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Research Article

Estimation of Trace Elements in Gingival Crevicular Fluid and Serum- Comparative Study in Healthy and Periodontitis

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ABSTRACT

Background: This study aims to determine and compare the levels of trace elements copper, zinc, selenium and chromium in GCF and serum of patients with periodontitis and healthy individuals. Methods: This cross sectional study includes 24 study subjects recruited from the patients reporting to the Department of Periodontics , Tagore Dental College Chennai. All the selected patients were subjected to a clinical examination done by a single examiner. The estimation of trace elements Copper, Zinc, Selenium and Chromium in GCF and serum is performed using Perkin Elmer optima 5300 Inductively Coupled Plasma Emission Spectrometer (ICPOES). Results: GCF and serum copper levels showed no significant difference in both periodontitis and healthy groups . Selenium levels tend to be the same in both groups. Serum zinc levels are more in periodontitis patients than healthy subjects ($p<0.01$). GCF chromium levels are found to be more in patients with periodontitis than healthy. Conclusions : More research is therefore needed to monitor the role of these trace elements C with an increased sample size to ascertain whether they are associated with a reduced risk of periodontitis.

Keywords Periodontitis, Trace elements, Health status.

INTRODUCTION

Periodontitis is an inflammatory disease of bacterial origin resulting in attachment loss and bone loss. Though the tissue destruction seen in the disease is mainly due to the interaction between the periodontopathic bacteria and host immune response,the nature and severity of the disease is also determined by other factors like hormonal changes, balance between antioxidant and oxidants mechanisms , aging, nutritional deficiencies and other systemic disorders[1,2].

The integrity of body tissue mainly depends on the adequate source of both macro and micro nutrients available to the host. Additionally they are also essential for the better functioning of the various biochemical processes operating within a human system[3]. A chronic deficiency of one or more nutrients may produce pathological alterations leading the tissue loss resistant to injuries.

Nutrients can be classified either as macro or micro depending on their requirements[4]. The micronutrients mainly include vitamins and minerals and adequate amount required are to be taken in diet for the performance of biochemical functions. Minerals like iron, zinc, copper,iodine, selenium, chromium etc have been found to be essential to perform various biological activities.

Several studies have shown that there is an alteration in the level of these nutrients in disease conditions like Diabetes Mellitus, rheumatoid arthritis, obesity, cardiovascular disease[5-7]. A study by Frithof L[8] has shown that decreased serum zinc levels might be related to increased alveolar bone resorption. Similarly a study by Enwonwu[9] has shown that malnutrition which usually involves concomitant deficiencies of macro and micro nutrients has the potential to adversely influence the prognosis of periodontal infections.

The role of selenium in the maintenance of oxidative stress was well proved and its role in periodontal disease was also established[10]. Predominantly all these minerals were assessed in the serum. Gingival crevicular fluid though an ultra filtrate of serum is more reliable than serum itself as it depicts the pathogenic changes that occur in the periodontal micro environment. Unfortunately, studies showing the levels of minerals in periodontitis subjects using GCF as a marker is scarce. Hence the main aim and objective of this study is to determine and compare the levels of trace elements mainly, copper, zinc, selenium and chromium in the GCF and serum of patients with periodontitis and healthy individuals.

MATERIALS AND METHODS

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This cross-sectional study was conducted from March 2013 to May 2014. 24 study subjects were recruited from the patients reporting to the Outpatient department of Periodontics, Tagore Dental College, Chennai. The subjects were allowed to participate in the study if they have at least 20 teeth, excluding third molars. Both the sexes were allowed to participate in this study. Patients with history of any systemic disease,subjects with history of intake of anti-inflammatory, antibiotics, antioxidants or multi-vitamin supplements in the previous six months, smokers, pregnant and lactating females were excluded.

The study was performed according to the Declaration of Helsinki, as revised in 2000, and was approved by the Institutional Ethical committee (IEC), Tagore Dental College. Informed consent was obtained from the participants who volunteered to participate in the study after explaining to them the study protocol in their regional language.

Clinical examination

All the selected subjects were subjected to a clinical examination done by a single examiner[11]. Each subject underwent a full-mouth periodontal examination including the calculation of probing depth (PD), clinical attachment level (CAL) with a University of North Carolina (UNC)-15 periodontal probe (Hu-Friedy, Inc. Chicago, Illinois (IL), USA). The oral hygiene status and gingival health were assessed by taking Oral hygiene index[12] –simplified, gingivitis index and plaque index (TureskyGillmore Glickman modification of Quigley-Hein plaque index)[13-15.] Radiographic bone loss was recorded from intra oral periapical radiographs by long cone technique to differentiate chronic periodontitis group from the healthy individuals. The diagnosis of chronic periodontitis was made as per the criteria bySwatiPradeep Patel 2012 et al.[16] The participants were then categorized into two groups; group I (healthy) consisted of 12 subjects with clinically healthy periodontium, with PD< 3mm and no evidence of attachment loss and group II (chronic periodontitis) consisted of 12 subjects who had signs of clinical inflammation, presence of PD>4mm in 30% of sites, clinical attachment loss> 1mm in 30% of sites and radiographic evidence of bone loss.

Selection sites for collection of GCF

Samples were collected from only one site in each subject. GCF was pooled from one or more than two sites without any inflammatory signs in healthy participants to get an adequate volume.In periodontitis patients, sites were identified using a University of North Carolina (UNC)-15 periodontal, probe (Hu-Friedy, Inc. Chicago, Illinois (IL), USA) and the site showing highest clinical signs of inflammation and highest CAL along with radiographic confirmation of bone loss was selected for sampling. Supragingival plaque was removed with sterile curetteswithout touching the marginal gingiva. The area was isolated with using cotton to avoid the contamination of salivaand the GCF was collected by placing the micro capillary pipettes at the gingival sulcus, gently touchingthe marginal gingiva.A standardized 5 µl volume of GCFwas collected from each site using 1-5 µlcalibratedvolumetric microcapillarypipettes (Sigma – Aldrich, St.Louis , MO, USA). The sites from which there were no possibility of GCF sample collection within the same time in healthy group were excluded from the study. The contaminated micropipettes with blood and saliva were also not used in this study.The GCF collected was immediately transferred to Eppenndorf plastic vials and stored at -20c till further analysis.

Collection of Serum

Venous blood samples (2 ml) were collected from the antecubital fossa by venipuncture using 20 gauge needle with 2ml syringes without EDTA and allowed to clot at room temperature. The samples were then centrifuged after one hour; the serum obtained was transferred to plastic vials and stored at -20°c until further analysis of trace elements Copper, Zinc Selenium & Chromium.

Biochemical Analysis

The estimation of trace elements Copper, Zinc, Selenium & Chromium in GCF& serum was performed at Sophisticated Analytical Instrument Facility (SAIF) IIT Madras, by using Perkin Elmer optima 5300 DualViewing (DV) Inductively Coupled Plasma Optical Emission Spectrometer(ICPOES).

ICPOES

In inductively coupled plasma optical emission spectrometry the sample is usually transported into the instrument as a stream of liquid sample. The liquid is then converted into an aerosol through a process known as nebulisation. The sample aerosol is then transported to the plasma where it is desolvated, vaporized, atomized and excited and /or ionized by the plasma. The excited atoms and ions emit their characteristic radiation which is collected by a device that sorts the radiation by wavelength. The radiation is detected and turned into electronic signals that are converted into the concentration information.

Dual viewing (DV) indicates lowest detection limits with widest dynamic range. The Optima 5300 DV series offers the lowest detection limits and the greatest concentration in a single system. It can determine ultra trace and percentage concentrations levels in samples in the same run automatically, without the time-consuming search for alternate wavelengths[17,18]. Hence simultaneous detection of more than an one element is possible.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Wilcoxon Signed Rank test was used for comparison within groups and Mann Whitney U test between the groups. A probability value (p value) less than 0.05 is considered statistically significant.

RESULTS

This study comprised of 24 subjects divided into two groups with twelve each:those with healthy periodontium and those with chronic periodontitis .The distribution of baseline characteristics among healthy and periodontitis are shown in Table 1.The crevicular fluid copper levels are found to be lower than serum copper in both the groups and this is statistically nonsignificant(Table 2&3). Intra group comparison shows that copper levels are

Table 1: Distribution of Baseline characteristics among Healthy and Periodontitis.

	Healthy	Periodontitis
	Mean (SD)	Mean (SD)
Age	25.8(6.54)	43.69(12.92)
Gender		
Females	3.00	3.00
Males	9.00	9.00
Ohi(s)ndex	0.33(0.30)	1.20(0.73)
Plaque index	0.22(0.13)	1.18(0.56)
Probing depth	1.82((0.17)	3.59(0.22)
Gingivitis	0.14(0.24)	1.32(0.81)

Table 2: Mean Distribution of Trace Elements in GCF of Healthy and Periodontitis.

Trace elements	Healthy	Periodontitis	P' value
µgm/ml	Mean(SD)	Mean(SD)	
Copper(cu)	0.05(0.05)	0.10(0.09)	0.09
Zinc (zn)	0.07(0.07)	0.56(1.13)	0.01
Selenium (se)	0.03(0.04)	0.03(0.03)	1.00
Chromium (cr)	0.32(0.54)	0.34(0.57)	0.68

Table 3: Mean Distribution of Trace Elements in Serum of Healthy & Periodontitis

Trace elements	Healthy	Periodontitis	P value
µgm/ml	Mean(SD)	Mean(SD)	
copper(cu)	0.73(0.33)	0.83(0.40)	0.62
zinc (zn)	0.35(0.16)	0.47(0.22)	0.16
selenium (se)	0.48(0.32)	0.37(0.21)	0.48
chromium (cr)	0.12(0.32)	0.17(0.33)	0.70

Table 4: Comparison of mean Trace elements in GCF&Serum of Healthy Subjects.

Trace Elements	GCF	Serum	p value
µgm/ml	Mean(SD)	Mean(SD)	
copper(cu)	0.05(0.05)0	0.73(0.33)	0.00
zinc (zn)	0.07(0.07)	0.35(0.16)	0.00
selenium (se)	0.03(0.04)	0.48(0.32)	0.00
chromium (cr)	0.32(0.54)	0.12(0.32)	0.21

found to be higher in serum of both healthy and those with periodontitis, which is statistically significant(p>0.00)(Table 4&5).

Inter group comparison shows, crevicular fluid and serum zinc levels are more in patients with periodontitis than the healthy controls(Table 2&3). Intra group comparison shows serum zinc levels are more in patients with periodontitis, statistically non significant(Table 5).

Selenium levels in the crevicularfluid are same in both groups (Table 2). Serum selenium levels are more in patients with healthy periodontium, though statistically nonsignificant (Table 3). When the crevicular fluid selenium levels are compared with the serum selenium levels, amongst the groups , the serum levels are more than the crevicular fluid selenium levels (p>0.00)(Table 4&5).

The values of chromium in the crevicular fluid and serum of patients with periodontal health and disease follows a different trend than seen with the other metals . Inter group comparison reveals a statistically nonsignificant difference exist between the two groups in both crevicular fluid and serum samples(Table 2&3) ; whereas intra group comparison shows an increase in the level of chromium in the crevicular fluid than the serum in patients with periodontal health and disease than the healthy(p<0.02)(Table 4 &5) . This shows a marked difference than the other metals studied.

DISCUSSION

Thenutrients required to carry out the normal day to day metabolic activities in our body can be classified as macro and micronutrients, based on their requirement by our body; where as carbohydrates, lipids, fats, proteins come under macronutrients[4] (needed in relatively large amounts), vitamins, trace elements, polyunsaturated fatty acids are classified as micronutrients (required in small quantities as micrograms or milligrams per day). All these components are required for optimal health, proper growth and other physiologic activities.

The involvement of trace elements in the pathogenesis and progression of periodontitis was fairly reported in the literature. A study by Biju Thomas 2010[19] showed that the serum levels of zinc was decreased and copper was increased in diabetic patients with periodontitis compared to healthy individuals. A study by Huda Shakir Ahmed in 2014[20] has shown that the serum levels of copper and zinc was significantly decreased in diabetic patients with periodontitis disease than in the healthy controls. Similarly Panjamurthy et al[10] (2005) demonstrated that periodontitis patients had reduced levels of plasma glutathione peroxidase along with lowered vitamin C and vitamin E compared with healthy controls. All these studies, though with contradictory results indicates that an imbalance of these levels of micronutrients occurs in periodontal disease. However, the exact role of these trace elements in the pathology and pathogenesis remains unknown.

Trace elements are indispensable for the functioning of many physiologic and biochemical reactions. Many of them are metals and their exact role in the pathophysiology of periodontal disease are less evident and debatable. However their role in the maintenance of metabolic homeostasis especially that of glucose, oxidative stress, effective functioning of enzymes and proper functions of immune system were clear and proved[10,21,22,23].

In this study, we tried to compare the levels of Copper(Cu), Zinc (Zn), Selenium (Se) and Chromium(Cr) in the gingival crevicular fluid and serum samples of chronic periodontitis with those having healthy periodontitis.

Copper status is evaluated usually by assessing the levels of plasma copper and caeruloplasmin. The synthesis of caeruloplasmin, inturn depends on the adequate supply of

Table 5: Comparison of mean Trace elements in the GCF& Serum of Periodontitis.

Trace elements	GCF	Serum	P value
µgm/ml	Mean(SD)	Mean(SD)	
copper(cu)	0.10(0.09)	0.83(0.40)	0.00
zinc (zn)	0.56(1.13)	0.47(0.22)	0.25
selenium (se)	0.03(0.03)	0.37(0.21)	0.00
chromium (cr)	0.34(0.57)	0.17(0.33)	0.02

copper and the level, of circulating copper is dependent on that caeruloplasmin[4]. The levels of copper in gingival crevicular fluid is more in patients with periodontitis than the healthy controls, though the results are not statistically significant. This study results are similar to a study done by Freeland etal[24] where an increased level of plasma copper levels are shown in patients with periodontal disease. Also a study by Manea and Nechifor (2014)[25] has shown that there exists a connection between salivary copper levels and periodontitis.

A study by Martin et al(2010)[26] has shown that cementum of periodontitis-affected teeth has increased level of copper and zinc. Thesefindings suggests a possible link between the copper levels and periodontitis.

It is to be noted that these studies has used serum and saliva to assess the copper status respectively whereas gingival crevicular fluid was used by us in this study. Since the periodontal microenvironment can be better assessed by GCF than plasma metal levels our study results depict the trace element imbalance that can occur in periodontal disease.

When the serum copper levels are compared between healthy and periodontitis subjects, though statistically significant increased serum copper levels are found in patients with chronic periodontitis. Our findings are supported by the study done by Freeland[24] et al.This hypercupremic state has been reported to be present in other chronic inflammatory diseases like rheumatoid arthritis and diabetes mellitus[27]. Copper levels reduce several aspects of immune response including neutrophil numbers, lymphocyte proliferation and antigen specific antibody production all interfering with the host defense against pathogenic organisms (28&29). Hence this hypercupremic state might alter the response of periodontitis to microbes. However it is to be determined whether periodontitis increases the plasma copper levels or hypercupremic state is a risk factor for periodontitis as seen in other chronic inflammatory diseasesas mentioned. Further studies with increased sample size will be done in the future to get a clarity in this regard.

Zinc is a divalent cation and plays an important role in the functioning of hundreds of enzymes[30] in insulin metabolism and acts as an efficient antioxidant[31,32].

The relation between the plasma zinc levels and periodontitis is contradictory. Studies by Frithof et al[8,33]. Biju et al (2013) show reduced serum zinc levels in periodontitis subjects than in healthy controls, whereas study by Martin et al[26] has shown an increased zinc levels in the cementum of periodontitis affected teeth than the healthy controls. This is also supported by animal[34] and human studies (Pushparani 2014)[35]. It should be remembered here that we have used gingival crevicular fluid along with the serum, contrasting with the other studies which have analysed only the serum or other structures like cementum. Our study results show that statistically significant increase in the level of zinc is found in the crevicular fluid of patients with periodontitis. Though zinc is usually considered to be an antioxidant[36], increased serum concentration paradoxically can have pro oxidant activities[37]. An excess zinc is well known to inhibit various zinc dependent enzymes such as copper – zinc superoxide dismutase or thioredoxinreductase, and enzymes of DNA repair like glycosylases or endonucleases[38]. Also, it enhances production of reactive oxygen species. Additionally higher zinc levels can also favourapoptosis by the degradation of the antiapoptotic protein Bcl-2/ Bax[39]. Hence a combination of enhancing the production of reactive oxygen species, inhibition of zinc dependent antioxidant enzymes and inhibition of apoptosis aids in the progression of periodontal disease. Thus, within the limitations of our study, it can be concluded that periodontitis patients has increased zinc levels than the healthy controls.

Selenium acts as an antioxidant in the form of selenoprotein containing selenocysteins[40]. It is relatively well absorbed from the diet, better if it is in an organic form[41]. Selenium is involved in the complex system of defence against oxidative stress through selenium dependent antioxidant enzymes like glutathione peroxidase and thioredoxin peroxidase.

Various studies have estimated the level of glutathione peroxidase in periodontitis. A study by Biju et al (2013)[42] has shown that levels of glutathione peroxidase is more in periodontal health than disease . This was also confirmed in the gingival crevicular fluid in a study by Pradeep et al[16]. The former study also has estimated the serum selenium level and it showed no significant difference exists between health and disease. In our study, plasma selenium level is more in healthy controls than in patients with periodontal disease, though statistically nonsignificant. This confirms with the common view that the increased oxidative stress seen in periodontal disease utilizes selenium and the selenium dependent enzymes leading to reduced serum selenium levels. This is also supported by the fact that the concentration of selenium is more in the serum than in the crevicular fluid of subjects with both periodontal health and disease. All these studies have measured only the plasma selenium levels and this is the first study to estimate the selenium levels in the crevicular fluid. Our study does not identify any difference in the crevicular fluid selenium levels between healthy controls and periodontitis patients. The less sample size in our study might account for this finding and future studies with increased sample size might give a clear status.

Chromium is the most abundant metal in the crust of the earth and can exist in a divalent, trivalent and hexavalent elemental forms. The importance of chromium for glucose metabolic regulation has been seen in clinical states of relatively severe chromium deficiency, characterized by impaired glucose tolerance, fasting

hyperglycemia and eventually lipid disorders[43,44]. Chromium also decreaseoxidative stress, glycosylation and lipid peroxidation in erythrocytes and monocytes under hyper glycemic states[21].

Our study results showed the crevicular fluid of chromium is more in periodontitis subjects than in healthy controls in both the body fluids studied though the difference is very less in magnitude and also statistically nonsignificant. Interestingly, this is the only metal whose levels are more in crevicular fluid than the serum in both the groups (excluding zinc which is more in the serum of only in patients with periodontitis). This raises the possibility that gingiva can be the local reservoir for chromium. This hypothesis is stated due to the fact that chromium has a tendency to accumulate in the body tissues spleen, liver and kidney has been found to concentrate chromium[45,46]. The importance of gingiva acting as a reservoir for chromium is thought to be ascertained by future studies.

The gingiva being proposed as a reservoir also has the following reason, chromium increases the glucose transport by enhancing the activity of hormone sensitive GLUT-4 transporters. GLUT -4 is aninsulin sensitive that is primarily exposed in the adipose tissue, skeletal muscles. As postprandial glucose levels rise, the subsequent increase in the circulating insulin activate the intracellular signaling cascades that culminate in GLUT -4 translocation to the cell membrane[20]. It is now proved that gingiva expresses GLUT 4 and the relation between chromium and GLUT 4 was also explained.The above fact might be the reason to find increased chromium levels in the gingival crevicular fluid in both the healthy controls and periodontitis subjects.

Since our study is the first study to assess the levels of chromium in the gingival crevicular fluid in healthy controls and periodontitis subjects and no other studies to compare with our hypothesis must be proved by future studies.

ONE SENTENCE SUMMARY

Chromium levels are found to be increased in GCF of both the healthy controls and periodontitis subjects when compared to copper, zinc and selenium.

CONFLICT OF INTEREST

Nil

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