RESEARCH ARTICLE

DIAGNOSTIC UTILITY OF BCL-2, E-CADHERIN AND PSA IN THE PROGRESSION OF MALIGNANT PROSTATIC LESIONS

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ABSTRACT: **Background and aim**: Monitoring and diagnosis of benign or premalignant lesions as it progresses to a malignant condition is very necessary as its importance is the probability of itsabilityto predict or curb progression to the malignant condition and Immunohistochemistry could be a useful tool in achieving this as immunohistochemical markers are specific in nature. The purpose of this study is to demonstrate the expression of the immunohistochemical markers; BCL-2, E cadherin and PSA (prostate specific antigen) in BPH and PCa and their ability to be useful in the prediction of the progression to Prostatic malignant lesions. **Sample and method:** A total of 60 tissue blocks were retrieved from the pathology archive, previously collected from males histologically confirmed to be present with previously untreated BPH and malignant cases (PCa), consisting 10 normal cases (control), 20 BPH cases and 30 PCa cases which consisted of both LGPIN and HGPIN. Immunohistochemical analysis was carried out on the samples. The immunohistochemical staining was evaluated and the results were considered. **Result:** Cytoplasmic BCL-2 staining was expressed, Membrane staining of E-cadherin was expressed and cytoplasmic PSA was expressed within the cells of normal, BPH and PCa tissues. There was an upregulation of BCL-2 and down regulation of E-cadherin in the progression to the malignant condition and PSA showed a reduced expression in undifferentiated malignancy. **Conclusion:** The expression of BCL-2, E-cadherin and PSA in Normal, BPH and malignant prostatic tissue has confirmed the ability of immunohistochemistry (its markers) tosurveil the progression of prostate cancer.

KEYWORD: Prostate cancer, Benign prostatic hyperplasia, BCL-2, E-cadherin, PSA

INTRODUCTION:

Cancer of the prostate is now recognized as one of the main medical problems facing the male population as a whole. The disease accounts for 6% of all cancer related deaths among the male population [1]. In prostatic cancer, there are different varieties of precursors and benign lesion of which prostatic intraepithelial neoplasia (PIN) is an example of a precursor [2]. Prostatic Intraepithelial Neoplasia is comprised of prostatic ducts, which were existing previously, and cytologically atypical cells lining the acini and is further divided into low grade prostatic intraepithelial neoplasia (LGPIN) and high grade prostatic intraepithelial neoplasia (HGPIN) [3]. All through the method of malignant transformation, cells, step by step, evolve from the benign to the malignant phenotype [4].

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Benign Prostatic Hyperplasia (BPH) is defined as a progressive hyperplasia of glandular and stromal tissues around the urethra. It is characterized by a four-fold increase of the stromal component and a nearly doubling of the glandular elements of the prostate and due to these changes, BPH has been perceived as majorly a proliferative stromal disease. More than two-thirds of men older than 50 years have histological evidence of BPH and, after age 70, the proportion increases to 80 [5]. A variety of predisposing factors are highlighted in the progression of BPH. As aging occurs, the anticipated increase of estrogen and decrease in testosterone, androgen level, are noted to be the cause of the condition (BPH) to develop. An increase in the fibromuscular gland proliferation is another known cause of [6].

Immunohistochemical biomarkers are used throughout the scope of clinical progression from early detection and diagnosis through clinical endpoint determinations. Ideal biomarkers should have high sensitivity and specificity [7]. Among the better-characterized biomarkers, BCL-2, E-cadherin and PSA (Prostate Specific Antigen) are widely reported to be prognostic in prostate cancer. Each of these markers becomes dysregulated during the progression of prostate cancer [8, 9]

BCL-2 is an oncogene that codes for a protein that suppresses apoptosis [8]. The BCL-2 oncoprotein is known to promote tumor growth in several neoplasms, including prostate adenocarcinoma. Some reports suggest that overexpression of BCL-2 positively correlates with higher tumor stage, and it accompanies hormone-resistant disease [10]. The BCL-2 oncogene plays an important role in carcinogenesis by inhibiting cell death (apoptosis) [11] as damage to the BCL-2 gene has been identified as a cause of a number of cancers, including prostate [12].

E-cadherin is a transmembrane glycoprotein that mediates cell–cell adhesion [8]. Its basic role is to induce and maintain polarization and organization of normal epithelium. Disorders of epithelial cell adhesion are significant in tumor progression, and they may cause development of more invasive and metastatic phenotypes [10]. Suppression of expression or cleavage of this adhesion molecule may result in cells acquiring a mesenchymal morphology and increased motility. During cancer progression to an invasive state, intercellular adhesions between tumor cells are disrupted. Thus, aggressive tumor cells were hypothesized to have loss of E-cadherin [13].

Prostate specific antigen (PSA) is the most relevant protein for the management of men with suspected or diagnosed prostate cancer. The PSA level is largely proportionate to the quantity of prostate epithelial cells in the body this indicates that an increased serum PSA level is the most common cause for prostate cancer suspicion and subsequent prostate biopsy. PSA analysis is also common in histo-cytopathology [14]. Due to its perceived prostate specificity, immunohistochemical PSA analysis is routinely used to determine whether tumor bulks of unknown origin can be assigned to a prostate cancer [14]. Despite all these, Immunohistochemical expression of PSA has also been reported in a myriad of non-prostatic tissues and tumors, most rampantly in breast malignant tissues and salivary gland malignancy. In most of the other non-prostatic tissues and tumors, there are only sporadic cases of PSA positivity that may have not been validated or confirmed and immunoreactivity is often weak and half hazard [15]. However, cellular PSA expression can be substantially reduced in poorly differentiated prostate cancers, which can result in PSA negative immunohistochemistry and widespread metastatic prostate cancers with very low serum PSA levels [14].

MATERIALS AND METHODS:

TISSUE SAMPLE SELECTION

A total of 60 formalin fixed, paraffin embedded tissue blocks were obtained from the pathology archives of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife (OAUTHC). These obtained tissue blocks including; 10 normal prostatic tissue, 20 BPH and 30 previously untreated confirmed prostate carcinoma cases (containing HGPIN and LGPIN), were cut at 5 microns. Samples were collected from only male subjects according to the age group of 50-80 years.

METHODOLOGY

The expression of the biomarkers, BCL-2, E-cadherin and PSA, were demonstrated immunohistochemically using the immunoperoxidase method.

IMMUNOHISTOCHEMISTRY

The expression of the biomarkers, BCL-2, E-cadherin and PSA, were demonstrated immunohistochemically using the Avidinbiotinimmunoperoxidase method.

Procedure: : Sections, on adhesive coated glass slides, were deparaffinized in xylene and rehydrated using different gradients of ethanol. The sections were pretreated in a pressure cooker for antigen retrieval, using antigen retrieval buffer at 95 degrees Celsius for 30 minutes, 90 degrees Celsius for 10 seconds and 10 degrees Celsius for 10 minutes. Endogenous peroxidase activity was blocked by immersion in 3% hydrogen peroxidase solution for 5 minutes. Non-specific binding was blocked with the use of blocking buffer (horse non-immune serum) for 15 minutes. 200µl of diluted primary antibody (BioGenex mouse monoclonal primary antibodies) for Bcl-2 oncoprotein E-cadherin and PSA sequentially was added to slides and incubated at room temperature for 80 minutes. The slides were incubated with biotinylated rabbit anti-mouse secondary immunoglobulins for 15 minutes at room temperature. They were subsequently incubated with avidin-biotin peroxidase complex. 3,3 diaminobenzidine was used as a chromogen. The sections were counterstained with hematoxylin [16].

IMMUNOHISTOCHEMICAL ANALYSIS

Results will be presented in figures and tables; pictures (micrographs) will also be used where necessary. BCL-2, E-cadherin and PSA staining will be evaluated using regular light microscope at mg x40.

PHOTOMICROGRAPHY

The Stained sections were examined under a LEICA research microscope (LEICA DM750, Switzerland) interfaced with digital camera (LEICA ICC50). Digital photomicrographs of stained sections for the histomorphology, immunohistochemistry on the organs studied were taken at various magnifications, and reported for Morphological changes.

IMMUNOSTAINING ASSSESMENT

BCL-2, E-cadherin and PSA immunohistochemical staining will be evaluated by a semi-quantitative method using a 0-3 (+++, ++, +, 0) scale as a combination of intensity and distribution. Where 0 will be classified as no expression, no detectable expression or localized staining observed in less than 10% of cells; 1 (+) represents mild or weak but detectable discontinuous expression/localized staining observed in 10-39% of cells, 2 (++) represents moderate and clearly positive staining discontinuous expression/localized staining present on 40-79% and 3 (+++) represents strong and intense continuous expression greater than 79% [17, 18]

RESULTS:

Table 1a. Expression of BCL-2 protein in indicated cases.

Groups	Total cases(n	-	+	++	++	Positive rate (%
Normal/Control	10	10	-	-	-	0
BPH	20	10	3	7		50
PCa	30	-	-	10	20	100

A table 1a showing the semi-quantitative expression of BCL-2 in normal, BPH (benign prostatic hyperplasia) and PCa (prostatic cancer). Normal cases showed a positivity rate of 0% where expression on the slide wasn't up to 10%, BPH showed a positivity rate of 50% where ten (10) cases showed inadequate staining, three (3) cases showed weak staining and seven (7) showed moderate staining, PCa had a positivity rate of 100% as all of the cells showed significant expression.

Table 1b. Expression of BCL-2 protein in indicated cases.

Groups	N	Negative (n%	Positive (n%)
Normal/Control	10	10 (100)	-
BPH	20	10 (50)	10 (50)
PCa	30	-	30 (100)

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A table 1b showing the expression of BCL-2 in normal, BPH (benign prostatic hyperplasia) and PCa (prostatic cancer) where in Normal, all 10 slides showed no significant positivity; BPH had ten (10) slides with insignificant reactions (negative) and ten (10) with significant reaction (positivity); PCa had all thirty (30) slides showing varying degrees of significant reaction.

Table 2a. Expression of E-Cadherin protein in three groups of patients.

Groups	Total cases(n)	'	+	‡	‡‡	Positive rate (%)
Normal/Control	10	-	-	-	10	100
BPH	20	-	-	7	13	100
PCa	30	10	18	2	-	66.7%

A table 2a showing the semi-quantitative expression of E-Cadherin in normal, BPH (benign prostatic hyperplasia) and PCa (prostatic cancer). Normal cases showed a positivity rate of 100% where all slides showed strong positivity, BPH showed a positivity rate of 100% as they all showed significant expression; where seven (7) cases showed moderate staining and thirteen (13) cases showed moderate staining, PCa had a positivity rate of 66.7% where 10 slides showed inadequate expression, 18 showed weak expression and 2 showed moderate expression.

Table 2b. Expression of E-Cadherin protein in indicated cases.

Groups	N	Negative (n%)	Positive (n%)
Normal/Control	10	-	10 (100)
BPH	20	-	20 (100)
PCa	30	10(30)	20(67)

A table 2b showing the expression of E-Cadherin in normal, BPH (benign prostatic hyperplasia) and PCa (prostatic cancer) where in Normal, all ten (10) slides showed significant positivity; BPH had all twenty (20)

slides with significant reaction (positivity); PCa had twenty (20) showing significant reaction and ten (10)cases showing insignificant reaction.

Table 3a. Expression of PSA protein in indicated cases.

Groups	Total cases(n)	'	+	‡	‡‡	Positive rate (%)
Normal/Control	10	-	-	-	10	100
BPH	20	-	-	3	17	100
PCa	30	1	8	17	5	96.7

A table 3a showing the semi-quantitative expression of PSA in normal, BPH (benign prostatic hyperplasia) and PCa (prostatic cancer). Normal cases showed a positivity rate of 100% where expression highly significant, BPH showed a positivity rate of 100% where three (3) cases showed moderate staining and seventeen (17) showed strong staining, PCa had a positivity rate of 96.7% where one (1) showed an insignificant expression, eight (8) showed weak reactions, seventeen (17) showed moderate and five (5) showed strong expression.

Table 3b. Expression of PSA protein in indicated cases.

	Normal (n%)	BPH(n%)	PCA(n%)
Intensity Weak	-	-	9 (30)
Moderate	-	3 (15)	17 (56.7)
Strong	10 (100)	17(85)	4 (13.3)
Total	10	20	30
Percentage <10	-	-	-
10–60	-	3 (15)	25 (83)
≥60	10 (100)	17 (85)	5 (17)
Total	10	20	30

A table 3b showing the intensity and percentage reactivity of PSA in Normal cases, BPH and PCa, where the intensity consists of weak, moderate and strong and the percentage consists of (<10)%, (11-60)%, and (≥60)%.

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Table 4. Mean percentage reactivity of immuno-histochemical markers in PIN

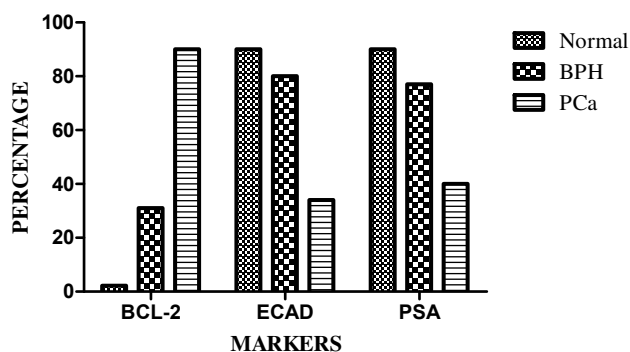|	Grades	BCL-2 Pos (%)	E-CAD Pos (%)	PSA Pos (%)
PIN	LGPIN	57.5	67	70
	HGPIN	80	50	52

A table 4 showing the mean percentage reactivity of BCL-2, E-cadherin and PSA on LGPIN (Low grade prostatic intraepithelial neoplasia) and HGPIN (High grade prostatic intraepithelial neoplasia). All areas of LGPIN present showed a mean percentage of 57.5% when stained with BCL-2, 67% when stained with E-cadherin and 70% when stained with PSA. All areas of HGPIN present showed a mean percentage 80% when stained with BCL-2, 50% when stained with E-cadherin and 52% when stained with PSA.

Table 5. Mean percentage reactivity of immuno-histochemical markers in indicated cases.

| Groups	BCL-2	E-CAD	PSA
Normal/Control	2.2%	90.3%	90.2%
BPH	30.8%	80%	77.1%
PCa	90%	34%	45%

The above table shows the mean percentage reactivity of BCL-2, E-cadherin and PSA in normal, BPH and PCa. It shows an increase in reactivity in BCL-2 from normal to PCa, and a decrease in both E-cadherin and PSA.

in the percentage reactivity of BCL-2 and gradual decrease in the percentage reactivity of E-cadherin and PSA.
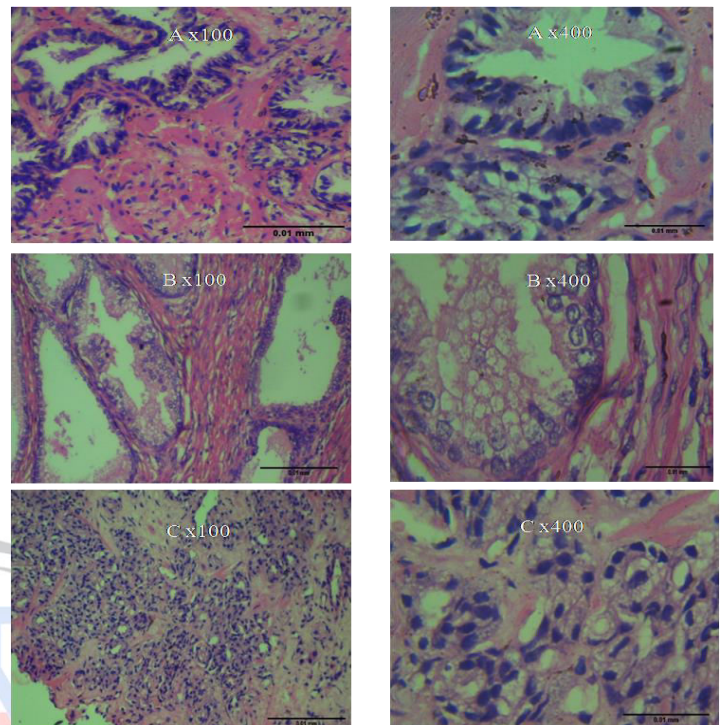

Figure 1. Haematoxylin and Eosin plates. H&E plates of normal at x100 and x400 (plate A); BPH at x100 and x400 (plate B); PCA at x100 and x400 (plate C). (plate A) shows the normal prostatic epithelium, (plate B) shows apparent hyperplasia of glandular cells and (plate C) shows signs of malignancy which include cellular atypia.

GRAPH SHOWING MEAN PERCENTAGE REACTIVITY

Graph 1. The above graph shows the gradual increase

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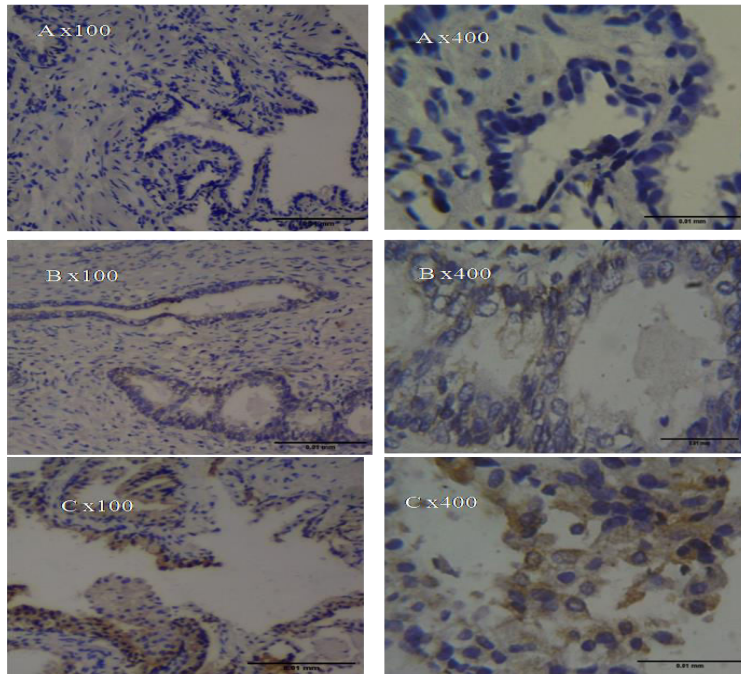
Figure 2. BCL-2 Immunohistochemistry Plates
Cytoplasmic BCL2 stained sections of normal at x100 and x400 (plate A); BPH at x100 and x400 (plate B); PCA at x100 and x400 (plate C). insignificant BCL-2 immunohistochemical staining in normal prostatic tissue (plate A), moderate immunohistochemical staining within the cytoplasm of BPH (plate B) and moderate to weak immunohistochemical staining in PCa (plate C).
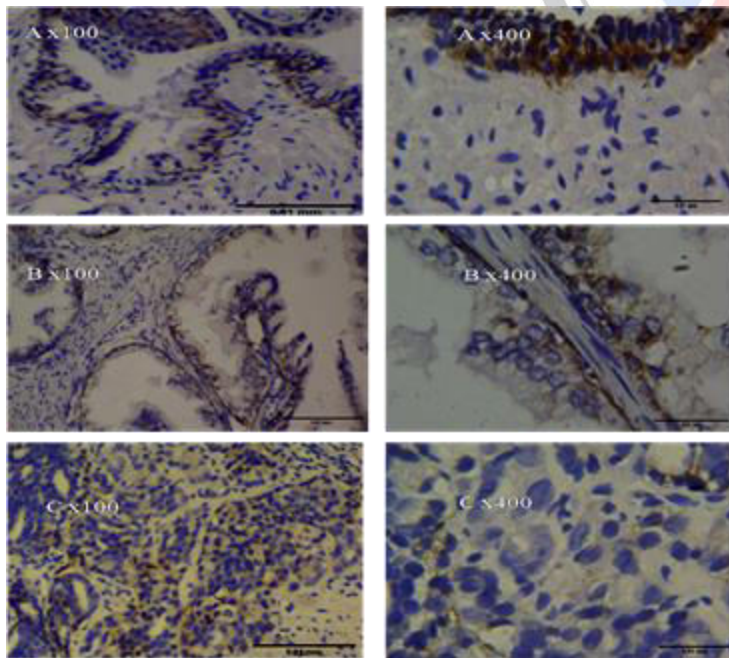
Figure 3. E-cadherin Immunohistochemistry Plates
Membranous ECAD immunohistochemical staining on sections of normal at x100 and x400 (plate A); BPH at x100 and x400 (plate B); PCA at x100 and x400 (plate C). strong membranous immunohistochemical staining observed in the epithelial cells in normal (plate A), significant immunohistochemical staining observed in BPH (plate B) and weak immunohistochemical staining observed in PCa (plate C).
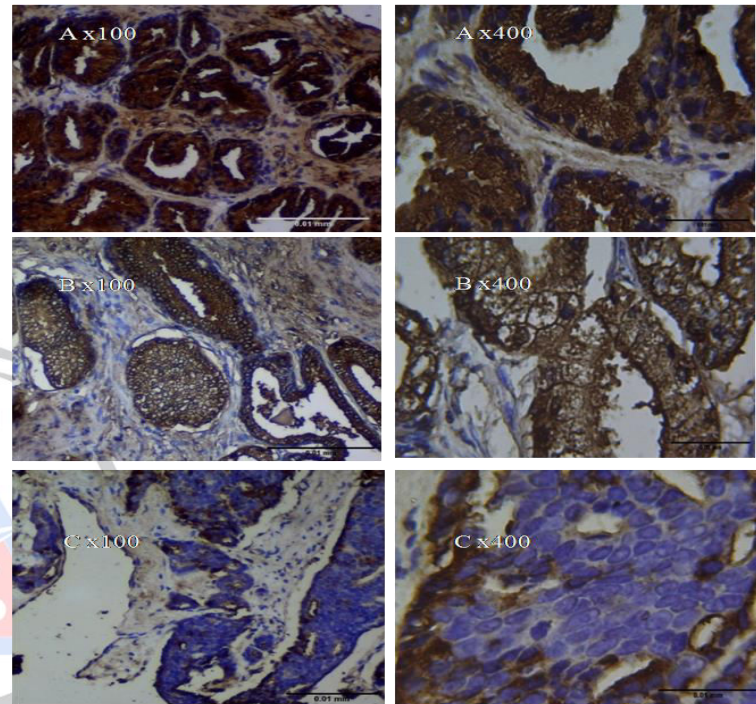
Figure 4. PSA Immunohistochemistry plates
Cytoplasmic PSA immunohistochemical staining on sections of normal at x100 and x400 (plate A); BPH at x100 and x400 (plate B); PCA at x100 and x400 (plate C). strong cytoplasmic immunohistochemical staining observed with the intensity higher in normal (plate A), a decrease is observed in BPH (plate B) and further decrease in PCa (plate C).

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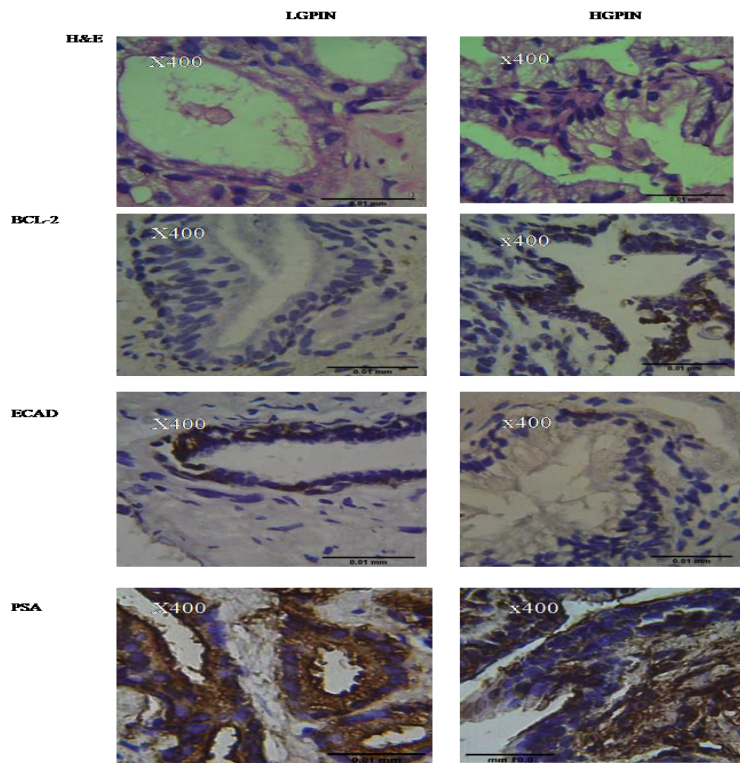
Figure 5. LGPIN and HGPIN plates. H&E stain sections showing LGPIN and HGPIN, with their characteristics evident. LGPIN still has its basement membrane intact while HGPIN doesn't. Hyperchromatia is observed also, the cribriform pattern of HGPIN is also observed. BCL-2 immunohistochemical stained section of LGPIN showing to few cytoplasmic staining, therefore not notable; HGPIN showing high degree of BCL-2 stain. E-cadherin immunohistochemical stained section of LGPIN and HGPIN with LGPIN showing moderate degree of membranous e-cadherin immunohistochemical staining and HGPIN showing weak to nonexistent immunohistochemical staining of membrane. PSA immunohistochemical attained sections of LGPIN and HGPIN, with both LGIN and HGPIN showing moderate cytoplasmic staining.

DISCUSSION:

Prostatic carcinoma which is known to be responsible for nearly 6% of all male cancer deaths is a common cancer worldwide therefore toaid in preventing avoidable fatalities, diagnosis is an effective tool for the reduction of this incidence [18]. It is a well-known fact that E-Cadherin molecules as well as BCL-2 contribute to the pathogenesis of several malignant tumors including the prostate [10, 12]. and the protein, PSA, has its function in the malignant condition [14].

From this study, our findings reveal that BCL-2 is expressed significantly in BPH and PCa with PCa having higher immunohistochemical expression and degree of reactivity while in the normal Prostatic tissue, weak immunohistochemical staining was observed within basement membrane and the glandular epithelium was classified as negative. The positivity rate of BCL-2 among the cases was zero (0%) in normal, fifty percentage (50%) in BPH and one hundred percent (100%) in PCa and the mean percentage reactivity was 2.2%, 30.8% and 90% in normal, BPH and PCa respectively. For LGPIN, the mean percentage reactivity was 57.5% and 75% for HGPIN. It has proven to be a useful predictive marker and upregulated in agreement with previous work by [10, 19]. who also explained the mechanism of action that in PCa, BCL-2 is overexpressed which enhances progression of PCa by having an active role in aiding tumor cells avoid augmenting angiogenesis and apoptosis. Its ability to hinder apoptosis leads to the presence of oncogenic alterations including uncontrolled proliferation mainly. Given that apoptosis blockage is a key oncogenic mechanism in malignancies. The anti-apoptotic BCL-2 acts by stopping the process of Mitochondria outer membrane permeabilization (MOMP) using these pathways; the binding and sequestration of the active forms of BAK and BAX, and/or the sequestration of the direct activators BH3-only proteins [20].

From the results, E-cadherin (ECAD) immunohistochemistry was found to be highly expressed within the normal epithelium and in BPH, but less expressed in PCa. It has also proven to be a useful predictive marker. The positivity rate for ECAD was 100% in normal cases, 100% in BPH cases and 66.7% in PCa and the mean percentage reactivity was 90.3%, 80% and 34% in normal, BPH and PCa respectively. For LGPIN, the mean percentage reactivity was 67% and 50% for HGPIN. According to the micrographs, the normal prostatic epithelium and BPH showed a higher degree of ECAD expression, showing that the epithelium is still intact, in normal and BPH the integrity of the epithelium is upheld unlike PCa where there is a

significant reduction in the expression of ECAD therefore letting the cells migrate from the epithelium and then further invade the surrounding

glandular tissue [14]. It is noted that according to the results, the protein E-cadherin is downregulated in the progression of the malignant condition which is in agreement with previous works such as Musalam in 2019, amongst others such as Ogundele in 2014. ECAD expression within the prostatic epithelium, describes the gradual progression of tumors from benign to malignant. It has been reported that when it becomes unregulated, cellular adhesion deteriorates and cells gain invasive properties [10, 13, 18].

PSA shows strong immunohistochemical stain in normal and BPH but a steady decline is noted as PCa progresses and cancer becomes differentiated, although a percentage of the slides show strong to moderate staining (i.e. undifferentiated cancers). The positivity rate of PSA among the cases was one hundred percent (100%) in normal, one hundred percent (100%) in BPH and ninety-six-point seven percent (96.7%) in PCa where the singular case without comparatively significant immunohistochemical expression was a poorly differentiated malignancy. The mean percentage reactivity was 90.2 %, 77.1% and 40.4% in normal, BPH and PCa respectively. For LGPIN, the mean percentage reactivity was 70% and 52% for HGPIN. The decline in the expression/ intensity of PSA in prostate cancer in comparison with the BPH tissue sections was also in line with a previous work by Veveris-Lowe in 2005 and Ghods in 2014. PSA production may be one of the most important functions of normal prostate glandular cells [9, 21]. One can thus speculate that a measurable deficiency in this function might represent a subtle sign of cellular dedifferentiation [12]. An increase in serological PSA levels have

been observed in conditions such as neoplasia or inflammation where the prostate glands structure is disrupted leading to the leakage of PSA into the blood stream. Progression to malignancy basically requires majorly the feature of avoiding growth inhibitory signals and proliferation. The protein, PSA catalyzes IGFBPs, to release insulin growth factor-1 (IGF-1) (a proliferative factor found in various cancers including prostate cancer) [22]. The

mean percentage reactivity of BCL-2, E-cadherin and PSA on LGPIN (Low grade prostatic intraepithelial neoplasia) and HGPIN (High grade prostatic intraepithelial neoplasia) were calculated by immuno-assessing the reactivity of various slides containing LGPIN and HGPIN. The expression of the markers in these premalignant lesions were similar to BPH in a few cases, and were consistent will the upregulation of BCL-2 and down regulation of E-cadherin and expression of PSA.

BCL-2 showed majorly cytoplasmic staining and minor nuclear staining across the epithelium and basement membrane while E-cadherin showed consistent membranous staining across the epithelial cells and PSA showed strong cytoplasmic staining in the epithelial cells except in the prostatic cells that had lost their cell polarity and cytoplasmic components. BCL-2 was up regulated while both PSA and E-cadherin were down regulated. E-cadherin showed the most amount of consistence among the markers used and PSA showed the least. BCL-2 also showed prognostic significance and is recommended to be used alongside E-cadherin.

CONCLUSION:

This study has established the usefulness of BCL-2, E-cadherin and PSA immunohistocheical markers in studying the prostatic tissue from normal to Benign prostatic hyperplasia and then to PCa. BBCL-2 and E-cadherin were found to be more useful in studying the progression as characterized in BCL-2's increase and E-cadherin's decrease along the progression.

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