Quest Journals
Journal of Medical and Dental Science Research
Volume 8~ Issue 2 (2021) pp: 41-51
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Prognostic Value of Amacr and Ki-67 in Progression of Prostate Cancer

Oladele Abraham Ayowole[1], Yusuf Nabil Hassan[1], Ekundina Victor Olukayode[1] , Wilson Hillary Toluwalope [1], and Aremu Adegoke

Departments of [1]Medical Laboratory Science (Histocytopathology Unit) college of medicine and health sciences, Afe Babalola University, Ado-Ekiti, Ekiti state and [2]Department of Morbid Anatomy, Obafemi Awolowo university teaching hospital, Osun state, Nigeria.

ABSTRACT
Prostate cancer is the second most frequently diagnosed cancer and the fifth leading cause of cancer death in male Incidence increases from 20% in men in their fifties to approximately 70% in men between the age of 70 and 80 years. Prostate cancer is not only significant for its lethality but also for the extremely high morbidity associated with it. Immunohistochemistry has proven to be an extremely important tool in the monitoring of the progression prostatic carcinoma as the immunohistochemical markers are known to be specific in nature. The purpose of this study was to evaluate the relevance of Ki-67 & AMACR expression in the progression to prostatic carcinoma prognosis. A retrospective study in which IHC analysis of the expression of Ki-67 and AMACR was performed 60 confirmed formalin fixed, paraffin-embedded tissue blocks retrieved from pathology archives, This tissue blocks were divided into normal prostatic tissue(10) , BPH(20) and Pca(30) according to their confirmed histologic diagnosis. The staining was evaluated and the results were considered. Cytoplasmic AMACR staining was expressed within the cells of BPH and PCa. Nuclear Ki-67 staining was expressed within the cells of BPH and PCa. The mean percentage reactivity of Ki-67 and AMACR in normal, BPH and PCa was calculated and presented as; 10% in normal, 45% in BPH and 100% in PCa in Ki-67, and 10% NO, 15% BPH and 100% PCa in AMACR. The mean percentage immunoreactivity for LGPIN and HGPIN was also calculated as 60% in LGPIN and 80% in HGIN when stained with Ki-67, and 50% in LGPIN and 75% in HGPIN when stained with AMACR. The expression of Ki-67, and AMACR in Normal, BPH and Pca tissue has therefore confirmed the ability of this markers effectiveness in predicting the progression of normal prostatic tissue, to prostatic carcinoma.

KEYWORDS: *IHC, Pca,AMACR& Ki-67.*

Received 03 Feb, 2021; Revised: 15 Feb, 2021; Accepted 17 Feb © The author(s) 2021.
P*ublished with open access at www.questjournals.org*

I. INTRODUCTION

Prostate cancer is a leading cause of cancer-related deaths among men. It is the most commonly diagnosed cancer among Nigerian men (Mohammed *et al.*,2008). An estimated hospital prevalence of 127 per 100,000 in Lagos, Nigeria was reported in 1997(Adeloye *et al*.,2016). A recently published data from southwestern Nigeria also reported a hospital prevalence rate of 182.5 per 100,000 male admission in the hospital (Ikuerowo *et al.*, 2013). However, the true prevalence in the Nigerian community is not known. BPH or benign prostatic hyperplasia is a non-cancerous enlargement of the prostate gland, affecting more than 50 percent of men over the age of 60. BPH is linked to hormonal changes as a man gets older. The balance of hormones in the body changes as men get older and this may cause the prostate gland to grow (Chapple ,2002). benign prostatic hyperplasia (BPH) occurs mostly in the transition zone of the prostate(Mikolajczyk *et al*.,2000).The prostate consists of glandular and connective tissue, but nearly all prostate cancers develop from glandular cells, which make fluid that becomes part of semen(Young, 2000). Prostate cancer cells can spread by invading nearby organs and tissues, such as the bladder or rectum, or by traveling through the blood or lymph to other parts of the body. This is known as metastatic prostate cancer.(Mustafa *et al*.,2016). prostate cancer is found in the peripheral zone(Mikolajczyk *et al*.,2000). While the incidence of prostate cancer is rising, the rate of prostate cancer-specific mortality in patients with high-risk disease has remained relatively unchanged(Kelly *et al.*,2017). This is partly due to the early diagnosis, Both digital rectal examination (DRE) and prostate-specific antigen (PSA) testing form two of the key components of the assessment of the prostate gland. The

limitations of PSA as a biomarker for prostate cancer are well known, PSA is not tumor specific in the prostate(Walsh *et al.*,2014). PSA levels can increase due to a number of other factors like BPE (benign prostatic enlargement), older age ; PSA levels normally increase with age. But this limitation can be overcome with the aid of with staining techinique like immunohistochemical (IHC) staining(You,2011). IHC involves specific antigen–antibody reactions with chromogen, it has apparent advantage over traditionally used special enzyme staining techniques that identify only a limited number of proteins, enzymes, and tissue structures. Therefore, IHC has become a crucial technique and is widely used in many medical research laboratories as well as clinical diagnostics(Ramos-Vara,2014).

The definite cause of Pca has not been established but, Studies have proven that gene mutations plays an etiologic role in prostatic cancer(Yang *et al.*,2017). Some gene mutations can be passed from generation to generation (inherited) and are found in all cells in the body. Inherited gene changes are thought to play a role in about 10% of prostate cancers(Xavier *et al.*,2019).

The Ki67 is a nuclear protein and it is the most widely recognized marker of proliferating cells(Jurikova *et al.*,2016). Ki67 is encoded by the *MKI67* gene. During interphase the Ki67 antigen can be detected in the cell nucleus whereas in mitosis most of the cell protein is located at the surface of the chromosomes(Sun *et al.,* 2018). Ki67 is present during all active phases of the cell cycle but absent in resting cells. The antigen appears to be degraded after mitosis, thus it could not be detected in resting (G0) cells. During the S phase, Ki-67 staining increases. This increase becomes more remarkable during the G2 phase, and staining intensity is highest in metaphase. As the cells move through the end of mitosis (i.e. anaphase/telophase) the intensity of Ki-67 staining begins to decrease(Gil *et al*.,2018). Ki67 seems have a significantly marked expression in Pca, but has a lower expression in BPH and minimal to no expression in normal prostatic tissue(Velonas *et al.*,2013).

Alpha-methylacyl-CoA racemase (AMACR), formerly known as P504s, is a mitochondrial and peroxisomal enzyme involved in the beta-oxidation of branched fatty acids and bile acid intermediates(Langner *et al.*,2006). This gene encodes a racemase. The encoded enzyme interconverts pristanoyl-CoA and C27-bile acylCoAs between their (R)- and (S)-stereoisomers. The conversion to the (S)-stereoisomers is necessary for degradation of these substrates by peroxisomal beta-oxidation. Encoded proteins from this locus localize to both mitochondria and peroxisomes(Kim *et al*.,2012). is used as a confirmatory stain for prostate cancer in conjunction with morphology and a basal cell–specific marker. Using AMACR as a positive marker alone might be misleading because weak expression of AMACR might be seen in benign glands and expression of AMACR is seen in high grade prostatic intraepithelial neoplasia (PIN) (Jiang *et al.* 2004) and atypical adenomatous hyperplasia (AAH) (Yang *et al.* 2002). Therefore, using AMACR as a positive marker along with basal cell-specific negative marker (p63) will enhance the diagnostic accuracy in minimal prostate cancer and reduce the chance of misdiagnosis(Pértega-Gomes *et al*.,2013). AMACR has been consistently overexpressed in prostate cancer epithelium; hence it becomes an ideal specific biomarker for cancer cells within the prostate gland (Rubin *et al*., 2005). Over-expression of AMACR may increase the risk of prostate cancer, because its expression is increased in premalignant lesions (prostatic intraepithelial neoplasia)(Lin *et al*.,2012).

The ability of ki67 and AMACR as IHC markers capable of predicting prostatic prostrate carcinoma from normal to prostatic carcinoma samples was assessed by correlating their expression of IHC with the progression of normal prostatic tissue to prostatic carcinoma. If they are found to be predictors of prostatic carcinoma, these IHC markers may prove to be useful in clinical practice.

II. MATERIALS AND METHODS

Case Selection: prostatic tissue blocks will be selected from the pathology files of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC). All samples were fixed in formalin and embedded in paraffin wax by conventional techniques. Haematoxylin and eosin stained slides of all samples were reviewed and classified. Confirmed prostatic tissue blocks of non-malignant, BPH and prostatic carcinoma were selected. In total, 60 biopsy samples were taken. Among these, 10 prostatic tissue blocks were non-malignant, 20 prostatic tissue blocks had BPH diagnosis, 30 prostatic tissue blocks were diagnosed with PCa .

PREPARATION OF SECTIONS FOR IMMUNOHISTOCHEMISTRY

All of the specimens were formalin-fixed and paraffin-embedded. 5µm thick serial sections were cut, and the end sections were stained with H&E to ensure that the lesions were still present in the serial sections. The sections were processed for immunohistochemical analysis as followed;

Deparaffinization was carried out with xylene followed by rehydration through graded alcohols. Epitope retrieval was performed by heating the sections for 10 min in citrate buffer (pH6.0) at 121°C. The sections were incubated in 3% hydrogen peroxide (H_2O_2) in methanol for 5 min to block endogenous activity, followed by blocking of nonspecific binding of primary antibodies to epitopes by a preincubation step with 5% normal goat serum for 10 min at 37°C. The primary antibodies used in this study are Ki-67 (Dako, M7240, 1:100) and AMACR antibody Monoclonal rabbit Anti Human AMACR-clone 13H4 Incubation with antibodies

was done for 30 min at room temperature. Colour development will be carried out with diaminobenzidine (DAB). The slides was counterstained with haematoxylin, dehydrated in ascending grades of alcohol, cleared in xylene and mounted in Dibutylphtalate polystyrene xylene (DPX) mountant (Specht, *et al*., 2001).

Staining expression was evaluated optically using the light microscope at ×100 magnification. Photomicrographs of fields of relevance were taken at × 100 magnifications with the use of a light microscope and camera. Expression of ki-67 and AMACR was determined through a semi-quantitative method.

IMMUOSTAINING ASSESMENT

Alpha-methylacyl CoA racemase and ki-67 immunohistochemical staining is evaluated by a semi-quantitative method using 0-3 scale as a combination of intensity and distribution. Where 0 is classified as absent or negative expression, 1 represents mild or weak expression, 2 represents moderate expression and 3 represents strong expression.

No expression, no detectable staining in < 10% of the membrane, weak but detectable discontinuous staining present in 10-39% of the membrane, moderate, clearly positive discontinuous staining present in 40-90% of the membrane, intense continuous staining of membrane creates a honeycomb pattern(Tolonen,2011).

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 tissue blocks studied were taken at various magnifications, and reported for Morphological changes.

DATA ANALYSIS

Analysis of observed data carried out using Graph pad prism software program.

Table 1 shows the comparison of the immunochemistry staining expression of KI-67 in the various stages in the progression to Prostatic carcinoma along with their percentage reactivity.

Group	Total cases	-	+	++	+++	Mean percentage reactivity
Normal/Control	10	9	1	0	0	10%
BPH	20	11	9	0	0	45%
PCA	30	0	0	10	20	100%

Table 1 shows the grading of the expression of KI-67 observed in various tissue sections which have been grouped into the various stages of the progression to Prostatic carcinoma according to their confirmed histologic diagnosis. 1 out of 10 cases diagnosed as normal Prostatic tissue was positive for KI-67, 9 out of 20 cases diagnosed of BPH was positive for Ki-67. while 100% of the 30 cases diagnosed to Prostatic carcinoma were KI-67 positive.

Table 2: shows the comparison between the percentage negativity and percentage positivity of Ki-67

Groups	N	Negative (n%)	Positive (n%)
Normal/Control	10	9(90)	1(10)
BPH	20	11(55)	9(45)
PCA	30	-	30(100)

Table 3 shows the comparison between the percentage negativity and percentage positivity of Alpha-methylacyl CoA racemase (AMACR)

Groups	N	Negative (n%)	Positive (n%)
Normal/Control	10	10(100)	-
BPH	20	14(70)	6(30)
PCA	30	0(0)	30(100)

Table 4 shows the correlation between histological diagnoses of the progression to Prostatic carcinoma and staining intensity of Alpha-methylacyl CoA racemase (AMACR)

Intensity	Normal	BPH	PCA
Negative	10(100)%	14(70)%	-
Weak	-	6(30)%	-
Moderate	-	-	5(17)%
Strong	-	-	25(83)%
Total	10	20	30

Table 5 shows the increasing staining intensity from cases with normal Prostatic tissue to Prostatic carcinoma Weak, moderate and staining patterns were observed. A positive correlation between lesion severity and staining intensity of Alpha -methylacyl CoA racemase (AMACR) was noticed.

Table 5: A table showing a the mean reactivity of immunohistochemical marker of PIN

GRADES	KI-67 Pos(n%)	AMACR Pos(n%)
LOW GRADE PIN	60%	50%
HIGH GRADE PIN	80%	75%

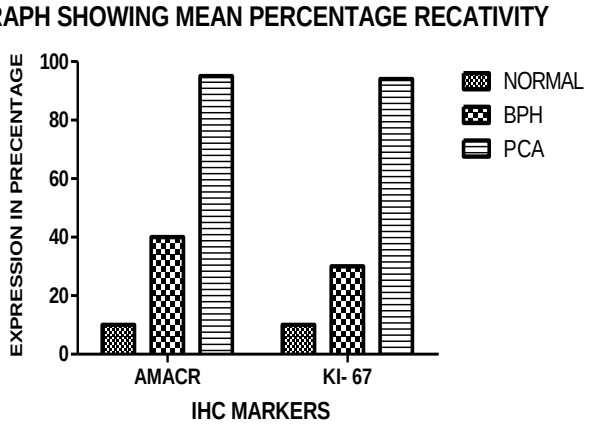
Table 5: showing the mean percentage reactivity of Ki-67 and AMACR on LGPIN (Low grade prostatic intraepithelial neoplasia) and HGPIN (High grade prostatic intraepithelial neoplasia). All areas of LGPIN present showed a mean percentage of 60% when stained with Ki-67 and 50% when stained with AMACR. All areas of HGPIN present showed a mean percentage 80% when stained with Ki-67 and 75% when stained with AMACR.

Table 6 shows the comparison between the Mean percentage reactivity of KI-67 and AMACR in the various stages in the progression to Prostatic carcinoma.

Groups	KI-67	AMACR
Normal/Control	10%	10%
BPH	30%	40%
PCA	95%	95%

Table 6 illustrates the correlation between the Mean percentage reactivity rate of tumour biomarkers KI-67 and AMACR in the various stages in the progression to Prostatic carcinoma. It was observed that there was an up regulation in the Mean percentage reactivity rate as the condition progressed from a state of pre-malignancy to malignancy in the tissues stained with both markers.

Figure 7 a graph illustrating shows the comparison between the Mean percentage reactivity of KI-67 and AMACR in the various stages in the progression to Prostatic carcinoma



HISTOPATHOLOGICAL MORPHOLOGY
HAEMATOXYLIN AND EOSIN STAINING

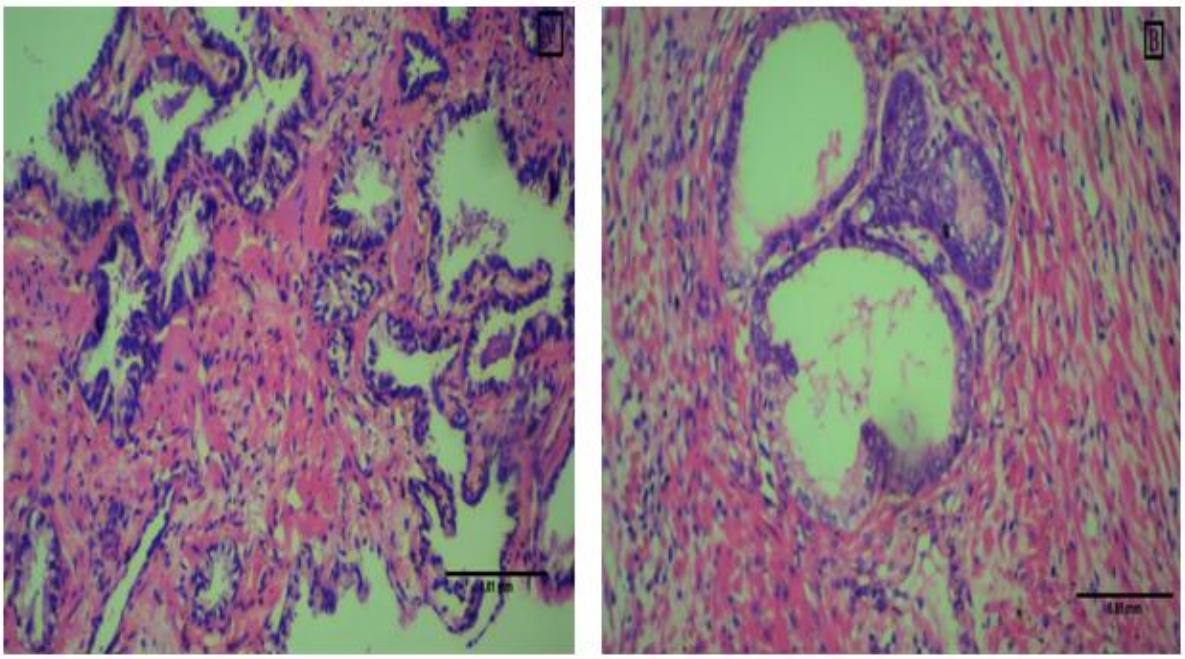


Plate 1: *micrographs of Prostatic sections stained with H&E showing: (A) (H&E ×100) showing showing the entire duct acinar system lined by pseudostratified columnar epithelum with secretory cells, Plate 2: micrographs of Prostatic sections stained with H&E showing: BPH (B) (H&E ×100) showing hyperplasia of glandular and stromal tissue with papillary buds infoliding*

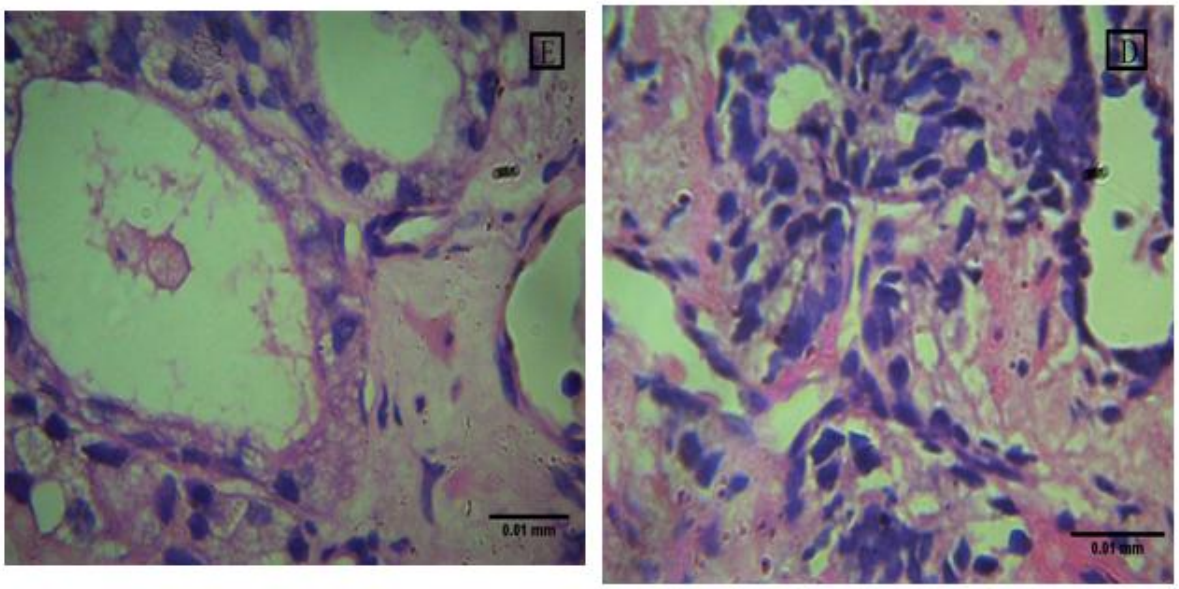


Plate 3: micrographs of Prostatic sections stained with H&E showing: Low grade PIN(E) (H&E ×400) showing epithelial cells that are irregularly spaced and pleomorphism. Plate 4: micrographs of Prostatic sections stained with H&E showing High grade PIN (D) (H&E ×400) Showing hyperchromatic and pleomorphism.

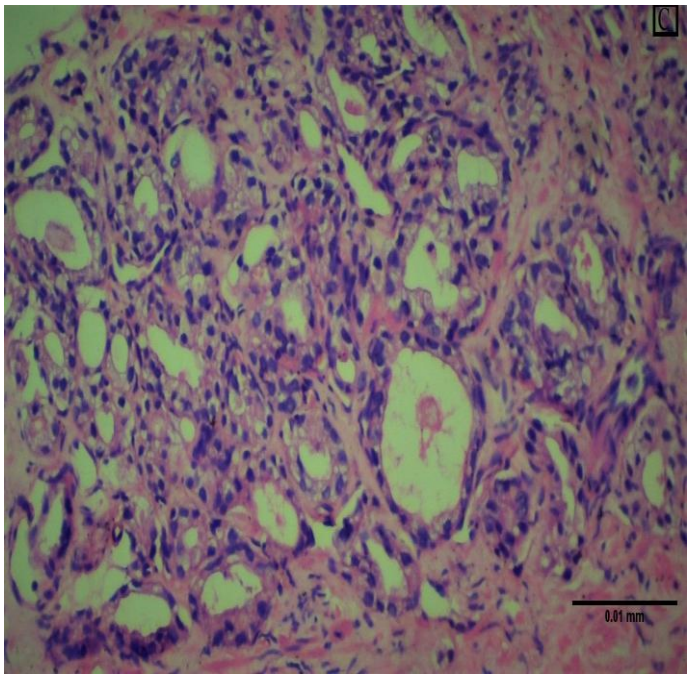


Plate 5: micrographs of Prostatic sections stained with H&E showing: prostratic carcinoma(C) (H&E ×100) showing hyperchromatic nuclei that have a single prominent nucleolus and invasion of cells at the basement membrane.

4.4 IMMUNOHISTOCHEMISTRY STAINING REACTION
4.4.1 Ki-67 STAINING REACTION

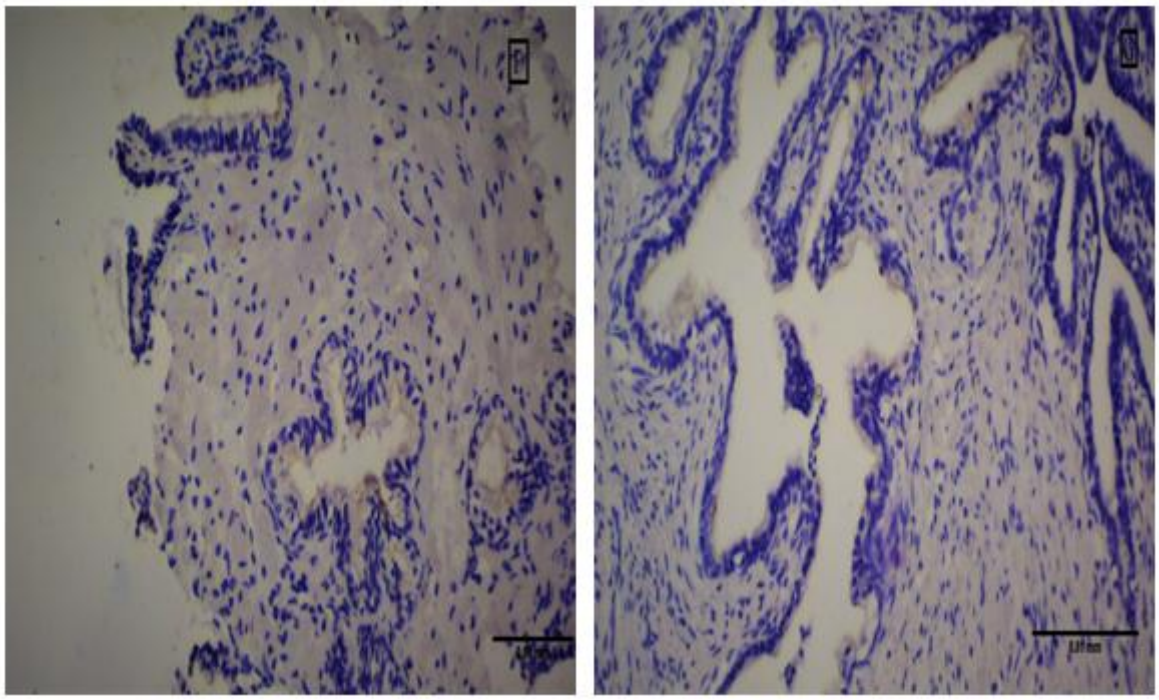


Plate 6: micrographs of prostatic sections stained with Ki-67 showing: (F) normal prostatic tissue (KI-67×100) illustrating the basement membrane (BM) in normal prostatic tissue with cells having nuclear expression Ki-67 being <10%. Plate 7: micrographs of prostatic sections stained with Ki-67 showing: (G)BPH(KI-67×100) Showing 10% of cells showing nuclear expression of Ki-67.

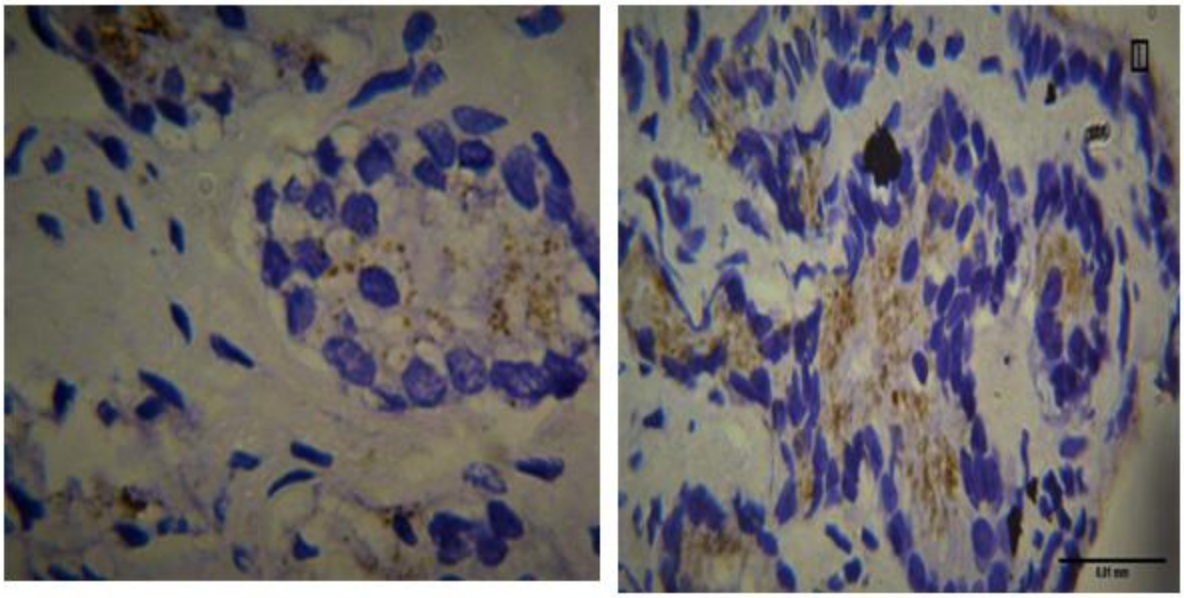


Plate 8: micrographs of prostatic sections stained with Ki-67 showing: (J) (KI-67 ×400) showing cells with hyperchromatic nuclei and pleomorphism in Low grade PIN. Plate 9: micrographs of prostatic sections stained with Ki-67 showing: (I) (KI-67 ×400) showing epithelial cells crowding and stratification with prominent nucleoli in High grade PIN.

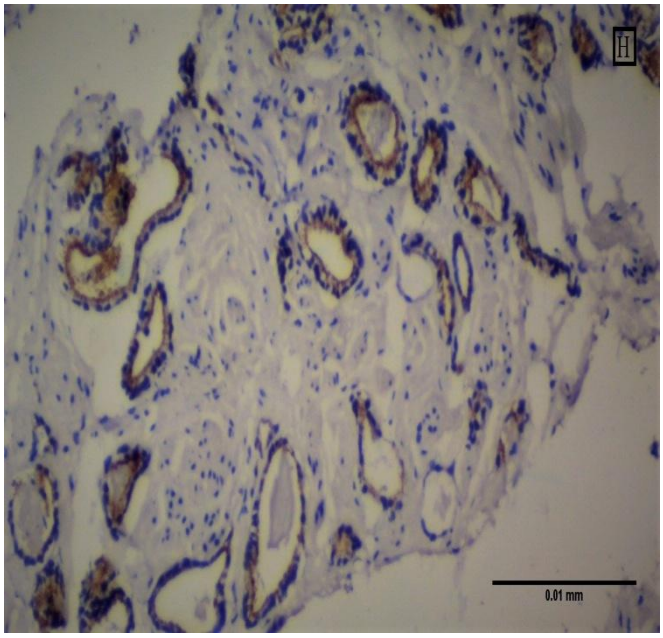


Plate 10: micrographs of prostatic sections stained with Ki-67 showing: (D) PCA (KI-67×100) Showing 90% of cells showing nuclear expression of Ki-67.

4.4.2 AMACR STAINING REACTION

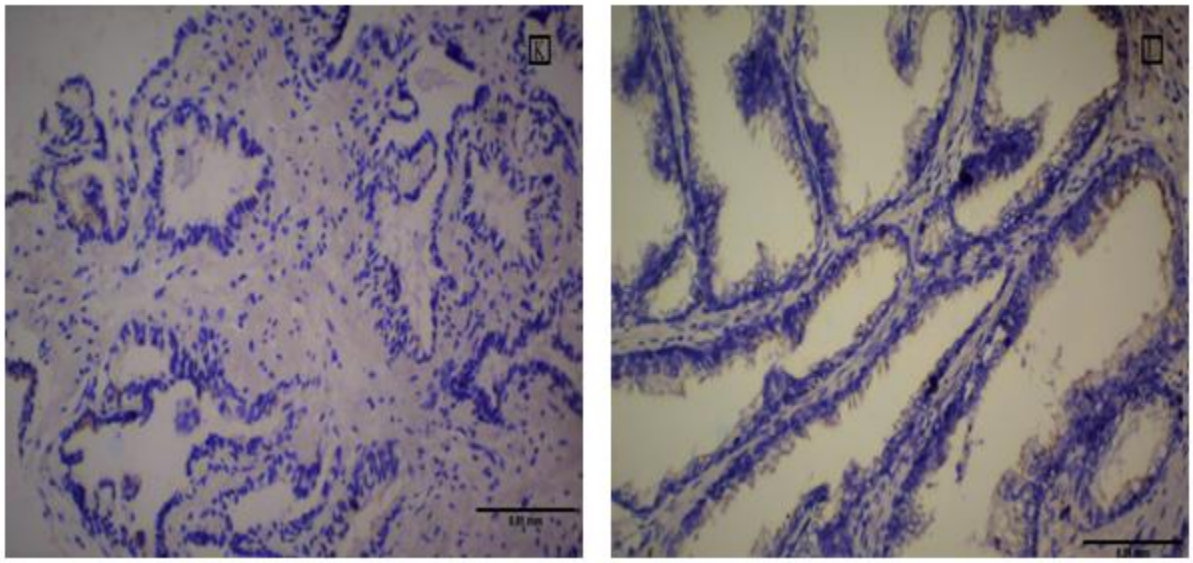


Plate 11: micrographs of prostatic sections stained with AMACR showing: (K) normal prostatic tissue (AMACR×100) illustrating the basement membrane (BM) in normal prostatic tissue with cells having nuclear expression AMACR being <10%. Plate 12: micrographs of prostatic sections stained with AMACR showing :(L)BPH(AMACR×100) Showing 10% of cells showing cytoplasmic expression of AMACR

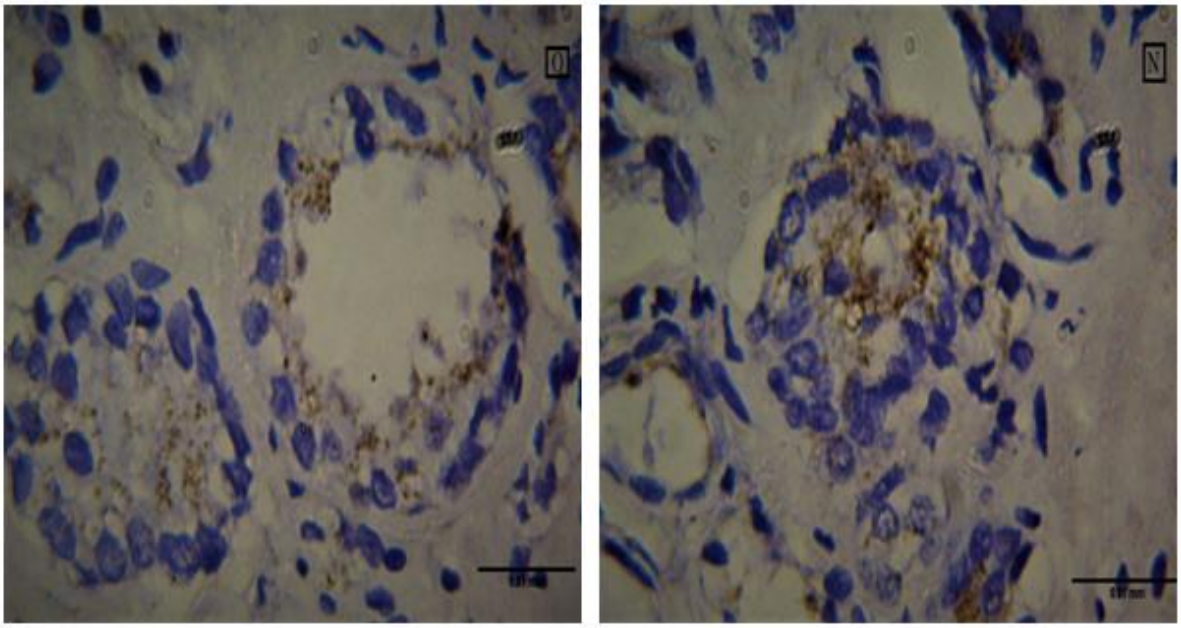


Plate 13: micrographs of prostatic sections stained with AMACR showing: (O) (AMACR ×400) showing cells with hyperchromatic nuclei and pleomorphism in Low grade PIN. Plate 14: micrographs of prostatic sections stained with AMACR showing: (N) (AMACR ×400) showing epithelial cells crowding and stratification with prominent nucleoli in High grade PIN.

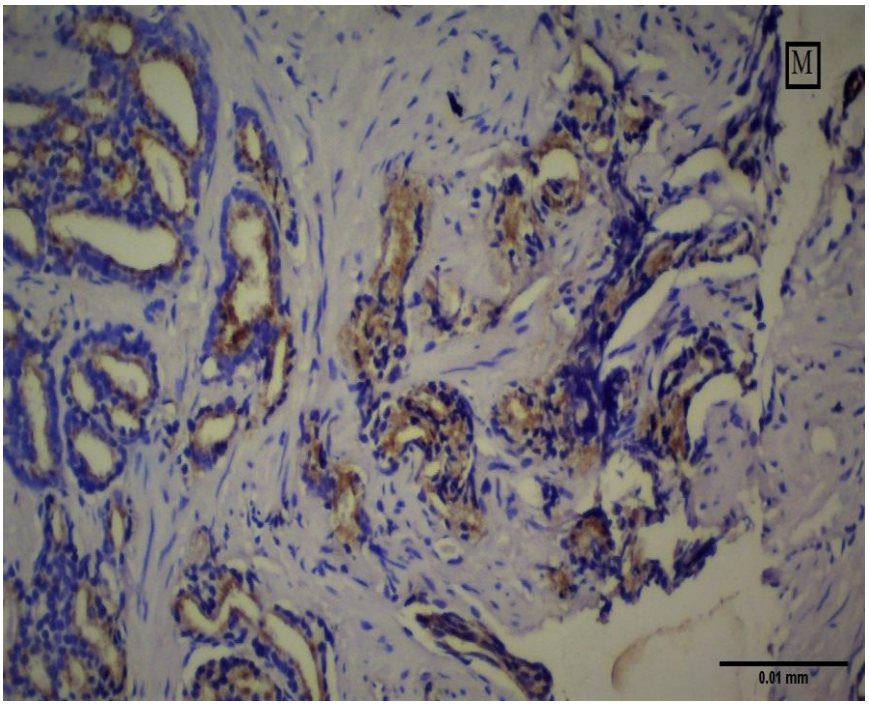


Plate 15: micrographs of prostatic sections stained with AMACR showing: (M) PCA (AMACR×100) Showing 95% of cells showing cytoplasmic expression of AMACR.

III. DISCUSSION

Prostate cancer is the second most frequently diagnosed cancer and the fifth leading cause of cancer death in males(Kimura, 2018). Incidence increases from 20% in men in their fifties to approximately 70% in men between the age of 70 and 80 years(Epstein,2005). Prostate cancer is not only significant for its lethality but also for the extremely high morbidity associated with it(Stephenson *et al.*,2009).

The Ki-67 protein is a nuclear antigen associated with cell proliferation and can be used as a marker for cell proliferation assay(Cidado *et al.*,2016). Results of this study showed that Ki-67 expression was minimal in normal prostatic tissues with only 10% of the distribution having a significant positive expression, this findings is in agreement with (Zhong *et al.,* 2008)in which the percentages of positive cells in BPH and normal prostate tissues were significantly lower than that in PCa tissues. From the results of immunohistochemistry, it can be seen that in BPH 45% of the distribution having a positive expression, this findings is in agreement with Bjartell *et al* that says 60% BPH cases were negative for Ki-67 staining, going with the fact that BPH is a disease associated with decreased rate of apoptosis rather than increased proliferation. As the condition progresses to prostatic carcinoma, more cases in the distribution were noted to have >50% positive nuclear staining reaction. 30 of 30 Pca sections showed highly significant positive reaction giving that distribution a positivity rate of 100%,this findings is in agreement with (Zhong *et al.,* 2008) which says that ki67 will be expressed high in Pca than other progression. The findings in this study are in concurrence with the studies of (Verma *et al.*,2015*)* and Zhong *et al* there is a directly proportional relationship between progression of prostatic cancer and percentage reactivity rate was observed in this group that was stained with ki-67.

AMACR. α-Methylacyl-CoA racemase (AMACR) has recently been reported as a vital solid tumor marker and is an attractive target for designing anti-tumor agents(Wajid Abbasi *et al.*,2015).It is a mitochondrial and peroxisomal enzyme which plays a central role in the oxidation of cholesterol metabolites and branched chain fatty acids. In this study, amongst the 10 normal prostatic tissues stained with AMACR 1 case displayed up 10% cytoplasmic staining expression of the marker which enabled it to be classified as positive, , this findings is in agreement with (Rubin *et al.*,2002) where high percentage of AMACR in normal stain negative. In the BPH cases, 30% of the 20 cases exhibited significant positive cytoplasmic reaction, this finding is in agreement with (Mahmoud *et al.,*2016) where the percentage expression of AMACR in BPH was low. In Pca cases, an almost uniform cytoplasmic staining was observed in 30 of the 30 selected cases, giving it a 100% reaction rate. Similar to this finding, (Ozgur *et al.,*2013). evaluated AMACR expression in the majority of their cases. However, they have determined a nonsignificant relationship with tumor grade and AMACR expression. The findings in this study are in concurrence with the studies of (Wright *et al.*,2009) and (Jiang *et al.*,2013) there was a direct relationship between the lesion severity, percentage positivity and staining intensity. There was a significant increase in the values of these parameters as the condition progressed from the premalignant phases to a state of malignancy.

IV. CONCLUSION

The expression Ki-67 and AMACR was observed in the transition from normal prostatic tissue to PCa are relevant markers in predicting whether a normal prostatic tissue will progress to PCa. While these markers have proven to be effective in predicting the progression of normal prostatic tissue to PCa, none of these markers can stand on its own to give a fully definitive result and should, as such, be used in concordance with each other in order to compensate for their limitations and obtain relevant results.

REFERENCES

[1]. Adeloye, D., David, R. A., Aderemi, A. V., Iseolorunkanmi, A., Oyedokun, A., Iweala, E. E., ... & Ayo, C. K. (2016). An estimate of the incidence of prostate cancer in Africa: a systematic review and meta-analysis. *PloS one*, *11*(4), e0153496.

[2]. Bjartell, A., Montironi, R., Berney, D. M., & Egevad, L. (2011). Tumour markers in prostate cancer II: diagnostic and prognostic cellular biomarkers. *Acta Oncologica*, *50*(sup1), 76-84.

[3]. Chapple, A., & Ziebland, S. (2002). Prostate cancer: embodied experience and perceptions of masculinity. *Sociology of Health & Illness*, *24*(6), 820-841.

[4]. Cidado, J., Wong, H. Y., Rosen, D. M., Cimino-Mathews, A., Garay, J. P., Fessler, A. G., ... & Zabransky, D. J. (2016). Ki-67 is required for maintenance of cancer stem cells but not cell proliferation. *Oncotarget*, *7*(5), 6281.

[5]. Epstein, J. I. (2005). The lower urinary tract and male genital system. *Robbins and Cotran Pathologic Basis of Diseases*.

[6]. Gil, R. S., & Vagnarelli, P. (2018). Ki-67: more hidden behind a 'classic proliferation marker'. *Trends in biochemical sciences*, *43*(10), 747-748.

[7]. Ikuerowo, S. O., Omisanjo, O. A., Bioku, M. J., Ajala, M. O., Mordi, V. P. N., & Esho, J. O. (2013). Prevalence and characteristics of prostate cancer among participants of a communitybased screening in Nigeria using serum prostate specific antigen and digital rectal examination. *Pan African Medical Journal*, *15*(1).

[8]. Jiang, N., Zhu, S., Chen, J., Niu, Y., & Zhou, L. (2013). A-methylacyl-CoA racemase (AMACR) and prostate-cancer risk: a meta-analysis of 4,385 participants. *PLoS One*, *8*(10), e74386.

[9]. Jiang, Z., Wu, C. L., Woda, B. A., Iczkowski, K. A., Chu, P. G., Tretiakova, M. S., ... & Krausz, T. (2004). Alpha-methylacyl-CoA racemase: a multi-institutional study of a new prostate cancer marker. *Histopathology*, *45*(3), 218-225.

[10]. Jurikova, M., Danihel, Ľ., Polák, Š., & Varga, I. (2016). Ki67, PCNA, and MCM proteins: Markers of proliferation in the diagnosis of breast cancer. *Acta histochemica*, *118*(5), 544-552.

[11]. Kelly, S. P., Rosenberg, P. S., Anderson, W. F., Andreotti, G., Younes, N., Cleary, S. D., & Cook, M. B. (2017). Trends in the incidence of fatal prostate cancer in the United States by race. *European urology*, *71*(2), 195-201.

[12]. Kim, B. J., Kim, S. M., Cho, M. K., Yu, H. S., Lee, Y. S., Cha, H. J., & Ock, M. (2012). Expression and Characterization of α-Methylacyl CoA Racemase from Anisakis simplex Larvae. *The Korean journal of parasitology*, *50*(2), 165.

[13]. Kimura, T., & Egawa, S. (2018). Epidemiology of prostate cancer in Asian countries. *International journal of urology*, *25*(6), 524-531.

[14]. Langner, C., Rupar, G., Leibl, S., Hutterer, G., Chromecki, T., Hoefler, G., ... & Zigeuner, R. (2006). Alpha-methylacyl-CoA racemase (AMACR/P504S) protein expression in urothelial carcinoma of the upper urinary tract correlates with tumour progression. *Virchows Archiv*, *448*(3), 325-330.

[15]. Lin, P. Y., Cheng, K. L., McGuffin-Cawley, J. D., Shieu, F. S., Samia, A. C., Gupta, S., ... & Liu, C. C. (2012). Detection of alpha-methylacyl-CoA racemase (AMACR), a biomarker of prostate cancer, in patient blood samples using a nanoparticle electrochemical biosensor. *Biosensors*, *2*(4), 377-387.

[16]. Mahmoud, N. N., Abuelfadl, D. M., Abbas, N. F., Abdelaal, W. E., Badawi, M. A., & El-Sharkawy, S. L. (2016). Immunohistochemical expression of α-methylacyl coenzyme-A racemase in prostatic carcinoma: correlation with image morphometric parameters. *Journal of The Arab Society for Medical Research*, *11*(2), 56.

[17]. Mikolajczyk, S. D., Millar, L. S., Wang, T. J., Rittenhouse, H. G., Wolfert, R. L., Marks, L. S., ... & Slawin, K. M. (2000). "BPSA," a specific molecular form of free prostate-specific antigen, is found predominantly in the transition zone of patients with nodular benign prostatic hyperplasia. *Urology*, *55*(1), 41-45.

[18]. Mohamed, A. A., Abbas, M. Y., Alharbi, H., & Babiker, A. Y. (2018). Assessment of Expression of Ki-67 in Benign and Malignant Prostatic Lesions among Sudanese Patients. *Open Access Macedonian Journal of Medical Sciences*, *6*(10), 1809.

[19]. Mohammed, A. Z., Edino, S. T., Ochicha, O., Gwarzo, A. K., & Samaila, A. A. (2008). Cancer in Nigeria: a 10-year analysis of the Kano cancer registry. *Niger J Med*, *17*(3), 280-4.

[20]. Mustafa, M., Salih, A. F., Illzam, E. M., Sharifa, A. M., Suleiman, M., & Hussain, S. S. (2016). Prostate Cancer: Pathophysiology, Diagnosis, and Prognosis. *Journal of Dental and Medical Sciences*, *15*(6), 4-11.

[21]. Ozgur, T., Atik, E., Hakverdi, S., & Yaldiz, M. (2013). The expressions of AMACR and iNOS in prostate adenocarcinomas. *Pakistan journal of medical sciences*, *29*(2), 610.

[22]. Pértega-Gomes, N., Vizcaíno, J. R., Gouveia, C., Jerónimo, C., Henrique, R. M., Lopes, C., & Baltazar, F. (2013). Monocarboxylate transporter 2 (MCT2) as putative biomarker in prostate cancer. *The Prostate*, *73*(7), 763-769.

[23]. Ramos-Vara, J. A., & Miller, M. A. (2014). When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry—the red, brown, and blue technique. *Veterinary pathology*, *51*(1), 42-87.

[24]. Rubin, M. A., Bismar, T. A., Andrén, O., Mucci, L., Kim, R., Shen, R., ... & Kantoff, P. W. (2005). Decreased α-methylacyl CoA racemase expression in localized prostate cancer is associated with an increased rate of biochemical recurrence and cancer-specific death. *Cancer Epidemiology and Prevention Biomarkers*, *14*(6), 1424-1432.

[25]. Rubin, M. A., Zhou, M., Dhanasekaran, S. M., Varambally, S., Barrette, T. R., Sanda, M. G., ... & Chinnaiyan, A. M. (2002). α-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. *Jama*, *287*(13), 1662-1670.

[26]. Specht, K., Richter, T., Müller, U., Walch, A., Werner, M., & Höfler, H. (2001). Quantitative gene expression analysis in microdissected archival formalin-fixed and paraffin-embedded tumor tissue. *The American journal of pathology*, *158*(2), 419-429.

[27]. Stephenson, A. J., Kattan, M. W., Eastham, J. A., Bianco Jr, F. J., Yossepowitch, O., Vickers, A. J., ... & Scardino, P. T. (2009). Prostate cancer–specific mortality after radical prostatectomy for patients treated in the prostate-specific antigen era. *Journal of Clinical Oncology*, *27*(26), 4300.

[28]. Sun, X., & Kaufman, P. D. (2018). Ki-67: more than a proliferation marker. *Chromosoma*, *127*(2), 175-186.

[29]. Tolonen, T. (2011). *Molecular biomarkers and histopathological parameters in prostate cancer diagnostics and prognostics*. Tampere University Press.

[30]. Velonas, V. M., Woo, H. H., Remedios, C. G. D., & Assinder, S. J. (2013). Current status of biomarkers for prostate cancer. *International journal of molecular sciences*, *14*(6), 11034-11060.
[31]. Verma, R., Gupta, V., Singh, J., Verma, M., Gupta, G., Gupta, S., ... & Ralli, M. (2015). Significance of p53 and ki-67 expression in prostate cancer. *Urology annals*, *7*(4), 488.
[32]. Wajid Abbasi, S., & Sikander Azam, S. (2015). Structural Characterization of Alpha-methylacyl-CoA Racemase: Comparative Structural Modeling, Molecular Docking and Dynamic Simulations Studies. *Current cancer drug targets*, **15**(9): 822-835.
[33]. Walsh, A. L., Considine, S. W., Thomas, A. Z., Lynch, T. H., & Manecksha, R. P. (2014). Digital rectal examination in primary care is important for early detection of prostate cancer: a retrospective cohort analysis study. *Br J Gen Pract*, *64*(629), e783-e787.
[34]. Wright, M. E. (2009). *Dietary Influences on Alpha-Methylacyl-CoA Racemase (AMACR) Expression in the Prostate*. ILLINOIS UNIV AT CHICAGO.
[35]. Xavier, M. J., Roman, S. D., Aitken, R. J., & Nixon, B. (2019). Transgenerational inheritance: how impacts to the epigenetic and genetic information of parents affect offspring health. *Human Reproduction Update*, *25*(5), 519-541.
[36]. Yang, X. J., Wu, C. L., Woda, B. A., Dresser, K., Tretiakova, M., Fanger, G. R., & Jiang, Z. (2002). Expression of α-methylacyl-CoA racemase (P504S) in atypical adenomatous hyperplasia of the prostate. *The American journal of surgical pathology*, *26*(7), 921-925.
[37]. Yang, Y., Ji, C., Guo, S., Su, X., Zhao, X., Zhang, S., ... & Chen, H. (2017). The miR-486-5p plays a causative role in prostate cancer through negative regulation of multiple tumor suppressor pathways. *Oncotarget*, *8*(42), 72835.
[38]. You, J. (2011). *Discovery of novel potential protein diagnostic biomarkers for Prostate Cancer in serum and tears* (Doctoral dissertation, The University of New South Wales).
[39]. Young, R. H. (2000). *Tumors of the prostate gland, seminal vesicles, male urethra, and penis*. Amer Registry of Pathology.
[40]. Zhong, W., Peng, J., Wu, D., Han, Z., Bi, X., & Dai, Q. (2008). Ki-67 and PCNA expression in prostate cancer and benign prostatic hyperplasia. *Clinical and investigative medicine*, E8-E15.
[41]. Zhong, W., Peng, J., Wu, D., Han, Z., Bi, X., & Dai, Q. (2008). Ki-67 and PCNA expression in prostate cancer and benign prostatic hyperplasia. *Clinical and investigative medicine*, E8-E15.