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Research Article

ROLE OF RETICULOCYTE HEMOGLOBIN CONTENT IN DIAGNOSIS OF IRON DEFICIENCY ANEMIA

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ABSTRACT

Objectives: Anemia is a global problem of immense health significance affecting persons of all ages and economic groups. Iron deficiency anemia (IDA) is the most common type of anemia met with in clinical practice. For IDA diagnosis, estimation and treatment, many indices such as serum iron (SI), total iron binding capacity (TIBC), serum ferritin (SF), and soluble transferrin receptor assay are used. But reticulocyte hemoglobin content (CHr) is called as the gold standard for diagnosing IDA as it is the most valuable screening tool for identifying IDA with a sensitivity of 94% and specificity 80% and differentiates IDA from anemia of systemic disease. The present study was undertaken to evaluate CHr as a most efficient marker in diagnosing IDA.

Methods: This prospective observational study was carried out in the Department of General Medicine of M.K.C.G. Medical College and hospital, Berhampur, Odisha, India, from October 2017 to October 2019. Sixty microcytic hypochromic patients of either sex >18 years of age admitted in the medicine ward fulfilling the inclusion and exclusion criteria were included in this study. After taking detailed history and clinical examination, laboratory investigations including complete blood count, SI, serum, ferritin, serum transferrin saturation, TIBC, CHr, and bone marrow aspiration with iron stain were done in all patients.

Results: In the study group of 60 patients, 10 (16.66%) patients had mild anemia, 17 (28.33%) had moderate anemia, and 33 (55%) had severe anemia. Mean hemoglobin of the patients was 6.86 g/dL and SD was 1.95 g/dL. Nineteen (31.66% patients) had TIBC in the range of 351–400 µg/dl. Mean±SD of serum TIBC was 333.91±67.26 µg/dL. Thirty-nine patients (65%) had transferrin saturation in the range of 0.1–10%. The mean±SD of the study group was 13.68±3.22%. Fifty (83.33%) patients had SF in the range of 0–100 µg/dL. Twenty-three patients (38.33%) had CHr concentration between 15.1 and 20 pg followed by 19 (31.66%) between 20.1 and 25 pg and 18 (30%) between 25.1 and 30 pg. The mean±SD of this study was 22.14 pg±3.92.

Conclusion: CHr is found to be a potential biomarker that can be used to differentiate IDA from other causes of anemia.

Keywords: Anemia, Iron deficiency anemia, Reticulocyte hemoglobin content.

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INTRODUCTION

Anemia is defined as "Hemoglobin level in blood below the lower extreme of the normal range for the age and sex of individual" [1]. It is a global problem of immense health significance affecting persons of all ages and economic groups. It has been estimated that 20% of the world's population is iron deficient. It occurs at all ages, but is especially common in women of child bearing age, in whom it is an important cause of chronic fatigue and ill health [1]. During the reproductive life of the female, menstruation, pregnancy, parturition, and lactation the physiological requirements of iron increase significantly [1,2].

Iron deficiency anemia (IDA) is the most common type of anemia throughout the world and according to Idris *et al*., when iron deficiency is widespread and severe, the prevalence of morbidity and effects on the individual's resistance to infectious disease are significant [3].

The screening procedure used most often is blood hemoglobin determination. Values falling below the cutoff point are considered abnormal. The normal range of hemoglobin values for adult males is 13.5–17.5 g/dL and that for adult females is 12.5–15 g/dL. The World Health Organization (WHO) defines anemia as a hemoglobin level <13 g/dL in men, <12 g/dL in non-pregnant women, and <11 g/dL in pregnant women. Anemia in pregnancy is common. This is related to increased demands of iron during pregnancy, pre-existing negative iron balance due to frequent pregnancies, menstrual blood loss, dietary inadequacies, helminthiasis, and amoebiasis are important contributory factors [2,4,5]. In India and other developing countries, incidence of nutritional anemia in reproductive age groups ranges from 60% to 80% compared to 10–20% in developed countries [6]. In Asia, the prevalence of nutritional anemia is particularly high in countries such as Bangladesh (74–80%), Indonesia (37–73%), India (34–69%), and the Philippines (42–47%) [7].

For IDA diagnosis, estimation and treatment, many indices such as serum iron (SI), total iron binding capacity (TIBC), serum ferritin (SF), and soluble transferrin receptor assay are used. Gorden *et al.* have shown that SF is the best investigation for distinguishing those with iron deficiency from those who were not iron deficient. Appropriate use of SF would refute diagnosis of iron deficiency without a bone marrow aspirate in 70% of patients [8].

Reticulocyte hemoglobin content (CHr) is a measurement of hemoglobin inside the reticulocyte. It correlates directly with the functional availability of iron in bone marrow. Just like any other perfect laboratory test it is accurate, simple, and less expensive [9]. Reticulocytes are newly produced, relatively immature red blood cells. Reticulocyte count or percentage is an indicator of ability of persons bone marrow to produce adequate RBCs (erythropoiesis).

Reticulocytosis reflects responsive marrow. Reticulocytopenia suggests nonfunctional bone marrow. Automated reticulocyte counts have greater precision, accuracy and reproducibility than manual counts [10]. The reference range for reticulocyte count for adults is 0.5–1.5%. Corrected reticulocyte count (CRC) or reticulocyte index (RI) give more accurate assessment of marrow function. The reference range for CRC in adults is 0.5-1.5%. Modern automated particle cell counters utilize flow cytometry technique and measure reticulocyte cellular characteristics, that is, immature reticulocyte fraction (IRF) and CHr [11,12]. CHr is most valuable screening tool for identifying IDA with a sensitivity of 94% and specificity of 80% [13,14]. In anemia of systemic disease (ASD) functional iron deficiency may be seen because of poor absorption from GI tract of increased production of hepcidin which in turn trap Fe in reticuloendothelial system (RES). This condition (ASD) confuses with IDA. CHr differentiates IDA from ASD. The discriminatory power of CHr both with respect to sensitivity and specificity is better than mean corpuscular volume (MCV) and ferritin [11]. CHr predicts early response to treatment of IDA whereas Hematocrit, RBC indices take weeks to predict response to treatment.

Today CHr is called as the gold standard for diagnosing IDA replacing both of these. Despite its simplicity and utility, it is rarely used in clinical practice. In our state particularly southern Odisha IDA is very common problem which needs to be diagnosed early. Hence, CHr can be an extremely valuable recent addition for diagnosis and assessment of therapeutic response and also it differentiates from ASD which many time confuses with IDA.

The present study was undertaken to evaluate reticulocyte hemoglobin content (CHr) as a most efficient marker in diagnosing IDA.

MATERIALS AND METHODS

A prospective observational study was designed and conducted in the Department of General Medicine in MKCG Medical College and Hospital, Berhampur, Odisha, India, over a period of 2 years from October 2017 to October 2019. A total number of 60 microcytic hypochromic patients of either sex above the age of 18 years fulfilling the inclusion criteria were taken in this study. The study was conducted after the study protocol was approved by the Institutional Ethics Committee (No. 630/ Chairman-IEC, M.K.C.G. Medical College, Berhampur-4). Informed consent was obtained from all the patients and the study was done in accordance with the guidelines of the Declaration of Helsinki 2008.

Inclusion criteria

The following criteria were included in the study:

- Patients with microcytic hypochromic anemia
- >18 years of age
- Patients agreed to undergo bone morrow aspiration for diagnosis.

Exclusion criteria

The following criteria were excluded from the study:

- Pregnant women
- Patients who had received blood transfusion, oral, or IV iron supplementation within a month
- Patients suffering from any inflammatory disorders
- Patients suffering from hemoglobinopathy, leukemia, or myelodysplastic syndrome (MDS)
- Patients having mean MCV >80 fl.

All the 60 patients were subjected to detailed history and clinical examinations. They underwent the routine laboratory investigations such as complete blood count (CBC), comment on perepheral smear (CPs), SI, TIBC, SF, serum transferrin saturation, reticulocyte hemoglobin content (CHr), bone marrow iron estimation, C-reactive protein, erythrocyte sedimentation rate, serum electrolytes, renal function test, liver function test, FBS, PPBS, HBSAg, ICTC, and urine examination.

Venous blood was collected in all patients with aseptic precaution in EDTA anticoagulant for hematological investigation. Separate blood sample was collected for biochemical investigations. The hematological investigations were performed on sysmex KX-21 with standard calibration using fresh whole blood. As a part of CBC, red blood cell indices (MCV, MCH, and MCHC), PCV, RDW, white blood cell count, and platelet count were obtained by sysmex KX-21.

Test methodology for reticulocyte hemoglobin content (CHr)

The test methodology used to measure CHr was flow cytometry. The cellular hemoglobin content of the reticulocytes was measured on a per cell basis by dual angle light scatter and the mean was reported as the CHr.

Specimen collection

Two milliliters of whole blood in a lavender top tube were taken. Specimens were collected and handled carefully, especially when collecting from a central venous catheter.

RESULTS

To ensure accurate results

- Contaminating blood with heparin or saline was avoided.
- Luer adapter to collect specimen directly into lavender top tube was used. Use of syringes was avoided.
- Samples were by inverting gently 5 times after collection.
- Specimens were refrigerated promptly while awaiting shipment. Freezing was avoided.

Interpretation of results

The normal limits for CHr are: 24.5–31.8 pg.

Values <26 pg may be indicative of iron deficiency.

Bone marrow biopsy for evaluation of iron status

To estimate the iron stores directly, usually requires sampling one of the two principal storage depots, the bone marrow or the liver.

Aspiration and biopsy of marrow is usually preferred. More often, the specimen is stained by the Prussian blue method, which renders hemosiderin blue.

Procedure for bone marrow iron estimation

- Under strict aseptic precaution bone marrow aspirates were collected from posterior iliac crest.
- Bone marrow smear were freshly collected and contained marrow fragments because iron was demonstrable only in marrow particles.
- The smear was fixed in methanol for 15 min and dried.
- Equal volume of 2% potassium ferrocyanide and N/5HCL were mixed in a Coplin jar. The stain was warmed to 55°C just before use.
- The stain was then washed in running water for 15 min which was followed by washing in distilled water.
- The smear in the slide was counterstained with 1%nuclear fast red or 0.1%neutral red that stained the nuclei red.

Findings

- The smear on the slide was examined and looked for marrow particles with the help of a scanner objective.
- The marrow particles were at first examined in low power objective and then using high power objective for presence of iron granules in reticuloendothelial cells and also for free iron.

Grade	Criteria	Iron content (μg/g)
0	No iron granules observed	43±23
1+	Small granules in the reticulum cells, seen only in oil immersion lens.	130±50
2+	Iron granules visible with low power lens.	223±75
3+	Numerous small granules in all marrow particles	406±131
4+	Large granules in small clumps	762±247
5+	Dense large clumps of granules	1618±464
6+	Very large deposits obscuring marrow details	3681±1400

Criteria For Grading Iron Stains In Bma

Sensitivity and specificity of CHr, SF, SI, TS, and TIBC in males and females in diagnosing IDA were calculated using the following formula.

Sensitivity = True positive/(True positive+False negative) × 100

Specificity = True negative/(True negative+False positive) × 100

Positive predictive Value = True positive/(True positive+False positive) × 100

Negative predictive Value= True negative/(True negative+False negative) × 100

Statistical analysis

Data were entered using Microsoft Excel and exported to SPSS version 17.0. Receiver operating characteristic curve analysis was performed to identify the optimal CHr cut off value for predicting IDA. $p<0.05$ was considered statistically significant.

RESULTS

In the study group of 60 patients, of microcytic hypochromic anemia, 36 (60%) are female and 24 (40%) are male. Ratio of male: female is 1:1.5 (Table 1). Table 2 and Fig. 1 show that patients' age is ranged from 21 to 70 and maximum incidence of 31 (51.66%) patients is seen in age group of 21–30 years. Mean age ±SD of patients in the study group is 36.03±14.21 years.

In the present study, 10 (16.66%) patients had mild anemia, 17 (28.33%) patients presented moderate, and 33 (55%) with severe anemia. Mean hemoglobin of the study group is 6.86 g/dl and standard deviation is 1.95 g/dL (Table 3 and Fig. 2).

Incidence of mild anemia in male 9 (15%). Ten (16.66%) patients have mild anemia. including one female. Moderate anemia was found to be in 8 (13.33%) male patients and 9 (15%) female patients; severe anemia in 7 (11.6%) males and 26 (43.33%) females (Table 4 and Fig. 3).

MCV value of the patients ranging from 40 to 80 fl was found and maximum incidence of 25 (41.66%) patients was seen in range 60–70 fl. Mean±SD of MCV in the study group was 64.74±7.8 fl (Table 5 and Fig. 4).

Table 1: Sex distribution of the study population (n=60)

Sex	Number of patients (%)
Male	24 (40)
Female	36 (60)

Table 2: Distribution of study population as per age (n=60)

Age (years)	Number of patients (%)
21–30	31 (51.66)
31–40	12 (20)
41–50	5 (8.33)
51–60	8 (13.33
61–70	4 (6.66)

Table 3: Distribution of study population according to severity of anemia (n=60)

Grade of anemia (Hb in g/dL)	Number of patients (%)
Mild (9.1–11)	10 (16.66)
Moderate (7.1–9)	17 (28.33)
Severe (≤7)	33 (55)

Hb: Hemoglobin

Forty (66.66%) patients had SI below 50 µg/dL, 17 (28.33%) patients had SI between 51 and 100 µg/dL, and 3 (5%) had SI above 100 µg/dL. The mean and standard deviation of SI in the study group was 40.06±33.87 µg/dL (Table 6 and Fig. 5).

Maximum patients (31.66%, n=19) had serum TIBC in the range of 351–400 µg/dL. Mean±SD of serum TIBC is 333.91±67.26 µg/dl (Table 7 and Fig. 6).

Maximum patients (n=39, 65%) had transferrin saturation in the range of 0.1–10%. The mean±SD of the study group was 13.68±13.22% (Table 8 and Fig. 7).

Maximum patients (n=50, 83.33%) had SF in the range 0–100 µg/dL. The mean of the SF of study population was 40.69 µg/L (Table 9).

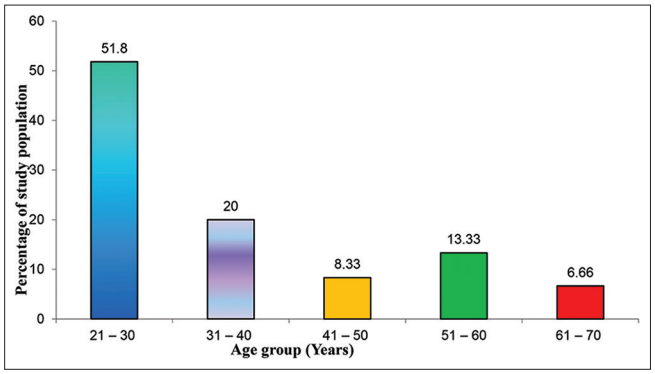

Fig. 1: Distribution of study population as per age (n=60)

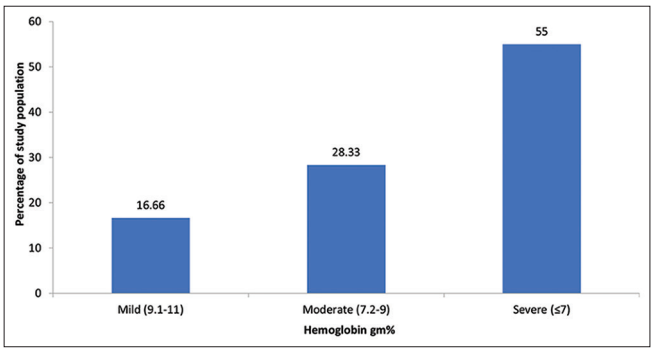

Fig. 2: Distribution of study population according to severity of anemia (n=60)

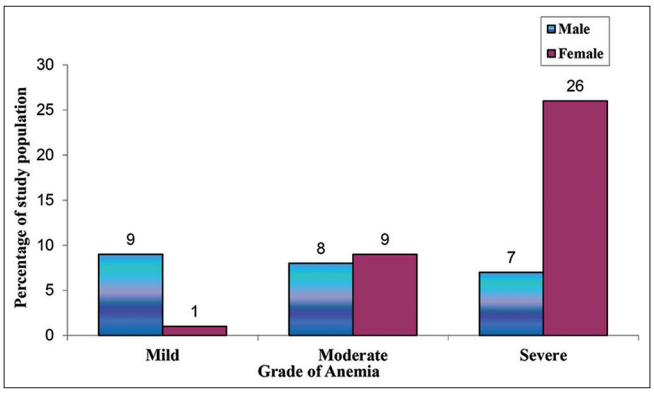

Fig. 3: Comparison of severity of anemia between the sexes (n=60)

Twenty-three (38.33%) had CHr concentration between 15.1 and 20 pg followed by 19 (31.66%) between 20.1–25 pg and 18 (30%) between 25.1 and 30 pg. The mean±SD of CHr of the study group was (22.14±3.92 pg) (Table 10 and Fig. 8).

Out of 24 male patients, 9 (37.5%) had CHr concentration between 15.1 and 20 pg, 5 (20.83%) between 20.1 and 25 pg, and 10 (41.66%) between 25.1 and 30 pg. Out of 36 female patients 14 (38.88%) had CHr concentration between 15.1 and 20 pg, 14 (38.88%) between 20.1-25pg, and 8 (22.22%) between 25.1 and 30 pg (Table 11).

Table 4: Comparison of severity of anemia between the sexes (n=60)

Severity of anemia	Male (number of patients)	Female (number of patients)
Mild	9	1
Moderate	8	9
Severe	7	26

Table 5: Distribution of study population as per MCV (n=60)

MCV (f1)	Number of patients (%)
40–40.9	3 (5.00)
50–59.9	14 (23.33)
60–69.9	25 (41.66)
70–79.9	18 (30.00)

MCV: Mean corpuscular volume

Table 6: Distribution of SI (n=60)

SI (µg/dL)	Number of patients (%)
0–50	40 (66.66)
51–100	17 (28.33)
101–150	3 (5.00)

SI: Serum iron

Table 7: Distribution of serum TIBC (n=60)

Serum TIBC (µg/dL)	Number of patients (%)
201–250	7 (11.66)
251–300	17 (28.33)
301–350	4 (6.66)
351–400	19 (31.66)
401–450	13 (21.66)

TIBC: Total iron binding capacity

Table 8: Distribution of serum transferrin saturation (n=60)

Serum TSAT (%)	Number of patients (%)
0.1–10	39 (65.00)
10.1–20	3 (5.00)
20.1–30	7 (11.66)
30.1–40	9 (15.00)
40.1–50	2 (3.33)

TSAT: Transferrin saturation

Table 9: Distribution of SF (µg/L) (n=60)

SF (µg/L)	Number of patients (%)
0–100	50 (83.33)
101–300	7 (11.66)
201–300	3 (5.00)

SF: Serum ferritin

Out of 60 patients, 20 (33.33%) had body mass index (BMI) of Grade 0; 20 (33.33%) had BMI of Grade 1; 1 (1.66%) had BMI of Grade 2; 12 (20%) had BMI of Grade 3; and 7 (11.66%) had BMI of Grade 4 (Table 12).

From Table 13 and Fig. 9, sensitivity and specificity of SI in diagnosing IDA were calculated and found to be 70% and 40%, respectively.

From Table 14 and Fig. 10, sensitivity and specificity of TIBC were calculated and found to be 60% and 40%, respectively.

The sensitivity and specificity of transferrin saturation were calculated and found to be 70% and 40%, respectively (Table 15).

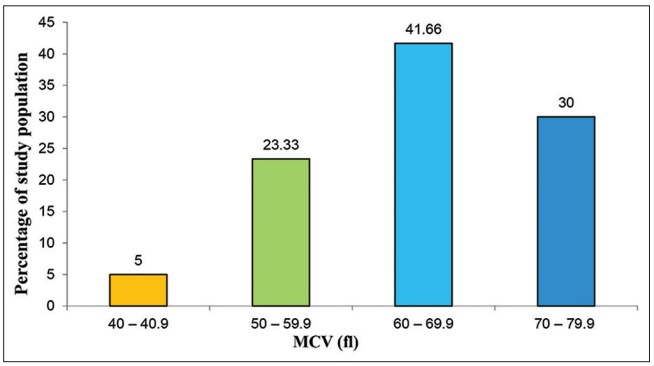

Fig. 4: Distribution of study population as per mean corpuscular volume (n=60)

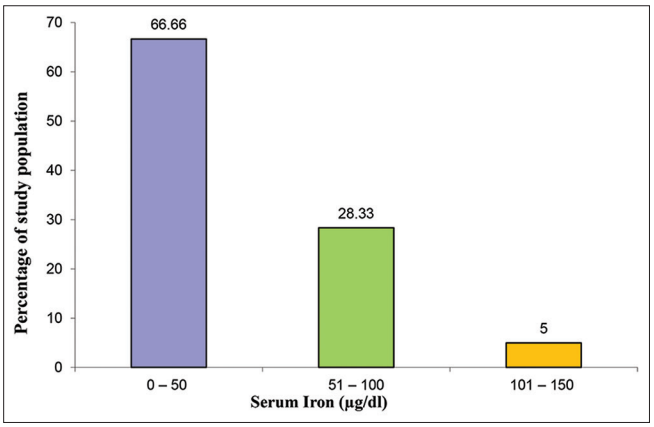

Fig. 5: Distribution of serum iron

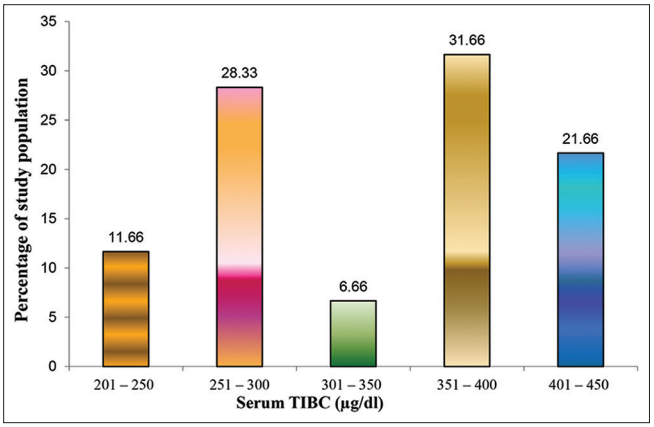

Fig. 6: Distribution of serum total iron binding capacity

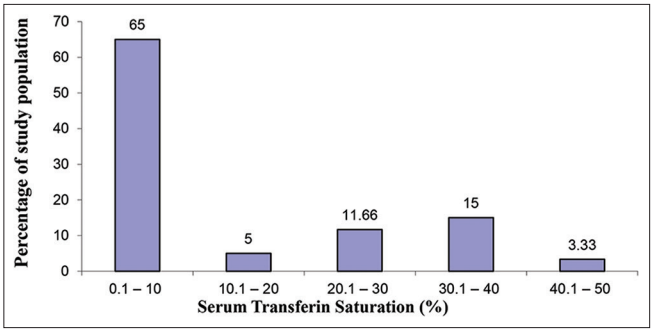

Fig. 7: Distribution of serum transferrin saturation

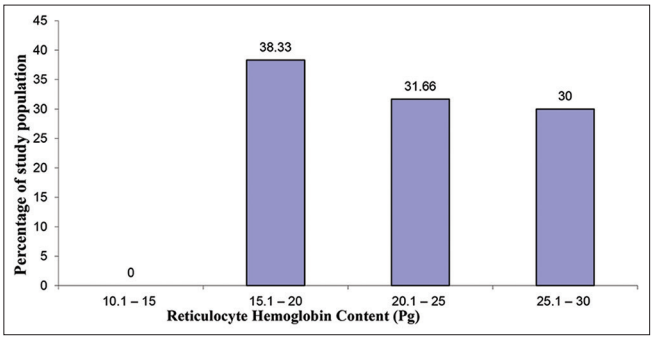

Fig. 8: Distribution of reticulocyte-hemoglobin content

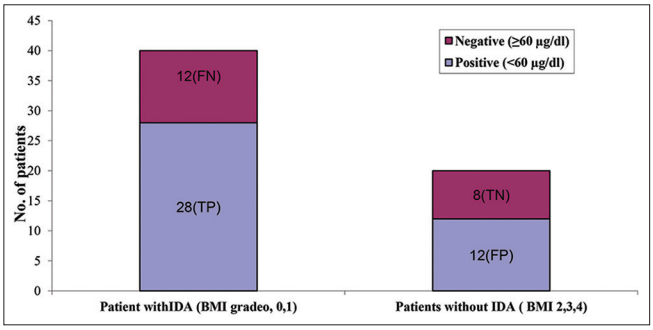

Fig. 9: Sensitivity and specificity of serum iron. TP: True positive, FN: False negative

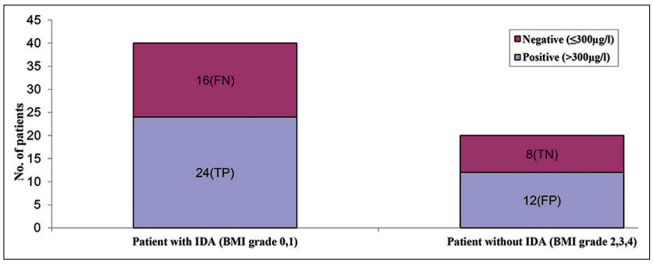

Fig. 10: Sensitivity and specificity of total iron binding capacity in diagnosing iron deficiency anemia. TP: True positive, FN: False negative

The sensitivity and specificity of transferrin saturation were calculated and found to be 70% and 40%, respectively (Table 15).

The sensitivity and specificity of serum ferritin were calculated and found to be 90% and 80%, respectively (Table 16 and Fig. 11).

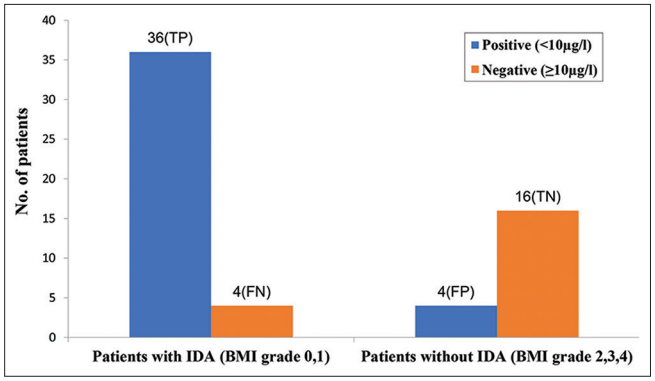

Fig. 11: Sensitivity and specificity of serum ferritin in diagnosing iron deficiency anemia. TP: True positive, FN: False negative

Table 10: Distribution of reticulocyte-hemoglobin content (n=60)

Reticulocyte hemoglobin content (pg)	Number of patient (%)
10.1–15	0
15.1–20	23 (38.33)
20.1–25	19 (31.66)
25.1–30	18 (30)

Table 11: Comparison of reticulocyte-hemoglobin content between the sexes

CHr (pg)	Male (number of patients)	Female (number of patients)
15.1–20	9	14
20.1–25	5	14
25.1–30	10	8

CHr: Reticulocyte hemoglobin content

Table 12: Distribution of bone marrow iron (n=60)

BMI grading	Number of patient (%)
Grade-0	20 (33.33)
Grade-1+	20 (33.33)
Grade-2+	1 (1.66)
Grade-3+	12 (20.00)
Grade-4+	7 (11.66)

BMI: Body mass index

Table 13: Sensitivity and specificity of serum iron

Serum iron result	Patients with IDA (BMI grade 0, 1)	Patients without IDA (BMI grade2, 3, 4)	Total
Positive (<60 µg/dL)	28	12	40
Negative (≥60 µg/dL)	12	8	20
Total	40	20	60

IDA: Iron deficiency anemia, BMI: Body mass index

The sensitivity and specificity of reticulocyte hemoglobin content (CHr) were calculated and found to be 95% and 80%, respectively (Table 17 and Fig. 12).

The sensitivity and specificity of reticulocyte hemoglobin content (CHr) in males were found to be 91.66% and 75%, respectively (Table 18 and Fig. 13).

The sensitivity and specificity of reticulocyte hemoglobin content (CHr) in females were calculated and found to be 96.42% and 87.50%, respectively (Table 19 and Fig. 14).

The sensitivity and specificity through this analysis were computed as 95% and 80%, respectively, for CHr; 90% and 80%, respectively, for SF; 70% and 40%, respectively, for SI and transferrin saturation; and 65% and 40%, respectively, for TIBC in diagnosing IDA (Fig. 15).

Table 14: Sensitivity and specificity of TIBC in diagnosing IDA

TIBC result	Patient with IDA (BMI grade 0, 1)	Patient without IDA (BMI grade 2, 3, 4)	Total
Positive (>300 µg/dL)	24	12	36
Negative (≤300 µg/dL)	16	8	24
Total	40	20	60

IDA: Iron deficiency anemia, BMI: Body mass index, TIBC: Total iron binding capacity

Table 15: Sensitivity and specificity of transferrin saturation in diagnosing IDA

TS result (%)	Patients with IDA (BMI grade 0, 1)	Patients without IDA (BMI grade 2, 3, 4)	Total
Positive (<16)	28	12	40
Negative (≥16)	12	8	20
Total	40	20	60

IDA: Iron deficiency anemia, BMI: Body mass index, TS: Transferrin saturation

Table 16: Sensitivity and specificity of SF in diagnosing IDA

SF result	Patients with IDA (BMI grade 0, 1)	Patients without IDA (BMI grade 2, 3, 4)	Total
Positive (<10 µg/L)	36	4	40
Negative (≥10 µg/L)	4	16	20
Total	40	20	60

IDA: Iron deficiency anemia, BMI: Body mass index, SF: Serum ferritin

Table 17: Sensitivity and specificity of reticulocyte hemoglobin content in diagnosing IDA

CHr result	Patients with IDA (BMI grade 0,1)	Patients without IDA (BMI grade 2,3,4)	Total
Positive (<26 pg)	38	4	42
Negative (≥26 pg)	2	16	18
Total	40	20	60

IDA: Iron deficiency anemia, BMI: Body mass index, CHr: Reticulocyte hemoglobin content

Table 18: Sensitivity and specificity of CHr in male sex in diagnosing IDA

CHr result	Patients with IDA (BMI grade 0, 1)	Patients without IDA (BMI grade 2, 3, 4)	Total
Positive (<26 pg)	11	3	14
Negative (≥26 pg)	1	9	10
Total	12	12	24

IDA: Iron deficiency anaemia, BMI: Body mass index, CHr: Reticulocyte haemoglobin content

DISCUSSION

The present study had an age distribution of 21–70 years and maximum incidence of 31 (51.66%) patients seen in the age group 21–30 years. Mean±SD of patients in study group is 36.03±14.21 years which correlates with observation by Ahmad *et al.* [15].

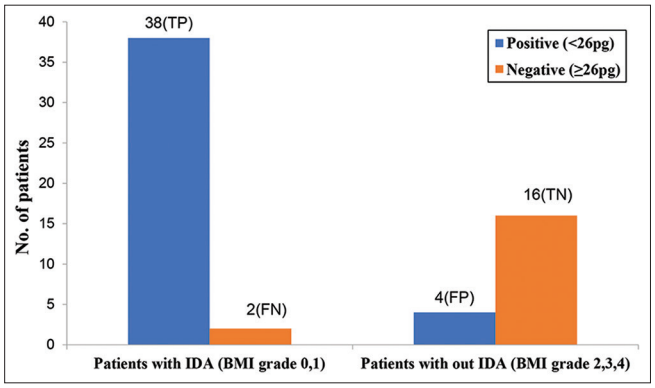

Fig. 12: Sensitivity and specificity of reticulocyte hemoglobin content in diagnosing iron deficiency anemia. TP: True positive, FN: False negative

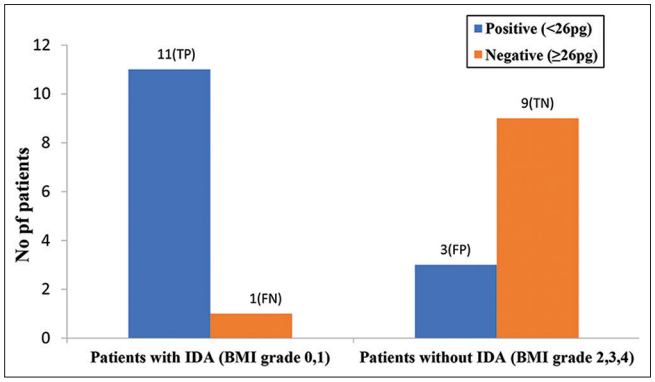

Fig. 13: Sensitivity and specificity of reticulocyte hemoglobin content in males in diagnosing iron deficiency anemia. TP: True positive, FN: False negative

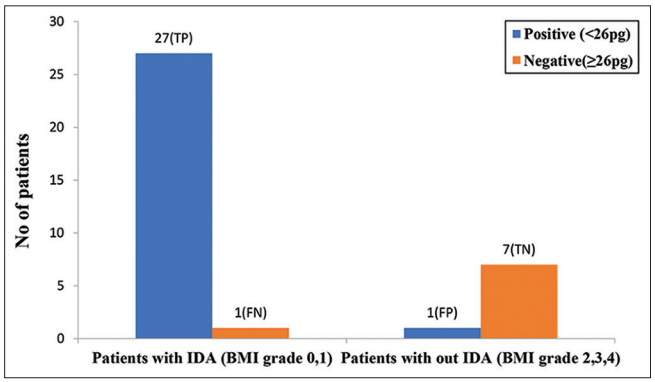

Fig. 14: Sensitivity and specificity of reticulocyte hemoglobin content in females in diagnosing iron deficiency anemia. TP: True positive, FN: False negative

Table 19: Sensitivity and specificity of CHr in females in diagnosing IDA

CHr result	Patients with IDA (BMI grade 0, 1)	Patients without IDA (BMI grade 2, 3, 4)	Total
Positive (<26 pg)	27	1	28
Negative (≥26 pg)	1	7	8
Total	28	8	36

IDA: Iron deficiency anaemia, BMI: Body mass index, CHr: Reticulocyte haemoglobin content

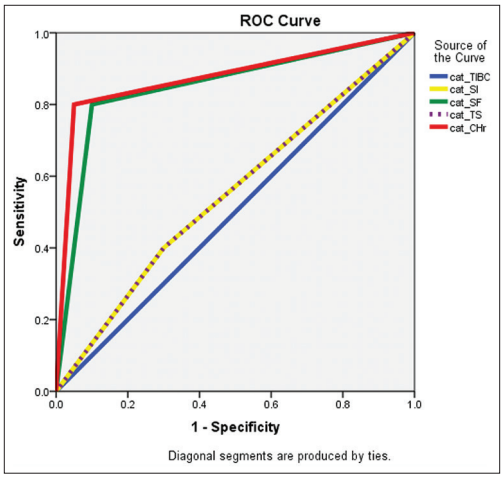

Fig. 15: Receiver operating characteristic curve analysis for sensitivity and specificity of reticulocyte hemoglobin content, serum ferritin, serum iron, transferrin saturation, total iron binding capacity in diagnosing iron deficiency anemia

Out of 60 patients 36 (60%) were females and 24 (40%) were males similar to Praveen *et al.* which shows 41% male and 58% female [16].

In the present study, 10 (16.66%) patients had mild anemia, 17 (28.33%) had moderate anemia and 33 (55%) had severe anemia. Mean hemoglobin of the study group was 6.86 g/dL and SD was 1.95 g/dL. Maximum no of cases was classified as severe anemia in contrast to other studies such as Ahmad *et al.* and Abel *et al.* where maximum numbers were in moderate anemia group [15,17]. This disparity is because of decrease care and health seeking behavior with females due to various factors such as poverty and socioeconomic dependency in our state.

MCV value ranging from 40 to 80 fl and maximum incidence of 25 (41.66%) patients were seen in the range 60–70 fl in the present study. Mean±SD of MCV in the study group was 64.74±7.8 fl.

Out of sixty patients, SI in 40 (66.66%) patients had below 50 µg/dL, 17 (28.33%) had between 51–100 µg/dL, and 3 (5%) had above 100 µg/dL. The mean SI in the study group is 40.06 µg/dL which is similar to Tater *et al.* study where the mean SI was 50.9 µg/dL [18].

In this study group of 60 patients of microcytic hypochromic anemia, maximum patients (n=19, 31.66%) had TIBC in the range of 351–400 µg/dL. Mean±SD of serum TIBC 333.91±67.26 µg/dl which is similar to Tater *et al.* study [18].

Maximum patients (n=39, 65%) had transferring saturation in the range of 0.1–10%. The mean±SD of the study group is 13.68±3.22% which is similar to Tater *et al.* study [18].

Maximum patients (n=50, 83.33%) had SF in the range of 0–100 µg/dl which is similar to Thoradeniya, Zeben VD, and Mast AE [19-21].

Twenty-three (38.33%) had CHr concentration between15.1 – 20 pg followed by 19 (31.66%) between 20.1 and 25 pg and 18 (30%) between 25.1 and 30 pg. The mean±SD of this study is 22.14±3.92 pg which is similar to studies such as Karagülle *et al.* and Karlosson [22,23].

Sensitivity and specificity of SI of this study is 70% and 40%, respectively, at a cutoff value off 65 µg/dL compared to studies like Asif *et al.* [24] where sensitivity and specificity 63.5% and 38.6%, respectively, in diagnosing IDA.

Sensitivity and specificity of serum TIBC of the study is 60% and 40%, respectively, at a cutoff value of 300 µg/dl compared to studies like Asif *et al.* [24] where sensitivity and specificity 64.5% and 42.8%, respectively, in diagnosing IDA.

Sensitivity and specificity of serum transferrin saturation 70% and 40%, respectively, at a cutoff value of 16% compared to other studies like Fishbane *et al.* [25] where sensitivity and specificity 81% and 63%, respectively, at a cutoff value off 21%, Tessitone *et al.* [26] where sensitivity and specificity 59% and 78%, respectively, at a cutoff value of 19%, Kalantar-Zadeh *et al.* [27] where sensitivity and specificity 88% and 63%, respectively, at a cutoff value of 20% in diagnosing IDA. The variation in sensitivity and specificity is due to different sample sizes.

Sensitivity and specificity of SF 90% and 80%, respectively, at a cutoff value of 10 µg/L compared to other studies like Fishbane *et al.* [25], where sensitivity and specificity 48% and 75%, respectively, Tessitone *et al.* [26], where sensitivity and specificity 35% and 78%, respectively, Kalantar-Zadeh *et al.* [27], 88% and 100%, respectively, in diagnosing IDA. The variation in sensitivity and specificity is due to variations in cutoff values and prevalence of IDA.

In the study group of 60 patients, 20 (33.33%) had Grade 0; 20 (33.33%) had BMI of Grade 1; 1 (1.66%) had BMI of Grade 2; 12 (20%) had BMI of Grade 3 and 7 (11.66%) had BMI of Grade 4, in contrast to other studies like Dharwadkar *et al.* [28] where out of 55 patients 8 (14.54%) had BMI of grade 0; 35 (63.63%) had BMI of Grade 1; 8 (14.54%) had BMI of Grade 2; 4 (7.27%) had BMI of Grade 3; and 0 (0%) had BMI of Grade 4. This discrepancy is due to prevalence of IDA more in states like Odisha.

In the study group of 60 patients BMI of Grade 0 has mean CHr±SD of 17.96±1.09 pg; BMI of Grade 1 has mean CHr±SD of 22.37±2.00 pg; BMI of Grade 2 has mean CHr±SD of 28.3±0.00 pg; BMI of Grade 3 has mean CHr±SD of 26.79±2.08 pg; and BMI of Grade 4 has mean CHr±SD of 24.62±3.47 pg. From this it can be concluded that patients with low grade of BMI have lesser mean CHr.

From the above analysis p value is found to be very highly significant for CHr, which is <0.001.

CONCLUSION

Reticulocyte hemoglobin content is found to be a useful biomarker in the diagnosis of IDA besides the conventional biomarkers which are used in routine practice.

AUTHORS' CONTRIBUTIONS

The authors declare that all the named authors have contributed equally to this article.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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