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Salivary Ferritin is a Diagnostic Marker in Iron Deficiency Anaemia in Children

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Abstract

Introduction: Iron deficiency anemia is the most common nutritional problem in children in Bangladesh. We need to establish a less invasive, easy method to diagnose iron deficiency anemia in children. Saliva contains ferritin and it is higher than the normal level in iron deficiency anemia. Salivary ferritin level changes even before hematological changes, and it is convenient, painless, easy to collect. In Bangladesh, most of the patients are unable to bear the costly investigations. Salivary ferritin assay may be beneficial both for the pediatric patients and the physicians in our country.

Methodology: This cross-sectional study was carried out in the Department of Clinical pathology, in collaboration with Department of Pediatrics and Pediatric Hematology and Oncology, BSMMU from March '2013 to February '2014. From the outdoor patient department with age group 3-18-year-old of patients were included in the study.

Results: In this study, clinically suspected 50 iron deficiency anaemic patients were included as per inclusion criteria. Their CBC, PBF, CRP, serum ferritin and salivary ferritin were done. Serum ferritin and salivary ferritin were done by ELISA method. Statistical comparison of the mean values of salivary ferritin and serum ferritin levels was done. Validity of salivary ferritin was done by calculating sensitivity, specificity, PPV, NPV and accuracy.

Conclusion: In this study, salivary ferritin had sensitivity 90.9% and specificity 83.3% in the detection of iron deficiency. Salivary ferritin can be used as a reliable and useful marker for detection of iron deficiency anemia in children.

Keywords: Iron-deficiency Anemia, Serum Ferritin, Salivary Ferritin, Children

1. Introduction

Anemia is the most common nutritional problem all over the world. It is defined as reduction of hemoglobin level below the lower extreme of the normal range for the age and sex of the individual [1]. Anemia affects approximately 25% to 50% of the world population in which 40%-60% is children [2]. It occurs in all stages of the life but more prevalent in children [3]. Anaemia is a widespread public health problem in Bangladesh affecting the lives of 27 million children, adolescents and women[4]. Prevalence of anemia in girls are slightly higher than boys [5]. In Bangladesh, anemia affects approximately 64% of children aged 6-23 months, 42% of children aged 24-59 months, 29% of adolescent girls, 17% adolescent boys, 48% in pre-school children and 34% in school aged children [6].Iron deficiency anemia is the most common cause of anaemia [7-8]. In Bangladesh, iron deficiency is most common when iron requirement is high due to rapid growth, menstruation and reproduction, hookworm infestation and diarrhoea [3, 9, 10]. It causes lowered resistance to infection, poor cognitive development and long-term motor impairment, poor learning capacity and school performance [4, 11, 12]. Anemia makes adolescent girls and women weaker during pregnancy and increased the risk of pregnancy associated complications [13]. So, effective strategies should be taken during early childhood for proper management and prevention of iron deficiency anaemia [14]. For the diagnosis of iron deficiency anemia, patient's history, signs and symptoms give the diagnostic clue [15]. There are several hematological and biochemical tests used such as hemoglobin estimation, red cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean

corpuscular hemoglobin concentration and peripheral blood film, serum iron, serum ferritin, total iron-binding capacity, transferrin saturation, serum transferrin receptor. There is no reliable, sole biochemical indicator that is confirmatory of iron deficiency anemia except the bone marrow Perl's stain [16]. Bone marrow examination is an invasive procedure, painful and has some complications, so it is not used in routine diagnostic test. Stained marrow iron may not be seen in children due to rapid growth [17] procedure is rarely done to diagnose the iron deficiency anaemia [18].

Among the various laboratory tests serum ferritin is the most important test to diagnose iron deficiency anaemia [19]. Body iron store is reflected directly by the ferritin level and decreased serum ferritin levels are used as a marker for iron deficiency anaemia [20]. Low serum ferritin is highly specific for iron deficiency anemia and is less invasive than the gold standard method of obtaining a bone marrow Perl's stain [21]. It is now known that there are several factors like inflammation, infection and malignancy may elevate serum ferritin and thus complicating interpretation [22]. Moreover, drawing of venous blood increases the risk of contamination. In children, serum ferritin concentration is a less useful guide to detect iron deficiency anemia than in adult because of rapid change in iron status [23]. Repeated measurement of ferritin level is required for diagnosis and follow-up in iron deficiency anaemia [24]. So, this procedure may not be appropriate for paediatric patients. All these evidences indicate to establish a less invasive approach for assessment of ferritin with a high sensitivity and specificity [14]. Saliva is an exocrine secretion from the salivary glands in which there is approximately 99% of water and the remaining parts are electrolytes, and proteins like enzymes, immunoglobulins [29]. It is a complex fluid composed of various organic and inorganic components at a gradient comparable with the serum [30]. Saliva contains ferritin and it is higher in iron deficiency anemia than in normal individual[31]. Ferritin is endocytosed by the duct of salivary glands and is secreted through the saliva. Ferritin is internalized by the intercalated ducts in parotid gland in the form of lysosomes is a possible mechanism for the increased salivary levels in iron deficient patient [32]. Salivary iron dependent enzymatic function conserves iron through saliva, which biologically maintains the high ferritin level in saliva in iron deficient patients [26]. Medical practitioners, laboratory specialists, researchers are using saliva for diagnosis of iron deficiency anemia, thereby making it more acceptable and accessible [28]. Salivary ferritin can be used as a valuable tool in monitoring the iron status as the salivary ferritin level changes even before hematological changes occur [26]. Oral epithelial abnormalities are frequent in iron deficiency anemia before the significant alterations in red cell morphology or hemoglobin level [27]. It is painless, easy and convenient to collect [28]. Saliva contains ferritin and it is increased in iron deficiency anemia. It can be detected in early stage of iron deficiency anemia before any changes in hematological parameter. Repeated sample also can be taken easily. Salivary ferritin assay is a simple, reliable, quick, less expensive, noninvasive method which can be carried out easily in peripheral hospital. Bangladesh is a developing country, most of the patients are unable to bear the costly investigations. Salivary ferritin can be a valuable marker in iron deficiency anemia which can be beneficial both for the patients and the physicians in our country. For this reason, salivary ferritin may be used as a diagnostic marker for iron deficiency anemia in children. So, aim of this study was to compare the salivary ferritin level with serum ferritin in iron deficiency anemia in children and assess its reliability as a predictive marker of iron deficiency anemia.

2. Methodology

Study Population & Settings: It was cross-sectional study conducted at the Department of Clinical Pathology, in collaboration with the Department of Paediatrics and Department of Paediatric Hematology and Oncology, BSMMU, Shahbag, Dhaka. Clinically suspected iron deficiency anemic patients attended in the outpatient department of Pediatrics and Pediatric Hematology and Oncology in BSMMU. Sample size was 50. Purposive sampling. As per inclusion criteria the patient was enrolled in this study. The particulars of the patients and clinical data was recorded in a pre-designed data-sheet and was kept until the end of the study. The whole procedure was explained to the participant and informed written consent was taken. Data was collected by a pre designed preform. Saliva and blood sample was obtained from patients suspected of iron deficiency anemia. Patient information was obtained through using patient's information sheet which involves questionnaire, clinical findings. The Exclusion criteria was patients with acute infection, inflammation, patients on iron therapy, diagnosed or suspected case of haemoglobinopathies. And the inclusion criteria: Suspected case of iron deficiency anaemia, based on significant pallor of the conjunctiva, nail bed and hemoglobin level as follows [33].

- ✓ Children upto 4 years <11.0 g/dL
- ✓ Children 5-11 years <11.5 g/dL
- ✓ Children 12-14 year <12.0 g/dL
- ✓ Children 15-18 year (female) <12.0g/dl
- ✓ Children 15-18 year (male) <13.0g/dl
- Age: 3-18 years.
- Sex: both.
- CRP: < 6 mg/L

Specimen collection:

Collection of saliva: About 2.0 ml of unstimulated saliva was collected from the study patient. Patients were asked to stop eating or drinking for at least 60 minutes prior to each collection. Eating, drinking, chewing gum were also prohibited during this hour. They were asked to rinse the mouth with distilled water and were supplied with a sterile container. After rinsing mouth with water, saliva was allowed to accumulate in the floor of the mouth for approximately 2 minutes and they were asked to expectorate the unstimulated saliva into the container. Then the samples were aliquot and centrifuged at 3000 rpm for 5 minutes. Centrifugation causes free of large particulate debris, allowing more accurate sample. Supernatant was subsequently stored at -30 degree until analysis was performed.

Collection of blood sample: About 3.0 ml blood was collected from each patient through an aseptic venipuncture from antecubital vein, of which 1.0 ml of blood was taken in EDTA tube for complete blood count. Complete blood count was done within 2 hrs of collection. 2 drops of blood were taken in 2 glass slides for preparation of PBF. About 2.0 ml blood was taken in a clean, dry plain test tube for serum

ferritin and CRP. Sample was centrifuged within 30 minutes at 3000 rpm for 5 minutes and serum was carefully removed and transferred to a micro centrifuge tube using a micropipette. Sample was stored at -30 degree until analysis was performed.

Laboratory Investigations:
All laboratory investigations were done in the department of Clinical Pathology, BSMMU, Dhaka. The Laboratory assay are Complete blood count (CBC) and Peripheral blood film (PBF), Serum ferritin, Salivary ferritin, CRP. And the test procedure,

For CBC: 1.0 ml blood was taken into a vial containing ethylene diamine tetra acetate (EDTA) and mixed well to prevent clot formation and analyzed in Haematology auto analyzer (SYSMEX-XT 4000i).

For Peripheral blood film: For examination of PBF, uniform blood smear was made on a glass slide with the help of spreader. Leishman`s staining was done by standard technique. It was examined by binocular microscope at first 10x objective then 40 x objectives. Total count of WBC and platelet, differential count of WBC, morphology of RBC was done. Then the findings of laboratory investigation were recorded in a predetermined data collection sheet.

For Serum Ferritin and Salivary Ferritin: The salivary ferritin and serum ferritin were analysed using enzyme linked immunosorbent assay technique using ferritin kit (DRG Rerritin: EIA-1872; DRG international, Inc, USA).

For CRP: Latex agglutination test was done (Semiquantitative)

Statistical Analysis:
Data editing, clearing and analysis was done by statistical package for social science SPSS (17.0). Microsoft Excel 2007. Qualitative variables are expressed as percentages and quantitative variables as means, standard deviation (SD), and range. Data and result was presented in the form of tables and graph where applicable.

3. Results

Out of total 50 patients, 30 (60%) patients were female and 20 (40%) were male patients.

Table 1: Distribution of the study patients by age and sex (n=50)

Age (in years)	Number of patients n=50 (%)	Male n=20	Female n=30
≤5	15 (30.0)	9	7
6-10	24 (48.0)	8	15
11-15	5 (10.0)	2	2
16-18	6 (12.0)	1	6
Mean ± SD	8.47	±4.4	
Range	(3	-18)	

Table 1 shows age of the study patients; it was observed that 78% of the patients belonged to age under 10 years. Overall mean age was 8.47±4.4 years.

Table 2: Distribution of the study patients by serum ferritin and salivary ferritin (n=50)

Parameter	Normal (%)	Positive (%)	Total
Serum Ferritin(ng/ml)	6 (12%)	44 (88%)	50
Salivary ferritin (ng/ml)	5 (10%)	45 (90%)	50

*Positive indicate below threshold level

Serum ferritin Up to 15 years for both sex normal range was (7-140 ng/ml) and positive (<7 ng/ml). For >15 Years male normal range was (20-250 ng/ml) and positive (<20 ng/ml). For >15 years' female normal range was (10-120 ng/ml) and positive (<10 ng/ml). Salivary ferritin normal range was (9.5-10.5 ng/ml) and positive range was (>10.5ng/ml).

Table 3: Comparison between serum ferritin, salivary ferritin and sex (n=50)

Parameter	Normal Male n (%)	Positive Male n (%)	Normal Female n(%)	Positive Female n(%)	P- Value
Serum Ferritin (ng/ml)	2 (33.3)	18 (40.9)	4 (66.7)	26 (59.1)	**0.544^{ns}**
Salivary Ferritin (ng/ml)	2 (40.0)	18 (40.0)	3 (60.0)	27 (60.0)	**0.690^{ns}**

s=significant; P value reached from fisher's exact test

Table 4: Comparison between Hb and serum ferritin, salivary ferritin (n=50)

Parameter	Normal n (%)	Below Normal n (%)	P- Value
Hb	0 (0.0)	50 (100)	**0.013^{s}**
Salivary Ferritin (ng/ml)	6 (12)	44 (88.0)	

Hb	0 (0.0)	50 (100)	
Salivary Ferritin(ng/ml)	5 (10.0)	45 (90.0)	**0.028^{s}**

s=significant; P value reached from McNemer test

Table 5: Relationship between hemoglobin, serum ferritin and salivary ferritin

Hemoglobin levels	Serum ferritin		Salivary ferritin		Serum ferritin:
	Mean	±SD	Mean	±SD	Salivary ferritin ratio
Mild IDA (10.0 to lower normal according to age and sex) [n=36]	8.1	±6.6	16.2	±7.1	**1:2**
Moderate IDA (7-9.9 g/dl) [n=14]	3.3	±2.2	19.3	±11.3	**1: 5.9**

Table 6: Comparison between serum ferritin and salivary ferritin (n=50)

Salivary ferritin	Serum ferritin				P value
	Positive (n=44)		Normal (n=6)		
	n	%	n	%	
Positive (45)	40	90.9	5	83.3	**0.001^{s}**
Normal (5)	4	9.1	1	16.7	

s=significant; P value reached from Fisher exact test

Table 7: Sensitivity, specificity, accuracy, positive and negative predictive values of the salivary ferritin evaluation for prediction of iron deficiency anemia in children

Test of validity	Percentage
Sensitivity	90.9
Specificity	83.3
Accuracy	90.0
Positive predictive value	97.6
Negative predictive value	55.6

Table 7 shows the validity of salivary ferritin evaluation for iron deficiency anemia in children is measured by calculating sensitivity, specificity, accuracy, positive and negative predictive values. The sensitivity, specificity, accuracy, positive and negative predictive value were 90.9,83.3, 90.0, 97.6 and 55.6 percentage respectively.

Table 8: Receiver-operator characteristic (ROC) curve of salivary ferritin for prediction of iron deficiency anemia in children

	Cut of value	Sensitivity	Specificity	Area under the ROC curve	95% Confidence interval (CI)	
					Lower bound	Upper bound
Salivary ferritin	>10.5	90.9	83.3	0.530	0.289	0. 772

Receiver-operator characteristic (ROC) curve of salivary ferritin for prediction of iron deficiency anemia in children. (Table: 8, Fig 1). The area under the receiver-operator characteristic (ROC) curves for the prediction of iron deficiency anemia in children is depicted in the following table (Table-XI). Based on the receiver-operator characteristic (ROC) Salivary ferritin had the best area under curve 0.530. Receiver-operator characteristic (ROC) were constructed (fig-8) using Salivary ferritin value of the Children, which gave a cut off value of 10.0 as the value with a best combination of sensitivity 90.9% and specificity 83.3% for iron deficiency anemia in children.

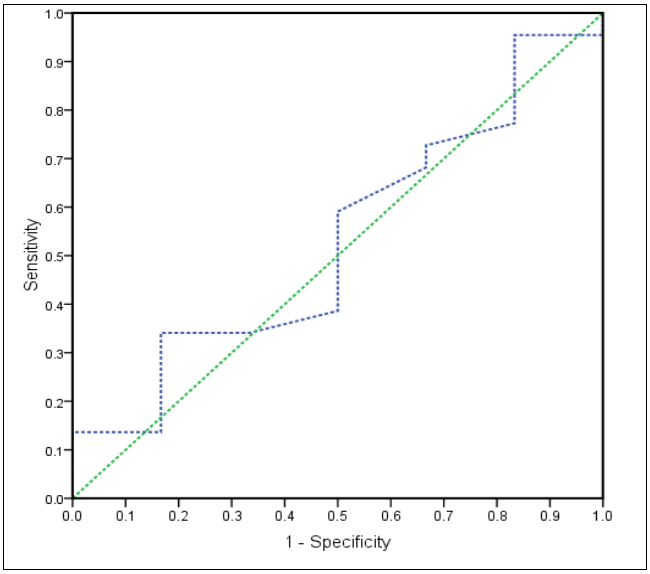

Fig 1: Receiver-operator characteristic (ROC) curve of salivary ferritin for prediction of iron deficiency anemia in children

4. Discussion

To diagnose the iron deficiency anemia, assessment of ferritin promises to be the most useful tool. So, we need to establish a less invasive, easy method for assessment of ferritin to diagnose iron deficiency anemia in children than serum ferritin. Saliva contains ferritin which is increased in iron deficiency anemia. Hence, the present study was conducted to assess the salivary ferritin in the diagnosis of iron deficiency anemia which demonstrated that there was significant increase of salivary ferritin in iron deficient children.

In our study, sensitivity and specificity, positive predictive value and negative predictive value of salivary ferritin in diagnosing iron deficiency anemia were 90.9%, 83.3%, 97% and 55.6% respectively. Other study has also found sensitivity and specificity, positive predictive value and negative predictive value of salivary ferritin were 100%, 88.24%, 87.6% and 100% respectively [14]. Sensitivity and specificity, positive predictive value in our study is almost nearer to this study. In this study, in mild iron deficiency anemia, mean±SD of serum ferritin was 8.1±6.6 and mean salivary ferritin ±SD was 16.2±7.1. The ratio of serum ferritin and salivary ferritin was found 1:2 in mild iron deficiency anemia. In moderate iron deficiency anemia, mean serum ferritin ±SD was 3.3±2.2 and mean salivary ferritin ±SD was 19.3±11.3. The ratio of serum ferritin and salivary ferritin was found 1:5.9. The other study found the serum ferritin: salivary ferritin was found 1:1.65 in mild iron deficiency anemia and 1:3.77 in moderate iron deficiency anemia and 1:9.55 in severe iron deficiency anaemia [14]. So, result of our study is consistent with this study.

In present study, all patient had hemoglobin level below normal. Serum ferritin was below normal in 44 and normal in 4 patients. The difference was statistically significant (<0.05) between hemoglobin and serum ferritin. The studies have also found hemoglobin was statistically significant (<0.05) with serum ferritin [34-36]. So, observation of this study is within international norms. In our study, serum ferritin was positive in 18 and normal in 2 male patients. In female patient, 26 had positive and 4 normal serum ferritin. In comparison between serum ferritin and sex, the difference was not statistically significant. Others have also found that serum ferritin is not significant between male and female patient [37]. So, finding of our study is similar with other.

Few studies have been done in different countries but so far, my knowledge, no study has been done yet in our population. In India, a study suggested that salivary ferritin can be used as a good marker for assessing the iron deficiency anemia with sensitivity and specificity of 100% and 88.24% respectively [14]. Iron deficiency anemia negatively affects cognitive functioning so early diagnosis and management is needed that should be started from childhood [25]. Serum ferritin is not a useful guide for children, it is falsely elevated in infection and also it has a wide reference range. Moreover, repeated sample is needed for follow up and management. So, we need such a specific and sensitive marker which is easy to collect from children

and is cost effective [14]. One study has done on "Salivary iron status in children with iron deficiency and iron overload". They had taken forty anemics and 10 non-anemic children as a control aged from 8 months to 10 years and Hemoglobin, hematocrit, serum iron, salivary iron were measured. The salivary iron was significantly higher in iron deficient and iron overload conditions compared to controls. They concluded that, salivary iron is increased in iron deficiency anaemia [26]. The other one attempted a cross sectional study on "Serum ferritin to detect iron deficiency anemia in children below five years of age". Hemoglobin and serum ferritin were performed on children aged 6-59 months. The prevalence of iron deficiency was 32%. The sensitivity and specificity and positive predictive value of serum ferritin (<12 µg/L) were 17%, 93%, 56% respectively. They concluded that the diagnostic value of serum ferritin is modestly capable of detecting iron deficiency anaemia [38].

According to our study, salivary ferritin is increased in iron deficiency anemia. Our data indicates that salivary ferritin may be a good diagnostic marker for diagnosis of iron deficiency anemia in children.

5. Conclusion

Our study showed that salivary ferritin is significantly higher in iron deficiency anemia in children. Salivary ferritin may be used for assessment of iron deficiency anemia in children from whom blood collection is very difficult. We may conclude that salivary ferritin might be an important diagnostic marker in iron deficiency anemia in children.

6. Limitation of the study

- This study was done in limited time of span.
- The sample size was small.
- Due to lack of budget, Perls' stain was not done and no specific investigation was done to exclude coexistence of hemoglobinopathies.
- The study was confined to one hospital (BSMMU) only.

7. Financial support & sponsorship: None.

8. Conflicts of Interest: None.

Ethics: This study was approved by the BSMMU institutional review board. A written informed consent was obtained from all patients.

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