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Comparative Evaluation of Serum Ceruloplasmin in Chronic and Aggressive Periodontitis Patients: A Cross-Sectional Study

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Abstract
Background: Pro-inflammatory markers have come a long way as indicators of periodontal disease. One such marker which can be detected in the serum is the ceruloplasmin. The aim of this study was to evaluate the serum levels of cerruloplasmin in both aggressive and chronic periodontitis patients.
*Materials and Methods: Blood samples were collected from aggressive periodontitis patients(*n = *20), chronic periodontitis patients(*n = *20) and periodontally healthy patients (*n = *20). The serum was extracted from all the blood samples and ceruloplasmin levels were spectroscopically evaluated.*
*Results: Periodontally healthy patients did not show increase in the levels of serum ceruloplasmin. Serum ceruloplasmin levels were found to be significantly higher in aggressive periodontitis patients (*P > *0.05) than in chronic periodontitis patients (*P > *0.05) even though increase in the level of ceruloplasmin was found in chronic periodontitis. The levels of serum ceruloplasmin also increased with the disease severity.*
Conclusion: Serum ceruloplasmin levels increased in both aggressive and chronic periodontitis patients, but more in aggressive periodontitis patients. Thus it can be used as a potential marker for the diagnosis of periodontitis.
Keywords: *Ceruloplasmin,hypoferremia,hypoxia,periodontitis.*

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I. Introduction

Periodontitis is a multifactorial inflammatory disease which generally affects the connective tissue attachment and supporting bone present around the teeth. It is generally caused by interactions between periodontal microflora and host response.[1] The two common types of periodontitis are the chronic periodontitis and aggressive periodontitis. Chronic periodontitis is more common in adults whereas aggressive periodontitis most commonly affects young individuals. Aggressive periodontitis is a rapidly progressing disease that affects otherwise healthy individuals. The main characteristic of this disease is that it is episodic and the destruction of periodontal tissues is very rapid, resulting in early tooth loss.[2] Moreover, aggressive periodontitis is seen to be faster in progression than the chronic periodontitis, even in the presence of minute amount of microbial deposits.

Currently, periodontitis is diagnosed almost entirely on the basis of an array of clinical measurements including clinical attachment level (CAL), bleeding on probing (BOP), probing depth (PD), and radiographic findings .[3] Additional information obtained by medical and family history, and specific characteristics of clinical presentation, such as quantity of local factors and location of lesions, are helpful in the differential diagnosis of specific types of periodontitis.[4] These clinical parameters are the best currently available indicators for determining disease status; however, they only provide information about past periodontal tissue destruction and do not elucidate current disease activity nor predict future activity due to low sensitivity and positive predictive value .[5]

Therefore, one of the major challenges in the field of periodontics is to identify a quick, efficient, and objective diagnostic and monitoring method, with the ability to screen for susceptibility to periodontal disease, diagnose periodontal disease, evaluate response to treatment, predict future tissue destruction, and identify disease progression. Therefore, advances in oral and periodontal disease diagnostic research are moving toward methods, whereby, periodontal risk can be identified and quantified by objective measures like biomarkers.[6] One of these biomarkers that can be detected in several inflammatory conditions is ceruloplasmin (CP).[7]

Ceruloplasmin a 122-kD multi-copper binding plasma protein containing ferroxidase activity necessary for ferric ion saturation of transferrin. Ceruloplasmin helps in transferring of copper within our body and also influences the uptake of iron into the cells because of its property of conversion of ferrous form of iron to the ferric form, due to which alterations in serum iron are often accompanied by changes in serum ceruloplasmin.[8,9] It thus leads to a state of hypoferremia. Ceruloplasmin is also an acute phase reactant seen to increase in inflammatory conditions.[9,10]

Thus the present study was conducted to evaluate the serum levels of cerruloplasmin in both aggressive and chronic periodontitis patients.

II. Materials and methods

The present study was conducted in the department of Periodontology, Indira Gandhi Govt. Dental college, Jammu. A total number of 60 subjects were included in the study between 16-60 years of age(Male - 35 and Female - 25) . These were divided into three groups wherein Group A constituted 20 subjects with untreated generalized aggressive periodontitis (Age 16-30 years, Male - 14 and Female - 6), Group B constituted 20 subjects (Age 35-60 years, Male - 12 and Female - 8) with untreated generalized chronic periodontitis and Group C constituted 20 subjects (Age 24-50 years, Male - 9 and Female -11) who were periodontal healthy. Individuals who had taken antibiotics in the previous 3 months, those having any systemic condition, smokers,pregnant or lactating women were excluded. Ethical clearance was obtained from the ethical committee of the institution. All subjects were verbally informed and written informed consent was obtained for their participation in the study.

Clinical measurements were taken at 6 sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual/mesiopalatal, lingual/palatal and distolingual/distopalatal) of every tooth present, except third molars, with a University of North Carolina-15 (UNC-15) probe. The clinical parameters measured were percentage of sites that bleed on probing (BOP), probing depth (PD), clinical attachment level (CAL) and Ramfjord's periodontal disease index. Percentage of bleeding was evaluated by multiplying number of sites involved with hundred and dividing the result by number of sites present. PD was measured as the distance the UNC-15 probe penetrated the depth of the gingival sulcus/periodontal pocket. CAL was measured as distance of the marginal gingival from the cementoenamel junction. Full-mouth orthopantamograph were recorded to evaluate the bone condition.

The cases diagnosed as generalized aggressive periodontitis and generalized chronic periodontitis were further divided into mild cases (BOP ≥ 40%, CAL 0-3 mm > 30% sites), moderate cases (BOP ≥ 40%, CAL 3-5 mm > 30% sites) and severe cases (BOP ≥ 40%, CAL > 5 mm > 30% sites).

Sample collection and ceruloplasmin analysis

Serum was extracted by centrifugation from the blood samples collected from all the 60 subjects. Serum ceruloplasmin levels were assessed using a new kinetic technique wherein phenylenediamine has been replaced by norfloxacin based reagent which is more stable, cheaper and physiological.The ceruloplasmin detection test is based on the detection of the ferroxidase activity of ceruloplasmin and the unit of measurement used here is IU/ml. Spectroscopic analysis of the serum samples for detection of ceruloplasmin levels were carried out. The levels of serum ceruloplsmin obtained from the selected subjects were tabulated then statistically analyzed. The results were analyzed keeping in mind the normal serum ceruloplasmin level, which is less than 1000 IU/ml in any healthy individual.[11]

Experimental results are presented as mean ± standard deviation. The analysis of variance (ANOVA) was used for the analysis and comparison of results between the groups. $P < 0.05$ was considered as statistically significant.

III. Results

The periodontal parameters recorded showed that the Group A patients had BOP in 49% of sites, PD of 6.8 mm (±0.80) and CAL of 4.0 mm (±0.20). Group B patients had BOP in 72% of sites, PD of 5.8 mm (±0.50) and CAL of 2.9 mm (±0.40). Group C patients had BOP in 6% sites, PD of 2.2 mm (±0.40) and no CAL[Table-1].

Table-1: The clinical parameters recorded.

Group	Sample size	Bleeding on probing-% (average)	Probing depth-mm (average)	Clinical attachment level-mm (average)
A	20	49	6.8±0.80	4.0±0.20
B	20	72	5.8±0.50	2.9±0.40
C	20	6	2.2±0.40	0.0

*****A- Generalized aggressive periodontitis, B- Generalized chronic periodontitis, C- healthy.

The serum levels of ceruloplasmin reveal that the patients of Group A having generalized aggressive periodontitis had a higher mean level of serum ceruloplasmin (1372 ± 215.60 IU/ml, min level-910 IU/ml, max level 1856 IU/ml) when compared to patients of Group B having chronic generalized periodontits (1190 ± 205.75 IU/ml, min level - 1015 IU/ml, max level 1698 IU/ml) and patients of Group C not having any form of periodontitis (926 ± 180.10 IU/ml, min level - 760 IU/ml, max level 1090 IU/ml)[Table-2].

Table-2: Serum ceruloplasmin levels

Group	Mean serum ceruloplasmin level(IU/ml)	Standard Deviation(IU/ml)	Minimum level(IU/ml)	Maximum level(IU/ml)
A	1372	215.60	910	1856
B	1190	205.75	1015	1698
C	926	180.10	760	1090

*****A- Generalized aggressive periodontitis, B- Generalized chronic periodontitis, C- healthy.

On correlating the serum ceruloplasmin levels with the disease severity (Increase in BOP and CAL), it revealed that the levels of ceruloplasmin increased as the disease severity increased. In generalized aggressive periodontitis cases, it was seen that that the ceruloplasmin levels in mild, moderate and severe cases were 1275 ± 185.5 IU/ml, 1355.00 ± 130.0 IU/ml and 1480.00 ± 175.5 IU/ml respectively. In generalized chronic periodontitis cases, it was seen that that the ceruloplasmin levels in mild, moderate and severe cases were 1072.00 ± 75.0 IU/ml, 1176.00 ± 110.5 IU/ml and 1275.00 ± 160.00 IU/ml respectively [Table 3].

Table-3: Correlation between serum ceruloplasmin levels and disease severity

Group	Mean serum ceruloplasmin level(CAL:0-3mm)	Mean serum ceruloplasmin level(CAL:3-5mm)	Mean serum ceruloplasmin level(CAL:>5mm)
A	1275.00±185.5(n=6)	1355.00±130.0(n=8)	1480.00±175.5(n=6)
B	1072.00±75.0(n=4)	1176.00±110.5(n=12)	1275.00±160.0(n=4)

*****A- Generalized aggressive periodontitis, B- Generalized chronic periodontitis

The statistical analysis in the form of ANOVA revealed that the level of significance in between groups, within groups and the sum total were all not statistically significant (>0.05).

IV. Discussion

The present study was conducted to evaluate the serum levels of cerruloplasmin in both aggressive and chronic periodontitis patients. It has long been established that simple and non-invasive 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.[12] 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.[6]One such potential marker recognized is the ceruloplasmin.

Ceruloplasmin is a 132-kDa pro-inflammatory marker protein with multiple copper-binding domains [13]seen to increase in systemic infections. Ceruloplasmin has the ability to create a state of hypoferraemia, which increases the natural resistance of the body to fight the disease.[14] 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).[15] Ceruloplasmin functions an anti-inflammatory agent and it can also work as a proinflammatory molecule.[16]

The present study revealed that periodontally healthy patients did not show increase in the levels of serum ceruloplasmin. Serum ceruloplasmin levels were found to be significantly higher in aggressive periodontitis patients than in chronic periodontitis patients even though increase in the level of ceruloplasmin was found in chronic periodontitis. The results of our study are in agreement to the study conducted by Harshwardhana B et al. in 2013[7] which showed similar results.

Study by Iwata *et al.* has already proved that ceruloplasmin causes priming of the neutrophils in localized aggressive periodontitis.[15] This can be attributed to the fact that 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. Ceruloplasmin mediated conversion of ferrous ion to ferric ion increases the intracellular ferric ion content, leading to increased binding of $gp91^{phox}$.[17]Thus, as previously proved PMNs can produce a faster and more response to secondary challenges and present a "primed" phenotype. In the presence of these primed PMNs, we expect a faster and severe form of tissue destruction. As proven in our study, it is this reason why the ceruloplasmin content is drastically increased in aggressive periodontitis.

The potential pathogens acquire the iron necessary for growth through mechanisms, which are extremely complex. It is significant that inspite of bacterial iron acquiring mechanisms, the natural resistance to infection operates effectively in the normal low iron environment.[14] 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,[18,19]which can be regarded as virtually zero. 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.[14]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.[20]So, it can be postulated and has also been proved by the present study 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.

As the disease severity increases, it is natural for any proinflammatory marker to increase. The present study has revealed that the level of ceruloplasmin was more in periodontal disease with higher CAL. We observed that as the CAL increased corresponding to the percentage of bleeding sites, the serum level of ceruloplasmin showed higher values. Even though the results were clinically significant, they were not statistically significant. This can be explained by the fact that the sample size selected for this study was very small. Further studies with an increase in the sample size will surely help in making it a statistically significant result.

V. Conclusion

Biomarkers have come a long way as indicators of periodontal disease. Increased tissue damage in the locally inflammed tissues of the periodontium in periodontitis patients and increased host resistance in periodontitis are very much attributable to ceruloplasmin. Serum ceruloplasmin levels increased in both aggressive and chronic periodontitis patients, but more in aggressive periodontitis patients. Thus it can be used as a potential marker for the diagnosis of periodontitis.

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