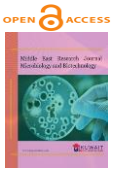

Middle East Research Journal of Microbiology and Biotechnology
ISSN: **2789-8644 (Print & Open Access)**
Frequency: Bi-Monthly
DOI: https://doi.org/10.36348/merjmb.2026.v06i03.007

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Estimation of Ferritin and Complete Blood Picture of Patients in Kirkuk City, Iraq

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Abstract: ***Background:*** Anemia is a major public health problem worldwide attacking both high- and low-income countries. It is characterized by decreased hemoglobin (HB) levels and a reduced number of circulating erythrocytes (RBCs), resulting in an insufficient erythrocyte mass needed for physiological demands**.** ***Materials and Methods:*** This study was carried out in General Hospital of Kirkuk city, Iraq. 48 patients were included and they were complaining of anaemia. Age and gender were recorded for all participants. Complete blood counts and ferritin estimation were carried out. ***Results:*** Erythrocytes numbers ranged between 3.5 to 6.4 x 10^6/µl. The highest concentration of haemoglobulin was found among age group ≥ 51-60 years old. Haematocrit (HCT) values ranged from 37.42% to 41.43% across age groups and the highest mean Corpuscular Volume (MCV) was 84.1Fl. Mean corpuscular haemoglobin (MCH) ranged between 26.3 to 28.5 pg among different age groups. The present study revealed also that mean corpuscular haemoglobin concentration (MCHC) value ranged between 33.15 to **33.95** ± 0.46 g/dL among age groups studied. Ferritin levels among the studied age groups varied with the highest level observed in ≥51-60 age group and the value was 57.83 ± 22.12 ng/mL whereas the lowest mean was seen in 41-50 years age group with value equal to 32.25 ± 19.65 ng/Ml. ***Conclusions:*** Comparison of haematological parameters among the five age groups revealed no statistically significant age-related differences ($P > 0.05$). Although ferritin, lymphocyte count, MCV and haematocrit showed some numerical fluctuations between age groups, these variations were not statistically different. Erythrocyte count was the only parameter showing a statistically significant gender difference ($P < 0.05$) utilizing Mann-Whitney U test. Because only summary statistics were available, these data would be regarded as descriptive comparisons rather than indication of significant correlations.

Keywords: Anaemia, ferritin, blood picture, age, gender, Iraq.

Research Paper

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How to cite this paper:
Al-Jebouri, M. M., Hassan, M. S., Darweesh, Q. R., & Hakim, R. (2026). Estimation of Ferritin and Complete Blood Picture of Patients in Kirkuk City, Iraq. *Middle East Res J. Microbiol Biotechnol., 6*(3), 193-202. https://doi.org/10.36348/merjmb.2026.v06i03.007

Article History:
| Submit: 12.07.2026 |
| Accepted: 15.08.2026 |
| Published: X |

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INTRODUCTION

Anemia is a major public health problem worldwide attacking both high- and low-income countries. It is characterized by decreased hemoglobin (HB) levels and a reduced number of circulating erythrocytes (RBCs), resulting in an insufficient erythrocyte mass needed for physiological demands. Laboratory findings typically indicate decreased HB, hematocrit (HT), and RBC count. Anemia represents an indication of an underlying disorder. Iron deficiency represents one of the most relevant underlying conditions and still among the most common nutritional deficiencies in Europe, affecting a substantial proportion of the population [1,2]. Old ages are at increased risk of iron deficiency due to inadequate dietary intake, poor gastrointestinal absorption often related to proton pump inhibitor use, gastrointestinal malignancies, chronic blood loss due to angiodysplasia or using of

antithrombotic therapy [3]. Complete blood count parameters are cheap, widely available, and routinely used in clinical practices. It is important to consider that erythrocyte indices such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) reflect alterations in erythropoiesis and may hence provide indirect evidence for iron-restricted erythropoiesis before biochemical abnormalities become evident. Globally, anaemia affected almost 37% of pregnant women in the reproductive age group in 2019 [1]. Widely regarded as the first choice for assessing iron deficiency in pregnancy, serum ferritin is considered the most useful initial test for diagnosing absolute iron deficiency, despite its susceptibility to elevation during active infection or inflammation [4,5].The serum ferritin concentration or level always serves as the most sensitive and specific test for diagnosing iron deficiency, typically indicated by a level below 15 ng/mL, with a higher cut-off below 30 ng/mL recommended in settings of inflammation [6,7]. World Health Organization (WHO) revealed that the normal references for hemoglobin levels are 13 to 18 g/dL in adult men and 12 to 16 g/dL in adult women who are not pregnant [8,9,10]. The etiology of anemia is varied and may be due to blood cell destruction, blood loss, deficient production, or defective production.[11,12,13,14]. Erythrocytosis may be due to dehydration or hemoconcentration or from elevated production of red cells, which may be due to a primary myeloproliferative pathology or secondary to cyanotic heart disease, high altitudes, respiratory disease, smoking, renal pathology, or erythropoietin-secreting tumors.[15]. The normal reference interval of haematocrit (HCT) is usually 40% to 54% in adult men and 36% to 48% in adult women. Similarly to Hb, HCT is reduced in anemia, increased in erythrocytosis, and is affected by changes in plasma volume.[16]. The normal reference value of erythrocyte count interval is usually 4.6 to 6.2 million cells/µL in adult men and 4.2 to 5.4 million cells/µL in adult women. Red cell indices are important in evaluating anemia[5]. MCH and MCHC are also widely used for quality control within laboratories.[17]. Mean corpuscular volume (MCV) definition is the average volume of the red blood cells present and is expressed in femtoliter (fL) or cubic micrometers (μm^3). The value is calculated by dividing HCT by RBC. The normal reference interval in adults is 80 to 100 μm^3. MCV may be elevated due to red cell agglutination, hyperglycemia, hypernatremia and hyperleukocytosis [5, 18, 19, 20, 21, 22,]. Mean corpuscular hemoglobin (MCH) measures the amount of hemoglobin per red blood cell and is expressed as picograms per cell (pg/cell). The value is calculated by dividing hemoglobin by red cell count. The normal reference interval value in adults is usually 27 to 32 pg/RBC. MCHC is the mean hemoglobin concentration per unit volume of red blood cells which is expressed as a gram per deciliter (g/dl) or percentage of Hb per cell. The value is calculated by dividing the hemoglobin by the hematocrit. White blood cell (WBC) is the number of white blood cells present per blood microliter (µL). The normal range value in adults is usually 4500 to 11000 cells/µL. Leukopenia is defined as a low WBC due to increased utilization, reduced production or increased destruction of white blood cells. The lymphocyte count is the number of lymphocytes per microliter of blood and is expressed as a percentage of the WBC. The normal reference interval in adults is usually 20% to 40% of the or 1000 to 4000 cells/µL. Lymphocytosis may be due to viral and/or bacterial infections whereas cytopenia may be due to infection, bacterial infection, autoimmune disease, malignancy, myeloproliferative disease, primary immunodeficiency diseases, lymphoproliferative disease, or medications [23-28].

Materials and Methods

Patients

This study was carried out in General Hospital of Kirkuk city, Iraq. 48 patients were included and they were complaining of anaemia. Age and gender were recorded for all participants. The acceptance for participation in the present study was taken from all the participants whose native language is Arabic. They were not mentally retarded and they were completely healthy considering hearing and speaking.

Complete blood counts

Complete blood picture was done using automatic haematological analyzer Quintus (Boulemedical AB, Sweden) as stated by Al-Jebouri and Taha [19].

Ferritin

Serum ferritin was carried out as described elsewhere [29].

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics such as means, standard deviations, and frequency distributions were computed to summarize the data. A paired sample t test correlation coefficient ® and coefficient of determination (R2) were calculated. [30,31,32,33,34].

Results

Age

The present study revealed that the mean values of white blood cells were ranging from 6.54 to 8.79 $\times 10^3$/ml among different age groups whereas the frequency of lymphocyte across different age groups ranged from 29.12% to 46.00% (Table 1). Erythrocytes numbers ranged between 3.5 to 6.4 x 10^6/µl. The highest concentration of haemoglobulin was found among age group ≥ 51-60 years old. Haematocrit (HCT) values ranged from 37.42% to 41.43% across age groups and the highest mean Corpuscular Volume (MCV) was 84.1Fl. Mean corpuscular haemoglobin (MCH) ranged between 26.3 to 28.5 pg among different age groups. The

present study revealed also that mean corpuscular haemoglobin concentration (MCHC) value ranged between 33.15 to 33.95 ± 0.46 g/dL among age groups studied. Ferritin levels among the studied age groups varied with the highest level observed in ≥51-60 age group and the value was 57.83 ± 22.12 ng/mL whereas the lowest mean was seen in 41-50 years age group with value equal to 32.25 ± 19.65 ng/mL.(Figure 1). Comparison of haematological parameters among the five age groups revealed no statistically significant age-related differences (P > 0.05). Although ferritin, lymphocyte count, MCV and haematocrit showed some numerical fluctuations between age groups, these variations were not statistically different. Overall, the haematological profile remained relatively stable across the studied age groups.

Table 1: Interrelationship of blood counts and ferritin in relation to age of patients

Parameter	Age group	Number	Mean	Std. Error	Minimum	Maximum
WBC	10-20	12	8.79a	0.9490	6.4	18.1
	21-30	16	8.30 a	0.4637	6.0	12.6
	31-40	10	8.37 a	0.5275	5.9	11.2
	41-50	6	6.54a	0.4944	4.8	7.9
	≥ 51-60	4	6. 90a	1.1598	4.8	9.5
Lymphocyte	10-20	12	35.40 a	3.30	19.5	54.7
	21-30	16	31.1 8a	2.0638	16.8	47.6
	31-40	10	29.19 a	3.9314	3.0	42.5
	41-50	6	29.1 2a	6.1602	2.2	43.6
	≥ 51-60	4	46.00 a	6.0960	36.3	63.8
Erythrocyte	10-20	12	4.63 a	0.1312	3.9	5.4
	21-30	16	4.62 a	0.0962	4.2	5.5
	31-40	10	4.44 a	0.1524	4.0	5.5
	41-50	6	4.71 a	0.3908	3.5	6.4
	≥ 51-60	4	4.9 3a	0.0883	4.8	5.2
Haemoglobin	10-20	12	13.0 3a	0.4310	10.2	14.7
	21-30	16	12.7 8a	0.2782	11.2	14.7
	31-40	10	12.50 a	0.6963	9.7	16.6
	41-50	6	12.40 a	1.0286	8.5	15.8
	≥ 51-60	4	13.8 3a	0.7878	12.2	15.5
Haematocrit	10-20	12	39.1 6a	1.2599	33.1	44.9
	21-30	16	38.3 9a	1.0137	31.6	46.8
	31-40	10	37.42 a	2.1650	28.0	48.9
	41-50	6	37.4 7a	3.2207	25.5	48.8
	≥ 51-60	4	41.4 3a	2.1500	35.3	45.3
MCV	10-20	12	81.54 a	3.2577	48.8	88.9
	21-30	16	79.90 a	3.0146	38.3	94.3
	31-40	10	83.64 a	2.5660	69.7	91.6
	41-50	6	79.4 2a	1.9429	72.9	86.3
	≥ 51-60	4	84.05 a	4.0956	71.9	89.8
MCH	10-20	12	28.24 a	0.7261	21.0	30.7
	21-30	16	26.4 3a	1.6501	2.4	30.3
	31-40	10	28.52 a	0.7405	24.2	30.3
	41-50	6	26.30 a	0.8165	24.3	29.7
	≥ 51-60	4	28.5 3a	1.3512	24.8	31.0
MCHC	10-20	12	33.34 a	0.4958	28.7	35.2
	21-30	16	33.49 a	0.3104	31.4	35.6
	31-40	10	33.32 a	0.2728	32.2	34.6
	41-50	6	33.15 a	0.3490	32.1	34.4
	≥ 51-60	4	33.95 a	0.4555	32.6	34.5
Ferritin	10-20	12	34.2 1a	5.3390	11.5	62.3
	21-30	16	34.14 a	9.8488	3.2	159.5
	31-40	10	54.4 4a	23.6292	4.8	194.9
	41-50	6	32.25 a	19.6461	8.3	130.0
	≥ 51-60	4	57.8 3a	22.1163	8.9	107.5

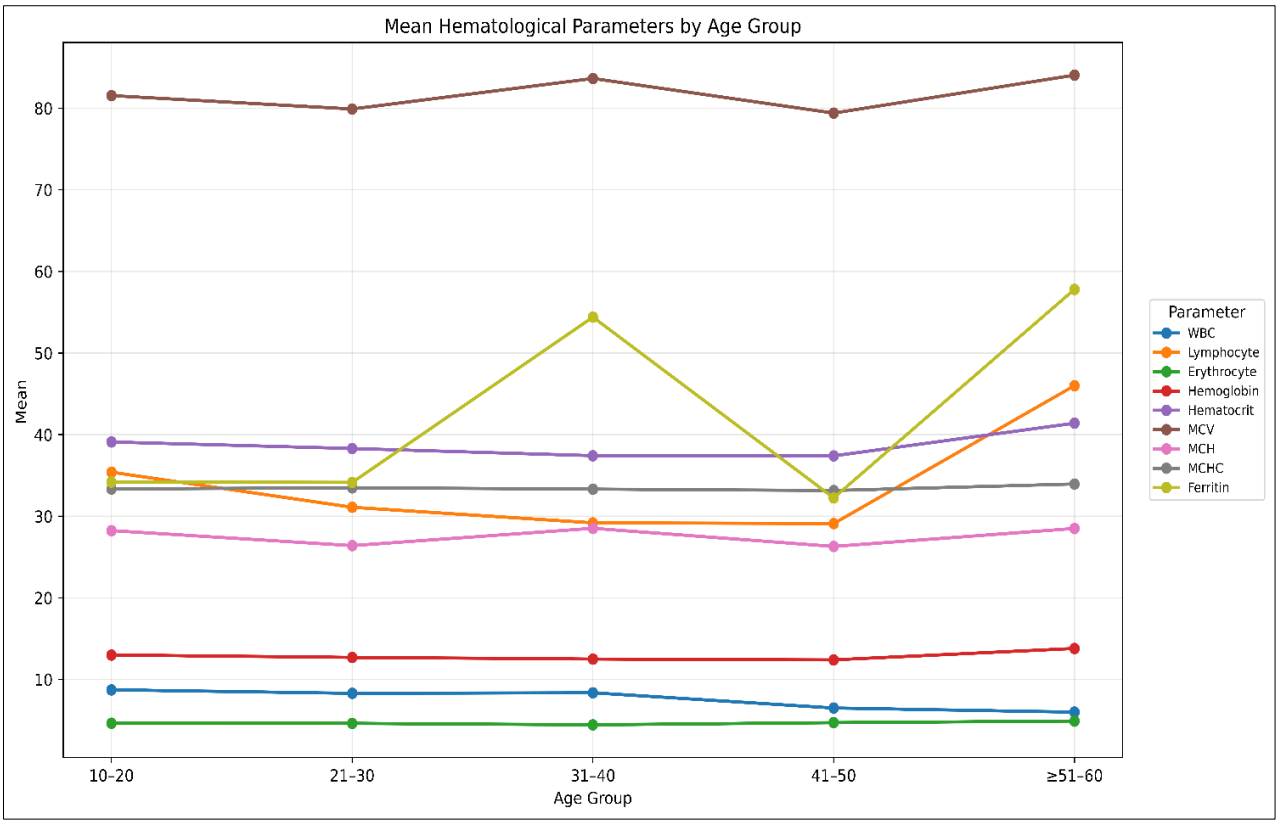

Figure 1: Age-related variation in mean hematological parameters among different age classes

Ferritin and gender

Ferritin values were numerically higher in male participants than in females. Among the hematological indices, erythrocyte count, hemoglobin, hematocrit, lymphocyte count, and MCHC possessed the same directional trend as ferritin, whereas WBC, MCV, and MCH showed opposite trends (Table 2, Figure 2). Erythrocyte count was the only parameter showing a statistically significant gender difference ($P < 0.05$) utilizing Mann-Whitney U test. Because only summary statistics were available, these data would be regarded as descriptive comparisons rather than indication of significant correlations.

Table 2: Interrelationship between blood counts and ferritin in relation to gender

Parameter	Gender	Number	Mean	Std. Error	Minimum	Maximum
WBC	Male	14	7.94 a	0.53	4.8	12.6
	Female	36	8.16 a	0.40	4.8	18.1
Lymphocyte	Male	14	35.10 a	4.25	2.2	63.8
	Female	36	32.19 a	1.70	3.0	54.7
Erythrocytes	Male	14	4.92 a	0.14	4.1	6.4
	Female	36	4.53 b	0.07	3.5	5.5
Haemoglobin	Male	14	13.16	0.69	6.9	16.6
	Female	36	12.55	0.25	8.5	15.0
Haematocrit	Male	14	39.06 a	2.01	23.4	48.9
	Female	36	37.92 a	0.79	25.5	46.0
MCV	Male	14	75.94 a	4.63	38.0	94.3
	Female	36	82.16 a	1.32	48.8	91.6
MCH	Male	14	26.09 a	1.92	2.4	31.0
	Female	36	27.98 a	0.35	21.0	30.7
MCHC	Male	14	33.92 a	0.30	31.4	35.6
	Female	36	33.23 a	0.20	28.7	35.2
Ferritin	Male	14	42.40 a	13.01	4.3	159.5
	Female	36	38.64 a	7.45	3.2	194.9

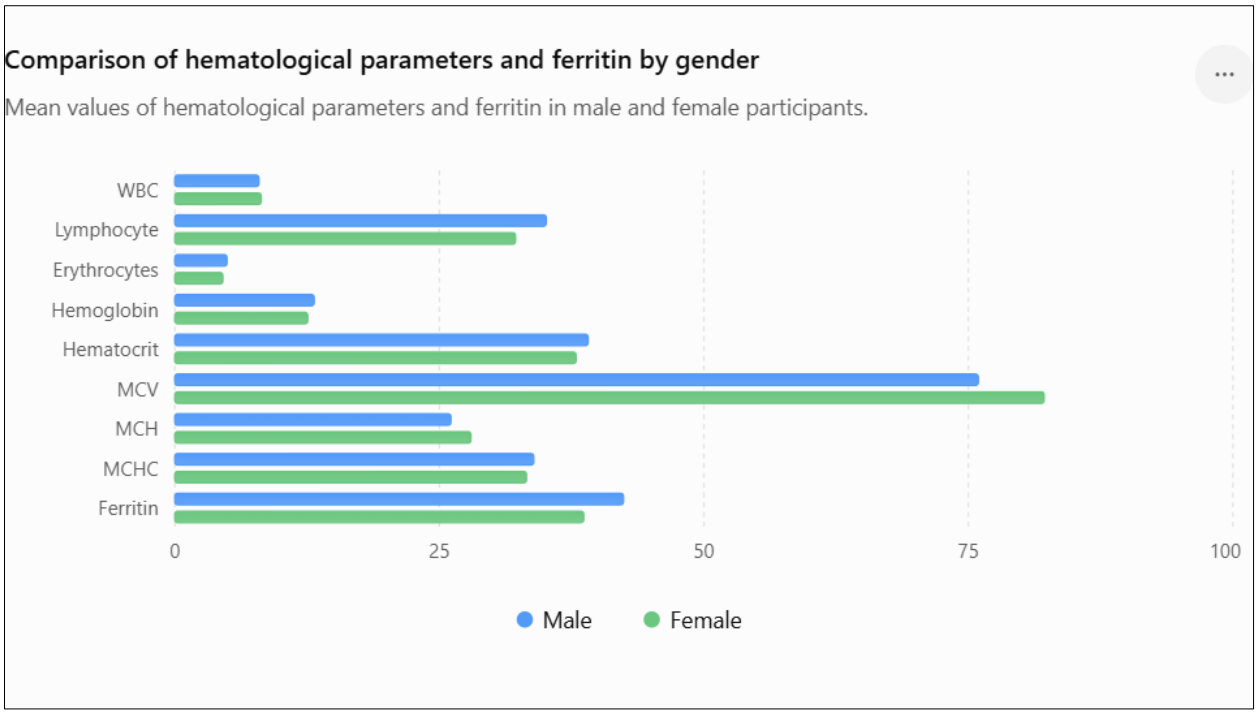

Figure 2: Comparison of hematological parameters and ferritin concentrations between male and female participants

DISCUSSION

The present study revealed that the mean values of white blood cells were ranging from 6.54 to 8.79 $\times 10^3$/ml among different age groups whereas the frequency of lymphocyte across different age groups ranged from 29.12% to 46.00%. Erythrocytes numbers ranged between 3.5 to 6.4 x 10^6/µl. The highest concentration of haemoglobulin was found among age group ≥ 51-60 years old. Haematocrit (HCT) values ranged from 37.42% to 41.43% across age groups and the highest mean Corpuscular Volume (MCV) was 84.1Fl. Other values were variable according to age and gender (Table 1). MCH emerged as the most influential predictor for the ferritin model. This finding is biologically supported by previous evidence showing a significant association between serum ferritin and MCH levels in patients with iron-deficiency anemia [35-40]. Reduced iron content directly limits HB biosynthesis, leading to a decrease in HB content per erythrocyte before more noticeable changes in cell size occur. Furthermore, MCH may reflect iron-restricted erythropoiesis at an earlier sensitive stage than other RBC indices. MCV ranked highest in importance. This observation is consistent with the progressive reduction in erythrocyte size observed in iron-deficient states, in which sustained impairment of HB synthesis ultimately leads to production of microcytes [41-44]. The differential ranking of MCH and MCV across the two targets suggests that distinct haematological expressions of iron deficiency may be preferentially captured depending on whether the biochemical reference marker reflects iron stores or circulating iron availability [2,45-48]. It has been concluded that MCV and MCH values were lower among latent iron-deficient women were not significantly different from the non-deficient or normal group which were similar with earlier studies. Rabindrakumar *et al.,* concluded a positive correlation between serum ferritin and MCV, MCH, and MCHC indicating that these indices decrease progressively with worsening iron depletion [6]. However, the changes may not statistical significance in latent deficiency when haemoglobin production is not yet compromised. Tiwari et al also found no significant association between ferritin levels and MCV or MCH in pregnant women [49-53]. The physiological explanation for these findings related to the gradual depletion of iron stores, which primarily affects the uniformity of erythrocyte production. As iron becomes insufficient for consistent haemoglobin production, erythropoiesis synthesizes red cells of variable size. Only with sustained deficiency do MCV and MCH decline significantly [54-57]. This early variability in red cell morphology may be used as a practical indicator of compromised iron supply to the marrow. Furthermore, the present study revealed that Ferritin values were numerically higher in male participants than in females. Among the hematological indices, erythrocyte count, hemoglobin, hematocrit, lymphocyte count, and MCHC possessed the same directional trend as ferritin, whereas WBC, MCV, and MCH showed opposite trends. However, it was found that male sex, feeding type, and postnatal weight gain were individually associated with increased iron deficiency (ID) risk. Male infants possessed approximately twice the ID risk of females (27.9% vs. 15.8%), confirming previous observations of mean corpuscular volume, lower hemoglobin, and serum ferritin in males at 4, 6, and 9 months [58,

59,60,61,62,63]. Regarding feeding types, breastfed infants demonstrated markedly higher ID prevalence (29.1%) compared with mixed-fed (17.6%) and formula-fed(12.0%) groups [40,42,64]. The highest burden of anaemia is observed in low-middle-income countries like Nigeria [64-66]. In some African countries like Nigeria, the prevalence of anaemia is 58% among pregnant women in the reproductive age group [2]. The cause of anaemia is multifactorial, but it has been mostly due to insufficient dietary intake of iron which results in iron deficiency [1]. Low iron stores in pregnancy have been associated with a number of unfavourable outcomes, such as anaemia in the mother, low birth weight, preterm birth, and enduring cognitive impairments in the offspring [10,67-70]. Serum ferritin, serum transferrin, total iron binding capacity, and transferrin saturation are examples of iron-specific biomarkers that can be used to differentiate iron deficiency anaemia from other types of anaemia [71,72,73,74,75,76]. Serum ferritin is the earliest indicator of decreasing iron stores and remains unaffected by recent iron consumption [77,78,79,80,81]. Widely regarded as the first choice for assessing iron deficiency in pregnancy, serum ferritin is considered the most useful initial test for diagnosing absolute iron deficiency, despite its susceptibility to elevation during active infection or inflammation [82-85]. Variety Laboratory investigations for diagnosing iron deficiency and anemia are used such as complete blood count (CBC), ferritin, serum iron, transferrin, and transferrin saturation. The diagnostic workup of anemia must follow a stepwise policy in which the complete blood count (CBC) serves as the initial investigation and guides further laboratory examinations, whereas iron estimations, including ferritin and transferrin parameters are carried out only when anemia or microcytosis is concluded, to ensure appropriate and cost-effective use of laboratory resources [86-90].

CONCLUSIONS

Comparison of haematological parameters among the five age groups revealed no statistically significant age-related differences ($P > 0.05$). Although ferritin, lymphocyte count, MCV and haematocrit showed some numerical fluctuations between age groups, these variations were not statistically different. Erythrocyte count was the only parameter showing a statistically significant gender difference ($P < 0.05$) utilizing Mann-Whitney U test. Because only summary statistics were available, these data would be regarded as descriptive comparisons rather than indication of significant correlations.

Statement of Ethics

All the procedures involving human participation were conducted in strict accordance with ethical standards of Institutional Research Committee, Department of Scientific Research, Al-Qalam University as well as the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical norms.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

The author declares that he has no conflicts of interest, financial or otherwise.

Funding Sources

The author extends his appreciation to the Department of Scientific Research of University of Al-Qalam

Financial disclosure

The authors declared that this study did not receive any financial support.

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