Original Article

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Pathology Section

Expression of ERG in Prostatic Acinar Adenocarcinoma Diagnosed on TRUS-guided Biopsy and its Association with WHO Grade Group- A Prospective Observational Study

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ABSTRACT

Introduction: Prostate cancer is a common malignancy affecting men and the second leading cause of cancer related death in India. Numerous molecular biomarkers have been evaluated for their potential role in predicting disease progression, their response to therapy and survival. Erythroblast Transformation Specific (ETS) related Gene (ERG) is one of the newest addition in the existing list of biomarkers of prostate cancer.

Aim: To analyse the expression of ERG in prostatic adenocarcinoma and to evaluate its association with World Health Organisation (WHO) grade group.

Materials and Methods: This was a prospective observational study was conducted in the Department of Pathology in association with Department of Urology, IPGME&R, SSKM Hospital, Kolkata, West Bengal, India. The duration of the study was 1.5 years, from January 2019 to June 2020. A total of 267 cases of Transrectal Ultrasound (TRUS) guided tru-cut biopsy was included. Clinical data including preoperative Prostate Specific Antigen (PSA) level, Digital Rectal Examination (DRE) were obtained. Histopathological reports were prepared by two pathologists along with Gleason scoring and WHO grading as per 2014 International Society of Urological Pathology (ISUP) consensus conference on gleason grading of prostatic carcinoma. Formalin Fixed Paraffin Embedded (FFPE) sections of representative blocks of each tumour was selected for Immunohistochemistry (IHC) study. Only the cases which had more than 10% nuclear staining were considered as positive. Statistical analysis was performed with help of Epi Info (TM) 7.2.2.2 and Chi-square test was used to test the association of different study variables.

Results: The mean age of the study participants was 65.55 years, and the age range was 45-93 years. Among the 80 malignant cases where, ERG immunostaining was assessed, 28 cases (35%) showed positive expression. Among these positive cases, 50% cases were weakly positive, 28.57% showed moderate positivity and 21.43% had strong positive expression. Highest positivity was observed in WHO grade group V (44.83%). The intensity of ERG expression was also higher in high grade group (13) than low grade group cancer patients.

Conclusion: ERG expression in the prostate cancer can be a prognostic factor as the expression and intensity of expression both increases with higher grade group of cancer.

Keywords: Carcinoma prostate, Erythroblast transformation specific related gene, Immunohistochemistry, Transrectal ultrasound, World health organisation

INTRODUCTION

Prostate cancer is the 8th most common cause of cancer death overall, and the 5th most common cause of cancer death worldwide for males [1]. The Indian scenario also follows the global trend with prostate being the second leading site of cancer among males in cities like Delhi and Kolkata. There is dispute regarding the use of serum levels of PSA as a tool of public health screening for prostate cancer. Definitive diagnosis is primarily based on core needle biopsy which is usually prompted by suspicious clinical presentation such as Lower Urinary Tract Symptoms (LUTS), elevated serum PSA level, suspicious DRE, and TRUS or Magnetic Renosance Imaging (MRI) findings [2]. Numerous molecular biomarkers have been evaluated for their potential role in predicting disease progression, response to therapy, and survival in prostate cancer patients [3]. These efforts have been greatly facilitated by the wealth of information garnered from gene expression array studies and by sophisticated bioinformatics tools evaluating the overwhelming data sets generated from genomic, transcriptomic, and proteomic studies. Genomic technologies are yielding new markers that can in turn be evaluated for clinical use in a high throughput manner using Immunohistochemistry (IHC) and Fluorescence In Situ Hybridisation (FISH) labelled tissue microarrays and state-of-the-art image analysis systems [4].

Transmembrane Serine Protease 2 (TMPRSS2)-ERG fusion is a frequent event in prostate cancer. PSA screened hospital based cohort studies detect a frequency of TMPRSS2-ERG fusion, ranging between 40% and 78% [5]. ERG IHC may offer an accurate, simpler, and less costly alternative for evaluation of ERG fusion status in prostate cancer on needle biopsy and radical prostatectomy samples [6-8]. In the present study, the expression of ERG oncoprotein expression in prostatic carcinoma using immunohistochemical staining was analysed and a relationship between ERG positivity and intensity with World Health Organisation (WHO) grade group was also assessed.

MATERIALS AND METHODS

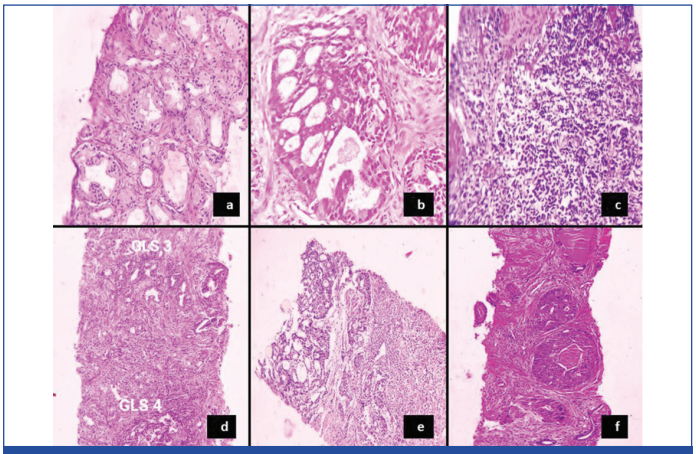
A prospective observational study was conducted in the Department of Pathology in association with Department of Urology at a tertiary care Hospital, Kolkata, West Bengal, India. The duration of the study was 1.5 years, from January 2019 to June 2020. The study was carried out in accordance with the ethical principles stated by the declaration of Helsinki principles and was approved by the Institutional Ethical Committee (IPGME&R/IEC/2019/004).

Inclusion criteria: Relevant patient particulars had been obtained along with history and documentation from bed head tickets. Thus, by feasibility method of non randomised sampling, 267 cases fulfilling the above mentioned inclusion criteria were included in the study.

Exclusion criteria: On sectioning and further processing, 29 cases with tiny TRUS cores did not yield sufficient tumour tissue for ERG interpretation and the patients refusing to give consent and insufficient biopsy samples on IHC study were excluded from the study.

Study Procedure

Whenever TRUS guided biopsy of a suspected case of prostatic carcinoma with high serum PSA level (>4 ng/mL) and prostatomegaly on (DRE had been received from Department of Urology, the particular patient was approached in the urology ward for necessary consent. All those samples underwent histopathological examination and out of total selected cases, 109 cases were diagnosed as prostatic adenocarcinomas. These biopsy proven prostatic adenocarcinoma cases were subjected for ERG immunostaining. Hence, interpretation of ERG expression was done on 80 biopsy proven prostatic adenocarcinoma cases. ERG expression status was compared with WHO grade group of prostatic adenocarcinoma as proposed by 2014 ISUP Consensus Conference on Gleason Grading of Prostatic Carcinoma [9]. The parameters examined during histopathological assessment were- tumour load, presence of prostatic intraepithelial lesion, presence of perineural invasion, Gleason score and WHO grade group [Table/Fig-1]. The slides were examined under the light microscopy (olympus magnus 2 CX20i). The reporting was done by two experienced pathologists. There was interobserver variability in one prostatic carcinoma case, which was interpreted by one pathologist as benign with severe prostatitis (kappa value=0.992763). Later, IHC staining for PSA and Tumour Protein 63 (P63) was done on that specimen and both pathologists agreed that, to be a prostatic carcinoma. Gleason scoring and WHO grade were calculated as per 2014 ISUP consensus conference on Gleason grading of prostatic carcinoma [9].



[Table/Fig-1]: a) Low magnification view showing prostatic adenocarcinoma with Gleason score 3+3 Haematoxylin and Eosin (H&E), 100x); b) High magnification view showing cribriform pattern of glands lined by malignant cells, Gleason score 4+4 (H&E, 400x); c) High magnification view showing diffuse sheet like pattern of malignant cells, Gleason score 5+5 (H&E, 400x); d) Low magnification view showing prostatic adenocarcinoma with both fused glands and separated glands, Gleason score 4+3 (H&E, 100x); e) Low magnification view showing prostatic adenocarcinoma with cribriform glands and sheet like growth pattern, Gleason score 5+4 (H&E, 100x); f) Low magnification view showing prostatic adenocarcinoma with cribriform glands with comedo-necrosis, Gleason score 5+5 (H&E, 100x).

Interpretation of IHC

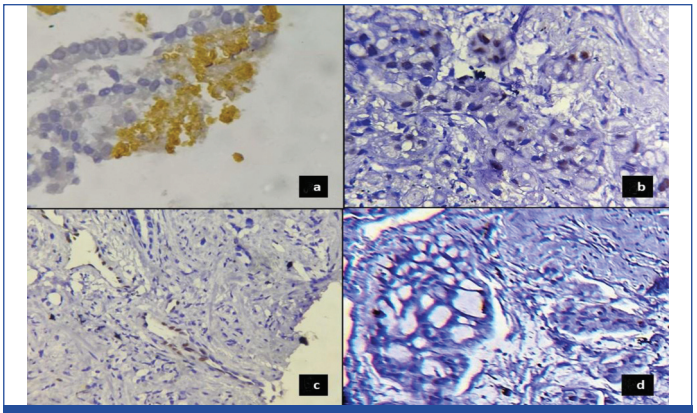
For immunohistochemical evaluation FFPE sections of representative blocks of each tumour was selected. The authors used anti-ERG monoclonal rabbit clone antibody EP111 (Dako, Denmark) for ERG.

Only the cases which had more than 10% positive nuclear staining for ERG were considered as positive [10].

The intensity of ERG positivity was scored as [11]:

- No staining (0)
- Weak staining (1+)
- Moderate staining (2+)
- Intense staining (3+)

Endothelial cells were considered as positive control [Table/Fig-2].



[Table/Fig-2]: a) High magnification view showing strong nuclear ERG positivity (400x); b) High magnification view showing weak nuclear ERG positivity (400x); c) High magnification view showing no nuclear ERG expression in tumour cells, endothelial cells act as positive control (400x); d) High magnification view showing ERG negative tumour cells (400x).

STATISTICAL ANALYSIS

Statistical analysis was performed with help of Epi Info (TM) 7.2.2.2 Epi info is a trademark of the Centre for Disease Control and prevention (CDC). Using this software, basic cross-tabulation, inferences and associations were performed. Chi-square test was used to test the association of different study variables. Z-test (Standard Normal Deviate) was used to test the significant difference between two proportions. The p <0.05 was considered to be statistically significant.

RESULTS

The mean age of the study participants undergoing TRUS-guided biopsy were 65.55 years, and the age range was 45-93 years. Out of total 267 TRUS-guided biopsy cases, 148 cases (55.43%) were benign, whereas, 109 cases were diagnosed as acinar adenocarcinoma (40.82%). Among other categories 5 cases were diagnosed as Atypical Small Acinar Proliferation (ASAP) (1.87%), 3 cases were diagnosed as High Grade Prostatic Intraepithelial Neoplasia (HGPIN) (1.13%), and biopsy was found to be inadequate in 2 cases (0.75%) [Table/Fig-3].

Histological findings	Number (n)	Percentage (%)
Benign	148	55.43%
Prostatic acinar adenocarcinoma	109	40.82%
Atypical Small Acinar Proliferation (ASAP)	5	1.87%
High Grade Prostatic Intraepithelial Neoplasia (HGPIN)	3	1.13%
No opinion possible	2	0.75%
Total	267	100%

[Table/Fig-3]: Distribution of all cases according to histological findings (n=267). *109 adenocarcinoma cases were subjected for ERG immunostaining, out of which 29 core biopsy samples were inadequate for interpretation as most of the tissues were lost during processing. So, those cases were excluded from ERG imuunostain interpretation and final study samples were 80 cases.

The prevalence of malignant cases was significantly higher among the patients with age between 65-74 years. The authors also found 49 malignant cases (44.95%) in that age range. Out of 80 prostatic adenocarcinoma cases, 6 cases were in Gleason grade group II (7.5%); 9 cases were in grade group II (11.25%); 12 cases were in grade group III (15%); 24 cases were in grade group IV (30%) and 29 cases were in grade group V (36.25%). Average tumour load was 56±4.567%. Associated HGPIN was identified in 10 cases (12.5%). Evidence of perineural invasion was identified in 55 cases out of 80 cases (68.7%). Most of the malignant cases 77 out of 80 cases, (96.25%) had the PSA level more than 4 ng/ml. Hence, PSA had high sensitivity in diagnosis of malignancy. The distribution of malignant cases according to the WHO grade group and PSA levels were not statistically significant (p-value=0.291) [Table/Fig-4].

Level of PSA (in ng/mL)	Malignant	WHO grade I	WHO grade II	WHO grade III	WHO grade IV	WHO grade V	p-value
≤4	3	1	0	1	1	0	0.291
>4	77	5	9	11	23	29	
Total	80	6	9	12	24	29	

[Table/Fig-4]: PSA values in the different WHO grade groups of prostatic carcinoma (n=80).
*PSA: Prostate specific antigen

ERG immunoreactivity and gleason grade: The ERG status was estimated in total 80 biopsy proven prostatic adenocarcinoma patients. A total of 28 cases (35%) were positive while 52 cases (65%) were negative. Among these positive cases, 50% cases were weakly positive, 28.57% showed moderate positivity and 21.43% had strong positive expression. The relationship between Gleason grade and ERG IHC is summarised in [Table/Fig-3]. ERG expression was detected in higher primary Gleason grade than in the lower grade (p-value=0.005). Highest positivity was in WHO grade group V (44.83%) [Table/Fig-5].

WHO (Gleason) grade	ERG positive	ERG negative	Percentage of ERG positive status (%)	p-value
I	01	5	16.67	0.005
II	02	7	22.22	
III	03	9	25	
IV	09	15	37.5	
V	13	16	44.83	
Total	28	52	100	

[Table/Fig-5]: ERG expression status in WHO grade group of prostate cancers (n=80).

Additionally, the strong intensity of ERG expression were mostly found in grade group V (66.7%) [Table/Fig-6]. The lower grade group (grade group I and II) prostatic adenocarcinomas were mainly negative for ERG immunostaining or weakly/moderately positive.

WHO grade group	ERG positive status	Weak 1+	Moderate 2+	Intense 3+	p-value
I	1	1	0	0	0.76
II	2	1	1	0	
III	3	2	0	1	
IV	9	4	4	1	
V	13	6	3	4	
Total	28	14	08	06	

[Table/Fig-6]: ERG positive status in WHO grade group prostate cancer (n=28).

DISCUSSION

Recurrent genetic fusion of TMPRSS2 and ERG in prostate cancer was first reported by Pettersson A et al., [12]. ERG (ETS-related gene) is an oncogene located on chromosome 21 (21q22.2). The ERG gene encodes for a protein, also called ERG, which functions as a transcriptional regulator. The most common TMPRSS fusions is with ERG, resulting in the TMPRSS2-ERG fusion, which has been identified in approximately 23% to 50% of prostate cancer cases in different cohorts [13]. The fusion gene is critical to the progression of cancer because, it prevents the androgen receptor expression and it binds and inhibits androgen receptors already present in the cells. Essentially TMPRSS2-ERG fusion disrupts the ability of the cells to differentiate into proper prostate cells creating unregulated and unorganised tissue [14].

Since, early days after the discovery of the TMPRSS2-ERG fusion gene, many groups have searched for its clinical implications on the prognosis of prostatic acinar adenocarcinoma. However, variability in study population, clinical profiles and the methods for gene fusion detection has led to divergent expression results. In a study by Barwick BG et al., TMPRSS2-ERG fusion was associated with the disease recurrence in multiple cohorts [15]. However, Hermans KG et al., found that, the TMPRSS2-ERG gene fusion is significantly related to a favorable prognosis [16]. Several articles showed TMPRSS2-ERG gene fusion expression as an independent predictor of poor outcome in prostate carcinoma [17,18]. However, other studies have indicated that, the ERG gene fusion has no significant clinical implications or prognostic value [19,20].

In the present study, ERG positivity rate was 35%, which is relatively higher than other prostate cancer based studies. In Japanese population study by Furusato B et al., and Miyagi Y et al., a relatively lower rate of ERG positivity has been reported (20.1% and 28%) [21,22]. Study by Hashmi AA et al., showed ERG protein expression in 39.7% cancer cases (31 cases out of 78 cases), which is near to the present study [11]. Among the positive cases of prostatic adenocarcinomas in the present study, 50% cases were weakly positive, 28.57% showed moderate positivity and 21.43% had strong positive expression. Association between TMPRSS2-ERG fusion gene status and clinicopathological profile remained variable in multiple studies. Some studies showed that, lower Gleason score and grade group is associated with highest expression of ERG fusion gene [23,24]. Few groups however, found no significant association between Gleason score and ERG expression [25,26]. Newer studies like Mannan R et al., revealed higher expression of ERG gene fusion with higher Gleason scores and grades [10]. In the present study, ERG expression was detected in higher primary Gleason grade than in the lower grade (p-value=0.005). Highest positivity was in WHO grade group V (44.83%) which is in concordance with recent studies.

In the present study, intensity of ERG expression in prostatic adenocarcinoma was also more in higher WHO grade groups. The high Gleason scores like 9/10 or grade group V had the highest intensity of ERG IHC expression (4 out of 6 strong positive ERG, 66.7%). A study by Hashmi AA et al., showed similar findings [11]. Although, IHC is a suitable surrogate for fluorescent insitu hybridisation/polymerase chain reaction based detection of ERG, it can not specify the mutation/fusion type or the fusion partner [27].

Limitation(s)

The present study was limited in the aspect of shorter sample size and limited duration in a single Institution-based study samples.

CONCLUSION(S)

The present study recommends that, the ERG expression is a prognostic marker of prostatic adenocarcinoma and its expression predicts the higher grade in prostate carcinoma patients. Whenever present, strong intensity of ERG expression is also associated with high histological grades of prostatic adenocarcinoma.

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