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Expression of Transforming Growth Factorα and Epidermal Growth Factor Receptor in Thyroid Carcinoma

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Abstract: The current study was performed on 178 paraffin embedded tissue specimens, included 111 of them were affected by thyroid carcinoma, 46 were benign neoplastic and 21individuals as control group. The study was carried out in Laboratory of College of Science, University of Wasit in collaboration with AL-Hussein Teaching Hospital and Ibn al-Bitar Laboratory Specialist in Thi Qar Province, IRAQ, from October 2015 to April 2016. Immunohistochemical technique was used to determination the expression of TGFα and its receptor EGFR in thyroid carcinoma in comparison with benign thyroid disease and control group. The aim of this study is to detect, quantify and analyze the clinicopathological correlation of those genes in patients with thyroid carcinoma. The results showed a significant increase of TGFα in thyroid patients comparing with control group and benign neoplasms, and between control group and benign neoplasms ($p<0.05$). On the other hand, the positive expression of EGFR was increased but did not reach to significantly difference in thyroid carcinoma in comparison with neither benign neoplasms nor control group ($p>0.05$). Our results concluded that there is a strong relationship between TGFα overexpression with thyroid carcinogenesis.

Keywords: Thyroid Cancer, TGFα, EGFR and Immunohistochemistry

1. Introduction

Thyroid carcinoma is the most widespread type among endocrine malignancy [1]. This cancer occurs in the thyroid gland, which it is importantly endocrine glands located at the lower part of the larynx, and responsible for the production of hormones that regulate the control: heart rate, blood pressure, body temperature, and basal metabolic rate [2]. The occurrence of Thyroid cancer has increased significantly around the world over the last few decades [3]. Which it is the fifth more commonly malignant tumor at women [4]. And risk factors of thyroid cancer are very high in Arab countries [5]. In Saudi Arabia, thyroid carcinoma was occupied the second ranked in females [6]. While in Iraq it was occupies the 11th ranked among females [7]. Thyroid carcinoma is more widespread in females at a rate of three times more than men, and is more predominant in the white persons than black persons, also common in Asian/Pacific Islander more than in other populations [8]. According to histopathological characteristics, thyroid carcinoma has been split into two types: well differentiated, which include papillary and follicular carcinoma, and poorly differentiated, and this include medullary and anaplastic carcinoma [9]. There are other categories of thyroid cancer but uncommon, such as: Hürthle cell carcinoma which is variants of follicular [10]. Also there are mucoepidermoid carcinoma, thyroid lymphoma, squamous cell thyroid carcinoma and sarcoma of thyroid [11]. The epidermal growth factor receptor EGFR is glycoprotein existing on the surface of some cells and composed of 1186 amino acid [12]. Human EGFR gene is located on chromosome 7p12.3-p12.1 [13]. EGFR is a member in the receptor tyrosine kinase family which members play a significant role in promoting cell growth, division,

apoptosis and cell survival [14]. The EGFR have sites allows it to attaching to other proteins, called ligands, in the outside of the cell and to receive signals that help the cell respond to the surrounding environment, and suitable together such as to locks and keys [15]. EGFR is activated by binding of its own ligands, including epidermal growth factor (EGF) and transforming growth factor alpha (TGFα), this kinase triggers diversely downstream signaling pathways and results in various biological responses, such as proliferation and differentiation [16]. Transforming growth factor alpha (TGF-α): is a protein contain of 50 amino-acids polypeptide, the cytogenetic location of human TGF-α gene is: 2p13.3, which is the short (p) arm of chromosome 2. at position 13.3 [17]. TGF-α becomes effective when it is linked to receptors capable of protein kinase effectively of cellular signaling [18]. TGF-α is a ligand for the EGF receptor, and because TGF-α is a member of the receptor tyrosine kinase family, so this gives them the similarity biological structural about 30%, therefore TGF-α and EGF are linked to the same receptor [19]. Loss of regulation of the TGF-alpha and its receptor EGFR could lead to many human diseases, most notably cancer [20]. In the recent period, the increased of thyroid cancer was affected by numerous factors such as genetic changes, growth agents, and physical factors such as radiation, so there are needed to helpful forecast factors to detect the biologic behavior, providing an initial help [21]. A large number of biomarkers have been used in differential diagnosis of thyroid cancer, which include: TGF-α, and EGFR have been translated in clinical applications that led to the emergence of a significant improvement in the preoperative diagnosis of thyroid cancer [22].

2. Materials and Methods

2.1. Patients and Tissue Samples

The study was conducted in the laboratories of College of Science, University of Wasit and in collaboration with AL-Hussein Teaching Hospital, Thi Qar Province and Ibn Al-Bitar National Laboratory in Thi Qar Province for the period from October 2015 to April 2016. One hundred and seventy-eight patients with diseases of the thyroid gland are collected randomly, which included three groups of thyroid gland diseases, first group was thyroid carcinoma 111 (62.36%) patient's, second group benign tumor account 46 (25.84%) patient's and 21 (11.80%) other thyroid diseases (non-cancer and non- benign), the rate of the patient's age between 11 - 80 years. All patients were diagnosed and confirmed by specialized histopathlogistis. All clinical information about samples was taken. Histological samples were taken treated with formalin and embedded with paraffin wax postoperative.

2.2. Immunohistochemistry (IHC)

Immunohistochemistry technique was used in this study. ABC staining system: sc-2017, TGFα: sc-374433 and EGFR: sc-373746 (mouse monoclonal antibodies) was provided by Santa Cruz Biotechnology, Inc.

Serial tissue sections were cut 4 - 5 μm thick and positioned on positive charged slides. The slides were backed in oven at 60°C overnight. The tissue sections were deparaffinized; then the slides were dehydrated by different ethanol concentration (100%, 95%, and 70%) and distal water. The slides were treated with citric buffer for 15. minute, and then washed in two changes of PBS solution for 5. minutes. Then slides were washed in distal water. The percentage of expression was calculated as 0 for negative, 1 for 1-25, 2 for 26-50, and 3 for 51-100 staining cells. Intensity was calculated as 1 (weak), 2 (moderate) and 3 (strong).

2.3. Ethical Consent

The study was submitted and approved by the College of Science, University of Wasit in collaboration with AL-Hussein Teaching Hospital and Ibn al-Bitar Laboratory Specialist in Thi Qar Province, Iraq.

2.4. Statistical Analysis

All the clinical, pathological, follow-up, expression data were computerized. Statistical analysis was performed using the SPSS for Windows (version 23.0). Fisher's exact test, chi-square test or likelihood ratio was used for categorical variables. Statistical significance level of the tests was taken at a p-value <0.05.

3. Results

3.1. TGFα Expression and Intensity

The results of immunohistochemistry showed, that the expression of TGFα was positively in 76 (68.47%) of thyroid carcinoma patients out of 111 cases, while in benign 20 (43.48%) patients has appeared positive out of 46 cases, as for the control group; 4 (19.05%) cases are positive expression from 21 cases.

When thyroid carcinoma were compared with benign neoplasms showed there was highly significant differences between thyroid carcinoma patients and benign neoplasms to TGFα expression (P= 0.002) table 1, as well as when comparing thyroid carcinoma with control group table 2, the results showed that also there was highly significant difference between thyroid carcinoma patients and control group (P= 0.000). Also benign patients and control group cases had appeared a significant difference (P= 0.046) table 3.

Regarding to the intensity evaluation of TGF-α expression in thyroid carcinoma patients revealed that 35 (31.53%) patients were negative (score 0), while the intensity of expression was positive in 76 (68.5%) of cases, which were distributed as follows: score +1; 19 (17.12%) cases, score +2; 43 (38.74%) cases and score +3; 14 (12.61%) cases. In benign neoplasms intensity of TGF-

α expression was: 26 (56.52%) cased were negative (score 0) and 20 (43.48%) cases were positive divided as follows: 3 (6.52%) cases were score +1, 15 (32.61%) cases were score +2 and 2 (4.35%) cases were score +3. In control group the intensity of TGF-α expression was: 17 (80.95%) cases were negative (score 0), while only 4 (19.05%) cases were positive and distributed as: score +1; 1 (4.76%) case, score +2; 3 (14.29%) cases, while no case in score +3 (Fig. 1).

When thyroid carcinoma were compared with benign neoplasms and with control group, there were no significant differences in relation to intensity of TGFα expression (P>0.05). As well as, in the case of TGFα expression intensity between benign neoplasms and control group, also no statistically significant differences were found (P>0.05).

Comparison of TGF expression intensity between thyroid carcinoma and benign patients and the control group are shown in Tables 1, 2 and 3

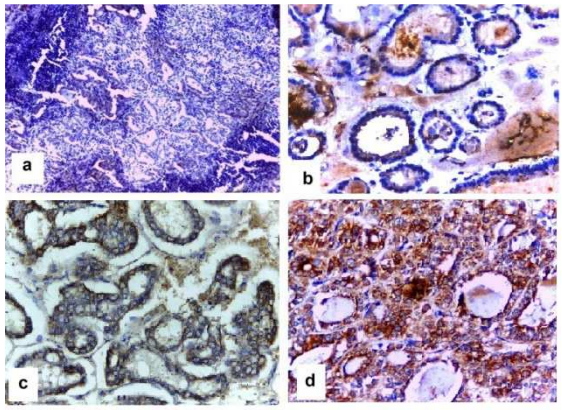

Figure 1. *Representative slides of TGF-α staining by immunohistochemistry in thyroid carcinoma, shows: (a) negatively staining (b) positively staining score +1 (c) positively staining score +2 (d) positively staining score +3. Magnification: 40X.*

Table 1. *TGFα expression and intensity in thyroid carcinoma compared with benign neoplasms.*

Cases	TGFα Expression		TGFα Intensity				Total No.%
	-ve	+ve	0	1+	2+	3+	
	No.%	No.%	No.%	No.%	No.%	No.%	
Thyroid carcinoma	35	76	35	19	43	14	111
	31.53%	68.47%	31.53%	17.12%	38.74%	12.61%	100.0%
Benign neoplasms	26	20	26	3	15	2	46
	56.52%	43.48%	56.52%	6.52%	32.61%	4.35%	100.0%
Total	61	96	61	22	58	16	157
	38.85%	61.15%	38.85%	14.01%	36.94%	10.20%	100.0%
P. value	highly significant P= 0.002		non-significant P= 0.378				

Table 2. *TGFα expression and intensity in thyroid carcinoma compared with control group.*

Cases	TGFα Expression		TGFα Intensity				Total No.%
	-ve	+ve	0	1+	2+	3+	
	No.%	No.%	No.%	No.%	No.%	No.%	
Thyroid carcinoma	35	76	35	19	43	14	111
	31.53%	68.47%	31.53%	17.12%	38.74%	12.61%	100.0%
Control group	17	4	17	1	3	0	21
	80.95%	19.05%	80.95%	4.76%	14.29%	0.00%	100.0%
Total	52	80	52	20	46	14	132
	39.39%	60.61%	39.39%	15.15%	34.85%	10.61%	100.0%
P. value	highly significant P= 0.000		non-significant P= 1.000				

Table 3. *TGFα expression and intensity in benign neoplasms and control group.*

Cases	TGFα Expression		TGFα Intensity				Total No.%
	-ve	+ve	0	1+	2+	3+	
	No.%	No.%	No.%	No.%	No.%	No.%	
Benign neoplasms	26	20	26	3	15	2	46
	56.52%	43.48%	56.52%	6.52%	32.61%	4.35%	100.0%
Control group	17	4	17	1	3	0	21
	80.95%	19.05%	80.95%	4.76%	14.29%	0.00%	100.0%
Total	43	24	43	4	18	2	67
	64.18%	35.82%	64.18%	5.97%	26.87%	2.98%	100.0%
P. value	significant P= 0.046		non-significant P= 1.000				

3.2. EGFR Expression and Intensity

The result of EGFR expression was demonstrated positive expression at 56 (50.45%) from 111 cases of thyroid carcinoma, and 15 (32.61%) cases of benign neoplasms were positive out of 46 case, while in control group 7 (33.33%) cases were positive expression from 21 case.

By comparing EGFR expression between thyroid carcinoma patients with benign patients and with control group, the results demonstrated there were no statistically significant differences (p> 0.05). Moreover, when comparing EGFR expression between benign neoplasms and control group, there was no significant difference (p> 0.05).

Regarding to intensity assessment of EGFR expression in thyroid carcinoma, the results were showed that: 55 (49.55%) cases were negative, score 0, 15 (13.51%) cases with score +1, 35 (31.53%) cases with score +2, and lowest percentage 6. (5.41%) cases were with score +3. As for intensity of benign were as follows: score 0; 31 (67.39%) cases, score +1; 5 (10.87%) cases, score +2; 9 (19.57%) cases, and only one case (2.17%) with score +3. In control group, 14 (66.67%) cases were score 0, 5 (23.81%) cases with score +1, 2 (9.52%) cases with score +2, and no case with score +3 (Fig. 2).

In relation to intensity of EGFR expression there were no statistically significant differences between thyroid carcinoma and benign patients (P>0.05), as well as, when the comparison between thyroid carcinoma and control group there were no significant differences (P>0.05), also is the situation between benign neoplasms and control group, no statistical significance differences were found (P>0.05).

Tables 4, 5 and 6 illustrate the comparison of EGFR expression and intensity between thyroid carcinoma and benign neoplasms and the control group.

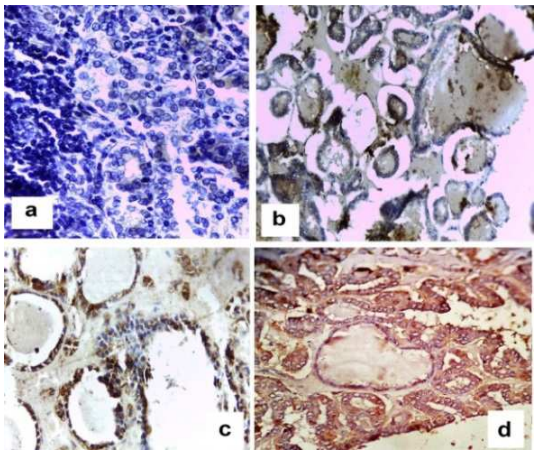

Figure 2. *Representative slides of EGFR staining by immunohistochemistry in thyroid carcinoma shows: (a) negatively staining (b) positively staining score +1 (c) positively staining score +2 (d) positively staining score +3. Magnification: 40X.*

Table 4. *EGFR expression and intensity in thyroid carcinoma compared with benign neoplasms.*

Cases	EGFR Expression		EGFR Intensity				Total No.%
	-ve	+ve	0	1+	2+	3+	
	No.%	No.%	No.%	No.%	No.%	No.%	
Thyroid carcinoma	55 49.55%	56 50.45%	55 49.55%	15 13.51%	35 31.53%	6 5.41%	111 100.0%
Benign neoplasms	31 67.39%	15 32.61%	31 67.39%	5 10.87%	9 19.57%	1 2.17%	46 100.0%
Total	86 54.78%	71 45.22%	86 54.78%	20 12.74%	44 28.02%	7 4.46%	157 100.0%
P. value	non-significant P= 0.3		non-significant P= 0.539				

Table 5. *EGFR expression and intensity in thyroid carcinoma compared with control group*

Cases	EGFR Expression		EGFR Intensity				Total No.%
	-ve	+ve	0	1+	2+	3+	
	No.%	No.%	No.%	No.%	No.%	No.%	
Thyroid carcinoma	55 49.55%	56 50.45%	55 49.55%	15 13.51%	35 31.53%	6 5.41%	111 100.0%
Control group	14 66.67%	7 33.33%	14 66.67%	5 23.81%	2 9.52%	0 0.00%	21 100.0%
Total	69 52.27%	63 47.73%	69 52.27%	20 15.15%	37 28.03%	6 4.55%	132 100.0%
P. value	non-significant P= 0.069		non-significant P= 0.079				

Table 6. *EGFR expression and intensity in benign neoplasms and control group*

Cases	EGFR Expression		EGFR Intensity				Total No.%
	-ve	+ve	0	1+	2+	3+	
	No.%	No.%	No.%	No.%	No.%	No.%	
Benign neoplasms	31 67.39%	15 32.61%	31 67.39%	5 10.87%	9 19.57%	1 2.17%	46 100.0%
Control group	14 66.67%	7 33.33%	14 66.67%	5 23.81%	2 9.52%	0 0.00%	21 100.0%
Total	45 67.16%	22 32.84%	45 67.16%	10 14.93%	11 16.42%	1 1.49%	67 100.0%
P. value	non-significant P= 0.219		non-significant P= 0.235				

4. Discussion

This study has shown that there is a high significant difference of TGFα expression between thyroid carcinoma and benign patients (P<0.05), Also there was a highly significant difference of TGFα expression between thyroid carcinoma and noncancerous control group, where the TGF-α overexpression was positive in (68.47%) of thyroid carcinoma patients, (43.48%) in benign patients and (19.05%) in noncancerous control group, this is compatible with results of Lam et al. [23], and Lau [24]. Overexpression of TGFα has been observed in a variety of human cancers, including hepatocellular cancer, a study done by Daveau, Scotte et al. [25], salivary duct cancer a study by Fan, Melhem et al. [26], and gastric cancer study by Konturek et al. [27]. On the other hand, there was no significant difference of EGFR expression between thyroid carcinoma and benign patients, as well, there was no significant difference of EGFR expression between thyroid carcinoma and noncancerous control group, although the percentage of EGFR overexpression was positively in (50.45%) of thyroid carcinoma and this is identical to the Lei Gong study [28], but the ratio was only (32.61%) in benign neoplasms and (33.33%) in noncancerous control group. In the study done by Lam et al. [23], they found that was a high expression of TGF-α noted in 77% (55 from 71) of thyroid cancers, and also a high level of EGFR expression was observed in 54% (38 from 71) of thyroid cancers. Additionally, in this study we compared TGFα and EGFR expression between benign neoplasms and noncancerous control group, whereas most of other studies were compared only among thyroid carcinoma patients with benign neoplasms or with noncancerous control group, in the current study we have noted there was significant difference of TGFα expression (P<0.05) between benign patients and noncancerous control group, while there was no significant difference of EGFR expression among benign neoplasms and noncancerous control group. In the study performed by Lau [24], 59 patients of papillary thyroid carcinoma and 10 benign thyroid neoplasm was taken, the results were showed, all cases of the papillary thyroid carcinoma positively for TGFα expression compared with only 20% in the benign thyroid neoplasm, and from 59 samples, 39 (66%) cases were higher expression and 20 (34%) cases were lower expression, as for the benign thyroid neoplasms all positive samples were classified as a lower expression. Moreover, there was no significant difference of TGFα intensity between thyroid carcinoma and benign neoplasms (P>0.05). The same applies for TGFα intensity between thyroid carcinoma and noncancerous control group were there no significant difference and this is consistent with results by Lau [24]. Also there was a no significant difference of the EGFR intensity between thyroid carcinoma and benign neoplasms. Our results concluded there is a strong relationship between TGFα expression and thyroid cancer.

5. Conclusion

Our study concluded that there is a strong relationship between TGFα overexpression with thyroid carcinogenesis, and this protein could serve as a biomarker tool to diagnosis the thyroid cancer.

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