



A comparative study of salivary and serum iron in normal and anaemic female patients in Udaipur city, Rajasthan, India

Estudo comparativo do ferro salivar e sérico em mulheres saudáveis e anêmicas da cidade de Udaipur, Rajasthan, Índia

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Abstract

Objective: To evaluate the concentration of serum iron and salivary iron in anemic and normal female patients and to compare and correlate the concentration of serum and salivary iron. **Material and methods:** The 20 Anemic and 20 non-anemic as control group female patients of age groups of 20-40 yrs were included in the study. Unstimulated whole saliva was collected and the morning blood sample was collected simultaneously. Salivary and serum iron was determined using automatic analyzer by Ferrozine method. **Results:** The salivary iron was significantly high in anemic female patients as compared to controls. The mean salivary:serum iron ratio was four times in anemic females as compared to control group. The correlation between salivary iron and serum iron was ($p<0.05$) in these cases. **Conclusion:** The iron in saliva is maintained at a higher level in iron deficiency anemia and it correlates well with serum iron in iron deficient anemic patients.

Keywords: Iron deficiency anemia. Females. Ferritin. Mouth. Saliva.

Resumo

Objetivo: Avaliar e comparar a concentração de ferro sérico e salivar em pacientes anêmicas e saudáveis do sexo feminino. **Material e métodos:** Vinte pacientes anêmicas e vinte pacientes saudáveis do sexo feminino, de idades entre 20 e 40 anos, foram incluídas neste estudo. Amostras de saliva total não estimulada e sangue matinal foram coletadas. Níveis de ferro sérico e salivar foram determinados utilizando o método analítico automatizado de Ferrozine. **Resultados:** A concentração de ferro salivar foi significativamente maior em pacientes anêmicas quando comparadas ao grupo controle. A média da razão entre ferro salivar e ferro sérico foi quatro vezes em pacientes anêmicas. A correlação entre ferro salivar e sérico foi $p < 0.05$ nestes casos. **Conclusão:** O ferro salivar se manteve em maior concentração em pacientes com anemia, apresentando correlação positiva com o ferro sérico.

Palavras-chave: Anemia por falta de ferro. Mulheres. Ferritina. Boca. Saliva.

Introduction

Iron deficiency anemia is common in the world and an enormous public health problem in developing countries. The most common reason for iron deficiency anemia in infants and children is the inadequate supply of iron in diet. This is particularly in developing countries where the level of intake of iron rich foods is low, and malaria and other intestinal parasites are common (1). The prevalence of iron deficiency anemia in industrialized countries has declined in recent decades, but there has been little change in the worldwide prevalence (2-4). It is currently estimated to affect more than 500 million people. Unfortunately, a great difference in the prevalence rate of iron deficiency anemia in the developed and industrialized countries exists; 25–35% in the developed countries compared to 5–8% in the industrialized ones (5-8).

Iron deficiency anemia is one of the most common health problems in India. The high-risk groups for anemia are females. According to WHO, the prevalence of Iron deficiency anemia is 50–90% and is common among females. Iron is stored in the reticuloendothelial cells, hepatocytes, and skeletal muscle cells as two iron-protein complexes, Ferritin and Haemosiderin. The ferritin molecule is an intracellular hollow protein shell composed of 24 subunits surrounding an iron core that may contain as many as 4000–4500 iron atoms (9). In the body, small amounts of ferritin are secreted into the plasma.

Under physiological conditions, there is a balance between iron absorption, iron transport, and iron storage in the human body. However, iron deficiency and iron deficiency anemia may result from the interplay of three distinct risk factors: increased iron requirements, limited external supply, and increased blood loss. In iron deficiency status, laboratory tests

may provide evidence of iron depletion in the body or reflect iron deficient red cell production. The salivary iron and serum ferritin levels can be used as a diagnostic test to evaluate the iron overload in the body. The normal serum ferritin level in male is 60–160 $\mu\text{g}/\text{dl}$ and among females is 37–145 $\mu\text{g}/\text{dl}$, whereas the normal salivary iron male is 5.4–28.7 $\mu\text{g}/\text{dl}$ and in female is 1.5–5.72 $\mu\text{g}/\text{dl}$. The serum iron level below 37 $\mu\text{g}/\text{dl}$ and the salivary iron level above 5.72 $\mu\text{g}/\text{dl}$ denotes iron deficiency anemia in female patients (10).

The trace elements present in the saliva and serum have been extensively studied in recent years. The altered serum trace elements are well documented and are considered as good biomarkers of malignancies (11). However, very few studies have been conducted to determine the role of trace element iron present in serum and saliva and its role in anemia. Thus, the present study was conducted aiming to determine the concentration of serum iron and salivary iron in anemic and normal female patients and to compare and correlate the concentration of serum and salivary iron.

Material and methods

A randomized clinical trial was conducted between July 2011 and August 2011. This study was approved by the Ethical Committee of the Darshan Dental College and Hospital and ethical clearance was obtained. Forty female patients with age groups between 20–40 yrs (20 anemic and 20 non-anemic) attending the department of oral and maxillofacial pathology participated voluntarily and were informed about the purpose

of the study, and a written consent was obtained. All participants had no history of any systemic disease and were not under any medication. A screening test was performed for hemoglobin estimation by Sahli's method prior to the study to determine the anemic and control group.

Unstimulated whole saliva was collected by spit method. All samples were collected between 8 and 11 am to minimize any effects of diurnal variability in salivary composition. Samples were collected at least 2 h after meals, and all the participants were abstained from eating or drinking for 2 h before their examination. The patients were asked to tilt head forward to allow saliva to dribble, and after 3 to 4 rinses of the mouth the whole saliva which accumulated in the floor of the mouth, in approximately 2 min. Saliva was repeatedly spit into ice chilled vial to collect about 2 ml.

Under sterile condition, the morning blood sample was collected between 8 and 11 am. The needle was gently inserted into the median cubital vein of the forearm by IV cannulation, and the patient's blood was collected in airtight vial tube and stored in the refrigerator until it was transported to the laboratory for investigation.

The laboratory test was carried out for the salivary and serum iron determination at Medicentre, Udaipur, and Rajasthan in a fully automatic analyzer

using Ferrozine method. We used the kit named Cobas C111. The reagents used were R1 and R2, citric acid, thiourea, detergent, sodium ascorbate, and Ferrozine.

The data were systematically analyzed using Microsoft Excel 2007. In order to evaluate reliability, chi-square analysis was used and the test was considered statistically significant when $p < 0.05$. Data was statistically computed using SPSS 15.0.

Results

A total of 40 patients participated, out of which 20 were anemic and 20 were non-anemic considered as control group.

The descriptive data show that the mean serum iron in control group was high (73.26 ± 15.64) as compared to the anemic group with 30.76 ± 4.5 . But the salivary iron in anemic group was high (8.07 ± 1.12) when compared to the control group with 1.78 ± 0.52 (Table 1).

To determine the reliability, the chi-square test was used to find the relation between the salivary and serum iron. The mean Salivary:Serum iron ratio in female anemic patient ($p=0.024$) is 4:1 compared to controls ($p=0.032$). The serum iron of control group is about twice that of anemic group (Table 1; Figure 1).

Table 1 - Distribution and relation of salivary and serum iron

Iron	Anemic	Control
Mean Serum Iron \pm SD	$30.76 \pm 4.5 \mu\text{g/dl}$	$73.26 \pm 15.64 \mu\text{g/dl}$
Mean Salivary Iron \pm SD	$8.07 \pm 1.12 \mu\text{g/dl}$	$1.78 \pm 0.52 \mu\text{g/dl}$
p value	0.024 (Significant)	0.032 (Significant)

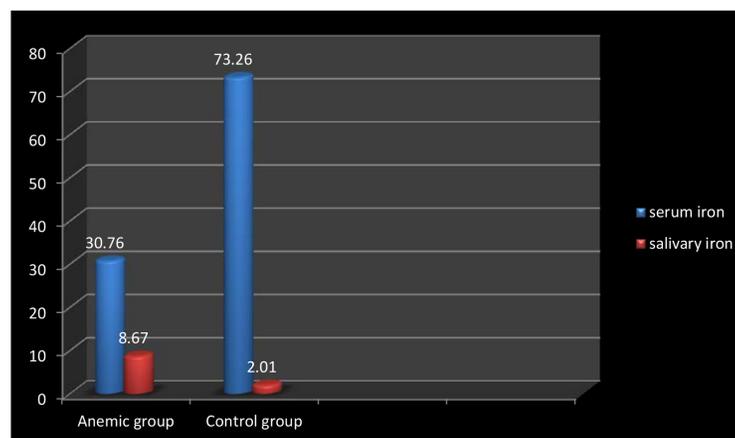


Figure 1 - Representation of the salivary and serum iron values

Discussion

In the present study, the concentration of serum iron and salivary iron in anemic and normal female patients was compared and the correlation was seen in the concentration of serum and salivary iron. Serum iron is the basic parameter for iron overload management and works as a diagnostic indicator for detection of inflammation and other diseases. The salivary iron gives remarkable result and can be used as a diagnostic aid. In the present study, there was an increase in salivary iron as compared to serum iron in anemic patients. Similar results were obtained from previous studies (12, 13) on the salivary iron status in children with iron deficiency and iron overload (12). A total of forty anemic patients were included in the study. The results showed that the mean salivary and serum iron ratio was the same in control and iron overload cases, whereas it was twice as high in iron deficient anemic children with ($r=0.7392$, $p<0.001$) as compared to the present study. The mean salivary and serum iron ratio in female anemic patient ($p=0.024$) is 4:1 compared to controls ($p=0.032$).

Similarly, a study conducted by Madanat et al. (13) on serum ferritin in assessing iron status was conducted among 192 preschool age children between the ages of 3 and 60 months. The mean serum ferritin for the iron deficiency anemia group was 39.1 ng/mg as compared to 41.7 ng/ml for the iron deficiency group and 84.7 ng/ml for the normal group, whereas in the present study the mean serum iron in control group was 73.26 $\mu\text{g}/\text{dl}$ and in anemic group was found to be 30.76 $\mu\text{g}/\text{dl}$. It was suggested that serum ferritin cannot be used alone for iron status determination. Multiple parameters will make the assessment more reliable. Another study (14) on serum ferritin in evaluation of iron status in 30% of children who had either iron deficiency or iron deficiency anemia found serum ferritin level of less than 12 ng/ml. Another study was conducted on the evaluation of essential trace elements in hair and saliva by Benson et al. (15). A total of 265 healthy children (7–9 years) were included in the study. The concentration of these trace elements in hair and saliva (Hair: Fe $28.47\pm 0.70\text{mg}/\text{kg}$; and Saliva: Fe $1.06\pm 0.03\text{mg}$) was determined. It was suggested that the cost-effectiveness of this method, when compared to blood analysis in disadvantage communities, has sparked the interest

in the potential evaluation of zinc, iron, copper, and manganese in hair and saliva as an assessment index for trace elements.

Similar to the present study, it can be observed in other saliva researches that more laboratories and clinics are relying on saliva for the diagnosis and treatment of different diseases due to the relative ease with which samples can be collected and analyzed. Other studies on serum and salivary patients with recurrent aphthous ulcer (RAU) showed that there are no significant differences between patients regarding levels of iron, vitamin B12, and folate (16). Barnadas et al. (17) reported that patients with RAU are prone to iron, vitamin B12, and folate deficiencies, although when the investigated factors were analyzed separately, there were no significant differences between patients with RAU and patients with other oral diseases. Significant difference between study and control group was found regarding serum folate levels.

However, serum vitamin B12, as well as haemoglobin and haematocrit levels, were within a normal range in both groups, which was reported by Thongprasom et al. (18). A study (19) conducted on iron supplementation for unexplained fatigue in non-anemic women, a randomized clinical trial showed serum ferritin concentrations were high in iron group (21.0 ± 9.2) as compared to control group (13.7 ± 6.9 ; $p<0.001$). Another study (20) on nickel, chromium, and iron levels the saliva of patients with fixed orthodontic appliances. Seventeen orthodontic patients undergoing treatment were compared with seven untreated individuals. The range of salivary metal levels found did not exceed those of daily intake. There was an increase in salivary iron levels (5.06 ± 0.76 ; $p<0.001$) similar to salivary iron, whereas in our study the difference between serum and salivary iron was 6.29 ± 0.6 ($p<0.05$).

This is probably the first study conducted in Udaipur city. However, there are only few studies and data available on the present context. The study attempts to show a significant ratio alteration between salivary and serum iron. In iron deficiency anemic female patients, the serum iron levels had decreased from normal whereas the salivary iron was significantly elevated from normal. Salivary iron estimation gives constant authentic results. It can be used for large population screening due to cost effectiveness and noninvasive nature. It can be used as a valid screening tool in children, older adults, and debilitated patients.

Since continuous monitoring and assessment of salivary serum iron is not currently available, several issues must be resolved to establish the study findings. However, further studies need to be conducted to establish the study findings.

Conclusion

The iron in saliva is maintained at a higher level in iron deficiency anemia, and there is a direct association between serum and salivary iron due to the significant decrease in blood volume of anemic patients. Thus, this screening tool will prevent further decrease of blood volume.

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Received: 02/06/2014
Recebido: 06/02/2014

Accepted: 03/06/2014
Aceito: 06/03/2014