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Analysis of immunohistochemical expression of CD10 in the malignant lesions of prostate

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Abstract:*Benign prostatic hyperplasiaand Carcinoma of the prostate are frequently increasing with advancing age. CD10 is a transmembranemetallo-endopeptidase that cleaves and inactivates avariety of peptide growthfactors.The aim of our study is to analyse the expression of CD10 and their pattern of expression in malignant lesions of prostate.Immunohistochemicalstaining forCD10 is performed on 20 cases of paraffin-embedded tissue from transurethral resected and core biopsy specimen of prostate. we observed differential pattern of expression according to Gleason score in the malignant glands thus empahsising the fact that CD10 plays a significant role in the development and progression of prostatic carcinoma.*
Key words:*CD10, malignant lesions,prostate*

I. Introduction:

The lesions of prostate are responsible for significant morbidity and mortality among the males worldwide (1).The age range of males presenting with symptoms due to prostatic lesions is 40- 90 years, with majority of the cases were in the age group of 60 – 70 years (1).

Prostatic lesions are broadly categorized as inflammatory and neoplastic lesions. The neoplastic lesions are inturnsubclassified as benign, in situ and malignant lesions .

Prostate cancer is the most aggressive malignant neoplasm with varied clinical presentations. This tumor does not show any warning signs in its early course of development.

The most widely used screening test for detecting prostatic cancer is the measurement of serum Prostate specific antigen (PSA) level , in conjunction with digital rectal examination for all the suspected cases.
Prostate Specific antigen is secreted by normal and malignant prostatic epithelial cells. Therefore their level in the serum increases significantly in men with prostate cancer. Though it gives the suspicion for the underlying tumor, it isnotspecific.There are many benign conditions like benign prostatic hyperplasia and prostatitis which increases the serum PSA levels.Therefore it is of at most significance to use a newer marker to identify the prostatic cancer at an early stage. 19

Cluster of Differentiation (CD) 10, also known as Common Acute Lymphoblastic Leukemia Antigen (CALLA) was first described on human leucocytes (20). Several studies on CD10 revealed that it isnot only seen in lymphocytes, but also found to be expressed in other human cells both in normal and in pathological states.

Regarding the prostate gland, CD10 is expressed constantly in the apical luminal surface of the normal prostatic epithelial cells.In various lesions of prostate the pattern of expression varies ranging from altered expression to loss of expression.

In prostatic cells CD 10 acts as a transmembrane peptidase .It plays an important role in the pathogenesis of prostatic cancer. Generally it cleaves the excessive growth factor from the stroma thereby it prevents the continuous and unwanted growth in the luminal epithelial cells.

Literature review shows that loss of CD10 expression is seen in lower Gleason score prostatic tumors whereas increased and altered expression in high Gleason score tumors, lymph node metastasis and in bone metastatic prostatic carcinoma. This concept signifies the use of CD10 as a diagnostic and prognostic marker in prostatic carcinoma.
Based on this we analysed the expression of CD 10 in the malignant lesions of prostate.

II. Materials and methods:

The present study is an observational study conducted in the Department of Pathology, Chengalpattu medical college, chengalpattu during the period of january 2016 to May2016.A total sample of 20cases of prostatic lesions wasanalysed.Tissue blocks of patients who were diagnosed as having malignant prostatic lesions were included in the study. Tissue blocks of patients who are diagnosed as prostatic carcinoma and underwent preoperative Radiotherapy or Chemotherapy were excluded.

III. Method:

Formalin fixed paraffin embedded blocksand haematoxylin eosin stained sections of 40 prostatic biopsies were analysed and were categorized as follows:

1. Prostatic intraepithelial neoplasia- high grade and low grade,
2. Prostatic adenocarcinoma, Gleason grade ranging from grade 1 to grade 5 according to modified Gleason grading system.

Immunohistochemical stainingwas performed on 5-mmthick,formalin-fixed, paraffin-embedded tissue sections mounted on gelatin-chrome alum coated slides .The slides were incubated at 58ºC for overnight. The sections were deparaffinized in xylene for 15 minutes for 2 changes and rehydrated through descending grades of alcohol and then washed in distilled water2 changes, 2 minutes each. Heat induced antigen retrieval was done with microwave oven at 150 degree Celsius with citrate buffer (pH 6.0) for 20 minutes.Thesections are cooled for 10 minutes followed by distilled water and Tris buffer saline wash 2 minutes each. Endoperoxidase blocking was done by adding hydrogen peroxide on the sections and kept for 5 minutes followed by washing in wash buffer 2 minutes twice. Primary antibody CD 10(Mouse monoclonal;prediluted) was added and kept for 30 minutes in a moist chamber then washed in wash buffer 2 times each. Poly excel target binder reagent was added and kept for 15 minutes and washed in two changes of buffer, 2 minutes each. Poly excel HRP (Horse Radish Peroxidase) was added and incubated for 15 minutes again washed with buffer for 2 minutes, 2 changes.Working DAB chromogen (1ml DAB buffer + 1 drop chromogen, mix well) was added and kept for 2-5 minutes. Then washed in distilled water and counter stained with hematoxylin for 30 seconds.

Sections containing normal prostatic gland were used as positive control.Negative control included Primary antibody replaced with PBS.

Immunostained sections were reviewed for CD 10 expression for Apical membranous positivity,Diffuse membranous positivity,Membranous and cytoplasmic positivity, and only Cytoplasmic positivity.

IV. Statistical analysis:

Data were compared between groups using Pearson Chi-square or Fisher‘s exact tests ($p<0.05$).All statistical analysis was performed using SPSS statistical software version 11. Charts were prepared using Microsoft excel 2007.

V. Results:

Among the 20 cases,5 cases were Prostatic intraepithelial neoplasiaand 15 cases were Prostatic adenocarcinoma. The mean age group Prostatic intraepithelial neoplasia -66yearsand Prostatic carcinoma -68years.

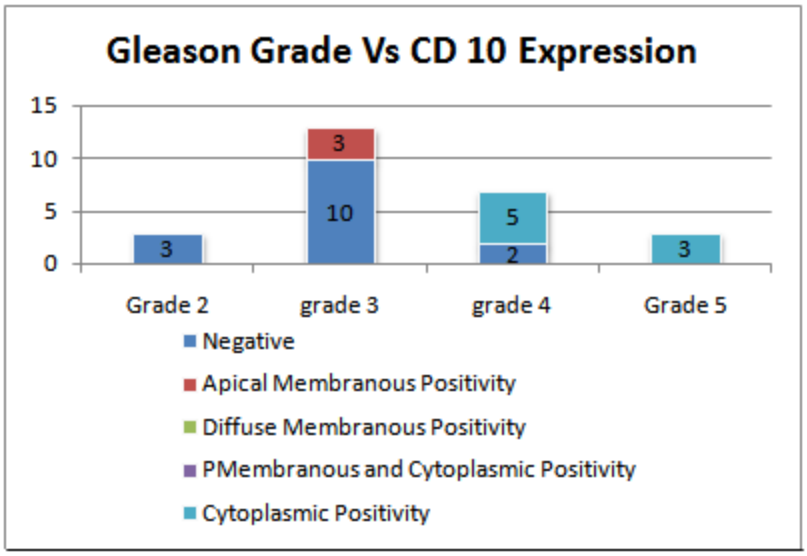
On categorisingthe prostatic adenocarcinoma according to Gleason score, we have 6 cases (40%) with the score of <3+3**,** 2 cases (13.33%) were having the score of 3+4,3 cases (20%) were having the score of 4+3, 4 cases (26.67%) were having the score of>4+4.On comparing serum PSA level with Gleason score,83.33% of Gleason score <3+3 have Serum PSA level of < 20 ng/dl whereas 75 to 100% of Gleason score of 7 and > 7 have serum PSA level >20 ng/dl.Using Fischer’s exact test, increased serum PSA level shows strong association with high Gleason score with the P value being <0.001. Upon analysing the proportion of different Gleason grade among the malignant lesions we have 7% of Grade 2, 53% of grade 3,34% of Grade 4 and 7% of grade 5.In our study, Gleason pattern 3 forms major proportion of cases.

In case of PIN 20% showed diffuse membranouspositivity, predominantly around 60 % showed both membranous and cytoplasmic positivity and 20 % showed only cytoplasmic positivity.

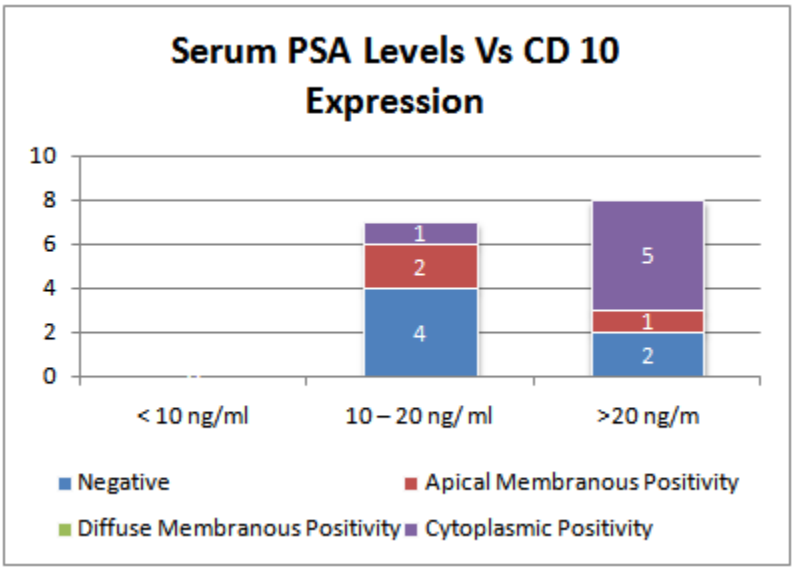
5.1Gleason grade and CD10 expression:

All the prostatic carcinoma cases were graded according to the Gleason’s grade and the percentage of each grade was estimated. Out of 15 cases grade 3 components was seen in 13 cases, grade 4 component in 7 cases, grade 2 and grade 5 component in 3 cases each.

Analyzing the expression of CD10, all the grade 2 components showed absence of expression(100%), 76.92% of grade 3 componentsshowed absence of expression and 23.07% showed apical membranous positivity.None of the grade 3 lesions showed combined or cytoplasmic positivity. Among grade 4 lesions, 71.43% showed intense cytoplasmic positivity and 28.57% showed absence of expression. All cases of grade 5 lesions (100%) showed diffuse cytoplasmic positivity with intense staining pattern.



5.2Serum PSA level and CD10 expression:
In the PSA range of < 20 ng/ml, 4 cases showed absence of expression, 2 cases showed apical membranous positivity and1 case with cytoplasmic positivity.
In cases with serum PSA level >20ng/ml, we had2 cases with negative expression



As the serum PSA level increases there is a shift from negative expression to cytoplasmic expression. P value showed significant association between increased serum PSA and increased cytoplasmic expression.

VI. Discussion:

Prostatic lesions (whether it is a nonneoplastic or neoplastic) are responsible for significant morbidity and mortality among the males worldwide (1).Our study is an attempt to use a hematological marker CD10 and to evaluate its expression and significance in various lesions of prostate. CD 10 is a transmembraneectopeptidase which generally cleaves the peptides is thought to have a role in prostatic cancer.

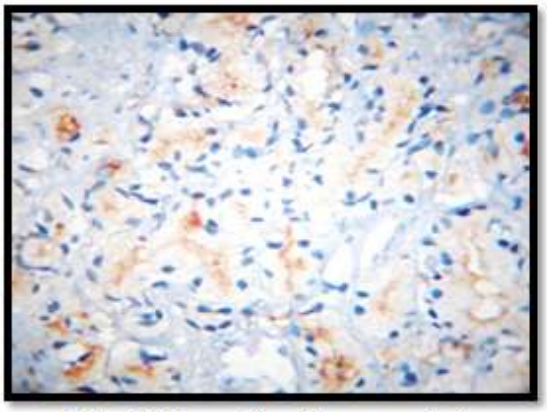


Fig 3: CD10 negative, Gleason grade 3, 40x.

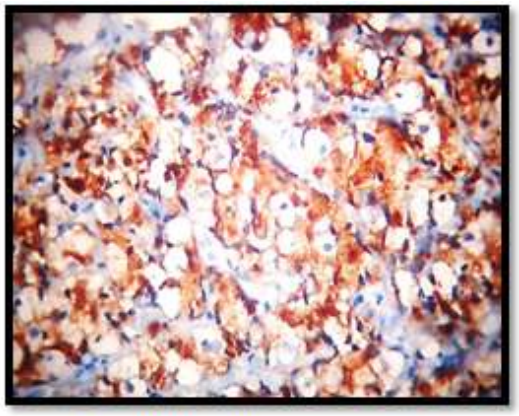


Fig 4: CD10 positivity, Gleason grade 4, cytoplasmic positivity, 40x

PIN cases shows variable expression pattern of CD10. 2 cases of Low grade PIN showed diffuse membranous positivity whereas high grade PIN showed negative in 2 cases and diffuse cytoplasmic positivity in 1 case.Sincelow grade PIN does not carry any risk for subsequent development of carcinoma, the expression pattern also displayed same feature as that of benign glands. No other study has taken low grade PIN into consideration.Studies by Freedland et al (7) and Zellweger et al (8**)**Sheriftawfic et al (14) showed absence of membranous positivity in High grade PIN which is similar to our study. Our study showed progressive loss of membranous CD10 expression from benign to premalignant conditions thus signifying its role in the pathogenesis

In normal glands and in benign conditions the extracellular peptidase activity of CD10 cleaves the unwanted peptides that could acts as a growth factor for the cells, thereby controlling the cell proliferation (9). In case of intraepithelial lesion their absence of expression leads to loss of cleavage activity thereby resulting in uncontrolled proliferation of cells.

In case of prostatic carcinoma, our studyshowed complete membranous and cytoplasmic loss of CD 10 expression in Gleason grade 2 and 3 (Fig 3), whereas the adjacent benign glands showed typical membranous positivity. Thus we can easily distinguish benign from the malignant counterparts even at a lower magnification itself.Sheriftawfic et al (14**)** and Mellisa et al (10) also observed similar pattern of expression in low grade tumors (grade 2 and 3). Study by Achim Fleischmann et al (11) showed variable expression patterns in grade 3.

In our study we observed that malignant cells of Gleason pattern 4 and 5 (Fig 4) showed increased cytoplasmic expression (71% and 100%) respectively. Thus there is a sharp alteration in the subcellular localisation of CD10, shifting from membranous in benign to cytoplasmic in malignant. Among the malignant lesions there is again a shift from absence of expression in lower grade to increased expression in higher gradetumors.

The reasons for the altered expression of CD10 in various lesions of prostate are only hypothesis arrived by prostate cancer cell specific microarray studies. Usmani et al.,stated that the loss of CD 10 expression could be due to hypermethylation of promoter region (12). Therefore CD 10 synthesis cannot take place thereby resulting in reduced expression in case of PIN (preneoplastic lesion) to absence of expression in the next stage of disease progression (Gleason pattern 2 and 3). The cytoplasmic localization of CD10 in high grade could be due to increased bound forms of CD10 with cytoplasmic heat shock proteins (13).This intracytoplasmic accumulation drives the cell to constant signaling pathway that is independent of the growth factor signaling.

Regarding serum PSA level and CD10 expression our study showed that as the PSA level increases, there is a shift from absence to cytoplasmic expression of CD10 thereby concluding that increased serum PSA level is associated with increased cytoplasmic expression of CD10 in malignant glands.Thus Serum PSA level directly correlated with increased cytoplasmic positivity, which is similar to the study byAchinm et al (11).Osman et al found that there is no significant association Of CD10 with serum PSA value thus contradicting our finding.

VII. Conclusion:

Since many studies have strongly shown an association between CD10 and outcome of prostate cancer, including disease free survival, lymph node metastasis (14) etc. This can be used as a potential target for the treatment by anti CD10 drugs. CD 10 can also process peptide prodrugs due to their cleavage activity thereby increasing the drug concentration around the malignant cells and by promoting the drug cytotoxicity (11)

By analyzing the CD10 expression on prostatic cancer biopsy specimen, we can categorise the prostatic adenocarcinoma as high grade and low grade tumors. It will help to follow up high grade tumors for lymph node metastasis and guide to treat them more aggressively.

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