

Significance of neutrophil to lymphocyte ratio in prostate lesions with special reference to prostate cancer

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Abstract

Aim: To study the significance of neutrophil- lymphocyte ratio (N:L ratio) in prostate lesions, especially in cases of prostate cancer (PCa).

Materials and Methods: Fifty cases of PCa and fifty cases of benign prostatic hyperplasia (BPH), diagnosed on transurethral resection of prostate biopsies were included in the study. Hematological parameters were recorded, N:L ratio was calculated in all cases and values were compared between BPH and PCa, further association between pathological characters of PCa and N:L ratio values was studied.

Results: In the present study, we found a statistically significant high N:L ratio in PCa cases as compared with BPH cases ($p=0.008$). We also found a significant difference in the PSA values ($p<0.001$) and ESR (0.001) between the benign and malignant groups. However hemoglobin, platelet counts and total leucocyte counts did not show a significant difference between the two groups. Sub group analysis failed to show an association between prognostic markers like PSA ($p=0.838$), gleason score ($p=0.161$), T ($p=0.052$) N ($p=0.225$) M ($p=0.529$) stage and N:L ratio.

Conclusion: In this study, significant difference in N:L ratio was noted between BPH and PCa, but no association could be demonstrated between N:L ratio and prognostic markers in PCa. Hence N:L ratio cannot be used as a prognostic marker in PCa.

Keywords: Prostate cancer (PCa), N:L ratio.

Introduction

Prostate cancer (PCa) is the second common type of cancer in men and stands sixth amongst cancer related deaths worldwide. The number of new PCa cases is steadily raising, and is expected to reach 1.7 million by 2030 with possible 4,99,000 new deaths.¹

Unlike the previous notion of PCa being an entity of the western world, the numbers are increasing drastically in India due to altered life styles, increased disease awareness, increased life expectancy and access to medical facilities. PCa is second common site of cancer among males in India.²

The well recognised risk factors for PCa include advancing age of the patient, positive family history, westernised life style habits, African American ancestry and environmental factors. The environmental factors mainly comprise of chronic inflammation in the prostate caused by infections (commonly E.coli and enterococcus species), hormonal changes (androgens), dietary factors (dietary mutagens like heterocyclic amines) and environmental exposures³.

With the raise in the number of PCa cases and increased disease burden, it is compelling to look for additional risk factors involved, to help in better understanding the aetiopathogenesis of PCa and apply this knowledge for the prevention and treatment.

Inflammation is the hallmark of cancer.⁴ Role of inflammation in few cancers has long been established and its role in other cancers is still debated. Increased

N:L ratio is proven to be associated with increased chances of recurrence in CIN,⁵ can be used as a prognostic marker in assessing the DFS and OS in breast cancer,⁶ is associated with poor prognosis in lung cancer,⁷ ovarian cancer⁸ and pancreatic cancer.⁹ Elevated N:L ratio is associated with prevalence of non neoplastic chronic conditions like diabetes mellitus, hypertension, asthma, arthritis and cardiovascular diseases.¹⁰

A variety of novel specific biochemical and hematological markers are available as measures of systemic inflammation like C-reactive protein, albumin, cytokines, platelet counts, platelet lymphocyte ratio, glasgow prognostic index and estimation of tissue inflammatory cell infiltrates, however these can be expensive and time consuming. N:L ratio is a simple, cost effective, reliable and reproducible alterantive marker which can be easily calculated. Tumor associated inflammation is evident in the peripheral blood in the form of neutrophilia and/or lymphopenia⁷. Pre-treatment hematological workup is done as a routine protocol in all cancer patients, the results of which can be extrapolated to estimate N:L ratio. N:L ratio is calculated by dividing the absolute neutrophil count by the absolute lymphocyte count.¹¹ Normal value of N:L ratio is 2.8 with a range of 1.2 to 4.4¹² which is different from the value in another study of 1.65 with a range of 0.78 to 3.53.¹³ After going through the literature, we found a disparity in the absolute value of N:L ratio, however the reference ranges were similar.

Inflammatory cells are known to play decisive roles in different stages of tumor development. Neutrophilia is associated with poor prognosis in cancers because of their tumor promoting actions like, they help in tumorigenesis, suppress antitumor effect of cytotoxic T cells,¹⁴ promote secretion of vascular endothelial growth factor (VEGF) and angiogenesis,¹⁵ escape of tumor cells into circulation and production of interleukins which help in metastasis.¹⁶ On the contrary, lymphocytes have a protective effect in cancers.¹⁴ This neutrophil lymphocyte interaction results in a fertile microenvironment for tumorigenesis. N:L ratio represents a balance between tumorogenic inflammation and the anti tumor immune mechanisms.⁶ Thus, the significance of N:L ratio estimation in cancer patients.

Clinicopathological significance of N:L ratio was studied in few tumors- for example, association between the N:L ratio and tumor invasion, lymph node metastasis in oesophageal cancer,¹⁷ response to chemotherapy in ovarian cancer (chemoresistant patients had high N:L ratio)⁸ thus expanding the clinical implications of N:L ratio.

Importance of assessing the prognostic significance of N:L ratio in solid tumors like PCa lies in the fact that, the cases can be stratified accordingly into different risk groups, to individualize treatment accordingly and to assess the response to therapy.

Few individual studies and meta-analysis have shown a positive association between elevated N:L ratio and poor prognosis, poor OS and disease free survival in PCa patients,^{18,19} have shown to influence outcome in patients treated with chemotherapy^{19,20} and in patients with metastatic PCa.⁷ On the contrary few other studies showed a lack of association,²¹⁻²³ however it was noted that these study population was not based in India. Hence, the present study was undertaken to find out the significance of N:L ratio in PCa in our population and to compare the values between BPH and PCa. This study, to the best of our knowledge is the first of its kind in Indian setup.

Materials and Methods

A total of 100 cases were included in the study, 50 of Benign prostatic hyperplasia (BPH) and 50 of PCa. These were diagnosed on biopsy specimens from TURP sent from the urology department attached to Sri Devaraj Urs Medical College. The clinical data of age, TNM stage, serum PSA levels were obtained from the clinical case file database and the pre-treatment hematological data (hemoglobin, ESR, total leucocyte count, platelet count, absolute neutrophil count, absolute lymphocyte count) and histopathological data were collected from pathology file database. Cases were divided into benign and malignant groups histologically.

1. Lack of complete hematological and histopathological data; 2. Known cases of systemic diseases associated with inflammation where the hematological data is expected to alter were the criteria followed for exclusion.

N:L ratio was calculated by dividing the absolute neutrophil count by absolute eosinophil count. After an extensive literature search for the normal range of N:L ratio, we finalised the cut-off value of N:L ratio at 2.5 for assessing its prognostic significance in PCa. Accordingly, values were stratified into two groups. Group 1- low L:N ratio if ≤ 2.5 and group 2- high L:N ratio if > 2.5 .

The study was approved by the institute ethics committee.

Statistical Analysis: SPSS software (version 20.0) was used to analyse the data by suitable tests. Variables were expressed as mean \pm standard deviation (SD) or as numbers and percentages depending on the type of variable in use. Students independent t-test was used for analysing quantitative data and chi-square test for qualitative data. Analysis between sub groups was done by Mann-whitney U test. P value of < 0.05 was considered to be of statistical significance.

Results

Table 1: Clinicopathological characters in BPH and PCa cases

Characters	BPH	PCa	P value
Age	63(36-82)	68(46-95)	0.022
PSA			<0.001
<20ng/ml	50(86.2%)	8(13.8%)	
>20ng/ml	0(0%)	42(100%)	
Hemoglobin (gm/dl)			0.064
<12	12(37.5%)	20(62.5%)	
12-16	34(53.1%)	30(46.9%)	
>16	4(100%)	0(0%)	
Platelet count (lacs/cumm)			0.824
<1.5	2(40%)	3(60%)	
1.5-4.5	45(51.1%)	43(48.9%)	
>4.5	3(42.9%)	4(57.1%)	

ESR			<0.001
Normal	39(84.8%)	7(15.2%)	
High	11(20.4%)	43(79.6%)	
TLC (cells/cumm)			1.000
<4000	0(0%)	0(0%)	
4,000-11,000	41(50%)	41(50%)	
>11,000	9(50%)	9(50%)	
N:L ratio			0.008
</=2.5	27(65.9%)	14(34.1%)	
>2.5	23(39%)	36(61%)	

Data expressed in numbers and percentages and analysed by chi-square test

The results were analysed. Out of the 100 cases, 50 cases belonged to the benign group and 50 to the malignant group. The mean age in BPH cases was 63 against the mean age of 68 in PCa cases. Malignant disease was more common in a slightly older age and this difference in age was statistically significant with a P value of 0.022. The serum PSA values and ESR values were high in PCa cases as compared with BPH cases and this observations were statistically significant with P values of <0.001 each.

However, the platelet count, hemoglobin percentage and total leucocyte count values did not statistically differ between the two groups (p=0.824, p=0.064, p=1.000).

The N:L ratio was significantly high in PCa cases as compared to the BPH cases with a statistically significant p value of 0.008. Further subgroup analysis was done to find association between N:L ratio and tumor parameters using Mann-Whitney U test.

Table 1 shows a summary of the analysis of significance of different clinicopathological variables in BPH and PCa cases (significant P value written in bold).

Table 2: Correlation between pathological characters and N:L ratio in PCa cases

Characters	Low N:L ratio (</=2.5)	High N:L ratio (>2.5)	P value
PSA			0.838
<20ng/ml	2(14.3%)	6(16.7%)	
>20ng/ml	12(85.7%)	30(83.3%)	
Gleason score			0.161
</=6	4(28.6%)	4(11.1%)	
7	4(28.6%)	10(27.8%)	
8-10	6(42.9%)	22(61.1%)	
Stage			
Tumor			0.052
</=T2b	6(42.9%)	6(16.7%)	
>/=T2c	8(57.1%)	30(83.3%)	
Node			0.225
N0	10(71.4%)	31(86.1%)	
N1	4(28.6%)	5(13.9%)	
Metastasis			0.529
M0	14(100%)	35(97.2%)	
M1	0(0%)	1(2.8%)	

Data was further analyzed to establish, if any association between the various tumor parameters and the low and high N:L ratio groups. Serum PSA, gleason score, tumor stage, node positivity and distant metastasis were stratified against the low N:L ratio group and the high N:L ratio group as shown in table 2. We did not find a statistically significant association between the groups for PSA (p=0.837), gleason score (p=0.161), tumor stage (p=0.052), node positivity (p=0.225) and distant metastasis (p=0.529) thus ruling out any prognostic impact of N:L ratio in PCa cases. (Table 2)

Discussion

Prostate cancer (PCa) is the second common cancer occurring in Indian men with a constant increase in the number of cases. PSA, TNM stage and gleason score are known prognostic markers of PCa.^{1,2}

Role of inflammation in various solid tumors has long been studied. Inflammation is characterised by local response in the form of tissue infiltration by inflammatory cells and systemic response in the form of release of systemic inflammatory mediators into the blood and alteration in the blood cell counts. These mediators can be used as measure of the inflammatory activity, for example- CRP, albumin, ESR. The

mediator assays are time consuming and expensive. Hence, the search for a simple, inexpensive alternative.

Alteration in the blood cell counts, especially absolute neutrophil and lymphocyte counts have been studied elaboratively as an alternative to mediator assays for quantifying the inflammation and thus indirectly quantifying the risk of cancer in various organ systems.

Neutrophils play a pivotal role in tumorigenesis by promoting the key steps of new blood vessel formation, escape of tumor cells into circulation and spread, whereas lymphocytes have an antitumor effect. Hence, the importance of N:L ratio in tumors.^{14,15}

In the Indian setup, where the socio-demographic, ethnic and racial characters are different from other setups where similar studies were undertaken, there is a paucity of studies which evaluate the significance of different hematological parameters in BPH and PCa cases with special reference to N:L ratio in patients with PCa. Hence the present study was undertaken, the first one of its kind in Indian setup.

In the present study, we found a significant difference in the N:L ratio values between BPH cases and PCa cases suggesting a strong role of inflammation in PCa. However on subgroup analysis there was no association between the PSA values, gleason score, TNM stage and N:L ratio. Hence, N:L ratio cannot be used as a prognostic factor in PCa cases.

Our findings concurred with findings of other studies in prostate^{9,24} and in other organs like thyroid.²⁵ However a majority of individual studies and meta-analysis reported N:L ratio as a poor prognostic factor in PCa. The possible explanations for this diverse results could be- 1. Lack of a uniform reference value for N:L ratio - different studies used different N:L ratio values as cut-off; 2. N:L ratio is highly variable, dependent on age, demographic characters; 3. Influence of socio-demographic and racial backgrounds- studies were conducted in different parts of the world; 4. Biological variations in tumor behaviour and tumor responses; 5. Hematological parameters were analysed by different cell counters.

We emphasize on the need for large data analysis to establish reference values for hematological parameters like N:L ratio and Platelet to lymphocyte ratio which are done on a daily basis and are cost effective tools. This helps in achieving comparable, reliable and reproducible results. There is also a valid need for large cohort studies and meta-analysis to establish the role of N:L ratio in PCa which can add to our knowledge of tumor biogenesis.

Studies have found an association between high N:L ratio and resistance to chemotherapy treatment in patients with PCa, expanding the importance of estimating N:L ratio further beyond prognostication into treatment,²⁰ however in the present study this could not be assessed due to lack of followups in patients due

to drop outs. Recurrence rates of PCa were also high in cases with high N:L ratio in few studies.²²

Limitations of the present study were 1. The study is a retrospective, observational study, the long term prognostic outcome of these patients was not analysed; 2. Inability to adjust for certain clinical confounders like presence of systemic inflammatory conditions that can influence the N:L ratio; 3. Small case number. Given the outcome of the present study, further research by longitudinal and multi-centre studies are needed to support/refute the results of present study.

Conclusion

We have concluded from our study that N:L ratio has no significance as a prognostic marker in PCa. However a significant difference in N:L ratio was seen between BPH and PCa cases. We urge for further studies in this regard to investigate the role of this simple and cost effective hematological parameter as prognostic marker in cancer.

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