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Short Communication

Expression of Heat Shock Protein 70 in thyroid gland tumors

Haider A. Hassan[a*], Saad Hasan Mohammed Ali[b], Athraa Y. Al-hijazi[b]

[a]*Biotechnology Department, Collage of Science, University of Baghdad, Baghdad, Iraq*
[b]*College of Dentistry, University of al-Mustaqbal, Babylon, Iraq*

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Abstract. Heat shock protein 70 (HSP70) is a crucial protein with vital biological tasks in cell continuation of life. The variation of HSP70 activation occurs as a consequence of stress that includes temperature states, toxicity, poisoning with heavy metals, and tumor-related conditions. One of the master jobs of the HSP family is the suppression of caspase-mediated apoptosis signals. A high level of the expression of HSP70 is accountable for tumorigenesis and resistance against chemotherapeutic drugs. For this reason, the detection of HSP70 may help to diagnose cancerous diseases. From the other side, targeting this chaperone might help in treatment by maintaining late caspase-dependent events. This study was conducted to detect the presence and the location of HSP70 in Iraqi thyroid tumor tissue specimens (25 samples), in addition to 10 samples of normal thyroid tissue. Using the immunohistochemical study (paraffin method), the protein was detected in 100% of follicular carcinoma or follicular adenoma (benign) in addition to 77.7 % of papillary thyroid carcinoma while, in normal thyroid tissue, the presence of protein was in 10 % of cases. Regarding protein location in the cells, it appeared in the nuclei and the cytoplasm of follicular carcinoma cases in comparison with just in the cytoplasm of other sections.

Keywords: heat shock protein 70, immunohistochemical expression, thyroid tumor

INTRODUCTION

Thyroid cancer (TC) is the most frequent type of endocrine malignancy (3.4% of all cancers diagnosed annually). The incidence rates of both males and females are five times higher in high and very high Human Development Index (HID) countries than in low and medium (HID) countries (Kitahara & Sosa, 2020; Seib & Sosa, 2019). Three chief histological types of TC were detected: differentiated (papillary and follicular) TC, undifferentiated (including poorly differentiated and anaplastic TC), and medullary TC (Kitahara & Schneider, 2022). Approximately 90% of all TC originate from thyroid follicular cells which considered the most common histological type of these cancers is papillary thyroid carcinoma (PTC) followed by follicular thyroid carcinoma (FTC) (Liu *et al.*, 2017) (Zarebczan & Chen 2010). Chaperone machines mediate many intracellular processes. One of the most important chaperone machines is the heat shock protein 70 (HSP70) which is an ATP-dependent large molecular weight chaperon. HSP70 mediates crucial processes such as protein folding, modification, and translocation, as well as cell survival. Hsp70s interact with many different polypeptides but do not work alone but with a team of co-chaperones such as J-domain proteins and nucleotide exchange factors (Mayer, 2013). When stress condition occurs, the cell must deal with raised concentrations of unfolded or collapsed proteins when the HSP70 level increases to help in this function (Rashmi *et al.*, 2004). Normally, HSP70 translocation occurs into nucleoli from the nucleus and cytoplasm using microtubules under kinase enzyme action. It is thought that the fast translocation of Hsp70, in particular during heat shock, provides fast folding and refolding of denatured and aggregated proteins (Avdalyan *et al.*, 2020). Thus, HSP70 is overexpressed in varied types of malignancies such as prostate, lung, pancreatic, urothelial, cervical, and breast cancers (Soleimani *et al.*, 2019).

*Author for correspondence: Haider Ahmed Hassan, Baghdad, 10071, Iraq
Email – hayder.ahmed@sc.uobaghdad.edu.iq

HSP70 prevents protein aggregation and assists the translocation of other proteins through the membrane (Blagosklonny, 2001). Furthermore, formulation of HSP70 in the cells can interfere with different biological processes such as inhibition of both apoptosis pathways; intrinsic and extrinsic cell death pathways (Murphy, 2013), senescence (Yaglom *et al.*, 2007), and autophagy (Nylandsted *et al.*, 2004, Daugaard *et al.*, 2007). Regarding the innate immune system, HSP70 may serve as a danger signal promoting receptor-mediated apoptosis. HSP70 is overexpressed in cells by the influence of stress which can cause acquired or inherent drug resistance (Rashmi *et al.*, 2004). Indeed, such overexpression may be considered for tumorigenesis and opposition chemotherapeutic agents used in treatment. This process occurs by prohibiting late caspase-dependent signals (Juhasz *et al.*, 2013). This work was done to investigate the expression pattern of HSP70 in TC patients to clarify whether it could be beneficial for diagnosis and treatment.

MATERIALS AND METHODS

Patients' characteristics

The study comprised 25 Iraqi cases treated in Baghdad Medical City (BMC), Iraq, 19 women and 6 men. All cases were examined histologically in the department of pathology in BMC, Iraq from 2020 to 2021. Twenty-five patients with thyroid tumors were diagnosed. Nine patients out of 25 were diagnosed with papillary thyroid carcinoma (PTC), in addition to ten were diagnosed with follicular carcinoma (FC), and six patients were diagnosed with follicular adenoma (FA). Patients were divided into groups of 10 years for statistical analysis (Girardi, 2017).

Statistical analysis

Statistical analyses were performed using the chi-square test. A p-value of <0.05 was considered statistically significant.

Immunohistochemically study

Immunohistochemical anti-heat shock protein antibody and Expose HRP/DAB detection IHC (mouse and rabbit) kits were supplemented by Abcam Company, UK. Senior pathologists verified the tissue specimens to include TC tissue for the study group to deal with the immunohistochemical study. Ten per cent formalin was used for 24 hours to fix all tissue samples, followed by treatment of the samples with 70% ethanol overnight. Dehydration of the samples was achieved using an increased graded series of alcohol (70%, 90% and 100% ethanol) for two hours for each concentration. All samples were treated with xylene for 2 hours to replace the ethanol (clearing). Melted paraffin (at 58°C) was used for tissue embedding for 2-3 hours at 60°C in an oven and cut into sections by a microtome for 5 μm thickness then floated on the water bath at 50°C then transferred onto a Superfrost Plus slides for next steps. Xylene was used to remove the paraffin from the slide in three steps for 10 minutes each. According to the procedure, the endogenous peroxidase activity must be blocked. For this purpose, three per cent hydrogen peroxide in methanol 100% for 25 minutes was used to do that. All sections were washed with triethanolamine-buffered saline (TBS) at pH 7.6. Then, citrate buffer (pH 6) was used to submerge the sections to activate the antigen retrieving and unmasking. Ten minutes was the time in which all samples were put in a microwave. All nonspecific reactions must be blocked, for this all sections are incubated in 20 % swine serum (Sigma, Aldrich, Germany) for 20 minutes. All sections were covered with antibody (primary) (at dilution 1/150 for HSP70) and incubated for 1 hour in a humidity chamber at 37°C followed by rinsing the sections gently in TBS. At room temperature, a secondary antibody was added for 1 hour followed by washing for 10 minutes. The Streptavidine-HRP antibody was added and washed to visualize the peroxide reaction. After that, hematoxylin was used to counterstain the slides for 30 seconds. Tap water was used to wash all slides followed by dehydration. The slides were mounted with a permanent-mounting medium (DPX). The slides were examined under the optical microscope at 10× and then at 40× magnifications. The above work steps were followed according to Khashman *et al.*, 2020.

Immunohistochemical evaluation

An experienced head and neck pathologist verified the immunohistochemical sections. If the average fraction of intensely staining cells

occupied more than 25% of the total slide, the section is considered positive for HSP70 (Syrigos *et al.*, 2003).

RESULTS AND DISCUSSION

Patients' characteristics

This study comprised 25 Iraqi patients treated in Baghdad Medical City (BMC), Iraq. Nineteen female and six male patients of average age of 44 years at surgery (range: 25–68 years) (Figure 1). According to the gender of patients that participated in this study, all three types of thyroid cancer had been detected in female patients mainly. However, there was no statistical difference at p-value < 0.05 between males and females in Papillary thyroid carcinoma cases. The differences in follicular carcinoma or follicular adenoma were statistically significant (Table 1). Also, no statistically significant differences were shown between the three types of tumors on gender basis (Figure 1). These results may belong to certain reproductive factors, especially in terms of estrogen receptor status (Liu *et al.*, 2021). In terms of age, patients between 20-29 years old (median age at diagnosis is 24.5 years old) followed by 40-49 years old (with median 44.5 years old) had the higher incidence of PTC. Regarding FC patients, the group of 60-69 years old followed by both 40-49- and 50-59-years old group had the higher incidence of FA (Figure 2). Follicular adenoma appeared to be the chief type in the 50-59 years group. There were no significant differences between the age groups for each case except in PTC where the p-value was 0.004.

These results might be due to both, the aggressiveness of disease and the usual decline of immune response in 60-69 years old group mainly. These changes might be associated mainly with molecular mutations (Xing *et al.*, 2013; Romei and Elisei, 2021). Also, the post-menopausal hormonal changes in females might play an important role in this incidence (Haymart, 2009).

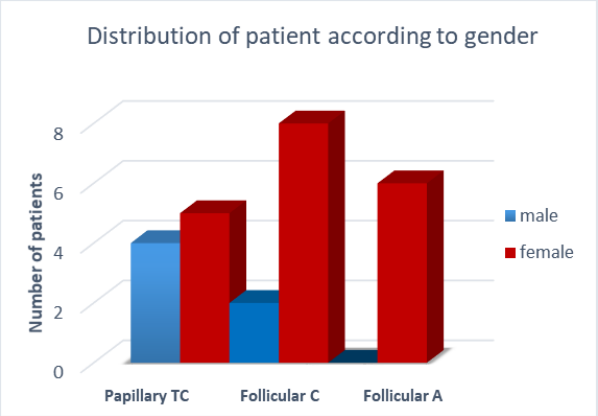

Figure 1. Distribution of patients according to gender

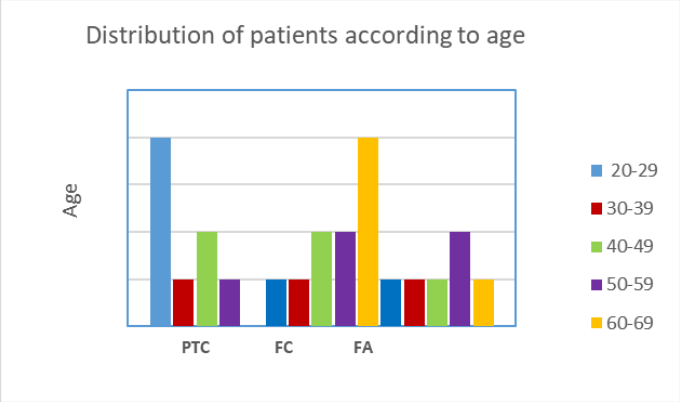

Figure 2. Distribution of patients according to age

Table 1. Summary of cases according to gender

Cases	Gender	No	p-value	Significance
Papillary thyroid carcinoma	Female	5	0.637	N.S.
	Male	4		
Follicular carcinoma	Female	8	0.013	S.
	Male	2		
Follicular adenoma	Female	6	0.013	S.
	Male	0		

Note: N.S.: not significance; S.: significance.

Immunohistochemistry

One of the main points in cancer pathology is to understand the mechanisms that belong to the origin of the loss of cell differentiation. Several studies pointed to the role of molecular chaperones in the maintenance of cellular and tissue homeostasis (Barna *et al*., 2018; Walsh *et al*., 1999). It is well known that an imbalance between cell proliferation and differentiation is crucial to ensure the correct growth and development of organisms and to maintain adult tissue and organ homeostasis. HSP70- was determined as a present or absent antibody in the tissues. Digital camera, light microscope and imaging computer software were used for histological analysis. Two criteria were used for the evaluation: intensity and localization of immunostaining in cells (nuclear or cytoplasmic). According to Table 2, nine papillary thyroid carcinoma (PTC) cases, ten follicular carcinoma (FC) and 6 follicular adenoma (FA) cases out of 25 TC patients were detected. HSP70 protein was determined in 7 out of 9 (PTC) (Figure 3 a, b), all (FC) patients (10 cases) (Figure 3 c, d), and in all (FA) patients (six patients) (Figure 3 e, f), in comparison with 2 healthy cases (Figure 3 g). No significant difference was determined between all the positive cases with a p-value of 0.202. This result is consistence with (Caruso *et al*., 2019). On the other side, different localization of HSP70 was detected in the patients which have been addressed in this study. It has been pointed in the nuclei of FA (Figure 3 f) and in the cytoplasm of PTC and FA (Figure 4 a, b). In this term, the localization of HSP70 was thought to be correlated with other molecules such as J-protein which might be the partner and functional regulator of HSP70 (Craig and Marszalek 2017; Kampinga and Craig 2010). Also, a histone chaperone (DNAJC9) was elucidated to be crucial for HSP70 catalysis (Hammond *et al*., 2021). In addition to the C-terminal domain (CTD) which affects the interaction of HSP70 (Faust *et al*., 2020). These results are consistence with the results of (Avdalyan *et al*., 2020). However, different localization in tumor cells might be due to the difference in the stages of the disease which may reveal the correlation of the HSP70 protein with the prognosis of the disease (Malusecka *et al*., 2006). Thus, targeting HSP70 might be a promising way to kill cancer cells (Moses *et al*., 2018; Alfano *et al*., 2010). However, due to our limited number of cases, conducting studies with a larger number of cases might reach coincided results.

Table 2. Expression of HSP70 between different types of thyroid cancer. The number of investigated cases and the percentage of positive and negative HSP70 are shown.

Specimen	Investigated cases (n)	HSP70 positive (n)
Normal thyroid tissue	10	1
Papillary thyroid carcinoma (PTC)	9	7
Follicular carcinoma (FC)	10	10
Follicular adenoma (FA)	6	6

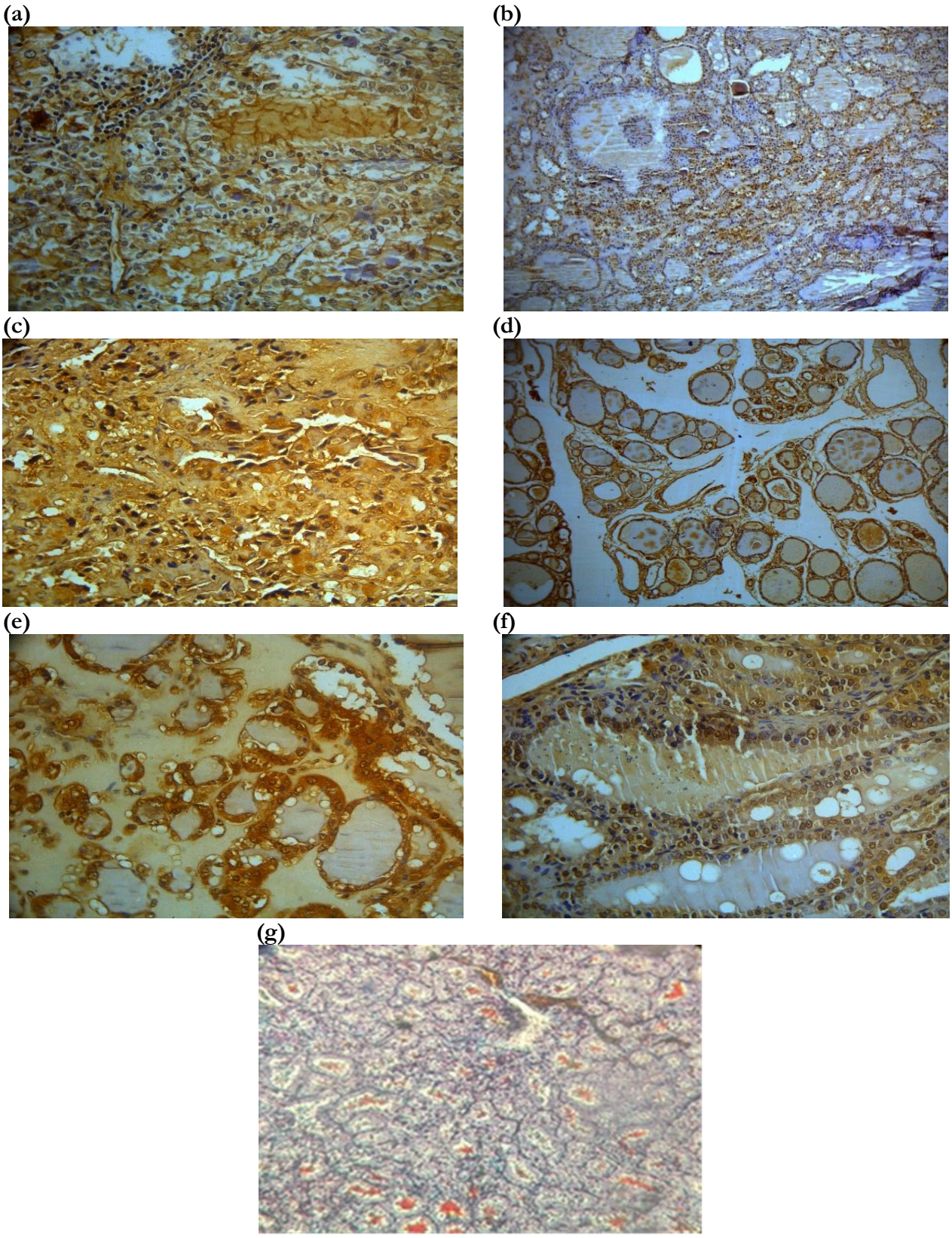

Figure 3. HSP70 immunostaining of different thyroid cancer cases. HSP70 was determined as a present or absent antibody in the tissues. (a) & (b): papillary thyroid carcinoma (PTC); (c) & (d): follicular carcinoma (FC); (e) & (f): follicular adenoma (FA); (g): normal section. Images (d) & (e) were taken with ×100, the rest were taken with ×40.

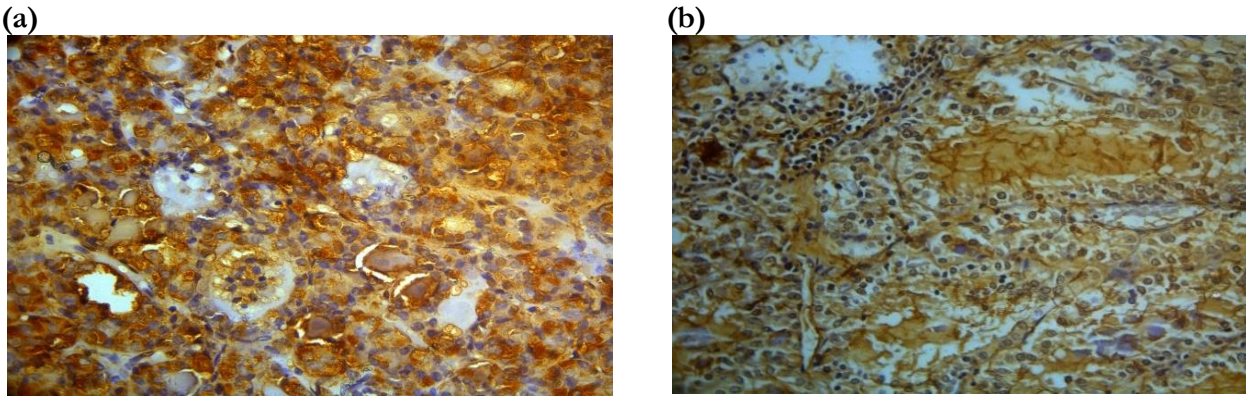

Figure 4. Immunohistochemical analysis of the expression of Hsp70 protein in thyroid cancer. (a) cytoplasmic localization, ×40; (b) nuclear cytoplasmic localization, ×40.

CONCLUSION

Molecular chaperones play a crucial role in the conservation of cellular and tissue homeostasis. HSP70 level increase occurs in thyroid cancer types (PTC, FC and FA). This indicates the biological effect of HSP70 in thyroid cancer. Thus, the HSP70 level can be exploited to detect the grade of the tumor in addition to predicting the patient's response to the treatment. This can help to more accurate diagnosis and finally to more successful treatment. In addition, to avoid the harmful side effects of many drugs that might not work with certain types of thyroid cancer.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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