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Gene Expression of Heat Shock Protein 90 (HSP90AA1 and HSP90AB1) in Thyroid Disorders Patients

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Abstract

The thyroid gland can produce a standard amount of thyroid hormone in response to serum Thyroid Stimulating Hormone (TSH). Hormones of the thyroid gland control the metabolism of cells and their activity speed. Also, they regulate the rate of oxygen consumption. During this study, the gene expression of HSP90AA1 and HSP90AB1 from non-treated hypo and hyperthyroid patients diagnosed by clinical examinations and paraclinical data were compared with a control group. The study included 120 samples collected from patients who suffer from thyroid disorders and 100 samples collected from healthy people as a control group. The age ranged from patients and healthy individuals having (17-79) years. The hormones were measured by ELISA methods; gene expression of HSP90 was measured by RT-PCR using SYBR Green Master after RNA was isolated and converted to cDNA. Significant changes were observed in the level of hormones, in hypothyroidism TSH increased while decreased in hyperthyroidism, whereas triiodothyronine (T3) and Thyroxine (T4) increased in hyperthyroidism and decreased in hypothyroidism. Results of HSP90AA1 gene expression showed the mean of folds was nonsignificantly increased in patients compared with control; while the results of HSP90AB1were high in hyperthyroidism and low in hypothyroidism but these differences were nonsignificant compared with a control group. The expression of HSP90 genes can be used as a risk factor in the diagnosis of thyroid disorders.

Keywords: Folding, TSH, Chaperone, HSP90, HSP90AA1 gene, HSP90AB1gene.

1. Introduction

In humans, the thyroid gland consists of Tow lobes that are lateral and inferior to the anterior part of the larynx and are connected by an isthmus across the larynx to create a U-shaped form, in adults the gland with an average weight of 30 gm (Chiasera, 2013). Thyroxine (T4) and 3,3 ', 5-triiod-L-thyronine (T3) hormones are secreted from the thyroid gland and stored therein. Thyroid hormones control homeostasis of energy, cell proliferation, and metabolism of carbohydrates, fats and proteins (Wallis *et al*., 2010). Among the most common endocrine diseases are thyroid gland disorders. Thus, the study of T3 and T4 thyroid hormones has important biological and medical implications (Demir *et al*., 2020).

Proteins called heat shock proteins (HSPs) are produced when cells exposed to high temperatures become temporarily resistant to subsequent heat shock. HSPs are molecular chaperones that are conserved and grouped by their molecular mass and a high degree of amino acid homology between microbes and humans (Kim and Yenari, 2017). The HSPs range from 15 to 110 kDa in molecular weight. There are groups of binding proteins with a high molecular weight of about: 100, 90, 70, and 60kDa. Also they are classified as small HSPs with a low molecular weight of 12-43 kDa. 80-100 amino acids contain small HSPs (Tkáčová and Angelovičová, 2012).

Heat shock protein 90 (HSP90) is essential for activating several signaling proteins in eukaryotic cells as a molecular chaperone. The structural and biochemical study of HSP90, which promotes the stimulation of HSP90's clientele, has shown a complex mechanism of ATPase-coupled conformation variations and interactions with cochaperone proteins. While recent progress has been made, key aspects of HSP90's ATP-coupled mechanism remain controversial, and therefore the nature of the changes produced by HSP90 in client defense are unknown (Pearl and Prodromou, 2006).

The present study aimed to evaluate changes in HSP90 gene expression and its relationship with thyroid disorders.

2. Methods

2.1. Blood Samples Collection

Ten ml of venous blood were collected from a suitable vein. 3 ml of blood samples were put in a dry EDTA tubes and shaken gently then used for moleculare diagnosis. The residual part of the blood sample was transferred to a glass tube (anticoagulation-free) and allowed to coagulate for serum separation for 5 minutes using a 4000 rpm centrifuge. In a sterile, clean white tube, the extracted serum was collected and stored at -20 °C for thyroid hormones measurement.

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2.2. Measurement of the Hormones levels

Accu-Bind ELISA microwell kit (Monobind Inc, USA) was used for the quantitative determination of Total Triiodothyronine (tT3), and total thyroxine (tT4) concentration in human serum by microplate Enzyme immunoassay. The quantitative immune enzymatic assay of TSH was based on the ELIFA technique by Mini VIDAS according to the manufacturer protocol (Bio Merieux, France).

2.3. Measurement of gene expression of HSP90 by quantitative real-time PCR

Total RNA was isolated from sample blood using Trizol reagent (Invitrogen, USA) according to the protocol of the manufacturer.; The concentration of extracted RNA was estimated by the protocol for quantitating RNA in a single tube using the Quantus fluorometer (Promega, USA). After that, RNA was reverse transcribed for use as a template in the PCR reaction into complementary DNA (cDNA). The reaction to Real Time (RT) was carried out using Accu Power Rocket Script RT PreMix (Bioneer, Korea). This kit is a ready-to-use lyophilized master mix containing all components from the RNA template for first-strand cDNA synthesis.

Table 1. Primer sets for genes analyzed by qRT-PCR.

GAPDH	F	5'-GAAGGTGAAGGTCGGAGTC-3'
GAPDH	R	5'-GAAGATGGTGATGGGATTTC-3'
HSP90AA1	F	5'- TGGAATGACCAAGGCTGACT -3'
HSP90AA1	R	5'- TGAGGACTCCCAAGCGTACT -3'
HSP90AB1	F	5'- TGATGAGGCAGAGGAAGAGAA -3'
HSP90AB1	R	5'- TCTGGTCCAAATAGGCTTGG -3'

The real-time fluorescent quantitative polymerase chain reaction (RT-qPCR) process with GAPDH as a control was used to study the mRNA expression levels of HSP90AA1 and HSP90AB1 in blood samples. The RT-qPCR was carried out according to the SYBR Green Master kit manufacturer's protocol. Table 1 lists the HSP90 gene primers for RT-qPCR. The dissociation curve was evaluated to ensure an apparent amplification peak and validate it. The expression levels of HSP90AA1 and HSP90AB1 were determined using the $2^{-\Delta\Delta}$Ct method and subjected to statistical analysis (Schmittgen and Livak, 2008).

2.4. Statistical

In order to detect the effect of different variables on study parameters, the Statistical Analysis System- SAS (2012) software was used. The least significant difference-LSD test (Analysis of Variation-ANOVA) was used to compare the means significantly.

3. Result

3.1. Levels of Hormones in hypo and hyperthyroidism

The present study showed (Table 2) a significant difference (P≥0.01) in the level of TSH in control, hypothyroidism, and hyperthyroidism. The mean of the TSH level of hyperthyroidism (0.288 µIU/ml) was lower than that of hypothyroidism (7.31 µIU/ml) and control group (2.78 µIU/ml), while the mean of TSH level of hypothyroidism was higher than that of control and hyperthyroidism. Also, the present study showed a significant difference in the level of T3 and T4 in control, hypothyroidism, and hyperthyroidism (P≥0.01). The mean of the level of the hormones in hypothyroidism was lower than that of hyperthyroidism and control group, while the mean of T3 and t4 levels in hyperthyroidism was higher than that of control and hypothyroidism; the T3 means for three groups were 1.71 ng/ml, 0.49 ng/ml, and 3.87ng/ml; and for T4 were 5.84 µg/ml, 1.41 µg/ml, and 10.81 µg/ml respectively.

Table 2. Distribution of the Hormones in Thyroid Disorders Patients

Group	T3 (ng/ml)	T4 (µg/ml)	TSH (µIU/m)
Control	5.84± 0.064 b	1.71± 0.053 b	2.78 ± 0.26 b
Hypothyroidism	1.41± 0.038 c	0.49± 0.062 c	7.31± 0.71 a
Hyperthyroidism	10.81± 0.12 a	3.87± 0.045 a	0.288± 0.021c

Means having the different letters in the same column differed significantly. ** (P≤0.01).

3.2. Gene Expression of HSP90 in hypo and hyperthyroidism

The positive results showed amplification at CT (threshold cycle) value were in range 15.9– 18.48 for housekeeping gene and 19.02- 21.69 for HSP 90AA1 gene and 17.71–20.66 for HSP 90AB1 gene in hyperthyroidism, while in hypothyroidism the CT value was in range 15.74–16.91 for housekeeping gene (Figure 1) and 18.97-20.06 for HSP 90AA1 gene and 17.13–24.67 for HSP 90AB1 gene. The results for the control group showed amplification at CT value was in the range 15.84– 16.34 for housekeeping gene and 18.96-19.94 for HSP 90AA1 gene and 17.67– 22.69 for HSP 90AB1 gene.

The melting temperature (Tm) values obtained for the patients samples of hyperthyroidism, hypothyroidism, and control; the melting temperature was in the range 85.23-85.93, 85.53-85.93, 85.73-86.23ºC respectively for housekeeping gene (Figure 1), HSP90AA1was in the range of 81.54-82.24, 81.64-82.04, 81.74-82.34 ºC respectively, HSP90AB1 was in the range of 79.15-79.84, 79.54-79.84, 79.44- 80.04 respectively.

The results of HSP90 genes expression in Figure (2) showed an increase in gene expression of HSP90AA1 in hypothyroidism and hyperthyroidism, but the increase was nonsignificant differences compared with control group. The mean of folds in hypothyroidism, hyperthyroidism, and control was 0.92±0.34, 0.96±0.33, and 0.82±0.28 respectively. While the results of HSP90AB1 (Figure 2) show a high in gene expression in hyperthyroidism and low gene expression in hypothyroidism, these differences were nonsignificant compared with a control group; the mean of folds was 1.82±0.43 (hypothyroidism), 4.84±2.72 (hyperthyroidism), and 3.81±1.29 (control).

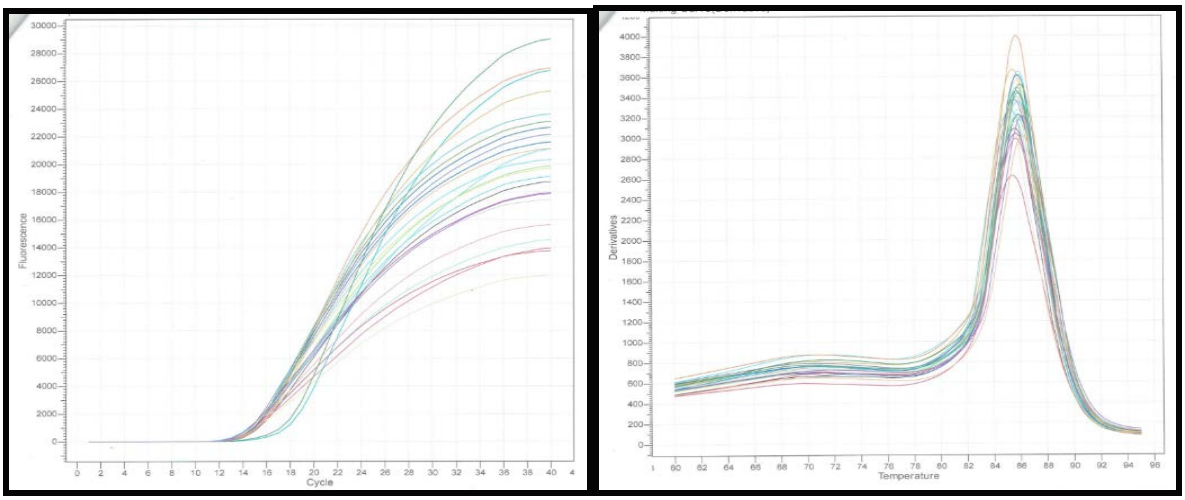

Figure 1. Curve of cycling and melting for housekeeping GAPDH gene

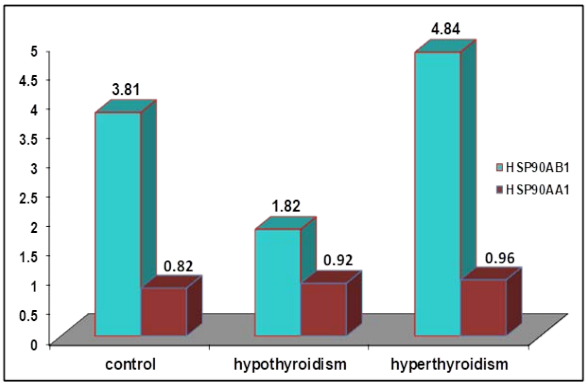

Figure 2. Expression levels of HSP90AA1 and HSP90AB1 gene

4. Discussion

Hyperthyroidism is a clinical condition due to an excessive increase in thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4). Research by Sugimoto and Mori (2012) showed that in hypothyroidism, TSH levels are raised, owing to the lack of a suppressive action of the T3 and T4. The most common cause of hyperthyroidism is toxic goiter or Graves' disease (Gilles *et al.*, 2008). The hypophysis tells the thyroid how much hormone is needed to produce. It will not be ready to give the thyroid the correct instructions if the pituitary is damaged by injury, a tumor, radiation, or surgery, and so the thyroid may stop producing enough hormones (Dunn and Turner, 2016). Iodine deficiency is the most common cause of hypothyroidism worldwide; and it is the most prevalent cause of hypothyroidism; and too much iodine can also cause or exacerbate hyperthyroidism (Al Hadid *et al.*, 2018).

HSP works on protein transport and assembly, and proper folding of peptide chains, thus playing a crucial role in protecting cells, and ultimately affecting cell survival (Chen *et al.*, 2018), and much research in recent years has demonstrated that HSP is related to cell apoptosis (Zhang *et al*., 2017). HSP90 exists in the thyroid follicular epithelial cells and follicular cavities (Calderwood, 2018; Yan *et al*., 2019).

HSP90 is a molecular chaperone that interacts with client proteins (Hertlein *et al*., 2010), thereby preventing their degradation. For example, because of the stress response to hypoxic, acidic, and nutrient-deprived hostile microenvironment characteristics, expression of HSP90 is increasing in many cancers and correlates with poor prognosis (Bagatell and Whitesell, 2004; Pick *et al*., 2007). HSP90 levels increase in stressed cells, and it is unclear whether cell stress is necessary for the efficacy of HSP90 inhibitors; and Yu-bao *et al*., (2014) suggest that HSP90 expression is relative to the disease.

5. Conclusion

The present work illustrates that the expression of the two HSP90 genes changes in both hypothyroidism and hyperthyroidism. Also, the HSP90AA1 gene and HSP90AB1 gene correlate with TSH and Thyroid hormones; thus these genes can be used as risk factors in the diagnosis of thyroid disorders.

Authors' Contributions

ASM, MMA, and NAM contributed to the study design and analyzed data. All authors contributed to the manuscript drafting and revising and approved the final submission.

Competing interests

The authors declare that they have no competing interests associated with this article.

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