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Research Article

SERUM AND SALIVARY IRON LEVELS IN TYPE II DIABETES MELLITUS

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ABSTRACT

Objective: To estimate and correlate serum iron, salivary iron and blood glucose levels in patients with diabetes mellitus.

Methods: Study group consisted of 15 diabetics and control group consisted of 15 non-diabetics. Blood sample of 2ml and salivary sample were collected from both the groups. Serum and salivary iron levels and serum glucose levels were estimated using colorimetric method with the help of reagents and semi auto analyser. Pearson's correlation coefficient test was applied to assess the correlation between salivary and serum iron levels.

Results: Results showed significant ($P < 0.001$) positive correlation between serum and salivary iron levels in diabetics where as in control group, though results showed positive correlation it was statistically non- significant.

Conclusion: Salivary and Serum iron levels showed positive correlation with significantly high levels in case of Diabetes Mellitus. Hence saliva can be used as a diagnostic tool to demonstrate the iron overload.

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic disorder of multiple etiological factors. It is characterized by chronic hyperglycemia with derangement of carbohydrate, fat and protein metabolism [Thomas B *et al* [1]]. Diabetes mellitus is perhaps one of the oldest diseases known to man. It was first stated in Egyptian manuscript about 3000 years ago [Abdulfatai B *et al* [2]][Ahmed AM *et al* [3]]. This condition results from defects in insulin secretion, insulin action, or both. It is expressed by abnormally high levels of glucose in the blood [Thomas B *et al* [1]]. The metabolism of several trace elements like iron is altered in diabetes mellitus. In exchange these nutrients have specific roles in the pathogenesis and progress of this disease and its complications [Thomas B *et al* [1]]. Iron as a potential catalyst is involved in various cellular reactions which produce Reactive Oxygen Species. These Reactive Oxygen Species induce oxidative stress and damage to tissues which alters the risk for Diabetes Mellitus [Tuomainen T P *et al* [5]] [Manikandan A *et al*[6]]. Increased serum iron levels are observed in diabetes and also in many other pathological conditions. Comparative estimation of serum iron levels and salivary iron levels is done in this study.

MATERIALS AND METHODS

Study Design and Subjects: The study was conducted on patients with type 2 diabetes mellitus attending the outpatient department of Department of Oral Medicine, at St Joseph Dental College, Eluru. Age and sex matched normal healthy individuals were considered as controls for the study.

Selection Criteria

Inclusion criteria: Study group included 15 type 2 diabetes mellitus patients whose post prandial blood sugar (PPBS) $\geq$ 140mg/dl in the age group 35-75 years and control group included 15 healthy subjects who were age and sex matched.

Exclusion criteria: Patients taking any iron and vitamin supplements, patients with secondary complications like diabetic nephropathy, patients who are smokers and alcoholics, pregnant and lactating women were excluded from this study.

Sample collection

2ml of venous blood was drawn using sterile disposable syringe under aseptic conditions from antecubital vein of all the subjects and dispensed into test tubes. The serum was separated by centrifugation at 3000 rpm for 3 minutes. Unstimulated

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salivary sample was collected through spitting method from the same patient simultaneously.

Serum iron and salivary iron were detected by colorimetric method using semiautomated analyser, 'Robonik Prietest' by Robonik India PVT. LTD. Iron reagent kit consisted of buffer, colour reagent and iron standard using ferrozine method was employed for this study.

Statistical Analysis

Mean values of serum glucose, serum and salivary iron levels were analysed using statistical software SPSS version 17. Pearson's correlation coefficient test was applied to assess the correlation between serum and salivary iron levels. The data was expressed as mean and in the entire tests P (probability) value ≤ 0.05 was taken to be statistically significant.

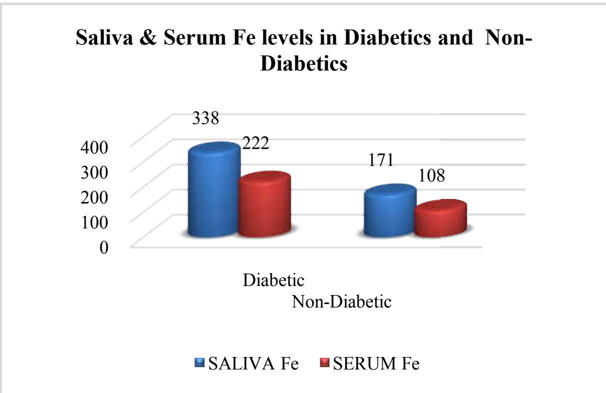
RESULTS

Significant positive correlation between salivary iron levels and serum iron levels was observed in diabetics where as the results showed insignificant positive correlation in healthy non-diabetic group [Table 1] [Graph 1].

Table 1 Correlation between Serum Fe and Saliva Fe in Diabetics and Non-Diabetics

Variable	Diabetic			Non-Diabetic		
	Mean±SD	Correlation coefficient	P Value	Mean±SD	Correlation Coefficient	P Value
SALIVA Fe SERUM Fe	337.67±96.34 221.55±103.33	0.868	<0.001;S*	171.38±38.17 107.79±31.67	0.067	0.8;NS

S*- Significant, NS – Not Significant



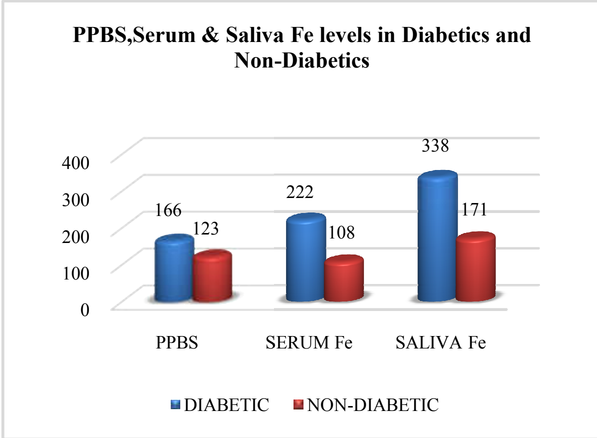
Graph 1 Salivary and Serum Fe levels in Diabetics and Non - Diabetics

Significant positive correlation of glucose levels with serum iron levels and salivary iron levels was observed in diabetes group whereas results showed insignificant positive correlation of glucose levels with serum iron levels and insignificant negative correlation of glucose levels with salivary iron levels in healthy non-diabetic group [Table 2] [Graph 2].

Table 2 Correlation of Glucose levels with Serum Fe, Saliva Fe in Diabetics and Non-Diabetics

Variable	Diabetic			Non-diabetic		
	Mean±SD	Correlation coefficient	P Value	Mean±SD	Correlation coefficient	P Value
PPBS SERUM Fe	166.13±29.94 221.55±103.33	0.769	0.001;S*	123.47±10.28 107.79±31.67	0.257	0.35;NS
PPBS SALIVA Fe	166.13±29.94 337.67±96.34	0.783	0.001;S*	123.47±10.28 171.38±38.17	-0.048	0.86;NS

S*- Significant, NS – Not significant



Graph 2 PPBS, Serum and Salivary Fe levels in Diabetics and Non-Diabetics

The mean serum iron levels in diabetic patients (221.55±103.33) was more than that in controls (107.79±31.67) which was statistically significant (p<0.0001). The mean salivary iron levels in diabetic patients (337.67±96.34) was significantly (p<0.0001) more than controls (171.38±38.17). The mean serum iron and salivary iron in diabetic patients and in controls were almost same with a mean difference of 0 which was statistically significant (p=0.003) [Table 3].

Table 3 Mean, Standard deviation, t-value and P-value of serum and salivary iron levels in Diabetics and Non-Diabetics

Variable	Diabetic				Non-Diabetic			
	Mean	SD	t-value	p-value	Mean	SD	t-value	p-value
Serum Fe Saliva Fe	221.6 337.7	103.3 96.3	3.182	0.003;S*	107.8 171.38	31.7 38.2	4.96	0.00003;S*

S*- Significant

DISCUSSION

Micronutrients like iron play an essential role in regeneration, for coping with oxidative stress and for an adequate immune response. Hence, this element is essential for maintaining health throughout life. Iron can cause diseases through deficiency, imbalance, or toxicity. Studies have shown that elevated iron levels may be a contributing factor in many inflammatory conditions. [Thomas B *et al* [1]]

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [Thomas B *et al* [1]]. Iron, in addition to being structural components of body tissues it is also involved in various physiological processes, such as proper metabolism and energy production. They also play a clear role in the synthesis, storage and secretion of insulin as well as its conformational integrity. Since iron affects metabolism of glucose and glucose metabolism impinges on several iron metabolic pathways, the relationship between them is bidirectional. Large number of experimental studies in humans has supported a significant association between type 2 diabetes mellitus and alteration in metabolism of several trace elements. Impaired insulin release, insulin resistance and glucose intolerance in type 2 diabetes mellitus have been linked to the compromised status of various trace elements viz; iron, magnesium, chromium etc required for proper insulin function [Manikandan A *et al* [6]].

Insulin is known to cause a rapid and marked stimulation of iron uptake by fat cells due to redistribution of transferrin receptors from intracellular membrane compartment to cell surface. Reciprocally, iron influence insulin action by inhibition of glucose production by liver. As iron stores increases, insulin metabolism and hepatic extraction reduces which leads to peripheral hyperinsulinemia [Manikandan A *et al* [6]]. In diabetes mellitus, there is decrease in uptake of iron and increase circulatory pool of catalytic iron. The increased blood glucose in diabetes mellitus stimulates nonenzymatic glycosylation of several proteins including hemoglobin. Glycosylation of hemoglobin also leads to increase in iron release from protein [Kapoor S *et al* [4]]. This may lead to mild iron overload in type 2 diabetes mellitus patients. All parenterally administered iron in excess accumulates in the liver as hemosiderin. Thus, the rise in the amount of iron in the serum of the diabetic patients might be either due to increased release of iron from the body storage depot into the systemic circulation or due to attenuation in the process of storage related to oxidative stress. All of these factors contribute to increase in serum iron levels. In the present study significant increase in the serum iron levels is noticed in diabetics.

Iron overload need not be a consequence of diabetes mellitus rather it may precipitate diabetes mellitus. This can be explained by the fact that iron is a transitional metal and a potential catalyst in cellular reactions that produce oxygen reactive species such as hydroxyl radical (OH^{-}) and superoxide anion ($O2^{-}$) that can initiate and propagate the cascade leading to oxidative stress and finally cell death [Fernandez-Real JM *et al* [7]][Kapoor S *et al* [4]]. Such an effect of oxidative stress on pancreatic beta cells may lead to cell death, decreased insulin secretion and deficiency favouring dissolved glucose metabolism. On the other hand, it is increasingly being recognized by few researchers that serum iron influence glucose metabolism even in absence of significant iron overload or even in a state of iron deficiency.

Saliva is a complex fluid composed of a wide variety of organic and inorganic substances in the form of protein, various enzymes, sodium, potassium, thiocyanates, and some minerals such as iron, copper, and chromium. These minerals are present in saliva at a gradient which is comparable with serum. They collectively act to modulate the oral environment. Agarwal and coworkers observed that saliva contains ferritin and changes in ferritin levels have been observed in iron deficiency and its levels in saliva were much higher than the normal [Sindhu S *et al* [9]]. In our present study too, salivary iron levels were introspective of serum iron levels.

Although many studies comparing the simulacrum, serum and saliva were commensurate, saliva has never been an exact replica of serum due to many factors. In the present study salivary iron levels were more than serum iron levels which could have been contributed by local factors. Epidemiological data confirm that diabetes is a major risk factor for periodontal diseases. There is a clear relationship between degree of hyperglycaemia and severity of periodontitis, with diabetes increasing the risk of periodontitis and periodontal inflammation negatively affecting glycaemic control. Diabetes increases inflammation in the periodontal tissues [Prshaw P.M *et al* [10]]. Lactoferrin, an iron binding protein is upregulated in the salivary secretions in gingival inflammation and periodontal diseases. Therefore it is consistent that salivary iron levels are more than serum iron levels [Sindhu S *et al* [9]].

Considering the applications of saliva, as a highly effective diagnostic tool in systemic conditions, viral infections, screening and early diagnosis of malignancies, present study was aimed to evaluate the salivary iron levels in diabetic patients [Anuradha A *et al* [8]]. It showed a significant increase in mean salivary iron levels in diabetics when compared to control group. Statistically significant correlation between saliva and serum iron suggests that both can be used parallely with equivalent efficiency. Protein composition of serum and saliva are same. 27% of whole saliva proteins are found in serum but varies with molecular function and biological process. Thus making saliva a surrogate of blood in diagnostic application with added advantage of being non invasive, safe and easy method. Moreover saliva can be used for long term and frequent monitor of patient health status, without much anguish [Anuradha A *et al* [8]].

CONCLUSION

Saliva is a biological fluid that offers several opportunities in diagnosis. In our study it is proved that salivary iron levels and serum iron levels were significantly high in cases of diabetes mellitus. Salivary iron levels increased synchronously with serum levels in diabetics crafting saliva as a diagnostic & screening tool as an alternative to invasive blood investigations.

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