

# Immunohistochemical Analysis of Prostein Expression in Archived Prostatic Core Biopsies from Prostate Cancer Patients in Western Kenya

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## Research Article

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# Abstract

## Background

Prostate cancer is the leading cause of cancer-associated mortality in men. Most of the current biomarkers for detection of the disease have low sensitivity and specificity. Prostein is a newly reported prostate cancer biomarkers whose diagnostic utility can help in early detection of the disease. Nonetheless, previous studies have utilized limited number of samples to evaluate its immunohistochemistry (IHC) and reports on the African population are not available. The current study aimed to determine the prostein expression in archived prostatic core biopsies from prostate cancer patients in Western Kenya.

## Materials and Methods

This was a retrospective study conducted on malignant and benign prostatic tissue core biopsies of 106 patients who underwent prostate core biopsy at Jaramogi Oginga Odinga Teaching and Referral Hospital and division of urology at Synergy Clinics, Kisumu between January 2018 to May 2021. Immunohistochemical technique was performed on each of the 106 samples and on the following non-prostatic male control biopsies; Testis, Penis, Liver and Esophagus. Cellular location of prostein staining was evaluated at X40, X100 and X400 magnification using a light microscope and was classified as cytoplasmic or nucleocytoplasmic. Intensity of prostein expression was assessed for each core biopsy at similar magnification and graded according the immunohistochemistry composite score.

## Results

The biopsies had been obtained from men whose mean (SE) age was  $72.00 \pm 0.93$  years. 95.3% (101) of the biopsies were malignant and 4.7% (5) were benign. Four non-prostatic male tissues were included. 97% of malignant and all the benign prostate tissue stained positive for prostein whereas the four non-prostatic male tissues were negative. Staining intensities were weak (24.5%), Moderate (17.0%), strong (55.7%) and non-stained (2.8%). The staining was highly immunolocalized within the cytoplasm (95.1% cases) as compared to nucleocytoplasmic (2.0% cases). The mean immunoreactivity composite score was  $1.91 \pm 0.96$  (0.0-3.14). Strongly stained sections had a punctate plasma membrane staining pattern clustered within the cytoplasm in a perinuclear location whereas the weakly stained sections had faint and punctate coarse brown cytoplasmic granular appearing.

## Conclusion

Prostein is exclusively expressed in benign and malignant prostate tissue with a higher cytoplasmic granular staining pattern in the present population. These findings suggest that prostein diagnostic utility

is applicable in the current study population and routine IHC diagnosis of prostate cancer may be recommended.

## Introduction

Prostate cancer (PCa) is the most frequent malignant tumor in the male population worldwide, and has remained one of the leading causes cancer-associated mortality in men (Bray *et al.*, 2018; Ferlay *et al.*, 2019; Siegel, *et al.*, 2020 & Cimadamore *et al.*, 2021). In East Africa, prostate cancer ranks third in both incidence and mortality, and leads to an estimated 9,000 (9% of all male cancers) cases and 7,300 (8.5% of all male cancer) deaths annually (Ferlay *et al.*, 2011). It is significant to note that PCa incidences increased by 64.5% between 1990 and 2010 (Lozano *et al.*, 2012). In 2019, the prevalence of Pca in Western Kenya was at 7.0% (Macharia *et al.*, 2019; Wambalaba *et al.*, 2019 & Luke, 2019). However, the incidences and the mortalities of the prostate cancer are extremely variable worldwide with higher incidence rate occurring most among the Africa-American Men as compared to the white (Panigrahi *et al.*, 2019). The clinical, natural history and pathological behavior of the disease has also been reported to be variable (Ouattara *et al.*, 2012).

Presently the diagnosis of prostatic cancer relies on histopathological features (Stage and Gleason score) supported by combination of Prostate Specific Antigen (PSA) levels and imaging techniques (Garudadri *et al.*, 2020). However, information such as PSA level, cancer stage, and Gleason score, are limited in their ability to determine the disease severity thus complicating most of the clinical decisions (Nayaran *et al.*, 2017). Both PSA and Gleason score are not able to clearly distinguish between indolent and aggressive cancers since tumors with similar histological patterns may have different clinical outcome (Shen and Abate, 2010; Squire *et al.*, 2011 and Schoenborn *et al.*, 2013). This has led to overtreatment and unnecessary biopsies in some cases. PSA also often fails to indicate accurately which patients are responding to a given treatment and is sometimes decreased or increased in higher grade or metastatic tumors. As a biomarker PSA lacks specificity and sensitivity (Epstein *et al.*, 2014 & Romero *et al.*, 2014). Due to increased number of specimens with limited number of suspicious glands and minimal atypia (Magi-Galluzzi, 2018), histomorphological findings in the biopsied tissues sometimes are difficult to report. Distinguishing between aggressive and indolent tumors is a major challenge (Sequeiros *et al.*, 2013). Moreover, differentiating between high-grade urothelial carcinoma (UC) and high-grade Prostate adenocarcinoma (PAC) is frequently a diagnostic and prognostic challenge. According to recent European guidelines, PCa validated biomarkers are urgently needed for guiding the pre-treatment decision processes (Mottet *et al.*, 2017). These scenarios can be improved by using Prostate specific and sensitive immunohistochemical biomarkers, which would adjunct PSA levels and help the pathologist to make much more accurate differential diagnosis.

Basal cell markers (high molecular weight cytokeratins, p63, CK5/6) and  $\alpha$ -methylacyl-CoA racemase (AMACR) are presently in use for confirmation malignancy. Prostate-specific antigen (PSA), prostate-specific acid phosphatase (PSAP), and prostate-specific membrane antigen (PSMA) are used to confirm the prostatic origin of the tumor (Adisa *et al.*, 2015 & Kuroda, 2014). Other innovative prostate cancer

diagnostic biomarkers include, 4K Score, a Kallikrein markers (Vickers *et al.*, 2010), DNA (hyper) Methylation via Confirm MDX<sup>R</sup> (Chan *et al.*, 2003), and prostate cancer specific Antigen 3 which measures PCA3 mRNA in first void and post DRE urine (Wei *et al.*, 2017). Although most the prostate markers show excellent specificity, the sensitivity and percentage positivity vary (Chuang *et al.*, 2007).

Prostein (also known as prostate cancer-associated protein 6 / P501S / SLC45A3) is a protein present in the Golgi apparatus of benign and malignant prostatic glandular epithelium. It is encoded by the Solute carrier family 45, member 3 (SLC45A3) gene, an androgen-regulated gene and a prostate specific marker expressed in prostatic glandular cells (Xu *et al.*, 2001). It shows perinuclear cytoplasmic localization in immunohistochemical experiments (Xu *et al.*, 2001; Sheridan *et al.*, 2007). Because it is highly specific for prostate glandular cells, this target is useful for differentiating prostatic metastases from other carcinomas such as urothelial carcinomas or colorectal carcinomas (Xu *et al.*, 2001; Lane *et al.*, 2008; Chuang *et al.*, 2007; Sheridan *et al.*, 2007). Although it may show diminished expression in some aggressive prostate cancers, this target is sometimes expressed in PSA-negative prostate carcinomas, and these two targets used in combination can lead to increased sensitivity in the identification of prostate cancer metastases (Perner *et al.*, 2013; Sheridan *et al.*, 2007). Most cohorts for evaluation of the current diagnostic biomarkers are restricted to the Caucasian Population with little or no representation of other geographic, ethnic population and then directly applied in general way to other population irrespective of their genetic variability (Narayan *et al.*, 2017).

Since prostate cancer is highly heterogeneous and its incidence and mortality vary strikingly among ethnic, racial, and national groups and this is an area worth exploring. native black African patients. There have been no reported studies on manual immunohistochemical diagnostic utility of Prostein in detection of prostate cancer among the African populations. The current study aims to evaluate manual immunohistochemical expression levels of Prostein, P501s, in archived formalin fixed paraffin embedded (FFPE) prostatic core biopsy specimens consecutively collected between January 2018 and May 2021 from prostate cancer patients in Western Kenya.

## Materials And Methods

This study involved 106 archived formalin fixed paraffin embedded prostatic core biopsy specimens consecutively collected between January 2018 and May 2021 from prostate cancer patients at Jaramogi Oginga Odinga Teaching and Referral Hospital, Pathology Department and Division of urology at Synergy Clinics, Kisumu. All the clinical prostate tissue samples used in this study were accompanied by demographic information and pathological reports such as Tribe, Age, and type of malignancy. Non-prostatic tissues from the neighboring organs such as Testis, Penile; distant organs such as Liver and Oesophagus were included in the study as control specimen.

Following approval by Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics and review committee (IERC/JOORTH/353/2021); the eligible patients or their families were contacted and consent obtained via recorded telephonic interview and their demographics obtained as well as their

histopathology reports retrieved from the hospital laboratory management information system. The tissue blocks, selected using non-probability approach, were retrieved from the laboratory archive, deidentified, sectioned and manually stained using protein immunohistochemical stains.

## **Immunohistochemistry**

Immunohistochemistry (IHC) was performed manually following manufacturer's instructions. The FFPE tissue specimens were cut into sections of 4 µm using Micros Razor rotary microtome. After preparation of the tissue, the sections were mounted on charged poly-L-lysine FLEX IHC Microscope Slides (Code K8020) flat and wrinkle-free. The tissue sections mounted on the slides were dewax at 60°C for 45 minutes in a hot oven then subjected to 3-in-1 pretreatment procedure with HIER using 1:50 diluted EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004) in Dako PT Link. Deparaffinization, rehydration and Heat induced epitope retrieval were performed in Dako PT Link at automated Pre-heat temperature: 65 °C; epitope retrieval temperature and time: 97 °C for 20 minutes; cool down to 65 °C. The slide rack was then removed from PT tank and immediately dipped into jar/tank (e.g., PT Link Rinse Station (Code PT109)) containing diluted room temperature EnVision FLEX Wash Buffer (20x) (Code K8007). The slides were left in Wash Buffer for 5 minutes.

The immunolabeling procedures and incubation times were carried out manually according to the manufacturers' instruction using Dako Envision Labelled monomer-HRP anti-mouse (Dako, Glostrup, Denmark). The protocol involved each slide being rinsed with wash Buffer for further cooling to room temperature and pressure. Thereafter, 100µL FLEX Ready to Use Primary Antibody was added to the slides and incubated for 5 minutes. This was again rinsed twice with Wash Buffer and then followed by addition of 100µL EnVision™ FLEX /HRP (RTU) and subjected to incubation for 20 minutes. The slides were rinsed in Wash buffer and incubated for 5 minutes then flooded with 200µL Envision™ FLEX Substrate working solution prepared by mixing 1ml of Substrate with one drop of 3,3'-Diaminobenzidine (DAB) and incubated further for another 20 minutes. The slides were further rinsed using wash buffer and finally counterstained using 100µL EnVision™ FLEX Hematoxylin (RTU) incubated for 20 minutes and rinsed in two changes of Deionized water and two changes of Distilled water. The incubations were done at room temperature.

After staining the slides were dehydrated in an increasing concentration of alcohol (70%, 90%, 95%, 100%, 100%), cleared in two changes of xylene and mounted using aqueous Dako Glycergel™ Mounting Medium, Code C0563 then examined and confirmed by a surgical pathologist and immunohistochemical features recorded.

A negative control, section treated with a tris-buffer solution instead of primary antibody, were run simultaneously using the same protocol as the patient specimens and control biopsies. Caution was taken not to allow tissue sections to dry out during the treatment or during the following immunohistochemical staining procedure.

## **Interpretation of the immunohistochemical stains**

Photomicrograph of prostein immunohistochemical staining pattern in PCa Benign, HGPIN and non-prostatic specimens was taken at x4, x10 and x40 objective lens using Euromex Oxion Microscope. The expression proportion and intensity of immunopositivity were scored, using a method similar to Yin et al.; Zhao et al. and Hao et al. (Yin *et al.*, 2007; Zhao, *et al.*, 2003 and Hao *et al.*, 2000). The intensity of immunopositivity was scored from 0 to 3 as follows: score 0 = non-stained; score 1 = weak; score 2 = moderate; and score 3 = strong. The percentage of positively stained cells for each staining intensity was then estimated in the respective biopsy section. The final composite score was determined after multiplying the intensity of positivity and percentage of positivity in the respective lesions (Yin *et al.*, 2007). For example, if 50% of tumor cells are scored 1 (1=Weak), 25% scored 2 (2= Moderate), and the remaining 25% scored 3 (3=Strong), the composite staining score of this case is  $[50\% \times 1] + [25\% \times 2] + [25\% \times 3] = 1.75$ . A board-certified surgical pathologists confirmed the evaluation of the specimens from this study. The data was presented as mean  $\pm$  standard errors (SE).

## Data Analysis

The frequency and descriptive statistic for age, tribe, tumor type, staining immunolocalization and intensity were analyzed using IBM SPSS version 23.0. For each dataset within groups, the distribution, mean and standard deviation within the 95% confidence limits are shown. Correlation between age and the intensity of prostein expression was evaluated by non-parametric Spearman correlation test.

## Results

In the present study, the median age of the subjects was  $72.00 \pm 0.93$  years (49.0 – 107.0) and Modal age group being 70.0 -79.0years. Majority of patients were Nilotic (81.1%). Other ethnic groups were Bantu (17.9%), and Cushites (0.9%) (Figure 1A-B).

The Population Mean Age  $72.00 \pm 9.62$  years with a range of 58 (49-107) Years. **(A)** The modal 70-79 years (35.8%). **(B)** Majority were Nilotes (81.1%), Bantu 17.9%. Prostein expression had a cytoplasmic immunolocalization in most of the prostatic core biopsies cases (95.1%) with varying degree of homogeneous staining pattern (Figure 2).

Prostein expression had a cytoplasmic immunolocalization in most of the prostatic core biopsies cases (95.1%) with varying degree of homogeneous staining pattern. Prostein was strongly expressed (55.7%), weakly expressed in 24.5%, moderately expressed in 17.0% and non-stained in 2.8% of the prostatic core biopsies. 95.3% of staining pattern was majorly cytoplasmic with 1.9% cases of Nucleocytoplasmic staining characteristics and 2.8% non-stained.

Most prevalent tumor was acinar adenocarcinoma (81.7%) followed by 5.7% intraductal Carcinoma, 1.9% High grade Prostatic Intraepithelial Neoplasms. 4.7% of the cases were Benign prostatic hyperplasia. The acinar adenocarcinoma was stained as follows; 51% strong, 23% weak, 16% moderate and 3% unstained. Intraductal carcinoma were stained as follows 2% strong, 4% moderate and 3% weak. The most common type of prostate malignancy among the prostatic core biopsies was acinar adenocarcinoma (91.2%)

followed by intraductal carcinoma (5.9%). 2.9% of the tissues had High grade Prostatic Intraepithelial Neoplasms (HGPIN) (figure 3).

Table 1  
Benign prostatic hyperplasia staining intensities

Cases	Intensities	Immunoreactive scores	Immunolocalization
1	Strong	1.3	Cytoplasmic
2	Strong	1.7	Cytoplasmic
3	Moderate	1.6	Cytoplasmic
4	Strong	2.91	Cytoplasmic
5	Strong	2.8	Cytoplasmic
Prostein was expressed within the cytoplasm in a perinuclear location of BPH biopsies.			

The sections were stained with Mouse monoclonal antibody 10E3-G4-D3 against Prostein. The intensity of the immunohistochemical analysis shows (**Arrows**) a strong punctate plasma membrane staining pattern with mouse monoclonal anti-prostein antibody, Clone 10E3. The stained areas are clustered within the cytoplasm in a perinuclear location. The staining characteristics has a completely random polarity from cell to cell but remained polarized within each cell (x400).

The intensity of the immunohistochemical analysis shows (**Arrows**) a moderate punctate plasma membrane staining pattern with mouse monoclonal anti-prostein antibody, Clone 10E3. Prostein is expressed in the cytoplasm showing circumscribed, cytosolic brown dense granular staining pattern.

The intensity of the immunohistochemical analysis shows (**Arrows**) a faintly stained punctate plasma membrane staining pattern with mouse monoclonal anti-prostein antibody, Clone 10E3. The granules are relatively faint and punctate showing focal loss of expression. However, the granules were still visible in the apical region of the cells using higher magnifications.

The intensity of the immunohistochemical analysis shows (**Arrows**) a moderately stained punctate plasma membrane staining pattern with mouse monoclonal anti-prostein antibody, Clone 10E3. The polarity of the staining characteristics appears remained polarized within each cell.

The immunohistochemical findings indicated that none of the four non-prostatic tissues (Liver Penile, Testis, and Esophagus) that were stained with mouse monoclonal anti-prostein antibody were positive for Prostein. Prostein was exclusively expressed only in the Prostatic epithelium tissues. The expression is specific for prostate and is not detected in other tumor tissues, including liver, penile, testis, and esophageal tissues (Figure 4e).

## Discussion

The findings of the present study have demonstrated that prostein, P501s, is expressed in both benign and malignant prostate tumor tissues with a brown punctate cytoplasmic staining pattern (Figure 4a-d; Table 1). The immunohistochemical staining showed stained areas clustered within the cytoplasm in a perinuclear location. This confirmed that Prostein has a cytoplasmic immunolocalization. The staining pattern corresponds to the location of Golgi complex as was similarly demonstrated by previous studies (Kalos et al., 2004 & Garudadri et al., 2020). The granular perinuclear cytoplasmic expression of prostein is pivotal feature in establishing the prostatic origin of the tumors (Srinivasan & Parwani, 2011). Prostein was expressed in majority of prostatic core biopsy tissues with different degrees of intensity ranging from weak, moderate to strong. This variation could be attributed to the tumor heterogeneity or different tumor progression. Previous high-throughput sequencing studies have demonstrated that prostate cancer is a heterogeneous disease (Kalos et al., 2004; Yin et al., 2007; Armenia et al., 2018 & Garudadri et al., 2020). The staining intensity appeared reduced in metastatic cases, a phenomenon which was similarly observed previously in studies conducted with PSA and NKX3.1 IHC stains (Bostwick et al., 1998; Renshaw *et al.*, 2000; Bowen et al., 2000 & Roudlier *et al.*, 2003). The polarity of the staining in moderately and strongly stained tissue sections were completely random within each cell (Figure 4a-b, d). However, in weakly stained cases, the granules were relatively faint and punctate, but were still visible in the apical region of the cells using higher magnifications (Figure 4c). The staining characteristics is consistent with the manufacturer's positive controlled pictorial atlas (Figure 5). The luminal epithelial cells show a moderate to strong granular cytoplasmic staining reaction. The staining characteristics from cell to cell was completely random but remained polarized within each cell.

None of the non-Prostatic specimens in our study including liver, penile, testicular and esophagus tissues showed prostein immunopositivity (Figure 4e). This is because prostein is a prostate specific biomarker and are only expressed by epithelial tissues of the prostate glands. These findings indicate that prostein is a potential biomarker for prostate cancer diagnosis. Previous studies demonstrated that prostein appears to be expressed immunohistochemically in an exclusively prostate-specific pattern and could not be detected even at mRNA expression levels in any of the non-prostatic tissues (Xu et al., 2001; Sheridan et al., 2007 and Wolfgang et al., 2000). Because it is highly specific for prostate glandular cells, it is useful as a target for differentiating extra-prostatic metastases from other carcinomas such as urothelial carcinomas or colorectal carcinomas (Xu et al., 2001; Lane et al., 2008; Sheridan et al., 2007 & Garudadri et al., 2020). Previous study by Chuang *et al.* involving 38 PCa cases, shown that prostein have an excellent specificity in differentiating prostate cancers from other urothelial cancers (Chuang et al., 2007). It is still expressed in poorly differentiated adenocarcinoma (Goldstein, 2002). Despite showing diminished expression in some aggressive prostate cancers, it's sometimes expressed in PSA-negative prostate carcinomas, and these two targets used in combination can lead to increased sensitivity in the identification of prostate cancer metastases (Sheridan et al., 2007; Perner et al., 2013 & Amin *et al.*, 2014). These results therefore confirm prostein as a prostate-specific marker with potential utility in the diagnosis of prostatic origin of metastatic adenocarcinomas among the African population.

There was no statistical correlation between age and the intensity of prostein expression as evaluated by non-parametric spearman's analysis. The age group was between 70 and 79 (mean 72.22±0.96) years



and is in contrast with earlier studies (Madani et al., 2011 & Rashed et al., 2012). The photomicrographs taken at different objective lenses showed excellent comparison of the cytoplasmic immunolocalization of the marker. However, preanalytical factors such as Ischemic time, amount and duration of fixation as well as tumor heterogeneity seems to affect the prostein staining characteristics. This results into non-staining features of some areas or sections (2.8%). Prescott and colleagues attributed 42.1% of the diagnostic discrepancies in immunohistochemistry to poor antibody selection (Prescott et al., 1995). Nevertheless, according to the findings from this study, prostein immunohistochemistry can be used even in resource limited setting as adjunct prostate cancer specific and sensitive biomarker. This will enhance both the accuracy and reduce the false positive and negative diagnostic rates of poorly differentiated prostate cancer. While Prostein immunostaining may be of value for the differential diagnosis of clinically significant Prostate cancer in H&E diagnostically challenging cases, a correlation with the Gleason grades should be considered.

## Conclusion

Our findings indicate that Prostein, P501S, is exclusively expressed with an excellent brown punctate cytoplasmic granular staining pattern in prostate tissues of African Men. We propose the utility of Prostein as an additional marker in the diagnosis of poorly differentiated prostatic carcinoma of unknown origin. Further studies can be conducted on the prostein expression and staging pattern of prostate adenocarcinoma and as a target for development of prostein-specific antibody therapeutic regimen for prostate cancer among the African population.

## Declarations

### Authors

1. Tyrus Omondi Swaya: Concept development, Design of the work; the acquisition, analysis, interpretation of data (Figure 1-3), and have drafted the work and substantively revised manuscript writing. Also ensured that all listed authors have approved the manuscript before submission.
2. Dedan Opondo: Supervision and Uro-oncologist; Design of work, Data analysis, Sample retrieval, substantively revised Manuscript reviewing and have substantially modified version that involves the author's contribution to the study. He has also agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.
3. David O. Atandi: Immunohistochemical staining protocol and analysis of the staining intensities and substantively revised manuscript writing. He has also agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of

the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

4. Benard Guyah: Supervision; Immunological concept development, Proposal writing, Data collection, Data analysis (Figure 1-3), and substantively revised manuscript writing and modified version that involves the author's contribution to the study. He has also agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

5. Ng'wena Gideon Magak: Supervision; Physiological concept development, Proposal writing, Data collection, Data analysis (Figure 1-3), and substantively revised manuscript writing and modified version that involves the author's contribution to the study. He has also agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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### **Conflict of interest**

The authors declares that there was no conflict or competing of interest.

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### **Ethical Statement**

All the methods were performed in accordance with the relevant guidelines and regularities as stipulated by Jaramogi Oginga Odinga Teaching and Referral Hospital Ethics and review committee (IERC/JOORTH/353/2021) approval. All the eligible participants or their families were contacted

and consent obtained via recorded telephonic interview. Their demographics were obtained for the retrieval of their tissue blocks as well as their histopathology reports retrieved from the hospital histology laboratory and management information system.

## Data Availability

The datasets generated and analyzed during the current study are available as additional supporting files, in spreadsheet format alongside other supporting documents. Part of the data contain information that would compromise research participant privacy thus are available on the manuscript as pictorials.

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## Figures

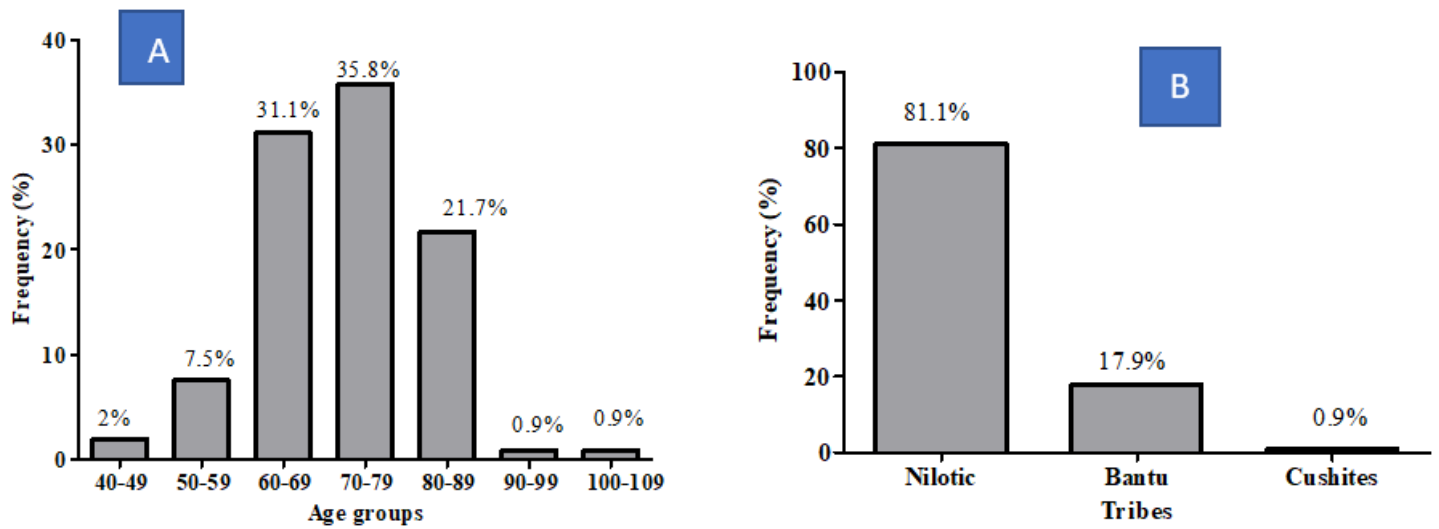


Figure 1

Demographic Characteristics of the population as percentages of the cases.

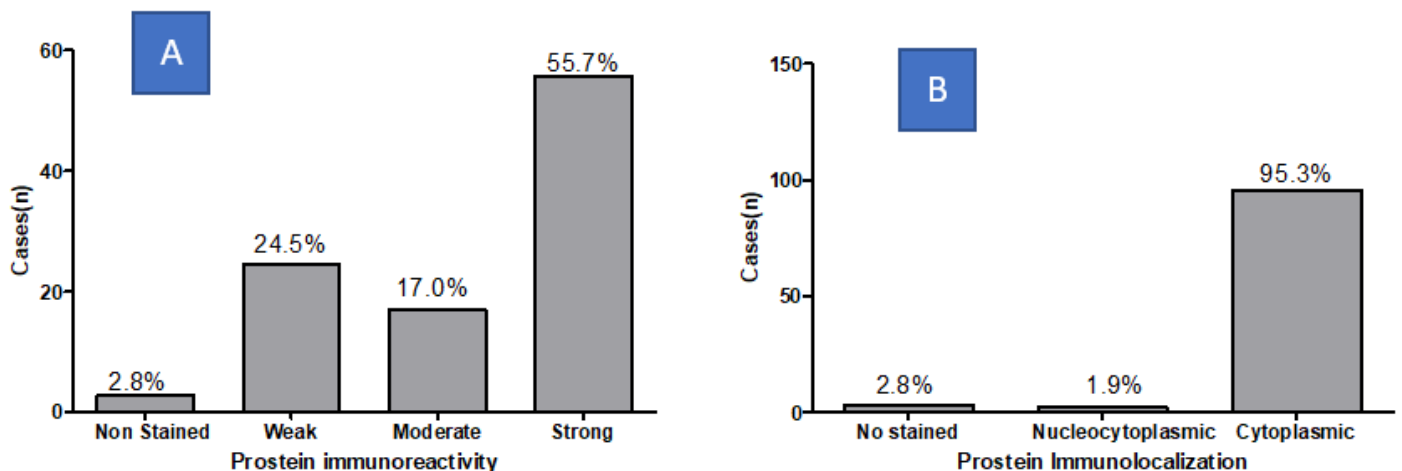


Figure 2

Frequency distribution of prostein immunohistochemical staining intensity (A) and location (B) in malignant biopsies

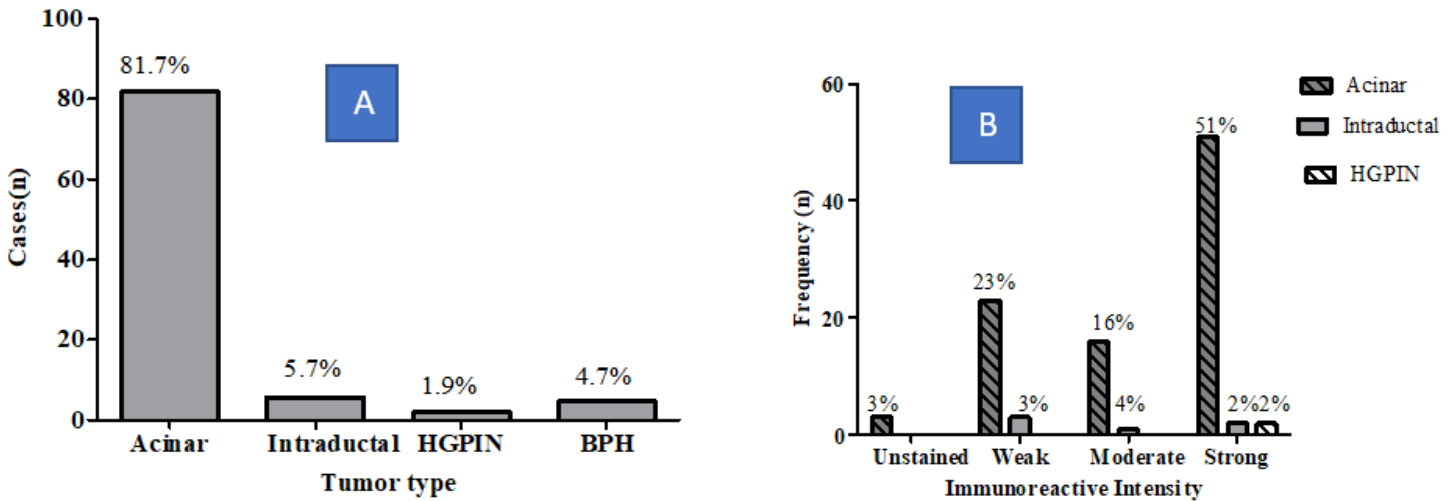


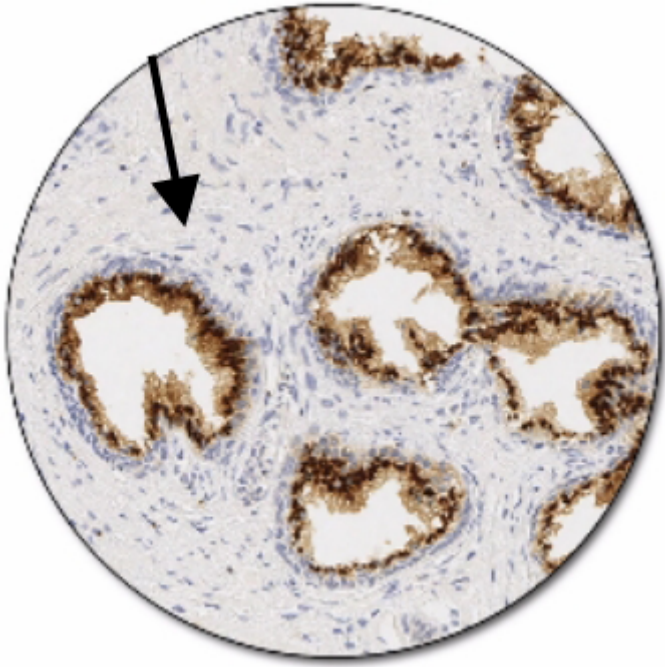
Figure 3

Tumor type (A) and Immunoreactivity (B).

Figure 4

a: Photomicrograph of Strong Prostein immunohistochemical expression pattern in PCa specimen at x40, x100 and x400 Magnification using Euromex Oxion Microscope. b: Photomicrograph of Moderate Prostein immunohistochemical staining pattern in PCa specimen at x40, x100 and x400 objective lens using Euromex Oxion Microscope. c: Photomicrograph of weak Prostein immunohistochemical expression in PCa specimen at x4, x10 and x40 objective lens using Euromex Oxion Microscope. d: Strong immunohistochemical expression pattern of prostein in Benign Prostatic hyperplasia core biopsy sections at x40, x100 and x400 objective lens using Euromex Oxion Microscope. e: Prostein Immunohistochemical staining on metastatic carcinomas of Penile, benign testicular tissue, benign tissue, esophageal squamous carcinoma, and hepatocellular carcinoma.





**Figure 5**

**Immunohistochemical expression pattern of prostein, Clone 10E in normal prostate:** Luminal epithelial cells show a moderate to strong granular cytoplasmic staining reaction ([www. Dako/Agilent/Prostein](http://www.Dako/Agilent/Prostein)).