Original Study Article

Comparison of serum heat shock protein 60 levels in patients with periodontal disease and cardiovascular disease

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ABSTRACT

Self-antigens such as heat shock protein 60 (HSP 60) has been implicated recently in the pathogenesis of both periodontal and cardiovascular disease. There are some evidence regarding HSP 60 and HSP 70 in circulation following periodontal disease and cardiovascular disease and its possible relationship. Forty-five peripheral blood samples were collected from two groups of patients. Group I – periodontal disease and group II – cardiovascular disease. Serum was used to detect and compare HSP 60 in both the groups by enzyme-linked immune sorbent assay (ELISA) technique. Statistical analysis was done student t test. Serum HSP 60 significantly increases in cardiovascular disease compared to periodontal disease.

Key words: Cardiovascular disease, ELISA, HSP 60, periodontitis, peripheral blood

INTRODUCTION

Periodontitis is defined as an inflammatory disease of the supporting tissue of the teeth caused by specific micro-organism or groups of specific microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both.[1] The inflammatory response in periodontal disease and cardiovascular disease (CVD) is evoked primarily in response to exogenous antigens (bacterial lipopolysaccharides, capsules, fimbriae, outer membrane proteins, etc.). Structural similarities of these exogenous antigens with host antigens like heat shock proteins (HSP) may result in molecular mimicry, thereby resulting in activation of the immune system.[2]

HSP are perhaps the most extensively investigated self antigens involved in periodontal disease and CVD. In response to stress stimuli, including high temperature, mechanical stress, infection, surgical stress, oxidant and cytokine stimulation, HSP are produced. HSP is otherwise called Molecular Chaperones. It is a mitochondrial chaperonin that assists in folding linear amino acid chains into the respective 3-dimensional structure.[3] HSP 60 has been reported to upregulate E- selection, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-I) expression, and interleukin (IL)-6 production in endothelial cells and thereby contribute to atheromatous plaque formation.[4]

Petit *et al.*, Ueki *et al.* have described a role for HSP 60 in periodontal disease and have demonstrated an upregulation of HSP 60 expression in gingival tissues affected by periodontal disease.[5,6]

In recent years, serum has been postulated to be capable of reflecting not only systemic disease such as CVD,

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rheumatoid arthritis, Sjogrens syndrome but also periodontal disease. The purpose of this study is to evaluate serum levels of HSP 60 in patients with periodontal disease and CVD.

AIM

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MATERIALS AND METHODS

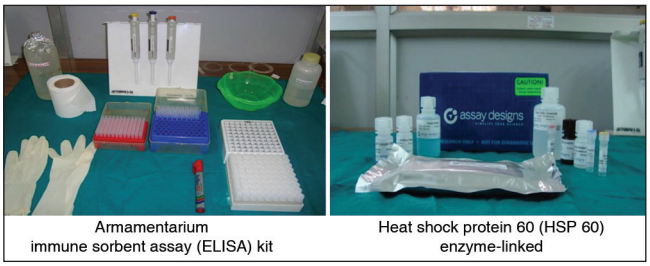
Forty-five patients (22 males And 23 females) who attended the outpatient department of Peridontology, Vivekanandha Dental College for Women and CVD block Vivekanandha Medical Care Hospital, Elayampalayam were enrolled in this study. Patients were divided into two groups according to the patients' periodontal and cardiovascular status. Informed consent was obtained from all the patients. The patients were informed that this research work was in no way directly related to the therapy or cure of the disease.

Group 1: Patients with periodontal disease: Patients with teeth exhibiting probing pocket depth >5 mm and clinical attachment loss >3 mm of radiographic evidence of bone loss in atleast 6 teeth.[7]

Group 2: Patients with CVD: Patients with positive coronary angiography.

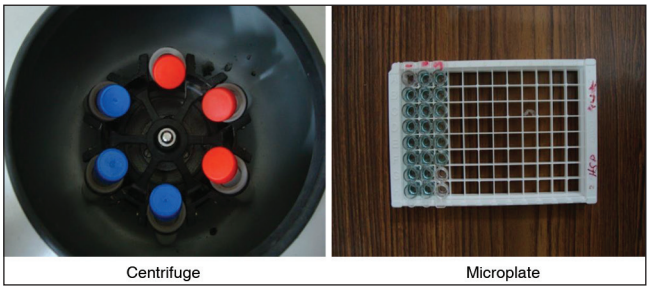
Sample preparation

Two milliliter of blood was drawn by vene puncture in anticubital fossa. The blood was allowed to clot at room temperature for 30 mins and centrifuged at 3000 rpm for 10 mins and stored at -70°C and used for further analysis.



Armamentarium immune sorbent assay (ELISA) kit | Heat shock protein 60 (HSP 60) enzyme-linked

Figure 1: Armamentarium, Hsp 60 Kit



Centrifuge | Microplate

Figure 3: Centrifuge, Microplates

RESULT

The present study assessed the serum levels of HSP 60 in patients with periodontal disease and CVD by sandwich enzyme-linked immune sorbent assay (ELISA) method. Absorbance was measured at 450 nm wavelength in terms of ng/dl and results were obtained. The mean serum HSP 60 in CVD was 80.89 ng/dl, whereas mean serum HSP 60 in periodontal disease was 53.54 ng/dl. There was a significant increase in the CVD when compared to periodontal disease at *P* value < 0.05 (0.044) [Table 1] [Figures 1-5].

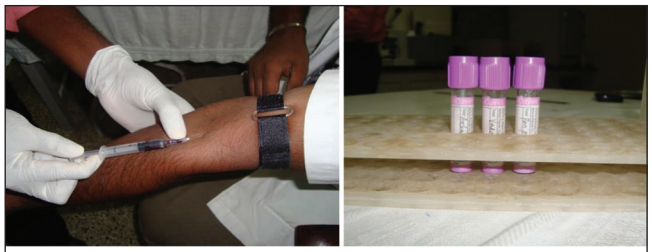
DISCUSSION

Although periodontal bacteria are the primary etiological agents in periodontal disease, the ultimate determinant of the disease progression and clinical outcome is the hosts immune response. The most studied self-antigen in periodontal disease is the heat shock protein, especially HSP 60. HSP 60 has been reported to be significantly elevated in gingival tissues affected by periodontal disease, but there is no conclusive evidence linking it to disease activity. HSP's are typically regarded as intracellular proteins but free serum

Table 1: Serum HSP-60 levels

Groups	Number of cases	Mean HSP-60 ng/dl	Standard deviation	*P* value
Periodontal disease	22	53.54	30.52	0.044*
Cardiovascular disease	23	80.89	49.57	

* = *P* < 0.05 – statistically significant, *P* > 0.05 – not significant
HSP-60 = heat shock protein 60

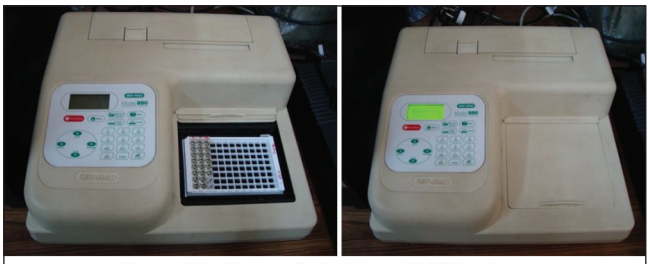


Venupuncture | Vaccutainer

Figure 2: Blood Collection and Storage

Automatic washervortex mixer

Figure 4: Atomatic Washer, Vortex Mixer



Enzyme-linked Immune Sorbent Assay (ELISA) reader

Figure 5: Elisa Reader

HSP 60 has been reportedly identified and linked to systemic inflammation and CVD.[8-11]

This study was undertaken to investigate if periodontal disease could contribute to the circulating HSP 60 levels in a manner similar to that of circulating proinflammatory cytokines.

The ELISA method was used to evaluate HSP 60 in preference to immunoblotting as it is versatile, accurate, reproducible, and economical. Previous studies done by Tabeta *et al*. (2001)[12], Watanabe S *et al*. (2003)[13], and Handley HH *et al*. 1995 [14] provide evidence that supports the use of this method.

The association between periodontal disease and CVD has gained enormous attention in recent years. Several investigators have found a strong association between the two diseases to the extent that joined consensus report between the association of American cardiologists and Periodontologists has addressed this issue. The numberof pathogenic mechanismshas been suggested by several authors through which periodontal disease may influence CVD. Our study indicate that HSP 60 could be considered another potential candidate through which periodontal disease influences the systemic inflammatory state and thereby CVD. The role of inflammation in the development of atheromatous plaque is now well established.[15]

There are several limitations in this study such as a small sample size which may affect statistical results, its cross-sectional nature; longitudinal studies are better suited to study the episodic nature of periodontal disease and CVD and its biomarkers, immunolocalization of HSP 60 in gingival tissues could have perhaps given a more complete picture.

These results certainly warrant a more detailed investigation which might help clarify the role of these proteins in periodontal and CVD.

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