Original Article

An immunohistochemical evaluation of tumor-associated macrophages (M1 and M2) in carcinoma prostate – An institutional study

ABSTRACT

Background: Tumor-associated macrophages (TAM) are the main component of inflammation along with leukocytes, endothelial cells and fibroblasts together form a tumor microenvironment, with immune cells representing its vital component. Many studies suggested that TAMs cumulating in tumors correlate with a poor prognosis. In prostate cancer, TAMs can increase cancer cell invasion by stimulating tumor angiogenesis, degrading the extracellular matrix, and also suppresses the antitumor functions of cytotoxic T cells resulting in poor prognosis.

Aims and Objectives: 1. To determine the expression of M1 (CD68) and M2 (CD163) in prostate carcinoma (Pca). 2. To find the association between M1, M2 macrophage with Gleason's score and stage of Pca.

Materials and Methods: This is a retrospective observational study. All transurethral resection prostatic (TURP) chips positive for Pca and the clinical details were collected. Radiologic findings with respect to stage of disease, size of lesion, were noted.

Results: Among the 62 cases studied, majority of the cases were in-between the age of 61–70 years. Highest cases were seen in Gleason's score 8, 9, and 10 (62%), prostatic specific antigen (PSA) levels 20–80 ng/mL (64%), tumor size 3–6 cm (51.6%), T3 stage (40.3%), N1 lymph node stage (70.9%). M1 stage of (31%). CD68 and CD163 expression was analyzed with Gleason's score, TNM stage and PSA levels. CD68 score 3 correlated with low distant and nodal metastasis 6.2% and 6.8%, respectively. CD163 score 3 correlated with high metastasis to lymph nodes and distant metastasis of 86.3% and 25%, respectively. On further analysis, statistically convincing association between the CD163 expression and Gleason's score, PSA levels, nodal and distant metastasis was found.

Conclusion: CD68 expression was correlated with good prognosis with less nodal and distant metastasis and Cd163 expression has poor outcome with increased chances of nodal and distant metastasis. Further exploration of TAM mechanisms and immune checkpoints in the prostate tumor microenvironment can furnish new light and motives for the treatment of Pca.

KEY WORDS: CD68, CD163, Gleason's score, prostate carcinoma, prostatic specific antigen, tumour-associated macrophages

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INTRODUCTION

Prostate cancer is a disease of the elderly men above 65 years of age. Studies showed that Pca is the second diagnosed carcinoma in older men.[1] Prostate cancer has been projected to have the largest corresponding increase in men in the upcoming years.[1] Studies show restricted data available on Pca and showed very convincing differences in, precipitating factors, incidence, and disease characteristics of Pca.[1] In India, restricted data available on true incidence of prostatic carcinoma as it is not a notifiable disease and very few populationbased cancer registries (PBCRs) in India.[1] Studies done on taking PBCRs, from 2009 to 2011, in different metro cities shows Annual Percentage Change of Karnataka 3.4%.[1] Study carried out in Kolar on prostate cancer cases showed 2.58% out of all other cancers[2,3]

Recurrent prostatic inflammation has been correlated with a high risk of cancer.

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Tumor-associated macrophages (TAM) are the main component of inflammation along with leukocytes, endothelial cells and fibroblasts together form a tumor microenvironment, with immune cells representing its vital component. Macrophages are innate immune cells, they have two main phenotypes – M1, M2, which correspond to T-Helper cells.[4]

IFN-gamma, activates M1 macrophages secrete cytokines IL-12 and tumor necrosis factor-alpha, support anti-tumor response. IL-4, IL-10, and IL13 activates M2 macrophages secrete anti-inflammatory cytokines – TGF-beta and IL-10 and angiopoietin and VEGF, promote tumor growth.[4] the pan macrophage marker is CD68 and plays the role of proinflammatory and antitumor response. CD163 is a scavenger receptor up-regulated by macrophages in an antiinflammatory environment and regarded as a highly specific monocyte/macrophage marker for M2 macrophages.[5] Studies have established that TAMs are linked with poor prognosis of human carcinoma such as hepatocellular cancer, gastric cancer, lung cancer, and breast cancer.[4-7]

Studies proposed that TAMs cumulation in tumors correlates with a poor prognosis. In prostate cancer, TAMs can intensify cancer cell invasion by stimulating tumor angiogenesis, degrading the extracellular matrix, and also suppresses the antitumor functions of cytotoxic T cells resulting in poor prognosis.[8-11]

Therefore, TAMs are an alluring target for therapeutic intervention by targeting their various functions. Hence the study is undertaken. In the H and E section, it is difficult to differentiate M1 and M2 phenotypes. Hence, immunostaining is used to identify M1 and M2 subpopulations of macrophages. CD68 is a marker for M1 macrophage and CD163 is a marker for M2 macrophage. Only few studies deciding expression of CD68 and CD163 have been done on prostate cancers and published in Indian literature so far. Hence the study is undertaken to decide the expression of CD68 and CD163 in Pca.

Aims and Objectives

- To determine the expression of M1 (CD68) and M2 (CD163) in prostate cancer.
- To find association between M1, M2 macrophage with Gleason's score and stage of the disease in Pca.

MATERIALS AND METHODS

This is a retrospective observational study.

Where we have taken transurethral resection prostatic (TURP) chips positive for Pca, received in Tertiary care Hospital from December 2019 to October 2021 and also the paraffin blocks of prostate cancer retrieved from Archives of Department of Pathology from the year January 2015 to November 2019 were included in the study.

Methodology

All TURP chips positive for carcinoma prostate confirmed by histopathological examination was added in the study. The clinical details (age, stage of the disease) were collected from the Medical Record Department. H and E slides were screened for histopathological types and Gleason's score of the tumor. Radiologic findings (USG, MRI, or CT findings) with respect to stage of disease and size of lesion were noted.

TUMOUR SIZE: Latest studies showed that tumor size along with other parameters such as prostatic specific antigen (PSA) and Gleason's score contribute in tumor progression and patient prognosis[12]

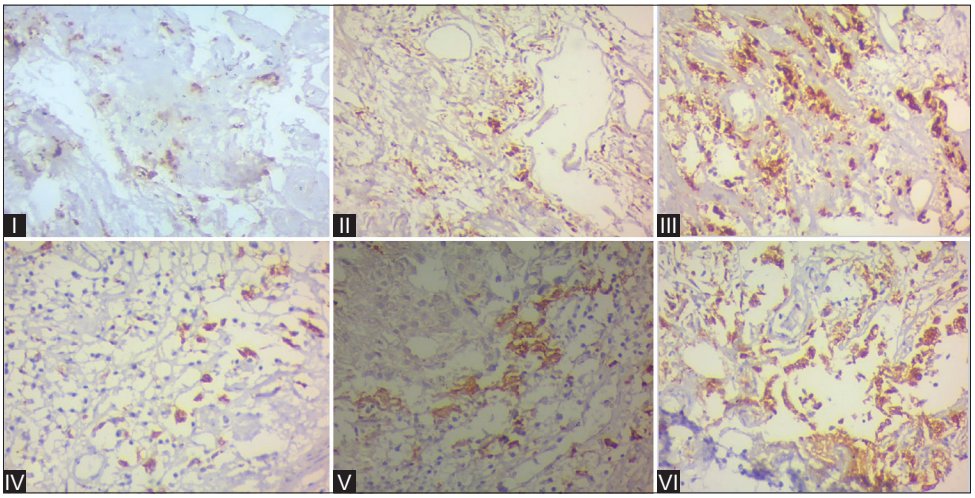

Figure 1: Showing IHC staining in 400x with CD163 expression Score 1 (0-30 macrophages), Figure II: Showing IHC staining in 400x with CD163 expression Score 2(31-60) macrophages), Figure III: Showing IHC staining in 400x with CD163 expression -Score 3(>60 macrophages)Figure IV: Showing IHC staining in 400x with CD68 expression -Score 1 (0-30 macrophages) Figure V: Showing IHC staining in 400x with CD68 expression Score 2 (31-60) macrophages) Figure VI: Showing IHC staining in 400x with CD68 expression Score 3(>60 macrophages)

In this study, we have divided the tumor size into three groups by MRI results taking the largest dimensions on MRI.[13]

Size
1-3 cm
>3- 6 cm
>6 cm

Immunohistochemistry scoring

The CD68 and CD163 immuno-stained smears were screened under low magnification (10X) and were looked for areas with maximum pronouncement of CD68 and CD163 by two observers and were called as "Hot spots." These hotspots were then viewed under higher magnification (40X), and CD68 and CD163 positive cells were counted and scoring was done on number of macrophages expressed by IHC as shown in Figure 1.[14]

CD68 and CD163 scoring[13]

- 0–30 macrophages – score 1
- 31–60 macrophages – score 2
- >60 macrophages – score 3.

All the scoring was done by two pathologists independently and both were oblivious of clinical data. All the decisions were taken by both the pathologists based on the consensus. In case of any discrepancies the case was referred to a third pathologist for a final decision which was acceptable to both.

Statistical analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) 22 version software from Microsoft excel data sheet. Categorical data was represented in the form of proportions and frequencies. Qualitative data for the test of significance was done by utilizing Chi-square test or Fischer's exact test (for 2 × 2 tables only).

Continuous data were represented as standard deviation and mean. To identify the mean difference between the two quantitative variables, an independent *t* test was used as a test of significance. Graphical representation of data, MS Excel and MS word was used to obtain various types of graphs. *P* value (probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests. Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

RESULTS

Nearly 62 cases (19 cases having distant metastasis and 43 cases were without any metastasis) confirmed with prostatic carcinoma were included in the study. To assess metastasis, radiological assessment using MRI, CT, or PET scans and ultrasound were considered. In the present study, 79% (49 cases) of patients came with chief complaints of urinary retention, 12.9% (8 cases) with urinary hesitation, and 8% (5 cases) of patients with burning micturition.

As shown in Table 1, in the present study more number of patients were in the age group of 61–70 years with median age group of 70 years.

Gleason's score and CD68 expression: As shown in Table 2, *P* value >0.005, showed statistically significant difference between Gleason's score and CD68. Here we can see that as the Gleason's score increases there is an increase in the expression of CD68.

PSA levels and CD68 expression: As shown in Table 2, *P* value >0.005, showed no statistically significant difference between CD68 expression and PSA levels. More number of cases were between the PSA levels of 20–80 ng/mL with more number of cases seen with score 2 and score 3.

Table 1: Distribution of subjects according to age group

Age group	Frequency	Percent (%)
41-50 years	2	3.2
51-60 years	5	8.1
61-70 years	28	45.2
71-80 years	22	35.5
81-90 years	5	8.1
Total	62	100.0

Table 2: Association between clinico-pathological parameters and CD68 expression

Parameters	Score 1 (0-30)	Score 2 (31-60)	Score 3 (>60)	Total	*P*
Gleason's score					
6	1 (11.1%)	6 (66.7%)	2 (22.2%)	9	
7	1 (6.7%)	4 (26.7%)	10 (66.7%)	15	0.130
8	1 (16.7%)	1 (16.7%)	4 (66.7%)	6	
9	1 (4.8%)	8 (38.1%)	12 (57.1%)	21	
10	0	3 (27.3%)	8 (72.7%)	11	
PSA levels (ng/mL)					
4-19	1 (10%)	5 (50%)	4 (40%)	10	
20-39	2 (15.4%)	4 (30.8%)	7 (53.8%)	13	
40-59	1 (7.7%)	2 (15.4%)	10 (76.9%)	13	0.319
60-79	0	5 (35.7%)	9 (64.3%)	14	
80-99	0	5 (71.4%)	2 (28.6%)	7	
>100	0	1 (20%)	4 (80%)	5	
Tumor size					
1-3 cm	0	3 (50%)	3 (50%)	6	
3-6 cm	3 (9.4%)	11 (34.4%)	18 (56.3%)	32	0.814
>6 cm	1 (4.2%)	8 (33.3%)	15 (62.5%)	24	
T stage					
T2	3 (16.7%)	7 (38.9%)	8 (44.4%)	18	
T3	1 (4%)	10 (40%)	14 (56%)	15	0.170
T4	0	5 (26.3%)	14 (73.7%)	19	
N stage					
N0	02 (11.1%)	02 (11.1%)	14 (77.7%)	18	
N1	35 (79.5%)	06 (13.6%)	03 (4.8%)	44	0.137
M stage					
M0	3 (6.9%)	5 (11.6%)	35 (81.3%)	43	
M1a	12 (75%)	2 (12.5%)	02 (12.5%)	16	0.102
M1b	2 (100%)	0	0	2	
M1c	1 (100%)	0	0	1	

Tumor size and CD68 expression: As shown in Table 2, *P* value of 0.814, showed statistically significant difference between tumor size and CD68 expression. In this table maximum number of cases were of tumor size 3–6 with score 2, in which 34.4% (11 cases) and 56.3% (18 cases) with score 3. Tumor size of >6 cm score 2 in which 33.3% (8 cases) and 62.5% (15) with score 3.

T Stage and CD68 expression: As shown in Table 2, *P* value of 0.170, showed statistically significant difference between clinical staging and CD68e expression. Maximum number of CD68 were expressed in T3 and T4 with score 2 being 40% (10 cases) and 26.3% (5 cases), respectively, and score 3 being 56% (14 cases) and 73.7% (14 cases), respectively.

N Stage and CD68 expression: As shown in Table 2, in this study there was no significant association between N Stage and CD68 expression with *P* value of 0.137. Lymph node metastasis was seen in a total of 44 cases of which 79.5% (35) cases having score 1, 13.6% (6) having score 2, 6.8% (3) with score 3. The present study concludes that as CD68 expression score increases there was no lymph node metastasis and patient has better prognosis.

M Stage and CD68 expression: As shown in Table 2, in this study there was no significant association between M stage and CD68 expression with *P* value of 0.102. In the present study, 44 cases show no distant metastasis with 81.3% (35) cases having score 3 in M0. Distant metastasis was seen in 18 cases in which 75% (12) cases seen in M1a stage with score 1. This study concludes that as the metastasis increases the expression of CD68 decreases and CD68 macrophages have no role in metastasis and provides protection against cancer spread.

Gleason's score and CD163 expression: As shown in Table 3, *P* value of 0.001 showed statistically significant difference between CD163 expression and Gleason's score. Here, we can see that the Gleason's score is directly proportional to the expression of CD163. Studies show that if the expression of CD163 is increased than the prognosis is bad with lymph node and distant metastasis.

PSA levels and CD163 expression: As shown in Table 3, *P* value of 0.001 showed statistically significant difference between CD163 and PSA levels. Maximum number of cases were between the PSA levels of 20–80 ng/mL with more number of cases seen with score 2 and score 3.

Tumor size and CD163 expression: As shown in Table 3, *P* value of 0.001 showed statistically significant difference found between tumor size and CD163. Maximum number of cases were of tumor size 3–6, 28.1% (9) cases with score 1, 21.9% (7) cases with score 2, and 50% (16) cases with score 3, followed by tumor size >6 cm having 91.7% (22) cases with score 3. This study concludes that as the tumor size increases the CD163 expression increases and has poor prognosis.

Table 3: Association between clinico-pathological parameters and CD163 expression

Parameters	Score 1 (0-30)	Score 2 (31-60)	Score 3 (>60)	Total	*P*
Gleason's score					
6	7 (77.8%)	1 (11.1%)	1 (11.1%)	8	
7	4 (26.7%)	7 (46.7%)	4 (26.7%)	15	0.001
8	0	0	6 (100%)	6	
9	1 (4.8%)	4 (19%)	16 (76.2%)	21	
10	0	0	11 (100%)	11	
PSA levels (ng/mL)					
4-19	6 (60%)	4 (40%)	0	10	
20-39	2 (15.4%)	3 (23.1%)	8 (61.5%)	13	
40–59	4 (30.8%)	3 (23.1%)	6 (46.2%)	13	0.001
60-79	0	1 (7.1%)	13 (92.9%)	14	
80–99	0	1 (14.3%)	6 (85.7%)	7	
>100	0	0	5 (100%)	5	
Tumor Size					
1-3 cm	3 (50%)	3 (50%)	0	6	
3-6 cm	9 (28.1%)	7 (21.9%)	16 (50%)	32	0.001
>6 cm	0	2 (8.3%)	22 (91.7%)	24	
T stage					
T2	8 (44.4%)	6 (33.3%)	4 (22.2%)	18	
T3	3 (12%)	4 (16%)	18 (72%)	15	0.001
T4	1 (5.3%)	2 (10.5%)	16 (84.2%)	19	
N stage					
N0	15 (83.3%)	01 (5.5%)	02 (11.1%)	18	
N1	02 (4.5%)	04 (9%)	38 (86.3%)	44	0.004
M Stage					
M0	32 (74.4%)	8 (18.6%)	3 (6.9%)	43	
M1a	1 (6.25%)	03 (18.7%)	12 (75%)	16	0.012
M1b	0	0	2 (100%)	2	
M1c	0	0	1 (100%)	1	

T Stage and CD163 expression: As shown in Table 3, *P* value 0.001 showed statistically significant difference between tumor staging and CD163. In the present study, majority of the cases seen in T3 stage with 72% (18 cases) expressing Cd163 score 3 and 84.2% (16) cases seen in T4 stage with score 3 concluding that tumor stage is directly proportional to the CD163 expression, showing the association of CD163 expression cases having poor prognosis.

N Stage and CD163 expression: As shown in Table 3, in this study, significant association between N Staging and CD163 expression was found with *P* value 0.004*. Lymph node metastasis was seen in a total of 44 cases of which 86.3% (38) cases having score 3, 9% (4) having score 2, and 4.5% (2) cases with score 1. Hence, this study concludes that as CD163 expression score increases there was more number of cases with lymph node metastasis and patient has poor prognosis

M Stage and CD163 expression: As shown in Table 3, In the present study 44 cases had no distant metastasis with 74.4% (32) cases having score 1, 18.6% (8) cases having score 2, and 6.9% (3) cases having score 3 in M0 stage. Distant metastasis was seen in 18 cases of which 75% (12) cases was seen in M1a stage with score 3. So, this study concludes that as the metastasis increases the expression of CD163 increases and CD163 macrophages have a role in metastasis and increases cancer spread with poor prognosis.

DISCUSSION

Many studies show that M2 macrophages and regulatory T-cells infiltration in prostatic carcinoma patients contribute tumor progression. So the possibility of these inflammatory cells creating an immunosuppressive environment is taken into consideration.[15-17] Few studies show that targeted blockade of interleukin-6 receptor (IL-6R) and high mobility protein-1 (HMGB-1) resulted in improvement of enzalutamide therapeutic effect in carcinoma prostate.[18] Therefore, TAMs are an appealing target for therapeutic intervention. The specific macrophage subtypes present in a prostatic adenocarcinoma have prognostic value, suggesting that the relative proportions of these populations are related to patient outcome. Understanding the relative contributions of these subtypes will not only inform patient prognostication, but will also help in immunotherapeutic strategies.[18-20]

As shown in Table 4, in this study the median age group of subjects were 70 years. which was similar to the study done by Lanciotti M *et al*.[14] and Erlandsson A *et al*.[13]

Clinical symptoms: In this study most frequent clinical symptom was urinary retention with 79.03% (49) cases, which is in contrast with the study done by Kitagawa *et al*.[21] which showed 22.2% of patients with urinary retention and Hamilton *et al*.[22] showed 33.3% with urinary retention and 47% of patients came with increased urinary frequency.

Studies have concluded that CD68 expression has better prognosis with less extra capsular invasion, metastasis to lymph nodes and distant metastasis. As shown in Tables 5 and 6, in the present study, we analyzed CD68 expression score 3 association with Gleason's score, TNM staging. We saw that as the Gleason's score increased the CD68 expression in the tumor cells increased, when this was compared to TNM stage number of cases was 14 (22.5%) in T3 and T4 but metastasis to lymph nodes and distant metastasis was seen in 3 (6.8%) and 01 (6.2%) cases, respectively.

So we concluded that as the CD68 expression increases there was reduced metastasis to lymph nodes and distant metastasis providing the protection against cancer spread, which was similar to the study done by Lanciotti *et al*.[14] Where they have come to the inference that M1 or CD68 expression was having better prognosis without extra capsular invasion. In this study, 31 (91%) cases were of Gleason's scores 6 and 7. In contrast with the present study, this can be because of early screening facilities in the western countries.

Many studies have concluded that CD163 expression has poor prognosis with extra capsular invasion, metastasis to lymph nodes and distant metastasis. As shown in Table 7, in this study we analyzed CD163 expression score 3 association with Gleason's score, TNM staging. We saw that as the Gleason's score increased the CD163 expression in the tumor cells increased, when this was compared to TNM stage number of cases was 18 (29%) in T3 and 16 (25.8%) in T4, but metastasis to lymph nodes and distant metastasis was seen in 38 (86.3%) and 15 (25%) respectively. So we concluded that as the CD163 expression increases there are more metastasis to lymph nodes and distant metastasis.

CD163 acts as pro inflammatory cells and promotes cancer spread and if CD163 expression in the tumor cells are more than the patient have poor prognosis having lymph node metastasis, bone metastasis, and distant metastasis.[23,24] This is similar to the study done by Lanciotti *et al*.,[14] which showed that pronouncement of CD163 is correlated with extra capsular invasion with metastasis to lymph nodes and have more tendency of distant metastasis.

CD163 TAM with various mechanism in the tumor microenvironment promotes the proliferation, growth, and

Table 4: Age distribution among other studies

Age	Lanciotti *et al*.[14]	Erlandsson *et al*.[13]	Present study
Median age group	67 years	74. 8 years	70 years

Table 5: Comparison of Gleason's score with other studies

Gleason's score	Lanciotti *et al*.[14]	Erlandsson *et al*.[13]	Present study
6	30 (32.2)	45 (20.0)	9 (14.5%)
7	40 (43)	44 (19.6)	15 (24.2%)
8-10	23 (24.8)	100 (44.4)	38 (62%)

Table 6: Comparison of Gleason's score and TNM stage with CD68 macrophage prevalence score 3 >60 cells with other studies

	CD68 expression (present study) >60 (score 3)	Lanciotti *et al*.[14] M1 prevalence
Gleason's score		
6	12 (19.3)	19 (55.9)
7		12 (35.3)
8-10	24 (38.7%)	3 (8.8)
TNM staging		
T2	8 (12.9%)	19 (55.9)
T3	14 (22.5%)	7 (20.6)
T4	14 (22.5%)	8 (23.5)
N1	03 (6.8%)	2 (5.9%)
M1	01 (6.2%)	2 (5.9%)

Table 7: Comparison of Gleason's score and TNM stage with CD163 macrophage prevalence score 3 > 60 cells with other studies

	CD163 expression (present study) >60 (score 3)	Lanciotti *et al*.[22] M1 prevalence
Gleason's score		
6	5 (8.06)	31 (52.6)
7		16 (27.1)
8-10	33 (53.2)	12 (20.3)
Tumor staging		
T2	4 (6.4%)	14 (23.8)
T3	18 (29.0%)	43 (47.4)
T4	16 (25.8%)	2 (3.4)
N1	38 (86.3%)	3 (5.1)

metastasis of Pca and also involves in the management of neuroendocrine differentiation and androgen deprivation therapy resistance in prostate cancer.[23] TAM mechanisms in the modulation of tumor proliferation and progression of Pca are very complex and many theories have been proposed on this. So, further exploration of TAM mechanisms and immune checkpoints in the prostate tumor microenvironment can provide new light and idea for the treatment of Pca.[23,24]

CONCLUSION

This study concludes that both M1 and M2 macrophages expressed in prostatic adenocarcinoma were associated with high Gleason's score. CD163 expression was expressed more in cases who have metastasis to lymph nodes and cases with distant metastasis. On further exploration, this study showed that there was a convincing statistical significance with the different parameters which were assessed. As the tumor microenvironment is considered a double edge sword and molecular changes at different stages of carcinoma cannot be simply assessed, more studies are required to establish the results. To the best of our knowledge, the literature did not reveal any Indian study on TAM on prostatic carcinoma. This study is a maiden attempt to understand the pathogenesis of TAM on Pca along with standardization of the scoring system of CD68 and CD163 in order to assess the effect of targeted immunotherapy on long-term prognosis of the patients.

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Conflicts of interest
There are no conflicts of interest.

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