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Saliva as Biomarker of Heat Shock Protein 70 in Radiotherapy

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ABSTRACT

Purpose: Human heat shock protein which contains 8 homologous chaperones, out of which 6 are found in the reticulum and mitochondria, and remaining in cytosol and nucleus. These proteins have tissue specific expression that suggest a distinct biological tasks. This paper gives in detail about the salivary Heat shock protein 70 levels in healthy individuals in comparison to patients undergoing radiotherapy for head and neck cancer.

Materials and methods: 40 individuals reporting to the department of oncology with head and neck cancer for radiotherapy were included in the experimental group and compared with 40 healthy individuals. Saliva and serum samples were collected and were analysed for Heat shock protein 70 levels with ELIZA (enzyme linked immunoassay for HSP 70) and statistical analysis was done with independent student 't' test. P<0.05 was considered statistically significant.

Results: There was a significant increase in Serum Heat shock protein 70 levels in Experimental group (6.525 ng/ml) in comparison with the control group (3.170 ng/ml). Salivary Heat shock protein 70 shock showed significant levels in experimental levels (5.694 ng/dl) compared to control group (2.641 ng/dl).

Conclusion: Salivary and serum Heat shock protein 70 showed a significant increase in individuals undergoing radiotherapy for head and neck cancer, thus is an efficient cell stress marker.

INTRODUCTION

Heat shock proteins are produced by cells in response to stress were called heat shock because they were initially expressed during temperature raise and provided protection from severe temperature change. But now scientifically proved to be expressed during other stresses including wound healing and tissue remodelling^[1]. The heat shock proteins perform chaperons function by stabilizing new proteins that were damaged by cell stress^[2].

The heat shock protein is regulated by the heat shock factor, which is found virtually in all living organism these proteins are named according to their molecular weight they also called as stress proteins^[3]. These proteins play important role in assisting establishment of proper protein shape and prevention of unwanted protein aggregates and help to stabilize partially unfolded proteins. It helps to transport proteins across membrane within the cell^[4].

Heat shock protein 70 which is named after its molecular weight is involved in binding antigen and presenting them to the immune system. Heat shock factor 1 a transcription factor maintains the expression of heat shock protein 70, it (HSF-1) is a powerful multifactor that modifies carcinogenesis. Heat shock protein 70 provides thermo tolerance and also provides protein folding during post translation import into the mitochondria, thus stress and thermal stress. HSP 70 prevents partially denatured proteins from aggregating and allows unfolding in instances of stress and thermal stress.

Hsp 70 interacts with endoplasmic reticulum sensor protein 1 alpha induced apoptosis their interaction prolongs the splicing of XBP-1 mRNA, thereby up regulating targets of spliced XBP-1 serving the cells from apoptosis. This stress response protein improves integrity increasing cell survival [5]. It binds to the partially synthesized peptide sequences tightly and prevents from aggregation and being rendered nonfunctional [6].

Ionizing radiation is a key therapy to head and neck cancer. It is said to enhance synthesis of variety of immune stimulator and modulating molecules like heat shock proteins [7]. Human heat shock protein which contains 8 homologous chaperones, out of which 6 are found in the reticulum and mitochondria, and remaining in cytosol and nucleus. These proteins have tissue specific expression that suggests distinct biological tasks. Hence HSP 70 could be used as a biomarker to analyse the cell stress response in individuals undergoing radiotherapy.

MATERIALS AND METHODS

After obtaining the institutional ethical clearance the study was conducted among the individuals reporting to the department of oncology, K.S. Hegde Charitable hospital, Mangalore for radiotherapy diagnosed with head and neck cancer.

The experimental group included 40 individuals, and test group included 40 healthy individuals. Individuals with other active infections, pregnant and lactating women's, smokers were excluded from the study.

Saliva collection

Salivary collection was done according to the technique by Navazesh. The 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 plate precoated 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 and individuals undergoing radiotherapy, 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 [6.525 ng/ml] in comparison with the control group (3.170 ng/ml) (Table 1).

Table 1. Independent sample t-test.

Hsp 70 (ng/ml)	Groups	N	Mean	SD	Mean difference (95% CI)	t	df	P-value
Saliva	Experimental	40	5.694	0.578	3.053 (2.564, 3.542)	13.107	18	<0.001*
	Control	40	2.641	0.456				
Serum	Experimental	40	6.525	0.234	3.355 (3.007, 3.704)	20.234	18	<0.001*
	Control	40	3.170	0.469				

* $P < 0.05$ statistically significant

Salivary Heat shock protein 70 showed significant levels in experimental levels [5.694 ng/dl] compared to control group (2.641 ng/dl) (Table 1).

DISCUSSION

The study shows an increase in HSP 70 levels in individual undergoing radiotherapy for head and neck cancer, which may be attributed to the cyto-protective effect of HSP's [8]. The eukaryotic cells activate variety of intracellular signaling pathways on exposure to ionizing radiation, thereby delaying cell cycle progression thus activating DNA repair by acting at the checkpoints G1, S and G2 [9].

Study conducted by Park et al. in 2000, suggested HSP 70 when induced rendered cells with radio resistance ^[10]. In comparison to Heat shock protein, HSPA1A synthesis is higher and rapid, it is a major stress inducible member usually overexpressed in aggressive tumour and metastasis. This HSPA1A which are membrane bound and extracellular HSP's protect cells against radiotherapy through IL-2 (interlukin) activated NK cells by secreting perforin/granzyme B present are in the lipid vesicle which get endocytosed through Mannose phosphate receptor and then taken up by HSP70, thus granzyme B is released via perforin thus granzyme B mediates caspase dependent apoptosis in tumour cells ^[11].

Work of Marguis group has shown that inducible HSP 70 has antitumour,therapeutic also reduces tumour size. Thus Heat shock protein 70 has a cytoprotective role and is a efficient marker for cells stress. The present study shows the increase in HSP 70, may be radiation induced or increase due to preexisting aggressive tumours, further studies with pre radiation and post radiation groups may give a clear identification of HSP 70 levels and their relevance with Radiation induced effects on the cells.

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