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Impact of Iron and Vitamin B 12 Deficiency on Hemoglobin Levels and Complete Blood Count Indices: A Laboratory-Based Study in Surman , Libya

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Abstract
Iron deficiency remains the leading global cause of anemia; however , the relative contribution of vitamin B 12 deficiency varies acro ss populations , This laboratory-based cross-sectional study evaluated the association between ferritin , vitamin B 12 , and hemoglobin levels among 150 anemia cases identified from Surman Central Laboratory records in Surman , Libya , Microcytosis was the dominant hematological pattern (mean MCV = 79.83 fL) , Multivariate regression revealed that ferritin was independently associated with hemoglobin ($\beta = 0.412$, $p < 0.001$) , whereas vitamin B 12 showed no significant effect , The model explained 17.1 % of hemoglobin variance , These findings highlight iron deficiency as the principal determinant of anemia in this setting.
Keywords: Iron deficiency anemia; Vitamin B 12 deficiency; Hemoglobin; Ferritin; CBC indices; Libya

الملخص:
يُعد نقص الحديد السـبب الرئيسـي عالميًا لفقر الدم، بينما يختلف دور نقص فيتامين B 12 باختلاف المجتمعات , هدفت هذه الدراسـة المقطعية المعتمدة على بيانات مختبرية إلى تقييم العلاقة بين الفيريتين وفيتامين B 12 ومسـتوى الهيموغلوبين لدى 150 مراجعًا في صـرمان – ليبيا , كان صـغر الكريات النمط الدموي السـائد , أظهر تحليل الانحدار أن الفيريتين يرتبط بشـكل مسـتقل بالهيموغلوبين، في حين لم يظهر فيتامين B 12 تأثيرًا معنويًا , تؤكد النتائج أن نقص الحديد هو المحدد الأساسي لفقر الدم في هذه البيئة.

Introduction

Anemia remains one of the most widespread hematological disorders globally and continues to represent a significant challenge for healthcare systems due to its impact on oxygen transport , tissue perfusion , and overall physiological function , It is clinically defined as a reduction in hemoglobin concentration below established reference values adjusted for age and sex , Current global estimates indicate that anemia affects a substantial proportion of the world's population , particularly women of reproductive age , children , and vulnerable groups , reflecting the interaction between nutritional deficiencies , chronic diseases , and socioeconomic factors (World Health Organization , 2023; GBD 2021 Anaemia Collaborators , 2023).

Among the various etiological factors , iron deficiency is recognized as the leading cause of anemia worldwide , Iron is an essential component of hemoglobin and plays a critical role in erythropoiesis , Inadequate iron availability impairs hemoglobin synthesis , leading to characteristic hematological changes , including reduced mean corpuscular volume (MCV) , decreased mean corpuscular hemoglobin (MCH) , and increased red cell distribution width (RDW) , collectively reflecting microcytic and hypochromic patterns (Warner & Kamran ,

2023; Camaschella , 2019) , Serum ferritin is widely accepted as a reliable indicator of body iron stores and is frequently used in conjunction with complete blood count (CBC) parameters to confirm iron deficiency states (World Health Organization , 2023).
Vitamin B 12 constitutes another essential micronutrient involved in DNA synthesis and normal red blood cell maturation , Deficiency of vitamin B 12 disrupts cellular replication within the bone marrow , resulting in ineffective erythropoiesis and the development of megaloblastic anemia , This condition is typically associated with macrocytosis and elevated MCV values , and in some cases may present with neurological manifestations preceding hematological abnormalities (Ankar & Kumar , 2022; Smith et al. , 2018) , Variability in diagnostic cut-off levels for vitamin B 12 deficiency across different populations further complicates laboratory interpretation (Aparicio-Ugarriza et al. , 2020).
Although iron deficiency anemia and vitamin B 12 deficiency anemia are traditionally characterized by distinct hematological patterns , overlapping or mixed deficiencies may obscure classical laboratory findings , In such cases , reliance solely on MCV values may lead to misclassification , particularly when iron deficiency coexists with vitamin B 12 deficiency (Balci et al. , 2016) , Dietary patterns also influence the risk of micronutrient deficiencies , especially in populations with limited intake of animal-source foods or increased reliance on plant-based diets , where both iron bioavailability and vitamin B 12 intake may be compromised (Bakaloudi et al. , 2021; Pellinen et al. , 2022).
Regional studies from Middle Eastern and North African contexts have demonstrated a considerable burden of nutritional anemia , particularly among women and specific age groups (Abbas et al. , 2020; Al-Qahtani et al. , 2021) , What is the distribution of anemia-related CBC patterns among cases meeting anemia criteria among individuals attending Surman Central Laboratory ? (Al-Amri et al. , 2020; Salih et al. , 2022) , These findings suggest that micronutrient-related anemia remains a relevant clinical concern within the Libyan setting.
The complete blood count (CBC) serves as the primary laboratory tool for the initial evaluation of anemia , Through parameters such as hemoglobin concentration , MCV , MCH , MCHC , and RDW , it allows preliminary classification of anemia into microcytic , normocytic , or macrocytic categories , However , accurate differentiation between iron deficiency and vitamin B 12 deficiency often requires integration of hematological indices with biochemical markers such as serum ferritin and vitamin B 12 levels (Warner & Kamran , 2023; Ankar & Kumar , 2022) , Establishing population-specific reference values and understanding local hematological patterns are therefore essential for improving diagnostic interpretation (Abbas et al. , 2024).
Within this broader clinical and epidemiological context , examining the interplay between iron status , vitamin B 12 levels , and CBC indices at the local laboratory level provides an opportunity to better characterize anemia patterns in specific communities and enhance evidence-based laboratory interpretation in routine practice.
Therefore , this study aimed to examine the association between serum ferritin , vitamin B 12 , hemoglobin , and CBC indices among laboratory attendees in Surman , Libya.

Problem Statement

Anemia remains highly prevalent worldwide and continues to pose diagnostic and therapeutic challenges , particularly in settings where laboratory investigations may be limited to routine complete blood count (CBC) testing , Although iron deficiency and vitamin B 12 deficiency represent two major nutritional causes of anemia , accurate differentiation between them cannot always be achieved based solely on red cell indices , Overlapping hematological patterns , early-stage deficiencies , and mixed micronutrient insufficiencies may obscure classical laboratory features , potentially leading to misclassification and inappropriate supplementation strategies.

In many regional laboratory settings , including Surman , clinical decisions are often initiated based on hemoglobin values and basic CBC parameters without systematic assessment of iron stores or vitamin B 12 levels , This approach may overlook subclinical deficiencies or underestimate the contribution of specific micronutrients to anemia severity , Furthermore , limited local data describing the quantitative relationship between serum ferritin , vitamin B 12 , and hemoglobin levels restrict the ability to develop evidence-based diagnostic protocols tailored to the population served.

Despite documented regional evidence of iron deficiency anemia and vitamin B 12 deficiency in Libya and neighboring countries , there remains insufficient local analytical data examining how these biochemical markers are reflected in CBC indices within the Surman population , The absence of such data hinders accurate interpretation of laboratory results and limits understanding of the dominant anemia pattern in this community.

Accordingly , the core research problem can be formulated as follows:

There is a lack of locally derived evidence clarifying the relationship between iron deficiency , vitamin B 12 deficiency , and alterations in complete blood count (CBC) indices among individuals attending Surman Central Laboratory , which may affect the accuracy of differential diagnosis and laboratory-based clinical decision-making.

Research Questions

Based on the problem statement and the statistical analyses performed in this study , the following research questions were addressed:

1. What is the distribution pattern of anemia pattern of anemia among individuals attending Surman Central Laboratory based on hemoglobin levels and CBC indices ?
2. What are the mean levels and distribution characteristics of serum ferritin and vitamin B 12 among the studied sample ?
3. Is there a statistically significant relationship between serum ferritin levels and hemoglobin concentration ?
4. Is there a statistically significant relationship between serum vitamin B 12 levels and hemoglobin concentration ?
5. To what extent do serum ferritin and vitamin B 12 levels predict variations in hemoglobin levels using multiple linear regression analysis ?
6. Do demographic variables (particularly sex and age) significantly influence CBC indices and anemia patterns in the studied population ?
7. What is the predominant hematological pattern of anemia (microcytic , normocytic , or macrocytic) observed in the Surman laboratory sample ?

Alignment with Study Findings

The statistical results demonstrated that:

- Serum ferritin showed a significant positive association with hemoglobin (Beta = 0.412 , $p < 0.001$).
- Vitamin B 12 did not show a statistically significant effect on hemoglobin levels ($p = 0.919$).
- The regression model explained 17.1 % of the variance in hemoglobin concentration.
- Microcytic anemia was the predominant pattern based on mean MCV values.
- Females represented the majority of anemia cases within the sample.

These findings directly answer the research questions and confirm that iron deficiency represents the primary driver of anemia in the studied population , whereas vitamin B 12 plays a less direct role in influencing hemoglobin concentration within this sample.

Significance of the Study

Anemia continues to represent a clinically relevant and epidemiologically significant condition , particularly in regions where nutritional deficiencies remain prevalent , Although complete blood count (CBC) testing is widely available and routinely performed , the interpretation of

hematological indices without confirmatory biochemical markers may limit diagnostic accuracy , Differentiating between iron deficiency anemia and vitamin B 12 deficiency anemia is essential to avoid inappropriate therapeutic interventions and to ensure targeted management strategies.

This study contributes to the existing body of knowledge by providing locally derived laboratory evidence from Surman , Libya , regarding the relationship between serum ferritin , vitamin B 12 levels , and CBC indices , Generating such local data is particularly important in settings where population-specific hematological reference patterns may differ due to dietary habits , socioeconomic conditions , and environmental factors.

From a clinical perspective , the findings help clarify the predominant etiology of anemia within the studied population and support laboratory-based differentiation between microcytic and macrocytic patterns , This enhances the precision of diagnostic decision-making and reduces the risk of empirical supplementation without biochemical confirmation.

From a public health standpoint , the study provides evidence that may guide nutritional awareness programs , screening strategies , and preventive interventions , especially among high-risk groups such as women of reproductive age , Furthermore , establishing quantitative associations between micronutrient status and hemoglobin levels may inform future research and support the development of locally adapted laboratory reference standards.

Objectives of the Study

General Objective

To evaluate the relationship between iron deficiency , vitamin B 12 deficiency , and alterations in complete blood count (CBC) indices among individuals attending Surman Central Laboratory.

Specific Objectives

1. To determine the distribution of anemia patterns based on hemoglobin concentration and red cell indices (MCV , MCH , MCHC , RDW) within the studied sample.
2. To assess serum ferritin and vitamin B 12 levels among laboratory attendees and describe their distribution characteristics.
3. To examine the association between serum ferritin levels and hemoglobin concentration.
4. To investigate the relationship between serum vitamin B 12 levels and hemoglobin concentration.
5. To evaluate the predictive effect of ferritin and vitamin B 12 on hemoglobin levels using multiple linear regression analysis.
6. To analyze the influence of demographic variables (age and sex) on anemia patterns and CBC indices.
7. To identify the predominant hematological pattern of anemia (microcytic , normocytic , or macrocytic) in the Surman laboratory population.

Null Hypotheses

Based on the study objectives and statistical design , the following null hypotheses were formulated:

H_{01}: There is no statistically significant relationship between serum ferritin levels and hemoglobin concentration among individuals attending Surman Central Laboratory.

H_{02}: There is no statistically significant relationship between serum vitamin B 12 levels and hemoglobin concentration among individuals attending Surman Central Laboratory.

H_{03}: Serum ferritin and vitamin B 12 levels do not significantly predict variations in hemoglobin concentration when analyzed using multiple linear regression.

H_{04}: There are no statistically significant differences in CBC indices (Hb , MCV , MCH , MCHC , RDW , RBC , HCT , WBC , PLT) between males and females.

H_{05}: Age has no statistically significant effect on hemoglobin levels or other CBC indices in the studied population.

Previous Studies

1- Study by GBD 2021 Anaemia Collaborators (2023) , entitled:"Prevalence , years lived with disability , and trends in anaemia burden by severity and cause , 1990–2021"

The study aimed to estimate the global prevalence of anemia by severity and underlying cause , and to analyze its trends over three decades , The study relied on secondary data from the Global Burden of Disease database , covering multiple countries and age groups worldwide. A large population-based dataset was analyzed using advanced epidemiological modeling techniques.

The study revealed the following findings:

- Anemia continues to affect approximately 24 % of the global population.
- Iron deficiency remains the leading cause of anemia worldwide , particularly among women and children.
- The burden of anemia remains high in low- and middle-income countries despite global health efforts.

2- Study by Al-Qahtani et al. (2021) , entitled:"Prevalence of iron deficiency anemia among female university students"

The study aimed to determine the prevalence of iron deficiency anemia among female university students and to assess its relationship with hematological indices , The study sample consisted of female university students , and the descriptive analytical approach was adopted , Laboratory blood investigations including CBC and serum ferritin were used as study tools.

The study resulted in the following findings:

- The prevalence of iron deficiency anemia among the participants was significant.
- Decreased MCV and MCH values were strongly associated with low ferritin levels.
- Iron deficiency was identified as the primary contributor to anemia in the studied population.

3- Study by Sahin et al. (2021) , entitled:"The rates of and relationship between anemia and deficiency of iron , zinc , vitamin B 12 and folic acid in hospitalized children"

The study aimed to investigate the prevalence of anemia and its association with micronutrient deficiencies among hospitalized children , The sample included more than 60 ,000 pediatric cases , and the study followed a descriptive cross-sectional design , Laboratory measurements included CBC , iron , vitamin B 12 , and folic acid levels.

The study concluded the following:

- A considerable proportion of children had anemia accompanied by iron and vitamin B 12 deficiencies.
- Iron deficiency showed a stronger association with low hemoglobin levels compared to vitamin B 12 deficiency.
- Mixed micronutrient deficiencies complicated hematological classification based solely on CBC indices.

4- Study by Salih et al. (2022) , entitled:"Vitamin B 12 deficiency among Libyan elderly: A cross-sectional study in Tripoli"

The study aimed to determine the prevalence of vitamin B 12 deficiency among elderly individuals in Tripoli , Libya , and to assess its hematological impact , The study sample consisted of elderly participants , and a cross-sectional descriptive design was used , Serum vitamin B 12 levels and CBC parameters were analyzed.

The study revealed the following results:

- A noticeable proportion of elderly participants had vitamin B 12 deficiency.
- Elevated MCV values were significantly associated with low vitamin B 12 levels.
- Vitamin B 12 deficiency may remain undetected if only hemoglobin values are considered.

5- Study by Sharma et al. (2024) , entitled:"Assessing the prevalence of iron deficiency anemia and risk factors among children and women: A case study of rural Uttar Pradesh"

The study aimed to assess the prevalence of iron deficiency anemia and identify associated risk factors among children and women , The study adopted a descriptive analytical approach and used hematological testing including CBC and ferritin measurements.

The study findings indicated:

- A high prevalence of iron deficiency anemia among women and children.
- A significant positive correlation between serum ferritin and hemoglobin levels.
- Nutritional factors were strongly associated with reduced hemoglobin values.

6- Study by Abbas et al. (2024) , entitled:"Determine complete blood count reference values among healthy adult populations"

The study aimed to establish reference ranges for CBC parameters among healthy adults , The sample consisted of adult participants , and laboratory-based hematological analysis was performed.

The study concluded that:

- CBC reference values may vary depending on demographic and regional factors.
- Establishing local hematological reference ranges improves diagnostic accuracy.
- Interpretation of anemia requires consideration of population-specific standards.

Research Gap

Despite the extensive international and regional literature on anemia , there is still limited locally generated evidence from Libya—particularly from Surman—linking iron status and vitamin B 12 status to complete blood count (CBC) patterns in routine laboratory attendees , Most available studies in the region focus on specific subgroups (e.g. , pregnant women , children , or the elderly) or report prevalence figures without integrating biochemical markers and CBC indices within a single analytical framework (Al-Amri et al. , 2020; Salih et al. , 2022; Al-Qahtani et al. , 2021).

In addition , many clinical settings rely primarily on CBC indices to classify anemia (microcytic vs , macrocytic) , yet mixed or early-stage deficiencies can mask classical patterns , making CBC-only interpretation potentially misleading , This limitation is particularly relevant in populations where dietary variability and micronutrient insufficiency may coexist , while ferritin and vitamin B 12 testing is not consistently incorporated into routine diagnostic workups (Balci et al. , 2016; Warner & Kamran , 2023).

Furthermore , there is a methodological gap in the Libyan context regarding the use of quantitative modeling (e.g. , multiple linear regression and ANOVA) to determine the extent to which ferritin and vitamin B 12 predict hemoglobin variation and explain anemia severity within local laboratory populations , Global data confirm iron deficiency as a major driver of anemia , but local determinants , laboratory patterns , and population-specific hematological distributions may differ and require evidence derived from the same setting (GBD 2021 Anaemia Collaborators , 2023; Abbas et al. , 2024).

Therefore , the research gap addressed by the present study can be summarized as follows:

There is a lack of locally derived analytical evidence from Surman , Libya that quantitatively examines how serum ferritin and vitamin B 12 levels relate to hemoglobin concentration and CBC indices , and how these relationships shape the predominant anemia pattern among individuals attending a central laboratory.

Theoretical Framework

Anemia is defined as a reduction in hemoglobin concentration below established reference thresholds , resulting in decreased oxygen-carrying capacity of the blood (World Health Organization , 2023) , Hemoglobin synthesis and red blood cell production depend on adequate availability of essential micronutrients , particularly iron and vitamin B 12 , Disruption in either

of these elements alters erythropoiesis and produces measurable changes in complete blood count (CBC) indices.

Iron and Erythropoiesis

Iron is a critical component of hemoglobin and plays a central role in oxygen transport , In conditions of iron deficiency , hemoglobin synthesis becomes impaired , leading to the production of smaller and less hemoglobin-rich red blood cells , This results in microcytic , hypochromic anemia characterized by reduced mean corpuscular volume (MCV) , decreased mean corpuscular hemoglobin (MCH) , and often elevated red cell distribution width (RDW) due to anisocytosis (Camaschella , 2019; Warner & Kamran , 2023).

Serum ferritin serves as a biomarker of body iron stores , When ferritin levels decline , hemoglobin concentration typically decreases as iron supply to the bone marrow becomes insufficient , Therefore , a positive relationship between ferritin and hemoglobin is biologically expected and supported by epidemiological evidence (GBD 2021 Anaemia Collaborators , 2023).

Vitamin B 12 and Red Blood Cell Maturation

Vitamin B 12 is essential for DNA synthesis and normal cell division , Its deficiency disrupts nuclear maturation within erythroid precursors , leading to ineffective erythropoiesis and the formation of enlarged red blood cells (macrocytes) , This condition is reflected by elevated MCV values and is commonly classified as megaloblastic anemia (Ankar & Kumar , 2022; Smith et al. , 2018).

Unlike iron deficiency , vitamin B 12 deficiency may not immediately reduce hemoglobin concentration in early stages , but it significantly affects red cell morphology , Consequently , its primary hematological manifestation is macrocytosis rather than microcytosis.

Complete Blood Count as a Diagnostic Model

The complete blood count (CBC) provides an integrated overview of erythrocyte quantity and morphology , Key indices include:

- **Hemoglobin (Hb):** indicator of anemia severity.
- **MCV:** classification tool (microcytic <80 fL , normocytic 80–100 fL , macrocytic >100 fL).
- **MCH and MCHC:** reflect hemoglobin content per cell.
- **RDW:** indicates variability in red cell size.

The theoretical relationship underlying this study assumes that:

- Iron deficiency primarily reduces Hb and MCV (microcytic pattern).
- Vitamin B 12 deficiency primarily increases MCV (macrocytic pattern).
- Serum ferritin and vitamin B 12 levels influence hemoglobin concentration either directly or indirectly through erythropoietic mechanisms.

However , mixed deficiencies may alter classical patterns and reduce the predictive power of MCV alone (Balci et al. , 2016) , Therefore , integrating biochemical markers (ferritin and vitamin B 12) with CBC parameters enhances diagnostic precision.

Conceptual Model of the Study

The present study is grounded in a biological model in which:

- **Independent Variables:**
  - Serum Ferritin (indicator of iron stores)
  - Serum Vitamin B 12
- **Dependent Variable:**
  - Hemoglobin concentration (Hb)
- **Supporting Hematological Indicators:**
  - MCV , MCH , MCHC , RDW , RBC , HCT

The model assumes that variations in micronutrient status influence erythropoiesis , which in turn alters CBC indices and hemoglobin concentration.

Materials and Methods

Study Setting and Period

This study was conducted at Surman Central Laboratory , Surman , Libya , Data were retrospectively collected from laboratory records covering the period from January 2024 to December 2025.

Inclusion and Exclusion Criteria

Inclusion Criteria:

Participants were included if they met the following conditions:

- Hemoglobin concentration below WHO reference thresholds (Hb < 13 g/dL for males and < 12 g/dL for females).
- Available complete blood count (CBC) results.
- Available serum ferritin and vitamin B 12 measurements.

Exclusion Criteria:

Cases were excluded if:

- Laboratory records were incomplete or missing key variables.
- Recent blood transfusion was documented (within 3 months) , when available in records.
- Known hematological malignancies were recorded.

Due to the retrospective nature of the study , information regarding pregnancy status , chronic kidney disease , inflammatory conditions (CRP levels) , hemoglobinopathies , or ongoing iron supplementation was not consistently available in laboratory records , These factors are acknowledged as potential confounders.

Research Design

This study adopted a **descriptive analytical cross-sectional design** to investigate the relationship between iron deficiency , vitamin B 12 levels , and alterations in complete blood count (CBC) indices among individuals attending Surman Central Laboratory.

The descriptive component was used to characterize hematological and biochemical parameters within the study population , while the analytical component examined statistical associations between serum ferritin , vitamin B 12 , and hemoglobin levels using inferential statistical methods.

The cross-sectional design was considered appropriate because data were collected at a single time point from laboratory records without longitudinal follow-up.

Population and Sample

Study Population

The study population consisted of all individuals attending **Surman Central Laboratory , Surman , Libya** , who were requested to undergo complete blood count (CBC) testing and biochemical assessment of serum ferritin and vitamin B 12 during the study period (2024–2025).

Study Sample

A **purposive sampling technique** was employed to select cases presenting with hemoglobin values below the normal reference range , consistent with World Health Organization criteria (Hb < 13 g/dL for males and < 12 g/dL for females).

The final sample included **150 participants** , both males and females , aged between 10 and 96 years.

The sample size (n = 150) was determined by the number of eligible laboratory records meeting the inclusion criteria during the study period (January 2024–December 2025).

This sample size was considered sufficient to support correlation and multiple regression analyses based on commonly accepted recommendations for multivariable modeling:

$$\frac{Z^2 \cdot P(1-P)}{{}^2d} = n$$

Where:
- Z=1.96Z = 1.96Z=1.96 (95 % confidence level)
- P=0.5P = 0.5P=0.5 (assumed prevalence for maximum sample size)
- d=0.08d = 0.08d=0.08 (margin of error ≈ 8 %)

Research Tools

The study relied on the following tools for data acquisition:
1. **Laboratory Records:** Official electronic and printed laboratory records were reviewed to extract hematological and biochemical data.
2. **Data Collection Sheet:** A structured data extraction form was designed to record:
   - Hemoglobin (Hb)
   - RBC count
   - Hematocrit (HCT)
   - MCV , MCH , MCHC
   - RDW
   - White blood cell count (WBC)
   - Platelet count (PLT)
   - Serum ferritin
   - Serum vitamin B 12
   - Demographic variables (age , sex)

Instrumentation

All laboratory analyses were conducted using standardized automated analyzers available at Surman Central Laboratory:
- **Complete Blood Count (CBC):** Performed using an automated hematology analyzer according to manufacturer specifications , measuring Hb , RBC indices , WBC , and platelet parameters using an automated hematology analyzer according to manufacturer specifications , which measures red cell indices , white blood cells , hemoglobin concentration , hematocrit , and platelet count through impedance and photometric techniques.
- **Serum Ferritin Measurement:** Determined using immunoassay-based laboratory techniques according to manufacturer protocols.
- **Vitamin B 12 Measurement:** Measured using automated immunoassay analyzers with standardized calibration procedures.

All equipment underwent routine internal quality control procedures to ensure measurement accuracy and reliability.

Data Collection Procedures

Data collection was carried out in the following steps:
1. Administrative approval was obtained from Surman Central Laboratory management.
2. Eligible cases meeting anemia criteria were identified from laboratory records.
3. Hematological and biochemical results were extracted and coded anonymously to ensure confidentiality.
4. Cases were categorized into three diagnostic groups based on laboratory criteria:
   - Iron deficiency anemia
   - Vitamin B 12 deficiency anemia
   - Mixed deficiency anemia
5. Data were entered into SPSS software for statistical analysis.

Case Definitions and Classification Criteria

For the purpose of this study , standardized laboratory criteria were used to define and classify anemia and micronutrient deficiencies:

- **Anemia:** Defined according to World Health Organization criteria as hemoglobin (Hb) concentration < 13 g/dL in males and < 12 g/dL in females.
- **Iron Deficiency:** Defined as serum ferritin level < 15 ng/mL in the absence of inflammatory conditions , In cases where clinical suspicion existed , ferritin < 30 ng/mL was considered of depleted iron stores.
- **Vitamin B 12 Deficiency:** Defined as serum vitamin B 12 level < 200 pg/mL.
- **Microcytic Anemia:** Defined as MCV < 80 fL.
- **Normocytic Anemia:** Defined as MCV between 80–100 fL.
- **Macrocytic Anemia:** Defined as MCV > 100 fL.

Based on these criteria , participants were categorized into three groups:

1. Iron deficiency anemia
2. Vitamin B 12 deficiency anemia
3. Mixed deficiency anemia

Procedures

The study procedures were conducted in the following steps:

1. Official administrative approval was obtained from Surman Central Laboratory.
2. Laboratory records were screened to identify individuals meeting the anemia criteria during the study period (2024–2025).
3. Eligible cases were selected according to predefined inclusion criteria.
4. Hematological (CBC) and biochemical (serum ferritin and vitamin B 12) results were extracted from the laboratory database.
5. Each case was assigned a confidential study code to ensure anonymity.
6. Data were entered into a structured database and reviewed for completeness and accuracy prior to statistical analysis.
7. Cases were classified according to anemia type based on laboratory findings.

Statistical Analysis

All statistical analyses were performed using the **Statistical Package for the Social Sciences (SPSS)** , version (XX).

The following statistical procedures were applied:

- **Descriptive Statistics:** Means , standard deviations , minimum and maximum values were calculated for continuous variables , Frequencies and percentages were used for categorical variables.
- **Pearson Correlation Analysis:** Conducted to assess the strength and direction of the relationship between hemoglobin (Hb) and serum ferritin , as well as between Hb and vitamin B 12.
- **Multiple Linear Regression Analysis:** Performed to evaluate the predictive effect of serum ferritin and vitamin B 12 on hemoglobin levels , The coefficient of determination (R^2) was used to assess the proportion of variance explained by the model.
- **One-Way ANOVA Test:** Used to determine the overall significance of the regression model.
- **Graphical Analysis (Box Plots):** Applied to visualize the distribution of CBC indices according to sex and to identify potential outliers.

A p-value of < 0.05 was considered statistically significant.

Prior to regression analysis , assumptions of linearity , normality of residuals , homoscedasticity , and absence of multicollinearity were assessed and considered acceptable.

Ethical Considerations

This study was conducted in accordance with ethical principles governing medical and laboratory research , Administrative approval was obtained from the management of Surman Central Laboratory prior to data collection.

As the study relied on retrospective laboratory records , no direct contact with participants was required , All extracted data were anonymized and coded to ensure confidentiality and privacy , Personal identifiers such as names , contact information , or national identification numbers were not recorded in the study database.

The study involved minimal risk to participants , as it utilized routine laboratory investigations performed for clinical purposes , No additional blood samples were collected for research purposes.

Data were used strictly for academic and scientific research objectives and were stored securely to prevent unauthorized access.

The study adhered to principles consistent with the Declaration of Helsinki regarding research involving human data.

Limitations of the Study

Despite the valuable findings generated by this study , several limitations should be acknowledged.

First , the study adopted a cross-sectional design , which limits the ability to establish causal relationships between iron status , vitamin B 12 levels , and hemoglobin concentration , The associations observed reflect relationships at a single time point rather than longitudinal changes.

Second , the sample was drawn from individuals attending a single laboratory in Surman , which may limit the generalizability of the findings to other regions in Libya, Laboratory attendees may not fully represent the general population , as they were referred for testing due to suspected clinical conditions.

Third , the regression model explained 17.1 % of the variance in hemoglobin levels , indicating that additional factors—such as folate deficiency , chronic inflammation , renal function , parasitic infections , dietary patterns , or genetic hemoglobinopathies—were not included in the analysis and may contribute to anemia severity.

Fourth , inflammatory markers were not systematically assessed; therefore , elevated ferritin levels in some participants could potentially reflect inflammatory states rather than true iron sufficiency.

Finally , dietary intake patterns were not quantitatively measured , limiting the ability to directly link nutritional habits with biochemical findings.

Despite these limitations , the study provides meaningful laboratory-based evidence regarding anemia patterns within the Surman population and offers a foundation for future multicenter and longitudinal research.

Results

Demographic Characteristics of the Sample

Distribution of Patients by Sex

Table 1 , Distribution of Participants by Sex

Sex	Frequency (n)	Percentage (%)
Male	42	28.0 %
Female	104	69.3 %
Missing	4	2.7 %
Total	**150**	**100 %**

The majority of participants were females (69.3 %) , while males represented 28.0 % of the total sample , This indicates that anemia-related laboratory investigations were more frequent among females during the study period.

Age Distribution

Table 2 , Descriptive Statistics of Age

Variable	N	Minimum	Maximum	Mean	Std , Deviation
Age	145	10	96	34.73	16.32

The mean age of participants was 34.73 years (SD = 16.32) , with a wide age range (10–96 years) , indicating representation across multiple age groups.

Complete Blood Count (CBC) Analysis

Table 3 , Descriptive Statistics of Hematological Parameters

Parameter	N	Minimum	Maximum	Mean	Std , Deviation
Hb (g/dL)	150	5.6	16.5	12.58	2.13
WBC	150	2.3	16.1	7.54	2.29
RBC	150	2.3	6.4	4.50	0.57
HCT (%)	150	18.6	50.3	36.63	5.25
MCV (fL)	150	55.0	118.0	79.83	11.99
MCH (pg)	150	16.8	35.2	27.84	3.93
MCHC (g/dL)	150	28.1	36.5	33.12	1.45
RDW (%)	150	11.2	28.9	15.41	2.76
PLT	150	84	587	270.22	77.07

The mean MCV (79.83 fL) was slightly below the normal lower limit (80 fL) , suggesting a predominantly microcytic tendency within the sample.

Effect of Sex on Hemoglobin Levels

Table 4 , Comparison of Hemoglobin by Sex

Sex	N	Mean Hb (g/dL)	Std , Deviation
Male	42	13.41	1.89
Female	104	12.11	2.17

Males demonstrated higher mean hemoglobin levels compared to females , Females showed greater variability and lower minimum values , suggesting higher anemia severity in this group.

Serum Ferritin and Vitamin B 12 Levels

Table 5 , Descriptive Statistics of Biochemical Parameters

Parameter	N	Minimum	Maximum	Mean	Std , Deviation
Ferritin (ng/mL)	149	2.2	451.0	63.72	80.78
Vitamin B 12 (pg/mL)	148	9.22	1221	435.24	196.68

Ferritin levels showed wide variability , indicating the presence of both iron deficiency and elevated iron stores , Vitamin B 12 levels also demonstrated broad dispersion across participants.

Relationship Between Hb , Ferritin , and Vitamin B 12

Table 6 , Multiple Linear Regression Analysis

Variable	B	Beta	t	Sig. (p)
Ferritin	0.012	0.412	5.378	<0.001
Vitamin B 12	0.000	0.008	0.102	0.919

Model Summary:

R	R^2	Adjusted R^2	F	Sig.
0.414	0.171	0.160	14.913	<0.001

The regression model was statistically significant , Ferritin showed a significant positive effect on hemoglobin levels ($p < 0.001$) , whereas vitamin B 12 did not demonstrate a statistically significant effect ($p = 0.919$) , The model explained 17.1 % of hemoglobin variability.

5 , Discussion

The findings of the present study provide important insights into the pattern of anemia among attendees of Surman Central Laboratory , particularly regarding the predominance of iron deficiency and its reflection on CBC indices.

The predominance of females in the sample (69.3 %) and the lower mean hemoglobin levels observed among women are consistent with findings reported by Al-Qahtani (2021) and Al-Amri (2020) , which demonstrated a higher burden of anemia-related laboratory findings among female participants , particularly within reproductive age groups , This observation is further supported by global epidemiological estimates reported by the GBD 2021 Anaemia Collaborators (2023) , highlighting the disproportionate impact of anemia on women worldwide , However , this finding contrasts with the results of Abbas et al. (2024) , who reported balanced hematological reference values among healthy adults without significant gender disparity , likely reflecting differences in study populations and underlying health status.

The mean MCV value (79.83 fL) , indicating a predominance of microcytic anemia , is consistent with the findings of (Gheriani , 2019) and (Sharma , 2024) , both of whom identified iron deficiency as the primary driver of microcytosis , Similarly , (Camaschella , 2019) emphasized that iron deficiency remains the most common cause of microcytic anemia worldwide , In contrast , this finding differs from (Salih , 2022) , who reported a higher prevalence of macrocytic anemia linked to vitamin B 12 deficiency among elderly Libyan populations.

The significant positive association between ferritin and hemoglobin (Beta = 0.412 , $p < 0.001$) agrees with the conclusions of (Warner & Kamran , 2023) and (Cox , 2023) , both confirming that iron stores are a key determinant of hemoglobin concentration , It is also consistent with global burden data identifying iron deficiency as the leading cause of anemia (GBD , 2023) , However , the lack of a significant relationship between vitamin B 12 and hemoglobin differs from findings reported by (Sahin , 2021) and (Ranjan , 2018) , who observed measurable hematological effects of combined micronutrient deficiencies , This discrepancy may be explained by differences in sample characteristics and the absence of severe macrocytic cases in the current study.

The relatively modest explanatory power of the regression model ($R^2 = 17.1$ %) supports the observations of (Kassebaum , 2014) , who highlighted the multifactorial nature of anemia , including nutritional , inflammatory , and chronic disease-related contributors , This suggests that while iron deficiency plays a dominant role in the Surman population , additional factors may contribute to hemoglobin variability.

Overall , the results reinforce regional and global evidence that iron deficiency remains the principal determinant of anemia in most populations , while vitamin B 12 deficiency appears less influential on hemoglobin concentration in this specific setting.

Conclusions and Recommendations

Conclusions

The present study evaluated the impact of iron deficiency and vitamin B 12 deficiency on hemoglobin levels and CBC indices among attendees of Surman Central Laboratory , Based on the statistical analysis , several key conclusions can be drawn:

1. **Iron deficiency appears to be the primary determinant of anemia** in the studied population , as evidenced by the significant positive association between ferritin and hemoglobin levels ($p < 0.001$).

2. **Microcytic anemia was the predominant morphological pattern** , reflected by a mean MCV slightly below the normal threshold (79.83 fL) , supporting the dominance of iron-related anemia.
3. **Vitamin B 12 did not show a statistically significant direct effect on hemoglobin levels** in this sample , suggesting that its hematological impact may be more related to red cell morphology rather than hemoglobin concentration itself.
4. **Females demonstrated lower hemoglobin levels and greater variability in hematological indices** , confirming their higher vulnerability to nutritional anemia.
5. The regression model explained 17.1 % of hemoglobin variability , indicating that anemia in this population is **multifactorial** and influenced by additional biological and environmental factors not captured in the current model.

Overall , the findings highlight iron deficiency as the dominant contributor to anemia in the Surman population , with vitamin B 12 deficiency playing a secondary role in hemoglobin reduction.

Recommendations

Based on the findings of this study , the following recommendations are proposed:

1. **Routine measurement of serum ferritin alongside hemoglobin** should be encouraged in laboratory evaluations to improve diagnostic accuracy for iron deficiency anemia.
2. **Targeted screening programs for women of reproductive age** should be implemented due to their increased vulnerability to anemia.
3. Nutritional awareness campaigns should emphasize **adequate intake of iron-rich and vitamin B 12–rich foods** , particularly in populations with dietary limitations.
4. Future research should incorporate additional biomarkers such as folate levels , inflammatory markers (CRP) , and chronic disease indicators to better explain hemoglobin variability.
5. Multicenter studies across different Libyan regions are recommended to establish **national hematological reference data** and better understand regional variations in anemia patterns.

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