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Comparative Evaluation Of Serum AmyloidA Protein LevelIn Subjects With Chronic Gingivitis And Chronic Periodontitis Before And After Scaling And Root Planing– A Clinical And A Serological Study

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Abstract:

The aim of the present study is to compare and evaluate the level of serum Amyloid A protein in subjects with chronic gingivitis and periodontitis before and after scaling and root planning. A total of 40 individuals were included in this study. 20 patients with chronic generalized gingivitis and 20 with chronic generalized periodontitis were selected. They were divided into 2 groups; Group1 included chronic generalized gingivitis and Group 2 chronic generalized periodontitis.Patients were assessed for levels of SAA. Blood samples were collected at baseline and 4 weeks after phase 1 therapy. To determine SAA levels, a sandwich enzyme-linked immunosorbent assay was utilized. Both groups showed increase in SAA Levels. Non-surgical periodontal treatment resulted in substantial reductions in the number of pockets, gingival bleeding index and oral hygiene index at 4 weeks in both the subjects with chronic gingivitis and chronic periodontitis. ($p<0.001$).However , Intervention therapy decreased the level of SAA in Chronic Gingivitis subjects as well as in chronic periodontitis subjects but Chronic Periodontitis subjects required additional surgical periodontal therapy to substantially reduce the inflammatory burden of the disease.Chronic gingivitis and periodontitis leads to elevated levels of SAA protein which may serve as a risk factor for cardiovascular disease.

Keywords: Serum amyloid A, cardiovascular disease, periodontitis , gingivitis, acute phase proteins

Introduction:

Chronic periodontitis is the consequence of interactions between certain subgingival microorganisms and multidimensional host reactions to infection. Numerous investigations have proposed that chronic periodontitis is a potential risk factor for atherothrombotic vascular disease.[1,2]The link between atherosclerotic vascular disease (ASVD) and inflammatory mediators in blood is well established, with consistent associations between levels of systemic inflammatory markers (such as CRP,SAA,Interleukins etc.) and increases in clinical event such as Myocardial infarction(MI) and non-hemorrhagic stroke, and in surrogate markers such as increased carotid intima-mediathickness(cIMT).[3] Acute phase proteins (APP) are defined as proteins whose serum concentrations is altered at least 25% in response to inflammation and includes proteins of the complement ,coagulation and fibrinolytic system , antiproteases ,transport proteins , inflammatory mediators and others. APP are the sensitive markers for evaluating the status ofinflammation. There are many acute phase proteins. Strong acute phase proteins include C- reactive proteins, α2- macroglobulin, serum amyloid A(SAA) which responds rapidly to inflammatory stimuli and their serum levels may increase several hundred folds[4].Serum amyloid A (SAA) proteins levels have been shown to positively correlate with the progress of atherosclerosis, implying that a prolonged elevation of SAA concentrations resulting from periodontitis may directly or indirectly contribute to the progress of cardiovascular disease.[5] Periodontitis being a chronic inflammatory disease results in increase in the acute phase reactant proteins and Serum amyloid A protein being one of them. It has been reported that elevated Serum amyloid A protein levels may be clinically valuable marker of cardiovascular risk[6].Hence this study was designed to evaluate and compare Serum amyloid A protein level as marker of systemic inflammation before and after scaling and root planing in subjects with gingivitis and periodontitis.

Material and Methods

Sample size calculation

The sample size was calculated to have a power of 80%. it was determined that ≥19 (for chronic gingivitis) or ≥19(for chronic periodontitis) group would be necessary.

Patients

In this study, 20 subjects with chronic gingivitis and 20 subjects with chronic periodontitis who visited the Out Patient Department of Periodontology And Oral implantalogy, were recruited . All subjects were informed

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individually about the objectives, risks, and benefits of the investigation and signed informed consent forms. The study design was approved by the Ethics Committee Systemically healthy patients in the age group between 25-60years with a diagnosis of chronic periodontitis with a probing depth of 5-6mm and chronic gingivitis according Loe and Silness gingival index,1963 were considered to be candidates for the study. Subjects who had not undergone scaling or root planing within last 6months were selected . The exclusion criteria, applied both to patients with periodontitis and to the patients with gingivitis, included a diagnosis of any systemic disorders. Pregnancy, the intake of systemic antimicrobials within the previous six months,tobacco users and subjects who had received any periodontal therapy in the past 6 months.

Clinical evaluation

The intraoral clinical examination was done at baseline and 4 weeks after phase 1 therapy.The relevant data and clinical findings were recorded on a special Performa designed for this study so as to have a systematic and methodical recording of all the information and observations. OHI-S Index (Greene and Vermillion,1964),Gingival Index (Loe and Silness, 1963) ,Probing pocket depth and clinical attachment level were recorded . At baseline, blood sample collection was done for both the groups, which was then analysed for SAA protein level estimation.Following sample collection, complete ultrasonic scaling and root planning was performed at day 1 for all patients. After 4 weeks from completion of Phase 1 therapy patients were reviewed for the clinical parameters and blood samples were collected and analysed again for SAA protein levels.

SAA estimation by ELISA Technique

To determine SAA levels, a double sandwich enzyme-linked immunosorbent assay (ELISA) was utilized. The RayBio® Human SAA (Serum Amyloid A) ELISA kit was used.Obtained samples were diluted 1:200 with a standard diluent buffer and were processed according to the manufacturer´s instructions. Optical densities were obtained at 450 nm . Standards and samples were pipetted into the wells. The wells were washed and biotinylated anti-human SAA antibody was added.After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution is added to the wells and color developed in proportion to the amount of SAA bound. The Stop Solution changed the color from blue to yellow.

Statistical Analysis

Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). The two groups were compared for parameter – OHI-S, GI, PPD, CAL using t-test. Using Mann Whitney U test, a pair wise comparison was done to analyse the differences within the groups at baseline was compared with that following between the groups. The differences within the groups at baseline were compared with that following phase 1 therapy at 4 weeks follow up.

Results

The Intragroup comparison for all clinical parameters showed higher values before phase 1 therapy and a **highly significant reduction** post phase 1 therapy in both chronic gingivitis and in chronic periodontitis group.($p<0.001$).For serum amyloid A protein values, Intragroup comparison was done using Wilcoxon Signed rank test. There was a statistically highly significant difference seen for the values .There was a statistically highly significant difference seen for the values between the time intervals ($p<0.001$) with higher values at the Baseline in both the groups (table 1)(table 2)

Table 1 : Table showing a comparison of SAA levels at baseline and after 4 weeks in Group 1

	Mean	Std. Deviation	Median	Mean diff	SD of diff	Z value	p value of **Wilcoxon Signed Ranks Test**
Baseline	94.50	7.409	93.5	9.750	4.993	-3.923	.000**
4 weeks	84.75	6.382	84				

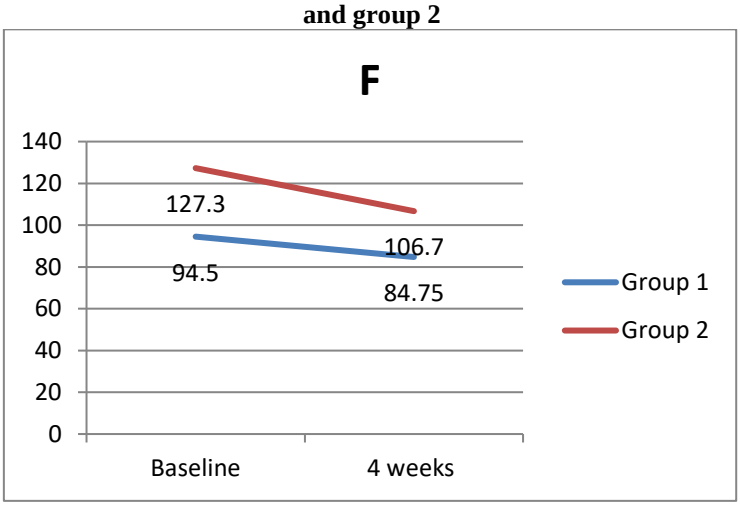
Table 2 : Table showing a comparison of SAA levels at baseline and after 4 weeks in Group 2

	Mean	Std. Deviation	Median	Mean diff	SD of diff	Z value	p value of **Wilcoxon Signed Ranks Test**

ISSN- 2394-5125 VOL 07, ISSUE 02,2020

Baseline	127.30	22.389	121	20.600	18.738	-3.922	.000**
4 weeks	106.70	6.736	107.5				

GRAPH 1: Diagrammatic presentation of pre and post treatment serum amyloid A levels in both group 1 and group 2



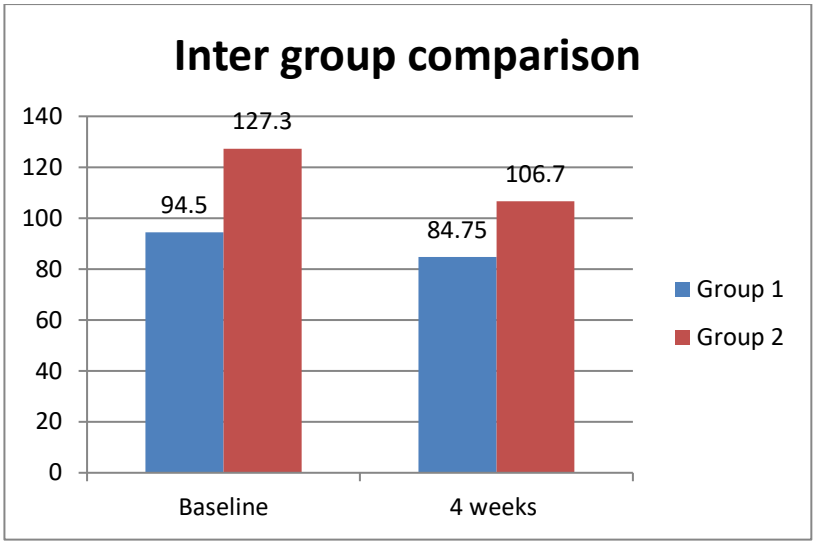
The Intergroup comparison for all clinical parameters showed **higher values pre and** post-phase 1 therapy in **group 2** as compared to groups 1($p<0.001$). For serum amyloid A levels, the intergroup comparison was done using the Mann Whitney test.

There was a statistically highly significant difference seen for the values between the groups ($p<0.001$) at baseline with higher values in group 2 and at 4 weeks there was a statistically significant difference between two groups with higher values in group 2($p<0.01$) (table 3)

	Group	N	Mean	Std. Deviation	Median	Mann-Whitney U value	Z value	p-value of Mann-Whitney U test
Baseline	1	20	94.50	7.409	93.5	3.500	-5.318	.000**
	2	20	127.30	22.389	121			
4 weeks	1	20	84.75	6.382	84	5.000	-7.281	.000*
	2	20	106.70	6.736	107.5			

GRAPH 2 : Intergroup comparison of pre and post-treatment serum amyloid A Level between group 1 and group 2

ISSN- 2394-5125 VOL 07, ISSUE 02,2020



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Discussion

During the destructive period of periodontitis, gingival inflammation increases proinflammatory cytokines and mediators, including various acute-phase proteins (APPs) such as TNF- α, α-2 macroglobulin, α-1 antitrypsin, and CRP in GCF (Ebersole&Cappelli, 2000)[7]. With the discovery of new acute phase proteins, their potential relationship with periodontal disease has provoked scientific interest. Studies have reported that APPs may be inflammatory markers of Periodontal disease (Keles et al., 2014, Pradeep et al., 2011, Balli et al., 2015)[8]for systemic diseases like cardiovascular disease and atherosclerosis. It has been reported that elevated serum amyloid A protein levels(SAA) may be a clinically valuable marker of cardiovascular risk, particularly in patients with periodontitis. Recent research has reported that elevated SAA protein levels may be a clinical valuable marker of cardiovascular disease.

GROUP 1 – GENERALIZED CHRONIC GINGIVITIS

The mean value for Serum Amyloid A (SAA) protein level at baseline was 94.50 with a standard deviation of 7.409 and 4 weeks after scaling and root planning was 84.75 with a standard deviation of 6.382. Using Wilcoxon signed-rank test, P-value was found to be <0.001, indicating that there was a statistically highly significant decrease in the Serum Amyloid A (SAA)levels from baseline to 4 weeks after completion of scaling and root planning in subjects with Generalized chronic gingivitis. The present study showed that chronic gingivitis and chronic periodontitis were discriminatively related to elevated levels of SAA.

Following are the studies which were in accordance to our study.

M. Kweider et al(1993)[9] conducted a study to evaluate Serum Amyloid A protein, white cell count and plasma fibrinogen and were compared in 50 patients with Gingivitis and in 50 controls with healthy periodontal tissues. Results showed that the patients had significantly higher levels of Serum Amyloid A protein, white cell count and fibrinogen. The dental indices correlated with these two cardiovascular risk factors on multivariate analyses. In our study the clinical parameters namely pocket probing depth (PPD), oral hygiene index simplified and gingival index (GI) were also evaluated and showed significant reduction after phase 1 therapy in both the groups.

Song et al 2020[10] SAA were up-regulated in inflammatory gingival tissues, which indicates that inflammatory gingival tissues are one of local sources of SAA. He concluded SAA is expressed strongly in inflammatory gingival tissues, and triggers inflammatory cytokine secretion via interacting with TLR2 pathway in human gingival fibroblasts.

However , a study conducted by **Vuletic et al. (2008)**[11] showed contrasting results as compared to our study. According to him the SAA levels did not show a statistically significant correlation with any pre-treatment periodontal disease parameters.

GROUP 2 – GENERALIZED CHRONIC PERIODONTITIS

The mean value for Serum Amyloid A (SAA) protein level at baseline was 127.30 with a
Standard deviation of 22.389 and 4 weeks after scaling and root planing was 106.70 with a
Standard deviation of 6.736 . Using Wilcoxon signed rank test, P value was found to be <0.001, indicating that there was a statistically highly significant decrease in the Serum Amyloid A (SAA)levels from baseline to 4 weeks after completion of scaling and root planing in subjects with generalized chronic periodontitis.

The results of our study correlated to the following study.

ISSN- 2394-5125 VOL 07, ISSUE 02,2020

Carlos Martín Ardila and Isabel Cristina Guzmán(2015)[12], conducted a study on the levels ofserum amyloid A (SAA) proteins and highly sensitive C reactive protein levels as a markersof systemic inflammation in patients with chronic periodontitis. They concluded that serumamyloid A (SAA) and highly sensitive C reactive protein concentrations in patients withchronic periodontitis were elevated, suggesting that both are good markers of inflammationin such patients.

Graziani F et al.(2010)[13]conducted a study to describe the kinetics of serum inflammatory markers after a course of treatment comprising surgical and non-surgical treatment of chronic periodontitis (CP). Blood markers of systemic inflammation including leucocyte counts, C-reactive protein (CRP), serum amyloid-A (SAA) and D-dimers and renal function (cystatinC) were determined Periodontal treatment resulted in substantial reductions of the number of pockets, gingival bleeding and plaque after non-surgical therapy($p<0.001$). Surgical therapy led to an additional reduction of periodontal pockets.

Aoki-Nonaka et al.(2014)[14]have shown that experimental periodontal infection induces elevated serum levels of SAA and P. gingivalis-specific IgG.

In contrast , Pussinen et al. (2004)[15]conducted a study in 30 otherwise healthy subjects with periodontal disease which showed no differences in SAA levels 3 months after periodontal treatment.

COMPARISON BETWEEN GROUP 1 AND GROUP 2

The Serum amyloid A protein level with a mean of 94.50±7.409 in the Group -1 and 127.30±22.389 in the Group-2 at baseline shows a highly statistically significant difference with a P value of P = 0.001 . The Serum amyloid A protein level were elevated in Group 2 (i.e., Generalized chronic periodontitis) as compared to Group1 (i.e., Generalized chronic gingivitis). The level was measured 4 weeks after phase 1 therapy in chronic Gingivitis patients (Group-1), 84.75 ± 6.382 and chronic periodontitis (Group-2), 106.70±6.736. There was statistical difference between the two groups. It was observed that phase1 therapy shows highly statistically significant improvement in clinical parameters as well as in the levels of serum amyloid A Protein. One of the possible reasons for greater level of SAA protein in chronic periodontitis patients as compared to chronic gingivitis patients could be the clinical characteristics of periodontitis that result from a microorganism infection and the host response. Production of SAA is stimulated by proinflammatory cytokines such as IL-6, IL-1, TNF, IFNγ, and transforming growth factorβ (TGFβ) (Nakamura, 2011, Migita et al., 2011).Direct measures of the infection, comprising the quantification of subgingival bacteria and antibody responses to these periodontopathogens, have been recommended as a more correct metrices of periodontitis for investigations of the relationship between periodontal and systemic disease.The results from the study byCarlos Martín Ardila and Isabel Cristina Guzmán showed that periodontitis and IgG2 antibody to P. gingivalis were independently associated with higher concentrations of SAA and hs-CRP, controlling for various potential confounder.

Türer, Ç.,Ballı, U., &Güven, B. (2017)[16] in their study showed increased levels of serum SAA were in the CP group. Moreover, the gingivitis group showed higher levels of SAA than the periodontal healthy group.

Results of our study showed that Group-1 subjects((i.e., Generalized chronic gingivitis) had statistically significant decrease in level of Serum Amyloid A protein,4 weeks after scaling and root planing. Group-2 subjects((i.e., Generalized chronic periodontitis)showed statistically significant decrease in level of Serum Amyloid A protein 4 weeks after scaling and root planing. In the current study, patients with chronic gingivitis and chronic periodontitis had SAA concentrations higher than 100 ug/ml, which are considered abnormally high values indicating systemic inflammation and possible infection.

However , after phase 1 therapy the levels significantly dropped in both the groups in range between 20ug/ml-100ug/ml indicating resolution process of inflammation and with an overall trend towards a reduction.[17]

Certain limitations of the study were taken into consideration, firstly, the severity of gingivitis and periodontitis was not considered. Secondly, as by the study performed by Graziani et al[13] , Phase 1 therapy resulted in substantial reduction in the SAA levels in chronic periodontitis group, however, additional reduction in the SAA levels could have been achieved if appropriate surgical intervention therapy was performed. Lastly, additional longitudinal studies are needed to confirm these findings.

Conclusion

Chronic gingivitis and periodontitis leads to elevated levels of SAA protein which may act as a risk factor for cardiovascular disease.

Intervention therapy may decrease the level in Chronic Gingivitis subjects as well as in chronic periodontitis subjects but Chronic Periodontitis subjects may require additional surgical periodontal therapy to substantially reduce the inflammatory burden of the disease thereby lowering the risk and improving the overall health and wellbeing of the patient. From the above findings, it is evident that SAA which is an important marker for cardiovascular disease, is also seen to increase in gingivitis and periodontitis. Thus, Periodontists will play a crucial role in imparting this knowledge amongst the medical fraternity. To elucidate the exact relationship between gingivitis, periodontitis and cardiovascular disease, further longitudinal and interventional studies with larger sample size should be carried out.

ISSN- 2394-5125 VOL 07, ISSUE 02,2020

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