JBAAR

Journal of Bioscience and Applied Research

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Comparative Analysis of Thyroid Function Tests in Pregnant Females Across Different Gestational Stages and Non-Pregnant Females.

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DOI: 10.21608/jbaar.2025.372385.1179

Abstract

Background and Objectives: Both hyperthyroidism and hypothyroidism are thyroid conditions that are essential to manage and are somewhat frequent during pregnancy. Proper management of these conditions is critical, particularly in the first trimester, as they can significantly impact maternal and fetal health. The objectives of this study are to determine the ideal levels of T3, T4, and TSH in each trimester of pregnancy, to investigate whether these values change as pregnancy progresses, and to compare the thyroid profile findings of non-pregnant individuals with those of pregnant women. **Materials and Methods**: This study included samples from 31 pregnant women, divided into groups according to the trimesters, and 19 age-matched control females with gynecological problems but normal thyroid function. The participants were recruited from Al Anbar, Iraq, at Al-Ramadi hospital in the Department of Obstetrics and Gynecology and from private laboratories. Serum blood samples were collected and analyzed for T3 (Triiodothyronine), T4 (Thyroxine), TSH (Thyrotropin-Stimulating Hormone), and Hemoglobin (Hb). **Results:** The analysis revealed significant variations in the levels of T3, T4, and TSH across different trimesters of pregnancy. Pregnant women exhibited altered thyroid profiles compared to non-pregnant controls, indicating the dynamic nature of thyroid function during pregnancy. **Conclusion:** The study concludes that thyroid function, as indicated by T3, T4, and TSH levels, undergoes significant changes during pregnancy. Regular monitoring of thyroid hormones is recommended to ensure optimal maternal and fetal health.

Keywords: Thyroid Hormones, T3, T4, TSH, Pregnancy, Hemoglobin, Maternal Health.

1-Introduction

Thyroid function is a cornerstone of metabolic regulation, particularly significant during pregnancy when the demands on maternal physiology increase substantially. The thyroid gland, located in the anterior neck, is responsible for the production of key hormones, namely Triiodothyronine (T3) and Thyroxine (T4). These hormones are meticulously controlled by Thyrotropin-Stimulating Hormone (TSH), which is released by the pituitary gland. T3 and T4 are essential in managing metabolism, growth, and development (1-3). During pregnancy, the maternal body undergoes profound physiological changes to

Received: April 1, 2025. Accepted: August 11, 2025. Published: September 20, 2025

support the developing fetus, and the thyroid gland adapts to meet the increased metabolic demands. This adaptation is vital for ensuring proper fetal development, particularly in the early stages when the fetus relies heavily on maternal thyroid hormones. The placenta also plays a role in modulating maternal thyroid function, with human chorionic gonadotropin (hCG) exerting a thyroid-stimulating effect, especially prominent in the first trimester (4). Thyroid dysfunction during pregnancy can have serious implications for both the mother and the fetus. Hyperthyroidism, characterized by an overproduction of thyroid hormones, can lead to complications such as preterm birth, preeclampsia, and even fetal growth restriction. On the other hand, hypothyroidism, marked by insufficient production of thyroid hormones, is associated with increased risks of miscarriage, low birth weight, and neurodevelopmental deficits in the child. Thus, maintaining euthyroid status during pregnancy is critical to minimize these risks (5). Given these considerations, this research seeks to assess the fluctuations in thyroid hormone concentrations, specifically T3, T4, and TSH, across different gestational stages in pregnant females. These findings will be compared with the thyroid function test results of non-pregnant females to establish a baseline and highlight the physiological changes induced by pregnancy. By conducting a comprehensive analysis across trimesters, this research intends to elucidate that the regulation of thyroid hormones during pregnancy is dynamic and subject to continuous adjustments (6). The study's methodology will involve longitudinal monitoring of thyroid function tests in a cohort of pregnant women at various stages of gestation—first, second, and third trimesters. A control group of non-pregnant women will also be assessed to provide a comparative baseline. The results are expected to shed light on the trajectory of thyroid hormone levels throughout pregnancy, offering insights into the timing and nature of thyroid adaptations (7). This investigation underscores the importance of regular thyroid function monitoring in pregnant women. Early detection and management of thyroid dysfunction can prevent potential adverse outcomes, promoting improved health outcomes for both the mother and the fetus. The findings of this study will contribute to a deeper understanding of thyroid physiology in pregnancy and potentially inform clinical guidelines for thyroid screening and management in prenatal care (8).

2-Materials and Methods

2-1-Patient's Sample Collection and Serum Preparation

Participants were meticulously selected and recruited from Al-Ramadi Hospital's Department of Obstetrics and Gynecology and private laboratories in Al-Anbar, Iraq. Blood samples were collected from 31 pregnant women at various gestational stages and 19 non-pregnant, age-matched women with no history of thyroid dysfunction. Venous blood was drawn using standard phlebotomy techniques, ensuring minimal patient discomfort and optimal sample integrity. The blood samples were left to clot at room temperature, then centrifuged at 3000 RPM for 10 minutes to isolate the serum, which was subsequently portioned and kept at −20°C for future analysis.

2-2-Laboratory Assay for Thyroid Hormones (TSH, T3, T4)

The serum levels of T3, T4, and TSH were quantified using a chemiluminescent immunoassay (CLIA) performed on a fully automated analyzer. This method ensures high sensitivity and specificity, providing reliable and reproducible measurements critical for accurate thyroid function assessment. The assay calibration was regularly verified using control standards to maintain analytical precision.

2-3-Inclusion and Exclusion Criteria

The study included pregnant women at various stages of gestation with no prior history of thyroid disorders and non-pregnant women aged 20-40 years

with no clinical signs of thyroid dysfunction, provided they gave informed consent. It excluded women with pre-existing thyroid diseases or those on thyroid hormone replacement therapy, participants with a history of autoimmune disorders, severe chronic illnesses, or medication affecting thyroid function, and individuals refusing to provide informed consent or unable to comply with study protocols.

3-Statistical Analysis

The data were analyzed using SPSS version 23.0 and Microsoft Office 2010. Descriptive statistics, such as the mean and standard error, were employed to summarize the data. ANOVA and post hoc tests were conducted to compare the groups, while Pearson's correlation coefficient (r) was used to evaluate the relationships, with statistical significance determined at $p \leq 0.05$.

4-Results

4-1. Classification of the studied groups

A total of fifty females participated in the current study. The participants were selected and classified into three groups: Group 1 consisted of 18 pregnant females in the first and second trimesters, Group 2 included 13 pregnant females in the third trimester, and Group 3 consisted of 19 non-pregnant females (Control group) (Figure 1).

4-2- A comparison of clinical data among the studied groups

It is presented in Table 1. According to the results, the weight of females was significantly higher in the third trimester of pregnancy (65.67 ± 2.93 vs. 73.08 ± 2.99 vs. 63.53 ± 1.35; p=0.031). In contrast, hemoglobin levels were notably higher in non-pregnant females (9.17 ± 0.15 vs. 9.92 ± 0.38 vs. 12.46 ± 0.30; p<0.001). However, there were no significant differences in age among the groups (p=0.261). Regarding thyroid function, T3 and T4 levels were significantly higher in first-trimester pregnant females (2.95 ± 0.32 vs. 1.63 ± 0.42 vs. 1.37 ± 0.21; p=0.001 and 17.23 ± 1.78 vs. 12.21 ± 2.84 vs. 10.20 ± 0.96; p=0.018, respectively). No significant differences were observed in TSH levels (p=0.123) between the three groups, as shown in Table 1 and Figure 2.

The post hoc test of ANOVA (Table 2) revealed significant differences in the levels of T3 and T4 between the first-trimester pregnant females (Group 1) and non-pregnant females (Group 3). Specifically, the differences were statistically significant for T3 levels (p=0.001) and T4 levels (p=0.006), indicating that pregnancy during the first trimester is associated with altered thyroid hormone levels compared to non-pregnant females.

4-3-Correlations between patients' age and weight with thyroid function status

There were significant negative correlations between patients' weight and T3 (r=-0.545, p<0.001) and T4 (r=-0.545, p<0.001). In contrast, a positive correlation was observed between patients' weight and TSH levels (r=0.822, p<0.001). Additionally, TSH exhibited a negative correlation with both T3 (r=-0.712, p<0.001) and T4 (r=-0.735, p<0.001). A significant positive correlation was also found between T3 and T4 (r=0.871, p<0.001), as shown in Table 3, Figure 3, and Figure 4.

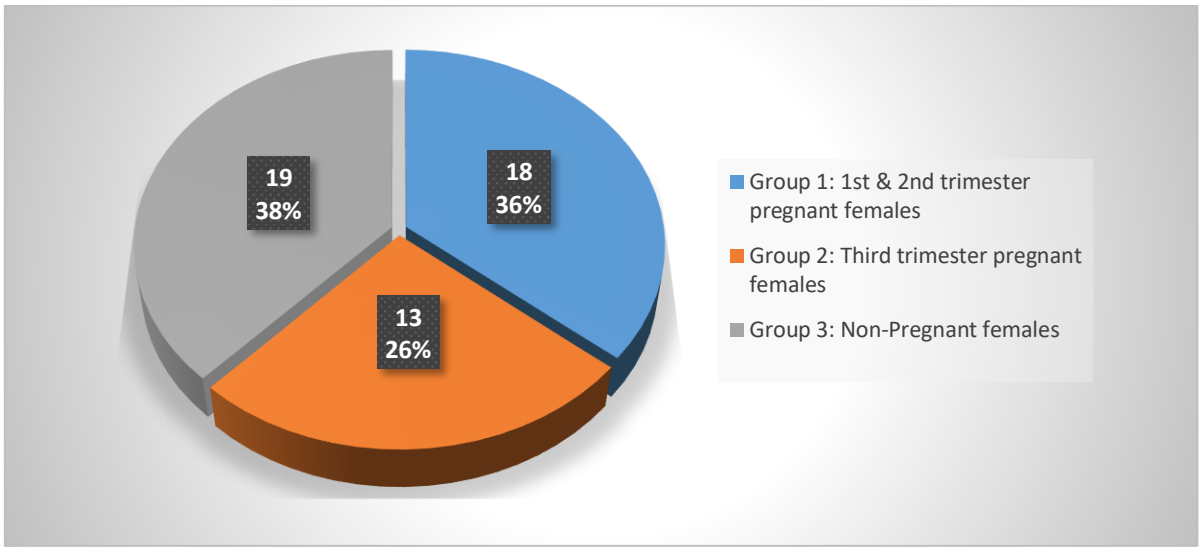

Figure 1: Categorization of the analyzed groups.

Table 1: Comparison of clinical data across the examined groups.

Parameters	Pregnant females at 1st & 2nd trimester (Group 1)	Pregnant females in the third trimester (Group 2)	Non-Pregnant females (Group 3)	*p* value
Age (Years)	29.17 ± 1.38	31.62 ± 1.81	28.26 ± 1.53	0.261
Weight (Kg)	65.67 ± 2.93	73.08 ± 2.99	63.53 ± 1.35	0.031 S
Hemoglobin (Hb) (g/dl)	9.17 ± 0.15	9.92 ± 0.38	12.46 ± 0.30	< 0.001
T3 (ng/ml)	2.95 ± 0.32	1.63 ± 0.42	1.37 ± 0.21	0.001 S
T4 (µg/dl)	17.23 ± 1.78	12.21 ± 2.84	10.20 ± 0.96	0.018
TSH(µIU/ml)	1.24 ± 0.84	1.39 ± 0.83	1.83 ± 0.32	0.123

T3: Triiodothyronine; T4: Thyroxine; TSH: Thyroid Stimulating Hormone; S: Significant ($p \leq 0.05$); NS: Not Significant ($p > 0.05$); V: Analysis of Variance (7).

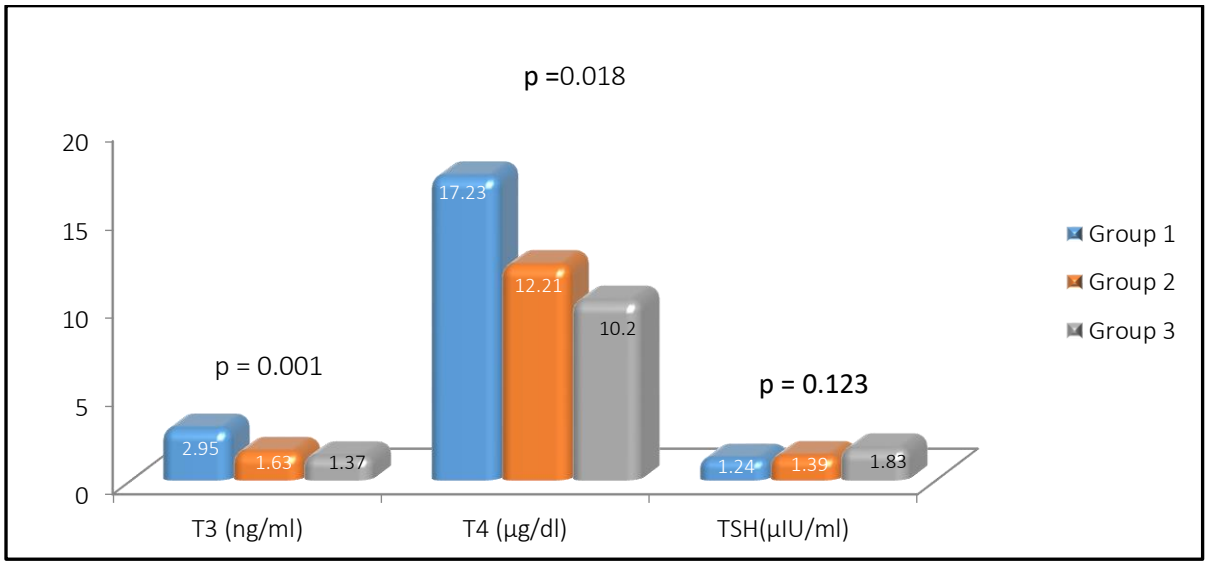

Figure 2: A comparison of demographic characteristics across the different study groups.

Table 2: Post hoc test of ANOVA of T3 & T4 for paired groups' comparison

Post hoc test for multiple group comparisons			*p* value
T3	Group 1	Group 2	0.102
	Group 1	Group 3	0.001
	Group 2	Group 3	0.468
T4	Group 1	Group 2	0.577
	Group 1	Group 3	0.006
	Group 2	Group 3	0.204

T3: Triiodothyronine; T4: Thyroxine; S: Statistically significant ($p \leq 0.05$); NS: Not statistically significant ($p > 0.05$).

Table 3: Correlations between patients' age and weight with thyroid function status

Parameters		Age	Weight	T3	T4	TSH
Age	r	1	0.171	-0.013	0.061	0.063
	p value		0.236	0.927	0.676	0.663
Weight	r	0.171	1	-0.545	-0.545	0.822
	p value	0.236		< 0.001	< 0.001	< 0.001
T3	r	-0.013	-0.545	1	0.871	-0.712
	p value	0.927	< 0.001		< 0.001	< 0.001
T4	r	0.061	-0.545	0.871	1	-0.735
	p value	0.676	< 0.001	< 0.001		< 0.001
TSH	r	0.063	0.822	-0.712	-0.735	1
	p value	0.663	< 0.001	< 0.001	< 0.001	

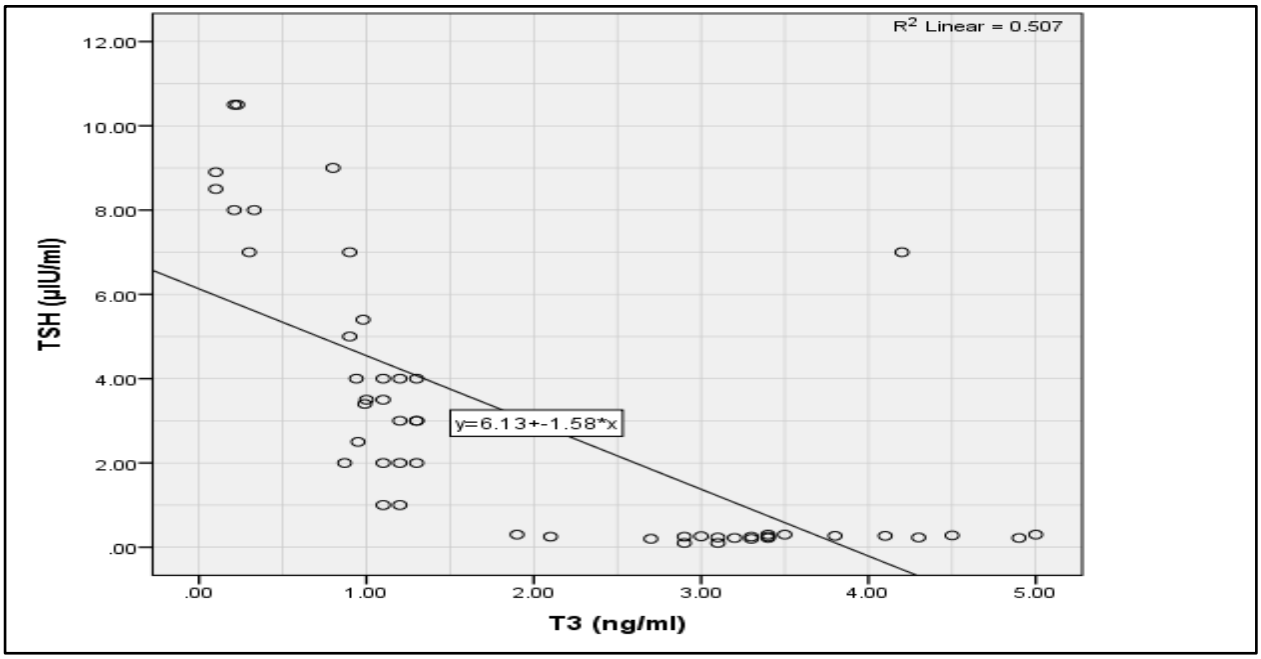

Figure 3: Correlation between T3 and TSH

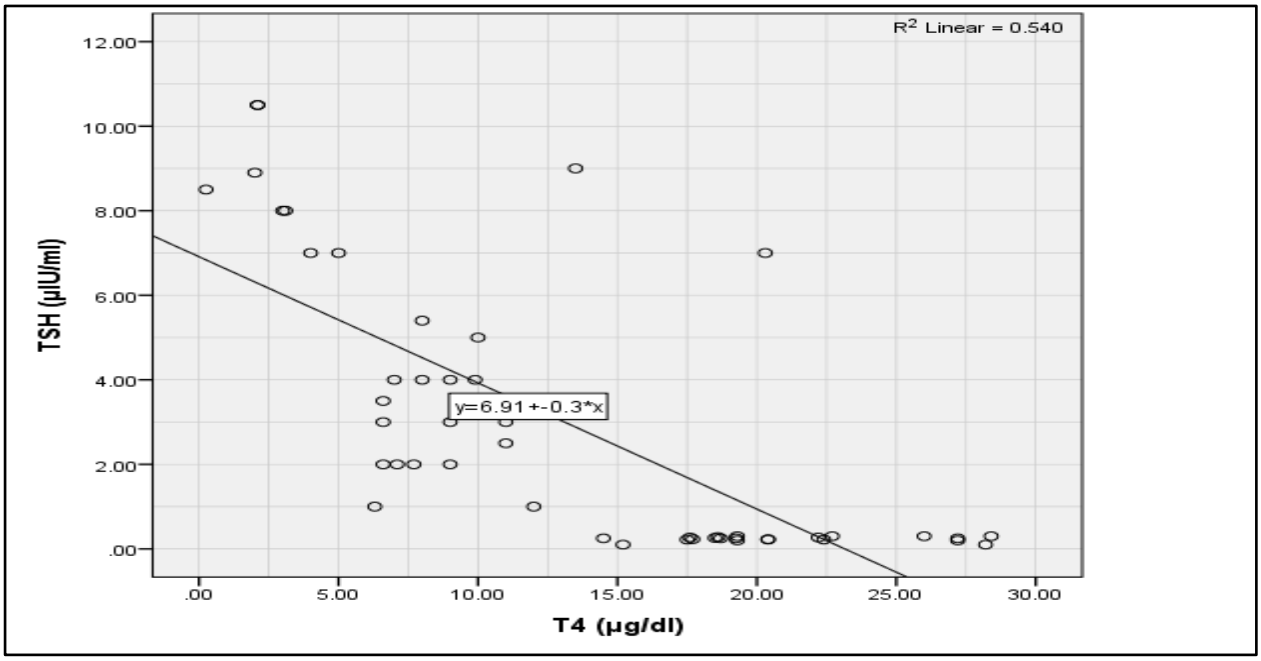

Figure 4: Correlation between T4 and TSH

5-Discussion

The current study's findings align with and expand upon previous research concerning weight gain and hematological changes during pregnancy (9). Our results indicate that the weight of pregnant females significantly increases during the third trimester compared to both earlier trimesters and non-pregnant females (73.08 ± 2.99 kg vs. 65.67 ± 2.93 kg and 63.53 ± 1.35 kg, respectively; p=0.031) (10). This increase is consistent with the well-documented physiological adaptations during late pregnancy, where there is substantial maternal weight gain due to fetal growth, placental development, and fluid retention. These findings

parallel the results of recent studies, such as those by (11). Which observed similar trends in third-trimester weight gain across diverse populations (12).

The hemoglobin concentrations observed in our study were notably higher in non-pregnant females (12.46 ± 0.30 g/dl) compared to pregnant counterparts across all trimesters, which is in concordance with the findings (13). They highlighted the prevalence of dilutional anemia in pregnancy, driven by increased plasma volume outpacing the rise in red blood cell mass(14). The statistical significance of this difference ($p<0.001$) underscores the clinical relevance of regular monitoring and managing maternal anemia to prevent adverse perinatal outcomes(15).

In terms of thyroid function, the elevated levels of T3 and T4 in the first-trimester pregnant group (2.95 ± 0.32 ng/ml and 17.23 ± 1.78 µg/dl, respectively) as compared to non-pregnant females, corroborate findings by (16). This is attributable to the surge in human chorionic gonadotropin (hCG), which exerts a thyrotropic effect, particularly evident in the first trimester. However, our study did not find significant variations in TSH levels across the groups ($p=0.123$), aligning with the studies of (17). Also noted that TSH often remains within the normal range due to effective thyroid hormone feedback mechanisms during pregnancy(18). These findings contribute to the growing body of evidence underscoring the necessity of trimester-specific reference ranges for thyroid function tests in pregnancy, as advocated by recent guidelines(19). They also emphasize the possible advantages of implementing early intervention strategies to reduce the risks linked to thyroid dysfunction, which could improve both maternal and fetal health outcomes (20).

The consistency of our data with contemporary research emphasizes the robustness of our study design and analytical methods, positioning our findings as a valuable addition to ongoing discussions about the management of maternal health during pregnancy(21). Future studies might build upon these insights by exploring the longitudinal impacts of thyroid function on pregnancy outcomes, integrating larger and more diverse cohorts to strengthen generalizability (22). The post hoc ANOVA test, presented in Table 2, revealed significant variations in T3 and T4 levels between first-trimester pregnant females (Group 1) and non-pregnant females (Group 3), with p-values of 0.001 and 0.006, respectively (23). These findings align with and expand upon existing literature, which consistently underscores the dynamic nature of thyroid hormone levels during pregnancy. Previous studies have established that early pregnancy induces notable changes in maternal thyroid function, largely driven by elevated human chorionic gonadotropin (hCG) levels, which stimulate the thyroid gland to produce more T3 and T4 (24). This physiological response is crucial to meet the increased metabolic demands of both the mother and the developing fetus. For instance, a study by (25). Demonstrated a significant rise in free T4 levels during the first trimester, a pattern similarly observed in our findings. Comparative research, such as that conducted by (26). Further corroborates these observations, indicating that maternal thyroid hormone levels typically peak in the early stages of pregnancy before gradually declining (27). The significant differences in T3 and T4 levels between early pregnant and non-pregnant females in our study reflect this early gestational surge, confirming the findings of these earlier investigations. Moreover, the relatively lower T3 and T4 levels in non-pregnant females are consistent with the baseline thyroid function typically observed in the absence of pregnancy-induced hormonal modulation (28). These differences highlight the essential role of thyroid hormones in facilitating early fetal development, especially in the formation of the fetal brain and nervous system, where maternal thyroid hormones offer a vital foundation during the early stages (29). Our study enriches the

existing body of knowledge by providing a detailed comparative analysis across different gestational stages and a control group, offering a broader perspective on thyroid function during pregnancy (24). This approach helps delineate the specific gestational changes in thyroid hormone levels, reinforcing the need for regular thyroid monitoring and management to mitigate potential adverse outcomes associated with thyroid dysfunction (8). Our results not only validate but also extend the understanding of thyroid hormone dynamics in early pregnancy, suggesting avenues for future research to further elucidate the complex interplay between maternal thyroid function and pregnancy outcomes (30). The findings from the data suggest several significant correlations between patient weight, age, and thyroid function markers, notably TSH, T3, and T4 levels. Specifically, the study indicates that weight correlates negatively with both T3 and T4, with correlation coefficients of -0.545 for both hormones ($p<0.001$), implying that higher body weight may be associated with lower levels of these thyroid hormones (31). This relationship could be due to several mechanisms, including altered thyroid hormone metabolism or changes in thyroid receptor sensitivity in overweight or obese individuals. A negative correlation between body weight and T3/T4 levels has been observed in other studies, suggesting a consistent trend in individuals with higher BMI (body mass index) demonstrating lower thyroid hormone levels (32). On the other hand, there was a significant positive correlation between weight and TSH levels ($r=0.822$, $p<0.001$), which aligns with findings from previous research linking increased body weight to elevated TSH levels (33). This could be interpreted as a compensatory mechanism, where the hypothalamus-pituitary-thyroid axis responds to the decreased thyroid hormones (T3 and T4) by stimulating higher secretion of TSH. This mechanism could be indicative of subclinical hypothyroidism, a condition that may not manifest with clear clinical symptoms but can influence weight and metabolic regulation (9). The negative correlations between TSH with both T3 ($r=-0.712$, $p<0.001$) and T4 ($r=-0.735$, $p<0.001$) are in line with the well-established negative feedback regulation within the thyroid axis. When T3 and T4 levels are low, the body compensates by increasing TSH secretion in an attempt to stimulate thyroid hormone production, which is a well-documented physiological response (34). Lastly, the strong positive correlation between T3 and T4 ($r=0.871$, $p<0.001$) further supports the expected physiological relationship between these hormones, which are both synthesized by the thyroid gland and closely linked in terms of function. This finding is consistent with the principle that T3 and T4 often fluctuate together, as both are crucial for regulating metabolism and energy production in the body (35). In conclusion, the present study offers valuable insights into the dynamic physiological changes that occur during pregnancy, with particular emphasis on the interactions between maternal weight, thyroid function, and hematological parameters (36). Our findings confirm the well-established pattern of increased maternal weight during the third trimester, largely due to the combined effects of fetal growth, placental development, and fluid retention, as observed in previous studies. The significant increase in weight during this period underscores the importance of monitoring maternal health and the potential implications for metabolic regulation during pregnancy (37).

Acknowledgment

We extend our sincere gratitude to the staff of the Obstetrics and Gynecology Department at Al-Ramadi Hospital and the private laboratories in Al Anbar, Iraq, for their invaluable assistance in specimen collection and data acquisition.

Consent

Informed oral consent was obtained from all participants, who were briefed on the study's objectives. Participants were assured that their data would be used exclusively for research purposes,

with stringent measures in place to ensure confidentiality and data protection.

Ethical Approval

The study protocol was thoroughly explained to participants and medical personnel. Ethical approval was secured from relevant hospital authorities, emphasizing the voluntary nature of participation. Participants were informed of their right to withdraw from the study at any point without repercussions to their medical care, ensuring their well-being and autonomy were prioritized.

Competing Interests

The authors declare that there are no competing interests that could have influenced the study's outcomes or interpretations.

Funding

This research was conducted without the support of external funding agencies.

Author Contribution

All authors made equal contributions to the conceptualization, data gathering, analysis, and preparation of the manuscript. Each author has reviewed and endorsed the final version of the manuscript, confirming its accuracy and consistency.

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