score			0.039
Low Stress (0-13)	2	9	
Moderate Stress (14-26)	16	15	
High Stress (27-40)	12	6	

**Table No. 3: Comparison of Serum Cortisol Levels, Thyroid Hormones (TSH, T3, T4), and Perceived Stress Scores in Pregnant and Non-Pregnant Women (Descriptive Statistics and Independent Samples T-Test)**

Variable	Group	Mean ( $\pm$ SD)	t-value	p-value	Levene's Test p-value
Cortisol Level ( $\mu\text{g/dL}$ )	'Pregnant (n=30)'	20.93 $\pm$ 2.449	4.074	0.001	0.049
	'Non-Pregnant (n=30)'	17.800 $\pm$ 3.42			
TSH (mIU/L)	'Pregnant (n=30)'	3.306 $\pm$ 0.4741	1.242	0.2	0.02
	'Non-Pregnant (n=30)'	3.120 $\pm$ 0.672			
T3 (ng/dL)	'Pregnant (n=30)'	148.13 $\pm$ 8.88	1.483	0.144	0.001
	'Non-Pregnant (n=30)'	145.40 $\pm$ 4.796			
T4 ( $\mu\text{g/dL}$ )	'Pregnant (n=30)'	166.96 $\pm$ 24.33	3.26	0.002	0.18
	'Non-Pregnant (n=30)'	140.10 $\pm$ 37.97			
PSS Score	'Pregnant (n=30)'	20.16 $\pm$ 5.06	2.779	0.007	0.39
	'Non-Pregnant (n=30)'	16.800 $\pm$ 4.28			

## DISCUSSION

Our study report that pregnancy effect significant physiological and psychological stress, as showed by the elevated cortisol levels and higher Perceived Stress Scale (PSS) scores among pregnant women. In accordance with the literature shown pregnancy was associated with increased stress<sup>(2)</sup>. A study by Morgan et al. (2022) found that pregnant women show elevated cortisol levels, which have been associated with increased risks of gestational complications, hypertension and preterm birth<sup>(13)</sup> The physiological

response may be future intensify psychological stressors, including concerns about pregnancy outcomes and society pressures, particularly in environments of Pakistan, where limited access to healthcare resources and economic challenges may exacerbate stress levels<sup>(14)</sup>.

The cardiovascular changes, the statically significant increase in pulse rate and systolic blood pressure reported in pregnant women was in accordance with studies that reported elevated cardiac output and vascular resistance during pregnancy<sup>(15)</sup>. These findings reflect the body's adaptation to support the growing

fetus, but they also highlights the importance of monitoring for possible hypertensive disorders, preeclampsia, which was common in women with elevated systolic blood pressure during pregnancy<sup>(16)</sup>. Our study the lack of significant change in diastolic blood pressure suggests that the cardiovascular strain is not undeviating, with specific elevations in systolic pressure due to increased blood volume and hormonal changes, a pattern<sup>(17)</sup> this was supported by da Silva et al. (2023)<sup>(18)</sup>.

Futhermore, thyroid function in pregnant women, while generally stable in terms of TSH and T3 levels, showed a significant elevated in T4 levels.in accordance to study by Bohn et al. (2021), which also showed increased T4 levels in pregnant women, imitating the increased metabolic demands of pregnancy<sup>(19)</sup>. Thyroid hormones are important for fetal development, mostly for neurological and metabolic functions, and increased T4 levels are a normal part of pregnancy both the mother's and the fetus's requirements. Though, the stability of TSH levels was reassuring, indicating that thyroid dysfunction, hypothyroidism, was not prevalent in this study. This is important, as 'thyroid dysfunction during pregnancy has been associated to adverse outcomes, miscarriage and preterm delivery'<sup>(20)</sup>

The higher perceived stress in pregnant women, as imitated by their higher PSS scores, was particularly concerning. A stud by Waqas et al. (2020), reported that perceived stress was a significant predictor of adverse perinatal outcomes, that were low birth weight and preterm birth<sup>(21)</sup>. The relationship between high perceived stress and increased cortisol levels further supports the need for stress management interventions during pregnancy. In Pakistan, where cultural and economic factors added to heightened stress levels, targeted interventions that address both psychological and physiological stressors are important for improving pregnancy outcomes<sup>(22,23)</sup>.

Our study supports existing research on the physiological and psychological changes associated with pregnancy. The findings underscore the need for comprehensive prenatal care that includes stress management and regular monitoring of hormonal levels, predominantly cortisol and thyroid hormones, to ensure ideal maternal and fetal health. Moreover, these findings highlight the importance of sociocultural and economic stressors in Pakistan, which may further intensify the physiological stress burden experienced during pregnancy.

## CONCLUSION

In conclusion, pregnant women experience significantly increased levels of physiological and psychological stress compared to non-pregnant women, as imitated in elevated cortisol levels and perceived stress scores. The cardiovascular system also experiences adaptations, with higher pulse rates and systolic blood pressure, the

thyroid function remains stable, the significant increase in T4 levels highlights the body's increased metabolic demands during pregnancy.

### Author's Contribution:

Concept & Design of Study: Maheen Shah  
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