

Iron Profile and Serum Electrolytes Among Patients with Microcytic Hypochromic Iron Deficiency Anemia Attending a Tertiary Care Center at Kathmandu, Nepal

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ABSTRACT

Introduction: Iron deficiency anemia is a significant public health concern in developing countries, including Nepal, and contributes considerably to morbidity. Beyond impaired hemoglobin synthesis, iron deficiency may influence red blood cell membrane function and electrolyte homeostasis through alterations in Na⁺/K⁺-ATPase activity. **Aims:** To evaluate serum iron profile, red cell indices, and serum electrolyte levels in patients with iron deficiency anemia and to assess the correlation of hemoglobin with serum ferritin, iron and serum electrolytes. **Methods:** A cross-sectional study was conducted at Nepal Medical College and Teaching Hospital from September 2024 to March 2025, among 323 patients aged 15–70 years diagnosed with iron deficiency anemia. Demographic data, complete blood count, red cell indices, serum iron profile, peripheral blood smear, and serum electrolytes (sodium, potassium, chloride, and bicarbonate) were analyzed using automated analyzers. Data analysis was performed using SPSS version 17, applying descriptive and inferential statistics, with $p < 0.05$ considered statistically significant. **Results:** Females constituted 65.6% of the study population, with the highest prevalence observed in the 15–24-year age group. Mean serum iron and ferritin levels were markedly reduced, confirming iron deficiency. While most serum electrolytes remained within normal reference ranges, hemoglobin showed significant correlation with bicarbonate, chloride, and Red Cell Distribution Width. **Conclusion:** Iron deficiency anemia is associated with significant alterations in iron profile, red cell indices, and serum electrolyte levels. Routine monitoring of serum electrolytes in individuals with iron deficiency anemia will aid in early detection of electrolyte imbalance and prevention of related complications.

Keywords: Electrolyte imbalance; Ferritin; Iron Deficiency Anemia; Serum Sodium; Serum Potassium

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INTRODUCTION

Iron deficiency anemia (IDA) is a highly prevalent nutritional disorder, particularly in developing countries, affecting nearly one-third of the global population, with iron deficiency accounting for about one-third of all anemia cases.^{1,2} Anemia-related complications result in approximately 800,000 deaths annually, in Nepal, IDA affects about 42.55% of the population, highlighting its major public health burden.³ IDA results from inadequate iron availability to sustain physiological functions representing a severe consequence of disturbed iron homeostasis, alongside hemochromatosis.^{4,5} Owing to its wide distribution across all ages, genders, socioeconomic groups, IDA re-

mains a critical global health problem.⁶ IDA is diagnosed by low serum ferritin or reduced transferrin saturation with increased iron-binding capacity.⁷ Beyond hematological impairment, IDA may be associated with disturbances in serum electrolytes, which are essential for cellular function, red blood cell (RBC) integrity, effective oxygen-carbon dioxide exchange.⁸ Calcium-sensitive potassium channels and the RBC membrane-bound Na⁺/K⁺-ATPase play key roles in erythrocyte survival and ionic homeostasis.^{9,11} Altered Na⁺/K⁺-ATPase activity in anemia, possibly as a compensatory response to hypoxia, may influence serum sodium and potassium levels.¹²⁻¹⁵ Electrolyte imbalances can cause clinical manifestations ranging from fatigue, muscle cramps to severe cardiac, neurological complica-

tions.¹⁰ While electrolyte alterations have been reported in other anemias, including sickle cell anemia and β -thalassemia major, findings in IDA are limited and inconsistent.^{10,15,20} Given the paucity of data on electrolyte disturbances in IDA, the present study aims to evaluate serum electrolyte alterations in individuals with iron deficiency anemia to aid in early detection, prevention of imbalance, reduction of associated complications.

METHODS

A hospital-based prospective cross-sectional study was conducted to evaluate the serum iron profile, red cell indices, and serum electrolyte levels among patients with iron deficiency anemia (IDA) and to assess the correlation of hemoglobin with serum ferritin, iron and serum electrolytes. The study was carried out at Medicine and Pathology departments at Nepal Medical College and Teaching Hospital (NMCTH), Jorpati, Kathmandu, Nepal, from September 2024 to March 2025. The study population comprised patients aged 15-70 years with laboratory-confirmed iron deficiency anemia attending the Outpatient Department (OPD) of NMCTH during the study period. Sample size included 323 patients.

Sample Size

A total of 323 eligible patients were included in the study.

$$n = z^2pq/d^2 = 1.96^2 \times 0.70 \times 0.30 / 0.05^2 \approx 323$$

where, $p = 70\% = 0.70$ = Prevalence of IDA and $q = 1 - p$

$$z^2 = 1.96 = \text{value of } z \text{ at } 5\% \text{ level of significance}$$

$$d = 5\% = 0.05 = \text{Margin of error}$$

$$\text{Prevalence of IDA } (p) = 70\%^{28}$$

A minimum of 323 patients with iron deficiency anemia (IDA), between 15 to 70 years of age was included. Participants were recruited using a convenience sampling technique. After obtaining written informed consent, venous blood samples were collected under aseptic precautions for complete blood count, serum iron profile, and serum electrolyte analyses at Pathology and Medicine departments of NMCTH. Demographic and laboratory data were recorded using a predesigned structured proforma. Ethical approval was obtained from the Institutional Review Committee of Nepal Medical College and Teaching Hospital (Ref. No. 22-0811082). Written informed consent was obtained from all participants before enrollment. In the proforma demographic details including name, age, gender, the area of residence was taken along with the following parameters: complete blood count (CBC), serum iron profile, peripheral blood smear (PBS), and serum electrolytes which include serum sodium, serum potassium, serum chloride and serum bicarbonate. CBC was analyzed in Celtac ES MEK – 7300 Hematology Analyzer. Serum electrolytes and iron profile were analyzed in Sensa Core ST-200 electrolyte Analyzer and Beckman Coulter AU 480 Chemistry Analyzer respectively. For peripheral blood smear Wright’s stain was performed. The normal reference ranges of the parameters measured at NMCTH are as follows:

Hemoglobin (Hb) concentration: 12-15 g/dl, Red cell distribution width (RDW): 10-15%, Serum ferritin level: 11-307 ng/ml, transferrin saturation: 20-50% in males and 15-45% in females, serum iron: 60-180 μ g/dl, total iron binding capacity: 250 – 450 μ g/dl, serum sodium: 135-145 mmol/L, serum potassium: 3.5-5.0 mmol/L, serum chloride: 98-107 mmol/L and serum bicarbonate: 22-26 mmol/L.

RESULTS

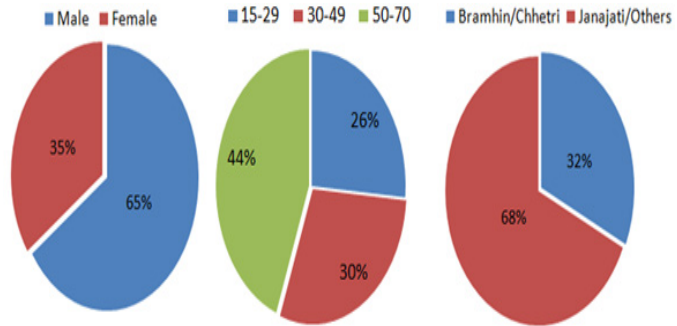


Figure 1: Distribution of study participants by gender, age group and ethnicity (n = 323)

Figure 1 depicts the demographic distribution of the study participants, with males (65%), individuals aged 50–70 years (44%), and Janajati/Other ethnicity (68%) representing the largest proportions of the study population.

Values of	Mean $\pm$ SD	Median	IQR	Minimum	Maximum
Iron	70.4 $\pm$ 33.51	57	53	9	170
Ferritin	50.6 $\pm$ 48.80	25	78	4.10	200
Sodium	138.4 $\pm$ 6.48	139	9	110	151
Potassium	4.03 $\pm$ 2.05	4	1	2.10	5.6
Bicarbonate	25.4 $\pm$ 1.53	26	3	20	29
Chloride	97.4 $\pm$ 6.41	97	3	35	110
Red cell width	14.3 $\pm$ 4.98	13.1	2.4	6	44.1

Table I: Descriptive Statistics of Serum Iron Profile and Electrolyte Parameters (n = 323)

	Shapiro-Wilk		
	Statistic	Df	Sig.
Iron_value	.906	323	.000
Ferritin_value	.787	323	.000
Sodium_value	.927	323	.000
Potassium_value	.219	323	.000
Bicarbonate_value	.952	323	.000
Chloride_value	.618	323	.000
Red_cell_width distribution	.634	323	.000

Table II: Tests of Normality (n = 323)

Normality testing using Shapiro–Wilk tests demonstrated that all study variables were not normally distributed ($p < 0.05$ for all variables). Therefore, non- parametric statistical tests were considered.

Serum values (Mean rank)	Gender		Mann-Whitney U
	Male (111)	Female (112)	
Iron	160.41	162.83	1.16, Z = -0.222, p = 0.825
Ferritin	155.31	165.50	11023.0, Z = -0.932, p = 0.351
Sodium	158.68	163.74	11397.3, Z = -0.466, p = 0.641
Potassium	177.50	153.88	10045.0, Z = -2.167, p = 0.030
Bicarbonate	173.14	156.17	10529.5, Z = -1.582, p = 0.114
Chloride	164.69	160.59	11467.5, Z = -0.378, p = 0.706
Red cell width	160.96	162.54	11651.0, Z = -0.145, p = 0.885

Table III: Serum values comparison in male and female (n = 323)

Mann-Whitney U test demonstrated significant gender differences in potassium levels ($p = 0.026$), with males showing higher mean ranks compared to females. However, no statistically significant differences were observed in serum iron, ferritin, sodium, bicarbonate, chloride, or red cell width between genders ($p > 0.05$).

Serum values (Mean rank)	Age group			Kruskal Wallis Test (df = 2)
	15-29 (85)	30-49 (95)	50-70(143)	
Iron	158.54	168.84	159.51	0.728, $p = 0.695$
Ferritin	147.77	155.57	174.73	5.082, $p = 0.079$
Sodium	173.82	162.38	154.72	2.259, $p = 0.323$
Potassium	166.62	148.528	168.21	2.344, $p = 0.504$
Bicarbonate	168.34	161.01	158.90	0.583, $p = 0.747$
Chloride	156.98	158.15	167.55	0.927, $p = 0.629$
Red cell width	160.77	156.28	166.53	0.714, $p = 0.700$

Table IV: Serum values comparison in different age group (n = 323)

Correlation Coefficient of serum values with Hemoglobin	Spearman's rho (ρ)	P value
Iron	1	0.055
Ferritin	1	0.251
Sodium	1	-0.033
Potassium	1	-0.038
Bicarbonate	1	0.190
Chloride	1	-0.098
Red cell width	1	-0.103

Correlation is significant at * 0.05 level, ** 0.01 level (1-tailed)

Table V: Spearman's correlation; hemoglobin level with ferritin, iron and serum electrolytes (n = 323)

Spearman's correlation analysis revealed a significant positive correlation of hemoglobin with ferritin ($\rho = 0.251$, $p < 0.001$)

and bicarbonate ($\rho = 0.190$, $p < 0.001$). Significant negative correlations were observed with chloride ($\rho = -0.098$, $p = 0.039$) and RDW ($\rho = -0.103$, $p = 0.032$).

DISCUSSION

Iron deficiency anemia (IDA) remains one of the most common nutritional disorders worldwide and constitutes a major public health concern in developing countries, including Nepal. Although the hematological consequences of iron deficiency are well established, its influence on serum electrolyte homeostasis remains inadequately understood. The present study evaluated iron profile parameters and serum electrolytes among patients with microcytic hypochromic IDA attending a tertiary care center in Kathmandu and explored their relationship with demographic characteristics and hemoglobin levels.

The study population was predominantly female, which is consistent with the recognized higher prevalence of iron deficiency among women due to menstrual blood loss, pregnancy, lactation, and increased physiological iron requirements. Similar findings have been reported by Rafiq et al²⁵ and Yortanlı et al²⁶ who observed a significantly higher prevalence of iron deficiency and IDA among females than males. This gender distribution reflects the continuing burden of nutritional anemia among women in South Asian populations. The mean serum iron concentration observed in the present study (70.4 ± 33.51 $\mu\text{g/dL}$) was within the lower range of normal values, while ferritin levels exhibited considerable variability among participants. Ferritin demonstrated a significant positive correlation with hemoglobin concentration ($\rho = 0.251$, $p < 0.001$), indicating that reduced iron stores are associated with declining hemoglobin levels. This finding is biologically plausible because ferritin serves as the primary intracellular iron storage protein and is regarded as the most sensitive marker of iron deficiency.^{7,32} The observed association confirms the central role of iron depletion in the pathogenesis of microcytic hypochromic anemia and supports the diagnostic utility of ferritin in assessing the severity of iron deficiency.

With regard to electrolyte status, the mean serum sodium, potassium, bicarbonate, and chloride concentrations were generally within established reference ranges. However, significant associations were observed between hemoglobin concentration and selected electrolyte parameters. Hemoglobin showed a significant positive correlation with bicarbonate levels ($\rho = 0.190$, $p < 0.001$) and significant negative correlations with chloride ($\rho = -0.098$, $p = 0.039$) and red cell distribution width (RDW) ($\rho = -0.103$, $p = 0.032$). In contrast, serum sodium and potassium levels were not significantly associated with hemoglobin concentration.

The positive association between hemoglobin and bicarbonate may be explained by the physiological role of erythrocytes in carbon dioxide transport and acid-base regulation. Hemoglobin acts as an important intracellular buffer and contributes substantially to the maintenance of acid-base balance through reversible binding of hydrogen ions and facilitation of carbon dioxide transport. Reduced hemoglobin concentrations may

therefore alter buffering capacity and bicarbonate homeostasis. Although the correlation observed was modest, it suggests a potential interaction between anemia severity and acid-base regulatory mechanisms.

The significant negative correlation between hemoglobin and chloride concentration may be related to alterations in erythrocyte membrane ion transport. Chloride participates in the chloride-bicarbonate exchange process (Hamburger shift), which is essential for carbon dioxide transport across erythrocyte membranes.⁸ Iron deficiency has been reported to affect erythrocyte membrane integrity and ion transport mechanisms, potentially leading to subtle changes in chloride distribution.^{9,10} However, the weak strength of the correlation indicates that the clinical significance of this association remains limited and requires further investigation. The inverse relationship between hemoglobin concentration and RDW observed in the present study is consistent with the characteristic hematological changes of IDA. As iron deficiency progresses, increasing anisocytosis results in greater variability in erythrocyte size and consequently elevated RDW values. Therefore, lower hemoglobin concentrations are expected to be associated with higher RDW values, reflecting increasing severity of iron deficiency and ineffective erythropoiesis.

An important finding of this study was the absence of significant correlations between hemoglobin concentration and serum sodium or potassium levels. Similarly, no significant differences in sodium, chloride, bicarbonate, ferritin, iron, or RDW were observed between male and female participants. The only gender-related difference identified was a significantly higher potassium level among males compared with females. The underlying reason for this observation is uncertain but may reflect differences in muscle mass, hormonal influences, dietary intake, or intracellular potassium distribution between sexes.

The findings regarding electrolyte parameters differ from those reported in several previous studies. Mansoor et al²² reported significantly lower sodium concentrations and significantly higher potassium and chloride levels among anemic patients compared with non-anemic controls. Likewise, Rafiq et al²⁵ observed lower sodium and bicarbonate levels and higher potassium and chloride concentrations in patients with IDA. Rajagopal et al²³ also demonstrated significant alterations in sodium, potassium, and chloride levels among individuals with IDA and reported significant correlations between electrolyte concentrations and hematological indices.

The discrepancies between the present findings and those of previous investigators may be attributable to differences in study design, sample characteristics, severity of anemia, nutritional status, ethnic variations, environmental influences, and laboratory methodologies. Notably, most previous studies compared anemic patients with healthy control groups, whereas the present study evaluated electrolyte patterns within a population already diagnosed with microcytic hypochromic IDA. Consequently, electrolyte differences that are apparent between anemic and non-anemic populations may not necessarily correlate with variations in hemoglobin concentration

among patients with established IDA.

The lack of significant age-related differences in iron profile and electrolyte parameters further suggests that the biochemical alterations observed in IDA may be relatively independent of age. Although ferritin demonstrated an increasing trend and sodium showed a decreasing trend with advancing age, neither reached statistical significance. These findings indicate that age alone may not substantially influence iron stores or electrolyte balance in patients with microcytic hypochromic IDA.

Several mechanisms have been proposed to explain electrolyte alterations in anemia. Previous studies have suggested that chronic tissue hypoxia may modify Na⁺/K⁺-ATPase activity as a compensatory response, thereby influencing transmembrane sodium and potassium transport.^{10–15} Experimental evidence has also demonstrated alterations in membrane potential and electrolyte handling in disorders such as sickle cell anemia and β -thalassemia.^{15–20,27} However, the largely normal electrolyte concentrations observed in the present study suggest that homeostatic mechanisms effectively maintain electrolyte balance in most patients with IDA despite impaired erythropoiesis and reduced oxygen-carrying capacity. The findings of this study have important clinical implications. Although major electrolyte disturbances were not observed, the significant associations of hemoglobin with ferritin, bicarbonate, chloride, and RDW indicate that iron deficiency may exert subtle effects on metabolic and membrane transport processes. Therefore, assessment of electrolyte status may be beneficial in patients with severe anemia, coexisting medical conditions, or clinical features suggestive of electrolyte imbalance. Early identification and correction of both iron deficiency and electrolyte abnormalities may contribute to improved patient outcomes.

The present study has several strengths, including a relatively large sample size and comprehensive evaluation of both iron profile and electrolyte parameters in a Nepalese population. However, certain limitations should be acknowledged. The cross-sectional design precludes causal inference, and the absence of a healthy control group limits direct comparison between anemic and non-anemic individuals. Furthermore, measurements of transferrin saturation, total iron-binding capacity, erythrocyte membrane enzyme activity, and acid-base parameters were not performed, which could have provided additional mechanistic insights into the relationship between iron deficiency and electrolyte homeostasis.

In conclusion, this study demonstrated significant positive correlations of hemoglobin concentration with serum ferritin and bicarbonate levels and significant negative correlations with chloride concentration and RDW among patients with microcytic hypochromic iron deficiency anemia. No significant associations were observed between hemoglobin and serum sodium or potassium concentrations, and most electrolyte parameters remained within normal physiological limits. These findings suggest that while IDA is associated with subtle alterations in selected biochemical parameters, overt disturbances in serum electrolyte homeostasis are uncommon. Further multicenter studies incorporating healthy controls and detailed

assessment of erythrocyte membrane transport mechanisms are warranted to clarify the complex relationship between iron metabolism and electrolyte balance.

LIMITATIONS

This hospital-based prospective cross-sectional study conducted at a single tertiary care center may limit the generalizability of the findings. Moreover, the cross-sectional design permits the assessment of iron profile and serum electrolyte status at a single point in time but does not allow causal relationships to be established.

CONCLUSION

Iron deficiency anemia is associated with significant alterations in iron profile, red cell indices, and serum electrolyte levels. Routine monitoring of serum electrolytes in individuals with iron deficiency anemia will aid in early detection of electrolyte imbalance and prevention of related complications.

REFERENCES

1. Kaur M, Singh A, Bassi R, Kaur H. Nutritional status and anaemia in medical students of SGRDIMSAR, Amritsar. *Nat'l J Physiol Pharm Pharmacol* 2015; 5: 45-9.
2. Wu AC, Lesperance L, Bernstein H. Screening for iron deficiency. *Pediatr Rev* 2002; 23: 171-8.
3. Mishra SK, Marasini S, Gupta BK, Agrawal KK, Gautam N. Prevalence of iron deficiency anemia in anemic patients: a hospital-based study. *J Uni Coll Med Sci* 2018; 02(18): 41-5.
4. Salsbury G, Cambridge EL, McIntyre Z, Arends MJ, Karp NA, Isherwood et al. Disruption of the potassium channel regulatory subunit KCNE2 causes iron-deficient anemia. *Exp Hematol* 2014; 42: 1053-8.
5. Mahantesha T, Reddy KM, Ellore VP, Ramagoni NK, litagi V, Anitha KS. Evaluation and association of iron deficiency anaemia with salivary pH and buffering capacity. *Natl J Physiol Pharm Pharmacol* 2014; 4: 229-32.
6. Antappanavar VB, Biradar SG, Patil V, Biradar PM, Mithare S, Sharma AK. A study of correlation between iron deficiency anaemia and serum lipid profile in Indian adults in Bidar. *Int J Adv Med* 2017; 1: 96-100.
7. Levi M, Rosselli M, Simonetti et al. Epidemiology of iron deficiency anemia in four European countries: a population-based study in primary care. *Eur J Haematol* 2016; 97: 583-93.
8. Cabantchick ZI. The Anion Transporter System of Red Blood Cell Membranes. *Blood Cell Biochemistry*. New York: Plenum Press; 1990. p. 337-59.
9. Lang E, Qadri SM, Lang F. Killing me softly-suicidal erythrocyte death. *Int J Biochem Cell Biol* 2012; 44: 1236-43.
10. Omar AK, Ahmed KA, Helmi NM, et al. The sensitivity of Na⁺, K⁺ ATPase as an indicator of blood diseases. *Afr Health Sci* 2017; 17: 262-9.
11. Totan AR, Greabu M. Effect of chronic hyperglycemia and vanadate treatment on erythrocyte Na/K-ATPase and Mg-ATPase in streptozotocin diabetic rats. *Acta Pol Pharm* 2002; 59: 307-11.
12. Mentzer WC, August CS, Nathan DG. The effects of androgen administration in sickle cell anemia. *Pediatr Res* 1969; 3: 378.
13. Koko A, Tannen RL. *Fluids and Electrolytes*. 2nd ed. Philadelphia, PA: WB. Saunders Company; 1990.
14. Shraf FA, Khanam A, Rizwan S. Evaluation of blood calcium and electrolytes in anemic women of Karachi. *World J Pharm Sci* 2017; 5: 103-5.
15. Tosteson DC, Shea E, Darling RC. Potassium and sodium of red blood cells in sickle cell anemia. *J Clin Invest* 1952; 31: 406-11.
16. Agoreyo FA, Nwanzen N. Plasma sodium and potassium changes in sickle cell patients. *Int J Genet Mol Biol* 2010; 2: 14-9.
17. Brugnara C. *Red Cell Dehydration in the Pathophysiology and Treatment of Sickle Disease*. Massachusetts: Department of Pathology and Laboratory Medicine, Harvard Medical School, Boston; 2000.
18. Joiner CH, Platt OS, Lux SE 4th. Cation depletion by the sodium pump in red cells with pathologic cation leaks. Sick cells and xerocytes. *J Clin Invest* 1986; 78: 1487-96.
19. Patil S, Biradar SM, Holyachi R, Devarmani S, Reddy S. Assessment of Serum Electrolytes and Glycated Hemoglobin Level in Non-diabetic Iron-Deficient Anaemic Patients. *Cureus* 15(5): e38656. doi:10.7759/cureus.38656.
20. Karim MF, Ismail M, Hasan AM, Shekhar HU. Hematological and biochemical status of beta-thalassemia major patients in Bangladesh: A comparative analysis. *Int J Hematol Oncol Stem Cell Res* 2016; 10: 7-12.
21. Anemia and Polycythemia. In: Jameson J, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J. eds. *Harrison's Manual of Medicine*, 20e. McGraw-Hill Education; 2020. Accessed July 27, 2024.
22. Mansoor F, Bai P, Kaur N et al. Evaluation of serum electrolyte levels in patients with anemia. *Cureus* 2021; 13: 18417. doi:10.7759/cureus.18417.
23. Rajagopal L, Ganesan V, Abdullah SM, Arunachalam S, Kathamuthu K, Ramraj B. Perturbations of serum electrolyte levels in iron deficiency anemia – A comparative analysis. *Natl J Physiol Pharm Pharmacol* 2018; 8: 370-5.
24. Baral KP, Onta SR. Prevalence of anemia amongst adolescents in Nepal: a community-based study in rural and urban areas of Morang District. *Nepal Med Coll J* 2009; 11(3): 179-82.
25. Rafiq M, Arooj A, Tahir Q, Fayyaz N, Sramad A, Bashir S. Evaluation of serum electrolyte levels in iron deficiency anemia patients. *Professional Med J* 2021; 28(5):691-6.
26. Yortanlı B, Ecirli S, Soykan SZ. Prevalence of Iron Deficiency and Iron Deficiency Anemia Among Nursing Students Working in the Internal Medicine Clinic. *Cureus* 2023; 15(12): 51212. DOI 10.7759/cureus.51212

27. Nnodim JK, Meludu SC, Dioka CE, Onah C, Chilaka UJ, Obi PC. Altered Membrane Potential and Electrolyte in Sick Cell Anemia. *J Krishna Inst Med Sci* 2014; 3(1): 70-3.
28. Baral KP and Onta SR. Prevalence of anemia amongst adolescents in Nepal: a community based study in rural and urban areas of Morang District. *Nepal Med Coll J* 2009; 11(3): 179-82.
29. Bekele A, Tilahun M, Mekuria A. Prevalence of Anemia and Its Associated Factors among Pregnant Women Attending Antenatal Care in Health Institutions of Arba Minch Town, Gamo Gofa Zone, Ethiopia: A Cross-Sectional Study. *Anemia* 2016; 2016: 1-9. doi: 10.1155/2016/1073192.
30. Kumar V, Abbas AK, Fausto Nelson. Red blood cell and bleeding disorders. In: Robbins and Cotran Pathologic basis of disease. 7th ed. Elsevier: Noida, India; 2005. pp 619–59.
31. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and mineral nutrition information system. Geneva, WHO, 2011 (WHO/ NMH/NHD/MNM/11.1)
32. World Health Organization. (2020). WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. World Health Organization. <https://iris.who.int/handle/10665/331505>.
33. Molla MD, Degef M, Bekele A, Geto Z, Challa F, Lejisa T et al. Assessment of serum electrolytes and kidney function test for screening of chronic kidney disease among Ethiopian Public Health Institute staff members, Addis Ababa, Ethiopia. *BMC Nephrol* 2020; 21(1): 494.
34. Bain BJ, Bates I, Laftan MA, Lewis SM. Dacie and Lewis Practical Haematology. In: Bates I, Lewis SM. 11th edition. China: Elsevier, Churchill Livingstone 2011; 14.
35. <https://www.msmanuals.com/professional/multimedia/table/typical-normal-serum-values-for-iron-iron-binding-capacity-ferritin-and-transferrin-saturation>
36. Lwanga SK, Lemeshow S. Sample Size Determination in Health Studies: A Practical Manual. Geneva: World Health Organization; 1991.