



A COMPARATIVE EVALUATION OF SERUM CERULOPLASMIN LEVEL BEFORE AND AFTER NONSURGICAL THERAPY IN CHRONIC PERIODONTITIS PATIENTS

Periodontics

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ABSTRACT

Introduction : Periodontal disease risk can be identified and quantified by objective measures, like biomarkers. One such marker and acute phase reactant, is Ceruloplasmin which causes a state of hypoferrremia leading to increase in the natural resistance of the body.

Materials and Method: A Randomised control study with 20 patients diagnosed generalized chronic periodontitis were divided into 3 groups according to the severity of the disease. Blood sample collected was sent to the lab for the assessment of ceruloplasmin enzyme. Clinical Parameters like CAL, bleeding on probing and Ramfjord index was collected. Non surgical therapy (SRP) was done and the patients were kept on follow up. After two months Reassessment of ceruloplasmin enzyme level was done. The statistical analysis was done using SPSS Version 16.0 Statistical Analysis Software. The significance of mean difference of serum ceruloplasmin and all clinical parameters at baseline and 60 days after nonsurgical therapy was tested.

Results: There was statistically significant difference of serum ceruloplasmin levels at baseline and 60 days after nonsurgical therapy in generalized chronic periodontitis patients. The serum ceruloplasmin level significantly reduced from baseline to 60 days. The levels of serum ceruloplasmin also increased with the disease severity which were increased bleeding on probing, increased pocket depth and increased attachment loss and significantly reduced 60 days after nonsurgical therapy.

Conclusion: Serum ceruloplasmin increases with severity of the disease and level reduces after nonsurgical therapy which can be considered as a biomarker for inflammatory disease in oral diseases apart from its role in systemic diseases.

KEYWORDS

Ceruloplasmin, Periodontitis

INTRODUCTION

Chronic periodontitis is an infectious inflammatory disease caused by the bacteria from dental plaque, which determines progressive destruction of the tissues that support the teeth (e.g., the gum, the periodontal ligament, cementum, and the alveolar bone). Periodontal disease consists of periods of exacerbation interspersed with periods of remission and presents a local microbial burden that initiates local inflammation and local tissue destruction.^{1,2}

Biomarkers play an important role in life sciences and have greater role in diagnosis, monitoring of therapy outcomes, and drug discovery.³ 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 one such biomarker is ceruloplasmin. Ceruloplasmin (CP) is a blue plasma glycoprotein that is synthesized primarily in hepatocytes which is involved in transport of copper throughout the body. It is an acute phase reactant seen to increase in inflammatory conditions, helps in transfer of copper in body and influences the uptake of iron into the cells because of its property of conversion of ferrous form of iron to ferric form, due to which alterations in serum iron are often accompanied by changes in serum ceruloplasmin. threshold change before a site can be identified as having experienced a significant anatomic event.⁴

Recent studies reported that Serum ceruloplasmin levels increased in both aggressive and chronic periodontitis patients, Major B Harshavardhana et al but more levels in aggressive periodontitis patients and is a potential marker for diagnosis of periodontitis.⁵

AIM

To compare and evaluate the level of serum ceruloplasmin before and after non surgical therapy (SRP) in chronic periodontitis patients.

METHODOLOGY

The study was conducted in the Department of Periodontology in kothiwal dental college and research centre Moradabad. The research protocol was ethically approved by the Ethical Committee and Review Board of the KDCRC, Moradabad. All subjects were verbally informed and written informed consent was obtained for their participation in the study.

A total of 20 patients were selected on the basis of diagnosis as generalized chronic periodontitis. The patients were divided into 3 groups (mild, moderate and severe) according to the severity of the

disease. 5ml of the blood sample was collected from each patient and was sent to the lab for the assessment of ceruloplasmin enzyme. Clinical Parameters like CAL, bleeding on probing and Ramfjord index was collected. Non surgical therapy (SRP) was done and the patients were kept on follow up after two months. After this period again the patient's blood sample was collected, clinical parameters were again recorded to assess the severity of the disease progression in blood sample was again sent to lab for reassessment of ceruloplasmin enzyme level. After two months of SRP assessment of ceruloplasmin enzyme in the blood was compared with the baseline assessment i.e. before SRP.

The distribution of mean $\pm$ SD of serum ceruloplasmin in all the groups (mild, moderate and severe) at base line (1063.40 $\pm$ 35.70, 1217.20 $\pm$ 45.40, 1465.80 $\pm$ 108.7) and 60 days after nonsurgical therapy (992.20 $\pm$ 61.80, 1121.80 $\pm$ 54.75, 1402.70 $\pm$ 91.70) respectively. The results of this study indicated that as the CAL increased corresponding to the increased percentage of bleeding sites and probing depth, the serum level of ceruloplasmin also increased at baseline and reduced in all the groups 60 days after nonsurgical therapy.

Subject Selection

Subjects were excluded if they had any systemic condition, which would influence the course of periodontal disease or treatment and medical conditions, which would require antibiotic prophylaxis for routine dental procedures. Individuals who had taken antibiotics in the previous 3 months or pregnant or lactating women and smokers were also excluded.

The clinical parameters measured were bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL) and OPG 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 $\geq$ 40%, CAL 0-3 mm $>$ 30% sites), moderate cases (BOP $\geq$ 40%, CAL 3-5 mm $>$ 30% sites) and severe cases (BOP $\geq$ 40%, CAL $>$ 5 mm $>$ 30% sites).

METHODOLOGY

Serum was extracted from the subjects' blood by centrifugation stored at 4 °C to 10 °C till being processed. Serum ceruloplasmin was estimated by a colorimetric enzymatic assay based on oxidation of a

dye, paraphenylene diamine Each sample was analyzed in triplicate and the mean was determined.

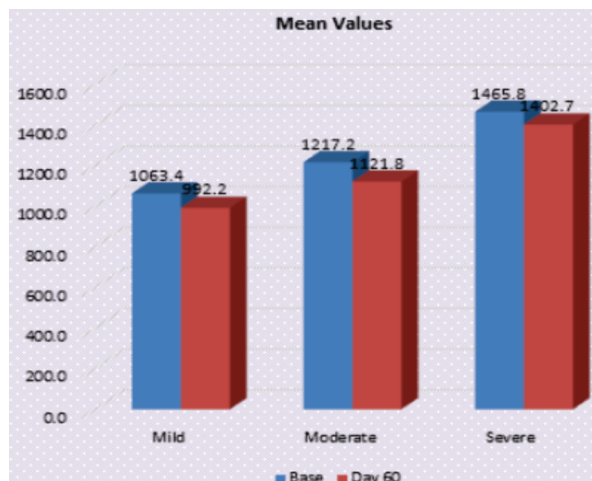
Principle:

The estimated value is considered as a baseline value with this following clinical parameters were recorded to check the present periodontal status of the patient (baseline). After assessing the initial clinical parameters scaling and root planing was done and were kept on follow up for 2 months after two months till then Patients were asked to maintain the oral hygiene. After two months again the patients were recalled 5 ml of blood sample was again collected from each patient to check the serum ceruloplasmin level and following this all the mentioned clinical parameters were again calculated. Serum ceruloplasmin level was then compared with the baseline value to check its significant increased or decreased values.

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 ceruloplasmin 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 and values of indices were compared to check the severity of the periodontal disease of the patients after non surgical therapy.

Table 1: Determination of linear correlation between different clinical parameters and the serum ceruloplasmin level at base line

Correlations		Level of ceruloplasmin	bleeding on probing	Clinical attachment loss	Clinical pocket depth
BASLINE					
Level Of Ceruloplasmin	Pearson Correlation	1	.813**	.967**	.927**
	p-values		<0.001	<0.001	<0.001
	N	20	20	20	20
Bleeding On Probing	Pearson Correlation	.813**	1	.824**	.889**
	p-values	<0.001		<0.001	<0.001
	N	20	20	20	20
Clinical Attachment Loss	Pearson Correlation	.967**	.824**	1	.948**
	p-values	<0.001	<0.001		<0.001
	N	20	20	20	20
Clinical Pocket Depth	Pearson Correlation	.927**	.889**	.948**	1
	p-values	<0.001	<0.001	<0.001	
	N	20	20	20	20



Graph : Comparison Of Serum Ceruloplasmin Level With Severity Of Disease

Table 2 :determination Of Linear Correlation Between Different Clinical Parameters And The Serum Ceruloplasmin Level 60 Days After Nonsurgical Therapy

Correlations		Level of ceruloplasmin Base	bleeding on probing at base line	Clinical attachment loss at baseline	Clinical pocket depth baseline
60 DAYS					
Level Of Ceruloplasmin	Pearson Correlation	1	.723**	.736**	.607**
	p-values		<0.001	<0.001	0.005
	N	20	20	20	20
Bleeding On Probing	Pearson Correlation	.723**	1	.541*	.572**
	p-values	<0.001		0.014	0.008
	N	20	20	20	20
Clinical Attachment Loss	Pearson Correlation	.736**	.541*	1	.913**
	p-values	<0.001	0.014		<0.001
	N	20	20	20	20
Clinical Pocket Depth	Pearson Correlation	.607**	.572**	.913**	1
	p-values	0.005	0.008	<0.001	
	N	20	20	20	20

DISCUSSION

Acute phase proteins are a class of proteins whose plasma concentration increase (positive acute phase proteins) or decrease (negative acute phase proteins) in response to inflammation'. This response is called as the acute phase reaction, also called as acute phase response, which occurs approximately 90 minutes after the onset of a systemic inflammatory reaction. In Periodontitis endotoxins released from gram negative organisms present in the sub gingival plaque samples interact with Toll- like receptors (TLR) that are expressed on the surface of Polymorphonuclear leucocytes (PMNs) and monocytes which are in abundance in periodontal inflammation. The complex formed due to interaction of Endotoxins and TLR activates the Signal transduction pathway in both innate and adaptive immunity resulting in production of Cytokines that co- ordinate the local and systemic inflammatory response. The pro inflammatory cytokines originating at the diseased site activates the liver cells to produce acute phase proteins as a part of non specific response.

The production of Acute phase proteins is regulated to a great extent by Cytokines such as IL-1, IL-6, IL-8, TNF- α and to a lesser extent by Glucocorticoid hormones. These proteins bind to bacteria leading to activation of complement proteins that destroys pathogenic organisms^{8,9,10}. Major B Harshavardhana et al (2013) conducted a study to evaluate the serum levels of ceruloplasmin in both aggressive and chronic periodontitis patients and concluded that 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. Periodontally healthy patients did not show increase in the levels of serum ceruloplasmin. The levels of serum ceruloplasmin also increased with the disease severity whose manifestations were increased bleeding on probing, increased pocket depth and increased attachment loss.

After clinical Observations and statistical analysis of the study, Following were the conclusion :

1. Serum ceruloplasmin is an active inflammatory biomarker.
2. The level of serum ceruloplasmin at baseline was greater as compared to 60 days after nonsurgical therapy.
3. Serum ceruloplasmin also increased with the severity of the disease as in mild group of generalised chronic periodontitis patient the level of serum ceruloplasmin was less as compared with the severe form of generalized chronic periodontitis patients. This positive correlation was also detected by Jyoti M Varghese et al (2012)⁶ who Conducted a study to evaluate the levels of Glutathione-S-transferase (GST) and Ceruloplasmin as diagnostic markers for patients with chronic periodontitis in gingival crevicular fluid (GCF) and the gingival tissues and concluded that Highly significant changes in GST between the diseased and normal patients ($P = 0.001$) were detected. There was a decrease in GST level in both gingival tissue & GCF in diseased patients when compared to the control patients. The ceruloplasmin levels in GCF and gingival tissues showed no difference between the control and diseased group. results indicated that a relationship suggesting that GST produced during chronic inflammation could be used as biomarker that indicate periodontal

disease.

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