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EVALUATION OF HEMATOLOGICAL PARAMETERS AND THEIR ASSOCIATION WITH THYROID HORMONE LEVELS IN WOMEN OF REPRODUCTIVE AGE: A COMPARATIVE STUDY

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ABSTRACT

Background

Thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4), are important for the regulation of metabolism and organ function, with imbalances linked to health issues such as hypothyroidism and hyperthyroidism. This investigation seeks to offer meaningful information on the clinical handling of hematological abnormalities linked to thyroid disorders, thereby contributing to improved health outcomes for those affected.

Methods

A one-year study was conducted at Maharshi Devraha Baba Autonomous State Medical College, including 500 non-pregnant female patients diagnosed with thyroid disorders. Participants were classified into hyperthyroid and hypothyroid groups based on clinical symptoms and laboratory results, with a control cohort of 100 age-matched females having normal thyroid function. Blood samples were analyzed for serum T3, T4, and TSH levels, along with hematological parameters, using a hematology analyzer.

Results

The study included 500 non-pregnant females, with 250 diagnosed with hypothyroidism, 250 with hyperthyroidism, and 100 euthyroid controls. Hypothyroid patients had significantly lower T3 (1.2 ± 0.35 nmol/L), T4 (54.40 ± 37.60 nmol/L), and elevated TSH (145.67 ± 192.40 mIU/L). Hematological analyses revealed lower hemoglobin (11.30 ± 1.10 g/dL) and RBC counts (4.02 ± 0.45 x10^6/µL) in hypothyroid subjects compared to euthyroid counterparts. No prominent variations were found in hematological parameters between hyperthyroid and euthyroid groups, although hypothyroid patients showed higher RDW and AEC.

Conclusion

The investigation demonstrates a significant correlation between thyroid dysfunction and hematological abnormalities, especially anemia in hypothyroid patients, emphasizing the need to evaluate thyroid hormone levels in women with unexplained anemia for better management.

Recommendation

Ongoing studies and collaborative endeavors are needed to enhance the understanding of hypothyroidism, ultimately improving patient treatment both within and outside of the community.

Keywords: *Thyroid Hormones, Hypothyroidism, Hyperthyroidism, Anemia, Hematological Parameters, Non-Pregnant Females.*
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INTRODUCTION

Thyroid hormones, mainly triiodothyronine (T3) and thyroxine (T4), are important for maintaining growth, overall development, and metabolism in the human body. They are essential for the optimal performance of various organ systems, affecting processes such as energy utilization and protein production. The thyroid gland's proper activity is critical for maintaining physiological balance, and any imbalance in hormone levels can result in numerous health complications. Conditions like hypothyroidism and hyperthyroidism represent significant endocrine disorders and are commonly observed in medical practice. These conditions not only affect metabolic processes but are also associated with several hematological abnormalities.

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Research has indicated that there is a complex interplay between thyroid hormones and blood parameters, with conditions such as anemia being commonly linked to hypothyroidism, while erythrocytosis is often associated with hyperthyroidism, particularly Graves' disease [1,2]. Women, especially during their reproductive years, are particularly vulnerable to thyroid disorders and hematological abnormalities. The incidence of these conditions tends to be higher in females, making it essential to investigate their relationship in this demographic. Anemia is notably prevalent among women of childbearing age, often exacerbated by underlying thyroid dysfunction. Moreover, evidence suggests that correcting thyroid hormone levels can lead to the normalization of hematological parameters, underscoring the importance of early diagnosis and treatment [3-8]. Due to the scarcity of extensive research in this part of the country, this study intends to assess the correlation between thyroid hormone levels—both within the normal range and deviating from it—and fundamental hematological factors in non-pregnant women of reproductive age. This investigation seeks to offer meaningful information on the clinical handling of hematological abnormalities linked to thyroid disorders, thereby contributing to improved health outcomes for those affected.

MATERIALS AND METHODS

Study setting

This investigation was conducted over one year at Maharshi Devraha Baba Autonomous State Medical College in Deoria. It encompassed 500 women participants who were not pregnant between 18 and 45 years and had various thyroid diseases. These patients were admitted to different clinical departments, allowing for a comprehensive assessment of thyroid-related health issues.

Patient Classification

The women were categorized into two cohorts as per their thyroid levels: hyperthyroidism (n=250) and hypothyroidism (n=250). Hyperthyroidism was identified by assessing clinical signs such as diffuse thyroid enlargement, exophthalmos, rapid heartbeat, tremors, palpitations, and excessive perspiration. This diagnosis was further confirmed through laboratory tests showing reduced serum TSH levels as well as elevated concentrations of T3 and T4, with thresholds defined as T3 greater than 3.6 nmol/L, T4 greater than 160 nmol/L, and TSH less than 0.35 mIU/L. On the other hand, hypothyroidism was diagnosed based on symptoms like weight gain and dry skin, corroborated by lab results indicating decreased T3 and T4 levels alongside increased TSH levels, with cutoffs set at T3 less than 1.6 nmol/L, T4 less than 60 nmol/L, and TSH greater than 3.5 mIU/L. Individuals who underwent testing for TSH, T4, and T3 were only included in the study classification process.

Control Group

In addition to the primary study groups, a control cohort comprising 100 age-matched females with normal thyroid function tests was established by including women who were suspected of having thyroid disorders but were confirmed to be serologically normal (T3 levels between 1.8 to 3.6 nmol/L, T4 levels between 60 to 160 nmol/L, and TSH levels between 0.35 to 3.5 mIU/L). The inclusion of this control group allowed for comparative analysis against those with diagnosed thyroid conditions.

Inclusion and Exclusion Criteria

Women aged 18 to 45 years were part of this investigation. Individuals with known cases of hypothyroidism or hyperthyroidism currently undergoing treatment, individuals with malignancies, those who have undergone surgeries or experienced major trauma within the last six months, patients with recurring health conditions, expecting mothers, and those with a history of bleeding diathesis were excluded from the study. These criteria ensured a clear and focused population for assessing the connection between thyroid function and hematological parameters.

Sample Collection and Laboratory Analysis

Peripheral blood samples ranging from 2 to 4 mL were collected from all 500 participants, with the serum separated as well as stored at -20°C for future studies. By using electrochemiluminescence technology on a COBAS C411 autoanalyzer, which has detection limits of 0.5 nmol/L for T3, 5 nmol/L for T4, and 0.05 mIU/L for TSH, the thyroid hormone levels were determined. Furthermore, total and differential leukocyte counts were conducted on EDTA-treated samples using the Sysmex XN550 six-part hematology analyzer. Core hematological parameters—such as hemoglobin (Hb), total leukocyte count (TLC), red blood cell (RBC) count, red cell indices, red cell distribution width (RDW), and platelet count—were validated through routine internal and external quality control protocols. Peripheral blood smears were prepared and stained with Leishman stain for additional analysis.

Statistical Analysis

The study's results were depicted as the mean ± standard deviation (SD). SPSS software version 20 was employed to conduct statistical analyses. The data was presented straightforwardly by utilizing Microsoft Word as well as Excel to create tables. The group means were compared using independent T-tests, with a p-value < 0.05 being considered statistically significant. Compliance with ethical research principles was ensured after the research progress

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was evaluated and approved by the institutional ethics committee.

Ethical Considerations

The study was carried out after receiving informed consent from all the participating women and ensuring that transparency was followed throughout the study.

RESULTS

The study population (n=500) was split into 2 test cohorts besides an additional control cohort of 100 patients, were categorized as hypothyroid patients (n=250), euthyroid individuals (n=100), and hyperthyroid patients (n=250) (Table 1).

Table 1: Distribution of Thyroid Hormone Levels in the Three Groups

Hormone	Hypothyroid cohort (n=250)	Euthyroid cohort (n=100)	Hyperthyroid cohort (n=250)
T3 (nmol/L)	1.2 ± 0.35	3.05 ± 0.41	4.02 ± 1.55
T4 (nmol/L)	54.40 ± 37.60	140.10 ± 10.32	218.25 ± 18.74
TSH (mIU/L)	145.67 ± 192.40	1.75 ± 0.62	0.04 ± 0.01

This research identified 250 newly diagnosed cases of hyperthyroidism and 250 cases of hypothyroidism based on the diagnostic criteria outlined in the methodology. A control group of 100 euthyroid women was randomly chosen to serve as a baseline for comparison, given the lower prevalence of thyroid disorders in males (Table 2).

Table 2: Comparison of Hematological Parameters between Hypothyroid and Euthyroid Subjects

Parameter	Hypothyroid cohort (n=250)	Euthyroid cohort(n=100)	p-value
Hemoglobin (g/dl)	11.30 ± 1.10	12.58 ± 1.50	0.001
Red Blood Cell Count ($x10^6$/µl)	4.02 ± 0.45	4.60 ± 0.50	0.001
Mean Cell Volume(fl)	84.50 ± 6.80	83.06 ± 5.80	0.273
Mean Cell Hemoglobin (pg)	27.40 ± 1.20	28.52 ± 1.30	0.042
Mean Cell Hemoglobin Concentration (g/dl)	32.14 ± 1.10	33.14 ± 1.05	0.002
Total Leukocyte Count ($x10^3$/µl)	6020 ± 1500	7032 ± 1300	0.001
Platelet Count ($x10^3$/µl)	2.48 ± 0.75	2.76 ± 0.67	0.67
Red Cell Distribution Width (%)	14.40 ± 1.50	13.02 ± 1.20	0.001
Absolute Eosinophil Count	410 ± 160	260 ± 82	0.001

Among the evaluated parameters, the most notable differences were observed in hemoglobin levels, MCHC, and RBC counts between hypothyroid and euthyroid individuals. Hypothyroid patients demonstrated significantly lower hemoglobin levels, with a mean value of 11.30 g/dL, reflecting a higher incidence of anemia in this group. Despite this, both euthyroid as well as hypothyroid groups showed normocytic cells, which is indicated with the help of the MCV values. Total leukocyte counts were significantly reduced in the hypothyroid group; however, these values remained within normal reference ranges and did not meet the criteria for leukopenia. The MCH parameter showed no significant differences amongst these cohorts. Additionally, RDW and AEC were notably elevated in hypothyroid patients, although AEC levels remained within the normal range (Table 3).

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Table 3: Comparison of Hematological Parameters between Hyperthyroid and Euthyroid Subjects

Parameter	Hyperthyroid cohort(n=250)	Euthyroid cohort(n=100)	p-value
Hemoglobin (g/dl)	12.80 ± 0.90	12.58 ± 1.50	0.191
Red Blood Cell Count (x10^6/µl)	4.75 ± 0.25	4.60 ± 0.50	0.740
Mean Cell Volume(fl)	83.80 ± 5.20	83.06 ± 5.80	0.797
Mean Cell Hemoglobin (pg)	28.50 ± 1.30	28.52 ± 1.30	0.928
Mean Cell Hemoglobin Concentration (g/dl)	33.10 ± 1.05	33.14 ± 1.05	1.000
Total Leukocyte Count (x10^3/µl)	7060 ± 1300	7032 ± 1300	0.950
Platelet Count (x10^3/µl)	2.98 ± 0.70	2.76 ± 0.67	0.169
Red Cell Distribution Width (%)	13.30 ± 1.10	13.02 ± 1.20	0.154
Absolute Eosinophil Count	240 ± 70	260 ± 82	0.052

In contrast, no significant disparities in hematological parameters were identified between hyperthyroid and euthyroid individuals, even though AEC levels were marginally higher in the former (Table 4).

Table 4: Comparison of Hematological Parameters between Hypothyroid and Hyperthyroid Subjects

Parameter	Hypothyroid cohort (n=250)	Hyperthyroid cohort(n=250)	p-value
Hemoglobin (g/dl)	11.30 ± 1.10	12.80 ± 0.90	0.0
Red Blood Cell Count (x10^6/µl)	4.02 ± 0.45	4.75 ± 0.25	0.001
Mean Cell Volume(fl)	84.50 ± 6.80	83.80 ± 5.20	0.713
Mean Cell Hemoglobin (pg)	27.40 ± 1.20	28.50 ± 1.30	0.051
Mean Cell Hemoglobin Concentration (g/dl)	32.14 ± 1.10	33.10 ± 1.05	0.008
Total Leukocyte Count (x10^3/µl)	6020 ± 1500	7060 ± 1300	0.0
Platelet Count (x10^3/µl)	2.48 ± 0.75	2.98 ± 0.70	0.002
Red Cell Distribution Width (%)	14.40 ± 1.50	13.30 ± 1.10	0.0
Absolute Eosinophil Count	410 ± 160	240 ± 70	0.0

Overall, the comparisons reveal that hematological indices are markedly lower in hypothyroid patients compared to those with hyperthyroidism. However, MCV and MCH values did not exhibit significant variations, indicating that red blood cells remain normocytic in both groups. Furthermore, AEC and RDW levels were prominently increased in hypothyroid patients, although both remained within normal limits.

DISCUSSION

Thyroid hormones are crucial for sustaining the metabolic balance of the human body. Thyroid hormone level imbalances are among the most common endocrine illnesses globally, with an estimated frequency of 2-5% [2,4]. These abnormalities are particularly significant for females of reproductive age, as they are essential for appropriate fetal growth and development. Additionally, thyroid hormones significantly influence erythropoiesis by enhancing the proliferation of erythroid progenitor cells in the bone marrow [1,2]. Furthermore, these hormones augment oxygen delivery to tissues by elevating levels of 2,3-diphosphoglycerate (2,3 DPG) [5].

The World Health Organisation (WHO) defines anemia in females as a hemoglobin concentration below 12 g/dL. The present investigation of 500 individuals revealed considerably lower hemoglobin levels in the hypothyroid group, underscoring the association between hypothyroidism and anemia. Erythropoietic progenitor cells include thyroid hormone receptors, which highlights the role thyroid hormones play in the synthesis of RBCs [6]. The RBC count in the hypothyroid cohort was lesser than that in the control cohort. Anaemia is a prevalent issue in developing nations such as ours, influenced by multiple variables, including hypothyroidism. Anemia prevalence in persons with hypothyroidism varies in the range of 20-65% [7]. Research conducted by Das et al. identified normocytic normochromic anemia as the major type, succeeded by microcytic hypochromic anemia attributed to iron shortage in hypothyroid individuals [8]. Studies demonstrate that the effectiveness and absorption of oral iron are enhanced in

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women with subclinical hypothyroidism following levothyroxine treatment [9]. Additionally, Ravanbod et al. exhibited that the efficacy of a combination of iron supplements and levothyroxine supplements in the treatment of subclinical hypothyroidism is superior to either treatment alone [10]. Research by Khan et al. [11] has also associated microcytic anemia with subclinical hypothyroidism. Our research demonstrated that the observed anemia was primarily of the normocytic normochromic kind, as seen by the normal MCV in the hypothyroid cohort. Moreover, when comparing the hyperthyroid and euthyroid groups, it was found that the red blood cell counts were found to be within acceptable limits. Hypothyroid individuals' anemia has been attributed to several factors, including decreased oxygen delivery to tissues, decreased erythropoietin levels brought on by inadequate thyroid hormone stimulation, and inadequate thyroid hormone stimulation of erythroid colony formation [13]. Treatment with erythropoiesis-stimulating drugs has demonstrated limited efficacy in anemic hypothyroid patients receiving dialysis for chronic renal failure [14]. While it has been indicated that Graves' illness correlates with anemia [15], a substantial cohort research by Omar et al. observed a significant prevalence of microcytosis (87.7%) in hyperthyroid patients, irrespective of hemoglobin concentrations [16]. In *this* investigation, hyperthyroid patients demonstrated normal hemoglobin concentrations. Limited data exist that establish the correlation between pancytopenia and Graves' disease, which generally improves markedly after therapy [17]. The suggested mechanism for pancytopenia in Graves' illness includes heightened destruction or sequestration of blood cells through an immune-mediated process. Prior studies have demonstrated decreases in various hematological parameters, including MCV, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in hypothyroid conditions, aligning with our results, and enhancements noted after the commencement of levothyroxine treatment [7,18]. Nevertheless, contradictory investigations have depicted no prominent variations in MCV between hypo- and hyperthyroid cohorts [18], indicating a necessity for additional research.

Thyroid hormones also significantly affect human hematopoiesis, which includes controlling the generation of WBCs. T3 has been demonstrated to promote normal B cell generation in the bone marrow [15]. The hypothyroid group in our study had the lowest total leukocyte count, which was not statistically lesser than that of the euthyroid cohort but was significantly lower than that of the hyperthyroid cohort. Jafarzadeh et al. observed no statistically significant variation in leukocyte counts among the three cohorts [6].

Numerous research have investigated the correlation between platelet counts and thyroid problems. Some studies revealed no significant alterations [6,18], whilst others indicated reduced platelet counts in hypothyroid conditions [15,20]. In the present investigation, while platelet counts were within normal ranges, they were markedly reduced in the hypothyroid cohort relative to both the hyperthyroid and euthyroid cohorts. Additionally, the hypothyroid group exhibited a statistically significant increase in absolute eosinophil count (AEC) in comparison to the hyperthyroid group, which was a noteworthy discovery. Serum IgE levels can indicate the immunopathogenesis of allergy diseases and correlate with absolute eosinophil numbers in peripheral blood. Increased serum IgE levels have been noted in both hypo- and hyperthyroid states, indicating a relationship between Th2 cell-mediated immunological responses and thyroid hormones. Moreover, it has been shown that the absolute quantities of pro-B, pre-B, and B cells in the bone marrow of hypothyroid mice are markedly diminished [6]. This necessitates additional thorough investigation and may signify a viable research direction in people.

The RDW parameter quantifies the heterogeneity in red blood cell dimensions, with an elevated RDW signifying the existence of erythrocytes of diverse sizes. Geetha and Srikrishna's research indicated that both hyperthyroid and hypothyroid individuals displayed elevated RDW in comparison to the euthyroid cohort, implying that thyroid hormone abnormalities may affect RBC size [12]. In our investigation, the hypothyroid group exhibited the maximum RDW, which is consistent with the results of Montagnana et al. and Aktas et al. [21,22]. However, the disparities were not statistically significant when contrasted with the hyper- and euthyroid groups. Research indicates that in patients with increased RDW who do not exhibit iron insufficiency, it is essential to evaluate thyroid function in conjunction with folate and Vitamin B12 levels.

A constraint of this investigation is its cross-sectional design, which restricts the capacity to demonstrate causal connections of hematological markers with thyroid problems.

CONCLUSION

Thyroid hormones are vital for the regulation of various hematological parameters. While other medical conditions can also affect these parameters, assessing thyroid function is crucial in cases of unexplained anemia among females of reproductive age. Correcting thyroid hormone imbalances may help normalize abnormal hematological values, thereby minimizing the need for unnecessary diagnostic tests and treatments for anemia. Considering the potential for confounding factors, further studies are required to improve the comprehension of the relationship of hematological parameters with thyroid hormones.

Limitations

The limitations of this study include a small sample population who were included in this study. Furthermore, the lack of a comparison group also poses a limitation for this study's findings.

Recommendation
Ongoing studies and collaborative endeavors are needed to enhance the understanding of hypothyroidism, ultimately improving patient treatment both within and outside of the community.

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Data Availability
Data is available upon request.

Author contributions
All authors contributed to the design of the research. PR collected and analyzed the data. PT wrote the manuscript. PR edited the paper. All authors read and approved the paper.

List of abbreviations
T3- triiodothyronine
T4- thyroxine
TSH- thyroid-stimulating hormone
RDW- red cell distribution width
AEC- Absolute Eosinophil Count
RBC- red blood cell
Hb- hemoglobin
TLC- total leukocyte count
SD- standard deviation
MCHC- Mean corpuscular Hemoglobin Concentration
MCV- Mean corpuscular Volume
MCH- Mean corpuscular Hemoglobin
DPG- diphosphoglycerate
WHO- World Health Organisation

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Conflict of interest
The authors have no conflicting interests to declare.

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