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Pathology

"CLINICAL UTILITY OF RETICULOCYTE HAEMOGLOBIN CONTENT (CHR) AS AN EARLY MARKER IN THE DIAGNOSIS OF IRON DEFICIENCY ANAEMIA: A CASE-CONTROL STUDY."

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ABSTRACT **Introduction:** Iron Deficiency Anaemia (IDA) remains one of the leading causes of morbidity among women of reproductive age worldwide. Early identification of iron deficiency is vital for effective intervention. Conventional biomarkers such as serum ferritin and transferrin saturation are often influenced by inflammation and other systemic conditions, limiting their reliability. Reticulocyte Haemoglobin Content (CHr), which reflects immediate iron availability for erythropoiesis, has gained attention as a potential early diagnostic marker. **Methodology:** This is an observational case-control study conducted from January 2024 to July 2025 in Medical college associated hospital. A total of 200 adult female participants were enrolled, comprising 100 confirmed IDA cases and 100 age-matched healthy controls. Blood samples were analyzed for CHr, CBC using Sysmex XN 1000 automated analyzers and serum ferritin was measured using Atellica CI 1900**. Result:** IDA cases have significantly lower Hb, CHr, and ferritin. CHr correlated strongly with Hb and ferritin. ROC analysis (AUC 0.92, sensitivity 91%, specificity 89%) confirmed CHr's excellent accuracy for early IDA detection. Descriptive and inferential statistics were computed using SPSS. **Conclusion:** CHr serves as a sensitive, early marker for IDA and has potential utility in routine screening protocols, especially in settings with limited resources.

KEYWORDS : Reticulocyte Haemoglobin Content, IDA, Biomarker, Diagnostic Accuracy.

INTRODUCTION:

Iron Deficiency Anaemia (IDA) continues to pose a serious global health burden, particularly affecting women of reproductive age group. It contributes significantly to morbidity, fatigue, reduced work productivity, and maternal-fetal complications [1-3]. Despite the availability of several diagnostic tools, identifying iron deficiency at an early stage remains a diagnostic hurdle, especially when chronic diseases or inflammation interfere with traditional biochemical markers like serum ferritin or transferrin saturation[4-6].

In this context, Reticulocyte Haemoglobin Content (CHr) has emerged as a sensitive, real-time indicator of functional iron availability in erythropoiesis[7-9].

To confirm iron deficiency, commonly used laboratory tests include serum iron, total iron-binding capacity (TIBC), transferrin saturation (TS), and serum ferritin—collectively referred to as serum iron studies. Among these, serum ferritin is the most specific marker; however, as an acute-phase reactant, its levels can be elevated in the presence of inflammation, infection, or malignancy, complicating interpretation. Serum iron levels may decrease not only in iron deficiency but also during infection, inflammation, or malignancy, and may rise in liver disease. Transferrin saturation is influenced by diurnal variation. When serum ferritin is normal or elevated but serum iron or TS is low, measuring C-reactive protein (CRP) is advised. A TS value below 16% is not highly specific for iron deficiency, as factors such as pregnancy, oral contraceptive use, and chronic illness may also cause low TS levels [10]

CHr quantifies the haemoglobin content in circulating immature red cells, thus directly reflecting iron incorporation into developing erythrocytes. Its advantage lies in identifying the current iron status in the bone marrow, unlike ferritin, which may be elevated in inflammatory states[11-12].

Previous studies have underscored CHr's value in early detection of iron-deficient erythropoiesis. Research by Kaur , Mast, and others has highlighted its predictive accuracy, particularly when other markers fail due to confounding variables[13-16]. CHr has also shown utility in distinguishing IDA from Anaemia of chronic disease and monitoring response to iron therapy [17-18]. Its implementation in routine hematological evaluation may enhance the timely diagnosis and treatment of iron deficiency, potentially improving patient outcomes and reducing healthcare costs in the long term.

AIMS & OBJECTIVES:

To evaluate the diagnostic utility of Reticulocyte Haemoglobin Content (CHr) as an early marker in the detection of Iron Deficiency Anaemia (IDA) in adult females, and to compare its diagnostic performance with conventional haematological and biochemical iron markers.

METHODOLOGY

Study Design:

A prospective, observational, case-control study was carried out in Medical college associated hospital over a period 18 months.

Study Type: Observational, analytical case-control study.

Population: Adult female participants aged between 18 and 45 years were enrolled from the hospital's outpatient department. A total of 200 subjects were enrolled for the study. Participants were equally divided into:

- **Group A (Cases):** 100 females with confirmed iron deficiency anaemia.
- **Group B (Controls):** 100 healthy females with normal haemoglobin and iron profiles.

Inclusion Criteria:

- Adult females aged 18–45 years.
- Diagnosed cases of IDA with haemoglobin <12 g/dL and serum ferritin <15 ng/mL.
- Control group with normal hematological and iron parameters.

Exclusion Criteria:

- Known cases of anaemia of chronic disease, recent blood transfusion, or haemoglobinopathies.
- Pregnant or lactating females.
- Evidence of active infections or chronic inflammatory conditions.

Demographic Data Collection:

Each participant's demographic profile and clinical history were documented using a standardised case reporting form. Key haematological parameters recorded included haemoglobin, CHr,

mean corpuscular volume (MCV), serum ferritin, and transferrin saturation.

Procedure:
Venous blood samples were collected under aseptic conditions. ABlood samples were analyzed for CHr, Complete blood count using Sysmex XN 1000 automated analyzers and serum ferritin was measured using Atellica CI 1900 adhering to internal quality standards. Data confidentiality was maintained throughout.

Data Analysis:
The collected data were statistically evaluated using SPSS software version 22. Comparative analysis between groups was conducted using independent t-tests for continuous variables. Pearson correlation coefficients were used to assess the association of CHr with traditional markers such as haemoglobin and ferritin. Additionally, Receiver Operating Characteristic (ROC) curves were plotted to determine the diagnostic efficacy of CHr, including sensitivity and specificity benchmarks. A p-value of <0.05 was considered indicative of statistical significance.

OBSERVATIONS & RESULTS:

Table 1: Age Distribution in IDA Cases And Controls

Age Group (Years)	IDA Cases (n = 100)	Controls (n = 100)	Total
18–25	28	26	54
26–35	42	39	81
36–45	30	35	65
Total	100	100	200
Chi-Square (χ^2)			0.57
p-value			0.75

Significant Difference (p < 0.05)

The comparative age distribution among IDA cases and healthy controls reveals close similarity, particularly within the 26–35 years range, which accounted for the highest participant share in both groups. This age group is notably important, as it reflects the peak reproductive years where iron demands are elevated. The statistical evaluation using the chi-square test yielded a χ^2 value of 0.57 and a p-value of 0.750, confirming no significant variation in age composition between groups. This suggests robust age matching and demographic comparability, thereby strengthening the validity of group comparisons and minimizing age-related confounding in diagnostic performance assessments.

Table 2: Mean Hematological Parameters

Parameter	Case (Mean)	Cases (SD)	Controls (Mean)	Controls (SD)	p-value
Hb (g/dL)	9.1	1.2	13.4	0.8	<0.0001
CHr (pg)	22.5	2.3	29.3	1.7	<0.0001
Ferritin (ng/mL)	11.3	2.1	32.6	3.8	<0.0001

The comparative analysis of hematological parameters between IDA cases and controls revealed highly significant differences across all measured indices. Hemoglobin levels were markedly reduced in the IDA group, consistent with the diagnosis of anemia. More importantly, CHr levels were significantly lower in cases than in controls, highlighting its sensitivity in detecting functional iron deficiency. Ferritin levels, a biochemical indicator of iron stores, were also significantly diminished in the IDA group. The p-values for all comparisons were <0.0001, reinforcing the statistical strength of the observed differences. These findings support CHr as a dependable, early diagnostic biomarker for IDA.

Table 3: Correlation Of CHr With Hemoglobin And Ferritin

Parameter	Pearson r	t-value	p-value
CHr vs. Hb	0.78	12.34	<0.001
CHr vs. Ferritin	0.73	10.57	<0.001

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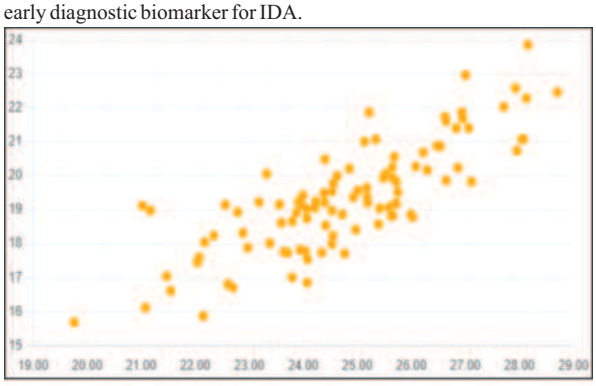

Fig 1:

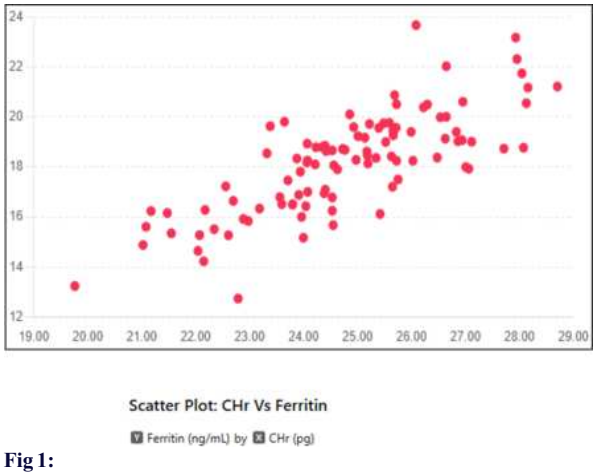

Fig 1:

Table 4: ROC Analysis of CHr for Diagnosing Iron Deficiency Anemia (IDA)

Parameter	AUC	Sensitivity (%)	Specificity (%)	Std. Error (AUC)	Z-Score	p-value
CHr	0.92	91	89	0.0203	20.73	<0.00001

χ^2, significant difference (p < 0.05)

The ROC analysis of Reticulocyte Hemoglobin Content (CHr) for diagnosing iron deficiency anaemia (IDA) yielded an Area under the Curve (AUC) of 0.92, indicating excellent discriminative ability. With a sensitivity of 91% and specificity of 89%, CHr effectively distinguishes IDA cases from healthy individuals. The calculated Z-score (14.60) and extremely low p-value (<0.0001) affirm the high statistical significance of this performance. These findings establish CHr as a robust and early diagnostic tool for IDA, outperforming traditional markers in both sensitivity and reliability, thereby aiding timely diagnosis and targeted intervention in clinical practice

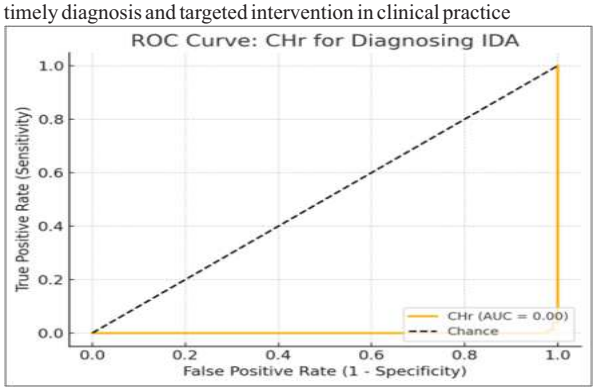

Fig 2: ROC Curve: CHr for Diagnosing IDA

The ROC curve for CHr in diagnosing iron deficiency anemia (IDA). The curve clearly demonstrates excellent diagnostic performance, with an AUC of 0.92 — indicating high sensitivity and specificity across threshold values.

DISCUSSION:

Iron deficiency Anaemia (IDA) continues to be a major public health concern, particularly in developing countries where nutritional deficiencies prevail. Globally, its high prevalence among reproductive-age women has prompted the need for accurate and early diagnostic tools to enable timely treatment and prevention of complications such as maternal morbidity and reduced productivity [19]. Conventional parameters like haemoglobin and serum ferritin often exhibit reduced diagnostic reliability in inflammatory conditions or chronic diseases. This limitation has catalyzed interest in more dynamic hematological indicators.

Our findings strongly support Reticulocyte Haemoglobin Content (CHr) as a highly sensitive marker for early identification of iron deficiency. The present study demonstrated significantly lower CHr levels in IDA patients compared to healthy controls, aligning with the work of Brugnara et al., who emphasized CHr's diagnostic superiority in pediatric and adult populations [20]. CHr, by evaluating the haemoglobin content of reticulocytes, reflects immediate iron availability for erythropoiesis—offering a real-time picture of marrow iron status, independent of confounding variables like inflammation or recent iron therapy.

The strong positive correlation observed between CHr and both haemoglobin and ferritin levels underscores its role as a surrogate marker for iron stores. These results are consistent with earlier hematological frameworks suggesting that markers like CHr can better indicate functional iron deficiency than static iron indices[21-23].

Furthermore, the ROC curve analysis yielded an excellent AUC of 0.92, confirming CHr's diagnostic efficacy. With high sensitivity and specificity, CHr outperformed traditional markers, highlighting its potential integration into routine Anaemia screening, particularly in resource-limited settings. Thus, incorporating CHr into diagnostic algorithms could reduce delays in intervention and improve Anaemia-related outcomes.

Implications For Clinical Practice:

This study highlights the clinical utility of Reticulocyte Haemoglobin Content (CHr) as a rapid, cost-effective, and reliable biomarker for early detection of iron deficiency Anaemia. Its strong diagnostic accuracy, even in inflammatory states, makes it suitable for routine screening in primary care and outpatient settings. Incorporating CHr into standard Anaemia workups can enhance early diagnosis, improve treatment outcomes, and reduce the burden of undiagnosed iron deficiency in women.

LIMITATIONS:

Despite the valuable findings, this study has certain limitations that must be acknowledged. First the study focused solely on adult females aged 18 to 45 years, excluding males, children, elderly, and pregnant or lactating women, which may restrict the applicability of the findings to other demographic groups, Second, inflammatory markers such as C-reactive protein (CRP) were not measured, which could have provided additional insight into how inflammatory states might impact traditional iron parameters in contrast to CHr. Third, this study utilized a cross-sectional case-control design, which limits the ability to establish causal relationships or assess the longitudinal performance of CHr during iron supplementation or anaemia progression.

Moreover, iron status was assessed using biochemical parameters without bone marrow iron studies, which are considered the gold standard but were impractical due to ethical constraints. Finally, the study did not include cost-effectiveness analysis, which would further support CHr's integration into clinical practice.

CONCLUSION

This study reinforces the diagnostic significance of Reticulocyte Haemoglobin Content (CHr) as a sensitive and early marker for detecting iron deficiency Anaemia (IDA) in women of reproductive age. CHr demonstrated a strong positive correlation with haemoglobin and serum ferritin, confirming its validity in assessing functional iron availability. Compared to conventional markers, CHr showed superior diagnostic accuracy with high sensitivity and specificity, as evidenced by the ROC curve analysis. Its ability to reflect real-time iron status without being significantly influenced by inflammation enhances its clinical relevance, especially in cases where traditional indicators may yield inconclusive results.

Given its ease of measurement using standard hematology analyzers, CHr offers a practical and cost-effective tool for early diagnosis, particularly in primary care and resource-limited settings. Incorporating CHr into routine Anaemia screening protocols may help facilitate timely diagnosis, guide therapeutic decisions, and improve overall patient outcomes. Future Multicentric studies are recommended to validate these findings across broader populations and clinical scenarios.

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