Original Article

CORRELATION OF SERUM & SALIVARY FERRITIN LEVELS IN IRON DEFICIENCY ANEMIA PATIENTS

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ABSTRACT

Keywords: Haemoglobin, Iron, Ferritin, TIBC, saliva, serum, iron deficiency anemia

Background: According to the World Health Organization (WHO), there exists two billion people in the world with anemia and half of them are due to iron deficiency. Iron deficiency anemia is one of the most important nutritional deficiencies in India. It is present in children <10 years of age, women after puberty and older adults. Among them, the prevalence is higher in children of age group between 5–14 years, which is 48%. This disease requires repeated measurement of iron and ferritin levels for the diagnosis and follow-up. Currently, the diagnosis is made by the assessment of iron and ferritin levels in serum through complete blood cell count, peripheral smear, iron indices and Bone marrow iron microscopy. But, this involves the drawing of venous blood, which is invasive and is physically, psychologically traumatic to the child patients. This study is done to estimate and correlate the iron and ferritin levels in blood and saliva of children with iron deficiency anemia. Thus, assessing the effectiveness of saliva as an alternative non-invasive diagnostic tool.

Aim: This study is done to estimate, compare and correlate the Ferritin, Iron, Total Iron Binding Capacity (TIBC) levels in serum & saliva of iron deficiency anemia patients, to determine whether saliva can be used as a predictive marker to monitor the iron levels in iron deficiency anemia.

Materials & methods: 50 subjects were chosen for the study. Quantitative estimation of ferritin was performed by direct chemiluminescence method. Total iron binding capacity was estimated by spectrophotometry. Percentage of saturation of iron is calculated, haemoglobin levels were also estimated to confirm the anemic status of the patient.

Results & Conclusion: Decreased levels of iron and ferritin in serum and saliva of iron deficiency anemia patients compared to normal controls. Increased TIBC in serum and saliva of iron deficiency anemia patients. As the iron and ferritin levels decreases, TIBC increases. Saliva can be used as an alternative diagnostic tool to monitor the iron levels in anemia patients especially in children upto 12 years of age, when the iron deficiency is at its peak, thus it will aid to improve the quality of life of iron deficient individuals.

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Inclusion criteria:
1. Iron-deficiency anaemia patients whose haemoglobin levels are less than 8 mg/dl.
2. The cases were selected on the basis of significant clinical features - pallor of the conjunctiva, nail beds and atrophic glossitis.
3. Freshly diagnosed cases of iron-deficiency anaemia not using any medication.
4. Subjects without any history of other systemic diseases, not taking any systemic drug therapy.

Exclusion criteria: Subjects with infections, thalassemia, sickle cell anemias, transfusions, hemoglobinopathies and other pre-existing medical illnesses, those who are on medications.

The study protocol was approved by the Ethical committee of the University.

A procedure of the study was clearly explained to all the participants in the study and a written informed consent was obtained. A predetermined data sheet was used to record the history and the clinical features were evaluated.

Sample collection:
Saliva: About 2 ml of unstimulated saliva was collected. Donors were asked to refrain from eating or drinking for at least 60 minutes prior to each collection. Smoking, chewing gum and intake of beverages were also prohibited. Mouth rinsed with deionized distilled water and unstimulated saliva was expectorated into a sterile container. After collection, the samples were aliquoted and then centrifuged at 2500 rpm for 5 minutes. The centrifugation resulted in free of large particulate debris and reduced viscosity, thereby allowing a more accurate and reproducible analysis. The supernatant was subsequently stored at -30°C until analysis was performed.

Blood: about 4.5 ml of venous blood was drawn using a 21 gauge needle with tourniquet application to prevent stasis. Sample collection was done with the help of iron free syringes and needles through venipunctures and was placed in iron free labelled 5 ml plastic evacuated vials containing 0.5 ml of EDTA. The content was immediately mixed by gently inverting the tube 5 times and the samples were stored at -50°C until analysis. The sample was centrifuged within 30 minutes at 2,500 rpm at 20°C for 15 minutes without brake. Plasma was carefully removed and transferred to a non-activating centrifuge tube using a plastic pipette and was centrifuged again using the same conditions. Platelet-free plasma was divided into aliquots and stored at -80°C until analysis.

Biochemical analysis:
Quantitative estimation of ferritin was done by - Chemiluminescence method
Iron & TIBC was measured by - Spectrophotometry
Percentage of saturation of iron - calculated
Hemoglobin percentage was estimated to confirm the anemic status.

RESULTS & DISCUSSION:

Table 1:

<table>
<thead>
<tr>
<th>Age</th>
<th>Group</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>25</td>
<td>7.0</td>
<td>16.0</td>
<td>11.28</td>
<td>2.46</td>
<td></td>
</tr>
<tr>
<td>Anemic</td>
<td>25</td>
<td>7.0</td>
<td>14.0</td>
<td>11.36</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>7.0</td>
<td>16.0</td>
<td>11.32</td>
<td>2.19</td>
<td></td>
</tr>
</tbody>
</table>

Table 2:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Group</th>
<th>Anemic</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>%</td>
<td>Count</td>
<td>%</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>96.0%</td>
<td>22</td>
<td>88.0%</td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td>6.0%</td>
<td>3</td>
<td>12.0%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100.0%</td>
<td>25</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Table 3:

The above chart shows the comparison of minimum, maximum, mean and standard deviation values of serum & salivary iron, serum & salivary TIBC, serum & salivary ferritin.

Table 4:

<table>
<thead>
<tr>
<th>Relation</th>
<th>r-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRON &amp; TIBC</td>
<td>-0.812</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IRON &amp; FERRITIN</td>
<td>0.844</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 5:

<table>
<thead>
<tr>
<th>Relation</th>
<th>r-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALIVA IRON &amp; SALIVA TIBC</td>
<td>-0.873</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SALIVA IRON &amp; SALIVA FERRITIN</td>
<td>0.799</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Iron is one of the most essential trace elements in the body. Daily requirement of iron: Children - 10–15 mg/day, male - 10 mg/day, female - 20 mg/day, pregnant women - 10 mg/day, lactating mothers - 25–30 mg/day. The total iron content in an adult male is about 3.8 gm, females is about 2.3 gm. In the human body iron exists as Essential/functional iron and storage iron. Essential iron - involved in the normal metabolism of cells; Storage iron - present in two forms - ferritin and hemosiderin. It is absorbed from the upper portion of the duodenum as ferrous or ferric salts. Absorption depends on the storage. If the tissues are depleted of iron sources, it is absorbed rapidly; if sufficient quantities are present, absorption is slight. Excretion of iron takes place by kidneys or alimentary canal - 1 mg/day. According to the World Health Organization (WHO), there exists two billion people in the world with anemia and half of them are due to iron deficiency. Depletion of iron stores in the body manifests as iron-deficiency anemia. This may arise due to various reasons - chronic blood loss, inadequate dietary intake, faulty absorption and increased requirements during infancy, childhood, adolescence and pregnancy. In the view of detecting an alternative non-invasive diagnostic tool for estimating the ferritin levels, researchers have focussed on saliva. But there are only a few such studies on salivary ferritin. Hence, this study was conducted to assess iron and ferritin levels in saliva, which can aid in the diagnosis of iron deficiency anemia.

The age range of iron deficiency anemia patients in our study was 7 years to 14 years. Age and gender matched controls were taken into consideration (Table 1). The study group comprised of 96% females, 4% males. Control group has 88% females and 12% males.

**In normal controls:**

The minimum and maximum values of *serum iron* levels was 56.0, 136.0 ng/dl respectively, with a mean value of 88.24±21.49. The minimum and maximum values of *salivary iron* was 68.0, 126.0 respectively, with a mean value of 90.32±16.91.

The minimum and maximum values of *serum TIBC* was 156, 306 respectively, with a mean value of 226±39.43. The minimum and maximum values of *salivary TIBC* was 156.0, 342 respectively, with a mean value of 231.44±45.25.

The minimum and maximum values of *serum ferritin* was 56.0, 154 respectively, with a mean value of 92.48±24.31. The minimum and maximum *salivary ferritin* was 69.0, 154 respectively, with a mean value of 102.56±24.63.

**In iron deficiency anaemia patients:**

The minimum and maximum serum iron level was 10.0, 42.0 respectively, with a mean value of 25.24±9.96. The minimum and maximum salivary iron level was 10.0, 42.0 respectively, with a mean value of 23.16±10.22.

Minimum and maximum serum TIBC was 305.0, 452.0 respectively, with a mean value of 372.32±45.25. Minimum and maximum salivary TIBC was 402.0, 826.0 respectively, with a mean value of 44.64±13.46.

The minimum and maximum serum ferritin was 16.0, 42.0 respectively, with a mean value of 26.56±7.81. The minimum and maximum salivary ferritin was 22.0, 70.0 respectively, with a mean value of 44.64±13.46.

There is a reduction in serum and salivary iron levels in iron deficiency anemia patients compared to normal healthy controls. But, there is an increase in TIBC. The mean values of all the parameters were represented in Graph-1. According to the results of this present study, there is a positive correlation between the iron

**Graph 1**

Multiple bar diagram representing the Mean values of different variables in normal and abnormal groups of both Serum and Saliva.

**Graph 2**

The above matrix scatter diagram representing that SERUM IRON had negative relation with SERUM TIBC and it was positively correlated with SERUM FERRITIN. SERUM TIBC was negatively correlated with SERUM IRON as well as SERUM FERRITIN. There was a positive correlation between SERUM FERRITIN and SERUM IRON but it was negative between SERUM FERRITIN and SERUM TIBC.

**Graph 3**

The above matrix scatter diagram representing that SALIVA IRON had negative relation with SALIVA TIBC and it was positively correlated with SALIVA FERRITIN. SALIVA TIBC was negatively correlated with SALIVA IRON as well as SALIVA FERRITIN. There was a positive correlation between SALIVA FERRITIN and SALIVA IRON but it was negative between SALIVA FERRITIN and SALIVA TIBC.
and ferritin levels in serum and saliva, and there is a negative correlation between iron and TIBC.

CONCLUSION:
Iron deficiency anaemia is most common in younger age group. Assessment of iron levels in serum would be painful & difficult in such patients and the iron markers are also altered by inflammation, which limits their applicability in areas where there is a high infection pressure. In such conditions, salivary ferritin assay may be useful. The advantages of using saliva are - avoids venipuncture, very much useful in young children when it is difficult to obtain several millilitres of peripheral blood, in epidemiologic surveys to determine the prevalence of iron deficiency and to evaluate the efficacy of intervention programs to alleviate it.

REFERENCES