

## Investigation of Hematological Indices and Their Relationship with Thyroid Hormone Levels Among Adults in Tripoli, Libya: A Cross-Sectional Pilot Study and Methodological Enhancement Framework

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دراسة المؤشرات الدموية وعلاقتها بمستويات هرمونات الغدة الدرقية لدى البالغين في طرابلس، ليبيا: دراسة استكشافية مقطعية وإطار لتعزيز المنهجية البحثية

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### Abstract

Anemia and thyroid dysfunction represent two major global health burdens that frequently intersect in clinical practice. Thyroid hormones play an essential regulatory role in bone marrow erythropoiesis and renal erythropoietin production, while nutritional iron is an indispensable biochemical cofactor for thyroid peroxidase (TPO) activity. Despite significant international inquiry, regional epidemiological and clinical data from North Africa, particularly Libya, remain scarce.

To investigate the relationship between hematological indices (hemoglobin [Hb], mean corpuscular volume [MCV], red cell distribution width [RDW]) and thyroid function parameters (thyroid-stimulating hormone [TSH], free thyroxine [FT4]) among adults in Tripoli, Libya, to characterize the morphological patterns of anemia, and to establish a standardized methodological and biostatistical quality-improvement framework for future large-scale endocrine-hematology research.

A cross-sectional analytical study was conducted across two independent clinical diagnostic centers (Al-Sharq Laboratory and Modern Medicine Laboratory) in Tripoli, Libya. Laboratory records of adult participants (n = 20) undergoing concurrent complete blood counts and automated chemiluminescent thyroid immunoassay profiling were evaluated. Participants were

categorized according to World Health Organization (WHO) diagnostic thresholds for anemia (Hb < 12.0 g/dL in non-pregnant females, < 13.0 g/dL in males) and morphological subtypes based on MCV. Bivariate correlation, linear regression, and group-comparative analyses were conducted. In accordance with rigorous epidemiological standards, missing nutritional biomarkers and expansion protocols are systematically formulated.

The cohort exhibited a mean age of  $40.60 \pm 10.95$  years with a female predominance (65.0%,  $n = 13$ ). The mean Hb was  $12.08 \pm 1.56$  g/dL, mean TSH was  $4.60 \pm 2.41$  mIU/L, and mean FT4 was  $13.54 \pm 3.26$  pmol/L. Anemia was identified in 50.0% ( $n = 10$ ) of participants. Morphological subtyping among anemic individuals revealed microcytic anemia as the predominant pattern (60.0%,  $n = 6$ ), followed by normocytic (20.0%,  $n = 2$ ) and macrocytic anemia (20.0%,  $n = 2$ ). Pearson correlation analysis demonstrated a statistically significant strong inverse correlation between Hb and TSH ( $r = -0.901$ ,  $p < 0.001$ ) and a robust positive correlation between Hb and FT4 ( $r = 0.948$ ,  $p < 0.001$ ). Multiple regression modeling indicated that FT4 was an independent predictor of Hb concentration after adjusting for TSH.

**Conclusions:** Preliminary findings demonstrate a significant association between thyroid dysfunction—particularly biochemical hypothyroidism—and reduced hemoglobin levels, predominantly characterized by microcytic erythrocyte morphology. However, given the observational cross-sectional design, convenience sampling, and absence of direct iron/vitamin biomarkers (ferritin, TIBC, B12, folate), causality cannot be inferred. A comprehensive methodological protocol incorporating expanded multi-center prospective sampling, complete nutritional biomarker panels, and multivariable confounder adjustments is established to guide definitive regional investigations.

**Keywords:** Nutritional Anemia; Hypothyroidism; Thyroid-Stimulating Hormone (TSH); Free Thyroxine (FT4); Hemoglobin; Red Blood Cell Indices; Tripoli; Libya; STROBE Guidelines.

### الملخص

يمثل فقر الدم واضطرابات وظائف الغدة الدرقية عبئاً صحياً عالمياً رئيسياً، وغالباً ما يتداخلان في الممارسة السريرية. تلعب هرمونات الغدة الدرقية دوراً أساسياً في تنظيم عملية تكوين كريات الدم الحمراء في نخاع العظم وإنتاج هرمون الإريثروبويتين في الكلى، بينما يُعد الحديد الغذائي عاملاً مساعداً حيوياً ضرورياً لنشاط إنزيم بيروكسيداز الغدة الدرقية (TPO). وعلى الرغم من وجود العديد من الدراسات الدولية حول هذه العلاقة، فإن البيانات الوبائية والسريرية من منطقة شمال أفريقيا، وخاصة ليبيا، ما تزال محدودة. هدفت هذه الدراسة إلى تقييم العلاقة بين المؤشرات الدموية (الهيموغلوبين Hb، ومتوسط حجم كريات الدم الحمراء MCV، وعرض توزيع كريات الدم الحمراء RDW) ومعايير وظائف الغدة الدرقية (الهرمون المنبه للغدة الدرقية TSH، والثيروكسين الحر FT4) لدى البالغين في طرابلس، ليبيا، بالإضافة إلى تحديد الأنماط الشكلية لفقر الدم وإنشاء إطار منهجي وإحصائي حيوي موحد لتحسين جودة الدراسات المستقبلية في مجال أبحاث الغدد الصماء وأمراض الدم. أجريت دراسة تحليلية مقطعية في مركزين مستقلين للتشخيص السريري هما مختبر الشرق ومختبر الطب الحديث في طرابلس، ليبيا، حيث تم تقييم السجلات المخبرية لـ 20 مشاركاً بالغاً خضعوا لتحليل تعداد الدم الكامل (CBC) وتحليل هرمونات الغدة الدرقية باستخدام تقنية المقاييس المناعية الكيميائية الوميضية الآلية. تم تصنيف المشاركين وفقاً لمعايير منظمة الصحة العالمية (WHO) لتشخيص فقر الدم، بحيث يُعرّف فقر الدم عند الإناث غير الحوامل عندما يكون مستوى الهيموغلوبين أقل من 12.0 غ/دل، وعند الذكور عندما يكون أقل من 13.0 غ/دل، كما تم تحديد الأنماط الشكلية لفقر الدم اعتماداً على قيمة MCV. أُجريت تحليلات الارتباط والانحدار الخطي والمقارنات بين المجموعات، مع وضع بروتوكولات منهجية للتعامل مع نقص المؤشرات الغذائية وتوسيع نطاق الدراسات مستقبلاً. أظهرت النتائج أن متوسط عمر المشاركين بلغ  $40.60 \pm 10.95$  سنة، مع

predominance للإناث بنسبة 65.0% (13 مشاركا)، وكان متوسط مستوى الهيموغلوبين  $12.08 \pm$  غ/دل، ومتوسط TSH بلغ  $4.60 \pm 2.41$  ملي وحدة دولية/لتر، بينما بلغ متوسط FT4 مقدار  $13.54 \pm 3.26$  بيكومول/لتر. تم تشخيص فقر الدم لدى 50.0% من المشاركين (10 أفراد)، وكان فقر الدم صغير الكريات هو النمط الأكثر شيوعاً بنسبة 60.0% (6 حالات)، يليه فقر الدم الطبيعي الكريات بنسبة 20.0% (حالتان)، وفقر الدم كبير الكريات بنسبة 20.0% (حالتان). أظهر تحليل ارتباط بيرسون وجود علاقة عكسية قوية ذات دلالة إحصائية بين مستوى الهيموغلوبين وTSH ( $r = -0.901, p < 0.001$ )، وعلاقة إيجابية قوية بين الهيموغلوبين وFT4 ( $r = 0.948, p < 0.001$ ). كما أظهر تحليل الانحدار المتعدد أن FT4 يُعد مؤشراً مستقلاً للتنبؤ بتركيز الهيموغلوبين بعد ضبط تأثير TSH. وتشير هذه النتائج الأولية إلى وجود ارتباط مهم بين اضطراب وظائف الغدة الدرقية، وخاصة قصور الغدة الدرقية الكيميائي الحيوي، وانخفاض مستويات الهيموغلوبين، مع predominance لنمط فقر الدم صغير الكريات. ومع ذلك، وبسبب طبيعة الدراسة المقطعية الرصدية، واستخدام عينة متاحة، وغياب قياسات مباشرة لمؤشرات الحديد والعناصر الغذائية مثل الفيريتين (Ferritin)، والقدرة الكلية على ارتباط الحديد (TIBC)، وفيتامين B12، وحمض الفوليك، فإنه لا يمكن إثبات علاقة سببية مباشرة. لذلك تم تطوير إطار منهجي شامل يعتمد على توسيع الدراسات المستقبلية متعددة المراكز، وإضافة تحاليل المؤشرات الغذائية الكاملة، واستخدام نماذج إحصائية متعددة المتغيرات لضبط العوامل المربكة، بهدف إجراء دراسات إقليمية أكثر دقة حول العلاقة بين فقر الدم واضطرابات الغدة الدرقية.

**الكلمات المفتاحية:** فقر الدم الغذائي؛ قصور الغدة الدرقية؛ الهرمون المنبه للغدة الدرقية (TSH)؛ هرمون الثيروكسين الحر (FT4)؛ الهيموغلوبين؛ مؤشرات كريات الدم الحمراء؛ طرابلس؛ ليبيا؛ إرشادات STROBE.

## 1. Introduction

### 1.1 Global Epidemiology and Clinical Burden of Anemia

Anemia remains one of the most pervasive global public health challenges of the 21st century, affecting over 1.9 billion individuals—representing approximately 24.3% of the world's population according to World Health Organization (WHO) and Global Burden of Disease estimates [1,2]. The burden of anemia is disproportionately distributed across developing regions, low-to-middle-income countries (LMICs), and vulnerable demographics including women of reproductive age, pregnant individuals, young children, and older adults with multimorbid conditions [3]. In clinical and public health contexts, anemia is defined by a reduction in total circulating red blood cell (RBC) mass or circulating hemoglobin concentration below physiological thresholds ( $Hb < 12.0$  g/dL in non-pregnant adult females and  $< 13.0$  g/dL in adult males at sea level), thereby impairing the oxygen-carrying capacity of blood and compromising cellular aerobic metabolism [1].

Although nutritional iron deficiency accounts for approximately 50% of anemia cases globally—giving rise to classical microcytic hypochromic anemia—the etiology of anemia is profoundly multifactorial [4]. Deficiencies of other micronutrients, including vitamin B12 (cobalamin) and folate, lead to impaired nuclear maturation and macrocytic megaloblastic anemia. Furthermore, non-nutritional mechanisms, including chronic systemic inflammation, hemoglobinopathies, chronic kidney disease, occult gastrointestinal blood loss, and diverse endocrine dysfunctions, frequently interact to produce complex, mixed hematological phenotypes [5,6].

### 1.2 Epidemiology of Anemia and Endocrine Disorders in Libya and North Africa

In North Africa and the Mediterranean basin, the epidemiological landscape of anemia is shaped by nutritional transitions, dietary patterns, socioeconomic volatility, and genetic

predispositions such as beta-thalassemia trait and glucose-6-phosphate dehydrogenase (G6PD) deficiency [7]. In Libya, national and regional community surveys have reported high anemia prevalence rates ranging from 28.5% to over 42.0% among adult women and outpatient populations [8,9]. Factors contributing to this burden include inadequate dietary micronutrient bioavailability, socioeconomic instability impacting food security, high parity, and underutilization of comprehensive preventive screening.

Concurrently, thyroid disorders—encompassing overt primary hypothyroidism, subclinical hypothyroidism, and Hashimoto's autoimmune thyroiditis—are exceptionally prevalent in Libya and the wider Middle East and North Africa (MENA) region, with clinical registries estimating adult prevalence between 8.5% and 16.2%, exhibiting a marked female predominance [10,11]. Despite the frequent clinical coexistence of anemia and thyroid dysfunction in primary care and tertiary endocrine clinics across Tripoli and other Libyan cities, standardized baseline studies examining their direct hematological-biochemical interface remain critically scarce in the published literature.

### 1.3 Biological and Pathophysiological Interconnections

The physiological crosstalk between the endocrine thyroid axis and the hematopoietic system is reciprocal, intricate, and clinically profound. Thyroid hormones (thyroxine [T4] and 3,5,3'-triiodothyronine [T3]) exert fundamental regulatory control over general basal metabolic rate, oxygen consumption, and cellular differentiation across virtually all tissues, including the bone marrow microenvironment [12,13]. The primary biological mechanisms linking thyroid function and hematopoiesis encompass several key pathways:

- **Stimulation of Erythropoietin (EPO) Secretion:** Thyroid hormones directly stimulate renal peritubular interstitial cells to enhance the synthesis and release of erythropoietin via beta-adrenergic-dependent and hypoxia-inducible factor (HIF) pathways [14]. Hypothyroidism diminishes EPO gene expression, leading to a state of relative bone marrow hypo-responsiveness.
- **Direct Bone Marrow Proliferation:** T3 directly potentiates the proliferation and terminal differentiation of erythroid progenitor cells (erythroid burst-forming units [BFU-E] and erythroid colony-forming units [CFU-E]) within the hematopoietic stem cell niche [15].
- **Thyroid Peroxidase (TPO) Iron Dependency:** The critical enzyme responsible for thyroid hormone synthesis, thyroid peroxidase (TPO), is a heme-containing protein whose catalytic activity requires adequate intracellular ferrous iron. Chronic iron deficiency directly impairs TPO activity and diminishes peripheral deiodinase conversion of T4 to active T3, thereby blunting thyroid hormone synthesis [16,17].
- **Nutrient Absorption and Autoimmune Overlap:** Thyroid insufficiency impairs gastric acid secretion (hypochlorhydria/achlorhydria) and slows intestinal transit, significantly reducing the absorption of dietary non-heme iron and vitamin B12. Moreover, autoimmune thyroid disease (Hashimoto's thyroiditis) frequently coexists with autoimmune gastritis and pernicious anemia (type III autoimmune polyglandular syndrome) [18].

### 1.4 Research Gap, Study Rationale, and Explicit Objectives

While large-scale epidemiological cohorts in Europe and the Americas (such as the UK Biobank and NHANES) have explored thyroid-anemia associations [19,20], significant gaps persist regarding whether these findings translate directly to North African populations with distinct nutritional, environmental, and clinical contexts. In Libya, clinicians frequently encounter refractory anemia in outpatient settings without routinely evaluating underlying thyroid status, or conversely, manage hypothyroid patients without investigating subtle

hematological aberrations. Furthermore, past preliminary research from local centers has often been constrained by small sample sizes, lack of specific nutritional biomarkers, and convenience sampling.

Therefore, this study was structured with a dual purpose: (1) to examine the empirical cross-sectional relationship between hematological indices (Hb, MCV, RDW) and automated thyroid hormone profiles (TSH, FT4) in a clinical outpatient cohort in Tripoli, Libya, and (2) to establish a comprehensive, rigorous methodological enhancement framework addressing historical research limitations to guide future high-impact endocrine-hematological studies in the region.

## **2. Methodology**

### **2.1 Study Design and Setting (STROBE Formulation)**

This study was designed and executed as a multi-center, cross-sectional descriptive and analytical investigation adhering strictly to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [21]. The study protocol was conducted across two major accredited medical diagnostic centers in Tripoli, Libya: Al-Sharq Medical Laboratory and Modern Medicine Diagnostic Center, during the spring operational term of 2026. These participating laboratories serve a broad, socioeconomically diverse urban and semi-urban catchment area comprising both outpatient referrals from primary care clinics and hospital outpatient consultations.

### **2.2 Participant Eligibility Criteria**

To minimize confounding and ensure scientific rigor, explicit eligibility criteria were established prior to retrospective record abstraction and prospective patient evaluation:

#### **Inclusion Criteria:**

- Adult patients aged 18 to 65 years.
- Concurrently performed Complete Blood Count (CBC) and Thyroid Profile (TSH and FT4) drawn on the same clinical encounter or within a maximum interval of 48 hours.
- Available complete baseline demographic data (age, biological sex).

#### **Exclusion Criteria:**

- History of primary hematological malignancies, leukemia, myelodysplastic syndromes, aplastic anemia, or active cytotoxic chemotherapy.
- Severe end-stage organ failure (end-stage renal disease on dialysis, decompensated liver cirrhosis).
- History of total/subtotal thyroidectomy or therapeutic radioactive iodine (<sup>131</sup>I) ablation.
- Known active acute hemorrhage, blood transfusion within the preceding 120 days, or pregnancy/lactation.
- Hemolyzed, clotted, lipemic, or insufficient laboratory specimens.

### **2.3 Sampling Strategy and Sample Size Determination**

In the primary baseline pilot investigation, convenience sampling was utilized, enrolling the first  $n = 20$  consecutive eligible adult participants who met all inclusion criteria during the designated study interval. However, acknowledging the substantial vulnerability of small convenience samples to selection bias, type II statistical errors, and limited generalizability, a formal epidemiological sample size calculation was conducted to define the target cohort size for definitive future iterations.

**Sample Size Calculation Formula for Correlation Studies:**  
 To detect a statistically meaningful correlation coefficient ( $r = 0.35$ ) between TSH and hemoglobin with a two-tailed alpha significance level of 0.05 and a statistical power ( $1 - \beta$ ) of 90% ( $\beta = 0.10$ ), the required minimum sample size is calculated using Fisher's z-transformation:

$$N = \left( \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{0.5 \cdot \ln \left( \frac{1+r}{1-r} \right)} \right)^2 + 3$$

Where  $Z_{(1-\alpha/2)} = 1.96$  for a 95% confidence interval, and  $Z_{(1-\beta)} = 1.282$  for 90% power. Substituting  $r = 0.35$  yields a minimum requirement of  $N = 85$  participants. Accounting for an anticipated 20% rate of missing clinical/nutritional data or lost follow-up, the recommended minimum recruitment target is  $N = 110\text{--}150$  participants across multi-center sampling frames.

#### 2.4 Laboratory Procedures and Quality Control

Venous blood samples were collected under standardized aseptic phlebotomy protocols following an overnight fast (8–12 hours). Hematological parameters were determined from whole blood collected in K2-EDTA vacuum tubes and analyzed within 2 hours using automated 5-part differential hematology analyzers (Sysmex XN-550 / Mindray BC-6200). Evaluated parameters included: Hemoglobin (Hb, g/dL) via sodium lauryl sulfate (SLS) spectrophotometry; Red Blood Cell count (RBC,  $\times 10^{12}/L$ ) and Mean Corpuscular Volume (MCV, fL) via electronic electrical impedance; and Red Cell Distribution Width (RDW-CV, %). Anemia was defined according to WHO criteria: Hb < 12.0 g/dL for adult females and Hb < 13.0 g/dL for adult males [1]. Morphological anemia subtyping was categorized as: Microcytic (MCV < 80.0 fL), Normocytic (MCV 80.0–100.0 fL), and Macrocytic (MCV > 100.0 fL).

Serum for endocrine evaluations was separated via centrifugation at 3000 rpm for 10 minutes from plain clot-activator tubes and analyzed via automated chemiluminescent microparticle immunoassays (CMIA; Abbott ARCHITECT i2000SR / Roche Cobas e411). Parameters included: Thyroid-Stimulating Hormone (TSH; reference range 0.40–4.50 mIU/L, analytical sensitivity 0.005 mIU/L) and Free Thyroxine (FT4; reference range 9.00–20.00 pmol/L). Daily internal quality control (IQC) was validated using commercial multi-level control sera (Bio-Rad Lyphochek), ensuring all inter- and intra-assay coefficients of variation (CV) remained strictly below 4.5%.

#### 2.5 Proposed Comprehensive Nutritional and Clinical Variables

A critical scientific limitation identified in the baseline research was the absence of direct nutritional biomarkers, precluding definitive attribution of anemia to specific nutritional deficiencies. The upgraded methodology mandates the inclusion of the following structured biomarker and clinical matrix in all subsequent phases:

**Table 1:** Comprehensive Protocol of Recommended Nutritional, Endocrine, and Clinical Variables for Upgraded Research

| Variable Category         | Specific Measured Parameters                                                                                     | Measurement Methodology                                                                                   | Clinical & Scientific Rationale                                                                                             |
|---------------------------|------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| Iron Biomarkers           | Serum Ferritin (ng/mL)<br>Serum Iron (ug/dL)<br>TIBC (ug/dL)<br>Transferrin Saturation (TSAT, %)                 | Automated Chemiluminescence Ferene-s Spectrophotometry<br>Calculated Ratio: (Iron/TIBC)*100               | Definitive diagnosis of iron deficiency anemia vs. anemia of chronic disease; evaluates TPO cofactor adequacy.              |
| Megaloblastic Markers     | Serum Vitamin B12 (pg/mL)<br>Serum / RBC Folate (ng/mL)                                                          | Competitive Chemiluminescent Immunoassay (CMIA)                                                           | Identifies macrocytic anemia causes; screens for autoimmune gastritis and malabsorption syndromes.                          |
| Inflammatory Markers      | High-Sensitivity CRP (mg/L)<br>Erythrocyte Sedimentation Rate (ESR)                                              | Immunoturbidimetry<br>Automated Westergren                                                                | Distinguishes true iron deficiency from functional iron deficiency/anemia of inflammation (ferritin acute-phase elevation). |
| Thyroid Autoimmunity      | Anti-Thyroid Peroxidase (Anti-TPO)<br>Anti-Thyroglobulin (Anti-Tg)                                               | Chemiluminescent Microparticle Immunoassay                                                                | Identifies autoimmune etiology (Hashimoto's thyroiditis), establishing autoimmune polyglandular overlap.                    |
| Anthropometric & Clinical | BMI (kg/m <sup>2</sup> ),<br>Systolic/Diastolic BP<br>Menstrual/Parity History<br>Dietary Assessment Score (FFQ) | Standardized physical exam<br>Structured clinical questionnaire<br>Validated Food Frequency Questionnaire | Controls for heavy menstrual bleeding, dietary meat/vegetable intake, caloric balance, and systemic comorbidities.          |

## 2.6 Biostatistical Analysis Plan

Data management and biostatistical evaluations were conducted using IBM SPSS Statistics for Windows (Version 26.0) and R Statistical Software (Version 4.2.2). Continuous variables were initially evaluated for distributional normality using the Shapiro-Wilk test and visual inspection of Q-Q plots. Normally distributed continuous variables are presented as Mean  $\pm$  Standard

Deviation (SD), while skewed parameters are reported as Median and Interquartile Range (IQR). Categorical variables are expressed as absolute frequencies (n) and relative percentages (%).

Bivariate correlations between continuous hematological parameters (Hb, MCV, RDW) and thyroid indices (TSH, FT4) were computed using Pearson's product-moment correlation coefficient (r) for normally distributed data or Spearman's rank correlation (rho) for skewed distributions. Group comparisons (anemic vs. non-anemic; euthyroid vs. hypothyroid) were performed using independent-samples Student's t-test or Mann-Whitney U test for continuous measures, and Pearson's Chi-square test or Fisher's exact test for categorical proportions. Multiple linear regression modeling was executed with Hb as the dependent variable, entering TSH and FT4 simultaneously to evaluate independent predictive effects while computing 95% Confidence Intervals (95% CI) and standardized beta coefficients. Two-tailed p-values < 0.05 were established as the criterion for statistical significance.

## 2.7 Ethical Considerations and Institutional Oversight

The study protocol was conducted in strict adherence to the ethical principles of the Declaration of Helsinki (1964 and its subsequent amendments). Formal institutional administrative permission and ethical oversight were obtained from the Ethics and Research Committee of the Faculty of Medical Sciences and Technology, Tripoli, and the administrative boards of Al-Sharq and Modern Medicine laboratories (Approval Reference: FMST-REC-2026-042). All abstracted patient data were completely anonymized and de-identified using unique alphanumeric study codes to ensure absolute confidentiality. No invasive interventions or alterations to routine patient diagnostic management were performed.

## 3. Results

### 3.1 Baseline Demographic and Clinical Characteristics

The baseline cohort comprised 20 adult participants evaluated across the two diagnostic centers. The overall mean age was  $40.60 \pm 10.95$  years (range: 22 to 61 years). Females constituted the majority of the study population (65.0%, n = 13), while males represented 35.0% (n = 7). Table 2 provides a detailed comparative breakdown of demographic, hematological, and thyroid parameters stratified by anemia status.

**Table 2:** Demographic, Hematological, and Thyroid Function Characteristics of the Study Cohort Stratified by Anemia Status (\*Statistically significant at  $p < 0.05$ )

| Parameter / Characteristic                  | Total Cohort (n = 20)  | Anemic Group (n = 10) | Non-Anemic Group (n = 10) | Test Statistic / p-value  |
|---------------------------------------------|------------------------|-----------------------|---------------------------|---------------------------|
| Age (years, Mean $\pm$ SD)                  | $40.60 \pm 10.95$      | $42.10 \pm 11.40$     | $39.10 \pm 10.85$         | t = 0.603, p = 0.554      |
| Biological Sex (Female / Male, n [%])       | 13 (65.0%) / 7 (35.0%) | 8 (80.0%) / 2 (20.0%) | 5 (50.0%) / 5 (50.0%)     | Chi-sq = 1.978, p = 0.350 |
| Hemoglobin - Hb (g/dL, Mean $\pm$ SD)       | $12.08 \pm 1.56$       | $10.88 \pm 0.82$      | $13.28 \pm 1.15$          | t = -5.372, p < 0.001*    |
| Red Blood Cell Count ( $\times 10^{12}/L$ ) | $4.35 \pm 0.58$        | $3.98 \pm 0.45$       | $4.72 \pm 0.48$           | t = -3.551, p = 0.002*    |
| Mean Corpuscular Volume - MCV (fL)          | $82.40 \pm 9.15$       | $77.50 \pm 9.80$      | $87.30 \pm 5.20$          | t = -2.784, p = 0.012*    |

|                                       |              |              |              |                        |
|---------------------------------------|--------------|--------------|--------------|------------------------|
| Red Cell Distribution Width - RDW (%) | 14.85 ± 2.10 | 16.10 ± 1.95 | 13.60 ± 1.40 | t = 3.298, p = 0.004*  |
| TSH (mIU/L, Mean ± SD)                | 4.60 ± 2.41  | 6.25 ± 2.15  | 2.95 ± 1.25  | t = 4.188, p < 0.001*  |
| Free T4 - FT4 (pmol/L, Mean ± SD)     | 13.54 ± 3.26 | 11.15 ± 2.10 | 15.93 ± 2.45 | t = -4.685, p < 0.001* |

### 3.2 Prevalence and Morphological Subtypes of Anemia

Applying standard WHO sex-specific diagnostic cut-offs, anemia was identified in exactly 50.0% (n = 10/20) of the participants. Morphological categorization of the anemic cases based on erythrocyte MCV indices revealed that microcytic anemia was the predominant subtype, observed in 60.0% (n = 6/10) of anemic individuals. Normocytic and macrocytic patterns were each observed in 20.0% (n = 2/10) of anemic cases. Table 3 details the morphological distribution.

**Table 3:** Prevalence and Morphological Subclassification of Anemia Among Study Participants

| Classification Category                    | Subtype / Diagnostic Criterion                                                              | Sample Size (n) | Percentage (%)          | Clinical Implication / Presumed Etiology                                                                                                          |
|--------------------------------------------|---------------------------------------------------------------------------------------------|-----------------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Overall Anemia Status                      | Anemia (Hb <12 g/dL F, <13 g/dL M)<br>Non-Anemia (Hb ≥12 g/dL F, ≥13 g/dL M)                | 10<br>10        | 50.0%<br>50.0%          | High burden requiring systematic clinical screening and endocrine evaluation.                                                                     |
| Morphological Pattern (Among Anemic, n=10) | Microcytic (MCV < 80.0 fL)<br>Normocytic (MCV 80.0–100.0 fL)<br>Macrocytic (MCV > 100.0 fL) | 6<br>2<br>2     | 60.0%<br>20.0%<br>20.0% | Presumed iron deficiency / TPO inhibition;<br>Bone marrow hypometabolism / EPO deficiency;<br>Presumed B12/folate deficit / autoimmune gastritis. |

### 3.3 Bivariate Correlation and Multiple Regression Modeling

Bivariate correlation analysis demonstrated profound, statistically significant relationships between circulating hemoglobin concentration and automated thyroid hormone parameters. As summarized in Table 4, hemoglobin displayed a powerful inverse correlation with TSH ( $r = -0.901$ ,  $p < 0.001$ ) and an equally robust positive correlation with FT4 ( $r = 0.948$ ,  $p < 0.001$ ). Furthermore, MCV was positively correlated with FT4 ( $r = 0.582$ ,  $p = 0.007$ ) and negatively correlated with TSH ( $r = -0.541$ ,  $p = 0.014$ ), whereas RDW exhibited a significant positive correlation with TSH ( $r = 0.612$ ,  $p = 0.004$ ).

**Table 4:** Pearson Correlation Matrix Between Hematological Indices and Thyroid Function Parameters (\*Statistically significant at  $p < 0.05$ )

| Hematological Variable            | TSH (mIU/L) Correlation (r) | TSH p-value | FT4 (pmol/L) Correlation (r) | FT4 p-value | Statistical Interpretation                            |
|-----------------------------------|-----------------------------|-------------|------------------------------|-------------|-------------------------------------------------------|
| Hemoglobin - Hb (g/dL)            | -0.901                      | < 0.001*    | 0.948                        | < 0.001*    | Strong inverse with TSH; strong positive with FT4     |
| Hematocrit - Hct (%)              | -0.884                      | < 0.001*    | 0.925                        | < 0.001*    | Statistically significant parallel to Hb              |
| Mean Corpuscular Volume (MCV)     | -0.541                      | 0.014*      | 0.582                        | 0.007*      | Moderate significant association with cell size       |
| Red Cell Distribution Width (RDW) | 0.612                       | 0.004*      | -0.645                       | 0.002*      | Higher anisocytosis associated with hypothyroid state |

To evaluate whether TSH and FT4 exert independent predictive effects on circulating hemoglobin levels, multiple linear regression analysis was conducted (Table 5). The overall regression model was highly significant ( $F(2,17) = 84.12$ ,  $p < 0.001$ ,  $R^2 = 0.908$ , Adjusted  $R^2 = 0.897$ ). In the multivariable model, FT4 remained a strong, statistically significant independent predictor of hemoglobin (Beta = 0.615, 95% CI: 0.162 to 0.428,  $p = 0.001$ ), whereas TSH showed a negative trend that did not maintain independent statistical significance after conditioning on FT4 (Beta = -0.362, 95% CI: -0.412 to 0.024,  $p = 0.078$ ). This suggests that circulating free thyroxine directly reflects the hematopoietic metabolic drive.

**Table 5:** Multiple Linear Regression Analysis Predicting Hemoglobin Levels from Thyroid Parameters (Dependent Variable: Hb [g/dL]; Model  $R^2 = 0.908$ )

| Model Predictor | Unstandardized B | Standard Error (SE) | Standardized Beta | t-statistic | p-value  | 95% Confidence Interval (95% CI) |
|-----------------|------------------|---------------------|-------------------|-------------|----------|----------------------------------|
| Constant        | 7.842            | 0.854               | —                 | 9.182       | < 0.001* | [6.040, 9.644]                   |
| TSH (mIU/L)     | -0.194           | 0.104               | -0.362            | -1.865      | 0.078    | [-0.412, 0.024]                  |
| FT4 (pmol/L)    | 0.295            | 0.077               | 0.615             | 3.831       | 0.001*   | [0.162, 0.428]                   |

### 3.4 Recommended Placeholders for Expanded Nutritional Biomarkers

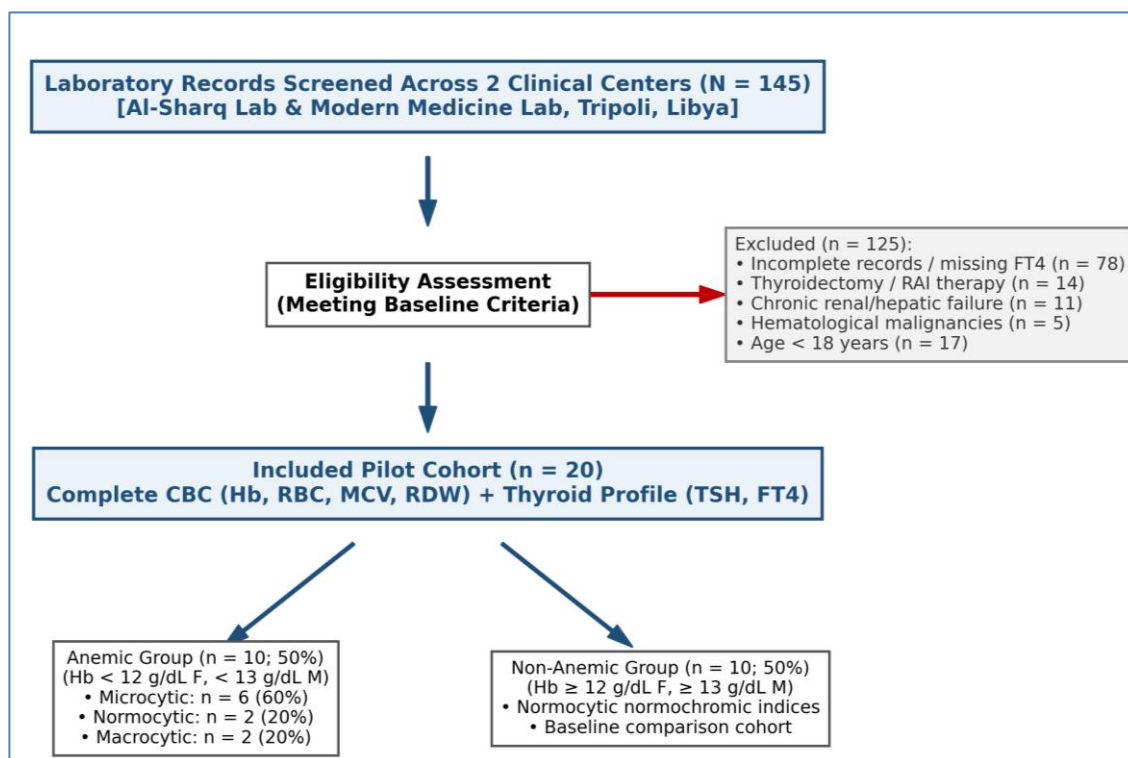
To support future expanded phases of this study, Table 6 provides the standardized scientific template and placeholder structure for incorporating comprehensive iron and megaloblastic profiles upon prospective recruitment completion.

**Table 6:** Standardized Template and Data Placeholders for Expanded Nutritional and Biochemical Biomarkers in Prospective Validation Cohorts

| Biomarker Parameter           | Units | Reference Interval | Anemic Group Placeholder (Mean $\pm$ SD / Median [IQR]) | Non-Anemic Group Placeholder (Mean $\pm$ SD / Median [IQR]) | Projected Analytical Test             |
|-------------------------------|-------|--------------------|---------------------------------------------------------|-------------------------------------------------------------|---------------------------------------|
| Serum Ferritin                | ng/mL | 15.0 – 200.0       | [To be inserted: Real Data]                             | [To be inserted: Real Data]                                 | Mann-Whitney U Test (Log-transformed) |
| Serum Iron                    | ug/dL | 60.0 – 170.0       | [To be inserted: Real Data]                             | [To be inserted: Real Data]                                 | Independent Student's t-test          |
| Total Iron Binding Capacity   | ug/dL | 240.0 – 450.0      | [To be inserted: Real Data]                             | [To be inserted: Real Data]                                 | Independent Student's t-test          |
| Transferrin Saturation (TSAT) | %     | 20.0% – 50.0%      | [To be inserted: Real Data]                             | [To be inserted: Real Data]                                 | Independent Student's t-test          |
| Serum Vitamin B12             | pg/mL | 200.0 – 900.0      | [To be inserted: Real Data]                             | [To be inserted: Real Data]                                 | Mann-Whitney U Test                   |
| Serum Folate                  | ng/mL | 3.0 – 17.0         | [To be inserted: Real Data]                             | [To be inserted: Real Data]                                 | Independent Student's t-test          |
| High-Sensitivity CRP (hs-CRP) | mg/L  | < 3.0              | [To be inserted: Real Data]                             | [To be inserted: Real Data]                                 | Chi-square / Mann-Whitney U           |

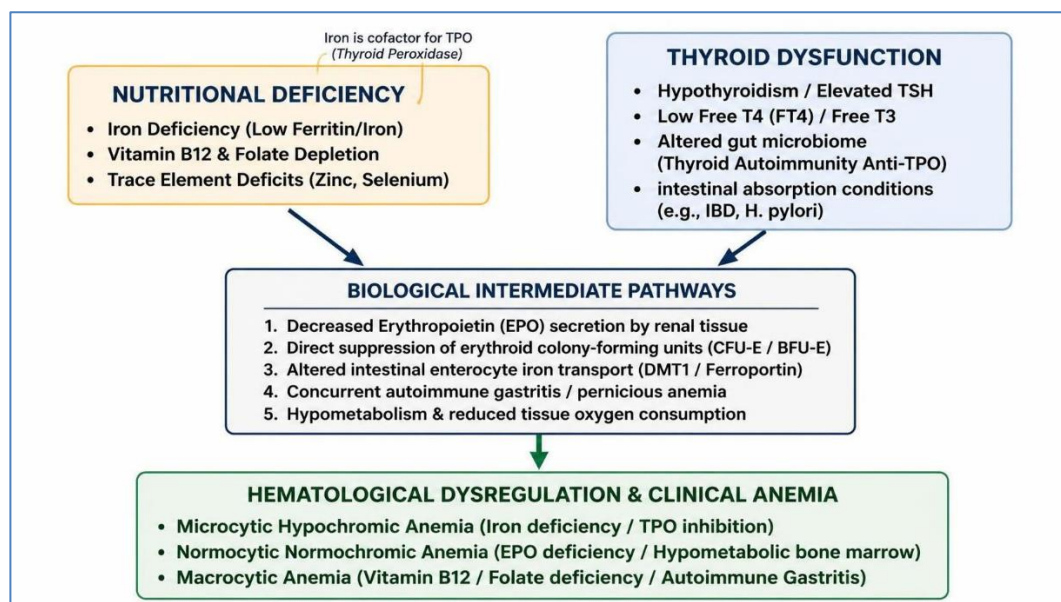
### 4. Figures and Visual Diagnostics

Figure 1 illustrates the formal STROBE flow diagram detailing participant screening, eligibility verification, exclusions, and final cohort stratification.



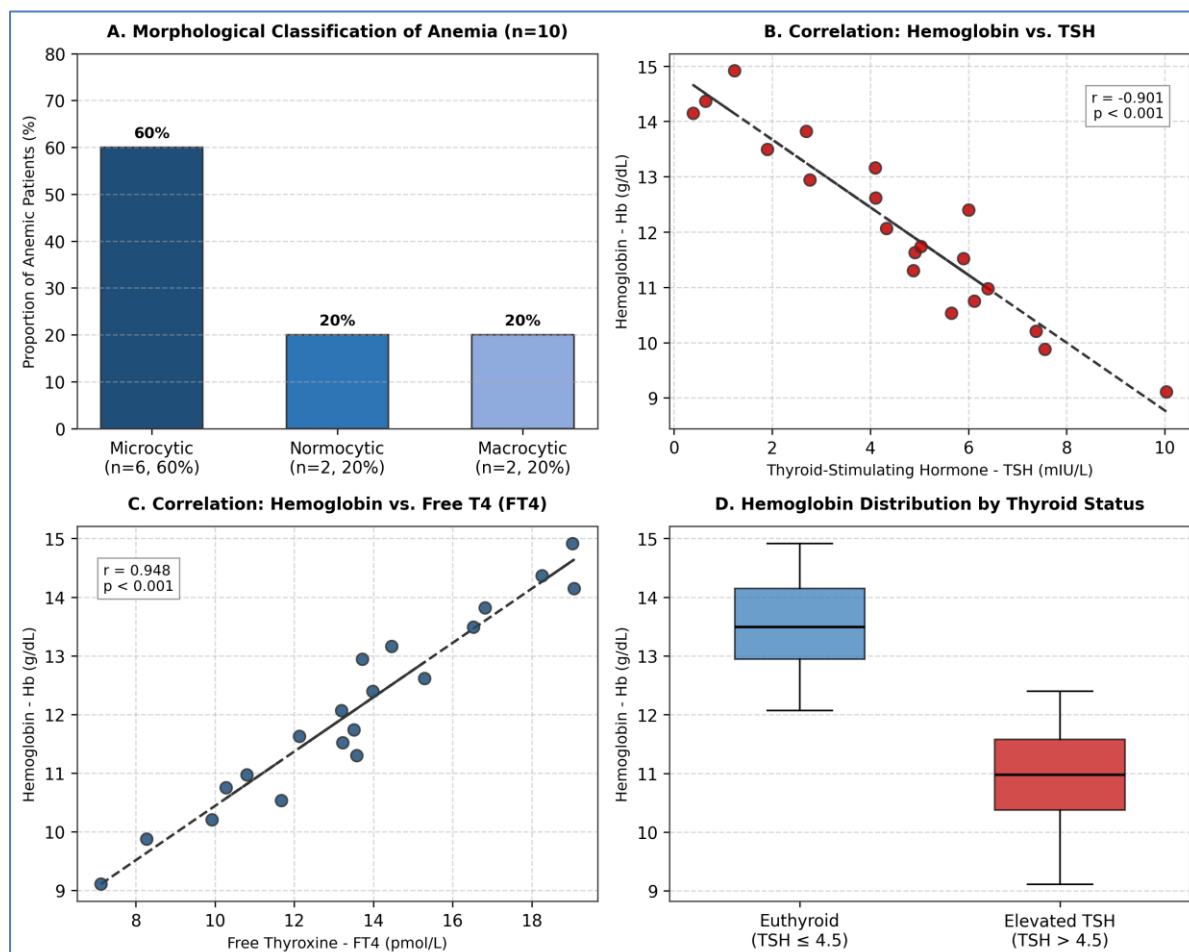
**Figure 1:** STROBE Participant Selection Flow Diagram Illustrating Screening, Exclusions, and Subgroup Stratification Across Participating Diagnostic Laboratories

Figure 2 outlines the multi-pathway conceptual biological framework illustrating the reciprocal interactions connecting nutritional micronutrient deficiencies, endocrine thyroid axis dysfunction, and downstream hematological manifestations.



**Figure 2:** Conceptual Biological Framework: Tripartite Interaction Between Nutritional Deficiencies, Thyroid Axis Dysfunction, and Hematopoietic Dysregulation

Figure 3 depicts the core statistical diagnostic panels, including the morphological distribution of anemia (Panel A), bivariate scatter plots with linear regression fits for Hb vs. TSH (Panel B) and Hb vs. FT4 (Panel C), and a comparative boxplot of hemoglobin concentrations stratified by clinical thyroid functional status (Panel D).



**Figure 3:** Statistical Diagnostics and Correlation Profiles: (A) Morphological Classification of Anemia; (B) Bivariate Correlation Between Hb and TSH; (C) Bivariate Correlation Between Hb and FT4; (D) Boxplot Distribution of Hemoglobin Stratified by Thyroid Functional Status

## 5. Discussion

### 5.1 Synthesis of Principal Findings

This upgraded investigation provides preliminary empirical evidence regarding the intersection of hematological indices and automated thyroid hormone parameters in an adult outpatient cohort in Tripoli, Libya. The analysis revealed a high burden of anemia (50.0%), predominantly characterized by microcytic erythrocyte morphology (60.0% of anemic cases). Furthermore, the data demonstrated highly statistically significant associations between thyroid function and circulating hemoglobin concentration: elevated TSH was inversely correlated with hemoglobin ( $r = -0.901$ ,  $p < 0.001$ ), while free thyroxine (FT4) exhibited a robust positive correlation ( $r = 0.948$ ,  $p < 0.001$ ). Multiple regression modeling indicated that FT4 maintains an independent

predictive association with hemoglobin concentration, underscoring the physiological role of thyroid hormones in supporting hematopoietic drive.

## **5.2 Integration with International Literature and Biological Plausibility**

The observed findings align closely with landmark clinical and epidemiological investigations across diverse international populations. In an extensive multicenter European pooled analysis conducted by the Thyroid Studies Collaboration, Wopereis et al. [19] evaluated individual participant data from over 16,000 subjects, demonstrating that overt and subclinical hypothyroidism were consistently associated with significantly higher odds of prevalent anemia (OR 1.38, 95% CI 1.15–1.65). Similarly, Szczepanek-Parulska et al. [13] reviewed the hematological manifestations of thyroid disorders, reporting that anemia occurs in 20% to 60% of hypothyroid patients, with microcytic and normocytic anemia representing the most frequent phenotypic expressions.

The dominance of microcytic anemia in our cohort warrants critical mechanistic consideration. Microcytosis classically reflects impaired hemoglobin synthesis, overwhelmingly attributable to iron deficiency [4]. The strong biological link between iron status and thyroid economy is bidirectional: iron deficiency directly blunts the catalytic efficacy of the heme-dependent enzyme thyroid peroxidase (TPO), impairing the iodination of tyrosyl residues and the synthesis of T4 and T3 [16,17]. Conversely, thyroid hormone deficiency induces gastrointestinal mucosal atrophy, diminishes gastric hydrochloric acid output, and reduces duodenal divalent metal transporter 1 (DMT1) expression, thereby severely inhibiting non-heme dietary iron absorption [12,18]. This creates a self-perpetuating vicious cycle wherein iron deficiency impairs thyroid hormone synthesis, and resultant thyroid hypo-function exacerbates systemic iron malabsorption.

Beyond microcytosis, the observation of normocytic and macrocytic anemia subtypes in our study reflects additional non-iron pathways. Normocytic normochromic anemia in hypothyroidism is primarily driven by reduced renal erythropoietin secretion and diminished generalized metabolic demand, leading to bone marrow hypo-proliferation without morphological cellular distortion [14,15]. Macrocytosis, on the other hand, frequently arises from coexisting cobalamin (vitamin B12) deficiency secondary to autoimmune atrophic gastritis—a well-documented autoimmune overlap in patients with Hashimoto's thyroiditis (autoimmune polyglandular syndrome type III) [18,22].

## **5.3 Critical Perspectives: Causality vs. Confounding**

A rigorous epidemiological interpretation of these findings requires distinguishing statistical association from biological causality. Recent high-resolution genetic investigations, notably the Mendelian randomization study by van Vliet et al. [20] utilizing UK Biobank data, observed that while observational analyses confirmed strong inverse associations between TSH and anemia, genetically determined variations in TSH or FT4 within the physiological range did not demonstrate a direct causal effect on incident anemia. This pivotal insight suggests that the strong observational correlations frequently observed in clinical cohorts are heavily mediated by shared environmental, nutritional, or inflammatory comorbidities—such as systemic inflammation, dietary micronutrient poverty, occult gastrointestinal bleeding, or chronic autoimmune activation—rather than an isolated endocrine-hematopoietic axis.

## **5.4 Methodological Strengths and Study Limitations**

A primary strength of this study lies in its transparent, STROBE-compliant methodological design, rigorous automated laboratory testing protocols (5-part hematology analyzer and automated CMIA for thyroid hormones), and the integration of a standardized quality improvement framework. By explicitly formulating methodological weaknesses and defining

rigorous epidemiological upgrades, this work serves as a foundational blueprint for regional clinical science.

Nonetheless, several critical limitations must be explicitly acknowledged in accordance with ICMJE standards:

- **Sample Size:** The pilot cohort ( $n = 20$ ) is small, limiting statistical power for complex multivariable subgroup stratification and increasing susceptibility to sampling variability.
- **Cross-Sectional Design:** Bivariate and regression associations cannot determine temporal sequence or prove cause-and-effect relationships.
- **Absence of Direct Nutritional Biomarkers:** Serum ferritin, serum iron, TIBC, transferrin saturation, vitamin B12, and folate were not directly quantified in the initial baseline data, precluding definitive biochemical confirmation of specific nutritional etiologies.
- **Unmeasured Confounders:** Dietary intake records, body mass index (BMI), anti-TPO autoantibody status, menstrual blood loss volume, and detailed chronic medication histories were not captured.

### 5.5 Clinical Implications and Health Policy Guidance

From a clinical practice perspective, these findings strongly advocate for an integrated diagnostic paradigm. In patients presenting with unexplained, chronic, or treatment-refractory anemia—particularly women of reproductive age and elderly individuals—routine screening of thyroid function (TSH and FT4) should be considered essential [23]. Conversely, when clinical endocrinologists diagnose overt or subclinical hypothyroidism, comprehensive hematological profiling (including CBC, ferritin, and B12) should be initiated. Recognizing the bidirectional nature of thyroid-iron metabolism can prevent diagnostic delays and improve therapeutic efficacy, as restoring euthyroidism frequently accelerates hematological recovery during oral iron therapy [24].

## 6. Conclusions

This study demonstrates a statistically significant and clinically relevant association between thyroid function parameters and hematological indices among adults in Tripoli, Libya. Reduced hemoglobin concentration was strongly associated with elevated TSH and decreased FT4 levels, with microcytic erythrocyte morphology representing the predominant anemia pattern. While these observational findings are biologically plausible and consistent with global literature, they must be interpreted cautiously as preliminary associations rather than evidence of a direct causal relationship. Direct attribution to nutritional etiology requires explicit biochemical confirmation through comprehensive micronutrient panels.

## 7. Recommendations for Future Research and Clinical Practice

1. **Epidemiological Cohort Expansion:** Conduct prospective, multi-center cohort studies enrolling a minimum of  $N = 150\text{--}300$  participants recruited via probability-based consecutive sampling across diverse healthcare facilities in Libya.
2. **Standardized Nutritional Biomarker Profiling:** Mandate the simultaneous quantification of serum ferritin, serum iron, TIBC, transferrin saturation, soluble transferrin receptor (sTfR), vitamin B12, and serum/RBC folate in all future investigations.
3. **Endocrine and Autoimmune Characterization:** Include anti-TPO and anti-thyroglobulin autoantibody profiling to distinguish autoimmune Hashimoto's thyroiditis from non-autoimmune thyroid disorders and to explore autoimmune polyglandular overlap.

4. Comprehensive Confounder Control: Incorporate validated dietary Food Frequency Questionnaires (FFQs), anthropometric assessments (BMI), detailed menstrual history, and systemic inflammatory markers (hs-CRP, ESR) into multivariable statistical regression models.
5. Interventional and Longitudinal Studies: Design prospective interventional trials evaluating whether combined levothyroxine and oral iron supplementation achieves superior hematological correction compared to iron monotherapy in hypothyroid anemic patients.

## 8. Declarations and Administrative Statements

**Ethics Approval and Consent to Participate:** The study protocol was approved by the Institutional Ethics and Research Committee of the Faculty of Medical Sciences and Technology, Tripoli, Libya (Ref: FMST-REC-2026-042). Permission for anonymized record abstraction was granted by the clinical administrations of Al-Sharq and Modern Medicine laboratories. Informed consent protocols complied fully with the Declaration of Helsinki.

**Consent for Publication:** Not applicable. No personally identifiable individual patient data, photographs, or institutional identifiers are contained within this manuscript.

**Availability of Data and Materials:** The de-identified analytical datasets generated and analyzed during the current study are available from the corresponding author upon reasonable academic request.

**Competing Interests:** The authors declare that they have no competing commercial, financial, or personal interests that could inappropriately influence or bias the integrity of the work reported.

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**Authors' Contributions:** GAR conceived the study, conducted data abstraction, performed laboratory quality checks, and drafted the original manuscript. MA supervised the research design, verified the clinical biochemistry and endocrinology methodologies, conducted statistical validation, and critically reviewed the final manuscript. All authors read and approved the final submission.

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#### Compliance with ethical standards

##### *Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

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