ASSESSMENT OF CD34, PSMA AND P53 IHC EXPRESSION IN NORMAL, BENIGN AND MALIGNANT PROSTATE LESIONS

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ABSTRACT

Background

Prostate cancer is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in the male worldwide, it include the transformation of normal to benign prostatic hyperplasia and then invasive cancer. Immuno-histochemistry has proven to be a very useful diagnostic tool in the study of this transformation.

Aim: Assessing the immunohistochemical expression of CD34, PSMA and P53 as diagnostic marker in normal prostate, benign prostatic hyperplasia (BPH) and prostate adenocarcinoma, and to determine the degree of the expression of these IHC biomarkers'

Methods: Confirmed prostate tissue blocks of non-malignant, BPH and prostate adenocarcinoma were obtained from the pathological archives of Obafemi Awolowo University Teaching Hospital Complex. In total, 50 prostate tissue blocks were retrieved. Among these, 10 prostate tissue blocks had non-malignant diagnosis, 20 prostate tissue blocks were diagnosed with BPH and 20 prostate tissue blocks were diagnosed with adenocarcinoma of the prostate. Sections were cut and immunohistochemical study were done using CD34, PSMA and P53 antibodies following standard protocols.

Results: Membranous CD34 staining was expressed; normal cases showed the positivity rate of 80%, benign prostatic hyperplasia showed a positivity rate of 60%, and prostate cancer showed a positivity rate of 90%. Cytoplasmic PSMA staining was expressed, the normal cases showed a positivity rate of 30%, benign prostatic hyperplasia showed a positivity rate of 50%, and prostate cancer showed a positivity rate of 90%. Nuclear p53 staining was expressed; normal cases showed the positivity rate of 3%, benign prostatic hyperplasia showed a positivity rate of 5% and prostate cancer showed a positivity rate of 80%. There was an upregulation in PSMA in the progression to malignant condition.

Conclusion: This study established the usefulness of CD34, PSMA and P53 immunohistochemical markers in the study of the prostate tissues from normal to BPH and to a malignant prostate. These markers provided differential diagnosis of different prostatic lesion, hence their use is recommended in histopathology laboratories alongside routine Hematoxylin and Eosin in the diagnosis of prostate biopsies.

Keywords: Prostate cancer, Benign Prostatic Hyperplasia, CD34, PSMA, P53.

INTRODUCTION

Cancer is described as an unregulated growth and consequent spread of the cells to other parts of the body (Roma-Rodrigues *et al.*, 2019). All types of cells can undergo such malignant changes and become cancers, however only epithelial cells can become carcinomas. The normal cell cycle is disrupted and the new "tumor" cells overgrow in a localized region at first, then spread to surrounding tissue and finally to other parts of the body via the lymphatic system and vascular system (Stuelten *et al.*, 2018).

Prostate cancer is the most common cancer diagnosis for men in the United States, with over 160 000 new cases diagnosed each year. Prostate cancer is the third-leading cause of cancer death in men.

As of 2012, prostate cancer is the second-most frequently diagnosed cancer,15% of all male cancers and the sixth leading cause of cancer death in males worldwide (Jemal *et al.*, 2011). In 2010, prostate cancer resulted in 256,000 deaths, up from 156,000 deaths in 1990 (Lozano *et al.,* 2012). Rates of prostate cancer vary widely. Rates vary widely between countries. It is least common in South and East Asia and more common in Europe, North America, Australia, and New Zealand. Prostate cancer is least common among Asian men and most common among black men, with white men in between (Lozano *et al.,* 2012). It accounts for 19% of all male cancers and 9% of male cancer-related deaths. Cases ranged from an estimated 230,000 in 2005 to an estimated 164,690 in 2018. Deaths held steady around 30,000 in 2005 and 29,430 in 2018 (Jemal *et al*., 2005). The specific objectives are to determine the expression of CD34, PSMA and P53 in non-malignant prostate, BPH and prostate adenocarcinomas, and to determine if CD34, PSMA and P53 are predictors of malignant transformation in the prostate.

Immunohistochemistry (IHC), which requires the use of monoclonal and polyclonal antibodies to identify particular antigens in tissue sections, is a very important tool in the diagnostic surgical pathologist's arsenal. IHC is a technique that uses monoclonal and polyclonal antibodies to assess the tissue distribution of a target antigen in healthy and diseased tissue. Since specific tumor antigens are expressed de novo or up-regulated in some cancers, it is commonly used for cancer diagnosis. The advantage of IHC is to explore specificity of antigen-antibody reaction of (Ramos-Vera *et al*., 2014). The principle of IHC is based on specific antigen antibody reactions in biological tissues (Cruz-Alonso *et al*., 2019).In IHC, sections are incubated with an appropriate antibody. The antibody binding site is then visualized by a marker such as fluorescent dye, enzyme, radioactive element, or colloidal gold, using an ordinary or fluorescent microscope (Orakpoghenor *et al.,* 2018).

CD34 is a single-pass transmembrane protein with a molecular weight of 105.120 kDa (Kumagai *et al.,* 2014). It is expressed on the surface of a variety of cells, particularly on vascular endothelial cells; therefore, CD34 is often used to label vascular endothelial cells (Ho et al., 2013; Foroozan et al., 2017). Moreover, CD34 is more likely to be expressed on newly-formed vascular endothelium (Ajili *et al*., 2012). Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein that is overexpressed in prostate cancer. Radio-labeled small molecules that bind with high affinity to its active extracellular center have emerged as a potential new diagnostic standard of reference for prostate cancer, resulting in images with extraordinary tumor-to-background contrast. Prostate-specific membrane antigen (PSMA) is expressed on the cell surface in normal prostate tissue and is overexpressed in prostate cancer by several orders of magnitude (Michael *et al*., 2018).

The P53 tumor suppressor plays a pivotal role in cancer and infectious disease. Many oncology treatments are now calling on immunotherapy approaches, and scores of studies have investigated the role of P53 antibodies in cancer diagnosis and therapy. This review summarizes the current knowledge from the preliminary evidence that suggests potential role of P53 as an antigen in the adaptive immune response and as a key monitor of the innate immune system, thereby speculating on the idea that mutant p53 antigens serve as a druggable target in immunotherapy (Beuzeboc *et al.,* 2009).

MATERIALS AND METHODS

Tissue Sample Selection

In this retrospective analysis, all tissue blocks were obtained from the pathology archive of Obafemi Awolowo University Teaching Hospital Complex Ile-ife (OAUTCH). Confirmed prostate tissue blocks of non-malignant, BPH and prostate adenocarcinoma were selected. In total, 50 prostate tissue blocks were taken.

Among these, 10 prostate tissue blocks were non- malignant, 20 prostate tissue blocks were diagnosed with BPH and 20 prostate tissue blocks were diagnosed with adenocarcinoma of the prostate.

Immunohistochemistry

All the specimens were formalin-fixed and paraffin-embedded. Four (4) microns thick serial sections were cut, and the end sections were stained with Hematoxylin and Eosin to ensure that the lesion was still present in the serial sections. The sections were processed for immunohistochemical analysis as follows; De-paraffinization was carried out with xylene followed by hydration through descending grades of alcohols. Epitope retrieval was performed by heating the sections for 10minutes in citrate buffer (pH 6.0) at 120^0C. The sections were incubated in 3% hydrogen peroxide (H_2O_2) in methanol for 5 minutes to block endogenous activities, followed by blocking of nonspecific binding of primary antibodies to epitopes by pre-incubation step with 5% normal goat serum for 10 minutes at 37^0C. The primary antibodies used in this study are CD34, PSMA and P53. Incubation with antibodies was done for 30 minutes at room temperature. The slides were counterstained with hematoxylin, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in DPX (Lee *et al*., 2003). Staining expression was evaluated optically using the light microscope at x100 and x400 magnification.

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 slides studied were taken at x100 and X400 magnifications, and reported for morphological changes.

Immunostaining Assessment

The tumor's immunohistochemical (IHC) profile was assessed by staining one segment from a representative block for CD34, PSMA and p53. IHC was performed using the streptoavidin-biotin immunoperoxidase technique on 4m thick parts from 10 percent formalin-fixed paraffin-embedded specimens (Dako-cytomation). Multiple slides were examined, and IHC staining was used on the ideal portion. The positive and negative controls were both run at the same time. Positive staining was characterized as strong brown nuclear immunoreactivity. The percentage of tumor cells that reacted with the antibody was used to conduct the immunoquantification. To find areas with the most positive cells, each slide was examined at a magnification of 40 times. The proportion of positive cells to total cells was determined after these areas were examined at x400 magnification. At least 500 cells were counted, and only the cells that were definitely positive for the desired marker was considered.

The percentage of positive cells was graded as follows;

0% cells are stained = negative (-), grade 0
0.1% are stained = positive (+), grade 1
10.1 - 50% are stained = positive (++), grade 2
50.1 – 80% are stained = positive (+++), grade 3
80.1% - 100% are stained = positive (++++), grade 4 (Ekundina *et al*., 2021).

RESULTS

Table 1a; Showing the expression of CD34 in normal, in BPH and in prostate cancer tissue; Where in normal, three (3) slides showed no significant positivity and seven (7) slides showed positivity. In BPH had fourteen (14) slides with insignificant reactions (negative) and six (6) slides with significant reaction (positive). Prostate cancer had two (2) slides that showed insignificant reaction and eighteen (18) slides showing several amounts of significant reaction.

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Table 1b; Showing the semi-quantitative expression of CD34 in normal, BPH and prostate cancer tissue; Where the normal cases showed a positivity rate of 70%, where three (3) slides showed insignificant reaction, two (2) slides showed mild reaction and five (5) slides showed moderate reaction. In benign prostatic hyperplasia showed a positivity rate of 30% where fourteen (14) cases showed insufficient expression and six (6) cases showed mild expression. In prostate cancer, the cases showed a positivity rate of 90% significant expression where two (2) slides showed no significant reaction, one (1) slide showed mild reaction, seven slides showed moderate reactions and ten (10) slides showed very strong reaction membranous reaction.

Table 2a; Showing the expression of PSMA in normal, in benign prostatic hyperplasia and in prostate cancer tissue where; In normal, seven (7) slides showed no significant positivity and three (3) slides showed weak positivity. In BPH had six (6) slides with insignificant reactions (negative) and fourteen (14) with significant reaction (positive). Prostate cancer had two (2) slides with no significant positivity and eighteen (18) slides showing marked cytoplasmic reaction.

Table 2b; Showing the semi-quantitative expression of PSMA in normal, BPH and prostate cancer tissue. The normal cases showed a positivity rate of 30% where seven (7) slides showed insignificant reaction and three (3) slides showed weak reaction. Benign prostatic hyperplasia showed a positivity rate of 70% where six (6) cases showed insignificant expression, ten (10) cases showed mild expression and four (4) cases showed moderate expression. In prostate cancer, the cases showed a positivity rate of 90% significant expression where two (2) slides showed no significant reaction, two (2) slides showed mild reaction, six (6) slides showed moderate reactions and ten (10) slides showed very significant cytoplasmic reaction.

TABLE 1a; Expression of CD34 in indicated cases

GROUPS	TOTAL CASES (%)	NEGATIVE (%)	POSITIVE (%)
NORMAL	10	30(100)	70(100)
BPH	20	70(100)	30(100)
PROSTATE CANCER	20	10(100)	90(100)

TABLE 1b: Expression of CD34 in indicated cases

	TOTAL CASES	_	+	++	+++	POSITIVITY RATE (%)
NORMAL	10	3	2	5		70
BPH	20	14	6			30
PROSTATE CANCER	20	2	1	7	10	90

TABLE 2a; Expression of PSMA in indicated cases

	TOTAL CASES	NEGATIVE (%)	POSITIVE (%)
NORMAL	10	70(100)	30(100)
BPH	20	30(100)	70(100)
PROSTATE CANCER	20	10(100)	90(100)

TABLE 2b; Expression of PSMA in indicated cases

	TOTAL CASES	–	+	++	+++	POSITIVITY RATE (%)
NORMAL	10	7	3			30
BPH	20	6	10	4		70
PROSTATE CANCER	20	2	2	6	10	90

Table 3a; Showing the expression of P53 in normal, in BPH and in prostate cancer tissue. In the normal case, nine (9) slides showed no significant positivity and one (1) slide showed weak positivity. In BPH had eighteen (18) slides with insignificant reactions (negative) and two (2) with weak reaction (positive). Prostate cancer had three (3) slides that showed insignificant reaction and seventeen (17) slides showing significant amount of significant reaction. Table 3b; Showing the semi-quantitative expression of P53 in normal, BPH and prostate cancer tissue; the normal cases showed a positivity rate of 10% where nine (9) slides showed insignificant reaction and one (1) slide showed mild expression. In Benign prostatic hyperplasia, the cases showed a positivity rate of 10% where eighteen (18) cases showed insignificant expression, two (2) cases showed mild expression. In prostate cancer, the cases showed a positivity rate of 85% significant expression where three (3) slides showing significant expression, a (1) slide showed a mild expression, six (6) slides showed a moderate expression and ten (10) slides showed marked expression. Table 4 shows the mean percentage reactivity of CD34, PSMA and P53 in normal, BPH and Prostate Cancer. It shows an increase in reactivity in PSMA and P53 from normal to Prostate Cancer, and a varying reactivity in CD34.

TABLE 3a: Expression of p53 in indicated cases

GROUPS	TOTAL CASES	NEGATIVE (%)	POSITIVE (%)
NORMAL	10	90(100)	10(100)
BPH	20	90(100)	10(100)
PROSTATE CANCER	20	15(100)	85(100)

TABLE 3b; Expression of p53 in indicated cases

	TOTAL CASES	–	+	++	+++	POSITIVITY RATE (%)
NORMAL	10	9	1			10
BPH	20	18	2			10
PROSTATE CANCER	20	3	1	6	10	85

TABLE 4: mean percentage reactivity for all antibodies used.

GROUPS	CD34	PSMA	P53
NORMAL	80%	30%	3%
BPH	60%	50%	5%
PROSTATE CANCER	90%	90%	80%

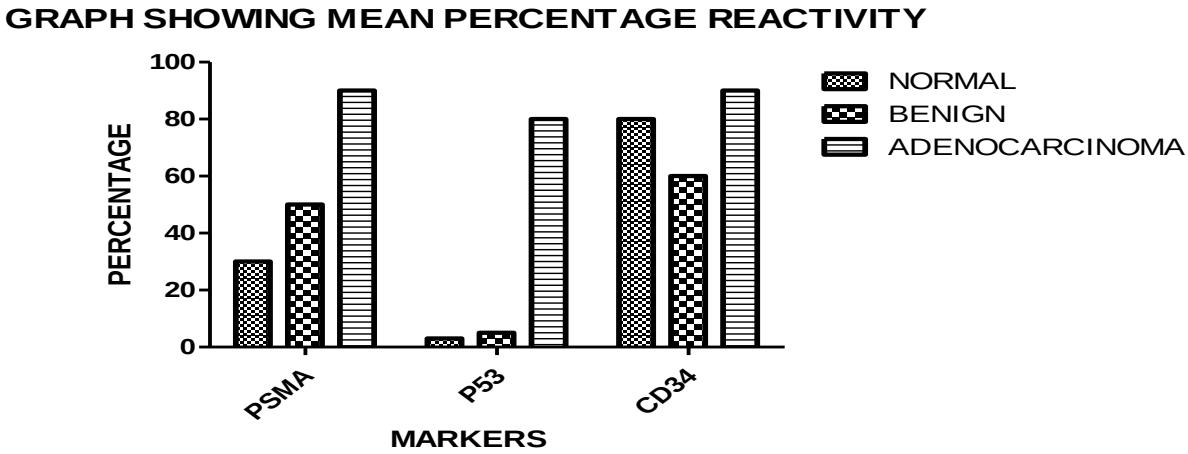

Figure 1: the above graph shows a gradual increase in PSMA and P53 and a varying percentage for CD34. It shows the difference between the normal, benign and malignant lesions. The graph is depicting the various increases among the lesions of the breast and how the immunohistochemical markers are expressed.

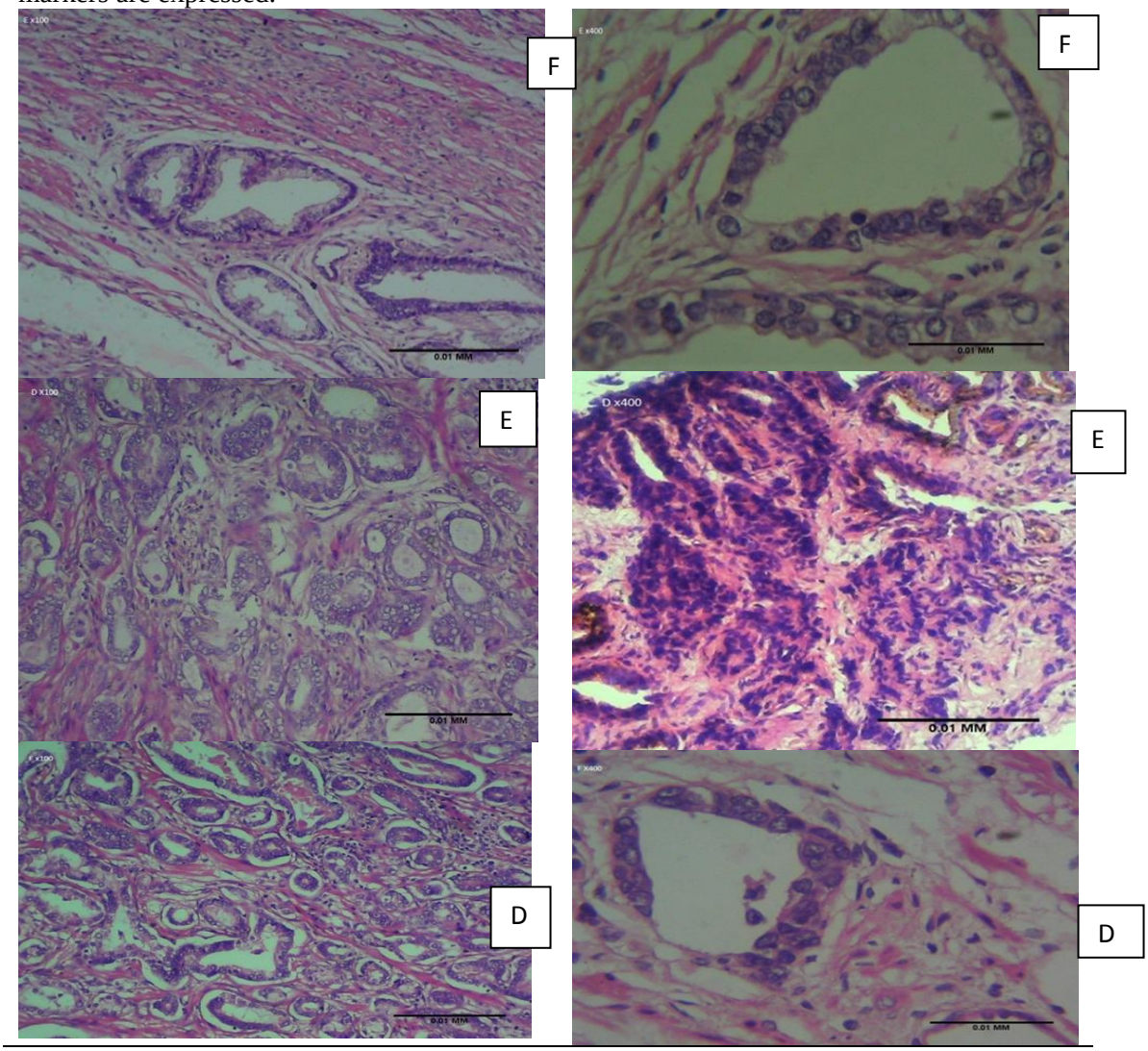

Figure 2; Hematoxylin and Eosin plates. H&E plates of normal at x100 and x400 (Plate F), BPH at x100 and x400 (Plate E) showing growth in the stroma, lined with double epithelial, prostate cancer at x100 and x400 (Plate D) showing occlusion of glands, hyperchromasia, anisonueclosis, high nucleus to cytoplasmic ratio.

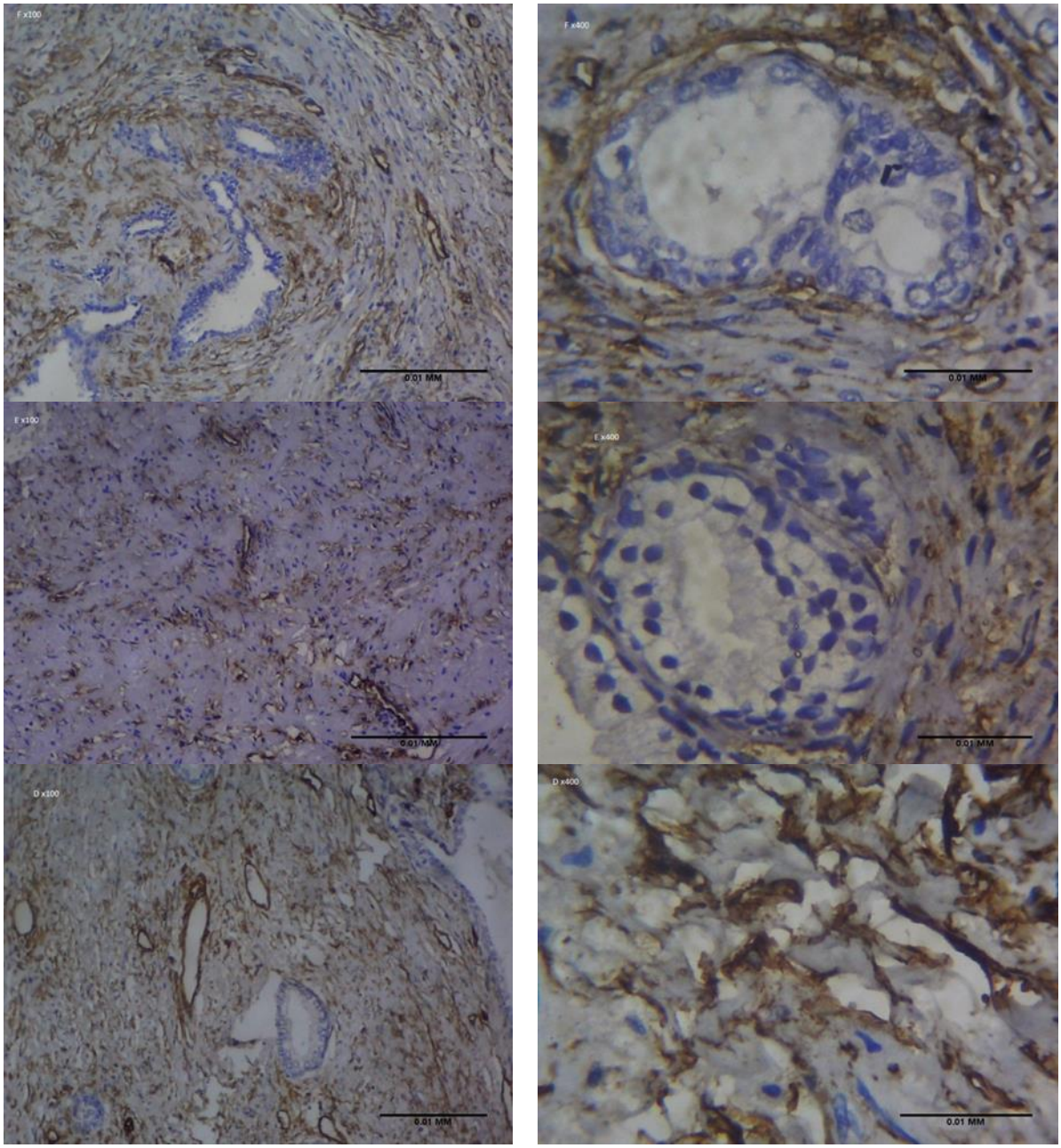

Figure 3:CD34 immunohistochemistry plates
Cytoplasmic CD34 stained sections of normal prostate tissue at x100 and x400 (plate F); BPH at x100 and x400 (plate E); prostate cancer at x100 and x400 (plate D). Mild immunohistochemical staining observed in the membrane of normal (plate F). Moderate membranousimmunohistochemical staining observed within the membrane of BPH (plate E). Strong membranousimmunohistochemical staining observed in Prostate Cancer (plate D)

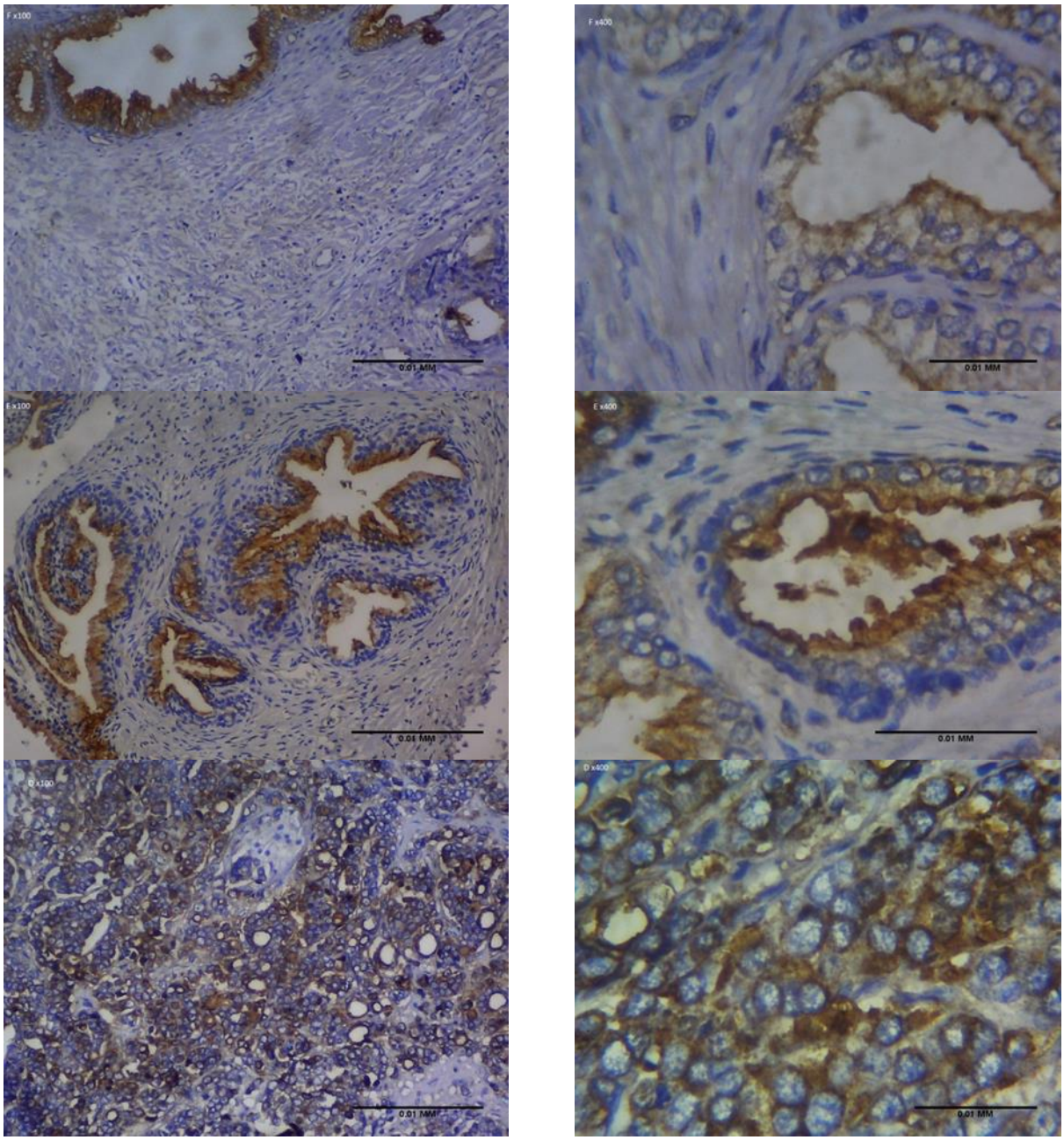

Figure 4: PSMA immunohistochemistry plates

Cytoplasmic PSMA stained sections of normal prostate tissue at x100 and x400 (plate F); BPH at x100 and x400 (plate E); prostate cancer at x100 and x400 (plate D). Mild immunochemical staining observed in the cytoplasm of normal (plate F). Mild to moderate cytoplasmic immunohistochemical staining observed within the cytoplasm of BPH (plate E). Strong cytoplasmic immunohistochemical staining observed in Prostate Cancer (plate D).

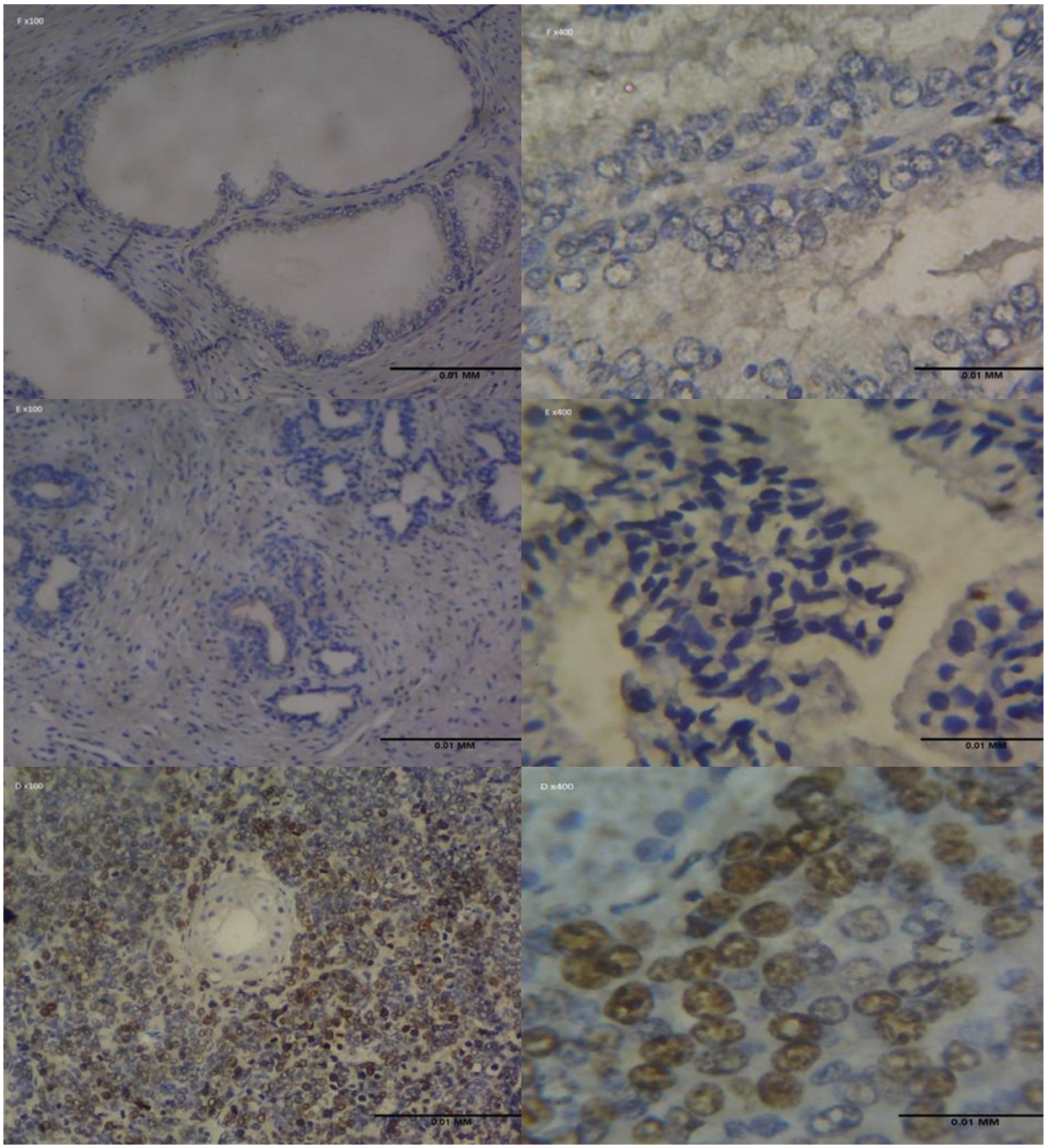

Figure 5. P53 immunohistochemistry plates

Nuclear P53 stained sections of normal prostate tissue at x100 and x400 (plate F); BPH at x100 and x400 (plate E); prostate cancer at x100 and x400 (plate D). Negative immunochemical staining observed in the nucleus of normal (plate F). Negative immunohistochemical staining observed within the nucleus of BPH (plate E). Strong nuclear immunohistochemical staining observed in Prostate Cancer (plate D).

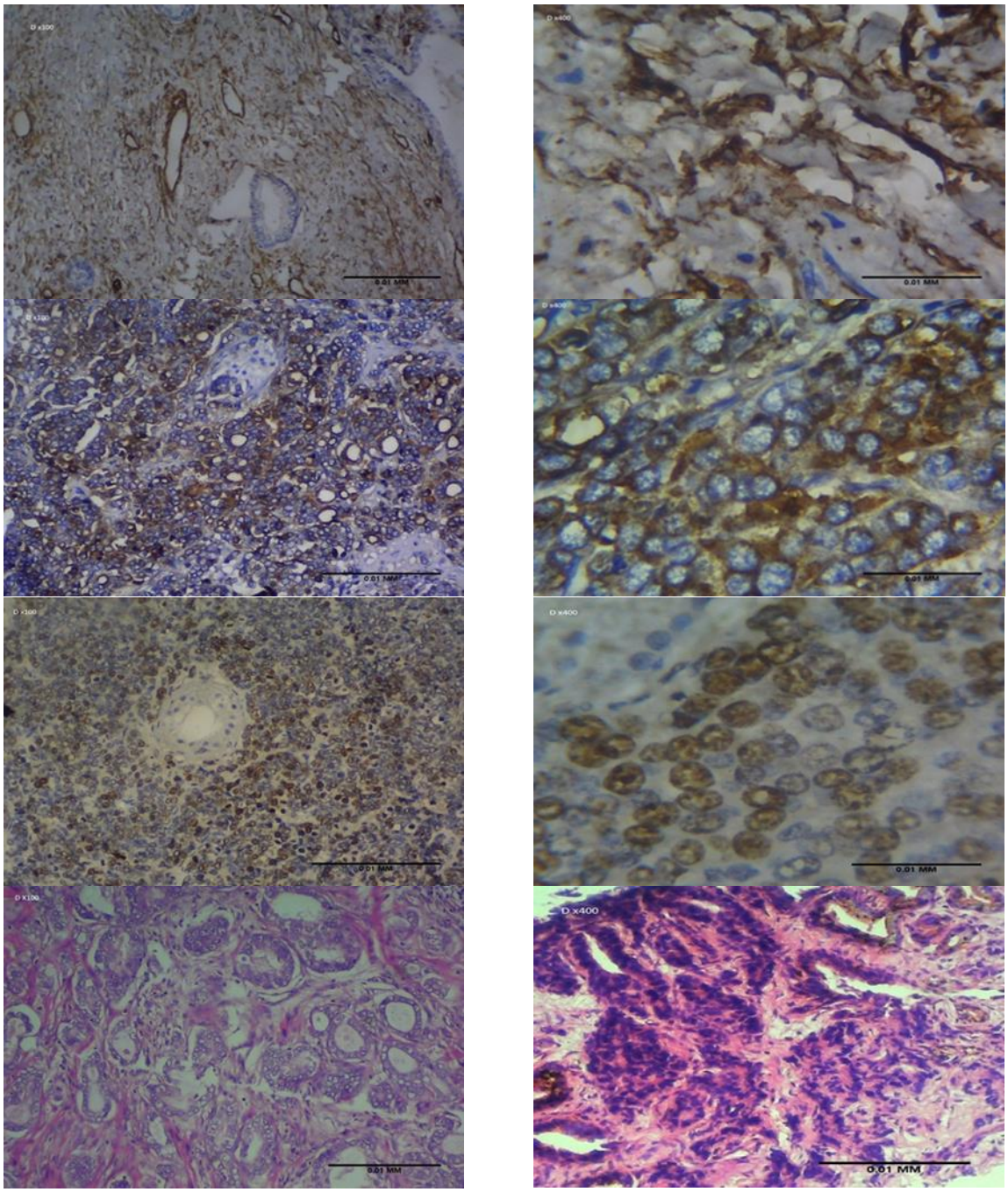

Figure 6; Prostate Adenocarcinoma plates.

CD34 immunohistochemical stained section of prostate cancer showing Strong nuclear immunohistochemical staining. PSMA immunohistochemical stained section of prostate cancer showing Strong cytoplasmic immunohistochemical staining. P53 immunohistochemical stained section of prostate cancer showing Strong nuclear immunohistochemical staining. H&E sections showed occlusion of glands, hyperchromasia, anisonueclosis, and high nucleus to cytoplasmic ratio.

DISCUSSION

Prostate Cancer is one of the most common health threats for men in the developed world and is described as unregulated growth and consequent spread of cells to other parts of the body (Elkahwaji, 2013). All types of cells in the prostate can undergo such malignant changes and become cancers, however only epithelial cells can become carcinomas. The normal cell cycle is disrupted and the new "tumor" cells overgrow in a prostate at first, then spread to surrounding tissue and finally to other parts of the body via the lymphatic system and

vascular system (Stuelton *et al*., 2018). Adenocarcinoma is a type of cancer arising from epithelial cells of the secretary glands lining the prostatic ducts. The cytological features include enlarged hyper-chromatic nuclei, as in PIN (Rani *et al*., 2020). The genetics of prostate cancer are poorly understood, we know cancers almost always arise from a single somatic cell that undergoes a number of genetic changes which cause a change in gene activity and therefore phenotype (Janiszewska, 2020). Cancer causing mutations usually arise in genes involved in the regulation of cellular growth or death (Reed *et al*., 2007). Prostate cancer is the third-leading cause of cancer death in men, exceeded by lung cancer and colorectal cancer. It accounts for 19% of all male cancers and 9% of male cancer-related deaths. Cases ranged from an estimated 230,000 in 2005 to an estimated 164,690 in 2018 (Jemal *et al*., 2005). Rates of prostate cancer vary widely. Rates vary widely between countries. It is least common in South and East Asia, and more common in Europe, North America, Australia, and New Zealand. Prostate cancer is least common among Asian men and most common among black men, with white men in between (Lozano *et al*., 2012).

CD34 plays a key role in the inhibition of hematopoietic stem cell differentiation, attachment of stem cells to bone marrow, angiogenesis and cell–cell adhesion (Sidney *et al*., 2014). CD34 is a single-pass trans-membrane protein with a molecular weight of 105.120 kDa (Kumagai *et al*., 2014). It is expressed on the surface of a variety of cells, particularly on vascular endothelial cells; therefore, CD34 is often used to label vascular endothelial cells (Ho *et al*., 2013; Foroozan *et al*., 2017). Moreover, CD34 is more likely to be expressed on newly-formed vascular endothelium (Ajili *et al*., 2012). A high expression of CD34 in tumor tissue indicates intensive tumor neovascularization and increased MVD. Among micro vascular immunohistochemical markers, CD34 has the best sensitivity and stability with a high positive rate and expression level. CD34 is expressed in the small blood vessels of tumor tissues (Teo *et al*., 2002). Moreover, the expression level of CD34 in the endothelium of newly-formed blood vessels is higher than that in old blood vessels, suggesting that CD34 is involved in tumor neovascularization (Miyata *et al*., 2015).From this study, our findings revealed that CD34 is expressed significantly in prostate cancer and in normal having higher immunohistochemical expression and degree of reactivity while in benign prostatic hyperplasia, a moderate immunohistochemical staining was observed within the membrane. The positivity rate of CD34 among the cases was eighty percent (80%) in normal, sixty percent (60%) in BPH and ninety percent (90%) in prostate cancer and the mean percentage reactivity was 80%, 60% and 90% in normal, BPH and in prostate cancer respectively. The immunohistochemical expressions and reaction was membranous. CD34 was expressed in normal prostate tissue with increased expression in the malignant prostate. In BPH, CD34 expression was moderate and in prostate cancer CD34 is overly expressed. This finding is in agreement with the result of Nassif *et al*., (2010), Foorozan*et al*., (2017) whose findings showed an increased expression of CD34 in prostate cancer samples compared to benign prostate tissue.

Prostate-specific membrane antigen (PSMA) is expressed on the cell surface in normal prostate tissue and is overexpressed in prostate cancer by several orders of magnitude. It is a type II transmembrane glycoprotein encoded by the folate hydrolase 1 (FOLH1) gene, also referred to as the glutamate carboxypeptidase II (GCPII) gene. The unique expression profile of PSMA provides an excellent target for prostate cancer imaging and therapy (Eder *et al*., 2012, Eder *et al*., 2014).

From the results, PSMA is expressed significantly in prostate cancer and in BPH having higher immunohistochemical expression and degree of reactivity while in normal, a mild immunohistochemical staining was observed in the membranes and cytoplasm. The positivity rate of PSMA among the cases was thirty percent (30%) in normal, seventy percent (70%) in BPH and ninety percent (90%) in prostate cancer and the mean percentage reactivity was 30%, 50% and 90% in normal, BPH and in prostate cancer respectively. The immunohistochemical expressions and reaction were cytoplasmic and membranous. PSMA in normal prostate is normally expressed and there is an increase in expression in normal prostate and in benign case PSMA expression is moderate and this was shown in agreement with bostwick et al., 1998. PSMA is overly expressed in prostate cancer and this was shown in agreement with the findings of Ghosh and Heston 2004, Jemaa *et al*., 2010, Hupe*et al*., 2018) who reported that Prostate-specific membrane antigen (PSMA) is expressed on the cell surface in normal prostate tissue and is over expressed in prostate cancer by several orders of magnitude. PSMA has a unique folate hydrolase activity whereas PSMA exists in the cell cytoplasm and has no enzymatic activity. PSMA plays a role in prostate carcinogenesis and that the enzymatic activity and/or the extracellular location of PSMA are important.

A suggested mechanism by which PSMA might be involved in prostate carcinogenesis is via its folate hydrolase activity. It is thought that this membrane folate hydrolase could give prostate cancer cells expressing PSMA a growth advantage in a low folate tumor microenvironment by allowing these cells to capture extracellular folates and deglutamate poly-γ-glutamated folates released by surrounding dead and dying cells. Folate taken up by these cells is an essential nutrient for growth and replication (Yao and Bacich, 2005).

P53 gene is located on the seventeenth chromosome (17p13.1) and it's known as the "guardian of the genome" thanks to its capacity to respond to outside stresses, which promotes transient or permanent cycle arrest and apoptosis, following different stress factors including hypoxia, DNA impairment, oxidative stress, hyper-proliferative signals, nutrient shortage (Jorde, 2000). P53 supports tumor suppression through its roles as transcription factor and mitochondrial membrane permeabilization (to trigger apoptosis) and, indeed, the most investigated biological activity of p53 is its transcriptional activator role (Beuzeboc *et al*., 2009). The loss of p53 gives way to the initiation and progression of malignancies, which are generally characterized by more malignant features such as intensified invasiveness and metastatic capability, genetic instability and poor cellular differentiation (Morigi *et al*., 2016).In this study, 85% cases of carcinoma revealed strong nuclear positivity with p53 immunostain. From the results, P53 is expressed significantly in prostate cancer having higher immunohistochemical expression and degree of reactivity, in normal it was negative (less than 10%) and BPH it was almost negative. The immunohistochemical staining was observed in the nucleus. The positivity rate of P53 among the cases was ten percent (10%) in normal, seventy percent (10%) in BPH and ninety percent (85%) in prostate cancer and the mean percentage reactivity was 3%, 5% and 80% in normal, BPH and in prostate cancer respectively. The immunohistochemical reaction of p53 was found in the nucleus and it is not expressed in normal prostate tissue, and almost absent in BPH but markedly expressed in prostate cancer, a finding in agreement with Jemal et al., 2007. P53 expression was up-regulated in prostate carcinoma (85%) as compared with benign prostatic tissue (10%) and this was shown in agreement with (jiang *et al*., 2005, Verma *et al*., 2015).

CONCLUSION

This study established the usefulness of CD34, PSMA and P53 immunohistochemical markers in the study of the prostate tissues from normal to BPH and to a malignant prostate. These markers provided differential diagnosis of different prostatic lesion, hence their use is recommended in histopathology laboratories alongside routine Hematoxylin and Eosin in the diagnosis of prostate biopsies.

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