Expression of 34β E 12 in prostatic lesion.

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Abstract

Introduction: Prostate cancer is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide, To diagnose prostate cancer, no specific single histologic feature is sufficiently available. It is a challenging task to accurately diagnose small foci of prostate cancer for pathologists and to distinguish cancer from its benign mimickers. **Material and Methods:** The present study was a prospective study. Establishing a definitive diagnosis of malignancy in prostate needle biopsies with very little foci of adenocarcinoma is a major diagnostic challenge for pathologists. A negative diagnostic marker specific for prostatic adenocarcinoma may enhance the ability to detect limited prostate cancer and reduce errors in diagnosis. The recent discovery of the 34βE12 in prostate cancer is a successful example of translating an advanced molecular finding into clinical practice. **Results:** Among 37 cases 19 were prostatic cancer, 5 were prostatic intraepithelial neoplasia, 1 case was atypical foci, and 9 were benign prostatic hyperplasia cases. 34βE12has been proven to be one of the few biomarkers that can help distinguish cancer from benign cells, with high sensitivity and specificity for prostate carcinoma. This study focuses on the study of 34βE12 expression in prostate cancer, premalignant lesions, benign prostate tissues, and other normal and malignant tissues and a discussion of its clinical usefulness. **Conclusion:** The present study recommends the interpretation of the 34βE12 immunohistochemical results in routine surgical pathology practice and also discuss the potential future applications of this marker in diagnosis of various lesions.

Keywords: 34βE12, Basal cell marker, Benign prostatic lesion, Immunohistochemistry, Prostate cancer.

Introduction

Prostate cancer is the second most frequent malignancy (after lung cancer) in men worldwide, In 2018 a total of 12,76,106 new cases and 3,58,989 deaths were reported worldwide which was 3.8% of all deaths caused by cancer in men [1,2]. Its incidence has steadily risen with time. This is attributed to the increased life span and also to the westernisation of lifestyle typified by diet with high calories and inadequate exercise to the body [3]. Serum PSA is increasingly being used as a screening tool.

Consequent to it, prostate needle biopsies are increasingly performed in men. Increased prostate-specific antigen levels increases needle biopsies, for the exclusion of prostate cancer. Prostatic needle biopsy is the preferred method.

It has fewer side effects, and helps with accurate information regarding degree of tumour extension. The grade of tumour is also diagnosed with precision.

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Prostate carcinoma – pathologic features

Gross findings

- Firm, solid, white grey to yellow orange in contrast to tan, Spongy benign prostatic tissue
- PSA-detected cancer often not grossly visible

Microscopic findings of Prostatic Carcinoma

Architecture features:

Haphazard glandular arrangement; infiltrative growth; less differentiated glands with cribriform, fused glands, cords, sheets, or single tumor cell. Typically, small glands with straight luminal border.

Cytologic features:

Pale to amphophilic cytoplasm; no lipofuscin pigment.

Nuclear features:

Enlargement, hyperchromasia, variably prominent nucleoli.

Cancer-specific features:

Mucinous fibroplasias (collagenous mironodules); glomeruloid formation; perineural invasion.

To diagnose prostate cancer, no specific single histologic feature is sufficiently available. The combination of architectural and cytologic change gives the diagnostic clue [4]. There are numerous benign mimickers posing as prostate cancer. These include benign conditions including atrophy, basal cell hyperplasia, small crowded glands and inflammatory atypia [4]. Wrong diagnosis leads to serious issues, like radiation induced adverse effects, prostatectomies done unnecessarily because of falsely positive diagnosis. Also, falsely negative results cause delay in early effective treatment. Hence, definitive diagnosis with the available specimen is essential for the benefit of patients [4].

The present study was formulated with aim to study the Expression and Diagnostic utility of Immunohistochemical marker 34βE12 in various Prostatic lesions.

Materials and methods

Study Design: Prospective study

Study Period: From August 2011- July 2012

Study Place: Coimbatore Medical College Hospital, Coimbatore

Sample size: A total number of 37 cases were taken up for the study. Samples were selected from case records, brief clinical data which included age, presenting complaints, digital rectal examination (DRE) findings, serum PSA levels and clinical diagnosis. Among 37 cases 29 needle biopsies and 8 TURP (Trans Urethral Resection of Prostate) specimens were analysed.

The following inclusion and exclusion criteria were adopted in selecting the samples

Inclusion criteria:

1. All prostatic specimens- needle biopsies, TURP-transurethral resection of prostate and radical prostatectomy specimens.
2. Patients in all age groups

Exclusion criteria:

1. Ill fixed samples
2. Inadequate sample

Methods:

The received samples were then fixed in 4% formalin, embedded in paraffin and stained with H&E. After eosin and haematoxylin staining all slides were reviewed by pathologists and assigned to the following groups - Benign prostatic hyperplasia (10), Basal cell hyperplasia (1), PIN (5), malignant (20) and suspicious (1).

Procedure of Immunohistochemistry

The blocks from control and selected cases were cut and mounted on poly l- lysine coated glass slides. Blocking of endogenous peroxidase activity was done by 0.3% hydrogen peroxide in methanol, freshly prepared, for twenty minutes. Then, epitope retrieval by heat was performed by using buffer of Tris EDTA at pH 9.

Immunohistochemistry was done by utilising a monoclonal anti-HMWCK antibody (clone no 34βE12 of 1:50 dilution.

Interpretation of Immunohistochemistry:

The basal cell marker of benign prostatic glands are high molecular weight Cytokeratin.

Positive staining - Cytoplasmic or membrane staining with discontinuous/continuous staining.

Negative staining - No cytoplasmic or membrane staining

Results

A total of 37 cases were selected as per inclusion and exclusion criteria. Among 37 cases 19 were prostatic cancer, 5 were prostatic intraepithelial neoplasia, 1 case was atypical foci, and 9 were benign prostatic hyperplasia cases. (Table 1) and graphically represented in Fig 1.

Prostate carcinoma

Immunostaining with 34βE12 confirmed that basal cells were absent in the cancer focus in all 19 cases of prostatic carcinoma.

Prostatic Intraepithelial Neoplasia

Staining with 34βE12 highlighted the basal cell layer in 3 out of 5 cases.

Atypical focus suspicious of malignancy

Negative staining with 34βE12 in basal cells. Thus it was diagnosed as positive for malignancy.

Benign prostatic Hyperplasia

Among 9 cases categorised as Benign prostatic Hyperplasia, 8 cases showed positivity for 34βE12 in benign glands and 1 out of 9 showed negativity for 34βE12.

Table 1: Expression of 34βE12in various prostatic lesions.

	No of cases	Positive	Negative
Prostatic Carcinoma	19	0	19
PIN	5	±3	2
ASAP	1	0	1
BCH	1	1	0
BPH	9	8	1

±indicates focal and discontinuous positivity, BPH: Benign Prostatic Hyperplasia, BCH: Basal Cell Hyperplasia, PIN: Prostatic Intra epithelial Neoplasia, ASAP: Atypical Small Acinar Proliferation.

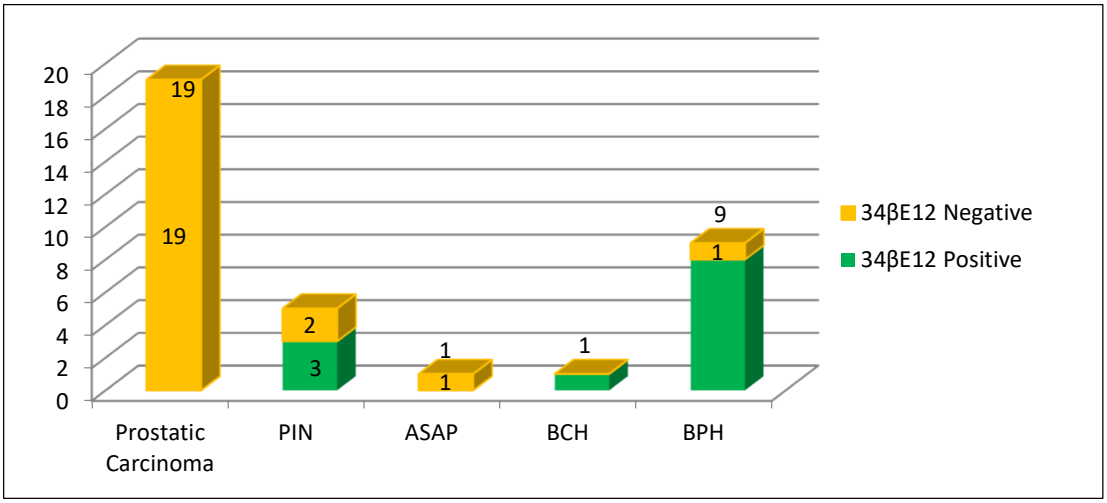

Fig-1: Expression of 34βE12 expression in various prostatic lesions.

The immunohistochemical profile of 34βE12 expression in various prostatic lesions is given in Figures 2, 3, 4, 5, 6, 7, 8, 9,10, 11,12 and 13.

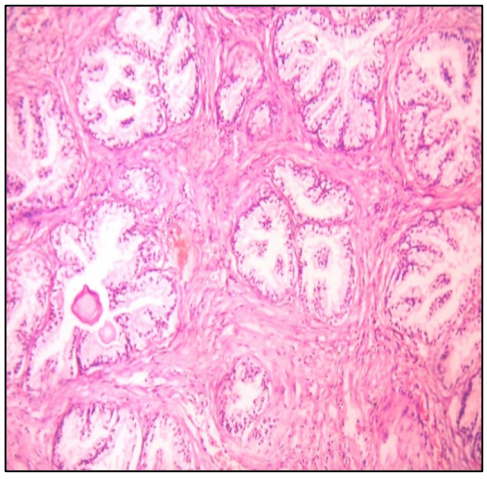

Fig-2: H & E shows Benign prostatic Glands with secretions inside it with fibro muscular stroma. 10X.

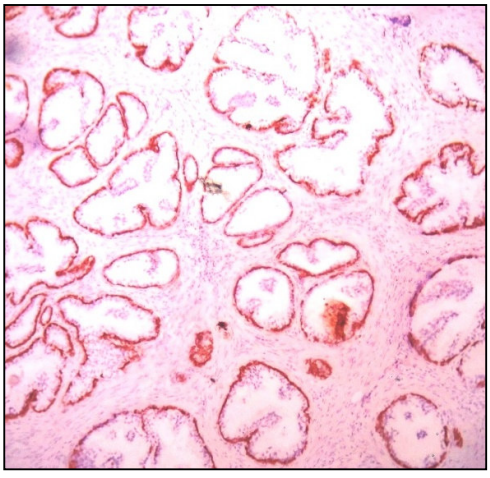

Fig-3: Continuous 34βE12 positivity of basal cell layer in Benign prostatic Glands. 10X.

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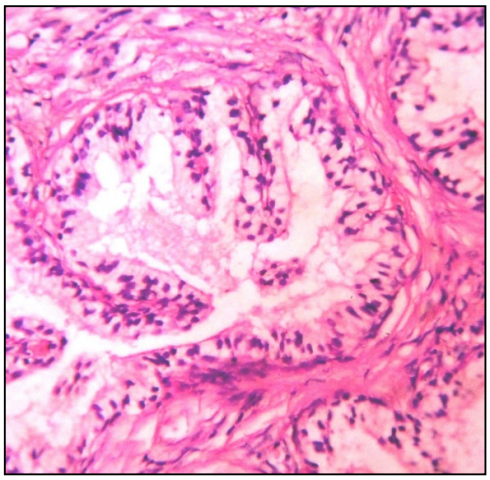

Fig-4: H&E shows Benign prostatic Glands with secretions inside it with fibro muscular stroma .40X.

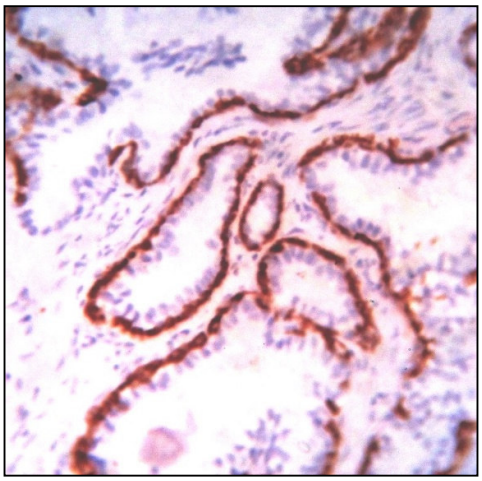

Fig-5: Continuous 34βE12 cytoplasmic positivity of basal cell layer in Benign prostatic Glands.40X.

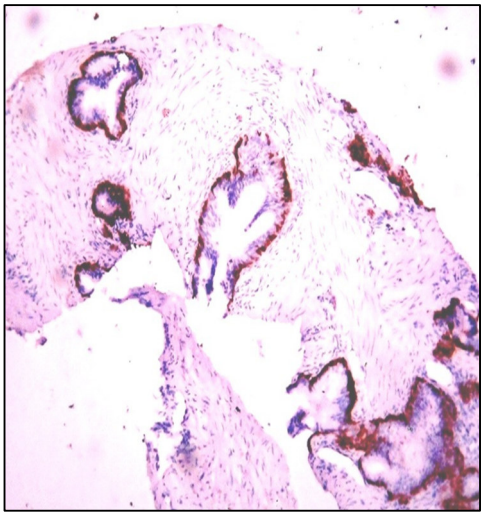

Fig.6: Continuous 34βE12 positivity of basal cells in benign prostatic glands.10X.

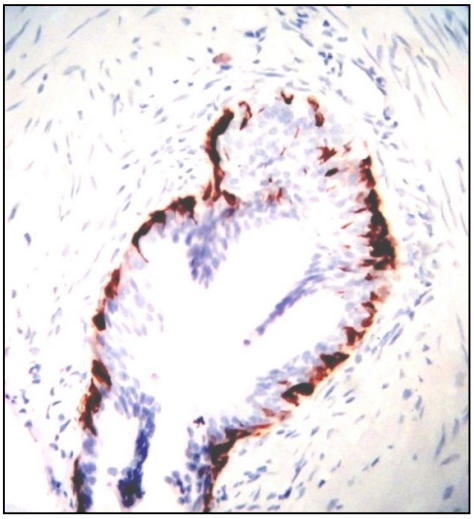

Fig.7: Continuous 34βE12 positivity of basal layer in benign prostatic glands.40X.

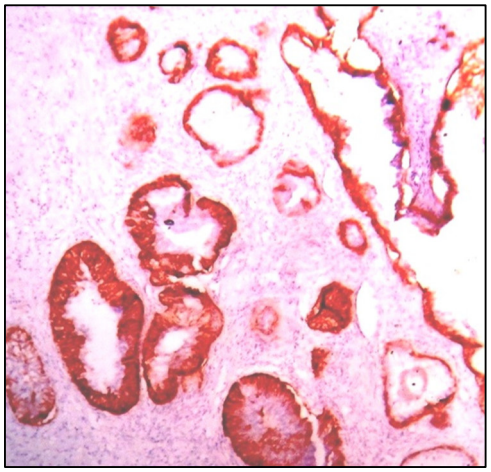

Fig.8: Continuous positivity of 34βE12 in Basal cell Hyperplasia shows multi layering of basal cells .10X.

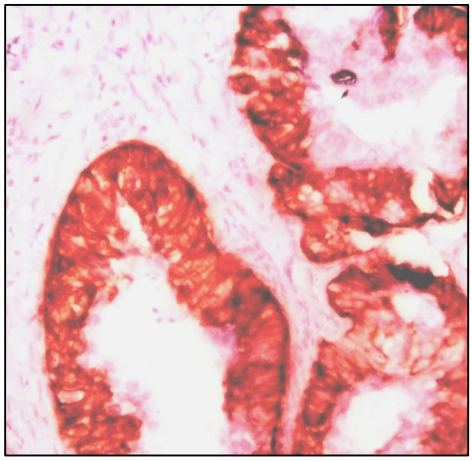

Fig.9: Continuous positivity of 34βE12 in Basal cell Hyperplasia shows multi layering of basal cells .40X.

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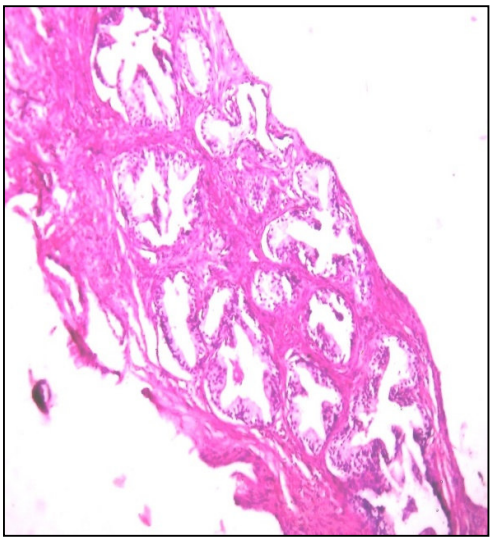

Fig.10: H&E. Low grade Prostatic Intraepithelial Neoplasia.10X

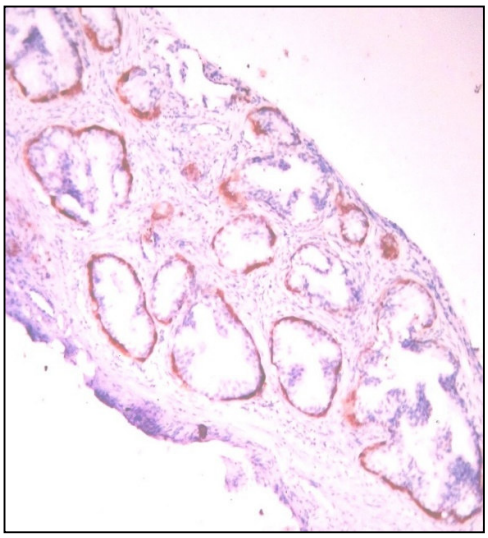

Fig-11: Discontinuous 34βE12 positivity of basal cell layer in Low grade Prostatic Intraepithelial Neoplasia.10X.

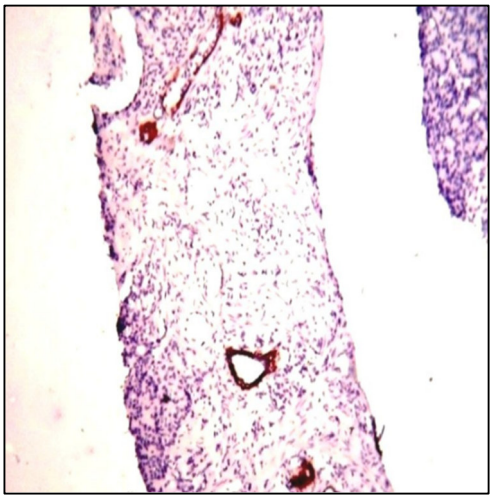

Fig-12: Continuous 34βE12 positivity of basal cell layer and adjacent malignant foci shows negativity of 34βE12 in Prostatic carcinoma.10X.

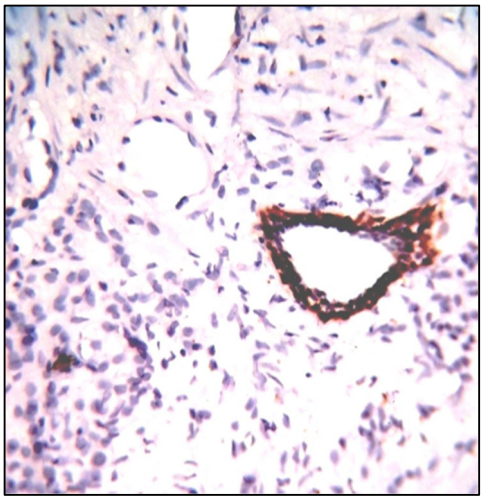

Fig-13: Continuous 34βE12 positivity of basal cell layer and adjacent malignant foci shows negativity of 34βE12 in Prostatic carcinoma.40X.

Discussion

Prostate carcinoma is the most common form of extra cutaneous cancer in men and the second leading cause of death [5]. The wide use of serum prostate-specific antigen (PSA) screening has resulted in an increased detection of patients with prostate cancer [6]. Tissue examination of a prostate needle biopsy or transurethral resection specimen of prostate is mandatory for the diagnosis of prostate cancer and permits patients to receive appropriate therapy.

However, tissue diagnosis can be difficult and inaccurate if the cancer is very limited, because the establishment of a pathologic diagnosis requires the presence of a combination of multiple histologic features of tumour cells such as pattern of growth, nuclear atypia, absence of basal cells, and the presence of characteristic extracellular material in malignant glands [7-9]. PSA is the most commonly used biomarker for the diagnosis and the prediction of prognosis in prostate cancer [10]. With the major effort in the early detection of prostate cancer by mass screening of men, there have been an increasing number of small foci of cancer encountered on prostate needle biopsy specimens. The diagnosis of these small foci of prostate cancer in needle biopsy specimens is one of the major diagnostic challenges in surgical pathology. Under diagnosis of a small focus of prostatic adenocarcinoma or the over diagnosis of a benign lesion mimicking cancer is not uncommon and can cause unfortunate consequences. Prostate cancer diagnosis is usually made using histological, traditional parameters, not

with any single diagnostic feature. They include nuclear features, tissue architecture and other features. In needle biopsies, tissue diagnosis of prostatic carcinoma is difficult. This is because of either the many benign mimickers of malignancy or a small focus of cancer. With the major effort in the early detection of prostate cancer by mass screening of men, there have been an increasing number of small foci of cancer encountered on prostate needle biopsy specimen [11].

Basal cells are noted in Benign glands. Prostate cancers do not contain basal cells. This helps in the diagnosis of specimens. Here comes the vital role of Immuno-histochemistry.

This is used by pathologists to diagnose suspicious lesions in small foci accurately. 34βE12 is a marker which is a high-molecular-weight cytokeratin, which takes positivity in benign glands. p63 is a newer basal cell marker. The diagnosis of prostate adenocarcinoma is supported by the basal cells' absence. However high-molecular-weight cytokeratin and p63 are negative markers for prostatic carcinoma

It has been shown that using 34βE12 as a positive marker can help to confirm the diagnosis when small atypical glands are identified by routine H&E staining [12].

Basal cell markers:

Cytokeratin is one of the main structural proteins about the human epithelial cell, and divided into the two groups; acidic type (type 1) and a (neutral-to-) basic type (type 2). The high molecular weight cytokeratin 34βE12 (CK34βE12) is recognized by the antibody, that is identified as human cytokeratin 1, 5, 10, and 14 of Moll's catalogue [13], is useful for the detection of epithelial basal cells, such as prostatic basal cells, and stratified squamous epithelium. 34βE12 is expressed in the cytoplasm of basal cells, and has been used as a marker of the basal layer to distinguish benign from malignant processes in prostate cancer [14], breast cancer [15], and basaloid carcinomas of the lung [16].

34 β E12

34 βE12 also known as CK34βE12 and keratin 903 (CK903), is an antibody specific for high weight cytokeratin (1, 5, 10 and 14) [17]. It is sometimes, less precisely, referred to as high-molecular weight keratin (HMWK) and high-molecular weight cytokeratin (HMWCK). 34βE12 is a high molecular weight cytokeratin immunochemical marker. It binds to high molecular weight cytokeratin, intermediate filament, not in luminal cells of prostate but in the basal cells' cytoplasm. 34βE12 is interpreted as positive / negative and continuous / discontinuous [18]. HMWCK (34βE12) and P63 and CK 5/6 are critical for demonstration of basal cells in benign glands. When they are seen, an invasive prostatic cancer diagnosis is very less likely. In the present study the incidence of prostatic carcinoma was common in the age group of 71-75 years. Incidence of prostatic carcinoma was 38%, prostatic intraepithelial neoplasia was 10%, benign prostatic hyperplasia was 48%. The sensitivity of 34βE12 detection of prostate carcinoma was 100% and specificity was 89%. Newer antibodies against prostatic tumour cells (p504s) and prostatic basal cells (34βE12, p63, CK 5/6) have proven to benefices.

The results showed that, for ambiguous lesions such as atypical small acinar proliferation, small foci of prostatic carcinoma not diagnosed, but suspected to be malignant can be benefited by the use of these markers. Immuno-histostaining with 34βE12 and p504s has improved diagnostic utility in microscopically difficult cases.

It helps to avoid newer and subsequent prostatic biopsies, which are costlier and causing morbidity in the patients. The application of these newer antibodies individually is less relevant than the combined use of these antibodies. Compared to a new series of biopsies, the cost of immunohistochemical techniques remains lower.

Limitations

For the diagnosis of prostate cancer, many limitations are noted in using basal cell markers. Stressing on absence of basal cell staining, a negative finding, to decide on a positive diagnosis of cancer is the most important. Also, some of benign prostatic glands (5% - 23%), some specimens of atrophy (23%), up to half of specimens of adenosis, 66% specimens of mesonephric hyperplasia, 44%-75% samples of nephrogenic adenoma may lack basal cell staining.

This is the reason why negative basal cell marker immunostaining in singularity cannot conclusively pinpoint carcinoma. Ejaculatory duct epithelium and seminal vesicle are invariably positive for basal cell markers. But the status of Cowper's glands is contradictory.

Conclusion

34βE12 has the potential to be a useful marker which can be used separately for diagnosis of prostate carcinoma in clinical pathology practice. In conjunction with the clinical scenario and morphology, use of prostatic epithelial marker 34βE12 is of better value in diagnosing the prostate carcinoma cases and other morphologically difficult lesions. The accuracy of diagnosis in prostate cancer is significantly increased. It can be inferred from this study that use of 34βE12 can be regarded as an inexpensive marker for assessing prostate carcinoma.

What the study adds to the existing knowledge?

The study shows the importance of 34βE12 in accurate diagnosis of prostate tumour which substantiates the importance of a relatively inexpensive method like 34βE12 immunohistochemical staining in tumour diagnosis and confirmation

Author's contribution

All Authors had equally contributed in every part of research like manuscript writing, data collection and statistical analysis.

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Conflict of interest: None declared
Ethical Approval: This study was approved by the Institutional Ethics Committee

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