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APPICATION OF GALECTIN3 , A NOVEL IMMUNOSTAIN, IN PROSTATIC CARCINOMA TO ASSESS ITS PATTERN OF EXPRESSION AND FUTURE POTENTIAL- CONDUCTED AS A TOOL OF MINI RESEARCH PROJECT

Pathology

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ABSTRACT

Carcinoma of prostate is the commonest type of cancer found in males of developed countries and is responsible for large number of cancer related deaths and significant morbidity .Gleason's grade and PSA level play pivotal role in decision making in the management of patients with prostate cancer. By modulating various aspects of tumour progression, Galectin 3 is emerging as a potential guardian of tumour microenvironment and studies indicate that it has important regulatory role in pathogenesis and progression of prostate cancer.

An observational cross sectional study was undertaken in the department of pathology of a tertiary care hospital in East India, of 6 months duration. Twenty nine samples diagnosed as acinar adenocarcinoma of prostate were taken by systematic random sampling as per the inclusion-exclusion criteria from the received specimens in the department and immuno-histochemical examination was done on the selected samples using monoclonal antibody against Galectin3 after obtaining thin sections from formalin fixed paraffin embedded blocks and retrieval of antigen. The data was interpreted by light microscopy using a semi-quantitative method with respect to prefixed parameters and statistical analysis was done using SPSS version 25.

Based on the prefixed cut off, 20.7% of total cases have shown positive expression of galectin3. Mainly the tumours with lower Gleason's grade have shown positive expression of this marker (62.5% of grade group 1 and 16.6% of grade group 2). None of the cases belonging to grade group 3, 4 or 5 have shown even minimal positivity. Positive expression of galectin3 appeared to decrease with progression of Gleason's grade and this association was found to be statistically significant. However, no significant association has been found between expression of this marker and percentage of the positive cores or the degree of maximum linear positivity.

KEYWORDS

Gleason's score, Galectins, Tumour microenvironment.

INTRODUCTION:

Carcinoma of prostate is the commonest type of cancer found in males of developed countries and is responsible for large number of cancer related deaths and significant morbidity.[1] The addictive nature of the tumour cells towards androgens and the role of it in the development, progression and recurrence of cancer, is now -a –days well established and substantiated by the effectiveness of androgen ablation (medically or by bilateral orchidectomy) in tumour regression. The discovery of PSA (prostate specific antigen) has made amenable this disease for screening, notwithstanding the question of sensitivity and specificity it shares like other tumour markers. And also in recent times, the universal screening of elderly male for raised PSA in the developed countries are raising big questions regarding therapeutic decisions in such cases as the risk of over treatment lingers;- addressing the unpredictable future behaviour of microscopic cancer foci.[2]

The peripheral region of prostate being the common origin of prostatic carcinoma, rarely it presents with micturition abnormalities unlike the cases of benign hyperplasia of prostate (BHP). The previous protocol of FNAC by Franzen needle has now been replaced by Trans rectal ultrasound (TRUS) guided biopsy of prostate. After a definitive diagnosis is established, most patients are managed conservatively by androgen ablation, the incidence of open prostatectomy (trans vesical or retro pubic) has largely decreased in this era.

BACKGROUND OF STUDY:

Among the cancers of epithelial origin, prostatic acinar adenocarcinoma is the most common variety, of which High grade prostatic intraepithelial neoplasia is a postulated precursor in core needle biopsies. Small crowded atypical glands arranged in linear pattern spanning the width of core biopsy or atypical glands on both sides of benign gland and intercalating isolated glands between and around benign glands are strongly suggestive of carcinoma.[2] The cytological features of acinar adenocarcinoma are often subtle, showing nuclear enlargement, nuclear hyperchromasia and presence of nucleoli, apoptotic bodies and mitotic figures.

Well recognized histopathological variants are there, each having separate prognostic significance[3]:

1. Atrophic 2. Pseudohyperplastic 3. Microcystic 4. Foamy gland 5. Mucinous (colloid like) 6. Signet ring like 7. Pleomorphic Giant cell rich 8. Sarcomatoid variant.

The PI3K, Myc and TP53 are the most common pathways deranged in prostate cancer. Recurrent gene fusion of ETS family of transcription factors, most notably TMPRSS2-ERG is also commonly encountered. The role of epigenetic regulation has also come forth on identifying the recurrent deletion of CHD1, a chromo-domain helicase necessary for chromatin remodelling.[3]

Gleason's scoring: Gleason's score, developed by Sir Donald Gleason in 1966-74 was based solely on architectural criteria and are expressed as the summation of the score of two most dominant patterns- the one having the greater proportion being the first. Pattern 3 is designated by well formed, variably sized, discrete glandular units. Poorly formed or fused glands, cribriform pattern, glomeruloid pattern are considered as pattern 4. Gleason's pattern 5 consists of sheets of cells, solid nests, cords, linear arrays and individual cells. Presence of comedo necrosis and karryorrhexis is considered as pattern 5, by definition.

Galectins are carbohydrate binding proteins having high affinity for beta galactosides and the members of this family are grouped structurally by the number and organisation of carbohydrate recognition domain (CRD).[4]Galectin 3 is the lone member of the chimeric group having a single CRD with a unique N-terminal domain. By having such characteristics like 1.positively regulating survival

signalling and suppressing stress pathways 2.blocking immune surveillance by inhibition of immune cells 3.modulating cell adhesion to regulate contact with stromal cells, promoting metastasis and skewing tumour cells homing to protective niches 4.suppressing tumor cell differentiation 5.controlling endocytosis of critical cell surface receptors 6.regulating cell survival cascades essential for tumour cells to survive changes in oxygen and metabolite content; galectin 3 is emerging as a potential guardian of tumour microenvironment [4] and may be worth targeting in different solid tumours.

The role of galectin 3 in various cancers is complex.[5] The elevated levels have been shown to prognostic for poor survival in cancers like lymphoma, leukaemia, breast cancer and thyroid cancer but decreased level appears to be detrimental to patients suffering from chronic lymphoblastic leukaemia and prostate cancer.[6][7][8] A possible explanation can involve the intracellular location of the marker. As an example, in neuroblastoma increased expression is favourable for good prognosis, only when the protein is localised inside nucleus.[9] In melanoma also elevated nuclear galectin 3 translates into good prognosis.[9]

Increased expression of galectin 3 in nucleus of clear cell renal carcinoma leads to a poor outcome in contrast, indicating that it is very likely that its contribution to survival of tumour cells may be type specific.[10] In acute lymphoblastic leukaemia it appears that the stromal derived galectin 3 is critical for chemo-resistance.[7] An important aspect regarding Galectin3 is that, not only the expression of this protein in neoplastic cells are to be kept in mind, but the expression in stromal cells and immune cells are also to be corroborated to get the whole scenario of tumour microenvironment.

Objective of research: To study the expression of Galectin3 in Prostatic carcinoma with respect to Gleason's grade

METHODOLOGY:

An observational, descriptive, cross sectional study was undertaken in the department of pathology, R G Kar medical college and hospital, Kolkata of 6 months duration. Twenty nine samples diagnosed as acinar adenocarcinoma of prostate were taken by systematic random sampling as per the inclusion-exclusion criteria from the received TRUS guided biopsy specimens in the department.

Ultrathin sections (3 micron) are obtained by microtomy from the formalin fixed paraffin embedded blocks. After floatation they are picked on poly-L-lysine coated slides, dried, deparaffinized and rehydrated in descending grades of alcohol.

Heat induced epitope retrieval (HIER) procedure was done by microwave method using TRIS Buffer, EMPARTA, pH 9.0. TRIS Buffer (EMPARTA, pH 7.2) was used for washing. Endogenous peroxidise activity was blocked with PolyExcel Peroxidase Block, (PATHNSITU). Incubation with primary antibody (Monoclonal antibody against galectin 3-Galectin-3-9 MIB, PATHNSITU) was done at 37°C for 60 minutes. For visualisation of result, serial incubation for 30 minutes each was carried out with PolyExcel Target Binder, PATHNSITU ;Poly HRP (PolyExcel HRP DAB Detection System, PATHNSITU) and chromogen ((Polyexcel Stunn DAB Buffer & Polyexcel Stunn DAB Chromogen, PATHNSITU).The sections were then counterstained with Harris Hematoxylin and mounted.

Sections of Benign hyperplasia of Prostate were taken as control group. For validation of galectin3 staining; section of Papillary Thyroid carcinoma was used as positive control. Expression of galectin3 was measured by semi quantitative method using immunohistochemistry. Intensity of the immunostaining were taken as 1+, 2+, 3+ depending upon the positivity. For statistical purposes 50% positivity of galectin3 in the neoplastic cell population was taken as positive.

RESULT AND ANALYSIS:

TABLE 1: Distribution of cases of prostate carcinoma according to grade group and galectin3 expression

Galectin3 Expression	Grade group 1	Grade group 2	Grade group 3	Grade group 4	Grade group 5	total
Positive	5	1	0	0	0	6
Negative	3	5	5	9	1	23
Total	8	6	5	9	1	29

TABLE 1S: Derived from Table 1 for the ease of statistical analysis

Galectin3 Expression	Grade group 1/2	Grade group 3/4/5	total
Positive	6	0	6
Negative	8	15	23
Total	14	15	29

P value: 0.0063 (by Fisher's exact Test) and so statistically significant.

TABLE 2: Distribution of cases of prostate carcinoma according to percentage of positivity of the cores and galectin3 expression

Galectin3 Expression	Percentage of positivity of cores				Total
	0-25%	25-50%	50-75%	75-100%	
Positive	1	3	0	2	6
Negative	2	5	6	10	23
Total	3	8	6	12	29

P value- 0.1638, so not significant.

TABLE 3: Distribution of cases of prostate carcinoma according to Maximum linear positivity of the cores and galectin3 expression

Galectin3 Expression	Maximum Linear positivity of the cores				Total
	0-25%	25-50%	50-75%	75-100%	
Positive	0	4	1	1	6
Negative	2	6	10	5	23
Total	2	10	11	6	29

P value 0.1981, so not significant.

DISCUSSION:

Twenty nine cases of Trans rectal ultrasound guided core biopsies diagnosed as acinar adenocarcinoma of prostate were included in our study. 31.0% cases belonged to Gleason's grade group 4(Fig 2), succeeded by 27% cases of grade group 1(Fig 1). Cases belonging to grade group 2, 3 and 5(Fig 3) comprised 20.7%, 17.2% and 3.5% respectively. Two specialised variants of adenocarcinoma, both belonging to the foamy cell variant were also included in the study.(Fig 4)

Galectin3 has mainly shown cytoplasmic expression in the cases of acinar adenocarcinoma of prostate dealt in our study. Strong cytoplasmic positivity of galectin3 in papillary thyroid carcinoma served as the positive control.(Fig 5) Based on the prefixed cut off, 20.7% of total cases have shown positive expression of galectin3.(Fig 7)

Mainly the tumours with lower Gleason's grade have shown positive expression of this marker(62.5% of grade group 1 and 16.6% of grade group 2). None of the cases belonging to grade group 3,4 or 5 have shown even minimal positivity.(Fig 8,9) It was apparent that expression of galectin3 decreases with progression of Gleason's grade and this association was found to be statistically significant.(Table 1,1S)

The case of Benign Hyperplasia of Prostate (obtained as TURP chips) run simultaneously as a non specific control in this study has shown strong positive expression of galectin3 both in the luminal cells as well as in the basal cells (Fig 6). However, no significant association has been found between expression of this marker and the percentage of the positive cores; or between the expression and the degree of maximum linear positivity.(Table 2 & 3)

So far, the existing literature has contradictory opinion regarding the role of this marker in progression of prostate cancer; some have shown a positive correlation between strong expression of this marker and increased tumour invasion, others demonstrated just the opposite.

Ste´phane Califice et al[11] generated transfectants using galectin3 negative prostate cancer cell line LNCaP , and observed that induced cytoplasmic galectin3 showed significantly increased Matrigel invasion, anchorage- independent growth, in vivo tumour growth and angiogenesis and decreased inducible apoptosis. The induction of nuclear galectin3 showed just opposite spectrum of changes. They concluded that role of galectin3 depends on cellular localization; nuclear localization of the marker hinders tumour progression, where as the cytoplasmic expression actually promotes it.

Y Wang et al [12] observed that galectin3 expression was always opposite to the expression of Androgen receptor or other luminal cell

markers in prostate cancer, but corroborates well with the expression of basal markers like Glutathione-s-transferase-p and BCL-2. They also showed that down regulation of galectin3 is associated with decreased tumour metastasis and increased apoptosis. They concluded that galectin3 can be served as a new marker defining the basal like subgroup of prostate cancer.

Vitaly Balan et al [13] showed that galectin 3, which has role in genesis and progression of prostatic carcinoma, when phosphorylated, becomes resistant by cleavage by PSA. The dephosphorylation reaction is catalyzed by PTEN. According to them, the ratio of phosphorylated/non-phosphorylated galectin-3 may be a complimentary indicator in addition to PSA level in prostatic carcinoma.

Avraham Raz et al [14] measured serum galectin3 levels by western blot and ELISA in cases of metastatic prostate carcinoma and non cancer patients. They observed that serum Gal-3concentrations were uniformly higher in patients with metastatic Prostate cancer as compared to non-cancer control patients. And they concluded that galectin3 can be regarded as an alternative biomarker to PSA to study cancer progression in prostate.

Yi Wang and Pratima Nangia-Makker et al [15] evaluated the role of galectin-3 during the progression of human prostate cancer using two approaches: staining human prostate cancer tissues with differential antibodies and silencing galectin-3 expression in human prostate cancer PC3 cells with siRNA. In human prostate cancer, galectin-3 expression was reported to be down-regulated with progressive stages. However they questioned if previous studies on galectin-3 expression in human prostate cancer using a single antibody provided the complete picture of the significance of this protein in prostate cancer, as cleaved galectin-3 is recognized by the polyclonal antibody, but not the monoclonal antibody.

Diego J Laderach et al [16] observed both nuclear and cytoplasmic positivity of galectin3 in prostate carcinoma. According to them, galectin3 expression significantly decreased from benign to premalignant and malignant lesions and it correlated with biochemical recurrence. Apart from this, the correlation between Age, Gleason's score, stage, seminal vesicle invasion, pre-operative PSA with biochemical recurrence was also noteworthy.

JS Knapp et al [17] demonstrated that median galectin3 staining scores significantly decreased from benign to adjacent benign and to tumour tissues. Apart from this, Gleason sum and galectin 3 expression was significantly associated with biochemical recurrence.

Van Den Brule et al [18] studied 145 cases of prostate carcinoma and found galectin3 expression was negative or decreased in tumour cells when compared against the staining pattern of surrounding normal glands. Positive expression of this marker, present in a subset of carcinoma is detected only in the cytoplasm, where as both nuclear and cytoplasmic staining was observed in benign cases. Specific cytoplasmic expression of galectin 3 is associated with disease progression and PSA relapse. However, no significant association was demonstrated between galectin 3 expression scores and traditional clinico pathologic parameters like Gleason's grade.

However, in contray to the findings of this study, we have found a significant association between galectin 3 expression and gleason's grade, which needs further confirmation in studies having larger sample size.

CONCLUSION:

Based on the prefixed cut off, 20.7% of total cases have shown positive expression of galectin3.Mainly the tumours with lower Gleason's grade have shown positive expression of this marker. None of the cases belonging to grade group 3,4 or 5 have shown even minimal positivity. Positive expression of galectin3 decreases with progression of Gleason's grade and this association was found to be statistically significant. However, no significant association has been found between expression of this marker and percentage of the positive cores or the degree of maximum linear positivity.

Owing to the small sample size, the results are to be confirmed in a wider set up, especially as there are conflicting opinions from different journals regarding the prognostic significance of expression of this marker in the progression of prostate carcinoma. Focussing on the robust activity of galectin3 in regulating almost all the hallmarks of the cancer, probably it will be difficult to draw any direct correlation between its expression and tumour behaviour.

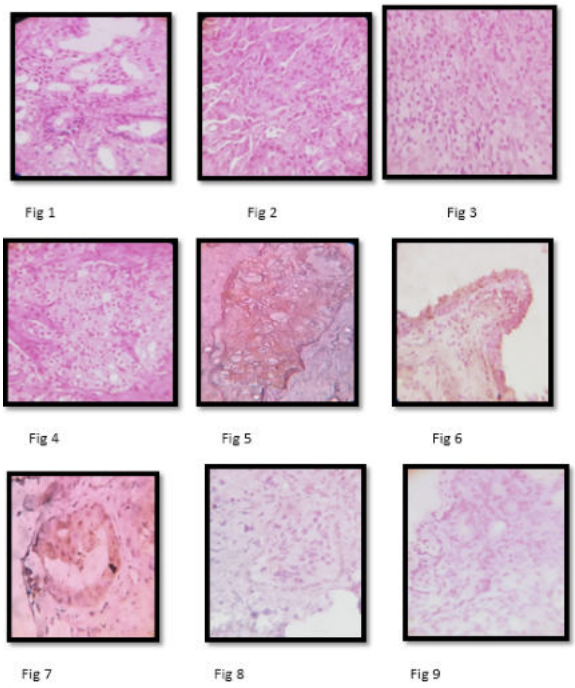

Fig 1: Acinar adenocarcinoma, Gleason grade group 1(3+3), H & E 100X Fig 2: Acinar adenocarcinoma, Gleason grade group 4(4+4), H & E 100X Fig 5: Gleason's 5+5 acinar adenocarcinoma, grade group 5, H & E 100X Fig 4: Prostate acinar adenocarcinoma, Foamy gland variety, H & E 100X Fig 5: positive control of galectin3 expression; Papillary thyroid carcinoma,400X Fig 6: positive cytoplasmic expression of galectin3 in Benign Hyperplasia of Prostate,400X Fig 7: positive expression of galectin3 in Gleason grade 3+3 acinar adenocarcinoma,400X Fig 8: Negative expression of galectin 3 in Gleason's 4+4 acinar adenocarcinoma,100X Fig 9: Negative expression of galectin3 in Gleason's 4+5 acinar adenocarcinoma,100X.

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