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Original Research Article

A study of tumour infiltrating lymphocytes and neutrophil to lymphocyte ratio in papillary carcinoma thyroid

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ABSTRACT

Background: Papillary thyroid carcinoma accounts for 70-80% of all diagnosed thyroid carcinomas. Immune response including systemic inflammation is thought to be essential for suppression of carcinogenesis. This study aims to correlate the tumour infiltrating lymphocytes (TIL) and neutrophil to lymphocyte ratio (NLR) in various demographic data and histomorphological features of papillary carcinoma thyroid.
Methods: A total of 60 cases of papillary carcinoma were taken from a period between January 2018 and July 2021. TIL was analyzed as diffuse or focal at the area of highest concentration and was graded subjectively as: absent (no lymphocytes), mild (1-10%), moderate (>10-50%) and dense (>50%). NLR was calculated as the neutrophil count divided by the lymphocyte count, based on the preoperative complete blood cell counts.
Results: The cases belonged to age group varying from 20 to 75 years with female preponderance (88.3%). In this study, there was significant correlation between TIL and tumour stage with a p value of 0.001. Lower the tumour stage, higher was the concentration of TIL. A cut of value of 2.17 was taken for NLR. Cases with diffuse, moderate to dense peritumoural lymphocytes had lower NLR and were statistically significant. High NLR was also seen in higher TNM stage. On the other hand, there were no association with gender, age, tumour size, histological subtype and focality with NLR.
Conclusions: As an index of inflammation, tumour infiltrating lymphocytes and NLR can be considered. We found increased TIL in lower tumour stage and increased NLR values in higher tumour stages.

Keywords: Papillary carcinoma thyroid, Tumour infiltrating lymphocytes, Neutrophil to lymphocyte ratio

INTRODUCTION

Papillary thyroid carcinomas (PTC) account for 70-80% of all diagnosed thyroid cancers. Though it has a good prognosis, 10-15% of patients have more aggressive progression with metastasis causing death.[1] They typically metastasize to regional lymph nodes. Distal organ involvement like lung and bone is uncommon and is often linked with a poor prognosis. Metastases to intra-abdominal organs are extremely uncommon.[2] Tumourigenesis is the result of imbalance between elaborate cancer-promoting and cancer-inhibiting molecular pathways. Cancer formation, progression, and metastasis are all impacted by inflammation. The intricate interaction between neoplastic cells and their inflammatory microenvironment may have a significant impact on a patient's prognosis.[3]

The human immune system is responsible for protecting our body from both the harmful pathogens from outside and also from enemies' within.[4] In 1863, Rudolf Virchow identified leukocytes in tumour tissues suggesting that inflammation has a role in development and progression of cancers. Immune response by innate and adaptive

immunity is essential for suppression of carcinogenesis and tumour spread. Effector T cells like cytotoxic T lymphocytes, natural killer cells and natural killer T cells have a property to induce apoptosis in tumour cells thereby preventing tumour growth.[4] Different types of tumour associated inflammatory cells like T cells, B cells and mast cells have been identified in thyroid cancers.[5]

Systemic inflammation also has a role in cancer development and progression and its degree can reflect the tumour burden. Leukocytosis and elevated C-reactive protein due to systemic inflammation are known to be poor prognostic factors in cancers. Neutrophils in peripheral blood are a part of innate immunity and indicate systemic inflammatory response. Lymphocytes being a cell of adaptive immunity provide a better and active defence following re-infection. So, the neutrophil to lymphocyte ratio (NLR) reflects the balance between the innate and adaptive immunity and can be used as a convenient and inexpensive prognostic marker.[3,6]

The aim of this study to evaluate a possible association with tumour infiltrating lymphocytes (TIL) and preoperative peripheral blood neutrophil to lymphocyte ratio with various pathological characteristics and stage of papillary thyroid carcinoma.

METHODS

This is a retrospective study conducted on 60 consecutive cases of PTC from January 2018 to July 2021 received in the Department of Pathology from the patients of Yenepoya Medical College, Mangaluru, Karnataka, India. All microscopically diagnosed cases of PTC of patients of both genders and of any age were included in the study. All cases where the complete blood count was not available pre operatively and if there was any documented infection during the time of thyroidectomy were excluded.

The thyroidectomy specimens were fixed in 10% neutral buffered formalin grossed according to the standard grossing protocol and then processed and paraffin embedded. Sections were cut at a thickness of 4-5 microns. Multiple sections were taken from each case and routine Haematoxylin and Eosin staining was done.

The demographical data, histomorphological features like tumour size, laterality, lymphovascular invasion, tumour stage and lymph node metastasis were assessed. The tumour size was determined as the greatest diameter of the lesion. Multifocal tumours were defined as 2 or more foci of tumour in the resected specimen. Unilateral tumours were defined as presence of tumour only in one lobe while bilateral means involvement of both lobes. These sections were also studied for lymphocytic infiltration. Peritumoural lymphocytic infiltration was analyzed as diffuse or focal at the area of highest concentration and was graded subjectively as: Absent (no lymphocytes), Mild (1-10%), Moderate (>10-50%) and Dense (>50%). The cases were grouped according to their tumour-node metastasis (TNM) classification. The pre-operative values of neutrophils and lymphocyte count of the patient were retrieved from the archives of the laboratory. Then, the ratio was calculated. Patient age, gender, tumour size, tumour focality, pathological stage and lymph node metastasis were correlated with peritumoural lymphocyte infiltrate and NLR. Statistical analysis was performed on SPSS version 27 using chi square test. Clinical significance was set to be $p<0.05$.

RESULTS

During the course of the study, 77 patients were diagnosed with papillary carcinoma thyroid in this hospital, but only 60 cases fulfilled the inclusion criteria and were included in the final analysis. Demographic characteristics, histopathological data and haematological data are shown in Table 1.

Table 1: Histomorphological data and haematological data of the cases.

Variables	No. of cases	Percentage
Age (years)		
1-30	13	21.7
31-40	42	70
>60	5	8.3
Gender		
Female	53	88.3
Male	7	11.7
Tumour lymphocytes		
Absent	4	6.7
Focal	22	36.7
Diffuse	34	56.7
Lymphocyte infiltrate		
Mild	20	33.3
Moderate	17	28.3
Dense	19	31.7
Absent	4	6.7
Lymphovascular invasion		
Present	27	45
Absent	33	55
Size (cm)		
<1	8	13.3
>1	52	86.7
Tumour focality		
Unifocal	36	60
Multifocal	24	40
Stage		
1	22	36.7
2	18	30.0
3	16	26.7
NLR		
4	4	6.7
<2.17	37	61.7
>2.17	23	38.3

The cases belonged to the age group varying from 20 to 75 years. The disease showed female preponderance as they constituted of 88.3% (53/60 cases). Classical variant of papillary carcinoma thyroid was the most common histological type among the cases studied and constituted 75% (45/60) cases, followed by follicular variant of PTC comprising of 18.3% (11/60) and the least common was oncocytic variant of PTC constituting of 6.7% (4/60) cases. Tumour size more than 1 cm was seen in 86.7% (52/60) cases and 60% (36/60) cases were having unifocal lesion. Lymphovascular invasion was present in 45% (27/60) cases.

Out of these 60 cases, 56 cases had peritumoural lymphocyte infiltrate, of which 22 cases had focal peritumoural lymphocyte infiltration and 34 cases had diffuse peritumoural lymphocyte infiltration (Figure 1).

This study showed significant correlation (p value 0.001) between the tumour stage and tumour infiltrating lymphocytes as the lower the stage was, higher were tumour infiltrating lymphocytes. There were no significant association between tumour infiltrating lymphocyte with the presence of lymphovascular invasion, tumour size as well as tumour focality. There was also no significant association between peritumoural lymphocyte infiltrates with different variants of Papillary carcinoma thyroid (Table 2).

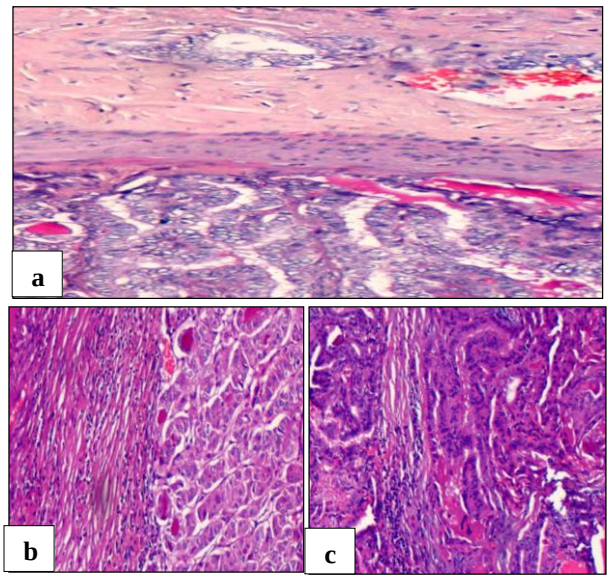

Figure 1: Cases of papillary carcinoma thyroid showing (a) mild, (b) moderate, and (c) and severe lymphocyte infiltration in the tumour section.

Table 2: Correlation of tumour infiltrating lymphocytes with demographic data and histomorphological features of the tumour.

Total case (60)	Tumour infiltrating lmphocytes				P value
	Mild	Moderate	Dense	Absent	
Age in years					
>30	4	4	3	2	0.306
30-60	14	13	14	1	
>60	2	0	2	1	
Gender					
Female	17	14	18	4	0.715
Male	3	3	1	0	
Size (cm)					
<1	2	2	4	0	0.755
>1	18	15	15	4	
Focality					
Unifocal	14	9	11	2	0.694
Multifocal	6	8	8	2	
LVI					
Present	10	7	7	3	0.553
Absent	10	10	12	1	
Variants					
Classical	14	11	16	4	0.439
Follicular	5	5	1	0	
Oncocytic	1	1	2	0	
Stage					
Stage 1	6	3	13	0	0.001
Stage 2	2	12	4	0	
Stage 3	11	2	1	2	
Stage 4	1	0	1	2	

LVI: Lymphovascular invasion.

NLR in this study varied from 0.75 to 18.25 and mean NLR was 3.42. The NLR of 2.17 was taken as cut off according to the study conducted by Manatakis et al.[3]

Table 3: Correlation of NLR with demographic data and histomorphological features of the tumour.

Variables	NLR		P value
	<2.17	>2.17	
Age (years)			
<30	8	5	1.000
30-60	26	16	
>60	3	2	
Gender			
Female	33	20	1.000
Male	4	3	
Focality			
Unifocal	24	12	0.419
Multifocal	13	11	
Size (cm)			
<1	7	1	0.138
>1	30	22	
Focality			
Unifocal	24	12	0.419
Multifocal	13	11	
LVI			
Present	17	10	1.000
Absent	20	13	
Extend of TIL			
Absent	3	1	0.048
Focal	9	13	
Diffuse	25	9	
Density of TIL			
Mild	7	13	0.026
Moderate	13	4	
Severe	14	5	
Absent	3	1	
Stage			
Stage 1	17	5	0.00
Stage 2	15	3	
Stage 3	4	12	
Stage 4	1	3	
Variants of PTC			
Classical	28	17	0.810
Follicular	6	5	
Oncocytic	3	1	

LVI: Lymphovascular invasion, TIL: tumour infiltrating lymphocytes, PTC: papillary thyroid carcinoma

NLR values did not display any association with gender or age as well as to the tumour size. Although, we observed tendency towards lower NLR in unifocal tumours, the difference did not attain statistical significance. NLR was also not significantly associated with lymphovascular invasion, though majority of cases without lymphovascular invasion had lower NLR. Cases with diffuse peritumoural lymphocyte infiltrate had lower NLR when compared to focal or absent peritumoural lymphocyte infiltrate and was statistically significant with a p value of 0.048. In this study, there was significant association with tumour stage and NLR. Higher the tumour stage, higher was the NLR, going as high as 18.25.

In our study, as the concentration of peritumoural lymphocyte infiltration increased, lower was the NLR and vice versa and the scenario was statistically significant with a p value of 0.026. Majority (72.9%) of cases with NLR<2.17 had moderate to dense peritumoural lymphocyte infiltration while 56.5% cases with mild peritumoural lymphocyte aggregation had NLR>2.17.

DISCUSSION

Hanahan et al proposed a few crucial features for the emergence of carcinoma which included the main mechanisms of cellular metabolism, which are reprogrammed to favour the progression of cancer by supporting the growth and the proliferation of cancer cells. Another mechanism involves active evasion by cancer cells from attack and elimination by immune system.[7] On their journey to developing a tumour, cancer cells should acquire the techniques of immune mechanism exploitation and immune surveillance evasion. The relationship between papillary carcinoma thyroid and inflammation is complex. The presence of lymphocytic infiltrate is usually significantly higher in patients with Papillary thyroid carcinoma than in those with benign thyroid lesions indicating that the presence of lymphocytes might favour cancer development.[8] However, some studies indicate that the presence of chronic lymphocytic thyroiditis in patients with Papillary carcinoma thyroid correlates with an improved prognosis.[9]

In this study, 94% cases had some peritumoural lymphocytic infiltrate, either diffuse or focal and found that lower the pathological stage (stage 1 and 2) of Papillary carcinoma, higher was the density of lymphocytic infiltrates suggesting that papillary thyroid cancers with coexisting inflammation had better outcome.[5,10] In a similar study conducted by Villagelin et al, it was concluded that more favourable outcome of PTC was seen in the presence of lymphocyte infiltration and supported the hypothesis that lymphocyte created an immune reaction to control tumour growth and proliferation.[1] Study by Kuo et al concluded that presence of tumour infiltration lymphocytes in papillary carcinoma thyroid is associated with up regulation of immune response and cytokine production with an overall survival benefit.[5]

The reason for better prognosis of papillary carcinoma thyroid with lymphocytic infiltration is probably due to cytotoxic T cells which activate and recruit natural killer cells or lymphocyte associated killer activity and performs as cancer cell killers.[11] Cytokines like interleukin (IL)-1 are thought to be secreted by these antitumour lymphocytes which inhibit thyroid carcinoma growth.[12]

Host immune responses are inhibited by these cells leading to tumour growth and proliferation through mechanisms such as major histocompatibility complex molecule expression regulation.[13] Homing of lymphocytes into the tumour stroma creates a milieu, in which the immune system homeostasis and control of self-tolerance are strongly modified. Due to their cytotoxic action and capacity to eradicate cancer cells, cytotoxic $CD8^+$ T cells (CTL), natural killer (NK), and NK T cells (NKT cells) are significant suppressors of tumour progression.[14] Other cell types, including the interleukin 10 (IL10)-producing $CD4^+$ T-helper 1 (Th1) cells, the TGF-b-producing $CD4^+$ Th3 cells, regulatory T (Tregs) cells, $CD4^-$ and $CD8^-$ (double negative (DN) T cells, have the capacity to suppress immune response, establish tolerance, and aid neoplastic cells in escaping immunological attack.[15]

In papillary carcinoma thyroid, some specific mutation can inhibit or enhance these responses. RET/PTC, a subtype of PTC can inhibit tumour growth by recruitment of inflammatory cells.[1] Serine/threonine- protein kinase (BRAF)- mitogen activated protein kinase (MAPK) signalling pathway induces synthesis and secretion of immunosuppressive cytokines like IL-10, VEGF and IL-6 by the PTC cells.[16,17]

In case of malignancy, tumour associated neutrophilia and lymphopenia in peripheral blood is seen due to cancer related inflammation and cytokine release.[6] In a similar study conducted by Feng et al on clinical and prognostic value of neutrophil lymphocyte ratio in thyroid cancer in 312 cases, showed that it can serve as an excellent marker for predicting tumour growth, prognosis and metastasis. Neutrophil to lymphocyte ratio was significantly associated with larger tumour size and increased metastasis.[18] Cheong et al studied on 432 patients regarding neutrophil to lymphocyte ratio in thyroid cancer and concluded that pre-operative neutrophil to lymphocyte ratio can be a significant predictor of thyroid cancer. It is also associated with tumour size and lymph node metastasis.[19]

Our study confirmed this relation of high NLR with larger tumour size and higher TNM stage. This study also showed that, higher the tumour infiltrating lymphocytes, lower was the neutrophil to lymphocyte ratio. It indicates the protective role of tumour infiltrating lymphocytes and the use of neutrophil to lymphocyte ratio in predicting the tumour profile. Systemic inflammatory response due to tumour- host reaction is generally not taken in most contemporary prognostic system. However, inflammatory biomarkers like white cell count, cytokines and CRP can be used as independent prognostic factors in various carcinomas of oesophagus, gastric, pancreas, colorectal and bladder.[3] The relatively low NLR values of papillary thyroid carcinoma in compared to other solid tumours is an intriguing feature that was also supported by this investigation.[20] This trend has been attributed to the comparatively indolent character of differentiated thyroid cancer, which results in a less potent systemic inflammatory response, as well as the possibility that inflammation may play a less significant role in the beginning of thyroid carcinogenesis.[21]

NLR is still primarily a non-specific biomarker of systemic inflammation in any scenario. Despite showing promise in terms of sensitivity, it is important to carefully exclude any individuals who have any illnesses that alter white blood cell differential counts (acute infections, allergic responses, and cardiovascular events).[22] The NLR is however widely accessible from standard blood tests and does not raise the expense of the preoperative diagnostic work-up.

Limitations

Larger sample size with follow up of patients over a long period of time could provide better information on the utility of assessing immune response to the malignancy.

CONCLUSION

Tumour infiltrating lymphocytes is associated with up regulation of immune response and cytokine production. In this study, higher the tumour stage was, less was the tumour infiltrating lymphocytes. There was also correlation between neutrophil to lymphocyte ratio and tumour infiltrating lymphocytes where when neutrophil to lymphocyte ratio was high, the tumour infiltrating lymphocytes were low. High neutrophil to lymphocyte ratio was also seen in larger tumour size and in higher stage indicating the presence of less lymphocyte count in larger tumours.

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