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The Evaluate level of heat shock protein 27 and gene expression of HSF1 in Tonsillitis Patients

Namareq Ataallah Mohemeed [1], Suha Maher Abed [2]
[1]Department of pharmacology and Toxicology, College of Pharmacy, Tikrit University, Iraq.
[2] Department of Biology, College of Science, Tikrit University, Iraq
Corresponding Author: namariq.ata.mohemed@tu.edu.iq

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Abstract

Introduction: Tonsillitis is inflammation of the tonsils , It is more prevalent in the winter and fall seasons during school age **.** children with tonsillitis have their health and quality of life impacted, and it causes major illnesses, and loss of school time **.**

Objectives: The current study was conducted to Evaluate level of heat shock protein 27 and gene expression of heat shock factor1 in Tonsillitis sufferer .

Methods: Ninety blood sample were collected from infected people with tonsillitis attending to Tikrit Teaching Hospital in Ear ,Nose and Throat unit , tonsillitis was diagnosed depending on physical examination by specialist physician, during the period from (December 2023- March 2024). and Thirty sample from healthy individuals collected as a control group . each samples were splitted into two tubes ,Gel tube for the purpose of immune assay and Eppendrof containing tranZole for whole blood for purpose of RNA extraction from sample **.**

Results : Revealed that a high noteworthy difference in the serum level of heat shock protein 27 (3.185 ±0.9062) (2.495±0.7558) ng/mL respectively,(P≤0.0005), between patients and healthy controls respectively**.** The relative expression of *HSF1* have a significant difference between patients compared to the controls (-0.761± 0.452) (7.75± 4.342) , respectively,(p= 0.0001).

Conclusions: difference in the concentration of heat shock protein 27(HSP27) between patients tonsillitis and healthy controls **.** the relative expression of *HSF1* have a significant difference between patients compared to the controls (-0.7615± 0.452)(7.975± 4.342) respectively

Introduction

There are two tonsils, and each tonsil is an oval mass of lymphatic tissue sited in the adjacent wall of oropharynx among anterior and posterior columns (1) Their primary role is to support the secondary immune system by identifying native pathogens and immunological selection antigens ; they are positioned to provide for constant lymphoid stimulation at the junction of the digestive and respiratory systems (2).

Tonsillitis is inflammation of the tonsils , It is more prevalent in the winter and fall seasons during school age (3). children with tonsillitis have their health and quality of life impacted, and it causes major illnesses, and loss of school time (4). The inflammation of palatine tonsils is caused by microorganisms such as bacteria ,viruses and fungi that enter by aerogenes and are foodborne (5) .Transmission happens via droplets from patients with acute tonsillitis or rarely by asymptomatic carries (6).

Tonsillitis happens if the activity and multiplication of pathogens in the tonsil lymphoid tissue surpasses the

defensive power of stimulated lymphoid and immunoglobulin –producing cells (7). There are diverse types of tonsillitis , Acute tonsillitis happened when the infection lasts less than three weeks , Chronic tonsillitis is defined by symptoms that persist for more than three months. Recurrent tonsillitis is defined as a recurrent episodes of acute tonsillitis that occur more frequently than once per year or two (8) .fever, sore throat , difficulty swallowing , red swollen tonsillitis ,head ache ,cough ,chills ,and swollen lymph nodes in the neck are among the more typical signs of tonsillitis (9).

Heat Shock protein 27 (HSP27) is also named (HSPB1) , heat shock in cells give rise to the synthesis of specific proteins called heat shock protein , these proteins produce in cells a transient state of thermotolerance (10).

In cellular activities that occur during and following exposure to oxidative stress caused by potentially harmful environmental and /or microbial agents, heat shock protein (HSP) is essential (11). The interaction of heat shock factor transcription factors and heat shock element protein in the promoter regions of the heat shock protein gene regulates the synthesis of heat shock proteins in eukaryotic cells (12).

Eukaryotic heat shock proteins (HSPs) are regulates by *Hsf1*, which is a transcript factor, that are highly preserved among diverse species (13) . Numerous cellular processes ,including oxidative stress, apoptosis ,autophagy ,the unfolded protein responses in the endoplasmic reticulum ,physiological development ,and multidrug resistance , have been linked to this transcription factor (14) .

Heat Shock factor1 , a major transcription factor triggered by heat shock , is mostly expressed in the majority of tissue and cell types . in the absence of stimuli , it is dormant (15). Because heat separates the *HSF1* inhibitory complex in the cytosolic compartment and generates a DNA-binding competent homotrimer complex , An increase in temperature is required for *HSF1* activation (16). The transcription of HSP27 involves *HSF1* activity . several recent reports even describe *HSF1*-mediated transcription as having important and diverse functions as regulation of mitochondrial energy homeostasis and metabolism (17).

Objectives: The current study was conducted to Evaluate level of heat shock protein 27 and gene expression of heat shock factor1 in Tonsillitis sufferer .

Materials and Methods

A total number 90 sample of peripheral blood ,the groups in this study consisted of 60 sample from tonsillitis patients(chronic 38 and acute 22)with age from 5-15year ,tonsillitis was diagnosed depending on physical examination by specialist physician ,collected from patients attending to the ENT unit at Tikrit Teaching Hospital during the period from (December 2023- March 2024) , and 30 sample from healthy individuals collected as a control group .blood sample from patients and control group have been gathered , it was divided into two tube ,the one eppendrof tube contain 750 µL of tranZol and mixing well with 250 µL of whole blood and storage in freeze to use for gene expression by using primer(F: *ATCTTCCGTGGACACCCTCT* , R : *GCTACGCTGAGGCACTTTTC*) . The second portion was placed in a test tube and centrifuge to get the serum and immediately stored in an eppendrof tube and kept frozen at -20 ℃ for ELIZA testing to measure the concentration of Heat shock protein 27(HSP27) according to (Elabscience ,USA) , This ELISA kit uses sandwich –ELISA as the method.

Gene Expression Analysis

RNA extraction

tranZol –preserved whole blood samples from 60 tonsillitis patients and 30 control were used to extract RNA sample .TranZol up plus RNA kit (transgenbiotech), which is suitable for the isolation of total RNA from cells and tissue .

cDNA synthesis

1 µg of RNA was transcribed revesely into cDNA by 100 U from iscript TM specialized kit for cDNA synthesis Bio Rad's depending to the guidelines providing by the manufacturer .

Quantitative Real Time – PCR

The expression level of *HSF1* was measured by using a qPCR assay . qPCR was approved in CFX .connect RT- PCR Detection system .

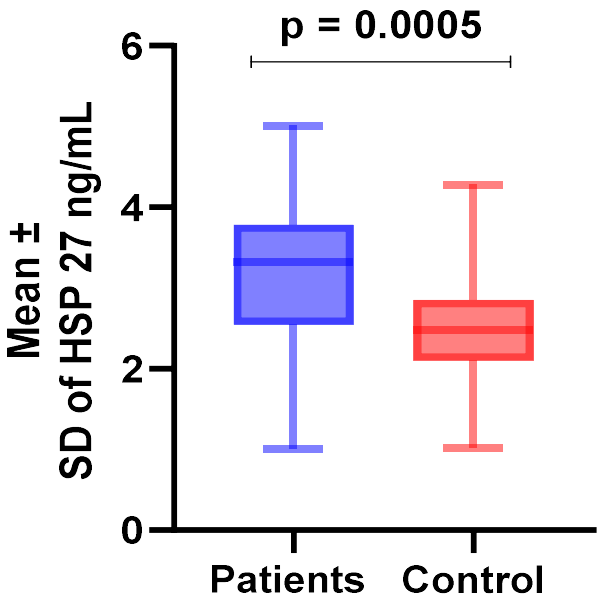
Statistical Analyses

P value of less than 0.05 were deemed statistically significant . The data were examined using the test and available as Means and arithmetical with standard error (S.E) . the relative expression of *HSF1* was computed using the precise ΔΔct method.

Results

Estimation the concentration of Heat Shock Protein 27 (HSP27)

The results showed that a noteworthy difference in the concentration of heat shock protein 27 between patients and healthy controls, values (Mean ±SD) were (3.185 ±0.9062) (2.495±0.7558) ng/mL respectively as shown

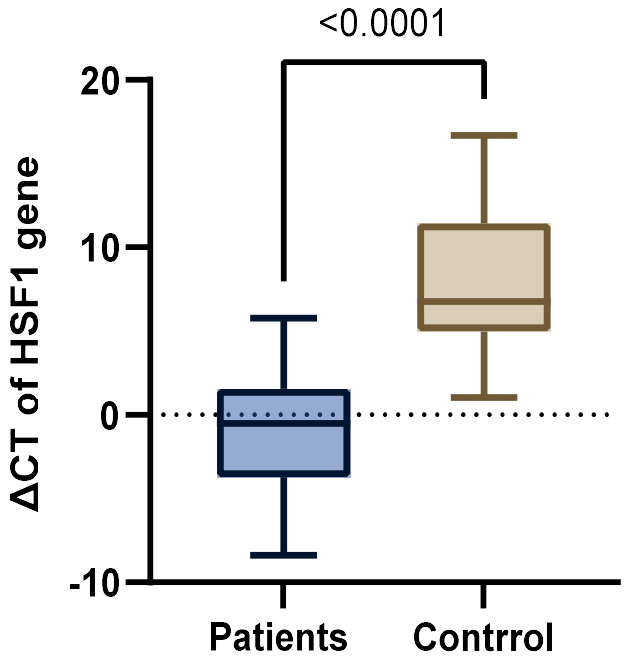


igure (1) .

Figure (1) show the level of HSP27 in patients and control

Relative expression levels of *HSF1* in Tonsillitis patients .

when comparing gene expression results between the group of patient and healthy people , the values of ΔCT(mean ± SD) were (-0.7615± 0.452) (7.975± 4.342) , respectively, the difference reach a significant value as shown in the figure(2) and table (1) . Roc curve values were as follows(AUC=0.9607) (sensitivity% =73) (specificity% =72.69) (P = 0.0001) as shown in ROC curve figure (3) .



Figure(2) Relative expression levels of *HSF1* in patients compared with controls, ΔCT = Delta cycle threshold.

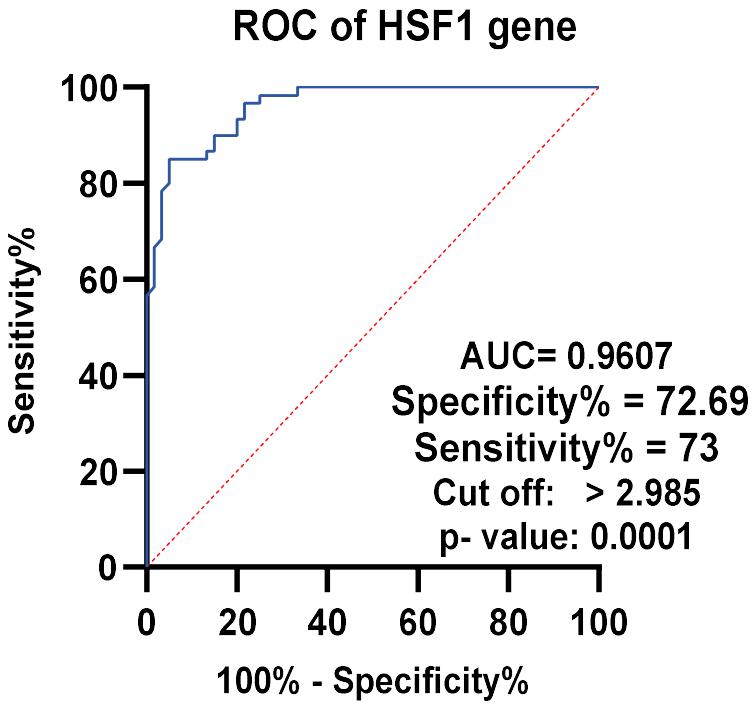


Figure (3) show ROC curve of *HSF1*

Table(1) Expression level in patients and control.

	ΔCT of patients (mean ± SD))	**ΔCT of control (mean ± SD))**	**P value**	**Expression fold ($2^{-\Delta\Delta ct}$)**
HSF1	-0.7615± 0.452	7.975± 4.342	0.0001	9.913

Discussion

Immunological Assay for Tonsillitis patients :

Role of Heat Shock protein27 in Tonsillitis patients

Intracellular heat shock proteins (HSPs) , are primarily involved in cell protection through the suppression of various stressor factors .this is achieved by either returning misfolded protein to the cell or overting apoptotic cell death (18). In this study , patients with tonsillitis has considerably greater serum levels of heat shock protein 27 (HSP27) than did controls who were in good condition (3.185 ±0.9062) (2.495±0.7558) ng/mL respectively,(P≤0.0005). this study agreed with (19).

Because antigenic peptides can be bound by HSPs and transfer them to T lymphocytes and antigenic –presenting cells, they serve a crucial role in promoting immunological response (20). HSP27 interacts with certain receptors to modulate the inflammatory response (21). High –level expression of heat shock proteins by bacterial pathogens occurs during adaptation to intracellular survival , Heat shock proteins from both hosts and pathogens support immunity by participating in the presentation of antigens for the adaptive immune response and by activating the innate immune response through immunity receptors (22).

the heat shock protein mediates the immune effector functions by induce activating natural killer cells, which lyse infected cells , and inducing Th2 type regulatory cytokines from macrophage ,which then assist in controlling the generation of pro-inflammatory cytokines brought on by infections (23).

Relative expression levels of *HSF1* in Tonsillitis Patients

The relative expression of *HSF1* have a significant difference between patients compared to the controls (-0.7615± 0.452) (7.975± 4.342) , respectively, (p= 0.0001). as shown in figure(2) and ROC curve (3).,*HSF1*amember of the heat shock factor(*HSF*),is activated under various stressors and subsequently induces up – regulation of heat shock genes (e.g., *Hsp27*), because this gene includes a wide number of stimuli stress, several studies have demonstrated that modifications to *HSF1* function affect protein homeostasis and are highly associated with illness (24). our study was the first to investigate gene expression of *HSF1* in Tonsillitis .*HSF1* is constitutively expressed in most tissues and cell types, Research has indicated that *HSF1* facilitates the appropriate operation of the immune system . *HSF1* inhibits several cytokines that cause fever and inflammation (25).

The stress response ,also known as heat shock gene expression ,is a fundamental and well-preserved cellular mechanism that protects organisms against a range of physical and chemical stresses ,including high temperatures, heavy metals ,toxins ,and oxidants ,as well as bacterial and viral infections, *HSF1* can be induced by the elevated temperatures encountered in inflamed tissues and in fever as well as by anti-inflammatory prostaglandins (26). Among the genes that the *HSF1* targets that have been found thus far are involved in the regulation of typical developmental processes. Research shows that *HSF1* acts in normal biological processes as well as stress-induced situations ,in contrast to its traditional name of heat shock (25).

The heat shock response is a key metabolic process that affects the resolution of inflammation ,especially in case of proteotoxic stress. this pathway powerful anti-inflammatory one ,and it is mediated by heat shock

transcription factor 1 ,which is required for expression of heat shock protein and other chaperones (27). The activation of *HSF1* drives that increase in HSPs in response to injury or stress (28). ,similar effect applied on microorganisms (29 , 30) .

Conclusion

The Results showed that a high substantial difference in the concentration of heat shock protein 27(HSP27) between patients tonsillitis and healthy controls **.** the relative expression of *HSF1* have a significant difference between patients compared to the controls .

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