ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2016; 2(4): 23-25

www.oraljournal.com
Received: 07-08-2016
Accepted: 08-09-2016

Dr. Mithra N Hedge
Vice Dean and HOD,
Department of Conservative Dentistry and Endodontics
A.B. Shetty Memorial Instituite of Dental Sciences, Nitte University, Deralakatte
Mangalore, Karnataka, India

Nireeksha
Department of Conservative Dentistry and Endodontics
A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Deralakatte
Mangalore, Karnataka, India

Dr. Suchetha Shetty
Professor, Department of Biochemistry K.S. Hegde Medical academy, Nitte University, Deralakatte
Mangalore, Karnataka, India

Correspondence
Nireeksha
Department of conservative Dentistry and Endodontics
A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Deralakatte
Mangalore, Karnataka, India

Saliva as a biomarker of heat shock protein 70 in poor oral health

Dr. Mithra N Hedge, Nireeksha and Dr. Suchetha Shetty

Abstract
Background: The Heat shock proteins are stress induced and there is increased evidence of presence of these chaperons extracellular which is considered to be intracellular. Their present cytokine like effect and help in immune recognition, thus indicate strong extracellular action of heat shock protein 70 in maintaining integrity of oral cavity.
Hypothesis: The aim of this study was to evaluate the Circulatory and salivary heat shock protein level 70 in healthy individuals and individuals with poor oral health.
Evaluation: Settings and Design: 80 patients attending to the outpatient department of A.B. Shetty memorial institute of dental sciences, Mangalore during the period of October 2015 to November 2015 were included in the study. Control group (N=40) includes healthy individuals with good oral hygiene (OHIS SCORE > 3) and Experimental group (N=40) includes individuals with poor oral hygiene (OHIS< 3).
Methods and Material: Saliva and serum samples were evaluated for Heat shock protein 70 levels in test and control group by ELISA kit method.
Statistical analysis used: Student's t test was used for statistical analysis, which was expressed in terms of mean and standard deviation. $P<0.05$ was considered to be statistically significant.
Results: There was a significant increase in Serum Heat shock protein 70 levels in Experimental group (5.87 ng/ml) in comparison with the control group (3.170ng/ml).
Salivary Heat shock protein 70 shock showed significant levels in experimental levels (4.21 ng/dl) compared to control group (2.641ng/dl)
Conclusions: Heat shock proteins 70 are efficient cell stress marker in poor oral health condition

Keywords: Cell stress, heat shock protein 70, poor oral health

Introduction
The Heat shock proteins are stress induced and there is increased evidence of presence of these chaperons extracellular which is considered to be intracellular. Their cytokine effect helps in immune recognition, thus indicate strong extracellular action of heat shock protein 70 in maintaining integrity of oral cavity [1].
Total salivary protein ranges from 0.5 to 3 mg/dl. Heat shock protein 60,70,90 are present in saliva due to passive transport from blood serum. Source of heat shock protein in saliva is largely from the salivary glands, mucosal cells, periodontal tissues either from exudate or direct blood contamination and other sources from the blood stream [2].
The various immunological and cytoprotective action of salivary HSP 70 is due to the defence action of these proteins by protecting the mucosal and periodontium by unspecific defensive alert system. Their presence show pronounced immune functions in poor oral health [3].
HSP 70 hasthe capacity to entrap gram positive and gram negative bacteria's and agglutinate them thus increasing the agglutinating capacity of saliva. Surface defence against toxins is one of the most important and major cytoprotective role of these proteins, thus decreasing cell apoptosis and necrotic liability. The facets of HSP 70 on immune activation may be due to release of pro-inflammatory cytokines from various immune cells, binding of un-complexed HSP 70 to other peptides and cross presenting their complex to cytotoxic T cells and NK cells helping in defence against bacteria's, tumour cells and virus infected cells. HSP 70 also exerts an opsonizing effect on bacteria, by acting on polymorph nuclear neutrophil granulocytes [4].
Thus, the aim of this study was to evaluate the Circulatory and salivary heat shock protein level 70 in healthy individuals and individuals with poor oral health.

Subjects and Methods

Study Population

After obtaining institutional ethical clearance, 80 patients attending to the outpatient department of A.B. Shetty memorial institute of dental sciences, Mangalore during the period of October 2015 to November 2015 were included in the study .Oral hygiene status was evaluated to divide them into test and control group. Oral hygiene index and DMFT index (WHO 2013) was used to evaluate the oral hygiene. Oral hygiene status was considered poor if the total score is more than 3(OHIS-Index).

Individuals under the age group of 35 to 60yrs, with poor oral health and willing to be a part of the study were included in the study. Individuals with any systemic condition, pregnant and lactating women's, Smokers were excluded from the study.

Control group (N=40) includes healthy individuals with good oral hygiene (OHIS SCORE > 3) and Experimental group (N=40) includes individuals with poor oral hygiene (OHIS< 3).

Saliva collection

Salivary collection was done according to the technique by Navazesh 1993 [5]. 3ml unstimulated saliva was collected in a sterile disposable plastic container and the samples were stored at −70 °C and used for further analysis.

Serum preparation

A volume of 3 ml of peripheral blood was drawn from patients using venepuncture from the antecubital fossa. Blood was allowed to clot at room temperature for 30 min and centrifuged at 3000 rpm for 10 min. The obtained serum was then divided into 2 aliquots and then transferred to a labelled poly propylene tube and stored at -70 °C and used for further analysis.

Enzyme -linked immunoassay for heat shock protein 70

Enzyme-linked immunosorbent assay kit (Assay Designs and Stressgen) was used. Serum, and saliva samples were analyzed using Elisa system according to the manufacturer's recommended procedure and 96 well plateprecoated with appropriate antibodies was used.

Serum, saliva samples and standards were added and incubated for 3 h. Then, the conjugate antibody was added and incubated 1 h at room temperature. The plates were washed again, and substrate was added to develop colour change and incubated for 30 min at room temperature in the dark. Finally, the optical densities were read at 450 nm, and the samples were compared to the standards. The results for HSP 70 were expressed at ng/ml.

Statistical Analysis

Student's t test was used for statistical analysis of the circulatory and salivary HSP 70 values healthy individuals with good oral hygiene and individuals with poor oral hygiene, which was expressed in terms of mean and standarddeviation. $P<0.05$ was considered to be statistically significant.

Results

There was a significant increase in Serum Heat shock protein 70 levels in Experimental group (5.87 ng/ml) in comparison with the control group (3.170ng/ml). (Table 1)

Salivary Heat shock protein 70 shock showed significant levels in experimental levels (4.21 ng/dl) compared to control group (2.641ng/dl)

Table 1

	Group	N	Mean (SD)	Mean difference (95% CI)	t	df	p-value
Saliva Hsp 70	EXPERIMENTAL GROUP	40	4.21 (0.58)	1.57 (1.07, 2.06)	6.68	18	<0.001*
	Control	40	2.641 (0.46)				
Serum Hsp	EXPERIMENTAL GROUP	40	5.87 (0.49)	2.69 (2.24, 3.15)	12.56	18	<0.001*
	Control	40	3.17 (0.47)				

Independent sample t test

*$P<0.05$ statistically significant.

Discussion

This study aimed at evaluating whether heat shock protein 70 is an efficient stress marker of poor oral health. Study showed a significant increase in HSP 70 in individuals with poor oral health which may be attributed to its role in stressful condition. Extracellular role of HSP 70 explains its cytoprotective property in events of pathological and physiological stress. It maintains pulpal health, periodontal health, aiding in repair of irritated dental hard and soft tissues and revert the inflammatory conditions [6].

A study conducted by Pileggi and Graham (2009) explains the expression of HSP 70 in 24 hour of pulpal injury as an early response to pulpal trauma and thus can be a method for measuring degree of trauma in dental pulp [7]. Ramya Netravathy *et al.* (2016) showed that Circulating HSP 60 levels may play a role in representing systemic inflammatory state produced by periodontal disease but salivary HSP 60 may not be used as a surrogate to determine systemic inflammation [8].

Role of HSP 70 have been established in various conditions. Over expression of HSP 70 was observed in oral squamous cell carcinoma [9] behcet's disease [10] and in oesophageal carcinoma [11]. Frank Tavassol *et al.* (2011) evaluated the prognostic significance of Heat shock protein 70 in oral cancer showed that the survival of patients suffering from T2 tumors with positive HSP70 expression was 8 times higher than that for patients with negative HSP70 expression, suggesting that T1-T2 tumors of OSCC with low expression of HSP70 require more radical treatment [12].

Thus, we conclude that Heat shock proteins 70 are efficient cell stress marker in poor oral health conditions, its efficiency to correct the altered state of protein assembly & function can be a harbinger to pleiotrophic effects which make them attractive targets for pharmacological intervention

References

1. Hsp70 is present in human saliva. Med Sci Monit, 2003; 9(1):BR62-65PMID: 12552239
2. Fabian TK, Fejerdy P, Csermely P. Salivary genomics, transcriptomics and proteomics: The emerging concept of the oral ecosystem and their use in the early diagnosis of cancer and other diseases. CurrGenom 2008; 9:11-21.
3. Tibor KárolyFábián, PálFejérdy, Minh Tú Nguyen, CsabaSőti, PéterCsermely. Arch Potential immunological functions of salivary Hsp70in mucosal and periodontal defense mechanisms. Immunol. Ther. Exp., 2007; 55:1-8.
4. Tibor KárolyFábián, Péter Hermann, Anita Beck. PálFejérdyand GáborFábián Salivary Defense Proteins: Their Network and Role in Innate and Acquired Oral

Immunity. Int. J Mol Sci. 2012; 13:4295-4320. doi:10.3390/ijms13044295.
5. Mahavash Navazesh. Methods for Collecting Saliva, annals of newyork academy of science, 1993. DOI: 10.1111/j.1749-6632.tb18343.x
6. Fábián TK, Gótai L, Beck A, Fábián1 G, Fejérdy P. The role of molecular chaperones (hspas/hsp70s) in oralhealth and oral inflammatory diseases: a review. European journal of inflammation. 2009; 7(2):0-0.
7. Roberta Pileggi, Graham Rex Holland. The expression of heat shock protein 70 in thedental pulp following trauma. Dental Traumatology. 2009; 25:426-428.
8. Ramya Nethravathy R, Swarna Alamelu, Arun KV, Kumar. TSS Evaluation of circulatory and salivary levels of heat shockprotein 60 in periodontal health and disease. Indian Journal of Dental Research., 2014, 25(3).
9. Thubashani M, Malathi N, Kannan L. Expression of heat shock protein 70 in oral submucous fibrosis and oral squamous cell carcinoma: An immunohistochemical study. Ind J Dent Res. 2011; 22:256-259. PMid:21891896
10. Direskeneli H, Saruhan-Direskeneli G. The role of heat shock proteins in Behcet's disease. Clin. Exp. Rheumatol 2003; 21:S44-48. PMid: 14727460.
11. Kawanishi K, Shiozaki H, Doki Y, Sakita I, Inoue M, Yano M *et al.* Prognostic significance of heat shock protein 27 and 70 in patients with squamous cell carcinoma of the esophagus. Cancer 1999; 85:1649-1651.
12. Frank Tavassol1, Oliver F Starke, Horst Kokemüller, Gerd Wegener, Corinna CM, Müller-Tavasso *et al*. André Eckardt Prognostic significance of heat shock protein 70(HSP70) in patients with oral cancer. Head & Neck Oncology. 2011; 3:10.