

# Heat Shock Protein-60 Levels in Serum and Saliva of Patients with and Without Periodontitis- A Comparative Study

Rajasekar S<sup>1\*</sup>, Anitha CM<sup>2</sup>, Senthil Kumar S<sup>3</sup>, Sethupathy S<sup>4</sup>, John William Felix<sup>5</sup>

<sup>1</sup>Professor & Head Department of Periodontics, Rajah Muthiah Dental College & Hospital, Annamalai University Chidambaram- 608 002, Tamil Nadu, India

<sup>2</sup>Assistant Professor, Department of Periodontics, SRM Dental College & Hospital, Ramapuram, Chennai, Tamil Nadu, India

<sup>3</sup>Dean & Professor, Department of Periodontics, Rajah Muthiah Dental College & Hospital Annamalai University, Chidambaram-608 002, Tamil Nadu, India

<sup>4</sup>Professor of Bio Chemistry Rajah Muthiah Medical College & Hospital Annamalai University Chidambaram-608 002, Tamil Nadu India

<sup>5</sup>Reader cum statistician Department of Community Medicine Rajah Muthiah Medical College & Hospita Annamalai University Chidambaram-608 002, Tamil Nadu India

DOI: [10.36348/sjodr.2021.v06i05.007](https://doi.org/10.36348/sjodr.2021.v06i05.007)

| Received: 09.04.2021 | Accepted: 17.05.2021 | Published: 23.05.2021

\*Corresponding author: Dr. S. Rajasekar

## Abstract

**Background and Objectives:** There is increasing evidence that mitochondrial dysfunction and oxidative stress may be central to both chronic periodontitis and type 2 diabetes mellitus pathogenesis. HSP 60 is a mitochondrial stress protein believed to be caused by mitochondrial dysfunction. The aim of this research was therefore to test the salivary and circulatory expression of HSP 60 in periodontitis patients. **Methods:** A total of 30 patients aged 35-50 were chosen and classified into two groups; (1) Healthy controls; (2) Chronic periodontitis and systemically healthy. HSP 60 in serum and saliva was estimated using a specific ELISA kit and correlated with periodontal parameters using statistical tests. **Results:** The serum and salivary levels of heat shock proteins were significantly higher in chronic periodontitis patients compared to healthy controls. **Conclusion:** Salivary levels of HSP 60 can be used as a biomarker to determine periodontitis severity. Patients with chronic periodontitis had higher salivary HSP 60 levels. **Key words:** Chronic periodontitis, diabetes mellitus, heat shock proteins, saliva, and serum.

**Copyright © 2021 The Author(s):** This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

## INTRODUCTION

There is a widespread interest in the cellular mechanisms utilized by an organism to cope with disruption in homeostasis. There is substantial evidence that Heat shock proteins (HSPs) are widespread in all species and play important physiological roles both under normal conditions and under conditions involving both systemic and cellular stress [1].

Cells produce HSPs on encountering physiological stress such as heat stress or chemical stimulation [2]. Different cells generate HSPs on stress and chemical stimulation, and these so-called stress proteins perform many cellular functions, including cell defense, intracellular assembly, folding and translocation of oligomeric protein [2].

Based on their size, structure and function, HSPs can be classified into five family groups (HSPs

110, 90, 70, 60, 27 and other small molecule HSPs). HSP60 family called GROEL in prokaryotes exists primarily in mitochondria, cytoplasm, with regular levels of expression in normal situations and is associated with intracellular protein translocation to mitochondria [2].

Periodontitis is a multifactorial disease that occurs in the presence of microbial challenge as well as genetic, environmental, and acquired risk factors. While periodontal bacteria are the causative agents in periodontitis, host immune responses are thought to determine subsequent progression and severity of disease. One of the target antigens of this auto immune response is heat shock protein. Periodontopathic bacteria such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Bacteroides forsythus*, and *Campylobacter rectus* have been found to

develop HSPs that are homologous to *Escherichia coli* GroEL [2].

It is also hypothesized that periodontopathic bacteria induce cells inside the periodontium to upregulate the expression of HSP 60, which in turn will stimulate macrophages and possibly other cells to supply proinflammatory cytokines. These pathways may be associated with periodontal disease chronicity and tissue destruction [3].

Periodontitis patients had considerably higher levels of seropositivity and titres of antibodies against human HSP60 and *Porphyromonas gingivalis* GroEL than periodontally stable test groups [3].

The current research sought to test the hypothesis that HSP 60 levels were higher in periodontitis patients than in periodontally stable subjects. The aim of this study was to compare the serum and salivary concentrations of heat shock protein 60 in patients with chronic periodontitis to those in periodontally stable controls.

## METHODOLOGY

This cross-sectional comparative study was performed from 30-1-2016 to 30-9-2016 by the Department of Periodontics. Ethical research approval was provided by the Institutional Human Ethical Committee and informed consent from all the patients involved in the experiment was obtained.

A total of 30 subjects (19 males and 11 females) in age range 35 -50 years attending the outpatient department were selected for the study. The sample size for the present study was determined by SYSTAT 12 power analysis where Kruskal Wallis test was applied to calculate the sample size based on the pilot study.

The selected patients who fulfilled the criteria were divided into

Group 1 (10) Healthy controls: Probing pocket depth (PPD)  $\leq$  3mm, no clinical attachment loss (CAL) and Bleeding on probing (BoP) at <10 % sites)

Group 2 (20) Moderate to severe chronic periodontitis and systemically healthy. (PPD and CAL > 3mm and BoP in >30% of sites)

### Exclusion criteria

1. Patients with any systemic disease.
2. History of hospitalization or intake of systemic antibiotics in the preceding 6 months.
3. Aggressive periodontitis.
4. Smokers and alcoholics.
5. Pregnant and lactating women.
6. Previous history of periodontal therapy in the last 6 months.

These were excluded as to avoid the added systemic effects and periodontal inflammatory burden that may interfere with the results of the present study.

### Clinical examination

The clinical examination was carried out by a single examiner using a sterile mouth mirror, dental explorer and Williams periodontal probe and following clinical parameters were recorded at the initial visit;

1. Plaque index (Silness and Loe 1963) [4]
2. Gingival bleeding index (Ainamo and Bay) [5]
3. Probing pocket depth [6]
4. Clinical attachment loss [6]
5. Anthropometric measurements

Body mass index (BMI) [7] was calculated as weight divided by square of height meters ( $\text{kg/m}^2$ )

Normal range: 19 – 24.9

Over weight : 25 – 29.9

Obese : > 30

### Collection of serum

Antecubital fossa venepuncture was used to collect three mL of blood from each patient for HSP 60 calculation. The samples were moved to test tubes and left at room temperature to coagulate. The serum and plasma were separated from the blood by centrifugation at 3000 rpm for five minutes. The serum and plasma were then moved to Eppendorf vials and stored at  $-20^\circ\text{C}$  until the assay was completed.

### Collection of saliva

The entire unstimulated saliva was collected in a sterile plastic disposable container, and the samples were stored at  $-20^\circ\text{C}$  and used for further testing. Saliva was collected in accordance with Navazesh's technique [8].

In serum and saliva samples, HSP 60 was measured using an appropriate ELISA package 'STRESS MARQ<sup>TM</sup>'. Biochemical analysis was carried out according to the manufacturer's instructions, and 96 precoated well plates with appropriate antibodies were used.

## STATISTICAL ANALYSIS

With the assistance of SYSTAT 12 software, data obtained from clinical and biochemical interpretations were analysed and the following findings were extracted, using student T test.

## RESULTS

The plaque scores as evident from table-1 were significantly higher in Group-2 (Chronic periodontitis)  $2.853 \pm 7.195$  compared to Group 1(healthy control)  $0.306 \pm 0.154$  with a p value <0.001.

Table 2 shows the mean Gingival bleeding index scores between group-1 ( $2.22 \pm 1.02$ ) and group-2 ( $74.27 \pm 9.06$ ) with a p value  $< 0.001$  which is statistically significant.

The probing depths were significantly less for group-1 ( $2.40 \pm 0.12$ ) than the group-2 ( $5.83 \pm 1.06$ ) with a p value  $< 0.001$  (Table 3)

There was no clinical attachment loss in group-1 patients whereas in group 2 the clinical attachment loss was  $6.06 \pm 0.87$  and this was statistically significant with a p value  $< 0.001$  (Table 4).

Table 5 displays the body mass index of subjects in group-1 ( $23.58 \pm 4.15$ ) and group-2 ( $24.38 \pm 4.13$ ) where the difference was non-significant (p value 0.521)

Table 6 revealed the comparison of HSP-60 levels in serum between the two groups. The levels were significantly higher for group-2 ( $4.66 \pm 2.31$ ) than for group-1 ( $2.34 \pm 0.77$ ) with a p value  $< 0.001$ .

Table 7 revealed the comparison of HSP-60 levels in saliva between the two groups. The levels were significantly higher for group-2 ( $30.68 \pm 19.81$ ) than for group-1 ( $4.51 \pm 1.65$ ) with a p value  $< 0.001$ .

**Table-1: Plaque Index Mean And Sd Groupwise**

GROUP	N	MEAN	SD	MINIMUM	MAXIMUM	P VALUE
1.(Healthy)	10	0.306	0.154	0.130	0.560	$< 0.001$ (s)
2. (CP)	20	2.853	7.195	2.350	3.780	

P $<0.05$ - Significant, P $< 0.001$ - Highly significant CP – chronic periodontitis

**Table-2: GINGIVAL BLEEDING INDEX Mean and SD Groupwise**

GROUP	N	MEAN	SD	MINIMUM	MAXIMUM	P VALUE
1.(Healthy)	10	2.22	1.02	3	4.47	$< 0.001$ (s)
3. (CP)	20	74.27	9.06	71	89	

P $<0.05$ - Significant, P $< 0.001$ - Highly significant CP – chronic periodontitis

### 3. Probing pocket depth

**Table-3: Mean and SD Groupwise**

GROUP	N	MEAN	SD	MINIMUM	MAXIMUM	P VALUE
1.(Healthy)	10	2.40	0.12	2.00	3.00	$< 0.001$ (s)
3. (CP)	20	5.83	1.06	5.00	7.00	

P $<0.05$ - Significant, P $< 0.001$ - Highly significant CP – chronic periodontitis

### 4. Clinical attachment loss

**Table-4: Mean and SD Groupwise**

GROUP	N	MEAN	SD	MINIMUM	MAXIMUM	P VALUE
1.(Healthy)	10	0.00	0.00	0.00	0.00	$< 0.001$ (S)
3. (CP)	20	6.06	0.87	5	7.00	

P $<0.05$ - Significant, P $< 0.001$ - Highly significant CP – chronic periodontitis

**Table-5: BODY MASS INDEX (BMI)**

GROUP	N	MEAN	SD	MINIMUM	MAXIMUM	P VALUE
1.(Healthy)	10	23.58	4.15	21	32	0.521 (NS)
3. (CP)	20	24.38	4.13	19	34	

CP – chronic periodontitis

**Table-6: HSP 60 LEVELS IN SERUM (mg/ml)- Mean and SD Groupwise**

GROUP	N	MEAN	SD	MINIMUM	MAXIMUM	P VALUE
1.(Healthy)	10	2.34	0.77	0.30	3.47	<0.001 (s)
3. (CP)	20	4.66	2.31	1.85	8.79	

P<0.05- Significant, P< 0.001- Highly significant CP – chronic periodontitis

**Table-7: HSP 60 LEVELS IN SALIVA (mg/ml)**

GROUP	N	MEAN	SD	MINIMUM	MAXIMUM	P VALUE
1(Healthy)	10	4.51	1.65	3.07	5.98	< 0.001 (s)
2.(CP)	20	36.68	19.81	21.29	64.44	

P<0.05- Significant, P< 0.001- Highly significant CP – chronic periodontitis

## DISCUSSION

Periodontitis is a chronic inflammatory disease, in which severe forms of the disease are associated with specific bacteria that have colonized the subgingival area regardless of the host's protective mechanisms [9].

Heat shock protein 60 belongs to a family of proteins that have survived evolution. Extracellular and membrane-bound heat shock proteins aid in antigen binding and presentation to the immune system. Heat shock protein 60s (HSP60s) are extremely immunogenic, with T-cell and antibody responses to HSP60 being identified in a variety of inflammatory conditions [3].

Immune reactions to HSP60s are thought to cause chronic inflammatory disorders, with the autoimmune reaction to human HSP60 being critical to pathogenesis [10].

HSP 60 has been reported to be implicated in the pathogenesis of a variety of chronic diseases, including Periodontitis and Diabetes mellitus [13]. The aim of this research was therefore to examine the serum and salivary expression of HSP60 in patients with chronic periodontitis [11].

In our study, all clinical periodontal parameters, such as plaque index, GBI, PPD and CAL, were significantly higher in the test group than in control. However, there was no significant difference observed in the body mass index scores between the two groups.

The results of our results are close to those produced by Kazuhisa Yamazaki *et al.*, who hypothesized that pathogenic bacteria induce periodontal cells to increase HSP60 production, which may, in effect, trigger macrophages to develop pro-inflammatory cytokines [12].

In the present study the HSP 60 level in both serum and saliva was significantly higher in the group 2 (chronic periodontitis) than the group 1(periodontally

healthy). These findings were in accordance with the observations of the previous study.

In our sample, the salivary HSP60 levels were 4 to 5 times higher than the serum HSP60 levels. This finding was close to the observations of Mathew B Fortes and Martin Whitham, who studied the impact of exercise on salivary and plasma HSP72 and found that there was a higher concentration of HSP72 in saliva than in plasma [13]. This suggests that passive blood exudates do not significantly increase the salivary level of HSP. Indeed, saliva serves as a first line of defense against pathogenic threats and contains a variety of proteins with anti-bacterial functions; thus, the presence of HSP60 in saliva at these elevated concentrations can aid in periodontal defense and repair. In our research, serum and saliva were used as tools because they represent systemic and local influences, respectively, and saliva collection is non-invasive.

HSP60 levels in saliva can be used as a biomarker for periodontal inflammation, and saliva can be a non-invasive method for testing this stress protein. To validate our results, more longitudinal and interventional research with a larger sample size is needed.

## REFERENCES

1. Farjam, A. S. (2011). Heat Shock Proteins as Modifying Factors in Physiological Stress Responses in Poultry (Doctoral dissertation, Universiti Putra Malaysia).
2. Yeh, Y. C., Kung, S. F., Hsieh, Y. D., Tsai, C. C., Jiang, Y. H., & Ho, K. Y. (2008). The Role of Heat Shock Protein 27/60 in Periodontitis and Cardiovascular Disease. *Journal of Dental Sciences*, 3(1), 1-12.
3. Yamazaki, K., Ueki- Maruyama, K., Honda, T., Nakajima, T., & Seymour, G. J. (2004). Effect of periodontal treatment on the serum antibody levels to heat shock proteins. *Clinical & Experimental Immunology*, 135(3), 478-482.
4. Silness, J., & Loe, H. (1964). Periodontal disease in pregnancy II. Correlation between oral hygiene and

- periodontal condition. *Acta odontologica scandinavica*, 22(1), 121-135.
5. Ainamo, J., & Bay, I. (1975). Problems and proposals for recording gingivitis and plaque. *International dental journal*, 25(4), 229-235.
  6. Armitage, G.C. (2000). The complete periodontal examination. *Periodontol*, 34, 22-23
  7. Zimmermann, G. S., Bastos, M. F., Dias Gonçalves, T. E., Chambrone, L., & Duarte, P. M. (2013). Local and circulating levels of adipocytokines in obese and normal weight individuals with chronic periodontitis. *Journal of Periodontology*, 84(5), 624-633.
  8. Navazesh, M. (1993). Methods for collecting saliva. *Annals of the New York Academy of Sciences*, 694(1), 72-77.
  9. Genco, R. J. (1996). Current view of risk factors for periodontal diseases. *Journal of periodontology*, 67, 1041-1049.
  10. Kiessling, R., Gronberg, A., Ivanyil, J. (1991). Role of HSP 60 during autoimmune and bacterial inflammation. *Immunol Rev*, 121, 91-112
  11. Grover, H. S., & Luthra, S. (2013). Molecular mechanisms involved in the bidirectional relationship between diabetes mellitus and periodontal disease. *Journal of Indian Society of Periodontology*, 17(3), 292.
  12. Yamazaki, K., Ohsawa, Y., Tabeta, K., Ito, H., Ueki, K., Oda, T., & Seymour, G. J. (2002). Accumulation of human heat shock protein 60-reactive T cells in the gingival tissues of periodontitis patients. *Infection and Immunity*, 70(5), 2492-2501.
  13. Fortes, M. B., & Whitham, M. (2011). Salivary Hsp72 does not track exercise stress and caffeine-stimulated plasma Hsp72 responses in humans. *Cell Stress and Chaperones*, 16(3), 345-352.