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Role of Interleukin-18 in thyroid tumorigenesis

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Original Article

Abstract

Purpose: Although the significance of Interleukin-18 (IL-18) has been studied in pathogenesis of different cancers including, ovarian, gastric, breast, lung carcinoma and melanoma, its role in thyroid cancer- the most common endocrine malignancy has not yet been looked at extensively. Hence, this study intended to examine the role of IL-18 in thyroid tumorigenesis. **Methods:** Sixty seven patients with benign thyroid diseases and 106 thyroid cancer patients (including 83 papillary, 6 follicular, 9 medullary and 8 anaplastic thyroid carcinoma patients) were enrolled in the study. To accomplish the aim, the circulating levels of IL-18 were estimated by enzyme linked immunosorbent assay (ELISA) from all patients and compared with controls. Further, protein expression of IL-18 was determined from the primary tumors of the patients using immunohistochemistry. **Results:** It was observed that the circulating levels of IL-18 were significantly higher in all patients: benign thyroid diseases (p = 0.006), papillary (p < 0.001), follicular (p = 0.023), medullary (p = 0.002) and anaplastic thyroid cancer (p < 0.001) than the controls. In addition to this, IL-18 could well discriminate papillary (AUC = 0.627, p =0.008) and anaplastic thyroid carcinoma patients (AUC = 0.777, p = 0.011) from patients with benign thyroid diseases. However, the difference between tumoral protein expression of IL-18 in patients with benign thyroid diseases and thyroid carcinoma was not significant. The Kaplan - Meier survival analysis revealed that neither the circulating nor the tumoral protein expression of IL-18 was the significant predictor of disease free survival (DFS) or overall survival (OS) in papillary thyroid cancer patients. **Conclusion:** Though not a significant prognosticator, circulating IL-18 may be useful as a differentiating factor in thyroid tumorigenesis and the increase in serum IL-18 levels may be provoked in response to the tumor. Thus, including IL-18 along with the current treatment practice may have a significant role in better management of the disease. However, further exploration of this interleukin is required in a larger series of patients with longer follow up period.

Keywords: IL-18, Thyroid tumorigenesis, Papillary thyroid cancer, Immuno-histochemistry, ELISA

1. Introduction

Interleukin-18 (IL-18), first discovered as Interferon-γ (IFN-γ) inducing factor, is a pleiotropic, proinflammatory cytokine with dual effects on tumor development and progression.[1] On one hand, it can induce Th1 immune response, which is generally regarded as the immune reaction acting against malignant tumors and on the other hand, it can promote Th2 immune responses that may inhibit recognition of cancer cells by immune cells, thus increasing the adhesion molecules, inducing production of angiogenic factors, and promoting a prometastatic micro-environment.[2,3]

IL-18 is secreted by multiple cell types, including T and B cells, monocytes, macrophages, and some tumor cells.[4] It can be secreted as both mature as well as inactive forms. IL-18 receptor (IL-18R) contains a ligand binding α chain and a signal transducing domain β chain.[5] On binding of IL-18 to IL-18Rα, the IL-18Rβ transduces its signal to activate the mitogen-activated protein kinase (MAPK) pathway.[1] IL-18 is also a costimulatory factor for the induction of IL–12 - mediated interferon-γ production by Th cells. In a potential direct negative

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feedback mechanism, IFN-γ induces the expression of the antagonistic IL-18 binding protein (IL-18bp), which acts to inhibit IL-18 activity, and this in turn controls IL-18 induced production of IFN-γ.[2] Thus, the activity of IL-18 is regulated by IL-18bp. On the other hand, downregulated IL-12 and IFN-γ would theoretically result in similarly reduced IL-18bp expression. Absence of this negative stimulus substantially explains that increased concentrations of IL-18 were correlated with advanced stages and were also found to be independently associated with shorter overall survival (OS) in various human cancers.[6]

Various studies indicated the role of IL-18 in pathogenesis of different cancers like, ovarian, gastric, breast, lung carcinoma and melanoma.[1, 7-12] However, its role in thyroid cancer - the most common endocrine malignancy has not yet been explored extensively. Hence, this study specifically aimed to examine the role of IL-18 in benign thyroid disease and thyroid cancer patients and correlate the results with clinicopathological parameters and disease outcome.

2. Methods and Materials

2.1. Patients

Patients with benign thyroid diseases (N = 67), and thyroid cancer (N = 106), (papillary thyroid cancer (PTC): N = 83, follicular thyroid cancer (FTC): N = 6, medullary thyroid cancer (MTC): N = 9 and anaplastic thyroid cancer (ATC): N = 8) enrolled at our institute, were included in the study. All these patients were without any history of autoimmune disease, did not receive any pre-treatment and none of them were taking any immunosuppressive or immunomodulant drugs. Sixty seven percent (45/67) patients with benign thyroid diseases, suspicious for having malignancy were operated at our institute. The thyroid cancer patients, (except 4/8 ATC patients who were unresectable) underwent surgery at the Department of Surgical Oncology of our institute. Only PTC patients which were similar as those cited in our previous articles,[13, 14] were considered for the correlation analysis. The clinicopathological characteristics of these PTC patients are depicted in Table 1.[13, 14] While the patients with other three types of thyroid cancers were included for incidental studies only, as the number of patients were very low for statistical analysis. The clinicians of the institute decided the treatment strategies. The primary treatment offered to the patients was either surgery or surgery followed by radioiodine ablation (RIA) therapy or surgery followed by RIA therapy and radiotherapy both. Surgery was performed by the oncosurgeons, while the RIA therapy and radiotherapy were instituted at the Nuclear Medicine department and Radiotherapy department, respectively. The clinical and histopathological details of the patients were noted from the case files maintained at the Medical record department of the institute. The 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. The patients were followed for a period of 4 years or until death within that period. For OS analysis, complete follow-up details were obtained in 92% (76/83) of PTC patients. Amongst them, 9% (7/76) patients had persistent disease and hence were excluded from the disease free survival (DFS) analysis. Thus, for DFS analysis, 69/76 PTC patients were included.

2.2. Sample collection

All samples were collected with informed consent from the subjects and the study was approved by Institutional Scientific and Ethical Committees. Pretherapeutic blood samples were collected from all patients as well as from 67 controls to determine the circulating levels of IL-18. Serum was separated after centrifugation and was preserved at -80˚C until analysis. For Immunohistochemical localisation of IL-18 protein expression, paraffin embedded tissue blocks of all the patients (who underwent surgery) were retrieved from the Histopathology department of the institute.

2.3. Circulating levels of IL-18 by Enzyme linked Immunosorbent Assay (ELISA)

The circulating levels of IL-18 were estimated from the serum samples of the subjects using commercially available ELISA kit from Krishgen Biosystems, as per manufacturer's instructions. The absorbance was read at 450 nm in Multiskan® Spectrum Microplate Spectrophotometer (Thermo Labsystems), within 30 minutes of stopping the reaction. A standard curve was plot in Graphpad prism 5 software with concentration of standards on X-axis and absorbance on Y-axis. The unknown concentrations were interpreted by the software from the standard curve generated. The concentrations of the diluted samples were multiplied by the dilution factor to determine the actual concentration. The circulating levels were expressed as mean ± standard error (M ± SE) and for survival analysis, the median value was used as cut-off to divide the PTC patients into low (≤ median) and high (> median) level groups, respectively.

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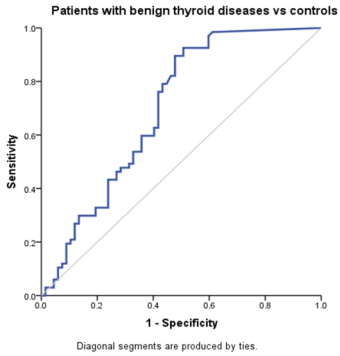
Table 1: Clinicopathological characteristics of PTC patients

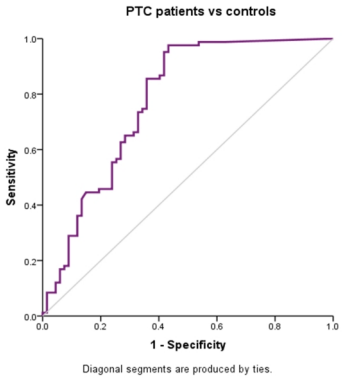
Characteristics	N (%)	Characteristics	N (%)
Age		Bilaterality	
<45 years	41 (49)	Unilateral	61 (74)
≥45 years	42 (51)	Bilateral	22 (26)
Gender		Haemorrhagic area	
Female	56 (68)	Absent	72 (87)
Male	27 (32)	Present	11 (13)
Tumour size		Necrosis	
T1 (N=16)+T2 (N=22)	38 (46)	Absent	67 (81)
T3 (N=30)+T4 (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		
Reccurrence/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)		

(a)



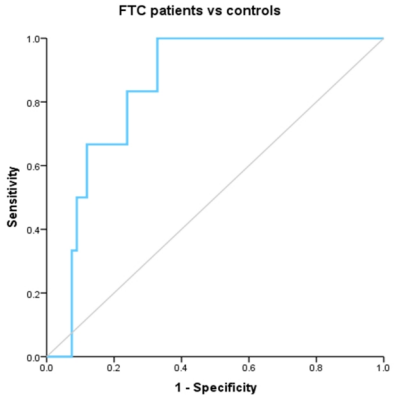
Test Result Variable	Area	Significance (P)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
IL-18	0.698	<0.001	0.607	0.788

(b)



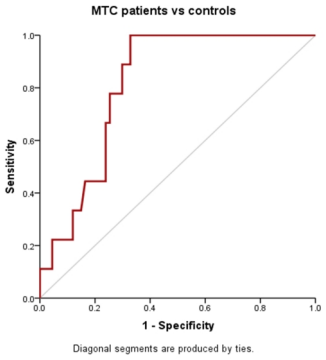
Test Result Variable	Area	Significance (P)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
IL-18	0.775	<0.001	0.696	0.854

(c)



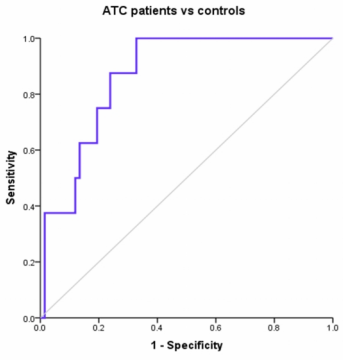
Test Result Variable	Area	Significance (P)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
IL-18	0.846	0.005	0.741	0.950

(d)



Test Result Variable	Area	Significance (P)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
IL-18	0.813	0.002	0.711	0.916

(e)



Test Result Variable	Area	Significance (P)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
IL-18	0.868	0.001	0.772	0.963

Figure 1: ROC curves of serum IL-18 in patients with thyroid diseases vs controls

Table 2: Significance of circulating levels of IL-8, IL-12, IL-18 and TNF-α in patients with benign thyroid diseases and thyroid carcinoma as compared to controls

Subjects	Circulating IL-18	
	M ± SE (pg/ml)	p value
Controls (N=67)	188.56 ± 31.82	
Benign thyroid disease (N=67)	305.81 ± 27.11	0.006*
Thyroid carcinoma (N=106)	430.18 ± 27.49	<0.001*
		0.003@
Papillary thyroid carcinoma (N=83)	402.17 ± 28.05	<0.001*
		0.016@
Follicular thyroid carcinoma (N=6)	439.22 ± 54.01	0.023*
		0.153@
Medullary thyroid carcinoma (N=9)	510.81 ± 144.99	0.002*
		0.026@
Anaplastic thyroid carcinoma (N=8)	617.04 ± 137.22	<0.001*
		0.001@

*Significance of circulating IL-18 in patients vs controls
@ Significance of circulating IL-18 in patients with thyroid cancer vs benign thyroid diseases

Table 3: Correlation of circulating levels of IL-18 with clinicopathological parameters of PTC patients

Parameter	N	IL-18	
		Mean ± SE	p value
Lymphatic permeation			
Absent	67	371.24 ± 29.12	0.020
Present	16	534.87 ± 72.45	
Haemorrhagic area			
Absent	72	374.22 ± 24.51	0.008
Present	11	589.68 ± 129.34	

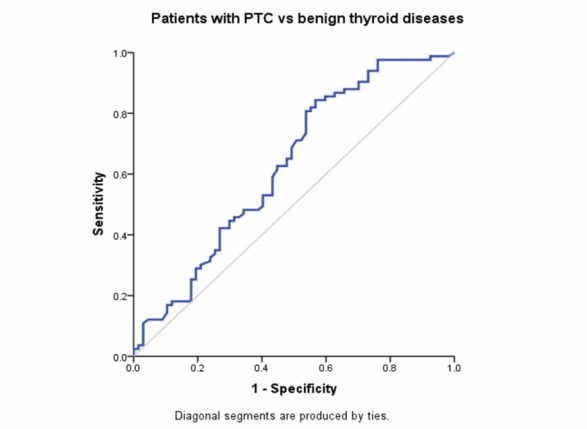
Table 4: Incidence of the cytokine expression in patients with benign thyroid disease and thyroid cancer

IL-18 expression		N (%)	Median IRS	Significance
Benign thyroid disease (N=45)		44 (98)		
	Cytoplasmic	44 (98)	6	
	Nuclear	33 (73)	4	
PTC (N=83)		83 (100)		
	Cytoplasmic	82 (99)	6	χ^2=3.032, r=+0.154, p=0.083
	Nuclear	63 (76)	4	χ^2=0.001, r=+0.003, P=0.973
FTC (N=6)		6 (100)		
	Cytoplasmic	6 (100)	5	χ^2=0.213, r=+0.129, p=0.645
	Nuclear	4 (67)	2.5	χ^2=0.000, r=+0.022, P=1.000
MTC (N=9)		9 (100)		
	Cytoplasmic	8 (89)	4	χ^2=0.000, r=+0.018, p=1.000
	Nuclear	6 (67)	4	χ^2=0.000, r=-0.017, p=1.000
ATC (N=4)		4 (100)		
	Cytoplasmic	4 (100)	4	χ^2=0.047, r=+0.110, p=0.829
	Nuclear	2 (50)	1	χ^2=0.000, r=+0.018, p=1.000

Table 5: Univariate survival analysis (of circulating and tumoral protein expression of IL-18) for DFS and OS in PTC patients (N=69)

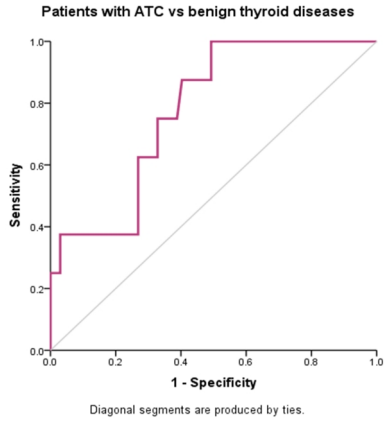
Disease free survival (DFS)			Overall survival (OS)		
Circulating IL-18	N	Patients relapsed N (%)	Circulating IL-18	N	Patients died N (%)
Low	34	3 (9)	Low	38	5 (13)
High	35	4 (11)	High	38	3 (8)
	Log rank=0.126, df=1, p=0.723			Log rank=0.589, df=1, p=0.443	
IL-18 expression					
Cytoplasmic			Cytoplasmic		
Low	37	3 (8)	Low	41	5 (12)
High	32	4 (12)	High	35	3 (9)
	Log rank=0.402, df=1, p=0.526			Log rank=0.285, df=1, p=0.594	
Nuclear			Nuclear		
Low	37	5 (13)	Low	41	5 (12)
High	32	2 (6)	High	35	3 (9)
	Log rank=1.020, df=1, p=0.313			Log rank=0.244, df=1, p=0.621	

(a)



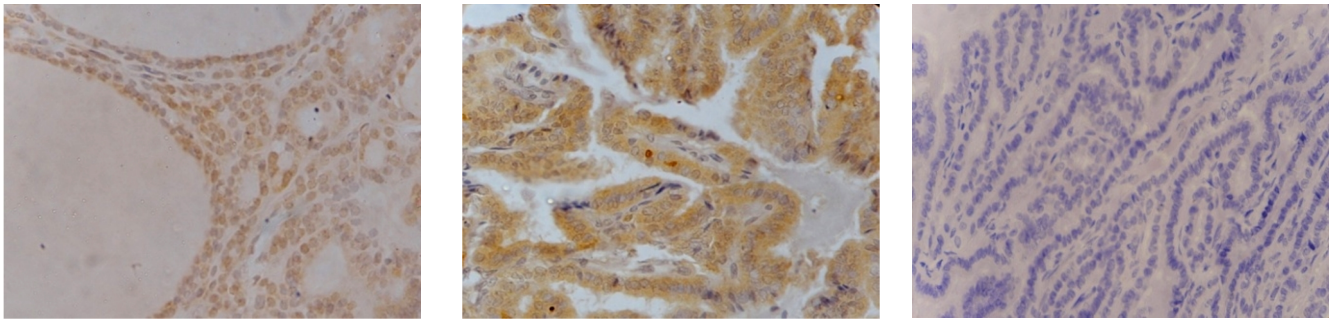
Test Result Variable	Area	Significance (P)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
IL-18	0.627	0.008	0.535	0.719

(b)



Test Result Variable	Area	Significance (P)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
IL-18	0.777	0.011	0.636	0.919

Figure 2: ROC curves of serum IL-18 in patients with thyroid cancer vs benign thyroid diseases



(a) (b) (c)

Figure 3: Photomicrographs showing staining for IL-18. Cytoplasmic and nuclear staining for IL-18 in (a) benign goitre, (b) Follicular variant of papillary carcinoma and (c) Negative control for IL-18 in PTC

2.4. Tumoral protein expression of IL-18 by Immunohistochemistry (IHC)

For detection of IL-18 protein expression from tumors, 3-5 µm thick sections were cut from the formalin fixed paraffin embedded tissue blocks and mounted on 3 - Aminopropyltriethoxysilane (APES) coated glass slides, followed by immunohistochemical staining with HRP polymerization technique performed using primary rabbit polyclonal IL-18 antibody from Santacruz Biotechnology, Inc. (sc - 7954) at 1:50 dilution and MACH4 Universal HRP-Polymer Detection System from Biocare Medicals, USA; as per manufacturer's protocol recommendations. For retrieval of antigen, the sections were heated in 10 mM sodium citrate buffer (pH, 6.0) for 20 minutes in a pressure cooker prior to application of the primary antibody. Scoring of the stained sections was done by two individual observers independently in a blinded fashion using a semiquantitative Immunoreactive Score (IRS) method of Remelle and Stegner (1987), based on staining positivity and staining intensity.[15] Staining positivity was scored as 0 for no stained cells, 1 for staining in 1% to 10% of cells, 2 for staining in 11% to 50% of cells, 3 for staining in 50% to 80% of cells and 4 for staining in > 80% of cells. The staining intensity was scored as 0 for no staining, 1 for weak/faint staining, 2 for moderate staining and 3 for intense/dark staining. The IRS score was then obtained as a product of staining positivity and staining intensity and therefore, theoretically the scores could range from 0 to 12. For statistical evaluation, cytoplasmic and nuclear expressions were scored independently and taken into account separately. The median IRS was used as cut-off value to divide the patients into low (≤ median IRS) and high (> median IRS) expression groups, respectively.

2.5. Statistical analysis

The data were analyzed statistically using the Statistical Package for Social Sciences (SPSS) software version 16 (SPSS Inc, USA). Independent Samples T-test was used to compare the means of circulating levels. Receiver's operating characteristic (ROC) curves were constructed to determine the discriminating efficacy of the circulating IL-18. Two- tailed $\chi 2$ test was used to compare the tumoral protein expressions. In case of less than five patients in the cells of 2 x 2 tables, Yate's continuity correction value alongwith its two-tailed significance was taken into consideration. Correlation between two parameters was calculated using Spearman's correlation coefficient (r) method. Univariate survival analysis was evaluated using Kaplan-Meier method and Log rank test was used to analyse difference in survival curves and to assess the prognostic significance of DFS and OS. p values ≤ 0.05 were considered to be significant.

3. Results

3.1. Circulating levels of IL-18 in healthy individuals, patients with benign thyroid diseases and thyroid cancer

The circulating levels (Mean ± Standard Error, M ± SE) of IL-18 in controls, in patients with benign thyroid diseases and thyroid cancer are depicted in Table 2.

The circulating levels IL-18 were significantly higher in all patients: benign thyroid diseases ($p = 0.006$), PTC ($p < 0.001$), FTC ($p = 0.023$), MTC ($p = 0.002$) and ATC ($p < 0.001$) than the controls [Table 2].

The ROC curves were generated to reveal the efficacy of significantly elevated IL-18 levels in order to differentiate the controls and patients with different thyroid diseases. The results demonstrated that serum IL-18 levels could significantly differentiate patients with benign thyroid diseases (AUC = 0.698, $p < 0.001$), PTC (AUC = 0.775, $p < 0.001$), FTC (AUC = 0.846, $p = 0.005$), MTC (AUC = 0.813, $p = 0.002$) as well as ATC patients (AUC = 0.868, $p = 0.001$) from controls [Figure 1a-1e].

Moreover, it was observed that, the levels of IL-18 were found to be predominantly higher in PTC ($p = 0.016$), MTC ($p = 0.026$) and ATC ($p = 0.001$) as compared to those in patients with benign thyroid diseases [Table 2].

Further, when ROC curves were generated to explore the power of significantly elevated serum IL-18 level to differentiate the benign and thyroid carcinoma patients, the results showed that IL-18 showed good discriminating efficacy between patients with PTC and benign thyroid diseases (AUC = 0.627, $p = 0.008$) [Figure 2a]. In addition to this, IL-18 could well discriminate ATC patients from patients with benign thyroid diseases (AUC = 0.777, $p = 0.011$) [Figure 2b].

The correlation of serum cytokine levels with the clinicopathological parameters of PTC patients have been depicted in Table 3. Significantly higher serum IL-18 levels were observed in patients whose tumors had presence of lymphatic permeation ($p = 0.020$) and haemorrhagic area ($p = 0.008$) relative to the tumors showing absence of lymphatic permeation and haemorrhagic area, respectively [Table 3] while, no such significant correlation of serum IL-18 was observed with rest of the clinicopathological parameters.

3.2. Tumoral protein expression of IL-18 in patients with benign thyroid diseases and thyroid carcinoma

Cytoplasmic and/or nuclear staining pattern was observed for IL-18. For statistical evaluation, cytoplasmic and nuclear expressions were scored independently and taken into account separately. The incidence of the cytokine expression in patients with

benign thyroid diseases and thyroid carcinoma has been depicted in Table 4. In PTC patients, the cytoplasmic IL-18 expression, was higher as compared to that observed in patients with benign thyroid diseases. However, the difference was not statistically significant ($\chi^2 = 3.032$, r = + 0.154, p = 0.083). While the incidence of cytoplasmic or nuclear IL-18 immunoreactivity was not significantly different in patients with FTC, MTC or ATC and benign thyroid disease, respectively [Table 4].

Figure 3, shows the representative staining of IL-18 protein expression.
The statistical analysis revealed that neither cytoplasmic nor the nuclear IL-18 expression exhibited significant relationship with any of the clinicopathological parameters in PTC patients indicating that the incidence of IL-18 immunoreactivity was nearly similar in the PTC patients when subgrouped according to the clinicopathological variables.

3.3. Survival analysis

Kaplan-Meier survival analysis was evaluated for DFS and OS in PTC patients. The median level of serum IL-18 (314.23 pg/ml), was used as cut-off value to divide the PTC patients into low (≤ median) and high (> median) level groups, respectively. It was observed that neither the circulating nor the tumoral protein expression of IL-18 was the significant predictor of DFS or OS in PTC patients [Table 5].

4. Discussion

The results of the present study perceptibly reveal that the serum IL-18 levels could significantly discriminate the patients with benign thyroid diseases as well as the PTC, FTC, MTC and ATC patients from controls. Additionally, it was efficient enough to distinguish the PTC and ATC patients from those with benign thyroid diseases. Further, in PTC patients, a significant positive correlation was obtained between circulating IL-18 and presence of lymphatic permeation and haemorrhagic areas in the tumors. In similarity with this, significantly higher concentration of IL-18 in the circulation of patients with different malignancies.[4, 7, 16-42] Moreover, Matveeva and Mosina,[43] through their study have recommended the use of serum IL-18 for diagnosis of atrophic gastritis, peptic ulcer disease and gastric cancer. Further, serum IL-18 levels were found to be significantly elevated in prostate cancer patients than both controls and benign prostate hyperplasia[44] and in gastric cancer patients than in gastric ulcer patients.[45] However, recently Bao *et al.*[46] have reported significant lower serum IL-18 levels in hepatocellular carcinoma patients compared to the control group. Additionally, its levels were also reported to be higher in polycystic ovarian syndrome[47, 48] and its possible correlation with artherosclerosis[49] and its role as a cardiovascular marker[50, 51] has also been documented.

Further, IL-18 immunoreactivity was observed in the cytoplasm and nucleus of the thyroid cells. Although not significant, a trend of increased cytoplasmic expression of IL-18 was observed in PTC patients as compared to patients with benign diseases, while the incidence of nuclear IL-18 immunoreactivity was almost similar in patients with benign thyroid diseases and PTC. Moreover, the cytoplasmic or nuclear IL-18 expression did not differ significantly in FTC, MTC or ATC patients and the benign disease patients. The IL-18 expression also did not correlate with any of the clinicopathological parameters in the PTC patients. In similarity with present observation, Ye *et al.*[11] also found that IL-18 was distributed in both cytoplasmic and nuclear compartments of the gastric tumor cells while; Orengo *et al.*[37] have reported only cytoplasmic staining of IL-18 in epithelial ovarian cancer cells. Further, the immunohistochemical analysis in a study by Okamoto *et al.*[33] revealed that IL-18 was strongly expressed in lung cancer cells and in bone metastasis. Srabovic *et al.*[52] have reported that IL-18 expression was significantly higher in breast cancer tumors relative to its expression in surrounding unchanged tissue of the same patient but it was not higher than that observed in patients with benign breast diseases. IL-18 was found to be overexpressed in tumor tissues of pancreatic carcinoma patients[28] and ovarian cancer patients.[12] Contrarily, Chia *et al.*[53] observed significantly higher incidence of IL-18 expression in hepatocytes of apparently normal surrounding tissue compared with the tumor tissue in hepatocellular carcinoma and Cui *et al.*[54] showed that expression of IL-18 increased in colorectal adenoma tissues than in coloreactal cancer tissues. Further, Chia *et al.*[53] and Srabovic *et al.*[52] did not find any association between IL-18 expression and histopathological factors in hepatocellular cancer and breast cancer patients, respectively, which is similar to our findings. Further, some researchers have observed that expression of IL-18 was significantly associated with poor prognosis.[6] However, the results of present study showed that IL-18 had no significant prognostic role in PTC patients. This may be because the overall incidence of disease relapse and mortality is very less in PTC patients and so it might be necessary to follow them for a longer period of time. Similar to current findings, IL-18 was not found to be associated with prognosis in patients with gastric cancer,[11, 55] esophageal carcinoma,[25] and lung cancer.[22]

In their study, Liu *et al.*[56] have reported that IL-18 played an important role in the inhibition of tongue squamous cell carcinoma and may be further investigated as a new therapeutic target against this cancer. Based on preclinical studies, many clinical trials of IL-18 are been conducted. Collectively, they show that IL-18 has low toxicity but has limited therapeutic effects as a single agent. However, IL-18 may be incorporated as an immune enhancing molecule in combinational therapies with other agents (e.g. mAb, cytotoxic drugs, or vaccines). Although recent literature has uncovered a

complex and divergent role of the IL–18 / IL-18R / IL-18BP system in different neoplastic conditions, more studies on the biologic role of cancer - related IL-18 are crucial.[57] Apart from this, very recently, Ma *et al.*[58] have suggested that although much needs to be done to expound the conflicting role of IL18, inclusion of IL18 in immune checkpoint blockade therapy may extend the evolving treatment regime of cancer.

5. Conclusion

Overall from current results it can be implicated that, although high serum IL-18 is not useful as a significant prognosticator, it may be useful as a differentiating factor in thyroid tumorigenesis and the increase in serum IL-18 levels may be induced in response to the tumor and is associated with host defence mechanism against the tumor cells. Thus, integrating IL-18 into the present treatment strategy may have impact in enhancing the immune system to conquer the disease.

Conflict of interest

The authors declare that they have no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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