Clinicopathological Evaluation of CD44 Expression as a Proliferative Marker in Prostatic Adenocarcinoma

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ABSTRACT:

BACKGROUND:

Prostatic cancer is the 6th commonest cancer worldwide, and the 2nd commonest cancer among men. In Iraq, it is the fourth most common cancer and cause of cancer deaths in males.

The commonest form of prostatic cancer is adenocarcinoma (75% of patients ≥ 65 years). Prevalence of prostate cancer rises with age and hormonal causes. Core needle biopsy is a standard technique and largely preferred for non-operative diagnosis since it permits architectural and cytological assessment.

OBJECTIVE:

Immunohistochemical evaluation of CD44 expression in prostatic adenocarcinoma as a proliferative marker and correlate it with clinicopathological parameters (age, PSA level, and tumor grade).

MATERIALS AND METHODS:

Prostatic specimens were obtained via different procedures (TURP, and tru-cut biopsy). Formalin fixed paraffin embedded blocks from 50 patients with prostatic adenocarcinoma, histological sections taken for hematoxylin and eosin staining to determine histopathological features, monoclonal antibody for CD44 used for immunohistochemical staining of tissue sections and CD44 expression correlated with clinicopathological parameters (age, PSA level, and tumor grade).

RESULTS:

There was a significant inverse correlation between CD44 expression and Gleason grade score (i.e. high expression in low grade tumors), while there was no correlation between age and PSA level with CD44 expression.

CONCLUSION:

CD44 expression in prostatic adenocarcinoma can be utilized as a prognostic marker as it implies less tumor aggressiveness with positive high expression which means lower grade tumor and better differentiation.

KEYWORDS: CD44, Proliferative, Marker, Prostatic, Adenocarcinoma.

INTRODUCTION:

Epidemiology

Prostatic adenocarcinoma is the 2nd commonest cancer worldwide[1]. It is the fourth commonest cancer and cause of cancer deaths in males In Iraq[2]. Its prevalence rises considerably with age; greater than 75% of patients are older than 65 years. Genetic and environmental factors play significant roles. Familial involvement is well-documented where men with multiple affected 1st-degree relatives have 5-10 times higher risk[3]. Gene amplifications are indicators of genetic instability and may be involved in the pathogenesis and prognosis of many malignancies including prostatic adenocarcinoma[4].

Majority of prostate cancers are adenocarcinoma (95%). Transitional cell morphology was shown in About 4%. less than 1% of cases are squamous cell carcinoma[5]. rare cases show neuroendocrine morphology. Prostatic adenocarcinoma is diagnosed at a median age of 67 years but are very rarely seen in young patients. After the diagnosis, ten years survival was 34% and 87% for high-grade and low-grade prostatic adenocarcinoma respectively[5].

Most cases of prostatic adenocarcinoma are asymptomatic, diagnosed by PSA screening, however, normal PSA levels were found in about 15% of patients[6].

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The diagnosis is usually made with light microscopic features, however, immunohistochemical studies may be useful in a few cases. The grade is one of the best prognosticators of biologic behavior in prostatic adenocarcinoma. The Gleason score is recommended routinely for grading[7].

Immunohistochemistry

Immunohistochemistry is crucial to define the histogenetic source of cancers needed for tumor classification whereby individual cellular antigens are detected on tissue sections made from frozen tissue or formalin-fixed paraffin-embedded tissue blocks or even from cytology specimens[8]. It is one of the most useful techniques for detecting minimal residual tumor cells in various places like surgical margins, lymph nodes, and bone marrow, which is essential for cancer staging and the therapeutic planning.

Immunohistochemistry can also be used to evaluate the sensitivity of various cancers to different therapeutic modalities. Furthermore, immunohistochemistry presents a variety of important prognostic and histopathological markers useful for research and follow up of tumors[8].

CD44

CD44 is a cell surface adhesion receptor largely expressed in various tumors and regulates metastasis through CD44 recruitment to cell surface. It stimulates migration and invasion processes implicated in metastases by communicating with proper extracellular matrix ligands [9]. Originally, it was recognized as a receptor for hyaluronan or hyaluronic acid and then to numerous other ligands (osteopenia, collagens, and matrix metalloproteinase). It was also defined as a stem cells marker for various cell types[9].

AIM OF THE STUDY:

Immunohistochemical evaluation of CD44 expression in prostatic adenocarcinoma as a proliferative marker and correlate it with clinicopathological parameters (age, PSA level, and tumor grade).

MATERIALS AND METHODS:

Sampling of cases

This retrospective study was carried out in Babylon training centre for Pathology in Al-Hilla teaching hospital, during the period from December 201٨ through 201٩ to determine CD44 expression. Blocks from 50 patients with different prostatic adenocarcinoma grades were revised in this study, those blocks were obtained from the histopathology departments of AL-Hilla teaching hospital in Babylon, Martyr Gazi Al-Hariri hospital for surgical specialities in Baghdad medical city, and the private sector in both Babylon and Baghdad. All these patients were previously diagnosed with prostatic adenocarcinoma, for whom prostatic specimens were obtained via different procedures (TURP, and tru-cut biopsy). All the clinicopathological parameters (age, PSA, and tumor grade) were obtained from the available histopathological reports.

All formalin-fixed paraffin embedded tissue sections were retrieved and re-stained with the routine H&E stain for verifying the morphological diagnosis and grading according to WHO classification 2016 and compared with previous pathological reports. Re-evaluation of all the slides was done by a pathologist to confirm the diagnosis and the grade.

Inclusion criteria

1- All ages with prostatic adenocarcinoma diagnosed by TURP and tru-cut biopsy
2- All grades

Exclusion criteria

1- Patients with benign prostatic hypertrophy
2- Patients with other types of prostate cancers

Tumour Marker

CD44: pathnSitu USA
Clone: EP44
Class: IVD
Isotype: Rabbit IgG
Reactivity: Human
Localization: Membranous
Control: Breast cancer, skin

Evaluation of immunostaining

The evaluation of CD44 immunostaining was performed semiquantitavely and expressed as percentage of positive cells by counting at least 1000 cell in selected hot spot area at high magnification (X 400).

Positive staining of CD44 was defined as membranous and/or cytoplasmic brown colour staining pattern of epithelial tumour cells, even if the staining was focal in the tumor cells.

CD44 immunohistochemical results were scored to four grades as follow[10]:

- No positive cells (score 0)
- Less than 25% positive cells (score 1)
- Between 25% - 75% positive cells (score 2)
- More than 75% positive cells (score 3)

Statistical Analysis

Statistical analysis was done by a statistician with SPSS v18.88 (Statistical package for social sciences). Data analysis was done using chi-square test for tables with frequencies, percentages, ranges, and means of standard

deviation. Values were considered statistically significant when p-value is equal to or less than 0.05.

RESULTS:

A retrospective study of 50 patients with prostatic adenocarcinoma to evaluate CD44 as a proliferative marker. Age of the patients studied ranged from 40 – 89 years with a mean age of 64 years (SD=11.9).

Table (1) showed a significant association between CD44 expression and Gleason grade score (P-value < 0.05). Within 18 CD44 positive cases, 10 patients (55.6%) were in Gleason grade 6, 5 patients (27.8%) in Gleason grade 7, and 3 patients (16.6%) in Gleason grades 8 - 10.

Table 1: CD44 expression with Gleason grade score.

Gleason grade score	CD44 expression		Total
	+ve	-ve	
6	10 (55.6%)	2 (6.25%)	12 (24%)
7	5 (27.8%)	10 (31.25%)	15 (30%)
8 – 10	3 (16.6%)	20 (62.5%)	23 (46%)
Total	18 (100%)	32 (100%)	50 (100%)

Pearson Chi-Square= 18.1, P- value = **0.0001**** **(significant).**

In table (2) there is no significant association between CD44 expression and PSA levels (P-value > 0.05). Within 18 positive cases, 4 patients (22.2%) were at PSA level < 4 ng/ml, 10 patients (55.6%) were at PSA level 4 – 10 ng/ml, while 4 patients (22.2%) were at PSA level > 10 ng/ml.

Table 2: Correlation of CD44 with PSA levels.

PSA level (ng/ml)	CD44 expression		Total
	+ve	-ve	
< 4	4 (22.2%)	2 (6.25%)	6 (12%)
4 – 10	10 (55.6%)	18 (56.25%)	28 (56%)
> 10	4 (22.2%)	12 (37.5%)	16 (32%)
Total	18 (100%)	32 (100%)	50 (100%)

Pearson Chi-Square = 3.6, P- value = 0.19 (not significant).

In table (3) there is no association between CD44 expression and age (P-value > 0.05). Within the positive cases, 4 patients (22.2%) aged 40 – 49 years, 4 patients (44.5%) aged 50 - 59 years, 4 patients (22.2%) aged 60 - 69 years, and 2 patients (11.1%) aged > 69 years.

Table 3: CD44 correlation with patients' age.

Patients' age (years)	CD44 expression		Total
	+ve	-ve	
40 – 49	4 (22.2%)	4 (12.5%)	8 (16%)
50 – 59	8 (44.5%)	12 (37.5%)	20 (40%)
60 – 69	4 (22.2%)	10 (31.25%)	14 (28%)
> 69	2 (11.1%)	6 (18.75%)	8 (16%)
Total	18 (100%)	32 (100%)	50 (100%)

Pearson Chi-Square = 1.44, P- value = 0.66 (not significant).

In table (4) there is no association between Gleason grade score and score of CD44 (P-value > 0.05). in Gleason grade 6, 5 patients (50%) were at CD44 score 1 (< 25%), and 5 patients (50%) at CD44 score 2 (25% - 75%); in Gleason grade 7, 2 patients (40%) were at CD44 score 1

(< 25%), and 3 patients (60%) were at CD44 score 2 (25% - 75%), while in Gleason grades 8 – 10, 2 patients (66.7%) were at CD44 score 1 (< 25%), and 1 patient (33.4%) was at CD44 score 2 (25% - 75%).

Table 4: Association between Gleason grade score and score of CD44.

CD44 score	Gleason grade score			Total
	6	7	8 - 10	
1 (< 25%)	5 (50%)	2 (40%)	2 (66.7%)	11 (61.1%)
2 (25% - 75%)	5 (50%)	3 (60%)	1 (33.3%)	7 (38.9%)
3 (> 75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	10 (100%)	5 (100%)	3 (100%)	18 (100%)

Pearson Chi-Square = 5, P- value = 0.5 (not significant).

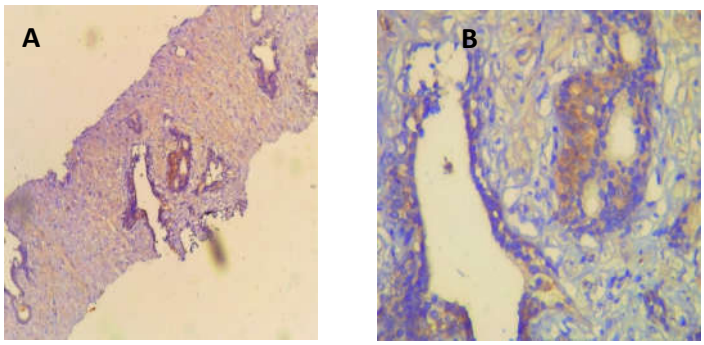

Fig 1: A section of CD44 in prostatic adenocarcinoma (25-75%), Gleason score 3+3=6, grade group 1 (well-formed gland). (A)100x, (B)400x.

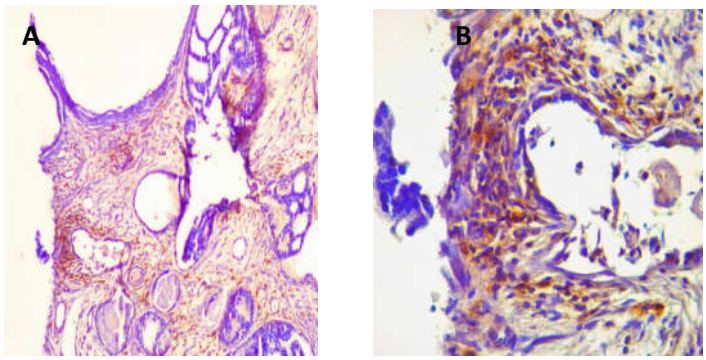

Fig 2: A section of CD44 in prostatic adenocarcinoma (25-75%), Gleason score 3+4=7, grade group 2 (well-formed gland predominantly with a less component of poorly formed, fused gland and cribriform). (A)100x, (B)400x.

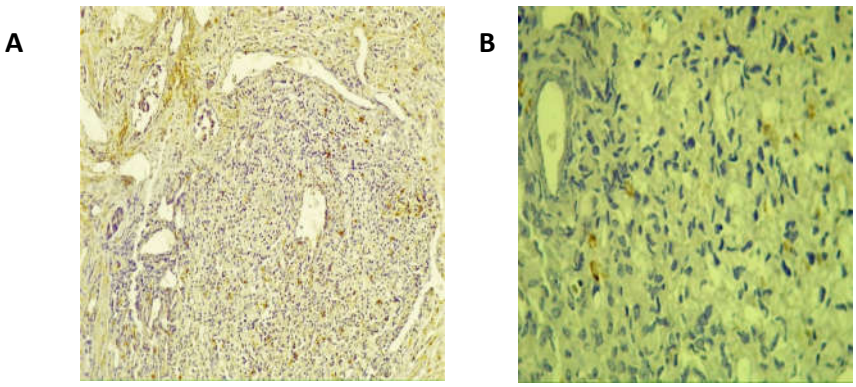

Fig 3: A section of CD44 in prostatic adenocarcinoma (25%), Gleason score 5+3=8, grade group 4 (sheet, single cell infiltration with less component of well-formed gland). (A)100x, (B)400x.

DISCUSSION:

Prostatic cancer is the 2nd commonest malignancy worldwide. It is the 6th leading cause of cancer-related deaths within men worldwide even with early detection and treatment[11].

CD44 has been identified as a putative cancer stem cell marker in a several solid tumors, it plays a major role in metastasis and progression of tumors, since the majority of tumor cells that exhibit high CD44 expression are considered low grade, so its tendency to progress or metastasize over a long time is low; whereas the tumor cells with low levels of CD44 expression mostly show high tendency for local invasion and metastasis, as their Gleason grade score is nearly always high (8 - 10) [11].

This study reviewed 50 patients of prostatic adenocarcinoma to evaluate CD44 expression as a proliferative marker in prostatic adenocarcinoma and correlate it with clinicopathological parameters (age, PSA level, and grade).

Our results showed that the percentage of CD44 expression in grade $\leq$ 6 was (55.6%), and in grade > 6 (44.4%), this agrees with C. G. Hirth[12] et al study who also observed an inverse correlation between CD44 expression and Gleason score. This was also similar to the study by E. Kalantari[11] et al within Gleason grade scores 7, and 8 – 10, but different in Gleason grade score 6; this difference can be due to variations in sample size (12 cases in our study compared to 26 cases in E. Kalantari et al[11] study) and objective variations in pathological readings where Gleason grade score 6 characterized by individual discrete well-formed gland (3 + 3).

There was a significant statistical correlation between CD44 expression and Gleason grade score (P-value = 0.0001), where lower expression of CD44 seen more frequently in tumors with higher Gleason scores (8 - 10), this means a decline in CD44 expression from Gleason group 1 to Gleason group 5, so we can regard CD44 as a marker of proliferative index in prostatic adenocarcinoma, since when the percentage of positive cells is high, the proliferative index is low.

Our results showed that CD44 expression in PSA < 4 ng/ml was (22.2%), 4 - 10 ng/ml (55.6%), and > 10 ng/ml (22.2%), this was nearly similar to C. G. Hirth et al[12] study. Also this was similar to the study by E. Kalantari et al[11] in PSA > 4 ng/ml and PSA > 10 ng/ml, while there was a difference in PSA 4 - 10 ng/ml, this difference can be due to variations in sample size (28 cases in our study had PSA 4 - 10 ng/ml compared to 40 cases in E. Kalantari et al[11] study), another explanation may be due to the fact that in the study by E. Kalantari et al[11], 34 cases from a total of 101 cases had unknown PSA levels.

In present study there was no statistically significant correlation between CD44 expression and PSA levels (P-value > 0.05), where the number of cases with PSA < 4 ng/ml (6), PSA 4 - 10 (28), PSA > 10 ng/ml (16); the above result show no significant p-value, this can be explained by the cases with PSA < 4 ng/ml which are regarded as benign (4 cases positive, 2 negative for CD44), and in cases with PSA 4 - 10 ng/ml which are regarded as a grey zone or suspicious of malignancy but most are benign (10 cases positive, 18 negative for CD44), while in cases with PSA > 10 ng/ml (4 cases positive, 12 negative for CD44). This means that PSA > 10 ng/ml is benign in some cases while in others it may reach 100 ng/ml which are definitely

carcinomas and most likely poorly differentiated adenocarcinoma that are almost always negative for CD44.

In current study, the mean age of patients with prostatic adenocarcinoma was (64) years and this agrees with the study by K. Korski et al[13] where the mean age was (63) years.

In the result of this study, CD44 was positive in cases aged < 66 years in (12) cases, while in cases aged ≥ 66 years in (6) cases. These results were similar to those in the study by E. Kalantari et al[11]. This means that CD44 as a stem marker is less active in older age group, also in the older age groups, the tumor is more likely to be poorly differentiated, which means higher grading and lower CD44 expression.

There was no significant statistical correlation between the age of the patients and CD44 expression (P-value > 0.05), this could be due to different age groups starting from 40 years to more than 80 years with different Gleason scores for each group.

In this study, when we assessed the scoring expression of CD44 in comparison with different Gleason scores, we found that the percentage of cases in Gleason score 6 with positive CD44 expression score 1 (< 25%) was (50%) compared to (40%) of cases in Gleason score 7 and (66.7%) of cases in Gleason scores 8 – 10, CD44 expression score 2 (25% - 75%) in Gleason score 6 was shown in (50%) of cases compared to (60%) of cases in Gleason score 7 and (33.3%) of cases in Gleason scores 8 – 10, CD44 expression score 3 (> 75%) was not shown in any of the Gleason grade scores within the cases of study. Those finding were nearly similar to those by M.A. Noordzij et al[14]. This means an inverse correlation between CD44 expression score and Gleason grade score.

Within our study there was no significant statistical correlation between Gleason grade score and CD44 expression score (P-value > 0.05).

CONCLUSION:

1. CD44 expression in prostatic adenocarcinoma is higher in well differentiated tumors than in poorly differentiated tumors, and correlated inversely with Gleason score which means higher expression in low grade tumors.
2. The level of CD44 expression denotes the extent of aggressiveness of tumor (the higher the score, the less aggressiveness of the tumor), so it can be used as a prognostic marker.

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