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Original Research Paper

Dentistry

EVALUATION OF HAPTOGLOBIN LEVELS IN CHRONIC GENERALIZED PERIODONTITIS PATIENTS WITH ANEMIA– A COMPARATIVE STUDY

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ABSTRACT

BACKGROUND: Anemia of chronic disease (ACD) is most seen in Indian population. Anemia occurring in patient having chronic infection, inflammatory conditions or neoplastic disorders and occurring despite the presence of adequate iron stores and vitamins.Haptoglobin is a marker in inflammation, and their systemic levels have been associated with anemia of chronic disease. This comparative study was conducted to assess the serum haptoglobin levels in chronic generalized periodontitis patients with anemia before and after non - surgical periodontal therapy. The aim of study was to evaluate serum haptoglobin level in healthy subjects, chronic generalized periodontitis with anemia, chronic periodontitis and anemia patients.

METHODS: A total of 120 subjects selected for the study. Blood samples taken from healthy controls (n - 30), chronic generalised periodontitis with anemia (n - 30), patients with chronic periodontitis (n - 30) and anemia (n - 30). The periodontal statuses of each patient were recording Periodontal Disease Index - (PDI), Probing Pocket Depth (PPD), Bleeding Index (BI) and Clinical Attachment Level (CAL). The levels of serum haptoglobin through mininephplusnephelometer using commercially available mininephplushaptoglobin test kit. Data description were done in the form of standard deviation and mean, whereas analysis of data was done using students t – test and one way ANOVA (Analysis of Variance) to test the statistical significance between groups.

RESULTS: The levels of serum haptoglobin were increased in patient with chronic generalized periodontitis with anemia when compared to healthy controls ($p<0.001$).Negative correlations were found to exist between levels of clinical parameters like BI, PDI, PPD and CAL when compared with serum haptoglobin as well as with the study groups.

CONCLUSIONS: Haptoglobin levels decreased as the clinical parameters of periodontitis decreased in the patients after non - surgical periodontal therapy but these values are statistically not significant after 6 months, which suggesting that the non - surgical periodontal therapy is not effective on periodontitis over longer period of time.

KEYWORDS : Haptoglobin, Anemia, Non - Surgical Periodontal Therapy.

BACKGROUND

Periodontitis is a chronic inflammation caused by facultative microorganisms that reside within the dental plaque. The host response to these pathogens produces persistent inflammation leading to clinical manifestation of disease [1]. Periodontitis has been documented as an etiological or modulating factor in systemic disease like cardiovascular disease, diabetes, anemia, respiratory diseases and adverse pregnancy outcome [2]. This could occur via host immunoinflammatory mediators which are elicited in response to bacterial toxin or local tissue response to cytokines.

Anemia of chronic disease (ACD) is most seen in Indian population. Anemia occurring in patient having chronic infection, inflammatory conditions or neoplastic disorders and occurring despite the presence of adequate iron stores and vitamins. [3].Patients suffering from severe periodontitis have increased production of pro - inflammatory cytokines like Interleukin - 1 (IL - 1), Interleukin - 6 (IL - 6), and Tumour Necrosis Factor - α (TNF - α) that can act on hepatocytes in the liver to produce reactive proteins including haptoglobin [4].

Haptoglobinis a moderate, type - II acute phase protein which is synthesized by hepatocytes and other types of cells like monocyte, fibroblast and endothelial cells [5]. The moderate acute phase protein responds rapidly to inflammatory stimuli and serum levels which may be raised up to two to tenfold.Haptoglobin belongs to a group of serum proteins, which have unique property of combining with haemoglobin. The estimation of haptoglobin levels depends upon measuring haemoglobin – haptoglobin complex formed. Raised levels of haptoglobin have been found in serum from patients with infection, anemia of chronic disease, malignancy, connective tissue disorders, acute rheumatic fever and myocardial infarction [6].

Chronic periodontitis is shown to raise inflammatory markers such as haptoglobin levels in the blood. Based on cross sectional and interventional studies, periodontitis has been linked to anemia, however mechanisms responsible for this association are obscure [5,6]. Therefore, this comparative study was conducted for the assessment of serum haptoglobin levels in chronic generalized periodontitis patients with anemia before and after non - surgical periodontal therapy. In past, not many studies have been done on the subject.

METHODS

Study Design

The present study was conducted in the Department of Periodontology, of a tertiary level health care centre at Bangalore. This was a comparative study which was carried out for a period of six months. Participants were interviewed to obtain medical and demographic information and were screened for periodontal examination. All periodontal clinical parameters were evaluated by a single investigator, who is blinded to the procedure. A total of 120 subjects were selected for the study which included 30 controls, 30 patients with chronic periodontitis, 30 patients with chronic periodontitis with anemia and 30 anemia patients. The nature and purpose

of study was explained to the patient and an informed consent was obtained.This study was put forward before the Ethical Committee and clearance was obtained. Subjects for this study comprised of:

Group A: Systemically healthy controls - Age group; 30 - 65 years. Individuals with PPD ≤3 mm, CAL <2 mm with no clinical signs of inflammation.

Group B: Chronic periodontitis patients - Age group; 30 - 65 years. Individuals having more than 30 % of their teeth with PPD ≥4 mm, CAL ≥2 mm.

Group C: Chronic periodontitis with anemia patients - Age group; 30 - 65 years. Individuals having more than 30 % of their teeth with PPD ≥4 mm, CAL ≥2 mm.

Group D: anemia patients - Age group; 30 - 65 years.. Individuals with PPD ≤3 mm, CAL <2 mm with no clinical signs of inflammation.Anemia patients were without chronic periodontitis and free from other systemic diseases.These patients were selected from the 'Dept of Internal Medicine' and same disease criteria were followed for all the patients.

INCLUSION CRITERIA

1. Patients with periodontitis, anemia and systemically healthy in group of 30 - 65 years of age were included in the study.
2. Patients not under systemic steroids, antibiotics or anti - inflammatory drugs.
3. Patients who have not undergone for periodontal treatment for 6 months prior to and during the period of study.

EXCLUSION CRITERIA

1. Patients with any systemic diseases except anemia.
2. Habits like smoking, alcohol consumption or drug abuse.
3. History of oral contraceptive use for the last 6 months.
4. Pregnant and lactating women.

METHODOLOGY

All clinical parameters were recorded by using UNC– 15 periodontal probe, which includes Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), Periodontal Disease Index (PDI) (Ramfjords), Bleeding on Probing (BOP) and Gingival Index (GI) (Loe H and Sillness J).

The serum samples were collected from all the patients selected in the four groups before starting the treatment. Group B and C patients had undergone non - surgical periodontal therapy that comprised of oral prophylaxis and 0.2% Chlorhexidine mouthwash (Figure 1). Group D patients were under treatment for anemia by medical specialist in the Hospital. All the four groups were provided with similar oral hygiene instructions.

Estimation of Serum Haptoglobin

Consent was taken from each subject.Venous blood was collected in the morning hours by venipuncture using 5ml of syringe with a 20 gauge needles (Figure 2). The blood was taken to non - EDTA containing test tubes and was allowedto clot and serums were extracted from the blood by centrifuging at 300 rpm for five min after one hour. The separated serum were immediately taken to Eppendorf vials and stored at - 20C till the time of assay. Quantitative nephelometric assay were used for measuring of haptoglobin in stored serum with minineph analyzer (The Binding Site Group Ltd, Birmingham, UK) (Figure 3). The test specimen was mixedin cuvette with 30□L of diluted sample,400□L of mininephhaptoglobin buffer and 40□L of minineph human haptoglobin antiserum and allowed to react (Figure 4).The cuvette placed in minineph instrument (Figure 5). Haptoglobin in the test specimen showed increased in turbidity which results in formation of insoluble complex,which were usually measured from the wavelength of 340nm.The concentration of haptoglobin in test specimen corresponds to the increase in turbidity. The calibration curve stored in the instrument which automatically calculated the haptoglobin levels.

For all the groups, blood sample collection was done on first day of study followed by serum samples from Group B and C after 6 months postnon - surgical periodontal intervention. Even in Group D, samples were collected 6 months after the treatment of anemia. After 6 months, a full mouth periodontal examination was performed in each subject and the UNC - 15 periodontal probe used to assess clinical parameters by a single examiner. The parameters comprised of GI, PD, CAL, PDI and haptoglobin. All the parameters were recorded prior to blood sample collection and repeated after six months.

STATISTICAL ANALYSIS

In four study groups a descriptive statistical analysis was carried out. The present study to explore the distributions of several characteristics of the cases studied. The statistical significance of difference of various variables (such as BOP, PPD, CAL, PDI and haptoglobin) across four study groups (Inter - group comparisons) were carried out using(ANOVA) one - way analysis with Post - Hoc Tukey's correction for multiple group comparisons. Pair - wise differences (intra - group comparisons) of several continuous variables was tested using paired 't' test in each study group. The variables which were not normally distributed were transformed to normality using appropriate mathematical transformations before subjecting them to ANOVA and paired't' test.The entire data were statistically analyzed using Statistical Package for Social Sciences (SPSS ver 11.5, Inc. Chicago, USA) for MS Windows. All the hypotheses were formulated using two tailed alternatives against each null hypothesis (hypothesis of no difference).

RESULTS

Periodontal Disease Index [PDI]

Intra Group

The mean PDI of Group A, B, C and D were 0.88 ± 0.09, 5.26 ± 0.15, 5.27 ± 0.16 and 0.887 ± 0.09 respectively at baseline [Table 1, Chart 1].The mean % change of PD of Group B, C and D were 13.1 %, 13.9 % and 0.056 % respectively [Table 1, Chart 2].

Inter Group

The intergroup comparisons for the PDI in between Group A and D (P - 0.984) and those % change between Group C and B (P - 0.079) were statistically non - significant (P>0.05). The Intergroup comparisons between Group "A and C', A and B', 'B and D'& C and D were statistically significant (P<0.05) [Table 2].

HaptoglobinParameters

Intra Group

The mean haptoglobinof Group A, B, C and D were 1.09 ± 0.04, 1.45 ± 0.06, 1.87 ± 0.08 and 1.41 ± 0.06 respectively at baseline [Table 3, Chart 3]. The mean % change of haptoglobin of Group B, C and D were 0.53 %, 0.58 % and 0.54 % respectively [Table 3, Chart 4].The statistical analysis showed average pre and post treatment haptoglobin levels did not differ significantly in group B, group C & group D (P - value>0.05).

Inter Group

The intergroup comparisons for the haptoglobin, the mean % change between Group B and D, B and C, C and D were statistically non - significant (P>0.05). The intergroup comparisons between Group A and C, A and B, and A and D were statistically significant (P<0.05)[Table 4].

The statistical analysis showed: -

1. The average pre - treatment haptoglobin level is significantly higher in groups B, C and D compared to group A (P - value<0.001 for all). The average pre - treatment haptoglobin level is significantly higher in groups C compared to groups B and D (P - value<0.001 for all). The average pre – treatment haptoglobin level is significantly higher in group B compared to group D (P - value<0.001).
2. The average post - treatment haptoglobin level is significantly higher in groups C compared to groups B and D (P - value<0.001 for both). The average post – treatment haptoglobin level did not differ significantly between groups B and D (P - value>0.05).
3. The average % change inhaptoglobinis not differ significantly across study groups B, C and D (P - value>0.05 for all).

DISCUSSION

Periodontitis is a chronic inflammation of the supporting tissues of the teeth, which usually have a feature of bone loss in alveolar bone. Periodontitis have a character of slowly progressive and destructive nature with a period of remission and exacerbation. Main factors contributing to periodontitis includes resident micro flora, oral hygiene and individual host response. The process in periodontal disease can involves bacterial product within vessel wall or involve of vasculature directly by interaction of organisms or through modulation of haemostasisor increased circulation of acute phase reactants such as CRP, fibrinogen and haptoglobin [7]. The increased in haptoglobin found to be risk factor for anemia of chronic disease [8]. Increased proinflammatory cytokines such as IL - 6 and TNF - α correlated with haptoglobin in excessive levels which helps in colonization and attachment of bacteria as well as migration of leucocytes at site of inflammation [9].

The aetiological pathways proposed for the association between anemia of chronic disease, systemic inflammation and periodontitis. In first pathways, oral microorganisms and byproducts pass through the circulating system. The second pathways, considers periodontal inflammation is a source of systemic inflammatory mediators. Whenever increased haptoglobin levels in periodontal disease patients exacerbate inflammatory process in blood vessels, thereby increasing the risk of anemia [10]. Therefore, Measurement of acute phase proteins help to identify a subset of patients who are risk for destructive disease or undergoing process of periodontal breakdown [11].

The mean value of haptoglobinat baseline was high in Gp B and Gp C. This can be due to the presence of cytokines at the site of pathology, which acts on hepatocytes to release acute phase proteins including haptoglobin [12]. In the present study, intragroup comparison of haptoglobinlevels showed statistically non - significant decrease in values in group B patients from baseline to 6 months after non - surgical periodontal therapy. This could be due to resolution of inflammation by nonsurgical periodontal therapy. These results were in accordance with the studies done by Buhlina K [10]. who showed decrease in haptoglobin value in first 3 months but after 6 months there was increase in haptoglobin value which was not statistically significant. Author mentioned that when non surgical periodontal therapy was carried out, there was a sudden decrease in bacterial load resulting in decrease inhaptoglobin levels. After this period it depends upon patient's oral hygiene maintenance. In the present study, mean haptoglobin level was found to be increased in patients with chronic periodontal disease with anemia when compared to patients with chronic periodontal disease and periodontally healthy subjects. This is in correlate with studies by Ebersole JL et al., who found elevated levels of mean haptoglobin in periodontal disease patients compared to control group[4].

In the present study intragroup comparison of Gp C patients, showed decrease in haptoglobin levels, the values of which were non - significant from baseline to 6 months post non - surgical periodontal therapy. This finding is in correlation with studies conducted by van Santen S [11] which showed non - significant improvement in haptoglobinlevels after non - surgical periodontal therapy.In the present study intergroup comparisons of haptoglobin showed statistically non - significant mean percentage change between groups 'B and C', 'B and D' & 'C and D'. The findings of our study clearly validate that haptoglobin levels increased by1.45 ± 0.06 in patients with periodontitis, whereas in periodontitis with anemia it increased by 1.87 ± 0.08. Further, haptoglobin levels were found virtually normal in healthy patients(1.09 ± 0.04).

This study shows that there is definite correlation between haptoglobin levels with periodontal parameters at baseline. However, after 6 months of non - surgical periodontal therapy there was decrease in haptoglobin levels, though it was statistically non - significant. Thus, prima facie evidence gives an indication towards no correlation between periodontitis, anemia and haptoglobin levels.

Limitation

Small sample size of population involved in study, it is difficult to evaluate the relationship of acute phase reactive proteins with periodontitis and anemia.Further, controlled prospective trials with larger sample size with definite time frame are required to establish the true nature of the relationship between periodontal disease and anemia.

Figure 1: Armamentarium

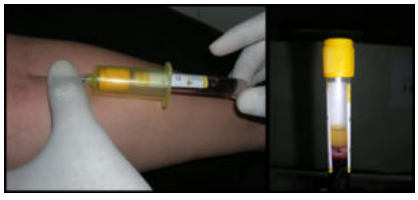

Figure 2: Blood sample collection & Serum Preparation

Figure 3: Mininephplusnephelometerand haptoglobinkit (swipe card, antiserum, buffer, controls)

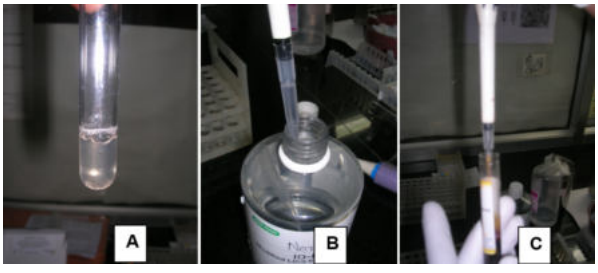

Figure 4: Serum dilution

A - Dilution of sample
B - Sample diluent
C- Serum

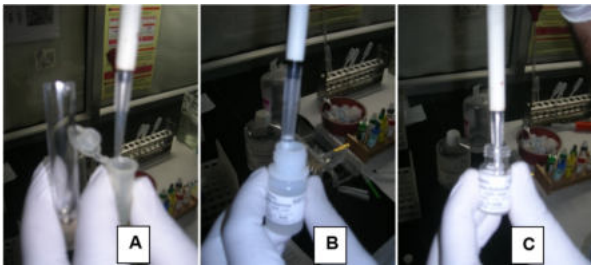

Figure 5: Addition of diluted sample and reagent into cuvette

A - Diluted sample
B - Buffer
C - Antiserum

Table 1: Intra group comparison of PDI in each study group.

Periodontal index (PDI)	Group A (n=60) [Healthy]	Group B (n=60) [Periodontitis]	Group C (n=60) [Periodontitis + Anemia]	Group D (n=60) [Anemia]
Pre-treatment	0.88 ± 0.09	5.26 ± 0.15	5.27 ± 0.16	0.887 ± 0.09
6-Months Post-treatment	--	4.57 ± 0.15	4.53 ± 0.15	0.886 ± 0.09
% Change	--	13.1%	13.9%	0.056%
Intra-Group Comparisons				
Pre-treatment v 6Months	--	0.001	0.001	0.483

Table 2: Inter group comparison of PDI (P-values).

	Inter Group Comparisons (P-values)					
Periodontal Disease Index (PDI)	Group A v Group B	Group A v Group C	Group A v Group D	Group B v Group C	Group B v Group D	Group C v Group D
Pre-treatment	0.001	0.001	0.984	0.973	0.001	0.001
6-Months Post-treatment	--	--	--	0.294	0.001	0.001
% Change	--	--	--	0.079	0.001	0.001

Table 3: Intra group comparison of haptoglobin levels in each study group.

Haptoglobin (g/L)	Group A (n=60) [Healthy]	Group B (n=60) [Periodontitis]	Group C (n=60) [Periodontitis + Anemia]	Group D (n=60) [Anemia]
Pre-treatment	1.09 ± 0.04	1.45 ± 0.06	1.87 ± 0.08	1.41 ± 0.06
6-Months Post-treatment	--	1.44 ± 0.06	1.86 ± 0.08	1.40 ± 0.06
% Change	--	0.53%	0.58%	0.54%
Intra-Group Comparisons				
Pre-treatment v 6Months	--	0.545	0.538	0.879

Table 4: Inter Group Comparison Of Haptoglobin Levels (P-values).

	Inter Group Comparisons (P-values)					
Haptoglobin levels	Group A v Group B	Group A v Group C	Group A v Group D	Group B v Group C	Group B v Group D	Group C v Group D
Pre-treatment	0.001	0.001	0.001	0.001	0.001	0.001
6-Months Post-treatment	--	--	--	0.001	0.124	0.001
% Change	--	--	--	0.679	0.962	0.512

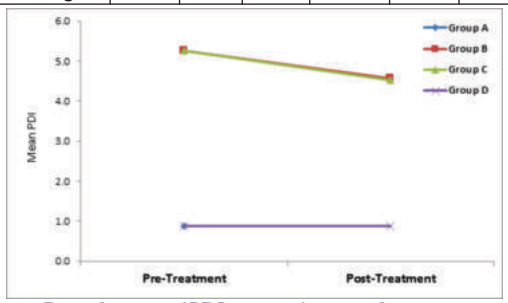

Chart 1: Distribution of PDI across four study groups.

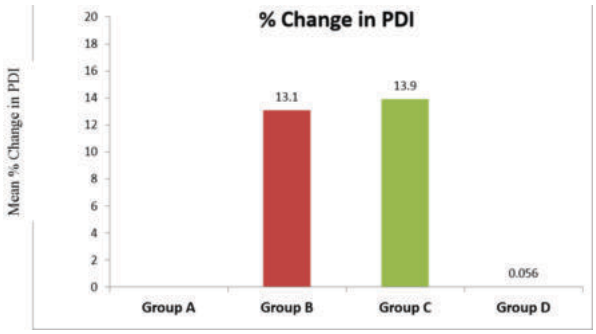

Chart 2: Distribution of % change in PDI across four study groups.

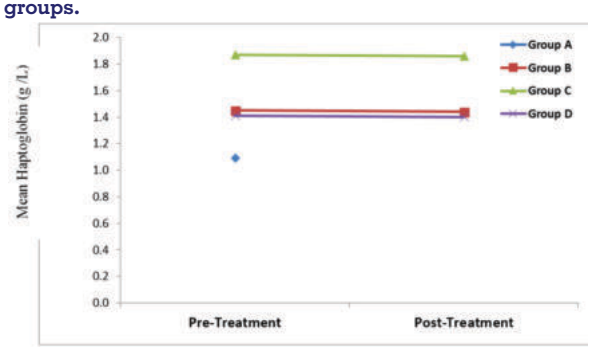

Chart 3: Distribution of haptoglobin levels across four study groups.

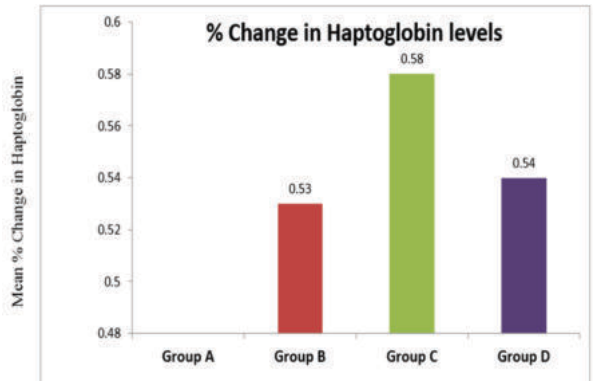

Chart 4: Distribution of % change in haptoglobin levels across four study groups.

CONCLUSION

Increases in levels of serum haptoglobin observed in study groups compared to healthy controls at baseline. The present study showed convincing evidence that haptoglobin is consistently elevated in chronic periodontitis patients and chronic periodontitis patients with anemia when compared with healthy controls, whereas haptoglobin levels decreased as the clinical parameters of periodontitis decreased in the patients after non - surgical periodontal therapy but these values are statistically not significant after 6 months. These results suggest that non - surgical periodontal therapy is not effective on periodontitis over longer period of time.Thus, it can be concluded that chronic periodontitis is not associated with anemia and elevated haptoglobin levels in periodontitis patients may exacerbate ongoing inflammatory processes. No correlation between systemic effects of anemia on severity of periodontitis has once again been confirmed.

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