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EGFR and CXCR1 expression in thyroid carcinoma in Qassim Region-Saudi Arabia: Correlation with clinicopathological parameters

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ABSTRACT

Aims: Recent evidence indicates an increased incidence of thyroid carcinoma, especially papillary thyroid carcinoma (PTC), in Saudi Arabia. EGFR and CXCR1 were reported to have increased expression in several human neoplasms. The goals of the present research was to investigate EGFR and CXCR1 expression in thyroid carcinoma and correlate the results to the established prognostic factors.
Methods: Immunohistochemical study for both EGFR and CXCR1 was performed on formalin-fixed paraffin-embedded thyroid carcinomas tissues sections applying Labeled Streptavidin-biotin method (LSAB).
Results: Remarkable high expression of EGFR and CXCR1 were observed in PTC cases (56% and 63% respectively). There was association between EGFR expression in PTC and each of histologic subtype, lymph node metastasis (LNM), distant metastasis (DM), TNM staging and tumor relapse. There was statistical significant correlation between CXCR1 expression in PTC and each of histologic subtype, LNM, and tumor relapse. A significant correlation was detected between concomitant EGFR and CXCR expression and LNM, DM, increasing stage and tumor relapse.
Conclusions: The results of the present study demonstrated, a statistically positive correlation of EGFR and CXCR1 expression in PTC compared to normal thyroid tissues and nodular hyperplasia in Qassim Region- Saudi Arabia. Concomitant high expression of both receptors were strongly correlated with LNM, DM, TNM stage and tumor relapse than did each alone. These findings suggest that EGFR and CXCR1 play crucial roles in PTC and serve as predictors of poor prognosis, biomarkers of tumor diagnosis, and potential targets of cancer therapeutics.

1. Introduction

Cancer of the thyroid gland represents the second most common malignancy after breast carcinomas in female patients in the Kingdom of Saudi Arabia [1]. A previous epidemiological study declared slight increase in the crude incidence rate of thyroid carcinoma among women of various regions of Saudi Arabia, contrarily Qassim region demonstrated dramatic increase [2]. There is argument about the precise etiology of this increase with the presence of discordant reports [3]. Increased incidence varied from one region of the world into another, as a result of environmental, genetic factors, as well as difference in the availability of the medical care [4]. Thyroid carcinomas affects young patients often peaks around the age of fifty years, however involvement of older age groups was observed in the recent decade carcinoma [5].

Epidermal growth factor receptor (EGFR) performs a crucial task in cancer development and progression. EGFR transmits signals from the cell surface to the inside of cell, causing several biological actions such as proliferation and migration of malignant cells [6]. Ligand binding to EGFR induces receptor dimerization followed by transphosphorylation resulting in activation of downstream signal transduction pathways. Abnormal EGFR expression may result in disturbance in cellular processes, causing malignant transformation [7]. Over expression of EGFR is often detected in carcinomas of the mammary gland [8], esophagus [9] and thyroid gland [10,11]. Increased EGFR in the late stage of malignancy is associated with metastatic potential and poor prognosis of the malignant tumors [12]. EGFR was found to contribute in proliferation, apoptosis' resistance and invasion of the malignant hepatocytes [12]. EGFR over expression was detected mainly in the greatly advanced thy-

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roid gland carcinomas [13]. EGFR over expression in many human malignancies indicates that its inhibition is essential therapeutic strategy [14]. The inhibition of EGFR kinase activity by anti-EGFR antibodies has shown anti-proliferative effects in thyroid carcinoma cell lines in vitro and in vivo [13]. Recently, the inhibitors of tyrosine kinase (EGFR targeted molecules) were efficiently used in EGFR-dependent malignant tumors' treatment [15].

CXCR1 is a receptor for IL-8 which acts as an important mediator of inflammatory and immune responses involved in many diseases, including neoplasia [16]. In inflammation, CXCR1 expressed in neutrophils cause chemotaxis of the white blood cells. However, in neoplasia, CXCR1 has been found to act on multiple cell types, playing an important role in invasion, migration and metastasis of carcinoma cells of the breast via crosstalk with other molecules [17]. Moreover, over expression of CXCR1 in several solid tumors is correlated with drug-resistance [18]. CXCR1 receptors were found to be expressed in normal epidermal cells, and malignant neoplasms [19–25] and have been correlated with tumor proliferation and metastasis [26–33] thus, forming the main targets for the development of novel therapies of malignant tumors [34–37]. Up till now, few studies were accomplished on EGFR and CXCR1 expression in thyroid carcinoma. Therefore, Immunohistochemical study for both EGFR and CXCR1 was performed on thyroid carcinomas to detect any correlation with prognostic parameters. The hypothesis of the current research is that EGFR and CXCR1 expression might be associated with progression, invasion and metastasis in thyroid cancer thus can be used as targets for development of novel therapies.

2. Materials and methods

2.1. Histopathology

This research was performed on eighty cases of papillary thyroid carcinoma which were collected from the surgical files of the Histopathology Department, King Fahd Specialist Hospital, Buraidah, kingdom of Saudi Arabia during the period from 2010−2017. Patients' data such as age, sex, size of tumor, pathologic subtype, TNM stage, extra thyroid extension (ETE), lymph nodes metastasis (LNM) and distant metastasis (DM) at presentation were revised and estimated from the histopathological reports. All cases were reassessed histologically to confirm the PTC histological subtype and then divided into 2 risk groups [38]. The low risk group consisted of cases diagnosed as conventional, follicular, cribriform-morular and oncocytic whereas the high risk group comprised cases diagnosed as tall cell, and micropapillary, respectively. Normal tissues of the thyroid were obtained from areas surrounding the tumor. Collected data from the reports were recorded and analyzed. This study was undertaken with full ethical approval of the *Regional Research Ethics Committee-Qassim Province- KSA under the number (H-04-Q-001).*

2.2. Immunohistochemistry

The expressions of EGFR and CXCR1 were evaluated by immunohistochemistry using the Labeled Strept Avidin Biotin (LSAB) immunodetection complex method. Three-micron formalin-fixed, paraffin-embedded sections of tissues were immunostained with EGFR (1:100 dilution, sc-03-G, Santa Cruz Biotechnology, USA) and CXCR1 (1:100 dilution, ab137351, abcam, USA). In brief, sections were cut and dried at 37 °C, deparaffinized, hydrated, washed in tap water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Behind washing the slides triple times in phosphate buffered saline (PBS, 0.01 M; pH = 7.2), they were immersed in 0.01 M citrate buffer solution (pH 6.0 in plastic Coplin jars), and heated for 4 cycles in a microwave oven (750 W for 4 min each), for heat antigen retrieval. After 25 min cooling period, the nonspecific protein binding was inhibited using normal goat serum (10% for 15 min). The sections were incubated overnight with primary antibody at 4 °C then treated with LSAB (Dako, Envision TM kit). After washing the slides triple times in PBS, diamino benzidine chromogen substrate solution (DAB) was added for 5 min to develop the color. Lastly, slides were stained with Mayer's HX, cleared, DPX mounted, then were examined using Olympus BX40 Research Microscope with digital camera (Hamburg, Germany). Cutaneous squamous cell carcinoma was used as a positive control for EGFR and neutrophils for CXCR1. Negative control (no primary antibody) were included simultaneously in the experimental runs for every batch.

2.3. Immunohistochemistry scoring

A semi-quantitative immunohistochemistry (IHC) scores were assessed depending on intensity of staining and percentage of positive staining cells in each block. The intensity of membranous staining for EGFR and the intensity of membranous and cytoplasmic staining for CXCR1 were assigned as 0 = negative staining, 1 = weak staining, 2 = moderate staining and 3 = strong staining. The percentages of positive cells were assigned as 0 = $\leq$ 5% positive cells, 1 = 6–25% positive cells, 2 = 26–50% positive cells, 3 = 51–75% positive cells and 4 = $\geq$ 76% positive cells. Multiplication of the intensity by percentage scores resulted in the final IHC staining scores: 0 (negative), 1+ (1–4), 2+ (5–8) and 3+ (9–12). For statistical analysis, a final IHC score of negative or 1+ was considered as low-expression group and a final IHC score of 2+ and 3+ was considered as high-expression group [39].

2.4. Statistical analysis

Results were statistically analyzed by utilizing SPSS 22. EGFR and CXCR1 expression in thyroid cancer were utilized to judge the role of histological pattern, age, gender, tumor size, LNM, DM, TNM stage and tumor relapse. Analysis of variance (ANOVA) and Chi Square coefficient tests with statistical significance p-value < 0.05 were used.

3. Results

3.1. Clinicopathological characteristics

In the whole group (80 cases), 59 patients were females (74%), and 21 patients were males. At the time of the diagnosis, the age of the patients ranged between 23 and 70 years old, with a median age of 44 years old. Surgical treatment consisted in total thyroidectomy with lymphadenectomy for 50 patients, and partial thyroidectomy for the remaining 30 cases. The histopathological exam revealed the tumor ETE in 15 cases, presence of LNM in 36 cases, DM in 26 cases and tumor relapse in 20 cases. The distribution of the PTC histological variants was as follows: conventional subtype – 50 cases, follicular subtype – 6 cases, cribriform – four cases, oncocytic variant – 2 cases, tall cell – ten cases, and micropapillary – eight cases.

3.2. EGFR and CXCR1 immunohistochemical expression in normal thyroid tissues, nodular hyperplasia and PTC

Immunohistochemical EGFR and CXCR1 expression were studied and showed few carcinoma cells with moderate staining for the two receptors in some PTC cases (Fig. 1A and 1B), and other cases demonstrated many carcinoma cells with strong staining for the two receptors (Fig. 1C and 1D). There were negative or weak

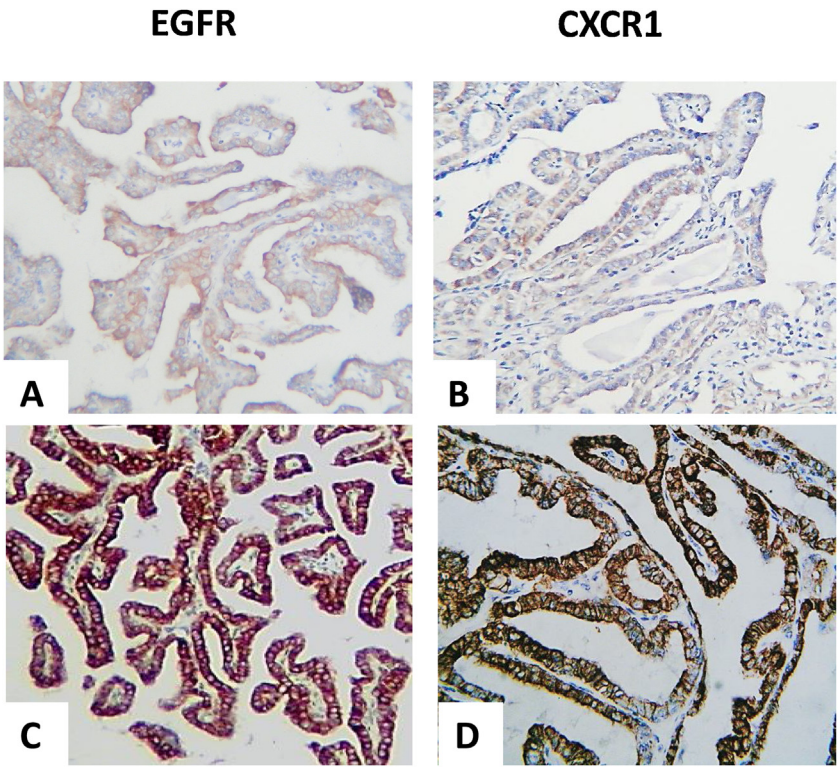

Fig. 1. Immunohistochemical staining for EGFR and CXCR1 in PTCs. Columns correspond to immunostaining for EGFR and CXCR1, respectively. The first row exhibits moderate staining (A–B) and strong staining (C–D) of EGFR and CXCR1 in PTCs. All the pictures are in high-power fields (×400). All the pictures are in high-power fields (×400) (DAB Chromogen & Hx counter stain).

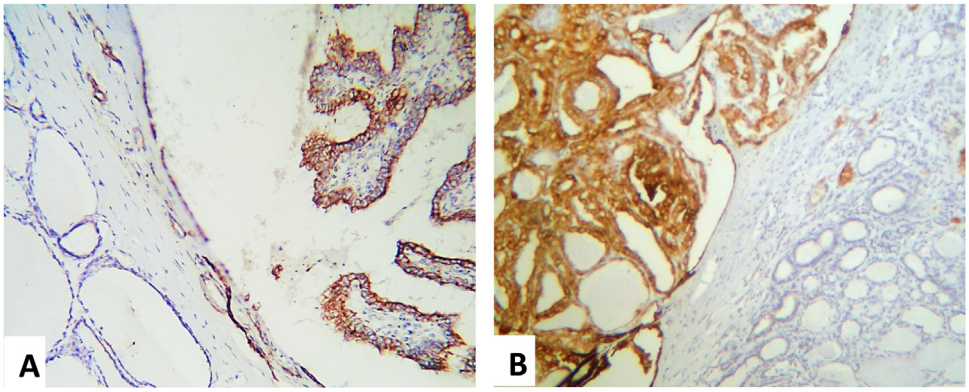

Fig. 2. Immunohistochemical staining for EGFR and CXCR1 in nodular hyperplasia tissues and PTCs. Figure A exhibits negative staining of EGFR in normal thyroid tissues to the left and strong staining in PTCs (conventional variant) to the right. Figure B exhibits negative staining of CXCR1 in nodular hyperplasia to the right and strong staining in PTCs (follicular variant) to the left. Both pictures are in high-power fields (×400) (DAB Chromogen & Hx counter stain).

staining for EGFR and CXCR1 in thyrocytes of both normal tissues and nodular hyperplasia of the thyroid gland as illustrated in Fig. 2 A and B. There were strong staining for EGFR and CXCR1 in high risk group such as tall cell and micropapillary variants of PTC as illustrated in Fig. 3A and B. Table 1 displayed that both the normal thyroid and nodular hyperplasia tissues had negative or 1 IHC score with no cases showed high expression (≥5) of the two molecules. However, in PTC, most cases had ≥3 IHC score with high expression (≥5) was present in 45 (56.3%) and 51 (63.8%) of 80 cases for both EGFR and CXCR1, respectively. The differences between protein expression levels of both receptors in normal tissues, nodular hyperplasia tissues and PTC were significant statistically ($P < 0.001$).

3.3. Correlation of EGFR and CXCR1 expression with clinicopathological parameters in PTC

The studied cases showed statistical significant differences in EGFR and CXCR1 expression between patients with different histologic subtypes of PTC ($P = 0.032$, $P = 0.009$, respectively), The studied cases showed no statistical significant differences in EGFR and CXCR1 expression between older patients (>45) and younger patients (≤45) ($P = 0.125$, $P = 0.070$, respectively), between male and female patients ($P = 0.118$, $P = 0.519$, respectively). Notably, PTC cases with LN metastasis showed significantly higher EGFR and CXCR1 expression than PTC cases without LN metastasis ($P = 0.001$ for EGFR and $P = 0.048$ for CXCR1). However, EGFR protein expression was correlated with DM ($P = 0.010$) and TNM stage ($P = 0.006$)

Table 1
The correlation of EGFR and CXCR1 protein expression with the clinicopathological parameters in papillary thyroid carcinoma (PTC).

		EGFR Expression			CXCR1 Expression		
	Total	Low	High	p*	Low	High	p*
Tissue type							
Normal thyroid tissue	40	40	0		40	0	
Nodular hyperplasia	35	35	0	< 0.001(HS)	35	0	< 0.001(HS)
PTC	80	35	45		29	51	
1. PTC (Subtypes)							
Low-risk group	62	31	31	0.032(S)	27	35	0.009(HS)
High-risk group	18	4	14		2	16	
2. Age (Years)							
< 45	48	24	24	.125	21	27	.070
≥45	32	11	21		8	24	
3. Gender							
Male	21	12	9	.118	8	13	.519
Female	59	23	36		21	38	
4. Pathologic tumor size (cm)							
< 2.3	36	20	16	.089	15	21	.249
≥ 2.3	44	15	29		14	30	
5. Extra thyroid extension (ETE)							
Absent	65	28	37	.801	23	42	.478
Present	15	7	8		6	9	
6. Lymph node metastasis							
Absent	44	27	17	.001(HS)	20	24	.048(S)
Present	36	8	28		9	27	
7. Distant metastasis							
Absent	54	29	25	.010(S)	22	32	.170
Present	26	6	20		7	19	
8. TNM stage							
I-II	30	19	11	.006(HS)	14	16	.104
III-IV	50	16	34		15	35	
9. Tumor relapse							
Absent	60	30	30	.044(S)	26	34	.019(S)
Present	20	5	15		3	17	

(S): Significant statistical correlation (HS): Highly significant statistical correlation.

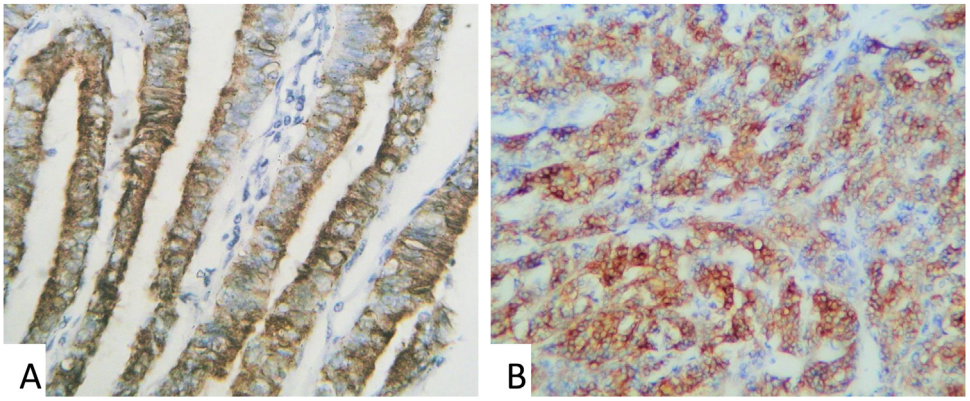

Fig. 3. Immunohistochemical staining for EGFR and CXCR1 in PTC variants. Figure A exhibits strong staining of EGFR in tall cell variant of PTC. Figure B exhibits strong staining of CXCR1 in micropapillary variant of PTC. The pictures are in high-power fields (×400) (DAB Chromogen & Hx counter stain).

whilst no correlation was detected between CXCR1 protein expression and DM (P = 0.170) or TNM stage (P = 0.104). Furthermore, PTC cases with tumor relapse showed significantly higher EGFR and CXCR1 expression than PTC cases without tumor relapse (P = 0.044 for EGFR and P = 0.019 for CXCR1) (Table 1).

3.4. Correlation of EGFR and CXCR1 protein expression with each other in PTC

Thirty four out of eighty PTC cases (42.5%) showed high expression and eighteen out of eighty PTC cases (22.5%) displayed low expression for both EGFR and CXCR1. A significantly positive statistical correlation between expression of EGFR and CXCR1 in PTC was present (P = 0.012) (Table 2).

Table 2
The correlation between the expression of CXCR1 and EGFR expression in papillary thyroid carcinoma (PTC).

	CXCR1 Expression			
Samples	**Total**	**Low (%)**	**High (%)**	**p***
PTC	80	29 (36.2)	51 (63.8)	
EGFR Expression				
Low (%)	35 (43.8)	18	17	0.012 (S)
High (%)	45 (56.2)	11	34	

(S): Significant statistical correlation.

3.5. Synchronal co-expression of EGFR and CXCR1 is correlated with LNM, DM, TNM stage and tumor relapse of PTC

In view of the fact that EGFR and CXCR1 expression were positively correlated with each other, and high expression of each

Table 3
Correlation of concomitant expression of EGFR and CXCR1 in PTC with LNM.

	Lymph Nodes metastasis			P value
	Absent (%)	Present (%)	Total	
Both GFR/CXCR1 low expression	14 (77.8)	4 (22.2)	**18**	0.001 (HS)
One of EGFR/CXCR1 high expression	19 (67.9)	9 (32.1)	**28**	
Both EGFR/CXCR1 high expression	11 (32.4)	23 (67.6)	**34**	
	Distant metastasis			
Both GFR/CXCR1 low expression	13(72.2)	5 (27.8)	**18**	0.002 (HS)
One of EGFR/CXCR1 high expression	25 (89.3)	3 (10.7)	**28**	
Both EGFR/CXCR1 high expression	16 (47.1)	18 (52.9)	**34**	
	TNM Stage			
Both GFR/CXCR1 low expression	11 (61.1)	7 (38.9)	**18**	0.028 (S)
One of EGFR/CXCR1 high expression	11 (39.3)	17 (60.7)	**28**	
Both EGFR/CXCR1 high expression	8 (23.5)	26 (76.5)	**34**	
	Tumor relapse			
Both GFR/CXCR1 low expression	17 (94.4)	1 (5.6)	**18**	0.008 (HS)
One of EGFR/CXCR1 high expression	22 (78.6)	6 (21.4)	**28**	
Both EGFR/CXCR1 high expression	21 (61.8)	13 (38.2)	**34**	

(S): Significant statistical correlation (HS): Highly significant statistical correlation.

receptor was related to LNM, consequent estimation of the association of synchronal high co-expression of both receptors with LNM, DM, TNM stage and tumor relapse in PTC was performed. The incidence of LNM is significantly higher in PTC cases (67.6%) with high expression of both receptors than in PTC cases (32.1%) with high expression of only one of these two molecules, or in PTC cases (22.2%) with low expression for these two receptors. Statistical analysis showed that concomitant high expression of both receptors was significantly associated with LN metastasis in comparison with cases not showing such expression ($P < 0.001$). PTC cases without LNM showing EGFR and CXCR1 high expression compared to absent/low expression in normal thyroid tissue and nodular hyperplasia tissues, respectively (Fig. 2A–B). Moreover, there was a statistically significant correlation between concomitant EGFR and CXCR expression and DM and increasing TNM stage and tumor relapse of PTC (0.002, 0.028, 0.008 respectively) (Table 3).

4. Discussion

In the recent years, the incidence of thyroid carcinomas has increased worldwide. Papillary thyroid carcinoma including its variants constitutes the most prevalent thyroid carcinoma type (more than 90%) [40]. PTC variants include classic/conventional, follicular, encapsulated, diffuse sclerosing, tallcell, cribriform-morular, columnar cell, oncocytic, and solid variant [41–43].

The activation of EGFR leads to downstream signaling events that are strongly correlated with various cancer cell lines invasion, metastasis, and drug resistance [44]. CXCR1 was detected to be correlated to drug resistance, invasion, and metastasis in many solid neoplasms [20]. EGFR and CXCR1 were up-regulated in several tumors including PTC [9–11,18,45]. However, few studies investigated EGFR and CXCR1 expression and its association with clinic-pathological features in PTC.

In this study, immunohistochemistry was utilized to investigate the expression of EGFR and CXCR1 in normal thyroid tissues, nodular hyperplasia and PTC. Normal thyroid tissue and nodular hyperplasia showed absence or low protein expression for both EGFR and CXCR1. In contrast, PTC showed high protein expression of EGFR and CXCR1 in 56.2% and 63.8% of cases respectively. The differences of receptors expression between normal thyroid tissues and nodular hyperplasia as well PTC were statistically significant ($P < 0.001$). These findings are in line with a previous study, demonstrating high EGFR and CXCR1 expression in PTC concluding that these receptors perform critical roles in PTC progression [39]. Recent study reported similar results in breast cancer showing absence or low CXCR1 expression in normal mammary tissues and breast fibroadenoma, but high expression in breast carcinomas [46].

Subsequently, the correlation of EGFR and CXCR1 expression with several clinicopathological indicators was estimated. The expression of these two receptors was insignificantly associated with many clinicopathological characteristics including age, gender, tumor size and ETE. However, there was statistically significant association between EGFR protein expression and histologic subtype, LNM, and DM, TNM stage as well as tumor relapse. Therefore, these results confirm that EGFR represent an indicator of PTC metastatic potential as its expression elevated with increased stage, LNM, DM and high risk histologic variants. On the other hand, CXCR1 protein expression was associated with LNM and tumor relapse and was not associated with DM and TNM stage. Remarkably, statistically significant correlations were found between EGFR protein expression and LNM ($P \leq 0.001$) and between the protein expression of CXCR1 and LNM ($P = 0.048$). The high expression of EGFR and CXCR1 was associated with LNM. These results suggest that EGFR and CXCR1 may have a major role in invasion and metastatic behavior of thyroid carcinoma.

This study was genuine to demonstrate a strong positive correlation between CXCR1 and EGFR expression in PTC ($P < 0.001$) compared to normal thyroid tissues and nodular hyperplasia in Qassim Region-Saudi Arabia. The existence of this positive correlation could be supported by previous studies showing that both EGFR and CXCR1 are highly expressed in PTC and associated with adverse pathologic features [11,38,47].

Based on the results of this study which illustrated that EGFR and CXCR1 expression were positively correlated with each other, and high expression of each receptor was related to LNM, consequent estimation of the association of concomitant high expression of both receptors with LNM, DM, TNM stage and tumor relapse in PTC was performed. The results revealed that synchronal high co-expression of the two receptors had stronger correlation with LNM, DM, high stage, and tumor relapse than did each receptor separately ($P = 0.001$, 0.002, 0.028, 0.008, respectively). These findings indicate that concomitant high expression of both receptors firmly correlates with LNM, DM, PTC stage and tumor relapse which additionally endorse the hypothesis of the correlation between EGFR and CXCR1 expression with the invasive and metastatic potential of several carcinomas (9–12, 24–27).

CXCR1, a G-protein coupled receptor (GPCR), stimulates many cellular functions mediating tumor cell proliferation, migration and differentiation. A previous study showed CXCR1 activate growth factor signaling cascades via receptor tyrosine kinases phosphorylation and interaction between CXCR1 and EGFR leads

to enhancement of cellular proliferation, migration and metastasis [48]. Another study revealed that CXCR1 induce phosphorylation and activation of downstream effector, focal adhesion kinase (FAK, a key regulator of cell spreading and migration) [49]. A recent study reported that overexpression of EGFR and FAK, correlates with papillary thyroid carcinoma lymphatic spread and tumor infiltration [50]. By this rational, the results of the current study reinforce data published previously showing that overexpression of EGFR and CXCR1 correlates with PTC progression.

A previous study demonstrated that reparixin, an inhibitor of CXCR1, inhibits aggressive behavior of human gastric carcinoma in vitro and in vivo [51]. Another study showed CXCR1 siege targets human breast cancer stem cells in vitro and xenograft in vivo [52]. Moreover, a recent study showed that reparixin alone or in association with other chemotherapeutics, constitutes a unique promising therapeutic strategy for aggressive carcinomas of the thyroid [53]. Reparixin impaired the viability of epithelial thyroid malignant cells and markedly suppressed proliferation, survival, epithelial to mesenchymal transition by xenotransplantation in nude mice [53].

In conclusion, the present study demonstrated a statistically significant positive correlation of EGFR and CXCR1 protein expression in papillary thyroid carcinomas compared to normal thyroid tissues and nodular hyperplasia. Moreover, high expression of both EGFR and CXCR1 were associated with histologic subtype, LNM and tumor relapse. On the other hand, concomitant high expression of both receptors were strongly correlated with LNM, DM, TNM stage and tumor relapse than did each separately. Consequently, aggressive behavior of papillary thyroid carcinomas such as of LNM, DM and high TNM stage can be predicted by concordant high expression of the both receptors. It is possible that transactivation of the EGFR via CXCR1, might be a mechanism that enhance cellular proliferation, migration and metastasis. Thus, it is recommended that EGFR and CXCR1 to be routinely added as negative prognostic markers in PTC patients. Future studies in larger sets of thyroid carcinoma patients emphasizing on the high and low risk group of papillary thyroid carcinoma would be necessary. Long term follow-up study on the clinically important prognostic parameters such as recurrence and metastasis is recommended to elucidate the utility of these receptors as biomarkers for diagnosis and prognosis of thyroid carcinomas and unveil potential targets for the therapy of PTC.

Author contributions

The author; Omran OM alone is responsible for the content; conception and design of the study; acquisition and analysis of data; and drafting the manuscript or figures and writing of the paper.

Disclosure statement

None declared.

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