A study to evaluate the status of vitamin C and iron in saliva and serum of patients with and without periodontal diseases

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Abstract

Background: The aim of this study is to evaluate status of vitamin c and iron in saliva and serum in patients with and without periodontal diseases.

Method: A continence quota sample of 75 patients comprising of three groups based on full mouth periodontal examination was taken where group A served as control, group B with gingivitis and group C with periodontitis. Saliva and serum samples were collected from above subjects for lab investigation. Statistical analysis was made to compare the status of vitamin c and iron in saliva and serum sample.

Statstics: ANOVA and Tukey multiple comparisons test.

Results: The concentration of vitamin C and iron in both saliva and serum showed the gradual decline from control to gingivitis to periodontitis serving a potential diagnostic tool.

Conclusion: The study shows that there is strong correlation of vitamin c and iron in saliva and serum in predicting the development of periodontal disease.

Introduction

Periodontal disease is a type of disease that affects one or more of periodontal tissues which comprises of - alveolar bone, periodontal ligament, cementum, gingival. It is an infection-mediated destruction of tooth supporting structures. Periodontal inflammation leads to gingival bleeding, pocket formation, destruction of alveolar bone and eventually loss of teeth.(1) Gingivitis is chronic plaque deposits on the teeth. Actually plaque is a sticky material made of bacteria, mucus, and food debris that builds up on the exposed parts of the teeth. It is also a major cause of tooth decay.(2-4) Vitamin c has long been a candidate for modulating periodontal diseases although the exact role of vitamin C deficiency is not known.(5) Serum iron and total iron binding capacity and transferring saturation is best indicator of nutritional deficiency arising from chronic infection, inflammation or chronic neoplastic diseases.(6) From above studies it can be hypothesized that there is decrease in Vitamin C and Iron level during inflammation since periodontal disease is an inflammatory disease. Relatively few studies have been done on the role of Vitamin C and Iron on periodontal tissue.

In view of this, we have made an attempt to investigate the salivary and serum levels of vitamin C and iron in periodontal disease.

Methodology

Institutional ethics committee approval was obtained from IEC of A.B Shetty Memorial Institute of Dental Sciences before conducting the study and informed consent was obtained from each subject enrolled.

Source of Data: About 75 (30-50years) patients attending Department of Periodontics, A.B. Shetty Memorial Institute of Dental Sciences were enrolled in our study

Exclusive Criteria

- Patients suffering from conditions condition requiring antibiotics prophylaxis prior to dental procedure.
- Intake of iron and vitamin supplement
- Women taking contraceptives

Groups

The selected patients were divided into

Subjects	Numbers
Patients without periodontal diseases	25
Patients with chronic periodontitis	25
Patients with chronic gingivitis	25

Inclusive Criteria

- Patients with minimum of 20 permanent teeth.
- Patients with Gingival Index between 1-2 (Loe and Silness gingival index) Gingival index of the control group, less than 0.5 Patient with probing depth > 5mm.
- Patient should not have undergone any periodontal treatment for past 1 year.

Examinations: A full mouth periodontal examination was conducted in all subjects using William's graduated periodontal probe. The following variables are determined

1. Oral hygiene status
2. Probing depth / attachment level: The tip of the instrument is placed with light pressure of 10-20 grams into the gingival sulcus, which is an area of potential space between a tooth and the surrounding tissue. It is important to keep the periodontal probe parallel to the contours of the root of the tooth and to insert the probe down to the base of the pocket.
3. Gingival index (Loe and Silness): 16 12 24, 44 32 36
4. Radiographic assessment

Sample collections

Blood: 2ml of blood samples was collected by venepuncture under aseptic conditions in anticubital fossa. The blood was transferred into EDTA containing bulb. The hematological parameters are accessed in patients.

Saliva: Unstimulated whole saliva was collected from all the subjects by direct expectoration. The subject were asked to spit 3-4 ml saliva into a container and is sent to laboratory for the analysis.

Statistical Analysis

Observations are mean±SD. ANOVA followed by Tukey multiple test.

Results

Table 1: Levels of Iron in the Serum

Group	Mean	Subject deviation	p value
Group- 1 Control	64.17	5.045	<0.001
Group-2 Gingivtitis	55.76	3.81	
Group-3 Periodontitis	47.8	4.092	

Observations are mean±SD. ANOVA test. There is a difference in mean serum iron levels between the subjects at 5% level of significance, p< 0.001- which indicates significance.

Table 2: Levels of Iron in the Serum

Pair	Mean Difference	p Value
Group- 1 Control Vs. Gingivitis	8.41	<0.001
Group-2 Control Vs. Periodontitis	16.36	
Group-3 Gingivitis Vs. Periodontitis	7.95	

Tukey test. There is difference in mean between control and gingivitis, control and periodontitis. p< 0.001- which indicates significance.

Table 3: Levels of Iron in the Saliva

Group	Mean	Subject deviation	P value
Group- 1 Control	53.37	4.291	<0.001
Group-2 Gingivtitis	44.41	2.501	
Group-3 Periodontitis	38.81	3.94	

Observations are mean±SD. ANOVA test. There is a difference in mean saliva iron levels between the subjects at 5% level of significance, p< 0.001- which indicates significance.

Table 4: Levels of Iron in the Saliva

Pair	Mean Difference	p Value
Group- 1 Control Vs. Gingivitis	8.95	<0.001
Group-2 Control Vs. Periodontitis	14.58	
Group-3 Gingivitis Vs. Periodontitis	5.63	

Tukey test. There is difference in mean between control and gingivitis, control and periodontitis. p< 0.001- which indicates significance.

Table 5: Levels of Vitamin C in the Serum

Group	Mean	Subject deviation	P value
Group- 1 Control	0.61	0.127	<0.001
Group-2 Gingivtitis	0.488	0.054	
Group-3 Periodontitis	0.3556	0.061	

Observations are mean±SD. ANOVA test. There is a difference in mean saliva iron levels between the subjects at 5% level of significance, p< 0.001- which indicates significance.

Table 6: Levels of Vitamin C in the Serum

Pair	Mean Difference	p Value
Group- 1 Control Vs. Gingivitis	0.122	<0.001
Group-2 Control Vs. Periodontitis	0.255	
Group-3 Gingivitis Vs. Periodontitis	0.133	

Tukey test. There is difference in mean between control and gingivitis, control and periodontitis. p< 0.001- which indicates significance.

Table 7: Levels of Vitamin C in the Saliva

Group	Mean	Subject deviation	P value
Group- 1 Control	0.574	0.141	<0.001
Group-2 Gingivtitis	0.394	0.058	
Group-3 Periodontitis	0.2783	0.067	

Observations are mean±SD. ANOVA test. There is a difference in mean saliva iron levels between the subjects at 5% level of significance, p< 0.001- which indicates significance.

Table 8: Levels of Vitamin C in the Serum

Pair	Mean Difference	p Value
Group- 1 Control Vs. Gingivitis	0.018	<0.001
Group-2 Control Vs. Periodontitis	0.296	
Group-3 Gingivitis Vs. Periodontitis	0.1158	

Tukey test. There is difference in mean between control and gingivitis, control and periodontitis. p< 0.001- which indicates significance.

Discussion

In the present study we found strong association between concentration of two parameters of our study i.e. vitamin C and iron in saliva and serum. In this study we had to collect unstimulated saliva and blood sample through venepuncture for laboratory analysis of our parameters. The sample chosen for this study is good for finding association between the two parameters.

Both, Gingivitis and periodontal disease commonly affect most of the adult population, with the prevalence of severe disease increasing with age Periodontal disease is a group of chronic inflammatory diseases caused by specific anaerobic Gram-negative bacteria that activate immuno-inflammatory mechanisms within the local periodontal tissues, leading to the destruction of collagen and bone supporting the teeth. Periodontitis occurs at greatly different rates in different participants. The chronic forms of the disease are widespread among the population, whereas the aggressive, destructive form of the disease affects ~ 10% of population, resulting in serious tooth loss before old age.

Gingival diseases are a diverse family of complex and distinct pathological entities found within the area of gingiva that are the result of a variety of etiologies. There are several clinical characteristics common to all gingival diseases and these features include clinical signs and symptoms of inflammation that are confined to the gingiva, but with a reversibility of the disease process by removing the etiology.

Gingivitis is characterized by inflammation of gums caused by plaque deposits with possible bleeding when brushed or probed. Periodontitis can be identified by hardening of plaque to form calculus, causing recession. Advanced periodontitis is distinguished by excessive tissue loss of gingival and alveolar bone and pocket greater than 5.5mm depth. This condition leads to tooth exfoliation due to the destruction of the tooth connective ligament according to L.C. Chappel et al.

Lamount & Jenkinson stated that the aetiology of periodontal disease is believed to be an imbalance in the bacterial species that colonize the oral cavity and the host immunological response to their bacterial pathogens. When stimulated by bacterial pathogens, host cell release pro inflammatory cytokines as a part of the immune response. These include IL-la and 13, IL-8 and TMF a. These cytokines recruit PMN to the site of infection.

Grain et al stated that the PMN's plays a major role in the periodontal disease as they are predominant host immune response to oral bacterial infection. Upon stimulation by bacterial antigens, cytokines such as IL-8 promote the PMN to express adhesion molecule and move out of circulation to the site of infection. Studies done by Asman PMN in periodontal disease patients display an increased number, adhesion and oxidative activity.

PMN produce the reactive oxygen species (ROS) superoxide via the respiratory burst as a part of the host response to infection. It is suggested that the proliferation results in a high degree of ROS release, culminating heightened oxidative damage to gingival tissue, periodontal ligament and alveolar bone.

Ascorbic acid performs numerous physiological functions in the body which include the synthesis of collagen, carnitine, and neurotransmitters; the synthesis and catabolism of tyrosine; and the metabolism of microsome. During biosynthesis ascorbic acid, it acts as a reducing agent by donating electrons and thus prevents oxidation in order to keep iron and copper atoms in their reduced states.

Ascorbic acid is well known for its antioxidant activity, as it acts as a reducing agent and hence reverses oxidation process. When there are more free radicals (reactive oxygen species, ROS) in the human body than antioxidants, called oxidative stress, which has an impact on hypertension, chronic inflammatory diseases and diabetes. Due to the irreversible nature of the destruction of tooth-supporting tissues, an non treated periodontitis increases the risk of infectious, even after the correction of the vitamin C deficiency. Regeneration of collagen to maintain the integrity of the tooth attachment elements is especially important for periodontal health.

In the study done by Pirkko I Pussiman, has shown that periodontitis may be associated with vitamin deficiency. A hypothetical association between periodontitis and vitamin c is supported by observation that additional vitamin c is required during infectious disease due to increased oxidative stress.

In contrast to the above, in the third national health & nutritional examination survey comprising of 12419 adult subjects, only weak association was found between the periodontal diseases as by clinical examination and a low vitamin intake as assessed by dietary information.

The analysis of our data revealed significant reduction in vitamin C level in saliva and serum between 3 groups **(Table 5-8).** This might be because of increased level of oxidative stress which may be due to excessive production of reactive oxygen species which are involved in the pathogenesis of periodontitis and vitamin C being a radical scavenger utilised to reduce destructive effect of ROS.

Anemia of the disease is the second most prevalent form of anemia, it is cytokine mediated anemia characterised by hypoferriemia with adequate reticuloendothelial iron stores and normal to elevated ferittin concentration. The pathogenisis is reported to be dysregulation of iron homeostasis, depressed erythropoiesis and a blunted erythropoeitin response caused by elevated levels of systemically circulating pro inflammatory process.

Subgingival microbiodata in patients with periodontitis thus pose a significant, long standing, 'gram -ve' bacterial challenge, thus results in a low grade systemic inflammation. Elevated levels of various systemic markers of inflammation have been noted in moderate to severe periodontal disease.

A tendency toward anemia in periodontitis has been reported. 16 As early as 1945, it was reported that periodontal therapy resulted in the resolution of anemia. 16 Also it was found that 58% of female patients with periodontitis and 30% of male patients with periodontis had depressed Htc levels. Nevertheless, Wakai et al did not observe a relationship between increased community periodontal index of treatment needs scores and Hb level. Within the limits of the study, our results indicate possible effect of severe periodontal inflammation on the severityof anemia, and suggest that periodontal therapy might have potential in improving role in anemic staus in periodontally diseased individuals. The analysis of our data revealed a significant reduction in the iron in the serum and saliva between the 3 groups **(Table 1-4).** In an anemic status, a relative decrease in oxygen into the tissues has been suggested to act as modifying factor in response of the periodontium to local irritation. The proinflammatory cytokines up regulate the expression of protein divalent metal transporter-1 causing increased uptake of iron into activated macrophages and reticuloendothelial cells. These proinflammatory stimuli also induce the retention of iron in macrophages by regulating the expression of ferritin, a transmembrane exporter of iron, thus blocking the release of the iron from these cells.[7-18]

Our results are in agreement with the above studies stating that the level of vitamin c and iron is markedly decreased in gingival and periodontal destruction.

Conclusion

The result of this study highlights the possible clinical value of unstimulated whole saliva and serum as a valid and convenient diagnostic tool. This novel approach to throws the light on the potential of salivary and serum iron and vitamin C level may prove to be useful in identifying patients with chronic inflammatory periodontal diseases, and may provide additional advantage in elucidating the role of oxidative stress and cytokines in the pathogenesis of periodontal destruction . However, further studies on a lagerer scale should be performed to clarify the exact role of iron and vitamin c in periodontal disease.

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