Original Article

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Assessment of Serum Ceruloplasmin Levels in Gingivitis, Chronic and Aggressive Periodontitis Patients- A Clinico-Biochemical Study

Dentistry Section

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ABSTRACT

Introduction: Pro-inflammatory mediators are mainly responsible for periodontal tissue breakdown in periodontitis. Ceruloplasmin is one such biomarker, the levels of which seems to be elevated in presence of inflammation. Hence, assessing biomarkers of inflammation is a valuable tool for determining periodontal disease activity.

Aim: To evaluate and compare the levels of serum ceruloplasmin in chronic generalised gingivitis, chronic generalised periodontitis and generalized aggressive periodontitis patients.

Materials and Methods: Patients visiting the dental Outpatient Department (OPD) during the period of January 2017 to March 2017 were screened for gingival index, probing pocket depth, bleeding on probing and categorised into the following four groups based on the clinical signs and symptoms; healthy controls, chronic generalised gingivitis, chronic generalised periodontitis and generalised aggressive periodontitis groups. Ten patients were enrolled in each group. Blood samples were collected from the antecubital vein of the subjects. The collected samples were taken for serum ceruloplasmin assessment with essential precautions. The test results were subjected to statistical analysis using Tukey's multiple post-hoc procedure, Karl pearson's correlation coefficient and One-way ANOVA.

Results: The results showed that serum ceruloplasmin level in chronic periodontitis was increased when compared to gingivitis group. Further, rise in levels of serum ceruloplasmin was comparatively higher in aggressive periodontitis subjects than chronic periodontitis subjects.

Conclusion: Inflammatory markers are being used extensively for the diagnosis of periodontal diseases. It can be inferred from the results of the present study that ceruloplasmin is one such biomarker which seems to be elevated with progress in attachment loss.

Keywords: Attachment loss, Biomarkers, Interleukins, Matrix metalloproteinases, Polymorphonuclear cells

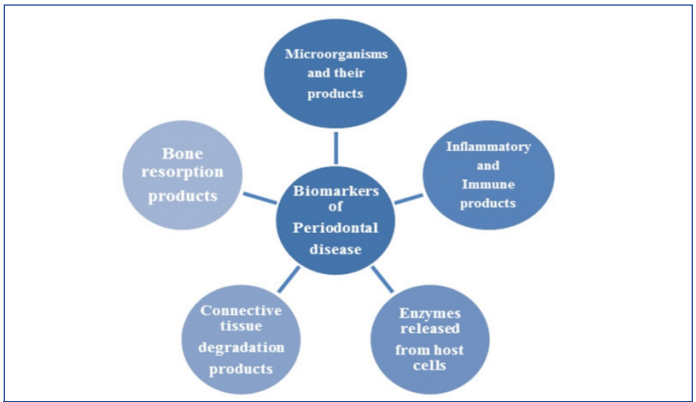
INTRODUCTION

Periodontium challenged with microbial load evokes a specific host response with release of certain inflammatory mediators and biomarkers into the adjacent area [1]. Periodontal diagnosis involves measuring probing depth or attachment level using a periodontal probe, or a measure of bone destruction through radiographs. However, they lack in both sensitivity and specificity. As the disease activity cannot be measured using conventional diagnostic methods, a biomarker that can assess periodontal disease activity is desirable [2].

Potential diagnostic biomarkers for diagnosis of periodontal disease in the latest modality are Tumour Necrosis Factor Alpha (TNF α), Interleukins (IL-1), IL-6, Matrix Metalloproteinases (MMPs), collagenases and certain enzymes [Table/Fig-1]. These biomarkers not only aid in identification of disease activity but also take part in assessing the treatment outcomes. Ceruloplasmin is one such biomarker [2].

Ceruloplasmin, an enzyme synthesised by the liver accounts for 95% of total copper in plasma of healthy individuals. Since, it contains six atoms of copper, it exhibits a similar oxidation activity to that of iron, and hence, is transported in plasma along with transferrin. Ferritin an acute phase reactant similar to ceruloplasmin, has demonstrated a strong association to periodontal disease activity [3].

Assessing the levels of ceruloplasmin would help in identifying early periodontal bone destruction, thus help in initiating corrective measures [4]. Since, not many studies have been done which correlate serum ceruloplasmin level with periodontal destruction. This study was planned to assess the level of serum ceruloplasmin in chronic generalised gingivitis, chronic generalised periodontitis and generalised aggressive periodontitis patients as compared to healthy controls.



[Table/Fig-1]: Different types of biomarkers used in diagnosing periodontal disease.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Periodontology, Sharavathi Dental College and Hospital, Shimoga, Karnataka, India between January 2017 to March 2017. After the study was approved by the Institutional Ethical Committee, patients visiting the dental OPD were screened for chronic generalised gingivitis, chronic generalised periodontitis and generalised

aggressive periodontitis using suitable clinical diagnostic criteria [5].

To enroll the subjects in to the study, following parameters were considered; gingival index by Loe H and Silness J, presence of a gingival bleeding on probing, probing pocket depth as measured with a William's graduated probe [6].

Patients who exhibited clinically healthy gingiva as demonstrated by an absence of bleeding on probing or probing pocket depth and a gingival index score of zero were enrolled as healthy controls.

Patients who demonstrated probing pocket depth greater than 5 mm in more than 30% of teeth, a gingival index score of one and above, and presence of generalised bleeding on probing were categorised as chronic generalised periodontitis patients.

Further, patients below 35 years who showed severe bone destruction as evidenced in Orthopantomogram (OPG) and had pockets ≥5 mm in more than three teeth other than incisors and first molars were grouped as generalised aggressive periodontitis patients [7].

Patients with systemic diseases, medically compromised patients, or patients who were on antibiotics, anti-inflammatory or anticoagulant therapy were excluded from the study as they could potentially affect the outcome. Similarly, pregnant and lactating mothers, smokers, alcoholics were excluded as these conditions and habits are well known to affect the periodontal status.

Forty patients (Age 16-45 years, male 22 and female 18) were selected amongst the screened subjects such that each group had 10 participants. Written informed consent was obtained from all the patients who were willing to participate.

A sample of 2 mL of blood was collected from antecubital vein of all the subjects using a syringe. The collected blood samples were taken to the biochemistry lab for serum ceruloplasmin assessment with essential precautions.

The serum was extracted from blood by centrifugation and the ceruloplasmin kit (APTEC Diagnostics nv™) was used to assess the levels of serum ceruloplasmin. The serum ceruloplasmin analysis was done using Memax™ spectrophotometer at 340 nm.

STATISTICAL ANALYSIS

The data entry was carried out using Microsoft Excel and intragroup and intergroup comparison was done using one way ANOVA and Tukey's multiple post-hoc procedure. Correlation between clinical parameters and ceruloplasmin levels (mg/dL) in all the groups was done by Karl Pearson's correlation coefficient and the result obtained was statistically significant (p-value<0.05).

RESULTS

The results showed that there was a considerable increase in the clinical parameters (gingival index, gingival bleeding index, probing pocket depth) with the severity of disease [Table/Fig-2-4]. From the data given in the above tables, it can be inferred that there was statistically significant difference between aggressive periodontitis patients and the other groups (p-value <0.001). On inter group comparison, maximum rise in ceruloplasmin levels were seen in aggressive periodontitis group followed by chronic periodontitis and chronic gingivitis groups [Table/Fig-5]. Further, with an increase in bleeding on probing which denotes disease activity, there was an increase in ceruloplasmin level in all the groups with the exception of healthy sites [Table/Fig-5]. With the progress in destructive activity, measured as probing pocket depth, it was noticed that ceruloplasmin levels steadily increased. There was statistically significant correlation between gingival index and ceruloplasmin level (p-value <0.001) in aggressive periodontitis when compared individually. Whereas, when correlation between clinical parameters and ceruloplasmin levels in all samples were assessed, these results were found statistically significant [Table/Fig-6,7].

Groups	Ceruloplasmin levels		
	Min	Max	Mean±SD
Healthy group	--	--	--
Gingivitis group	1.13	1.33	1.22±0.06
Chronic periodontitis group	1.30	1.43	1.38±0.04
Aggressive periodontitis group	1.43	1.75	1.61±0.11
F-value	68.1086		
p-value	0.0001*		
Pair wise comparisons by Tukey's multiple post-hoc procedures			
Healthy vs Gingivitis	--		
Healthy vs Chronic periodontitis	--		
Healthy vs Aggressive periodontitis	--		
Gingivitis vs Chronic periodontitis	p=0.0003*		
Gingivitis vs Aggressive periodontitis	p=0.0001*		
Chronic periodontitis vs Aggressive periodontitis	p=0.0001*		

[Table/Fig-2]: Comparison of four study groups (healthy, gingivitis, chronic periodontitis and aggressive periodontitis) with respect to gingival index scores by one way ANOVA.
*p-value <0.05 indicates significant difference between group

Groups	Ceruloplasmin levels		
	Min	Max	Mean±SD
Healthy group	--	--	--
Gingivitis group	0.28	0.57	0.39±0.11
Chronic periodontitis group	0.57	0.70	0.61±0.04
Aggressive periodontitis group	0.70	0.90	0.80±0.07
F-value	67.3520		
p-value	0.0001*		
Pair wise comparisons by Tukey's multiple post-hoc procedures			
Healthy vs Gingivitis	--		
Healthy vs Chronic periodontitis	--		
Healthy vs Aggressive periodontitis	--		
Gingivitis vs Chronic periodontitis	p=0.0001*		
Gingivitis vs Aggressive periodontitis	p=0.0001*		
Chronic periodontitis vs Aggressive periodontitis	p=0.0002*		

[Table/Fig-3]: Comparison of four study groups (healthy, gingivitis, chronic periodontitis and aggressive periodontitis) with respect to gingival bleeding index scores by one-way ANOVA.
*p-value <0.05 indicates significant difference between group

Groups	Ceruloplasmin levels		
	Min	Max	Mean±SD
Healthy group	--	--	--
Gingivitis group	--	--	--
Chronic periodontitis group	5.02	5.50	5.25±0.17
Aggressive periodontitis group	5.50	6.40	6.00±0.32
t-value	-6.5322		
p-value	0.0001*		

[Table/Fig-4]: Comparison of two study groups (chronic periodontitis and aggressive periodontitis) with respect to probing pocket depth scores by independent t-test.
*p-value <0.05 indicates significant difference between group

Groups	Ceruloplasmin levels		
	Min	Max	Mean±SD
Healthy group	15.99	69.32	39.24±16.29
Gingivitis group	20.26	73.59	51.20±14.27
Chronic periodontitis group	55.46	98.12	76.16±11.26
Aggressive periodontitis group	79.29	112.00	101.28±11.15
F-value	42.1917		
p-value	0.0001*		
Pair wise comparisons by Tukey's multiple post-hoc procedures			
Healthy vs Gingivitis	p=0.2095		
Healthy vs Chronic periodontitis	p=0.0002*		
Healthy vs Aggressive periodontitis	p=0.0002*		
Gingivitis vs Chronic periodontitis	p=0.0012*		
Gingivitis vs Aggressive periodontitis	p=0.0002*		
Chronic periodontitis vs Aggressive periodontitis	p=0.0011*		

[Table/Fig-5]: Comparison of four study groups (healthy, gingivitis, chronic periodontitis and aggressive periodontitis) with respect to ceruloplasmin level (mg/dL) by one-way ANOVA.
*p-value <0.05 indicates significant difference between group

Groups	Variables	Variables	r-value	p-value
Healthy	Gingival index	Gingival bleeding index	--	--
		Probing pocket depth	--	--
		Ceruloplasmin level (mg/dL)	--	--
	Gingival bleeding index	Probing pocket depth	--	--
		Ceruloplasmin level (mg/dL)	--	--
	Probing pocket depth	Ceruloplasmin level (mg/dL)	--	--
Gingivitis	Gingival index	Gingival bleeding index	0.8726	0.0010*
		Probing pocket depth	--	--
		Ceruloplasmin level (mg/dL)	-0.1016	0.7800
	Gingival bleeding index	Probing pocket depth	--	--
		Ceruloplasmin level (mg/dL)	-0.3766	0.2835
	Probing pocket depth	Ceruloplasmin level (mg/dL)	--	--
Chronic periodontitis	Gingival index	Gingival bleeding index	0.3616	0.3046
		Probing pocket depth	0.5999	0.0667
		Ceruloplasmin level (mg/dL)	-0.1106	0.7609
	Gingival bleeding index	Probing pocket depth	0.2639	0.4612
		Ceruloplasmin level (mg/dL)	-0.5183	0.1248
	Probing pocket depth	Ceruloplasmin level (mg/dL)	0.3869	0.2693
Aggressive periodontitis	Gingival index	Gingival bleeding index	-0.2138	0.5531
		Probing pocket depth	-0.0982	0.7872
		Ceruloplasmin level (mg/dL)	0.8885	0.0006*
	Gingival bleeding index	Probing pocket depth	0.0566	0.8766
		Ceruloplasmin level (mg/dL)	-0.0552	0.8796
	Probing pocket depth	Ceruloplasmin level (mg/dL)	-0.2220	0.5376

[Table/Fig-6]: Correlation between clinical parameters and ceruloplasmin levels (mg/dL) in four study groups (healthy, gingivitis, chronic periodontitis and aggressive periodontitis) by Karl Pearson's correlation coefficient.
*p-value <0.05 indicates significant correlation between them

DISCUSSION

Measurement of periodontal destruction by radiographs or clinical probing depths denotes only past or previous exposure to the disease. The present status of the disease, whether active destruction is in progress can be detected using biomarkers.

Variables	Variables	r-value	t-value	p-value
Gingival index	Gingival bleeding index	0.6808	3.9439	0.0010*
	Probing pocket depth	0.7078	4.2511	0.0005*
	Ceruloplasmin level (mg/dL)	0.8345	6.4262	0.0001*
Gingival bleeding index	Probing pocket depth	0.7455	4.7453	0.0002*
	Ceruloplasmin level (mg/dL)	0.5870	3.0765	0.0065*
Probing pocket depth	Ceruloplasmin level (mg/dL)	0.6373	3.5086	0.0025*

[Table/Fig-7]: Correlation between clinical parameters and ceruloplasmin levels (mg/dL) in all samples by Karl Pearson's correlation coefficient.
* p-value <0.05 indicates significant correlation between them

Further, these markers can be used for diagnostic or prognostic purposes and to evaluate the outcome of therapeutic procedures [2]. The present study utilised ceruloplasmin a new addition to the list of known biomarkers for evaluating periodontal disease activity. The present study revealed that ceruloplasmin is detected even in healthy individuals, and its level was significantly altered in presence of inflammation and tissue destruction.

The ceruloplasmin levels were normal among individuals with healthy periodontium as there was not much of inflammatory component clinically and intergroup statistical analysis among gingivitis and healthy periodontium group showed no statistically significant results, indicating that only advanced destruction of attachment apparatus is associated with elevated ceruloplasmin levels. However, in the periodontitis group whether chronic or aggressive, the level of ceruloplasmin was more when compared to control group or gingivitis group which were statistically significant. This could be due to the fact that ceruloplasmin level seems to rise considerably only in presence of active tissue destruction [8].

Ceruloplasmin was found to be elevated significantly more in aggressive periodontitis patients rather than chronic periodontitis group. This can be attributed to the extensive bone destruction seen in a short period of time amongst aggressive periodontitis patients [9]. Further, local hypoxia as a result of inflammation results in increased activation of Hypoxia Inducible Factor (HIF-1α), which in turn, activates ceruloplasmin to mediate iron ion conversion from ferrous ion to ferric ion. Ceruloplasmin mediated conversion of ferrous ion to ferric ion increases the intracellular ferric ion content leading to increased binding of $gp91^{phox}$ [10]. Thus, Polymorphonuclear (PMNs) cells can produce a faster and greater response to secondary challenges and present a "primed" phenotype. This might be the reason for increased ceruloplasmin level in aggressive periodontitis [8,11].

Studies have shown that elevated levels of free iron decreases tissue resistance to bacterial infection. Hence, ceruloplasmin level rises in serum due to acute phase reaction. The periodontal pathogens utilise free iron for their growth and colonisation leading to elevated levels of serum ceruloplasmin seen in chronic periodontitis [12]. This rise in ceruloplasmin further leads to rise in pathogenic flora due to decreased tissue resistance, resulting in an enhanced bone destruction which is manifested clinically as attachment loss. Findings of Harshavardhana B et al., study stated that elevated levels of ceruloplasmin was proportional to attachment loss. Further, they noted that with progression in disease severity there was rise in ceruloplasmin levels, which is similar to results of our study [4].

Hypoxia mediated oxygen free radical generation in PMNs and increased oxidative stress and neutrophil mediated tissue injury could be the reason behind elevated ceruloplasmin levels in our study [8]. Hence, it seems that ceruloplasmin could be used as a chairside diagnostic assay for assessing periodontal disease activity.

LIMITATION

The present study is only exploratory in nature as it utilised a small sample size, and further interventional treatment was not provided due to time and financial constraints. Hence, a study with interventional design whether surgical or non-surgical with a long duration and larger sample size would clearly point out the link between ceruloplasmin and bone destruction.

CONCLUSION

The present study demonstrated a significant relationship between periodontal bone destruction and considerable increase in ceruloplasmin level. Ceruloplasmin in the near future may be considered as an important biomarker for determining periodontal disease activity.

REFERENCES

[1] Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998;25(2):134-44.
[2] Taba M, Kinney J, Kim AS, Giannobile WV. Diagnostic biomarkers for oral and periodontal diseases. Dent Clin North Am. 2005;49(3):551-71.
[3] Iwata T, Kantarci A, Yagi M, Jackson T, Hasturk H, Kurihara H, et al. Ceruloplasmin induces polymorphonuclear leukocyte priming in localized aggressive periodontitis. J Periodontol. 2009;80(8):1300-06.
[4] Harshavardhana B, Rath SK, Mukherjee M. Evaluation of serum ceruloplasmin in aggressive and chronic periodontitis patients. J Indian Soc Periodontol. 2013;17(3):333-37.
[5] Newman M, Takei H, Klokkevold P, Carrranza F. Clinical Periodontology. 2013:11th ed; pp. 60-81.
[6] Loe H, Silness J. Periodontal Disease In Pregnancy, prevalence and severity. Acta Odontol Scand. 1963;21:533-51.
[7] Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999;4(1):01-06.
[8] Kantarci A, Oyaizu K, Van Dyke TE. Neutrophil mediated tissue injury in periodontal disease pathogenesis: Findings from localized aggressive periodontitis. J Periodontol. 2003;74(1):66-75.
[9] Armitage GC, Cullinan MP. Comparison of the clinical features of chronic and aggressive periodontitis. Periodontol. 2000. 2010;53:12-27.
[10] Martin F, Linden T, Katschinski DM, Oehme F, Flamme I, Mukhopadhyay CK, et al. Copper-dependent activation of hypoxia-inducible factor (HIF)-1: Implications for ceruloplasmin regulation. Blood. 2005;105(12):4613-19.
[11] Lang N, Bartold M, Cullinan M, Jeffcoat M, Mombelli A, Murakami S, et al. Consensus report. Aggressive periodontitis. Ann Periodontol. 1999;4:53.
[12] Griffiths E, Bullen JJ. Iron and infection: Molecular, physiological and clinical aspects. 2nd ed. 2007: pp. 01-25.

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