# *CK5/6, CK7 and* E-Cadherin*;* Molecular Differentiation of PCa and BPH Tissue Biopsies

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## Abstract

**Background**: Two major disorders of the prostate are prostate cancer (PCa) and Benign prostatic hyperplasia (BPH), both of which involve rapid proliferation of cells in the prostate. The clinical manifestations of prostate cancer result from the effects of local growth of the tumor, the spread to regional lymph nodes via the lymphatic system, and the hematogenous dissemination to distant metastatic sites. Although most patients with early-stage prostate cancer are asymptomatic, locally advanced disease can lead to obstructive or irritative voiding symptoms that result from local tumor growth into the urethra or bladder neck, extension into the trigone of the bladder, or both making it difficult to differentiate from symptomatic BPH. However, BPH arises as spherical masses of epithelial and stromal elements from the glands lining the proximal prostatic urethra. The ratio of epithelium to smooth muscle in the prostate can vary among individuals, from 1:3 to 4:1. However, larger prostates may contain more androgen-dependent epithelial elements than smaller glands, which contain a higher proportion of smooth muscle. In either case, the outcome of BPH may be urethral obstruction induced mechanically by epithelial overgrowth and dynamically by prostatic smooth muscle contraction, or combination of the two.

**Method:** Prostate biopsy samples were collected from the pathology laboratory (4 from patients clinically diagnosed as suffering from BPH and 4 from patients clinically diagnosed with PCa. The specimens were immunohistochemically analyzed using antibodies against E-Cadherin, Cytokeratin *5/6* and Cytokeratin *7* to distinguish cell proliferation of BPH from PCa and down regulation of ECAD.

**Results and Conclusion:** ECAD immunopositivity was greatly reduced in PCa biopsies while over expression were seen in BPH biopsies. Also the CK5/6 was reduced in the PCa biopsies, indicating the tumors are of epithelial origin rather than glandular. CK7 was immunopositive in BPH biopsies indicating that the cells are muscular rather than

glandular while CK5/6 was low in BPH biopsies further elucidating the fact that increased population of cells in BPH is not a cancer and is not glandular.

**KEYWORDS:** Prostate, BPH, CK5/6, CK7, ECAD, cytoskeleton, cell adhesion and epithelium.

**Abbreviations:** BPH (Benign Prostatic hyperplasia, PCa (Prostate Cancer), CK (Cytokeratin: Intermediate filaments), LUTS (Lower Urinary Tract Symptoms).

**Introduction:** The prostate gland is a pear shaped gland covering the prostatic part of the urethra just above the perineal membrane. It has been divided into the peripheral zone which lies mainly posteriorly and from which most carcinomas arises and a central zone which lies posterior to the urethral lumen and above the ejaculatory ducts as they pass through the prostate. There is a periurethral transitional zone from which most BPH arises. Smooth muscle cells are found throughout the prostate, but the upper part of the prostate and bladder neck, there is a separate sphincter muscle that sub-serves sexual function, closing during ejaculation [1]. BPH is the most common disease amongst prostatic disorders, followed by PCa [2].The actual relationship between PCa and BPH is unclear, but it is established that both co-exist in patients and age is a predisposing factor [3, 4].

One of the methods of assessing a patient with LUTS is by digital rectal examination (DRE) to locate tumors or nodular swellings which might have resulted from either PCa or BPH [5]. If there is a suspicious mass on DRE, and the Prostate Specific Antigen (PSA) is elevated, histological confirmation of biopsied tissue has been the gold standard for diagnosis [6]. However, the trucult needle used for the biopsy or even Trans rectal ultrasound [TRUS] may miss the site of some tumors despite the fact that multiple biopsies were taken [7].

Although prostate cancer is the most frequently diagnosed cancer and the second most common cause of cancer death in men, death from this disease has decreased in the United States and in the province of Quebec by up to 22% since 1991. Because even the best treatment for advanced metastatic disease, can only prolong life by a few months. The recent decrease in death rates from prostate cancer can only be due to the treatment of early disease, which of course requires early diagnosis or screening [8].

Any technique that can improve the early detection of PCa is highly desirable. Furthermore, BPH arises as spherical masses of epithelial and stromal elements from the glands lining the proximal prostatic urethra. The ratio of epithelium to smooth muscle in the prostate can vary among individuals, from 1:3 to 4:1. However, larger prostates may contain more androgen-dependent epithelial elements than smaller glands, which contain a higher proportion of smooth muscle. In either case, the outcome of BPH may be urethral obstruction induced mechanically by epithelial overgrowth and dynamically by prostatic smooth muscle contraction, or combination of the two [10] while in PCa, the clinical manifestations of prostate cancer result from the effects of local growth of the tumor, the spread to regional lymph nodes via the lymphatic system, and the hematogenous dissemination to distant metastatic sites. Although most patients

with early-stage prostate cancer are asymptomatic, locally advanced disease can lead to obstructive or irritative voiding symptoms that result from local tumor growth into the urethra or bladder neck, extension into the trigone of the bladder, or both [10,9].

It is important to study the origin and nature of cell proliferation in both PCa and BPH [4]. This will enable early differentiation and detection of early cancer for better management. Since tumor cells will often alter the surface proteins, a much more stable marker within or on the surface of the cell should be labeled in order to identify and characterize the cell. Alterations in shape of tumor cells have made this difficult. The Intermediate filaments of the cell (Cytokeratin) are of 20 types (CK1 to CK20), and are expressed in different cell types of the prostate [11]. CK7 can be observed in non-keratinized epithelium and is used in distinguishing endothelioma from clear cell adenocarcinoma; the glandular epithelial cell of the prostate does not express CK7 normally- from developmental origin, the glandular tissue will express CK7 [12, 13]. Since a major feature of cancer cell is alteration of adult mature proteins or re-expression of embryonic or neonate proteins, CK7 immunopositivity in the prostate is an important marker for PCa of glandular origin [14, 15]. Differential expression of CK7 in clinically diagnosed BPH biopsy is important in understanding possible cell dysregulation that may predispose a BPH patient to PCa, if any [16, 27].

Cytokeratin 5/6 (Ck 5/6) has been observed in vast majority of malignant tumors involving mesothelia cells. It is an important distinguishing factor between mesotheliomas and adenocarcinomas in the prostate gland [16]. CK 5/6 immunohistochemistry is thus an important tool for the identification and characterization of “Basal cell carcinoma” and can be used in the finger-printing of carcinomas in general [17]. Keratins are structural proteins found in epithelial cells and they represent the most stable class of intermediate filaments. They span the cell surface cell from one end to another contributing to cellular integrity thus having both basic and practical implications if compromised.

In this study, differential expressions of CK5/6 and CK7 have been used to demonstrate epithelial and secondary carcinomas of different origins [12]. E-Cadherin (ECAD) expression correlates with epithelial differentiation while loss of ECAD will cause epithelial dedifferentiation and invasion by cancer cells, absence of ECAD in immunostaining has been demonstrated in various animal models for prostate cancer [18]. The observation that E-Cadherin is often lost in most tumor cells prompted an examination of the functional role of ECAD in the progression of neoplasms [19, 20]. This study seeks to compare the differential expression of CK5/6 and CK7 between PCa and BPH biopsies so as to detect occult malignancy in a prostatic tissue. Also to examine the possibility of expression of this early tumor markers in BPH biopsies to confirm whether there is a predisposing factor or not and to examine ECAD expression in both PCa and BPH biopsies as a distinguishing factor for epithelial compromise typical of malignancy.

**Materials and Methods**

**Tissue Processing:** BPH and PCa samples (biopsies) were obtained from patients clinically diagnosed and histologically confirmed to have the condition(s) following ethical guidelines approved by the University Teaching Hospital. The biopsies were fixed in formalin (4BPH and 4PCa) and processed histologically to obtain paraffin wax embedded sections at the pathology laboratory of Ekiti State University Teaching Hospital, Ado-Ekiti,Nigeria.

**Histology:** Tissue sections were processed for routine Hematoxylin and Eosin following the methods of [21] to demonstrate the general morphology of the tissues and vessels in the tissue (small arteries).

***E-CAD*:** This was immunolabelled in the endothelium of the blood vessels and muscular part of the prostate. This was done using anti Human-*ECAD* Monoclonal antibody (Bioflow, India) [anti human ***ECAD-1*** monoclonal diluted in phosphate buffered saline (PBS) at 1:100].

**Anti Cytokeratin 5/6**: This was immunolabelled in the muscular, ductal system and glandular epithelium to distinguish the PCa as a mesotheliomas rather than an adenocarcinoma. Also to map the distribution of Ck 5/6 in PCa as against BPH.

**Procedure:** the paraffin wax embedded sections were mounted on a glass slide in preparation for antigen retrieval where the slides were immersed in urea overnight and then placed in a microwave for 45 minutes to re-activate the antigens and proteins in the tissue sections. Primary antibody treatment involved treating the sections with biotinylated goat serum for one hour following which the sections were transferred to 1% bovine serum albumin (BSA) to block non-specific protein reactions. Secondary treatment involved the use of diluted anti-ECAD, anti-CK5/6 and anti-*CK7* on the pre-treated sections for one hour. The immunopositive reactions were developed using a polymer 3'3' Diaminobenzidine Tetrachloride (DAB) with colour intensification involving the use of mathenamine silver kit. The sections were counterstained in Coomasie-G250 (brilliant blue) and treated in 1% acid alcohol (freshly prepared).

**Transformation:** Mathenamine silver intensification was used on the immunoperoxidase preparation after the peroxidase/$H_20_2$/DAB reaction has been carried out to give a brown deposit. The sections were then counterstained in Hematoxylin. The counterstained sections were washed in running tap water, thoroughly rinsed in distilled water, and placed in preheated methenamine silver solution at 60°C for five minutes. Although it could be occasionally longer if the intensification had been carried out at room temperature. In this study, to further increase the clarity, Hematoxylin was removed from counterstained nuclei with 1% acid alcohol before the silver intensification was carried out. The composition of the stock solution was 0.125% silver nitrate in 1.5% hexamine. The solution was stored at $4^oC$. Prior to use, 2ml of 5% tetraborate was added to 50ml of the stock silver solution giving a pH of 8.0, which was then filtered into a coupling jar and protected from sunlight.

**Results and Discussion:** Most of the human cancers originate from the epithelium because they consist of cells

that are characterized by a very high rate of proliferation [21]. The regular epithelium is organized by a series of intercellular junctional complexes usually the adherent junction and desmosomes that connects the cells to one another and to the cellular cytoskeleton of actin filaments and microtubules, such that the epithelium is organized into a framework of single cell layer barrier [22]. This cytoskeletal adhesion, however, is necessary to stabilize the epithelial structure. The cadherin molecules are important to maintaining the adherent junctions via calcium mediated cell-cell interaction; one of the most predominant cadherin molecules is E-Cadherin, a transmembrane protein that creates ECAD interaction between 2 cells (calcium dependent) [23]. It is logical to summarize that CK7 can mark for PCa of glandular origin, thus important in characterizing neoplasm tissue mass at the interphase of epithelium and glandular tissue (Figure 2 PCa). CK5/6 describes and differentiates epithelium cancer from glandular cancer and further reconfirms that BPH tissue which is only positive for CK7 and negative to CK 5/6 is a product of fibromuscular tissue proliferation rather glandular tissue proliferation (Figure 2 and 3 PCa). Finally, ECAD part positivity in glandular neoplastic tissue suggests tissue invasion by the cancer cells and the intact nature of others, while ECAD positivity in the epithelium of BPH tissues supports cell proliferation with intact epithelial interaction rather than malignancy and cell detachment (Table 1, Figure 4). Few cells in the BPH mass expressing CK7 suggests a triggering factor of glandular carcinoma by BPH rather than a transition of BPH tissue from hyperplasia to carcinoma or perhaps a missed focus of malignancy. Over expression of CK7 was seen in PCa biopsies (++) (Figure 2 PCa and Table 1) thus indicating the presence of a carcinoma. Based on this tissue analysis, it is possible to map the source of the tumor cells since each cell in the prostate carries a specific Cytokeratin. Also, it is important to note that CK expression in a specific tumor will be the same with that observed in their malignancies. Immunomapping of CK7 (a Type II Keratin) in BPH biopsies shows positivity at the epithelium of BPH tissues (Figure 2 BPH). This is similar to the observations that CK7 is always highly expressed around the luminal surface of epithelium found in glands like the salivary gland. It is important to state that although PCa and BPH showed positivity to CK7, the relative distribution of the CK 7 cells are in the glandular tissue of the PCa tissue while the epithelium of the BPH tissue showed increased CK7 expression (Figure 2 PCa and BPH). CK 7 was negative in the globular tissue of BPH. This further confirms that the cancer in PCa can be of glandular or epithelial origin, and if epithelial it can invade the glandular tissue as observed in Figure 2 PCa (S20). The absence of CK5/6 in certain PCa tissue site demonstrates the origin of the tumor cells are not basal cells as CK5/6 can be used in mapping basal cell carcinoma and distinguishing them from other types of cancers. This was also observed partially in the BPH biopsies, where CK5/6 positivity was restricted to specific tissue sites at the interface of the fibromuscular and glandular prostate. The positivity of Cytokeratin 5/6 was detected in 2 out of 4 cancer biopsies by immunohistochemistry while 4 out of 4 BPH biopsies were partly positive for

CK5/6 (Figure 3 PCa). This study further confirms the utility of CK 5/6 as a tool for distinguishing mesotheliomas from adenocarcinomas. The behavior of tumor cells in terms of modifying surface proteins distinguishes them from the BPH cells which remains intact in the epithelium (Brett *et al.,* 2013). It is important to compare the distribution of CK5/6, CK7 and ECAD in the PCa and BPH tissues. In PCa biopsies, ECAD distribution pattern is similar to that observed in CK7 (Figure 4 PCa and Figure 2 PCa), thus indicating that the cells have not become malignant. In BPH biopsies, ECAD showed strong immunopositivity with fine epithelial arrangement of BPH tissues (Figure 4 BPH), although CK7 expression was very positive. The ECAD+/CK7+/CK5/6-/+ nature of the BPH tissue is an indication that the BPH is of fibromuscular rather than of glandular origin and that the epithelial cells remains intact. While the ECAD+/CK7+/CK5/6- nature of the cancer cells shows that the cancer cells are of glandular origin rather than of epithelial or fibromuscular origin.

The ECAD is also a distinguishing factor for ductal and lobular cancers. Low molecular weight cytokeratins have been described as resembling a bag of marble in lobular proliferation, where ductal proliferation will show a more localized immunostaining pattern [24]. The role of ECAD and other CKs cannot be over elucidated as they play an important role in embryonic development and cell-to-cell adhesion required for synchronized growth and development [25]. They also play an important role in cell differentiation, shape, movement and communication [26]. The role of cadherin in tumorgenesis is important as tumor cells will often show deregulated cadherin expressions and constant replacement by other several cadherin family members. In this study ECAD expression levels indicates onset deregulation of cytoskeletal proteins in PCa and over expression of such in BPH; coinciding with excessive epithelial cell proliferation in BPH. CK5/6 and CK 7 are also important markers for glandular and fibromuscular cancer.

**In conclusion,** the CK5/6 and CK7 index is a very good distinguishing factor for BPH and PCa showing the difference in cell proliferation and ECAD provides the element of epithelial and tissue organization for both PCa and BPH, showing that epithelium is intact in BPH and absent in PCa, even though both are characterized by cell proliferation. This is important clinically in determining the rate of cell proliferation progression in PCa and BPH to screen tumorgenic cells. This can be used in the detection of early cancers in BPH patients where 'curative' radical prostatectomy or radiotherapy may be offered.

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**Conflict of Interest (COI) Statement:** The Authors hereby declare there is no conflict of interest associated with this study or any of the procedures and materials used for the purpose of the study

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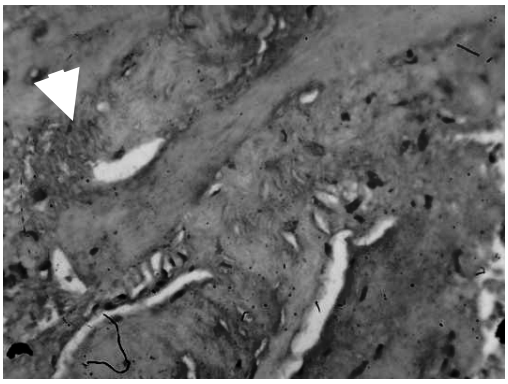
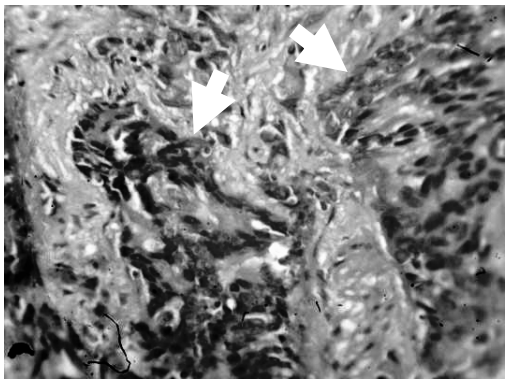
**ESULTS**



**S09**



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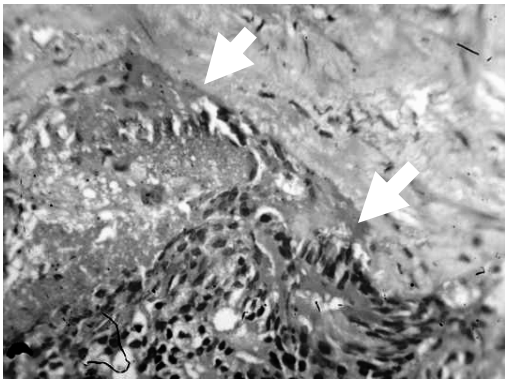

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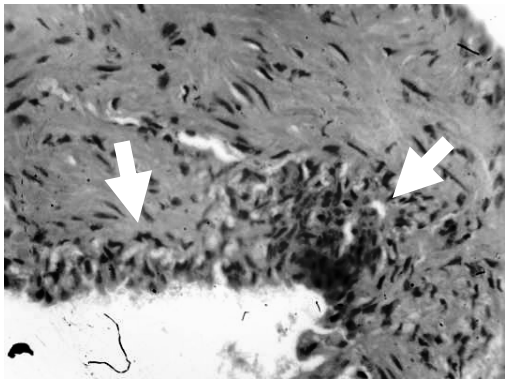

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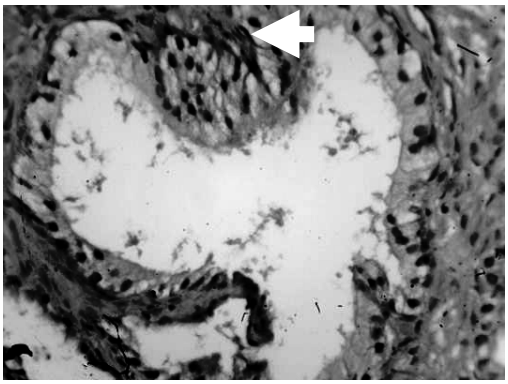
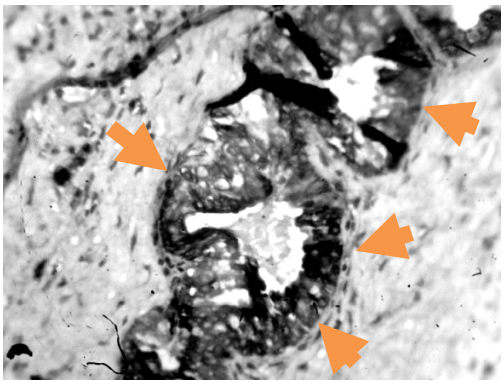


S40 (BPH)

**Figure 1**: H&E staining to demonstrate the general morphology of PCa and BPH biopsies. Proliferation and cell masses are associated with the epithelium in PCa biopsies (S09, S20 and S30), while Proliferation is seen in all parts of the tissue biopsy for BPH (S29 and S40). Magnification X400

**CK 7 PCa**



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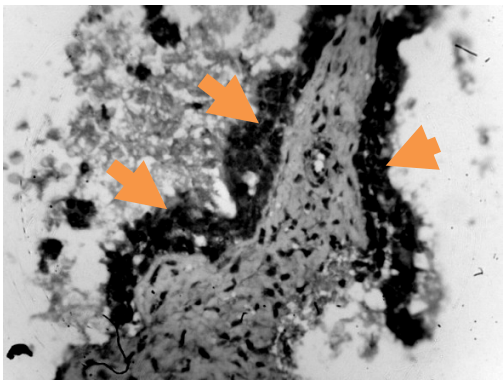

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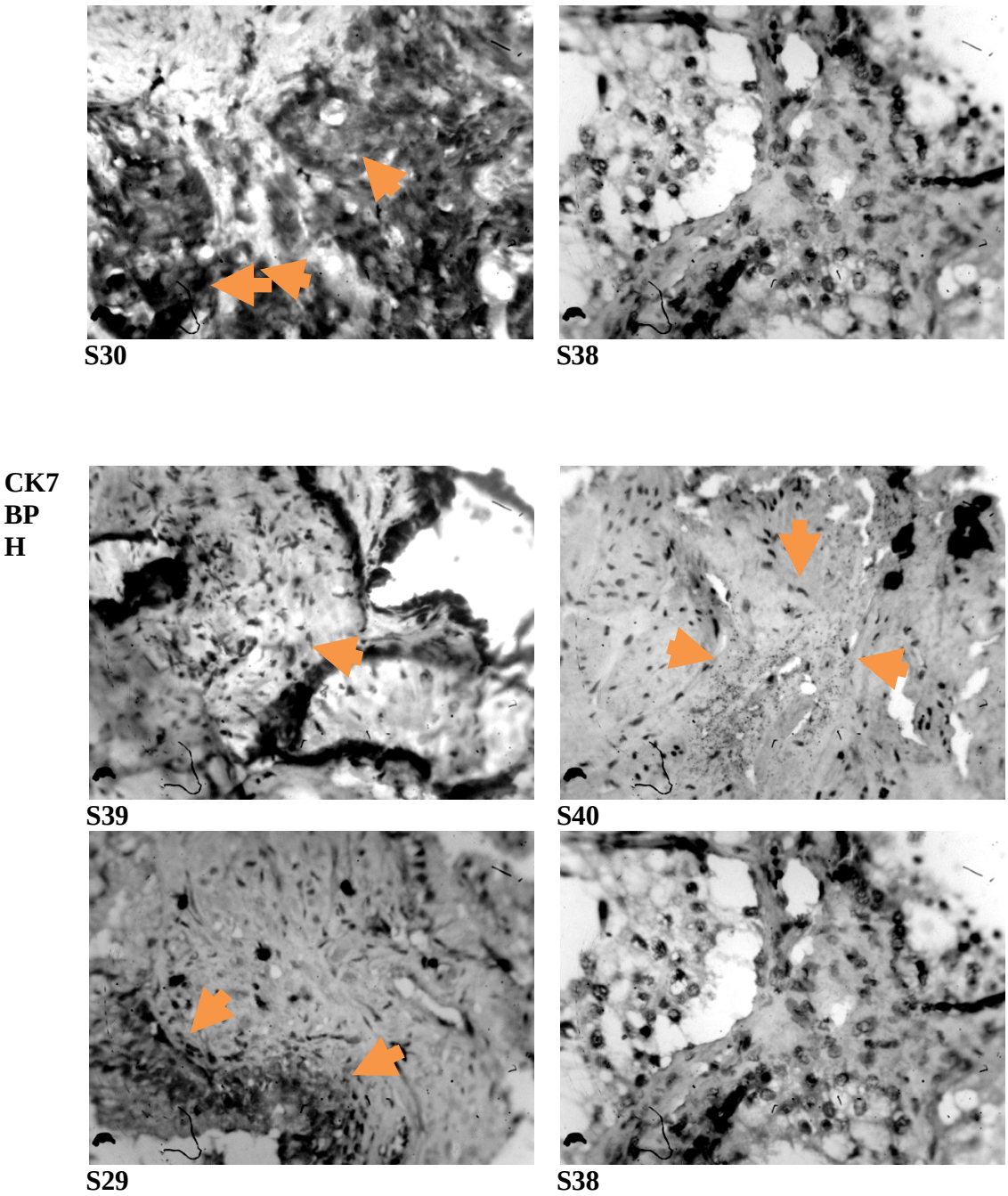
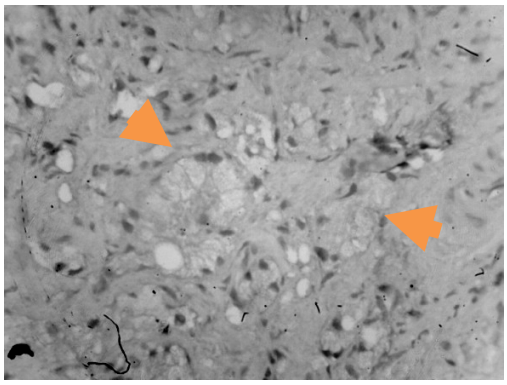



**Figure 2:** Immunohistochemical localization of CK7 in **PCa** biopsies; immunopositivity was observed in the epithelium of S09 and S20; while immunopositive cells were localized in the glandular tissue of S30 thus confirming the re-expression of CK7 in adult prostate cancer cells either of glandular or epithelial origin. S30 also shows certain level of invasiveness compared with S09 and S20 that are restricted to the epithelium. S38 represents the control testes of a patient. **BPH:** CK7 expression in BPH biopsies shows immunopositivity in cells interspersed within the BPH tissue especially around the fibro muscular layer of the prostate. CK7 expression Is peculiar to both PCa (glandular and

fibro muscular) and BPH (fibro muscular as it appears to be associated with cell proliferation in the prostate (Magnification X400).

**K5/6 PCa**



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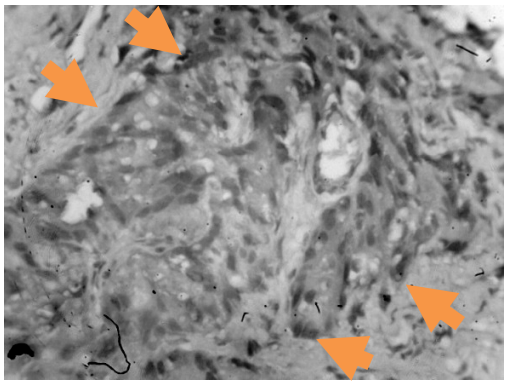

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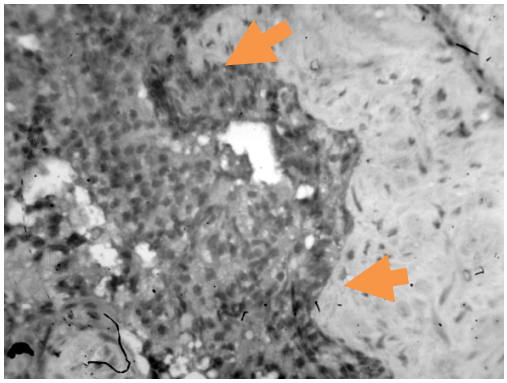
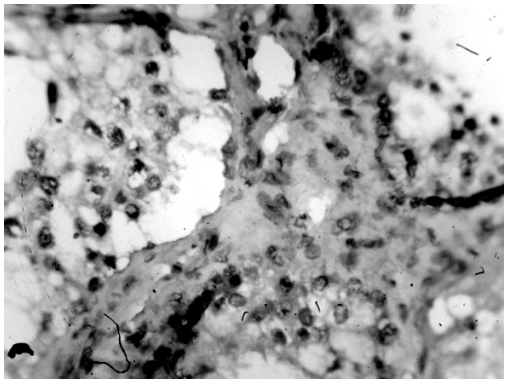
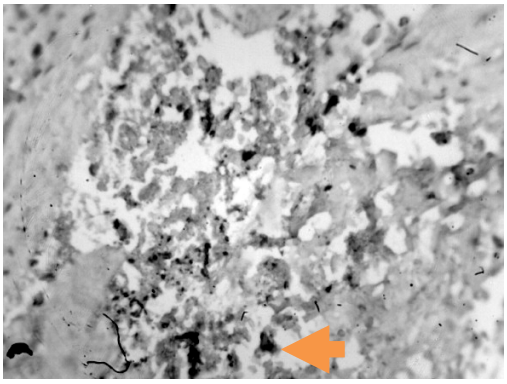


**S30**

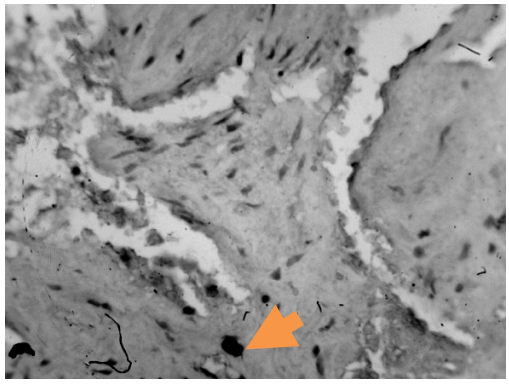


**S38**

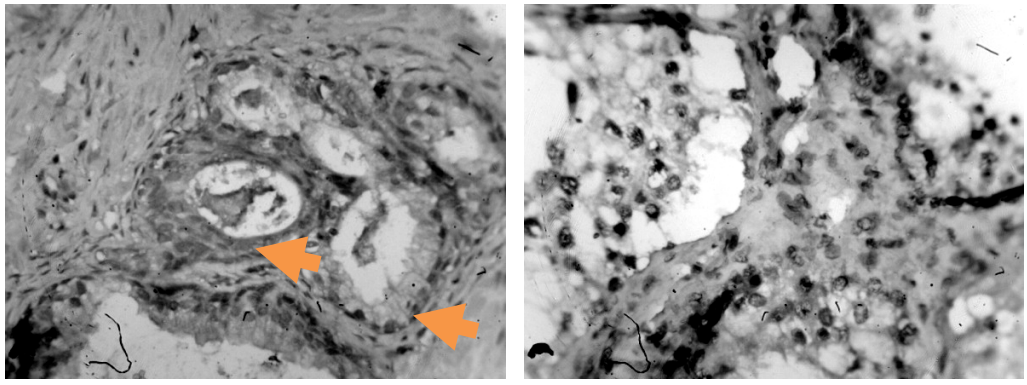
**CK5/6 BPH**



**S39**



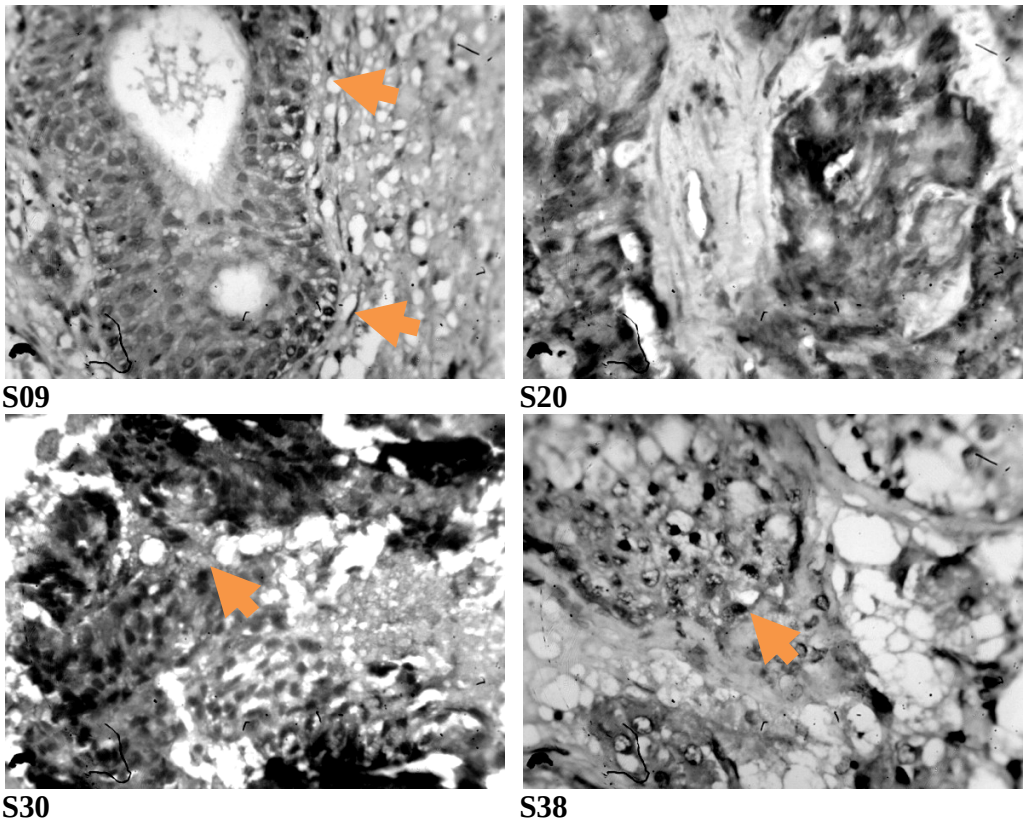
**S40**



**S29** **S38**

**Figure 3:** CK5/6 immunohistochemistry reveals immunonegativity of **PCa** biopsies compared to the control S38. Thus showing that the cancers are of glandular origin rather than from the epithelium as seen in CK7 positivity. S38 (control) shows expression of CK5/6 in normal testicular tissue. **BPH:** Immunopositivity of CK5/6 was however observed in S09, where the patient was also diagnosed with acute inflammation of the prostate, this was also observed BPH biopsies close to the epithelium around the tissue mass characterized by rapid cell proliferation (S29). (Magnification X400).

**ECAD PCa**



**S09** **S20**

**S30** **S38**

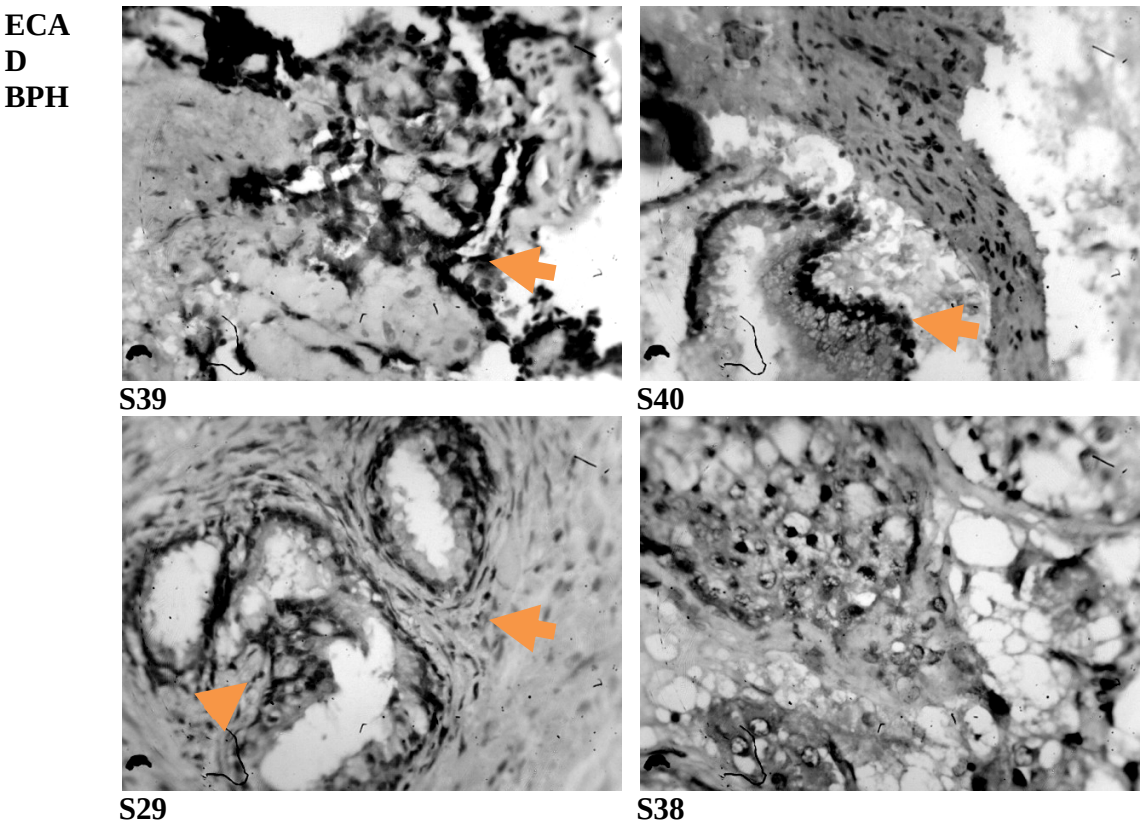



**Figure 4:** ECAD positivity was restricted to the epithelium close to the origin of the PCa. Certain cell in the neoplasm shows ECAD negativity showing the loss of desmosomes or hemi-desmosomes. Such a tumor is most likely going to become malignant as it has detached from the tumor mass within the tissue. **BPH:** ECAD positivity was observed in the epithelial tissue of BPH biopsies, showing proliferation but no evidence of detachment. This is important as a control factor; although certain cells in BPH biopsies were found to be CK7 positive, it is possible that inflamed cells resulting from stress factors in BPH contributes to such immunopositivity rather than PCa. (Magnification X400).

**Table 1:** CK5/6, CK7 and ECAD distribution in 4BHP and 4PCa Biopsies following immunohistochemistry using monoclonal antibodies against Human antigens (ECAD, CK7, CK 5/6).

| **PCa** | | | | **BPH** | | | |
|---|---|---|---|---|---|---|---|
| | *CK5/6* | *CK7* | *ECAD* | | *CK5/6* | *CK7* | *ECAD* |
| *S09* | --/+ | + | --/+ | *S39* | -/+ | ++ | ++ |
| *S20* | -- | ++ | ++ | *S40* | -/+ | ++ | ++ |
| *S30* | -- | ++ | ++ | *S29* | +/- | ++ | ++ |
| *S38 (C)* | -/+ | -/+ | -/+ | *S38 (C)* | -/+ | -/+ | -/+ |