

Estimation of Salivary and Serum Ceruloplasmin Levels in Subjects with and Without Chronic Periodontitis - A Comparative Biochemical Study

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Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Introduction

Periodontitis is a group of inflammatory diseases characterized by loss of connective tissue attachment and alveolar bone destruction. The initiation and the progression of periodontitis are dependent on the presence of complex sub gingival microbial communities. Although the bacteria are the etiological agents in periodontitis, the ecological interactions between the host and the pathogens determine the disease progression.¹⁻³ If left untreated, the disease progresses with the loss of collagen fibers, apical migration of the junctional epithelium, resulting in alveolar bone destruction, eventually leading to tooth mobility and finally loss of teeth.⁴

Diagnosis of periodontal diseases at an early stage reduces the morbidity rate of the teeth. However diagnosis of the periodontal diseases and identifying the patients who are at risk always possess a challenge to the clinicians. Conventional periodontal diagnostic parameters used clinically include probing pocket depths, bleeding on probing, clinical attachment levels, plaque index, and radiographs assessing alveolar bone level.⁵ The advantages of these conventional diagnostic tools are their ease of use,

their cost-effectiveness, and they are relatively non-invasive. Traditional diagnostic procedures have its own limitations of assessing only past tissue destructions and not the current disease status.⁶

Advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers.⁷ A biomarker is a substance measured objectively and evaluated as an indicator of normal biologic processes, pathological processes, or pharmacological responses to a therapeutic intervention. Biological media like Saliva, Serum and Gingival Crevicular Fluid are used to determine biomarkers in periodontal diseases. It has been a feature of periodontal diseases that it creates an area of local inflammation as well as systemic inflammation, which are indicated by elevated serum levels of various pro-inflammatory markers such as alkaline phosphatase, tumor necrosis factor α , interleukin-1, C-reactive protein, vascular endothelial growth factor,⁸⁻¹⁰ and reduced levels of various anti-inflammatory markers especially the interleukin-10.¹¹ One such pro-inflammatory marker for periodontal disease recognized in recent times is the Ceruloplasmin.

Ceruloplasmin was first described in 1948 by Holmberg and Laurell as the blue plasma protein.¹² This α -2 glycoprotein (mw,130kd) is the main copper metal binding protein in blood, binding upwards of 90% of total circulating copper.¹³ Elevated levels of Ceruloplasmin are associated with increased serum and synovial fluid copper and have been associated with inflammatory disease.¹⁴

The normal serum Ceruloplasmin levels ranges from 150 to 300, μ g/ml and can increase to as much as 900, μ g/ml during inflammation.^{15,16} The corresponding elevation in copper levels observed was believed to play a protective role, a view supported by an enhanced inflammatory response in copper-deficient rats.^{17,18} This protective role was assumed to be due to the ability of copper to affect prostaglandin production.¹⁹ Ceruloplasmin itself was recently described as an anti-inflammatory agent due to its ability to act as a scavenger of superoxide anion radicals, (O_2^-)^{20,21} which can be generated by PMNs during inflammation and lead to tissue damage.²²

A Study conducted by Iwata et al proved that Ceruloplasmin levels were increased in cases of localized aggressive periodontitis and caused priming of the neutrophils in localized aggressive periodontitis.²³ It was also observed that as the clinical attachment loss increased corresponding to the percentage of bleeding sites, the serum level of Ceruloplasmin showed higher values.²⁴

With this background this study aimed

1. To estimate and compare Salivary Ceruloplasmin levels in Subjects with Chronic Periodontitis and without Chronic Periodontitis.
2. To estimate and compare Serum Ceruloplasmin levels in Subjects with Chronic Periodontitis and without Chronic Periodontitis.

Methodology

Patients visiting the Department of Periodontics, AB Shetty Memorial Institute of Dental Sciences, NITTE

University, Deralakatte, Mangalore were included in the study after obtaining voluntary written consent.

Inclusion Criteria

1. Both male and female subjects aged 18-50 years.
2. Systemically healthy subjects.
3. Subjects who are not on long term medications.

Exclusion Criteria

1. Presence of systemic diseases.
2. A recent history or the presence of any other acute or chronic infection.
3. Pregnant or Lactating women.
4. Patients with history of any antibiotics therapy 3 months prior to study enrolled or any other regular medication.
5. Patients who has undergone periodontal therapy for last Six months.
6. Subjects with smoking or tobacco chewing habits.

Detailed case history was recorded along with thorough examination of the oral cavity. Individuals will be screened for Periodontal status by using following parameters :

1. Plaque Index by Sillness and Loe (1964).
2. Gingival Index by Loe and Sillness (1963).
3. Mean Probing Pocket depth.
4. Mean Clinical Attachment Level.

After screening 50 subjects were selected for the study and segregated into test group and control group consisting 25 patients in each.

Criteria for test group and control group were as follows:

Test group – Generalized Chronic Periodontitis.

- With 10% or greater number of sites with probing depth \geq 5mm.
- 30% of sites with clinical attachment loss of 5mm or more.

Control group – Periodontally Healthy Individuals.

- With Probing depth < 3mm.

- With no Clinical Attachment Loss.

Saliva and Venous blood samples were collected from both the groups for the estimation of Ceruloplasmin levels.

Collection of blood sample for estimation of Ceruloplasmin levels

Under aseptic measures, venous blood samples were drawn by venipuncture in antecubital fossa using 5ml syringe and collected in a plain vacuum tubes and transported to clinical laboratory.

Collection of Saliva sample by passive drool method for estimation of Ceruloplasmin levels

Ceruloplasmin levels were estimated by the diamine oxidase method based on the property of ceruloplasmin to catalyze an oxidative reaction and form a blue-violet complex which can be estimated at 532 nm spectrophotometrically.²⁵

Ethical clearance

Ethical clearance was obtained from the institutional ethical committee.

Statistical analysis

Descriptive statistical analysis has been carried out in the present study. Student “t” test was performed to find the significance of study parameters on categorical scale between two groups. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%).

Results

Total 50 patients aged between 18-50 years were examined. It was found that in both control group and test group with 25 subjects in each 30 (60%) were females and 20 (40 %) were males. Clinical parameters like PI, GI, PPD, CAL were recorded and segregated into test and control group. Biochemical parameters like Salivary Ceruloplasmin and Serum Ceruloplasmin levels were estimated between control and test group.

Table 1: Clinical parameters of the subjects (Mean ± SD).

	Control group (25)	Test group (25)	p value
GI	0.23±0.22	1.1±0.4	< 0.001**
PI	0.1±0.3	1.43±0.4	0.020*
PPD(mm)	1.12±0.4	2.7±0.9	< 0.001**
CAL(mm)	0.000	1.74±1.2	< 0.001**

Table no1 shows the mean Gingival (GI), Plaque Index, Probing Pocket Depth (PPD), Clinical Attachment Level (CAL) of control group and test group.

Mean Gingival Index (GI) in the test group was 1.1±0.4 which was highly significant with the p value < 0.001 when compared with control group in which mean GI was 0.23 ±0.22.

Results showed that mean Plaque index (PI) in the test group was 1.43±0.4 which was moderately significant with the p value 0.020 when compared with control group in which mean PI was 0.1±0.3.

The mean PPD in the test group is 2.7±0.9mm which was highly significant with the p value < 0.001 when compared to the control group in which the mean PPD score is 1.12±0.4mm.

The mean CAL in the test group is 1.74±1.3mm which is statistically highly significant with the p value < 0.001 when compared to the control group showing mean CAL score 0.

Table 2: Comparison of Mean values of Serum Ceruloplasmin in control and test groups.

Serum Ceruloplasmin Levels	Control group	Test group	p value
Mean	24.2236	31.9560	< 0.001**

Table 2 shows the comparison of Serum Ceruloplasmin levels between both the groups. The mean value in the test group was 31.9560 which were highly significant with the

p value of < 0.001 when compared with control group showing mean value of 24.2236.

Table 3: Comparison of Mean values of Saliva Ceruloplasmin in control and test groups.

Saliva Ceruloplasmin Levels	Control group	Test group	p value
Mean	15.2504	18.6720	< 0.001**

Table 3 shows the comparison of Saliva Ceruloplasmin levels between both the groups. The mean value in the test group was 18.6720 which was highly significant with the p value of < 0.001 when compared with control group showing mean value of 15.2504.

Discussion

The identification of susceptible individuals or sites at risk from disease and the diagnosis of active phases of periodontal disease represent a challenge for both clinicians and oral health researchers.²⁶

It has long been established that simple and noninvasive diagnostic tools that allows rapid screening, provides accurate predictive information and enables reliable evaluation of periodontal disease status would be of great value to both dentists and patients.²⁷

Many studies have shown that the determination of inflammatory mediator levels in biologic fluids is a good indicator of inflammatory activity. Potential diagnostic biomarkers for diagnosis of periodontal disease is the latest modality and mainly includes locally produced proteins of host and bacterial origin such as enzymes, immunoglobulins, cytokines and also includes genetic/genomic biomarkers such as deoxyribonucleic acid and messenger ribonucleic acid of host origin, bacteria and bacterial products, ions, steroid hormones and volatile compounds. One such potential marker recognized is the ceruloplasmin.

Ceruloplasmin is a ferroxidase enzyme. The normal Ceruloplasmin levels are 25-35mg/dl. The functions of ceruloplasmin are slightly confusing. Ceruloplasmin functions an anti-inflammatory agent and it can also work as a pro-inflammatory molecule.^{28,29} **Broadley C et al 1989, Lee KH 2007**

It plays a role as an anti-inflammatory agent, thus reducing the oxidative stress by its ability to scavenge superoxide anion radicals.⁸

Ceruloplasmin also acts as a downstream target for hypoxia inducible factor (HIF- 1α) which is created in an area of local inflammation during the infections. It is also seen to play a central role in excessive superoxide generation in phenotypically hyperactive and primed peripheral blood polymorphonuclear neutrophils (PMNs).²³

So, it is postulated that there is an increase in the level of ceruloplasmin level during these infections. Based on above reviews linking this enzyme with inflammatory processes within the body, the aim of this study was to evaluate the levels of Ceruloplasmin in both serum and saliva as diagnostic markers for patients with Chronic Periodontitis.

Study by **Iwata et al**²³ has already proved that ceruloplasmin causes priming of the neutrophils in localized aggressive periodontitis. Oxygen tension is generally lower in inflamed tissues. Local hypoxia as a result of this severe inflammation results in increased activation of HIF-1α. The outcome of this is that ceruloplasmin mediates iron ion conversion from ferrous to ferric form and hence down regulates this HIF-1α.³⁰

In the present study mean Serum Ceruloplasmin levels were high in subjects with Chronic Periodontitis than control group. The statistical results were highly significant.

This was in accordance with study conducted by **Harshavardhana et al** wherein Ceruloplasmin content is drastically increased in Aggressive Periodontitis and Chronic Periodontitis subjects when compared to subjects without Periodontitis.²⁴

It was also postulated that even the periopathogenic bacteria of Chronic Periodontitis can also lead to an increase in the activity of Ceruloplasmin in absence of any other disease leading to systemic infection.²⁴

Harshavardhana et al

The potential pathogens acquire the iron necessary for growth through mechanisms, which are extremely complex. It is significant that in spite of bacterial iron acquiring mechanisms, the natural resistance to infection operates effectively in the normal low iron environment.³¹

Bullen JJ et al, 2005

Only when iron is freely available these protective mechanisms are reduced. The factors contributing for the natural resistance to infection are ample, but it is now clear that these protective systems can only function successfully in an environment where the normal concentration of free ionic iron is about 10^{-18} M which can be regarded as virtually zero.³² **Bullen JJ et al 2006**

This low iron environment is due to the iron binding protein transferrin, which is normally only 30- 40% saturated with iron and its presence is regulated by ceruloplasmin. The ability of freely available iron to diminish or destroy normal resistance and increase bacterial virulence has been demonstrated repeatedly in experimental infections involving many different bacterial species.³²

As the disease severity increases, it is natural for any proinflammatory marker to increase.

This salient feature was noted in the study conducted by **Harshavardhana et al 2013** wherein the level of Ceruloplasmin was more in periodontal disease with

higher CAL. It was also observed that as the CAL increased corresponding to the percentage of bleeding sites, the serum level of ceruloplasmin showed higher values.²⁴

In contrast, Ceruloplasmin levels in GCF and gingival tissues showed no difference between the healthy subjects and Periodontitis subjects.³³ In the present study, Saliva Ceruloplasmin levels in the test group was increased with highly significant p value of < 0.001 when compared to the control group.

To our knowledge no report is available in the literatures comparing the level of salivary ceruloplasmin in generalized chronic periodontitis patients and healthy individuals. Hence, comparing the results with any previous study is not possible.

Due to the noninvasive and simple nature of their collection, analysis of saliva may be especially beneficial in the determination of current periodontal status and a means of monitoring response to treatment.³⁴ Therefore, studies related to the pathogenesis of periodontal diseases usually examine whether biochemical and immunologic markers in saliva might reflect the extent of periodontal destruction and possibly predict future disease progression.

Conclusion

Biomarkers have come a long way as indicators of periodontal disease. These indicators should not only depict the presence or absence of a disease but should also show some light on the severity of the disease. The findings of this study can guide us in using the ceruloplasmin as one such biomarker. Increased tissue damage in the locally inflamed tissues of the periodontium in periodontitis patients and increased host resistance in periodontitis are very much attributable to Ceruloplasmin. A newer epitope can be used just to detect

even a milder increase in the Ceruloplasmin level just to diagnose periodontitis in its early stages.

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