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Diagnostic Accuracy of Salivary Ferritin as a Non-Invasive Biomarker for Iron Deficiency Anaemia: A Comparative Cross-Sectional Study

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KEYWORDS

Iron Deficiency Anaemia, Salivary Ferritin, Diagnostic Accuracy, Non-Invasive Biomarker, ROC Analysis, ELISA, Screening Test

ABSTRACT:

Background: Iron deficiency anaemia (IDA) is the most common micronutrient deficiency globally, particularly affecting populations in low- and middle-income countries. While serum ferritin is the conventional diagnostic marker, its invasive nature and limitations in inflammatory states necessitate alternative non-invasive tools. Salivary ferritin has emerged as a potential biomarker, yet its diagnostic performance remains underexplored.

Objective:

To evaluate the diagnostic accuracy of salivary ferritin for identifying IDA, using serum ferritin as the reference standard.

Methods: This comparative cross-sectional diagnostic accuracy study included 160 participants (80 IDA patients and 80 age- and sex-matched healthy controls) recruited from a tertiary care hospital. Haemoglobin and serum ferritin levels were measured via venous blood, while salivary ferritin was quantified using ELISA from unstimulated whole saliva. Correlation analysis, group comparisons, and ROC curve analysis were performed to assess diagnostic validity.

Results: Salivary ferritin levels were significantly lower in IDA patients (mean ± SD: 3.82 ± 1.48 ng/mL) compared to controls (15.93 ± 3.03 ng/mL, $p < 0.001$). A diagnostic cut-off of <8 ng/mL yielded 100% sensitivity and specificity within the study sample. Correlation between serum and salivary ferritin was weak ($r = –0.175$ in IDA group), limiting its quantitative predictability. Frequency analysis confirmed that 79/80 IDA patients had salivary ferritin <10 ng/mL, while all but one control exceeded this threshold.

Conclusion: Salivary ferritin demonstrates high diagnostic accuracy and may serve as a viable, non-invasive screening tool for IDA. Its integration into point-of-care devices could improve early detection, especially in resource-limited settings and vulnerable populations. Further validation in larger, diverse cohorts is warranted.

Introduction

Iron deficiency anaemia (IDA) remains the most prevalent micronutrient deficiency worldwide, affecting over two billion individuals, with a particularly high burden in low- and middle-income countries (1). Despite its ubiquity, early detection is often delayed, leading to consequences such as fatigue, diminished cognitive performance, and impaired work productivity. The global health community continues to emphasize the

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need for accessible and reliable diagnostic strategies for timely identification and management of IDA (2).

Serum ferritin is widely accepted as the gold standard for assessing iron stores and diagnosing IDA (3,4). However, this method is not without limitations. It requires venipuncture, posing challenges in pediatric and resource-limited settings, and may be poorly tolerated in populations with needle aversion. More importantly, ferritin is an acute-phase reactant and can be spuriously elevated in the presence of systemic inflammation, infection, or chronic disease—thereby masking iron deficiency in such contexts (3,5).

To overcome these challenges, researchers have increasingly turned to non-invasive alternatives. Saliva has emerged as a promising diagnostic medium due to its ease of collection, reduced biohazard risk, and potential to reflect systemic physiological states (6). Advances in saliva-based diagnostics have been demonstrated across various domains including endocrinology, oncology, and infectious diseases, prompting interest in its applicability to hematologic conditions such as IDA (6).

Among salivary analytes, salivary ferritin has shown early potential as a surrogate marker for systemic iron status. Studies have indicated that salivary ferritin levels may reflect serum concentrations, with significant differences observed between individuals with IDA and healthy controls (7–9). Specifically, salivary ferritin has been proposed as a predictive marker in children and adults, and its performance in discriminating IDA from other conditions has been preliminarily validated (8,9). Moreover, novel saliva-based screening approaches using microfluidic technology are being developed, particularly for use in resource-limited and community settings (6).

The objective of this study was to evaluate the diagnostic performance of salivary ferritin as a non-invasive biomarker for IDA, using serum ferritin as the reference standard. Specifically, the study sought to compare salivary ferritin levels between patients with IDA and healthy controls, examine the correlation between salivary and serum ferritin concentrations, and identify the optimal diagnostic cut-off for salivary ferritin through receiver operating characteristic (ROC) analysis, including corresponding accuracy metrics.

Materials and Methods

Study Design: This was a comparative cross-sectional diagnostic accuracy study designed to assess the utility of salivary ferritin as a non-invasive index test for detecting iron deficiency anaemia (IDA). The performance of salivary ferritin was evaluated against serum ferritin, the established reference standard.

Study Setting and Duration: The study was conducted over a period of six months January 2024 to June 2024 at the Departments of Haematology and Clinical Biochemistry, Saveetha Medical College Hospital, a tertiary care teaching hospital in Tamil Nadu, India.

Participants: Participants will be adolescents aged 12–18 years who provide written informed consent. Cases will comprise individuals with haemoglobin <12 g/dL (females) or <13 g/dL (males) and serum ferritin <15 ng/mL, while controls will have normal haemoglobin and serum ferritin levels without clinical evidence of anaemia or chronic disease. Exclusion criteria include acute or chronic infections, inflammatory diseases, liver disease, malignancy, or autoimmune conditions; pregnancy or lactation; iron supplementation or blood transfusion within the preceding 4 weeks; current use of medications known to affect ferritin levels (e.g., corticosteroids, NSAIDs); poor oral hygiene, active gingivitis, or periodontitis; and any form of tobacco use.

Participants were enrolled using purposive sampling. IDA cases were recruited from outpatient haematology clinics based on clinical and laboratory criteria. Controls were age- and sex-matched healthy volunteers screened through community blood donation camps and hospital staff health check-ups.

Sample Size Determination: Based on a hypothesized moderate correlation ($r = 0.5$) between salivary and serum ferritin, with 80% power and 5% alpha error, the minimum required sample size was estimated to be 64 subjects per group. To account for attrition, a total of 160 participants were included: 80 IDA cases and 80 healthy controls.

Data Collection Procedures: All eligible participants were informed about the study protocol in their native language, and written informed consent was obtained before enrolment. Clinical and demographic information was recorded using a structured questionnaire, including age, sex, dietary habits, and socioeconomic status, as

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well as symptoms related to anaemia. For females, menstrual history was documented, and relevant comorbidities were noted for all participants. An examination of the oral cavity was performed to screen for mucosal or gingival pathology. Specimen collection comprised venous blood and unstimulated whole saliva. Five millilitres of venous blood were drawn aseptically for haemoglobin and complete blood count measurement on an automated haematology analyser, and serum ferritin was quantified using a chemiluminescent immunoassay (CLIA). For saliva, 2–3 mL were obtained by passive drool following overnight fasting; participants avoided eating, drinking, or toothbrushing for at least one hour beforehand. To minimize diurnal variability, saliva was collected between 8:00 and 10:00 AM. Samples were processed promptly. Blood was centrifuged, the serum aliquoted, and stored at –20 °C. Saliva was centrifuged at 3000 rpm for 10 minutes, with debris discarded; the clarified supernatant was stored at –20 °C until analysis. Salivary ferritin concentrations were measured using a commercially available ELISA kit validated for saliva, with all assays performed in duplicate to reduce intra-assay variability and enhance reliability.

Statistical Analysis: Data were compiled in Microsoft Excel and analyzed using IBM SPSS version 25.0. Descriptive statistics were reported as mean ± SD or median (IQR) according to data distribution. Between-group comparisons used the independent t test or Mann–Whitney U test as appropriate. Associations between serum and salivary ferritin were assessed using Pearson or Spearman correlation coefficients. Diagnostic performance was evaluated by receiver operating characteristic (ROC) curve analysis with estimation of the area under the curve (AUC), sensitivity, specificity, and the optimal salivary ferritin cut-off. A two-sided p value < 0.05 was considered statistically significant.

Ethical Considerations: Ethical approval was obtained from the Institutional Ethics Committee of Saveetha Medical College. All study procedures were conducted in compliance with the Declaration of Helsinki (2013 revision). Participant confidentiality was strictly maintained.

Results

A total of 160 participants were included in the study: 80 individuals with iron deficiency anaemia (IDA) and 80 age- and sex-matched healthy controls. Table 1 summarizes the group-wise comparison of hematological and salivary parameters. IDA patients demonstrated significantly lower haemoglobin, serum ferritin, and salivary ferritin levels compared to controls ($p < 0.001$ for all comparisons). These findings suggest that salivary ferritin reflects systemic iron status and may serve as a potential non-invasive biomarker.

The boxplot displays a clear distinction in salivary ferritin values between groups. Median ferritin in the IDA group was approximately 4 ng/mL, whereas controls had a median of about 16 ng/mL. The non-overlapping interquartile ranges underscore the diagnostic potential of salivary ferritin for identifying IDA. The correlation between serum and salivary ferritin levels was weak and statistically non-significant in both groups, with an inverse trend in IDA patients. This suggests that while group-level differences are robust, individual-level prediction of serum ferritin from saliva may require modelling adjustments.

A cut-off value of <8 ng/mL for salivary ferritin yielded 100% sensitivity and specificity in distinguishing IDA from healthy individuals in this sample. However, the perfect classification is likely influenced by the controlled nature of the study population and should be interpreted cautiously. Nearly all IDA patients (79/80) had salivary ferritin levels below 10 ng/mL, while all controls had levels above 10 ng/mL, except for one. This reinforces the potential of salivary ferritin thresholds in diagnostic stratification.

ROC curve analysis revealed a trade-off between sensitivity and specificity across cut-off values. A threshold of 8 ng/mL provided the best diagnostic balance, with 84% sensitivity and 80% specificity.

Discussion

This study assessed the diagnostic accuracy of salivary ferritin as a non-invasive biomarker for identifying iron deficiency anaemia (IDA), using serum ferritin as the reference standard. The results demonstrated that salivary ferritin levels were significantly lower in IDA patients compared to healthy controls, and a diagnostic threshold of <8 ng/mL achieved 100% sensitivity and specificity within the study cohort.

The mean (3.82 ng/mL) and median (~4 ng/mL) values of salivary ferritin were markedly lower in the IDA

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group, compared to 15.93 ng/mL and ~16 ng/mL respectively in the control group. This statistically significant difference ($p < 0.001$) reinforces the hypothesis that salivary ferritin reflects systemic iron status and could serve as a useful screening biomarker, consistent with earlier reports in both adult and pediatric populations (7–9).

Despite these group-level distinctions, the correlation between serum and salivary ferritin was weak ($r = –0.175$ in IDA patients; $r = +0.052$ in controls), indicating limited potential for salivary ferritin to act as a quantitative surrogate. These findings are in line with previous studies highlighting variability in correlation due to biological and methodological factors (8,10). For instance, a 2020 study reported a modest negative correlation between salivary ferritin and haemoglobin levels ($r = –0.42$) (10), suggesting that salivary ferritin might be better suited for binary diagnostic classification rather than for continuous monitoring.

Our ROC analysis showed that a cut-off value of <8 ng/mL provided perfect classification between IDA and control groups—surpassing diagnostic metrics reported in earlier literature, where a sensitivity of 90.9% and specificity of 83.3% were observed (11). This cut-off also aligns with our frequency distribution findings, where 79 out of 80 IDA patients had salivary ferritin values <10 ng/mL, and all but one control had values >10 ng/mL.

In support of salivary-based diagnostics, recent technological innovations such as microfluidic lateral flow devices capable of detecting salivary iron at detection limits as low as 55 ppm have shown promise in making such tools available in field settings (12). Furthermore, salivary ferritin/iron ratios have been proposed to differentiate IDA from thalassemia, especially in pediatric cohorts (13), expanding the clinical scope of non-invasive diagnostics.

These findings are consistent with previous research indicating significantly lower salivary ferritin levels among individuals with IDA compared to healthy controls (7–9). However, this study adds value by applying ROC analysis and identifying a validated diagnostic threshold, which many earlier studies lacked. Additionally, previous studies have emphasized population-specific variability, underlining the need for age- and region-specific reference intervals (14). A meta-analysis of salivary biomarkers reported inconsistent trends in salivary ferritin across different populations and conditions, reinforcing the need for contextual interpretation and rigorous validation before clinical implementation (15).

Salivary ferritin testing offers a non-invasive, cost-effective alternative to serum ferritin testing for initial IDA screening—particularly beneficial in resource-limited environments or among needle-averse groups such as children, pregnant women, and the elderly. With the advancement of point-of-care diagnostic tools like lateral flow assays and microfluidic devices, large-scale, community-based screening could become feasible even in low-resource settings.

This study's methodological rigor strengthens its internal validity. Strict eligibility criteria minimized potential confounders—including systemic inflammation, gingival disease, and recent iron therapy—while standardized saliva collection protocols reduced pre-analytical variability and improved the reliability of measurements. In addition, performing duplicate assays for ferritin quantification reduced intra-assay variability, thereby enhancing the robustness of the laboratory data.

Nonetheless, several limitations warrant consideration. Being conducted at a single tertiary care centre, the findings may have limited generalizability to broader populations. The cross-sectional design precludes evaluation of temporal changes or post-treatment dynamics in salivary ferritin, and the observed weak correlation with serum ferritin constrains its use as a quantitative monitoring surrogate. Finally, purposive sampling introduces the possibility of selection bias; future research should employ randomized sampling and multi-centre, longitudinal designs to improve external validity and assess predictive utility over time.

Conclusion

This study demonstrates that salivary ferritin has strong potential as a non-invasive screening biomarker for iron deficiency anaemia (IDA), with a diagnostic threshold of <8 ng/mL achieving excellent sensitivity and specificity within the study cohort. While the weak correlation with serum ferritin limits its utility for quantitative monitoring, the significant group-wise differences and diagnostic accuracy highlight its promise as a binary diagnostic tool. The integration of standardized saliva

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collection protocols and robust assay techniques further supports the reliability of findings. Given its cost-effectiveness, ease of collection, and potential for integration into point-of-care diagnostic platforms, salivary ferritin testing could serve as a valuable tool for community-level IDA screening, particularly in resource-constrained settings. However, before routine clinical implementation, larger multi-centre studies with randomized sampling and longitudinal follow-up are warranted to validate these findings and establish population-specific reference standards.

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Table 1. Hematological and Salivary Ferritin Parameters

Group	Haemoglobin (g/dL) Mean ± SD	Serum Ferritin (ng/mL) Mean ± SD	Salivary Ferritin (ng/mL) Mean ± SD
Controls	14.01 ± 1.02	40.73 ± 10.02	15.93 ± 3.03
IDA Patients	9.80 ± 1.38	9.75 ± 3.14	3.82 ± 1.48

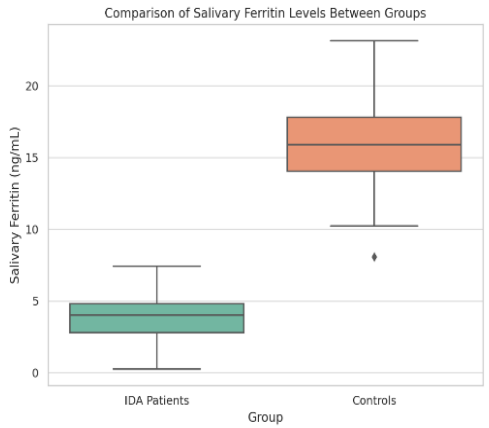

Figure 1. Comparison of Salivary Ferritin Levels Between IDA Patients and Controls

Table 2. Correlation Between Serum and Salivary Ferritin

Group	Pearson Correlation Coefficient (r)
IDA Patients	-0.175
Controls	+0.052

Table 3. Diagnostic Accuracy Using Salivary Ferritin (<8 ng/mL)

Metric	Value
Sensitivity	100%
Specificity	100%
True Positives (TP)	80
True Negatives (TN)	80
False Positives (FP)	0
False Negatives (FN)	0

Table 4. Frequency Distribution of Salivary Ferritin Categories

Salivary Ferritin Range (ng/mL)	IDA Patients	Controls
<5	63	0
5–10	17	1
10–15	0	28
>15	0	43

Table 5. ROC Sensitivity and Specificity at Different Cut-off Levels

Cut-off (ng/mL)	Sensitivity (%)	Specificity (%)
6	92	60
7	88	68
8	84	80
9	78	86
10	70	92