International Journal of Research and Review
Vol.7; Issue: 4; April 2020
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Interleukin-8: A Potential Marker for Differentiating Papillary Thyroid Cancer

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ABSTRACT

Background: Thyroid disorders represent relatively common medical conditions worldwide. Although better managed, it is often difficult to distinguish benign and thyroid cancer cells. To avoid inappropriate treatment decisions based on unconvincing results, it is inevitable to understand molecular mechanisms underlying thyroid carcinogenesis. Cytokines have a key role in an intricate relationship between inflammation and cancer. IL-8 is one such potent inflammatory mediator recognized to modulate proliferation, invasion and migration of tumor cells. Hence it was aimed to study expression of IL-8 in benign and thyroid cancer patients.

Methods: Circulating IL-8 levels (by ELISA) and tumoral IL-8 expression (by Immunohistochemistry) was studied in total 240 subjects: 67 healthy individuals, 67 benign thyroid diseases patients and 106 thyroid cancer patients with majority being diagnosed as papillary thyroid cancer (PTC: N=83).

Results: Circulating IL-8 was significantly elevated in benign and thyroid cancer as compared to controls. Both circulating and tumoral IL-8 expression were significantly higher in PTC as compared to benign thyroid diseases. Moreover, IL-8 expression was significantly associated with aggressive tumor characteristics of PTC patients.

Conclusion: IL-8 could be a potential marker for differentiating patients with benign thyroid diseases and cancer. IL-8 overexpression was not suppressed by Suppressors of cytokine signalling (SOCS) proteins and may induce expression of adhesion molecules: VCAM-1 and L-Selectin and thereby increase thyroid cancer progression. Further, significant association of IL-8 expression with shorter overall survival in PTC patients treated with surgery alone, suggests that conventional treatment strategies may be improved by additionally targeting IL-8 signalling in such patients.

Key words: Thyroid cancer, IL-8, SOCS, Immunohistochemistry, ELISA, Benign thyroid diseases

INTRODUCTION

'Thyroid'- an endocrine gland plays important role in regulating metabolism and helps maintain body temperature, heart rate and blood pressure. Thus, any problem that occurs in the thyroid gland, affects every cell of the body. Thyroid disorders represent relatively common medical conditions ranging from benign goitre to carcinoma. Although its incidence is lower, thyroid cancer represents the most frequent endocrine malignancy. Despite the fact that majority of patients with thyroid diseases are better managed, pathologists often find it difficult to distinguish benign and cancer cells. This may result in unconvincing treatment decisions by clinicians. Hence, it is important to decipher the molecular mechanisms underlying thyroid tumorigenesis, to avoid excessive treatment to the patients with indolent/low risk tumors and at the same time, guarantee effective management to the patients with aggressive disease.

Inflammation representing one of the hallmarks of cancer [1] includes the existence of inflammatory mediators-

cytokines. Shedding of cytokines by tumor cells into the local microenvironment is a key modulator of tumorigenesis. Overall, there is an intricate relationship between the immune system and cancer where cytokines have a significant role.[2] Cytokines modulate an antitumoral response in the tumor microenvironment, but during chronic inflammation, they can induce malignant cell transformation based on balance of pro- and anti- inflammatory type.

IL-8 is a cytokine recognized as potent neutrophil activator and chemotactic factor secreted by activated monocytes, macrophages, fibroblasts, lymphocytes, neutrophils, endothelial cells and a variety of normal and malignant epithelial cells.[3, 4] The increased secretion of IL-8 from tumor cells can have profound effect on the tumor microenvironment. IL-8- CXCR1/2 (IL-8 receptors) signalling mediates tumorigenesis and tumor progression that leads to subsequent activation of various pathways.[5, 6] The IL-8 signalling nexus directly influences the sensitivity of tumour cells to chemotherapies by altering pathways associated with apoptosis and multidrug resistance.[6]

Increased circulating IL-8 levels and its correlation with tumor burden and prognosis have been observed in patients with various malignancies.[7, 8] Moreover, statistically significant differences in IL-8 levels in patients with thyroid disease and normal reference group have also been demonstrated.[9-12] To date, immense research has been done to identify the role of IL-8 signalling in human cancers. Given that high expression of IL-8 is associated with tumorigenesis and progression of certain types of tumours, this study hypothesized that IL-8 may serve as useful biomarker in screening and evaluating prognosis in thyroid cancer patients as well. Hence it was aimed to study its expression in benign and thyroid cancer patients and thereby explore its role as a potential marker for differentiation and prognosis in thyroid carcinoma patients.

MATERIALS AND METHODS

Subjects

Total 240 subjects were included, of which 67 were healthy individuals, 67 were patients with benign thyroid diseases and 106 were thyroid cancer patients (PTC: N=83, follicular thyroid cancer (FTC): N=6, medullary thyroid cancer (MTC): N=9 and anaplastic thyroid cancer (ATC): N=8).[13-16] None of the subjects had any history of autoimmune disease, did not receive any pre-treatment and they were not on any immunosuppressive or immunomodulant drugs. Only 45/67 patients with benign thyroid diseases that were suspicious to be malignant were operated at our institute.

This study was approved by Institutional Scientific and Ethical Committees and informed consent was obtained from all subjects prior to sample collection. Except 4/8 ATC patients who were not resectable; all thyroid cancer patients underwent surgery at Department of Surgical Oncology of our institute. Only PTC patients were further considered for the correlation analysis (the number of patients with other three types of thyroid cancer were very low for comparative statistical analysis). Treatment strategies were decided by the clinicians of the institute. It included either surgery or surgery followed by radioiodine ablation (RIA) therapy or surgery followed by RIA therapy and radiotherapy both. Clinical and histopathological details of the patients were noted from the case files maintained at the Medical record department of the institute. Histological classification of the tumors was in accordance with the WHO classification. The PTC patients were staged according to the AJCC/UICC TNM staging system and were accordingly grouped into younger (<45 years) and elder (≥45 years) age groups. Clinicopathological characteristics of PTC patients are depicted in Table 1.[13-16] Follow up details of PTC patients were noted for a period of 4 years or until death within that period. Complete follow-up details were obtained in 92% (76/83) of PTC patients and hence were included for

overall survival (OS) analysis. Nine percent (7/76) of these patients had persistent disease and thus were excluded from disease free survival (DFS) analysis. Hence, 69/76 PTC patients were included for DFS analysis.

Table 1: Clinicopathological characteristics of PTC patients

Characteristics	N (%)	Characteristics	N (%)
Age		**Bilaterality**	
<45 years	41 (49)	Unilateral	61 (74)
≥45 years	42 (51)	Bilateral	22 (26)
Gender		**Haemorrhagic area**	
Female	56 (68)	Absent	72 (87)
Male	27 (32)	Present	11 (13)
Tumour size		**Necrosis**	
T1 (N=16)+T2 (N=22)	38 (46)	Absent	67 (81)
T3 (N=30)+T4 (N=15)	45 (54)	Present	16 (19)
Nodal status		**Calcification**	
Absent	30 (36)	Absent	32 (39)
Present	53 (64)	Present	51 (61)
Metastasis		**Extrathyroidal extension**	
Absent	73 (88)	Absent	52 (63)
Present	10 (12)	Present	31 (37)
Stage		**Fibrosis**	
Early [Stage I (N=37) + Stage II (N=12)]	49 (59)	Absent	61 (74)
Advanced [Stage III (N=11)+ Stage IV(N=23)]	34 (41)	Present	22 (26)
Lymphatic permeation		**Inflammation**	
Absent	67 (81)	Absent	46 (55)
Present	16 (19)	Present	37 (45)
Vascular permeation		**Differentiation**	
Absent	74 (89)	Well	76 (92)
Present	09 (11)	Moderate/ Poor	07 (08)
Capsular Invasion		**Multifocality**	
Absent	55 (66)	Absent	64 (77)
Present	28 (34)	Present	19 (23)
Encapsulation		**Residual Disease**	
Well encapsulated	76 (92)	Absent	24 (29)
Partially/Not encapsulated	07 (08)	Present	59 (71)
Treatment			
Surgery	29 (35)		
Surgery + RIA and/RT	54 (65)	Surgery + RIA	50 (60)
		Surgery + RIA +RT	04 (05)
Disease Status			
Recurrence/Distant Metastasis (N=69)		**Alive/Dead (N=76)**	
Absent	62 (90)	Alive	68 (89)
Present	07 (10)	Dead	08 (11)
Recurrence	3 (4)		
Distant metastasis	4 (6)		
Bone	1 (1.5)		
Lung	2 (3.0)		
Bone + Lung	1 (1.5)		

Enzyme Immunoassay (EIA) for circulating IL-8:

Pretherapeutic blood samples were collected from all subjects, and sera were separated and stored at –80°C until analysis. Circulating levels of IL-8 were estimated from the serum samples using commercially available kit (EIA IL-8: Immunotech-IM2237) using manufacturer's instructions. The unknown concentrations were determined through Graph pad prism 5 software.

Immunohistochemistry (IHC) for tumoral protein expression of IL-8:

Formalin fixed paraffin embedded tissue blocks of the patients were retrieved from Histopathology department. Four micron thick sections were taken and mounted on aminopropyl triethoxy silane (APES) coated glass slides. Immunohistochemical staining was performed using primary mouse monoclonal IL-8 antibody (R&D Systems-MAB208; dilution-1:50) and MACH4 Universal HRP-Polymer Detection System (Biocare Medicals, USA), as per manufacturer's

recommendations. Briefly, the Immunohistochemical staining procedure and semiquantitative scoring of the stained sections were performed as described earlier.[13]

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 16 (SPSS Inc, USA) was used to analyse the data. IL-8 levels between two groups were assessed by Independent Samples T-test. Discriminating efficacy of IL-8 was determined by Receiver's operating characteristic (ROC) curves. Tumoral protein expressions in benign and carcinoma patients and association with clinicopathological parameters of carcinoma patients were determined by Two-tailed $\chi 2$ test. Spearman's correlation coefficient (r) was used to find correlation between two parameters. Univariate survival analysis for DFS and OS was evaluated using Kaplan-Meier method and Log rank test. P values ≤ 0.05 were considered significant.

RESULTS

Circulating levels of IL-8

Circulating IL-8 levels in different subjects are depicted in Table 2. In patients with *benign thyroid diseases (N=67),* the circulating IL-8 levels were significantly higher as compared to that in *healthy individuals (P=0.003).* The total benign thyroid patients were further grouped as patients with *goitre (N=45) and autoimmune diseases (N=22).* It was observed that the patients with goitre had significantly higher circulating levels of IL-8 than the healthy individuals (P<0.001). Further, patients with *autoimmune diseases,* when sub grouped into *Hashimoto's thyroiditis (N=9)* and *Graves' disease (N=13),* it was noted that as compared to the healthy individuals, the circulating levels of IL-8 were significantly higher in both the subgroups of patients- Hashimoto's thyroiditis (P=0.002) and Graves' disease (P<0.001). In *Total thyroid carcinoma patients (N=106),* the circulating levels of IL-8 were significantly higher than the healthy individuals (P<0.001). These thyroid carcinoma patients were further sub grouped according to their histological subtypes as PTC, FTC, MTC and ATC. It was observed that in all these sub groups of thyroid carcinoma patients, the circulating levels of IL-8 were predominantly higher than the healthy individuals (PTC: P<0.001, FTC: P<0.001, MTC: P<0.001 and ATC: P<0.001).

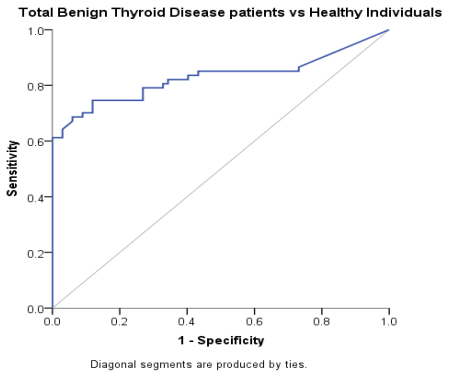
Table 2: Significance of circulating levels of IL-8

Subjects	**IL-8**	
	M ± SE (pg/ml)	**P value**
Healthy individuals (N=67)	2.76 ± 0.32	
Benign thyroid disease (N=67)	145.15 ± 46.72	**0.003***
Goitre (N=45)	144.53 ± 37.46	**<0.001***
Autoimmune diseases (N=22)		
Hashimoto's thyroiditis (N=9)	329.70 ± 296.95	**0.002***
Graves' disease (N=13)	19.53 ± 10.08	**<0.001***
Thyroid carcinoma (N=106)	353.97 ± 65.84	**<0.001*; 0.023#**
Papillary thyroid carcinoma (N=83)	355.37 ± 78.02	**<0.001*; 0.031#**
Follicular thyroid carcinoma (N=6)	566.72 ± 325.06	**<0.001*; 0.023#**
Medullary thyroid carcinoma (N=9)	415.09 ± 192.14	**<0.001***; 0.066#
Anaplastic thyroid carcinoma (N=8)	111.21 ± 60.92	**<0.001***; 0.806#

*Significance of circulating levels of IL-8 in benign and thyroid carcinoma patients as compared to healthy individuals.
#Significance of circulating levels of IL-8 in thyroid carcinoma patients as compared to benign thyroid diseases.

Further, the **ROC curves** revealed that IL-8 exhibited a good discriminatory efficacy between healthy individuals and patients with different thyroid diseases (Figure 1A-1H).

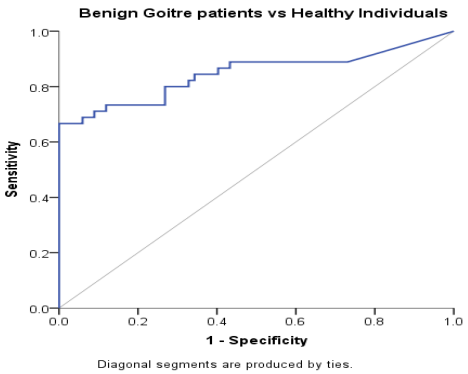
A



Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.828	<0.001	0.752	0.905

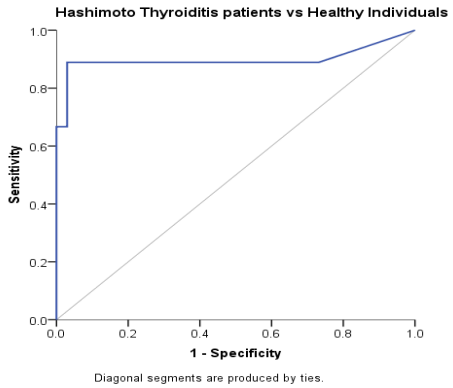
B



Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.846	<0.001	0.761	0.932

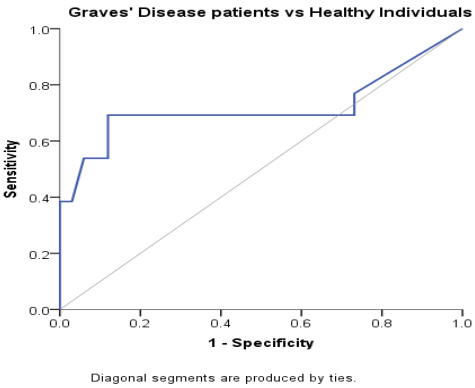
C



Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.897	<0.001	0.719	1.000

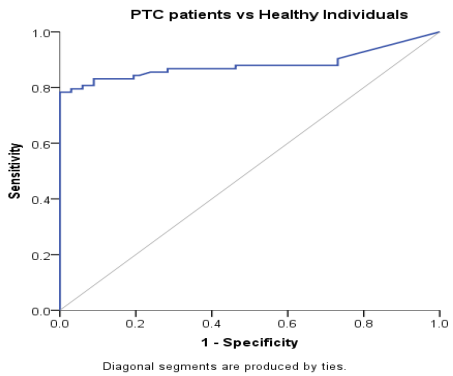
D



Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.719	0.013	0.513	0.924

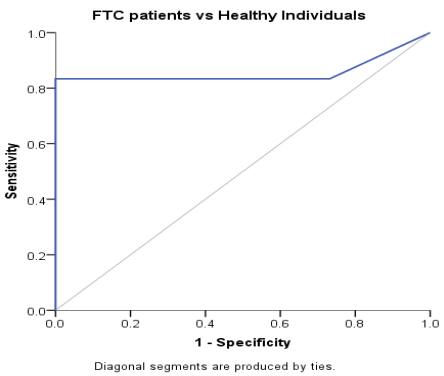
E



Area Under the Curve

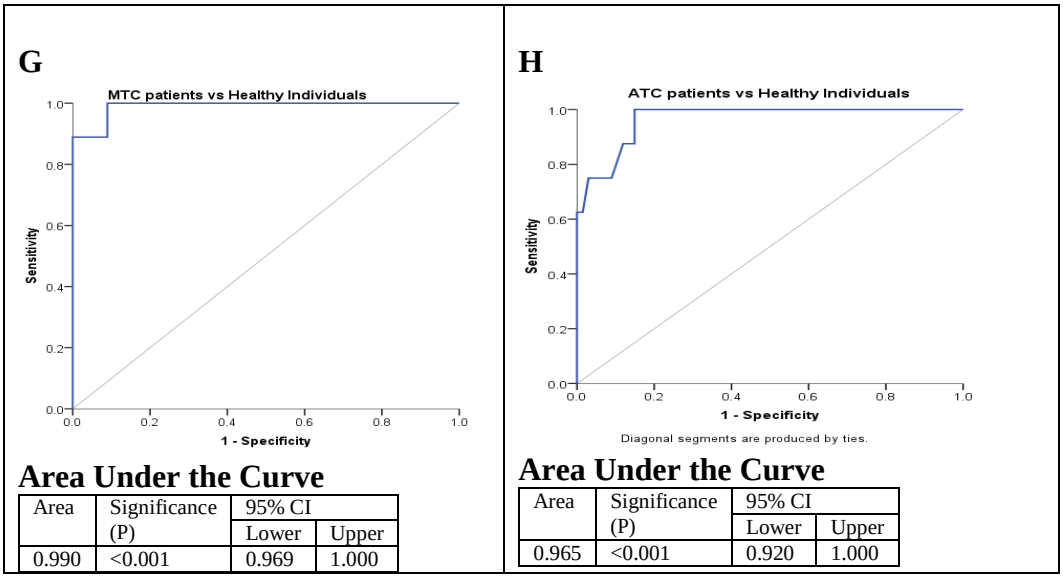
Area	Significance (P)	95% CI	
		Lower	Upper
0.882	<0.001	0.821	0.943

F



Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.856	0.004	0.596	1.000



Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.990	<0.001	0.969	1.000

Area Under the Curve

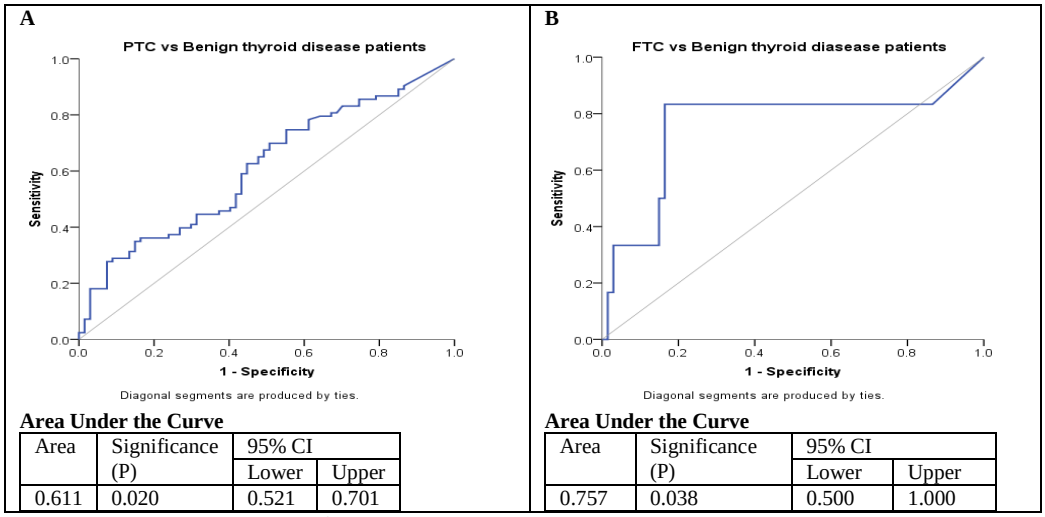
Area	Significance (P)	95% CI	
		Lower	Upper
0.965	<0.001	0.920	1.000

Figure 1: ROC curve for IL-8 in patients with thyroid diseases vs healthy individuals

A. Total benign thyroid disease patients vs Healthy individuals
B. Benign goitre patients vs Healthy individuals
C. Hashimoto thyroidtis patients vs Healthy individuals
D. Grave's disease patients vs Healthy individuals
E. PTC patients vs Healthy individuals
F. FTC patients vs Healthy individuals
G. MTC patients vs Healthy individuals
H. ATC patients vs Healthy individuals

Moreover, it was observed that IL-8 levels were significantly higher in thyroid carcinoma patients, as compared to patients with benign thyroid diseases (IL-8: P=0.023). Further, when sub grouped, the levels were found to be considerably higher in PTC and FTC patients as compared to patients with benign thyroid diseases (PTC: P=0.031 and FTC: P=0.023). [Table 2].

In agreement to this, ROC curves also showed that IL-8 could well discriminate between patients with benign diseases and PTC as well as between benign and FTC patients [Figure 2A and B].



Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.611	0.020	0.521	0.701

Area Under the Curve

Area	Significance (P)	95% CI	
		Lower	Upper
0.757	0.038	0.500	1.000

Figure 2: ROC curve for IL-8 in patients with thyroid cancer vs benign thyroid diseases

A. PTC vs Benign thyroid disease patients
B. FTC vs Benign thyroid disease patients

Tumoral protein expression of IL-8

Cytoplasmic and/or nuclear staining was observed for IL-8 (Figure 3). The immunoreactivity was either focal or scattered. For statistical evaluation, cytoplasmic and nuclear expressions were scored independently and taken into account separately.

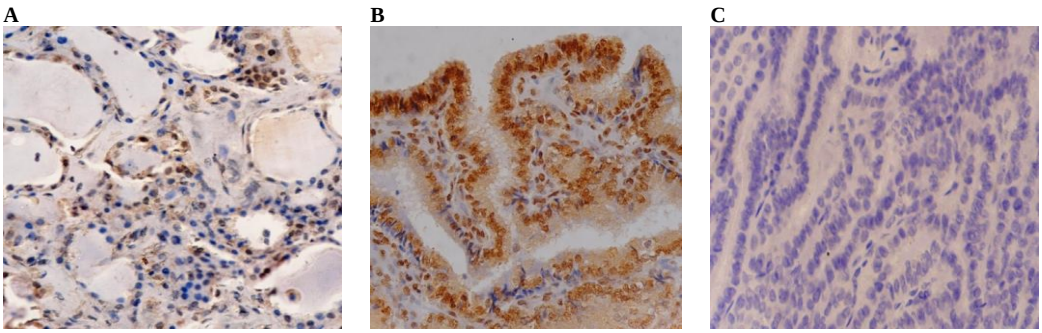


Figure 3: Photomicrographs showing staining for IL-8

A. Cytoplasmic and nuclear staining for IL-8 in benign goitre
B. Cytoplasmic and nuclear staining for IL-8 in PTC
C. Negative control for IL-8 in PTC

Median IRS of IL-8 expression was used as cut-off to divide the patients into low (≤median IRS) and high (>median IRS) expression groups, respectively. Accordingly, Table 3 depicts the comparison of IL-8 expression between the patients with benign thyroid diseases and thyroid carcinomas.

Incidence of cytoplasmic IL-8 immunoreactivity was significantly high in thyroid cancer patients as compared to the benign thyroid disease patients (χ^2=8.784, r=+0.244, P=0.003). In PTC patients too, the incidence of cytoplasmic immunoreactivity of IL-8 was found to be significantly high as compared to the benign thyroid disease patients (χ^2=8.474, r=+0.257, P=0.003). In FTC patients, the cytoplasmic IL-8 expression was higher than benign thyroid disease patients; but, this difference was not statistically significant (χ^2=1.624, r=+0.252, P=0.074).

Table 3: Comparison of cytokine expressions between the patients with benign thyroid diseases and total thyroid carcinoma patients

Cytokine expression	**BTD (N=45) N (%)**	**TTC (N=102) N (%)**	**PTC (N=83) N (%)**	**FTC (N=6) N (%)**	**MTC (N=9) N (%)**	**ATC (N=9) N (%)**
Cytoplasmic IL-8	Median IRS- 2	Median IRS- 4	Median IRS- 4	Median IRS- 1.5	Median IRS- 4	Median IRS- 2
Low	37 (82)	58 (57)	47 (57)	3 (50)	5 (56)	3 (75)
High	8 (18)	44 (43)	36 (43)	3 (50)	4 (44)	1 (25)
		χ^2=8.784, r=+0.244, **P=0.003**	χ^2=8.474, r=+0.257, **P=0.003**	χ^2=1.624, r=+0.252, P=0.074	χ^2=1.736, r=+0.239, P=0.188	χ^2=0.000, r=+0.051, P=1.000
Nuclear IL-8	Median IRS- 0	Median IRS- 0	Median IRS- 0	Median IRS- 0	Median IRS- 0	Median IRS- 1
Low	38 (84)	90 (88)	75 (90)	5 (83)	8 (89)	2 (50)
High	7 (16)	12 (12)	8 (10)	1 (17)	1 (11)	2 (50)
		χ^2=0.399, r=-0.052, P=0.531	χ^2=0.987, r=-0.088, P=0.324	χ^2=0.000, r=+0.010, P=1.000	χ^2=0.000, r=-0.047, P=1.000	χ^2=1.063, r=+0.244, P=0.302

BTD- Benign Thyroid diseases; TTC- Total Thyroid Cancer patients

Correlation of IL-8 with clinicopathological parameters of PTC patients

Preponderance of serum IL-8 levels was observed in male patients (P=0.035). Also IL-8 levels were observed to be higher in patients having larger tumor size (P=0.050), advanced stage disease (P=0.045) and presence of fibrosis (P=0.005).

Cytoplasmic IL-8 expression was significantly higher in males (χ^2=4.112, r=+0.223, P=0.043); in patients with larger tumor size (χ^2=3.970, r=+0.219, P=0.047) and presence of extrathyroidal extension of tumors (χ^2=9.006, r=+0.329, P=0.002). Moreover, a trend of higher cytoplasmic IL-8 immunoreactivity was evident in patients showing capsular invasion of tumors (χ^2=3.262, r=+0.198, P=0.072) and in those

having tumors in single lobe of the thyroid gland (χ^2=3.160, r=-0.195, P=0.077) as compared to their respective counterparts. On the other hand, higher nuclear IL-8 expression was predominant in patients with smaller tumor size than in patients with larger tumor size (χ^2=4.487, r=-0.273, P=0.034). Besides this, a trend of higher nuclear IL-8 immunoreactivity was seen in PTC patients with presence of distant metastasis (χ^2=3.081, r=+0.255, P=0.079) and those who had been postoperatively treated with RIA and/RT (χ^2=3.206, r=+0.239, P=0.073). Apart from these, IL-8 did not show significant correlation with rest of the clinicopathological parameters [Table 4].

Table 4: Correlation of IL-8 with clinicopathological parameters of PTC patients

Parameter	**Circulating levels**		**Tumoral protein expression**					
			Cytoplasmic			**Nuclear**		
	Mean ± SE (pg/ml)	P	Low N (%)	High N (%)		Low N (%)	High N (%)	
Gender								
Female	241.48 ± 76.66	**0.035**	36 (64)	20 (36)	**r=+0.223**			
Male	591.58 ± 173.36		11 (41)	16 (59)	**P=0.043**			
Tumour size								
Small (T1+T2)	189.64 ± 76.60	**0.050**	26 (68)	12 (32)	**r=+0.219**	31 (82)	7 (18)	**r=-0.273**
Large (T3+T4)	495.31 ± 125.65		21 (47)	24 (53)	**P=0.047**	44 (98)	1 (2)	**P=0.034**
Stage								
Early (I+II)	225.72 ± 67.74	**0.045**						
Advanced (III+IV)	542.21 ± 159.75							
Metastasis								
Absent						68 (93)	5 (7)	r=+0.255
Present						7 (70)	3 (30)	P=0.079
Fibrosis								
Absent	224.65 ± 47.43	**0.005**						
Present	717.81 ± 251.70							
Extrathyroidal extension								
Absent			36 (69)	16 (31)	**r=+0.329**			
Present			11 (35)	20 (65)	**P=0.002**			
Capsular Invasion								
Absent			35 (64)	20 (36)	r=+0.198			
Present			12 (43)	16 (57)	P=0.072			
Bilaterality								
Unilateral			31 (51)	30 (49)	r=-0.195			
Bilateral			16 (73)	6 (27)	P=0.077			
Treatment								
Surgery						29 (100)	0 (0)	r=+0.239
Surgery + RIA and/RT						46 (85)	8 (15)	P=0.073
r- correlation coefficient								

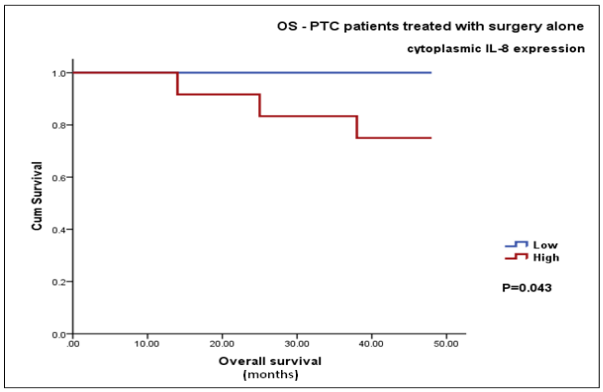
Survival analysis

The median level of IL-8 (34.20 pg/ml) and median IRS score was used as cut-off to divide the PTC patients into low (≤ median) and high (> median) level/expression groups, respectively. Univariate analysis revealed that neither circulating IL-8 nor the tumoral IL-8 expression was a significant predictor of DFS or OS in PTC patients [Table 5].

Table 5: Univariate survival analysis for DFS and OS in relation to IL-8 expression in PTC patients

	DFS (N=69)		**OS (N=76)**	
	N	**Patients relapsed N (%)**	**N**	**Patients died N (%)**
Circulating IL-8 levels				
Low	35	3 (9)	38	4 (10)
High	34	4 (12)	38	4 (10)
		Log rank=0.170, df=1, P=0.680		Log rank=0.001, df=1, P=0.969
IL-8 protein expression				
Cytoplasmic IL-8				
Low	39	6 (15)	42	4 (9)
High	30	1 (3)	34	4 (12)
		Log rank=2.572, df=1, P=0.109		Log rank=0.099, df=1, P=0.753
Nuclear IL-8				
Low	62	7 (11)	68	7 (10)
High	7	0 (0)	8	1 (12)
		Log rank=0.831, df=1, P=0.362		Log rank=0.056, df=1, P=0.813

However, cytoplasmic IL-8 expression was significant predictor of OS in subgroup of patients treated with surgery alone. In this group, 25% (3/12) patients with high cytoplasmic IL-8 expression had significantly shorter OS while, all the patients having lower cytoplasmic IL-8 expressions remained alive (Log rank=4.106, df=1, P=0.043) [Figure 4].



	PTC patients treated with surgery alone (N=27)	
CytoplasmicIL-8 expression	No.	Died No. (%)
Low	15	0 (0)
High	12	3 (25)
Log rank=4.106, df=1 P=0.043		

Figure 4: Significantly reduced OS observed in PTC patients treated with surgery alone having high cytoplasmic IL-8 expression as compared to those with low cytoplasmic IL-8 expression

Besides this, IL-8 expression when correlated with previous results on the expression of adhesion molecules (L-Selectin and VCAM-1)[14] and with SOCS proteins (SOCS-1, SOCS-2 and SOCS-3)[17] in PTC patients, it was observed that cytoplasmic IL-8 expressions exhibited a significant positive correlation with L-Selectin expression (r=+0.258, P=0.018) as well as with VCAM-1 (r=+0.437, P<0.001). It also showed significant positive correlation with the expression of all the three SOCS proteins (cytoplasmic IL-8 vs SOCS-1: r=+0.435, P<0.001; cytoplasmic IL-8 vs SOCS-2: r=+0.230, P=0.036; cytoplasmic IL-8 vs SOCS-3: r=+0.336, P=0.002). In addition, nuclear IL-8 expression (r=-0.244, P=0.026) showed a significant inverse correlation with SOCS-3 immunoexpression [Table 6].

Table 6: Correlation of IL-8 with adhesion molecules (L-Selectin and VCAM-1) and SOCS (SOCS-1, SOCS-2 and SOCS-3) proteins in primary tumors of PTC patients

	Circulating IL-8	Cytoplasmic IL-8 expression	Nuclear IL-8 expression
Circulating L-Selectin	r=+0.165,P=0.135	-	-
Circulating VCAM-1	r=-0.044, P=0.695	-	-
L-Selectin expression	-	**r=+0.258, P=0.018**	r=-0.062, P=0.579
VCAM-1 expression	-	**r=+0.437, P<0.001**	r=-0.124, P =0.266
SOCS-1 expression	-	**r=+0.435, P<0.001**	**r**=-0.184, P=0.096
SOCS-2 expression	-	**r=+0.230, p=0.036**	**r**=-0.066, P=0.551
SOCS-3 expression	-	**r=+0.336, p=0.002**	**r=-0.244, P=0.026**

r- correlation coefficient

DISCUSSION

Serum IL-8 levels were significantly elevated in all patients with thyroid disorders as compared to healthy individuals. Its levels were even elevated in the thyroid cancer patients as compared to the patients with benign thyroid diseases. Confirming the results, ROC curves also revealed that serum IL-8 showed good efficacy to discriminate between healthy individuals and patients with different thyroid diseases as well as between patients with benign thyroid diseases and thyroid cancer patients. Contrarily, studies of Krassas and colleagues found that IL-8 levels were not elevated in Graves' disease, toxic nodular goitre and Hashimoto's thyroiditis.[18] However, similar to our observation, some studies demonstrated statistically significant differences in IL-8 levels in patients with thyroid disease and normal reference group.[9-12] Our preliminary study revealed significant higher IL-8 levels in benign, autoimmune and thyroid carcinoma patients, and it was significantly associated with the advanced stage disease in PTC patients.[19] Increased circulating IL-8 levels have also been

observed in patients with various other malignancies.[20-33]

Moreover, high IL-8 levels were significantly positively correlated with larger tumor size, advanced stage and presence of fibrosis in PTC patients. Also the levels were found to be higher in the male patients who are more likely to be associated with aggressive tumor behaviour as compared to the female patients. Recently, Sanmamed and colleagues reported that serum IL-8 levels correlate with tumor burden and prognosis.[8] In colon cancer patients, its levels statistically correlated with tumor stage,[23] and in breast cancer, circulating IL-8 significantly increased with tumor size[29] which is consistent to the present results. IL-8 levels increased significantly in patients with more advanced stage in breast and uterine endometrial cancers.[28, 34]

Similar to present study, de Campos et al observed IL-8 immunostaining in cytoplasm and focally in nucleus of neoplastic cells in breast cancer patients,[35] while IL-8 protein expression was seen predominantly in cytoplasm of lung cancer cells.[36, 37] Jenkins et al had observed a significant increase in moderate IL-8 staining in Barrett's (premalignant) tissues and an increase in strong staining in adenocarcinoma tissue compared to adjacent squamous tissue.[38] Current study also demonstrated significantly higher cytoplasmic IL-8 expression in PTC patients as compared to benign thyroid diseases. Cytoplasmic IL-8 overexpression was significantly higher in male patients and was substantially positively correlated with larger tumor size and extrathyroidal extension of tumors, while higher nuclear expression was associated with smaller tumor size. This indicates that cytoplasmic expression might be related to more adverse tumor characteristics while; nuclear IL-8 immunoreactivity may be indicative of less hostile tumor behaviour in PTC patients.

In accordance to present study, Chen et al reported higher IL-8 expression in pancreatic cancer patients at both circulating and tumor tissue levels.[39] IL-8 expression correlated with disease progression in prostate, breast and ovarian cancers.[40-44] They observed increase in growth, proliferation, angiogenesis, adhesion and invasion with increased IL-8 overexpression and these effects were decreased on depletion of endogenous IL-8 expression by transfecting cells with plasmid encoding for antisense IL-8.[45]

IL-8 is found to be a prognostic marker in various human cancers.[46-49] In present study, higher cytoplasmic IL-8 immunoreactivity was associated with significantly reduced OS in the PTC patients who were treated with surgery alone. Thus, it can be suggested that the patients having higher IL-8 immunoreactivity may require more active treatment rather than surgery alone, for better prognosis.

Moreover, IL-8 expression exhibited significant positive correlation with the expression of adhesion molecules in PTC patients. It can be suggested that, an increased expression of adhesion molecules may be the result of neutrophil activation by inflammatory cytokines like IL-8.[50] In breast cancer cells, VCAM-1 expression was induced by cytokoine stimulation and its up regulation directly correlated with advanced stage.[51] It has been observed that addition of exogenous cytokines induced expression of endothelial adhesion molecules thereby increasing the adhesive property of cancer cells. This cell-cell adhesion leads to clustering of VCAM-1 which in turn activates the PI3K/AKT signalling that suppresses apoptosis and promotes survival signal in the tumor cells.[52]

These results indicate that production of IL-8 by cancer cells may be one of the factors leading to expression of adhesion molecules, which can facilitate tumor progression through activation of various signalling pathways and further substantiate the link between inflammation and cancer progression.

Further, the expression of SOCS proteins might be heterogeneously induced

by various cytoiknes including IL-8. Usually, SOCS expression is known to be stimulated by activation of cytokine signalling pathway. In turn, when SOCS are overexpressed, they tend to inhibit cytokine induced signal transduction in a negative feedback manner.[53, 54] Hence overall, cancer cells are sustained by several cytokines within the tumor microenvironment, which lead to activation of pathways that support cancer cell growth and survival. Expression of SOCS proteins may be a consequence of this.[54] Thus, in the present study, significant positive correlation of IL-8 with the SOCS proteins, is indicative of a possibility that, there might be failure of negative regulatory pathways acting upon the IL-8 induced pathways, which may overpower the capacity of SOCS proteins to suppress IL-8 expression and reduce the activity of downstream transcriptional factors.

Additionally, the varied effects of IL-8 signalling upon different cell types present within the tumor microenvironment has been suggestive of targeting of CXC-chemokine signalling including IL-8, in order to arrest disease progression and assist in sensitizing tumors to chemotherapeutic and biological agents. Repertaxin, is the CXCR1/2 inhibitor developed to prevent IL-8-induced injury.[55] Clinical trials are in progress to determine the safety and efficacy of repertaxin in combination with docetaxel chemotherapy in patients with advanced breast cancer.[56-58] Furthermore, evidence for IL8-CXCR1/2 axis in CSC has been reported by independent studies and offers a potential therapeutic target.[59-63] Currently, randomized, double blind phase 2 clinical trials aimed at testing the effective targeting of CSC through this axis are in progress.[56-58, 63]

CONCLUSION

IL-8 is not only produced by immune cells but also by the follicular cells of thyroid gland. Its overexpression at both circulating and tumor tissue levels may indicate an excessive production by tumor cells in an inflammatory microenvironment and subsequent release into the circulation. Overall, IL-8 has a role as a differentiating marker in patients with various thyroid diseases and in advancement of thyroid cancer. Further, IL-8 expression is not suppressed by the SOCS proteins and it may have a role in inducing the expression of adhesion molecules like VCAM-1 and L-Selectin and thereby increasing cancer cell proliferation and progression in PTC patients. Moreover, as its expression was able to predict OS in PTC patients treated with surgery alone, targeting IL-8 signalling along with conventional treatment strategies might be beneficial in such patients.

ACKNOWLEDGMENT

This work was financially supported by Gujarat Cancer Society (GCS), and it was approved by the GCRI/GCS ethics committee.

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How to cite this article: Kobawala T, Gajjar K, Ghosh N. Interleukin-8: A potential marker for differentiating papillary thyroid cancer. International Journal of Research and Review. 2020; 7(4): 369-382.

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