International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Index Copernicus Value (2015): 62.86 | Impact Factor (2015): 3.791

Relationship between TGF-α and its Receptor EGFR Expression with Clinicopathological Variables in Thyroid Carcinoma

Tahani Abduljabbar Nasser[1], Kareem Hamed Ghali[2], Hameed Naeem Mousa[3]

Department of Biology, College of Science, Wasit University, Iraq

Department of Pathology and Forensic Medicine, College of Medicine, Thi Qar University, Iraq

Abstract: ***Thyroid cancer is the one of the most common cancer worldwide.The understand of molecular events are still unknown yet, this is may be because of more complications in this disease. The current study included 111 thyroid cancer patients collected from January 2015 to April 2016, and designed to detection the relationship of TGFα and EGFR expression with pathological variables in thyroid cancer patients using immunohistochemical technique. The results showed significant relationship between TGFα expression with gender ($p<0.05$), while the results didn't show relationship between TGFα expression with histological type, stage, muscle and lymph nodes invasion and tumor size ($p>0.05$). Regarding EGFR expression, the findings demonstrated that there was a strong relationship with cell types affected ($p<0.05$), while no relationship with other variables. Our results concluded that the progression and metastasis of thyroid cancer are not depended on TGFα and EGFR expression in thyroid cancer.***

Keywords: Thyroid, cancer, immunohistochemistry, TGFα and EGFR

1. Introduction

Thyroid gland is one of the most significant endocrine in the human body (1). It is a butterfly-shaped gland, consists of two lobes separated by installation called isthmus (2). The thyroid located in the front of neck just below the larynx, which excreted two hormones; thyroxin (T4) and triiodothyronine (T3), these hormones regulated the metabolic rate in the body (3). Thyroid cancer occurs when nodules develop in the thyroid gland, although about 90% to 95% of thyroid nodules are noncancerous but there was increased in the number of thyroid cancer cases diagnosed in the worldwide (4). Thyroid cancer is the ninth widespread, where it is the fifth more common malignancy tumor in women at the United States, which is exemplified 3.8% of the new malignant tumor cases (5). In Iraq thyroid cancer was occupies the eighth place in women (6). Thyroid cancer occurs in women much more than men at a rate of 3:1 (7). There are several types of thyroid cancers can be categorized according to histopathological feature (8) The main category of thyroid carcinoma is papillary thyroid carcinoma (PTC), it represents about 75% to 85% of all thyroid malignancy, followed by the second category is follicular thyroid carcinoma (FTC), which represent about 10% to 20% of all thyroid malignant tumors, and both papillary and follicular thyroid carcinoma are arise from thyroid follicular cells so they called differentiated thyroid carcinoma (DTC) (9). Medullary thyroid carcinoma (MTC) represents approximately 3% to 8% of all thyroid malignancy, which arises from the C cells(10). Anaplastic thyroid carcinoma (ATC) or poorly differentiated thyroid carcinoma represents about 2% of all thyroid malignant tumors, this type of carcinoma is most aggressive and risky because it invade the surrounding lymph nodes and various sites of the body (11). Hürthle cell carcinoma uncommon type constitutes about 3% of all thyroid malignant tumors (12). There are other types of thyroid carcinoma but even rarer, such as: mucoepidermoid carcinoma (MEC) which is very rare, lymphoma, squamous cell thyroid carcinoma and Sarcoma of thyroid (13).

EGFR is a transmembrane protein found in some cell, and contains of 1186 amino acids (14). The cytogenetic location of human EGFR is: 7p11.2, which is the short (p) arm of chromosome 7 at position 11.2(UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly). EGFR is member of the epidermal growth factor family (EGF family) that plays an essential role in regulation cell differentiation, proliferation, survival and migration (15). Epidermal growth factor receptor connects with at least seven various ligands, this linking to a ligand give permission to the EGFR to link to a contiguous receptor protein, activating the receptors compound and signaling pathways in the target cell (16).

Transforming growth factor-alpha (TGF-α) is a stable protein for acid and heat has 50 amino acid, molecular weight of mature TGFα is 6 kDa, and human TGFα gene is located on chromosome 2p13.3 (17). This polypeptide shares by about 33% structural matching with epidermal growth factor (EGF), therefore competes for binds to the joint receptor, EGFR (18).

TGF-alpha and its receptor EGFR may play an important role in the pathogenesis and progression of different carcinomas types including thyroid carcinoma, as the increase in the expression of these genes are able to stimulate cell transformations and possibly could lead to carcinogenesis (19).

EGFR after linked with ligand, achieve activation by forming receptor complexes, the dimerization of EGFR represents the important mechanism that leading the transformation.

2. Materials and Methods

2.1 Patients and tissue samples

111 patients (84 women and 27 men) the subject of this study, medium age 35 years (13-80 yr), The patients are divided histologically to three groups ,the first group included 10 follicular patients, the second group were 94 papillary and the third group included 7 patients(distributed as, 1 anaplastic, 1 Hürthle, 4 medullary and 1 mucoepidermoid). The mean of tumor size was 8.34 cm (1.5 - 24) and subdivided into two groups, more than 10 cm and less than 10 cm.

Been using the TNM in classification of malignant tumors (tumors, lymph node and metastases) and MACIS staining system (metastases, age, completeness of resection, invasion and size) including: 74 patients with stage I, 22 with stage II, 6 with stage III and 9 patients with stage IV. Used histologic grade (G) in diagnostic grade of tumor, G1 well differentiated, G2 moderately and G3 poorly differentiated.

The samples were collected for the period from January 2015 to April2016,from Al- Hussein Teaching Hospital and Ibn Al-BitarPrivate Laboratory in the Province of Thi Qar.

2.2 Immunohistochemistry (IHC)

ImmunoCruz™ ABC Staining Systems sc2017 and primary antibodies were used in this study. All steps are completed at room temperature in a moisten chamber. Apply enough volumes of reagents to quite cover the section; 75-100 µl is usually acceptable, or 1–3 drops of working solutions. Use suck to remove reagents after any step, but evade drying of specimens between steps. The percentage (p) of staining cells was measured as zero for negative staining, plus 1 for 1-25, 2 for 26-50, and 3 for 51 to 100 staining cells. The procedure was involving the following, slides were packed for overnight, then incubated in 0.1–1% H2O2 diluted in PBS, deionized H2O or methanol to narrate endogenous peroxidase activity, then treated for 1 hr. in 1.5% blocking serum in PBS.

Primary antibody was added to sections slide for overnight at 4° C or 30 minutes at room temperature. Optimal antibody concentration should be decided by titration; recommended extent is 0.5–5.0 µg/ml, diluted in 1.5% blocking serum in PBS.Slides were incubated in biotinylated secondary antibody at approximately 1 µg/ml for 30 minutes. AB enzyme reagent added for 30 minutes, then sections were treated with 1–3 drops of peroxidase substrate for 30 seconds–10 minutes, sections were staining with Gill's formula #2 hematoxylin for 5–10 seconds. Finally, sections were dehydrated as follows: 2x 95% ethanol for 10 seconds all one, 2x 100% ethanol for 10 seconds all one, 3x xylenes for 10 seconds all one.

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 Private laboratory in Thi Qar Province.

2.4 Statistical Analysis

All the clinical, pathological, follow-up. Statistical analysis was performed using the Statistical Package for Social Sciences (version 23.0; SPSS) Fisher's exact test, chi-square test or likelihood ratio was used for categorical variables. Pearson correlation test was used for continuous variables. Survival analysis was tested using Significance level of the tests was $p<0.05$.

3. Results and Discussion

In this study we try to find if any relationship between the TGF-α and EGFR expression, and their relationship with the clinicopathological variables in thyroid carcinoma(Figs 1 and 2).

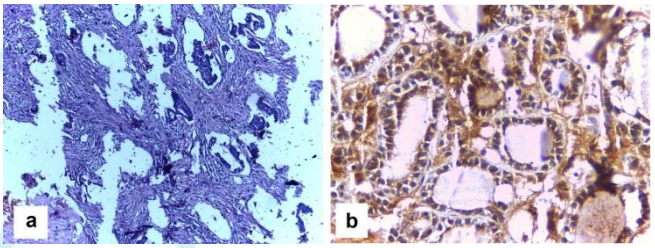

Figure 1: Representative images of EGFR staining by immunohistochemistry in thyroid patients cases show: (a) negatively staining (b) positively staining. Magnification: 40X.

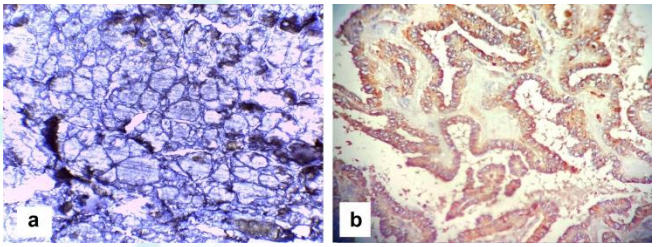

Figure 2: Representative images ofTGFα staining by immunohistochemistry in thyroid patients cases, show: (a) negatively staining (b) positively staining. Magnification: 40X.

111 cases of thyroid carcinoma have been studied, which were classified according to the histological type into three categories (Table 1). Immunohistochemical (IHC) analysis of TGFα expression was stained positively in 64 (68.1%)cases of papillary carcinoma out of 94, 6 (60.0%)cases of follicular carcinoma out of 10 and 6 (85.7%)cases of other types of thyroid carcinoma out of 7 cases. On the other hand, IHC analysis of EGFR expression was reported positive in 41 (43.6%)cases of papillary carcinoma, 9 (90.0%)cases of follicular carcinoma and 6 (85.7%) cases of other types of thyroid carcinoma. This result was compatible with the results of Lau (20) and Lam et al. (21).

Table (1) TGFα and its receptor EGFR expression in thyroid carcinoma according to histological type

Histological type	TGFα Expression		EGFR Expression		Total No.%
	-ve No. %	+ve No. %	-ve No. %	+ve No. %	
Papillary	30 31.9%	64 68.1%	53 56.4%	41 43.6%	94 100.0%
Follicular	4 40.0%	6 60.0%	1 10.0%	9 90.0%	10 100.0%
Other types	1 14.3%	6 85.7%	1 14.3%	6 85.7%	7 100.0%
Total	35 31.5%	76 62.5%	55 49.5%	56 50.5%	111 100.0%
P.value	non-significant P> 0.05		highly significant P= 0.002		

According to the gender (Table 2), there were 75.7 % (n=84) Females and 24.3 % (n=27) males of thyroid carcinoma patients, and this ratio was identical to National Cancer Institute (U.S.) (2015) about thyroid carcinoma which registered that the thyroid carcinoma is more widespread in females at a rate of three times more than males (22). IHC analysis of TGFα expression showed that 62 (73.8%) females were positive and 14 (51.9%) cases were positive from males. While the IHC analysis of EGFR expression was obvious positively only in 39 (46.4%) of females and 17 (63.0%) of males.

Table (2): TGFα and its receptor EGFR expression in thyroid carcinoma according to gender

Gender	TGFα Expression		EGFR Expression		Total No.%
	-ve No. %	+ve No. %	-ve No. %	+ve No. %	
Females	22 26.2%	62 73.8%	45 53.6%	39 46.4%	84 100.0%
Male	13 48.1%	14 51.9%	10 37.0%	17 63.0%	27 100.0%
Total	35 31.5	76 68.5%	55 49.5%	56 50.5%	111 100.0%
P.value	significant P= 0.031		non-significant P> 0.05		

Stage was classified according to the TNM staging system of the American Joint Committee on Cancer (AJCC) (23) and International Union Against Cancer (UICC) (Greene 2002) (24) (Table 3), with 66.7 % (n=74) were stage I, 19.8 % (n=22) were stage II, 5.4 % (n=6) were stage III and 8.1 % (n=9) were stage IV. TGFα expression was obtained positive in 51 (68.9%) cases of stage I, 13 (59.1%) cases of stage II, 3 (50.0%) cases of stage III and 9 (100.0%) cases of stage IV. On the other hand the EGFR expression was as follows: 35 (47.3%) were stage I, 10 (45.5%) were stage II, 4 (66.7%) were stage III and 7 (77.8%) were stage IV.

Table (3): TGFα and its receptor EGFR expression in thyroid carcinoma according to stage

Stage	TGFα Expression		EGFR Expression		Total No.%
	-ve No. %	+ve No. %	-ve No. %	+ve No. %	
S I	23 31.1%	51 68.9%	39 52.7%	35 47.3%	74 100.0%
S II	9 40.9%	13 59.1%	12 54.5%	10 45.5%	22 100.0%
S III	3 50.0%	3 50.0%	2 33.3%	4 66.7%	6 100.0%
S IV	0 0.0%	9 100.0%	2 22.2%	7 77.8%	9 100.0%
Total	35 31.5%	76 68.5%	55 49.5%	56 50.5%	111 100.0%
P.value	non-significant P> 0.05		non-significant P> 0.05		

With regard to grade of tumor, in our study there was not statistics computed because grade is a constant, where the all specimens was grade 1. Furthermore, invasiveness was found in 16.2 % (n=18) of samples while invasiveness was not found in 83.8 % (n=93) of samples (Table 4).Evaluation of TGFα expression in thyroid carcinoma in relation to invasiveness of tumor was positive in 15(83.3%) of invasiveness tumors and 61 (65.6%) of noninvasiveness tumors, while the estimate of positively EGFR expression was in 11(61.1%) of invasiveness tumors and 45 (48.4%) of noninvasiveness tumors.

Table (4): TGFα and its receptor EGFR expression in thyroid carcinoma according to invasiveness

Invasiveness	TGFα Expression		EGFR Expression		Total No.%
	-ve No. %	+ve No. %	-ve No. %	+ve No. %	
Yes	3 16.7%	15 83.3%	7 38.9%	11 61.1%	18 100.0%
No	32 34.4%	61 65.6%	48 51.6%	45 48.4%	93 100.0%
Total	35 31.5%	76 68.5%	55 49.5%	56 50.5%	111 100.0%
P.value	non-significant P> 0.05		non-significant P> 0.05		

Also in this study, thyroid cancer patients were divided according to lymph node invasion as follows (Table 5): 9.9 % (n=11) confirmed to have lymph node invasion and 90.1 % (n=100) not have lymph node invasion. So the TGFα expression reported positively in only 7 (63.6%) cases with lymph node invasive and 69 (69.0%) cases without lymph node invasion, whilst the positively expression of EGFR in cases have a lymph node invasion were 5 (45.5%) and in cases without lymph node invasion were 51 (51.0%).

Table (5): TGFα and its receptor EGFR expression in thyroid carcinoma according to lymph node invasion

Lymph node invasion	TGFα Expression		EGFR Expression		Total No.%
	-ve No. %	+ve No. %	-ve No. %	+ve No. %	
Yes	4 36.4%	7 63.6%	6 54.5%	5 45.5%	11 100.0%
No	31 31.0%	69 69.0%	49 49.0%	51 51.0%	100 100.0%
Total	35 31.5%	76 68.5%	55 49.5%	56 50.5%	111 100.0%
P.value	non-significant P> 0.05		non-significant P> 0.05		

Lastly, the mean tumor size was 8.34 cm (1.5 - 24) so, carcinoma patients were divided into two categories, the first category includes patients more or equal than 10 cm which were 71.2 % (n=79) cases, and the second category were include 28.8 % (n=32) patients less than 10 cm (Table 6). TGFα expression was positive in 57 (72.2%) of the patients with tumor size ≤ 10 and 19 (59.4%) of the patients with tumor size > 10. While the expression of EGFR appeared positively staining in 42 (53.2%) of the cases have been more or equal than 10 cm and 14 (43.8%) of the cases have less than 10 cm.

Table (6): TGFα and its receptor EGFR expression in thyroid carcinoma according to tumor size

Tumor size	TGFα Expression		EGFR Expression		Total No.%
	-ve No. %	+ve No. %	-ve No. %	+ve No. %	
≤ 10	22 27.8	57 72.2%	37 46.8%	42 53.2%	79 100.0%
> 10	13 40.6%	19 59.4%	18 56.3%	14 43.8%	32 100.0%
Total	35 31.5%	76 68.5%	55 49.5%	56 50.5%	111 100.0%
P.value	non-significant P= 0.075		non-significant P> 0.05		

In this study the results showed the positive expression of TGFα was not significantly associated with different histologic type of thyroid carcinoma tumor, between stages, invasiveness and between lymph nodes invasion (P>0.05). However, TGFα expression was associated marginally with tumor size (P = 0.075). But there was a high significance difference of TGFα expression correlated with gender of thyroid carcinoma cases (P = 0.031). In a study by Lam et al. (21), they found the TGFα expression had no significant differences associated with age, gender and lymph nodes invasion, but there significant differences associated with tumor size. According to a study conducted by Lau (20) reviewed that there was a significant difference to TGFα expression in lymph nodes invasion, while no significant differences were observed between the patient age, sex, stage and tumor size. These results are approximately identical to our study.And about the positively expression of EGFR, our results found that was no significance difference correlated with gender, stage of tumor, invasiveness, lymph nodes invasion and tumor size (P>0.05). Whilst, there was high significance difference of EGFR expression associated with different histologic types of thyroid carcinoma (P = 0.002). In a study by Tang et al. (25), there were not significant differences in EGFR expression between patients with different histologic subtype of thyroid carcinoma, age and gender. Not with standing, EGFR expression was associated with TNM stage and marginally with tumor size. In another study done by Lee and Lee (26), they found there were no significance difference associated of EGFR expression with age, or MACIS group, but was significant difference associated with gender and lymph node invasion, therefore they were concluded that the IHC assessment may be benefit for determining prognosis in thyroid carcinoma. Also, in the research of Lam et al. (21), they found the EGFR expression had no significant differences associated with age, gender, lymph nodes invasion and tumor size, and they concluded from that study: the overexpression of TGF-α and EGFR were associated with each other in thyroid carcinoma and these results provide information for management of thyroid carcinoma and targeting genetic therapy. Mizukami et al. recorded in their study, no significant correlations between EGF-receptor expression and tumor size, degree of invasion or cervical metastases in the thyroid carcinomas.)27).

4. Acknowledgement

We would like to thank YounusAtiyahKamil and Dr. Ali abidSaadoon Al-Ghuzy for helping us to accomplishment this research.

5. Conclusion

Our results concluded that the progression and metastasis of thyroid cancer are not depended on TGFα and EGFR expression in thyroid cancer.

References

[1] Larsen, P. R.; Kronenberg, H. M.; Melmed, S. and Polonsky, K. S. (2003).Williams textbook of endocrinology, tenth edition, tenth edition, Saunders Philadelphia, PA.
[2] Upadhyaya, M. (2012). Importance of inclusion of thyroid stimulating hormone (TSH) test in master health checkups –a survey, International journal of pharmacy and biological sciences, 2 (3): 216-222.
[3] Hall, J. E. (2016). Pocket companion to Guyton and Hall textbook of medical physiology, thirteenth edition, Elsevier Philadelphia, PA.
[4] Leung, A. M. and Mestman, J. H. (2015). Disorders of the thyroid gland, Global library of women's medicine, (ISSN: 1756-2228) 2015; DOI 10.3843/GLOWM.10308
[5] This chapter was last updated: November 2015
[6] Nguyen, Q.t.; Lee, E. j.; Huang, M. g.; Park, Y. i.; Khullar, A. and Plodkowski, R. a. (2015). Diagnosis and treatment of patients with thyroid cancer, American Health & Drug Benefits, 8 (1): 30-40.
[7] Iraqi Cancer Registry. (2011). Ministry of Health, Iraqi Cancer Registry Center.
[8] National Cancer Institute. Thyroid cancer treatment (PDQ). Updated July 11, 2014.http://cancer.gov/cancertopics/pdq/treatment/thyroid/HealthProfessional. Accessed January 12, 2015.
[9] National cancer institute (U.S.) (2006). The U.S. National Cancer Institute. [Bethesda, Md.], National Cancer Institute, http://purl.access.gpo.gov/GPO/LPS101377.
[10] Kumar, V.; Abbas, A.; Fausto, N. and Mitchell, R. (2007). Robbins Basic Pathology, 8th edition, Saunders Philadelphia.

[11] Cooper, D. S.; Doherty, G. M.; Haugen, B. R.; Kloos, R. T.; Lee, S. L.; Mandel, S. J.; Mazzaferri, E. L.; McIver, B.; Pacini, F.; Schlumberger, M.; Sherman, S. I.; Steward, D. L. and Tuttle, R. M. (2009). Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer, Thyroid official journal of the American thyroid association, 19 (11): 1167-1214.
[12] Fitzgerald, P. A.; Papadakis, M. A. and McPhee, S. J. (2013). Current medical diagnosis and treatment 2013, 52nd ed, US: McGraw-Hill Companies; 1126–1134.
[13] Luo, H.; Tulpule, S.; Alam, M.; Patel, R.; Sen, S. and Yousif, A. (2015). A rare silent killer: right atrial metastasis of thyroid Hürthle cell carcinoma, Case reports in oncology, 8(2): 233-237.
[14] Shindo,K.; Aishima, S.; Okido, M. And Ohshima, A. (2012). A poor prognostic case of mucoepidermoid carcinoma of the thyroid: A Case Report, Case Reports in Endocrinology, http://dx.doi.org/10.1155/2012/862545.
[15] K Shindo; S Aishima; M Okido; A Ohshima
[16] Mitrasinovic, P. M. (2012). Epidermal growth factor receptors: a functional perspective, CurrRadiopharm, 5: 29-33.
[17] Hallbeck, A-L. (2007). Studies of transforming growth factor alpha in normal and abnormal growth, Linkoping University Medical Dissertations.
[18] Cavalli, F. (2009). Textbook of medical oncology, 4th Edition, London, Informa Healthcare. http://site.ebrary.com/id/10373302.
[19] Ebadifar, A.; Hamedi, R.; Khorramkhorshid, H. R.; Kamali, K. and Moghadam, F. A. (2016). Parental cigarette smoking, transforming growth factor-alpha gene variant and the risk of orofacial cleft in Iranian infants, Iranian Journal of Basic Medical Sciences, 19 (4): 366-73.
[20] Ciana, P.; Ghisletti, S.; Mussi, P.; Eberini, I.; Vegeto, E. and Maggi, A. (2003). Estrogen receptor alpha, a molecular switch converting transforming growth factor-alpha-mediated proliferation into differentiation in neuroblastoma cells, The Journal of Biological Chemistry, 278 (34): 31737-31744.
[21] Yarden, Y. and Sliwkowski, M. X. (2001). Untangling the ErbBsignalling network, Nature Reviews. Molecular Cell Biology, 2 (2): 127-137.
[22] Lau, K. P. (2007). Clinicopathological Roles of Transforming Growth Factor Alpha (TGFα) in Papillary Thyroid Carcinoma, Hong Kong University Theses Online.
[23] Lam, A. K.; Lau, K. K.; Gopalan, V.; Luk, J. and Lo, C. Y. (2011). Quantitative analysis of the expression of transformation growth factor alpha (TGF- α) and epidermal growth factor receptor (EGFR) in papillary thyroid carcinoma: clinicopathological relevance, Pathology, 43 (1): 40–47.
[24] National Cancer Institute (U.S.) (2015). SEER, Surveillance, Epidemiology, and End Results: thyroid cancer, U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute.
[25] Edge, S. B.; Byrd, D. R. and Compton, C. C. et al. (2010). American Joint Committee on Cancer (AJCC): cancer staging manual, 7th ed, New York: Springer.
[26] International Union Against Cancer (UICC) (Greene 2002)
[27] Tang, C; Yang, L; Wang, N; Li, L; Xu, M; Chen, G.G. and Liu, ZM. (2014). High expression of GPER1, EGFR and CXCR1 is associated with lymph node metastasis in papillary thyroid carcinoma, International Journal of Clinical and Experimental Pathology, 7 (6): 3213-3223.
[28] Lee, Y. M. and Lee, J. B. (2013). Prognostic value of epidermal growth factor receptor, p53 and galectin-3 expression in papillary thyroid carcinoma, The Journal of International Medical Research, 41(3): 825-834.
[29] Mizukami Y1, Nonomura A, Michigishi T, Yokoyama K, Noguchi M, Hashimoto T, Nakamura S, Matsubara F.(1992). Immunohistochemical demonstration of epidermal growth-factor receptors in normal, benign and malignant thyroid tissues, Int J Oncol. 1992; 1(3): 331-335.