PROGNOSIS VALUE OF AMACR AND KI-67 IN PROSTATE CANCER PROGRESSION

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**CERTIFICATION**

This is to certify that the project proposal entitled “PROGNOSIS VALUE OF AMACR AND KI-67 IN PROSTATE CANCER PROGRESSION ” has been submitted by YUSUF NABIL HASSAN , with the matriculation number 15/MHS06/065 in partial fulfillment of the degree of Bachelor of Medical Laboratory Science (B.MLS) of Afe Babalola University Ado-Ekiti state, during the academic year 2019/2020.

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**CHAPTER ONE**

**INTRODUCTION**

Prostate cancer is the most common malignant tumor in men and also the second cause of death among malignant tumors in western countries. The etiology of prostate cancer is unknown but clinical and experimental observations suggest that hormonal, genetic, and environmental factors influence its pathogenesis. Specific molecular mechanisms are involved in the development and progression of prostate cancer. More than 95% of prostate cancers are adenocarcinoma (Nakata, *et al*.,2000).

Benign prostatic hyperplasia (BPH) is a nonmalignant enlargement of the prostate caused by cellular hyperplasia. It is a common age-associated disease affecting ∼70% of men aged 70 years or over. BPH can be a bothersome and potentially severe condition. Not only can it lead to lower urinary tract symptoms (LUTS) and diminish patients’ quality of life, but it may also be associated with certain male urologic cancers such as prostate cancer and bladder cancer. The mechanism between BPH and urologic cancers is not fully understood. Some studies suggested that hormones, inflammation, metabolic syndrome are likely to play a role in BPH and prostate cancer. For bladder cancer, a possible explanation for the association is that the residual urine in the bladder in patients with BPH may cause lower urinary tract damage and BPH may prolong the time of urothelial exposure to urinary excreted carcinogens( Dai *et al*.,2016).

Immunohistochemical analysis consists of using monoclonal or polyclonal antibodies to detect specific antigens in tissue samples, and it is a widely used technique that can be applied in diverse situations, such as cellular differentiation, characterization of a tumor's primary site, detection of metastases, prognostic factors, as a predictor of targeted therapy response and even in the identification of structures, organisms and materials secreted by cells of interest. In prostate cancer, immunohistochemistry has an important role in the diagnostic confirmation of borderline cases due to the presence (or absence) of basal cells, detected by specific antibodies against it combined with racemase expression in luminal epithelial cells. Currently, the identification of biomarkers capable of predicting the course of the disease has been gaining importance ( Jakobsen *et al*.,2016).

Ki-67 is a protein expressed in the nucleus of proliferating cells during mitosis interphase. It is expressed during late G1, S, G2, and Mphases, but not during G0 phase (Sulik *et al*.,2011). The Ki-67 protein is a cellular proliferation marker (Kim *et al*., 2017) and it is widely used given its high reproducibility (Sulik *et al*.,2011).

Alpha‐methylacyl CoA racemase (AMACR) is an enzyme involved in fatty acids metabolism. One of AMACRs primary substrates, phytanic acid, is principally obtained from dietary red meat/dairy, which are associated with prostate cancer (PCa) risk. AMACR is also a tumor tissue biomarker over‐expressed in PCa. In this study, we explored the potential relationship between *AMACR* polymorphisms, red meat/dairy intake, and PCa risk(Wright *et al*,2011)

**1.2 AIM OF STUDY**

The purpose of this study to detect and demonstrate the expression of the immunohistochemical markers; Alpha‐methylacyl CoA racemase and KI-67 for their ability to predict the progression of BPH to prostatic malignant lesion.

**SPECIFIC OBJECTIVES OF STUDY ARE:**

1. To determine and demonstrate the expression of KI-67 in normal, benign prostate hyperplasia and prostatic cancerous cases.
2. To determine and demonstrate the expression of Alpha‐methylacyl CoA racemase in normal, benign prostate hyperplasia and prostatic cancerous cases.
3. To compare the expression of KI-67 and Alpha‐methylacyl CoA racemase in the progression and prediction of prostatic carcinoma.

**STATEMENT OF THE PROBLEM**

The histological diagnosis of prostate cancer on a biopsy is based on architectural pattern, nuclear atypia and lack of basal cells. This can be a challenge when faced with a small focus of prostate cancer or in the presence of benign mimickers(Srigley,2004).

The mortality rate from prostate cancer globally could be reduced through a comprehensive approach that includes prevention, early diagnosis, effective screening and treatment programmes. This study will assist in early diagnosis and monitoring of already existing tumour through the expression of the selected tumour markers Ki-67 and AMACR.

Current screening algorithms are insufficient because of their lack of specificity and lack of selectivity for aggressive cancers that require treatment. The development of novel diagnostic and prognostic markers and imaging modalities is urgently needed to enhance the predictive value of screening tools(Abrahamsson *et al*.,2009).

Overdiagnosis of prostate cancer potentially leads to significant overtreatment of prostate cancer. Health professionals, especially urologists, should avoid overtreatment by developing safe methods of cancer surveillance and monitoring without resorting to invasive therapy. Invasive therapies should be tailored to patients’ needs and the prognosis of cancers diagnosed(Abrahamsson *et al*.,2009).

**1.4 JUSTIFICATION OF STUDY**

Alpha‐methylacyl CoA racemase and ki-67 have proven to be significant makers in diagnosing prostate cancer. The findings from the study will help observe/monitor the mechanism of action of this tumor makers as they progress from normal condition to benign prostate hyperplasia to prostatic cancer.

* 1. **HYPOTHESIS**

Null hypothesis (Ho); Ki-67 cannot be used as a predictive marker of prostate cancer.

Alternate hypothesis (H1); Ki-67 can be used as a predictive marker of prostate cancer.

Ho1; Alpha‐methylacyl CoA racemase cannot be used as a predictive marker of prostate cancer.

H11; Alpha‐methylacyl CoA racemase can be used as a predictive marker of prostate cancer.

**1.6 RESEARCH QUESTIONS**

* Is Ki-67 and Alpha‐methylacyl CoA racemase expressed normal, BPH and in Prostatic cancer?
* Can Ki-67 be predictive of prostatic cancer?
* Can Alpha‐methylacyl CoA racemase be predictive of prostatic cancer?

# CHAPTER TWO

## 2.0 Literature Review

Prostate anatomy

The human prostate is a pelvic gland located under the urinary bladder and in front of the rectum, and it is composed by glandular and non-glandular structures surrounded by one same capsule . It consists mainly of muscular-fibrous tissue, which it is subdivided into about 50 tubule-alveolar glands, at the lateral and posterior segment of the urethra, which drain to 20–30 small prostatic ductules opening in the prostate, or close to the posterior wall of the prostatic urethra . The prostatic secretion, which accounts for approximately 20% of the seminal fluid, confers a characteristic odor of this flowing, and participates in the activation of spermatozoa . The ducts of the prostatic glands open into a sulcus located on each side of the urethral ridge, called the prostatic sinus. The prostate is traversed throughout the prostatic portion of the urethra, from the base to the apex, with a slightly curved course in the anteriorposterior direction, and closer to its anterior face(Sakuramoto, *et al*.,2018).

The prostate is anatomically described as an inverted pyramid whose apex is the lowest portion, and which is located about 1.5 cm behind the lower border of the pubic symphysis and is directly related to the upper face of the urogenital diaphragm. The base of the prostate gland is in a horizontal plane that passes through the middle part of the pubic symphysis, and it is directly related to the cervix of the bladder and the inner ostium of the urethra. Inferiorlateral surfaces are convex and are separated from the superior fascia of the pelvic diaphragm by a venous plexus, and are related to the pubococcygeal muscles. The posterior surface is flattened and triangular, and it is related to the bladder of the rectum. The anterior surface is narrow and separated from the pubic symphysis by retropubic fat tissue. The upper part is related to the seminal glands and to the lower extremities of the vas deferens, and near its base presents small depressions for the entrance of the ejaculatory ducts(Sakuramoto, *et al*.,2018).

PATHOGENESIS OF PROSTATE CANCER

In prostate cancer, the cells of these prostate glands mutate into cancer cells. The prostate glands require male hormones, known as androgens, to work properly. Androgens include testosterone, which is made in the testes; [dehydroepiandrosterone](https://en.wikipedia.org/wiki/Dehydroepiandrosterone" \o "Dehydroepiandrosterone), made in the adrenal glands; and [dihydrotestosterone](https://en.wikipedia.org/wiki/Dihydrotestosterone" \o "Dihydrotestosterone), which is converted from testosterone within the prostate itself. Androgens are also responsible for secondary sex characteristics such as facial hair and increased muscle mass.

Most prostate cancers are classified as adenocarcinomas, or glandular cancers, that begin when normal semen-secreting prostate gland cells mutate into cancer cells. The region of prostate gland where the adenocarcinoma is most common is the peripheral zone. Initially, small clumps of cancer cells remain confined to otherwise normal prostate glands, a condition known as carcinoma in situ or prostatic intraepithelial neoplasia (PIN). Although there is no proof that PIN is a cancer precursor, it is closely associated with cancer. Over time, these cancer cells begin to multiply and spread to the surrounding prostate tissue (the [stroma](https://en.wikipedia.org/wiki/Stroma_(animal_tissue)" \o "Stroma (animal tissue))) forming a [tumor](https://en.wikipedia.org/wiki/Tumor" \o "Tumor)(Rajagopalan *et al.,*2011).

Eventually, the tumor may grow large enough to invade nearby organs such as the seminal vesicles or the rectum, or the tumor cells may develop the ability to travel in the bloodstream and lymphatic system. Prostate cancer is considered a malignant tumor because it is a mass of cells that can invade other areas of the body. This invasion of other organs is called metastasis. Prostate cancer most commonly metastasizes to the bones, lymph nodes, and may invade rectum, bladder and lower ureters after local progression. The route of metastasis to bone is thought to be venous as the prostatic venous plexus draining the prostate connects with the vertebral veins(Rider *et al*.,2016).

The prostate is a zinc-accumulating, citrate-producing organ. The protein ZIP1 is responsible for the active transport of zinc into prostate cells. One of the zinc's important roles is to change the metabolism of the cell in order to produce citrate, an important component of semen. The process of zinc accumulation, alteration of metabolism, and citrate production is energy inefficient, and prostate cells sacrifice enormous amounts of energy (ATP) in order to accomplish this task. Prostate cancer cells are generally devoid of zinc. This allows prostate cancer cells to save energy not making citrate, and utilize the new abundance of energy to grow and spread. The absence of zinc is thought to occur via a silencing of the gene that produces the transporter protein ZIP1. ZIP1 is now called a tumor suppressor gene product for the gene SLC39A1. The cause of the epigenetic silencing is unknown. Strategies which transport zinc into transformed prostate cells effectively eliminate these cells in animals. Zinc inhibits NF-κB pathways, is anti-proliferative and induces apoptosis in abnormal cells. Unfortunately, oral ingestion of zinc is ineffective since high concentrations of zinc into prostate cells is not possible without the active transporter, ZIP1.

Loss of cancer suppressor genes, early in the prostatic carcinogenesis, have been localized to chromosomes*8p*, *10q*, *13q*, and *16q*. P53 mutations in the primary prostate cancer are relatively low and are more frequently seen in metastatic settings, hence, p53 mutations are a late event in the pathology of prostate cancer. Other tumor suppressor genes that are thought to play a role in prostate cancer include PTEN (gene) and KAI1. "Up to 70 percent of men with prostate cancer have lost one copy of the PTEN gene at the time of diagnosis" Relative frequency of loss of E-cadherin and CD44 has also been observed. Loss of the retinoblastoma (RB) protein has been shown to induce androgen receptor deregulation in castration-resistant prostrate cancer by deregulating E2F1 expression(Huang *et al.,*2011).

RUNX2 is a transcription factor that prevents cancer cells from undergoing apoptosis thereby contributing to the development of prostate cancer.

The [PI3k/Akt signaling cascade](https://en.wikipedia.org/wiki/PI3K/AKT_pathway) works with the transforming growth factor beta/SMAD signaling cascade to ensure prostate cancer cell survival and protection against apoptosis. X-linked inhibitor of apoptosis (XIAP) is hypothesized to promote prostate cancer cell survival and growth and is a target of research because if this inhibitor can be shut down then the apoptosis cascade can carry on its function in preventing cancer cell proliferation. Macrophage inhibitory cytokine-1 (MIC-1) stimulates the focal adhesion kinase (FAK) signaling pathway which leads to prostate cancer cell growth and survival.

The androgen receptor helps prostate cancer cells to survive and is a target for many anti cancer research studies; so far, inhibiting the androgen receptor has only proven to be effective in mouse studies. Prostate specific membrane antigen (PSMA) stimulates the development of prostate cancer by increasing folate levels for the cancer cells to use to survive and grow; PSMA increases available folates for use by hydrolyzing glutamated folates(Leav *et al*.,2010)

**Prostate Cancer Risk Factors**

A risk factor is anything that raises your risk of getting a disease such as cancer. Different cancers have different risk factors. Some risk factors, like smoking, can be changed. Others, like a person’s age or family history, can’t be changed. But having a risk factor, or even several, does not mean that you will get the disease.Many people with one or more risk factors never get cancer, while others who get cancer may have had few or no known risk factors. Researchers have found several factors that might affect a man’s risk of getting prostate cancer.

Age :Prostate cancer is rare in men younger than 40, but the chance of having prostate cancer rises rapidly after age 50. About 6 in 10 cases of prostate cancer are found in men older than 65.

Race/ethnicity: Prostate cancer develops more often in African-American men and in Caribbean men of African ancestry than in men of other races. And when it does develop in these men, they tend to be younger. Prostate cancer occurs less often in Asian-American and Hispanic/Latino men than in non-Hispanic whites. The reasons for these racial and ethnic differences are not clear. Geography Prostate cancer is most common in North America, northwestern Europe, Australia, and on Caribbean islands. It is less common in Asia, Africa, Central America, and South America. The reasons for this are not clear. More intensive screening for prostate cancer in some developed countries probably accounts for at least part of this difference, but other factors such as lifestyle differences (diet, etc.) are likely to be important as well. For example, Asian Americans have a lower risk of prostate cancer than white Americans, but their risk is higher than that of men of similar ethnic backgrounds living in Asia.

Family history : Prostate cancer seems to run in some families, which suggests that in some cases there may be an inherited or genetic factor1 . Still, most prostate cancers occur in men without a family history of it. Having a father or brother with prostate cancer more than doubles a man’s risk of developing this disease. (The risk is higher for men who have a brother with the disease than for those who have a father with it.) The risk is much higher for men with several affected relatives, particularly if their relatives were young when the cancer was found.

Gene changes: Several inherited gene changes (mutations) seem to raise prostate cancer risk, but they probably account for only a small percentage of cases overall. For example: Inherited mutations of the BRCA1 or BRCA2 genes, which are linked to an increased risk of breast and ovarian cancers in some families, can also increase prostate cancer risk in men (especially mutations in BRCA2). ● Men with Lynch syndrome (also known as hereditary non-polyposis colorectal cancer, or HNPCC), a condition caused by inherited gene changes, have an increased risk for a number of cancers, including prostate cancer. ● Other inherited gene changes can also raise a man’s risk of prostate cancer.

Diet: The exact role of diet in prostate cancer is not clear, but several factors have been studied. Men who eat a lot of red meat or high-fat foods (especially dairy products) appear to have a slightly higher chance of getting prostate cancer. These men also tend to eat fewer fruits and vegetables. Doctors aren’t sure which of these factors is responsible for raising the risk. Some studies have suggested that men who consume a lot of calcium (through food or supplements) may have a higher risk of developing prostate cancer. Dairy foods (which are often high in calcium) might also increase risk. But most studies have not found such a link with the levels of calcium found in the average diet, and it’s important to note that calcium is known to have other important health benefits.

Obesity :Being obese (very overweight) does not seem to increase the overall risk of getting prostate cancer. Some studies have found that obese men have a lower risk of getting a low-grade (slower growing) form of the disease, but a higher risk of getting more aggressive (faster growing) prostate cancer. The reasons for this are not clear. Some studies have also found that obese men may be at greater risk for having more advanced prostate cancer and of dying from prostate cancer, but not all studies have found this.

Smoking : Most studies have not found a link between smoking and getting prostate cancer. Some research has linked smoking to a possible small increased risk of dying from prostate cancer, but this finding needs to be confirmed by other studies.

Chemical exposures: There is some evidence that firefighters can be exposed to chemicals that may increase their risk of prostate cancer. A few studies have suggested a possible link between exposure to Agent Orange, a chemical used widely during the Vietnam War, and the risk of prostate cancer, although not all studies have found such a link. The National Academy of Medicine considers there to be “limited/suggestive evidence” of a link between Agent Orange exposure and prostate cancer.

Inflammation: is often seen in samples of prostate tissue that also contain cancer. The link between the two is not yet clear, and this is an active area of research. Sexually transmitted infections Researchers have looked to see if sexually transmitted infections (like gonorrhea or chlamydia) might increase the risk of prostate cancer, because they can lead to inflammation of the prostate. So far, studies have not agreed, and no firm conclusions have been reached(Bostwick *et al*.,2004).

Benign prostate hyperplasia (BPH)

Benign prostate hyperplasia (BPH) is a common urological issue that causes prostate enlargement in men after 40-years-old. It is a noncancerous augmentation of the prostate gland size,with stromal and glandular epithelial hyperplasia in the transition zone. It is estimated that 50%of 50 year old, and 75% of 80 year old men could have some lower urinary tract symptom(LUTS). In such condition the urethra can be partially or totally blocked, resulting in urinary retention, weak urination stream, incomplete bladder emptying and hesitancy; and so carrying secondary problems as urinary tract infections, bladder stones and chronic kidney disease, culminating in kidney failure. The LUTS is reflection of the hormonal changes rising with age, and resulting in abnormal stromal and epithelial cell proliferation (hyperplasia) in the transition zone of the prostate. The molecular etiology of these events remains unclear, but few studies attempt to correlate it to sex steroids hormones, also known as gonadocorticoids andgonadal steroids, that interact with vertebrate androgen and estrogen receptors. It is important to mention that the BPH is generally not a precursor lesion to a prostate cancer (PCa) condition.Some animal models studies, including dogs and chimpanzees, have been performed in order tounderstand the prostate conditions. Chimpanzees sporadically suffer from age-associated BPH,

and are the closest match to human prostate gland. Throughout the time, dogs are like human

counterpart because they develop BPH containing distinct nodules of hyperplasia with diffuse

areas of compression of the rectum producing constipation, a symptom opposed to the urinary

retention in men. In order to supply these deficiencies, some transgenic animal modelsusing other normal mammal species were developed. Prostate-specific 15-LOX-2 transgenic mouse and PPAR\_ knockdown mice naturally develop increased prostate size with age, inaddition to epithelial-hyperplasia, and prostatic intraepithelial neoplasia progression(Kramer *et al.,*2007).

CLINICAL DIAGNOSIS AND BIOMARKERS FOR PCA

The diagnosis and follow-up of PCa patients are often difficult because of the absence of

specific markers that could change accordingly to the status of disease, the best therapy, and

the existence of future complications caused by the chosen treatment.

For several decades many researchers joined efforts to study biomarkers of prognosis and

treatment for PCa. Almost 50-years, PSA measurement represented the best marker for PCa.

The primary idea was to substitute the digital rectal examination by PSA screening; nevertheless

this was not possible despite the low specificity and false positive rate, as it is also

observed in BPH . No significant progress in the use of PSA as a precise biomarker of PCa was achieved during the past years.

Beyond this scenario, advances in genetic testing for PCa risk and new molecular diagnostic

assays have been designed to improve diagnostic accuracy and treatment decision beyond

prostate-specific antigen (PSA) testing. PSA is a protein of the kallikrein family synthesized in the prostatic epithelium and secreted in the seminal fluid. From its discovery in 1970 to the

present day, it is a diagnostic tool used as a tumor marker for early diagnosis, treatment and

monitoring of patients with neoplasia in conjunction with the rectal examination. However,

many studies have questioned the use of this biomarker for a diagnosis, due to the exponential increase in the diagnosis of PCa and, consequently, the increase of unnecessary hormonal, radiotherapeutic, chemotherapeutic and surgical treatments such as radical prostatectomy . PSA evaluation is performed by its measurement in serum using immunoassay (34kDa). Normal values vary according to the method used. In most tests, values of up to 2.5 ng/mL are allowed as normal. If this value is higher, it is indicated to request the dosage offractionated PSA, which relates total PSA to free PSA (fPSA). The result is expected to be equalto or greater than 20%; if it is lower, there is a probability that it is a PCa . However, this testdoes not have 100% of specificity or sensitivity, insofar as there is PCa whose PSA is not altered, and there are other transient factors that can raise serum PSA levels, such as prostatitis, benign prostatic hyperplasia , prostatic biopsies and trauma, due to prostatic cell lysis releasing PSA into the bloodstream .

Despite results enhancing detection at earlier stage and decreasing the number of metastatic

patients, the use of prostate-specific antigen (PSA) to detect PCa has low specificity, unnecessary biopsies and frequently mistaken diagnoses. Also, PCa has various features so prognosis following diagnosis is greatly variable. Hence, there is a requirement for new prognostic biomarkers, particularly to differentiate between inactive and aggressive forms of the disease, to improve clinical management of PCa patients. Research continues into finding additional markers that may allow this goal to be attained.

In order to improve the specificity of PSA as a tumor biomarker, tests called PHI (Prostatic

Health Index), that predicts the risk of having PCa and 4 K score™ (predicts the risk of having high-risk of PCa) were launched on the American and European markets [62]. 4 K score™blood test combines 4 prostatic biomarkers (total PSA, fPSA, intact PSA, and human kallekrein 2(hK2)) with the age of the patient, the digital rectal exam (DRE) findings (presence of a nodule or not), and the result of previous biopsies [63]. The higher the score, the greater the probability of finding tumor cells in a biopsy (Gleason \_ 7). This test combination is interesting because it does not allow unnecessary biopsies to be performed,whereas post-operative, as well as any surgery, has risks and can lead to future complications for the patient, affecting his quality of life. Another non-invasive test available is the ExoDxTM Prostate (IntelliScore) Test18, which, through urinalysis, assesses the risk of developing invasive PCa, and thereby target the best treatment by molecular analysis of three specific genes in exosome and microvesic RNAs released by tumor cells, called extracellular vesicles (further discussed in this chapter) . These related genes (ERG, PCA3 and SPDEF) are most commonly related to tumor progression and, consequently, its aggressiveness and invasion.

It is important to note that these tests are not accessible to the entire population, either because of the high cost of the technology, or because some countries have still not approved it. Thus, the main diagnostic method used nowadays for the screening and detection of the PCa remains PSA testing and rectal examination (DRE). If the results of these exams are altered, a biopsy is necessary to confirm the diagnosis, and determine the aggressiveness and prognosis of the cancer. This is done by histological analysis of the biopsied tissue, following classification according to the Gleason Scale. This system consists of the sum of 2 values that represent the degree of the tumor, and that determine the dominant cellular pattern and the most frequent cellular pattern, respectively. Tumor grades range from 1 to 5, the former representing more differentiated and prostate restricted tumors, while the latter represents totally undifferentiated tumors that have normally infiltrated the glandular stoma. The score, therefore, ranges from 2 (1 + 1) to 10 (5 + 5), and values below 4 on the Gleason Scale represent a well differentiated PCa; between 5 and 7, an intermediate PCa; and between 8 and 10, advanced PCa . The determination of the degree and stage of cancer allows classification into high, intermediate and low risk categories.

The clinical picture of castrated-resistant prostate cancer (CRPC) is quite heterogeneous, ranging from the asymptomatic increase in the PSA indices to the distant metastasis (commonly bone metastasis), with an important impairment of the patient’s quality of life. This is a reflection of the complexity and diversity of biomolecular alterations already found in biopsies.

Tumor progression is related to a number of genetic changes that can affect AR, signaling

cascades, apoptosis mechanisms and cell regulation, or, as in many cases, a combination of all of them.Biomolecular techniques, such as fluorescent in situ hybridization (FISH) and Microarray, for example, have identified a variety of key factors genes, oncogenes and tumor suppressor genes, related to the development and progression of PCa. The use of molecular

techniques also allowed the identification of some genes related to the suppressive function of metastasis, opening a new perspective for researching the phenomenon of tumor invasion to other tissues and, with that, to identify and elucidate new indicators of prognosis, or even PCa target therapies. As example, some studies have focused attention on the CDH1 gene and its protein expression, located on chromosome 16q22, which encodes the E-cadherin, a glycoprotein responsible for cell-cell adhesion, an important cellular function that prevents EMT in tumor progression. The Metastatic prostate adenocarcinoma (metPA) is diagnosed by immunohistochemistry. Nowadays very promising biomarkers have been used to determine prostatic origin of metPA, such as prostate specific membrane antigen (PSMA) and NKX3.1 . PSMA is a type II membrane protein not secreted and is expressed in all forms of prostate tissue, but it is expressed at high levels on malignant prostate cells with limited extraprostatic expression. Many approaches to target PSMA include DNA-based vaccines, as well as passive administration of monoclonal antibodies (PSMA-mAb), including 7E11.C5.3, that has already been approved by USA FDA (Food and Drug Administration); the medication is commercially available as ProstaScint®.

Compared to PSA, PSMA is upregulated with androgen deprivation, and its expression was

correlated with cancer aggressiveness and poor prognosis, while PSA decreases with androgen deprivation . PSMA was also evaluated in PCa using PET molecular imaging system. After all, PSMA is not specific only to prostate gland; it is expressed in other normal tissues (such as salivary glands, duodenal mucosa, renal tubular cells, and neuroendocrine cells in the colon), and in malignant cells (renal cell carcinomas, colon carcinomas, and endothelial cells that surround or are into the tumors).

Although multiple independent studies sought to demonstrate evidence that genetic variations

may be independent predictors of PCa risk in addition to family history and serum PSA levels, the challenge in the years to come will be to introduce these new gene-based diagnostic and prognostic tests in algorithms integrating the other known risk factors including age, ethnicity, family history and PSA level to better tailor diagnostic and therapeutic strategies for PCa.

**IMMUNOHISTOCHEMISTRY (IHC)**

**Immunohistochemistry** (**IHC**) is the most common application of [immunostaining](https://en.wikipedia.org/wiki/Immunostaining" \o "Immunostaining). It involves the process of selectively identifying antigens (proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. IHC takes its name from the roots "immuno", in reference to antibodies used in the procedure, and "histo", meaning tissue (compare to immunocytochemistry). Albert Coons conceptualized and first implemented the procedure in 1941(Coons *et al*.,1941).

**Principle**

The selection of antibodies for the immunohistochemical testing is made on the basis of their tumor specificity and the likelihood that they will react with the tumor under evaluation. After tissue sections are incubated with the prospective antibodies, positive reactions (tumor antigen- antibody binding) are identified through the application of one of several detection systems. Those that have the greatest sensitivity use a secondary antibody, reactive against the primary antibody, which is conjugated or linked to an enzyme marker. This system tends to be very sensitive because it allows for the attachment of a relatively large number of enzyme molecules, such as peroxidise, at the antigen site. The color of the reaction is determined by the selection of a precipitating chromogen, usually diaminobenzidine (brown) or aminoethylcarbazole (red), with which the enzyme react(Kabiraj et al.,2015).

IMMUNOHISTOCHEMICAL METHODS

The emphasis in diagnostic IHC has shifted in recent years from determination of the cell derivation of malignant tissues towards identifying prognostic markers of possible value in selection of therapy.9 Growing interest in immunohistochemical staining has led to the development of a wide range of highly specific immunostains which are of value to the surgical pathologist in diagnostic and investigative studies. The IHC technique includes the following steps:-

1. Deparaffinization of tissue sections akin on poly lysine coated slides (or else the aqueous solutions do not penetrate).
2. Quenching of endogenous enzymes (which otherwise react with IHC reagents giving false positive results). Alkaline phosphatises, peroxidises and biotin are examples of these endogenous enzymes. This is usually done by 3% H₂O₂or with free avidin.
3. Antigen retrieval.
4. Blocking of nonspecific binding sites.
5. Binding primary antibody.
6. Binding with biotinylated secondary antibody.
7. Detection methods using peroxidises- antiperoxidase methods, avidin biotin conjugates, peroxidises complexes or the more recently widely used polymer labelling two step method.
8. Addition of chromogen substrate, usually DAB.
9. Counterstaining, dehydrating and cover slipping the slide. There are numerous immunohistochemical staining techniques that may be used to localize and demonstrate tissue antigens (Kabiraj et al.,2015).

IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues. This has made it a widely used technique in the neurosciences, enabling researchers to examine protein expression within specific brain structures. Its major disadvantage is that, unlike [immunoblotting](https://en.wikipedia.org/wiki/Immunoblotting" \o "Immunoblotting) techniques where staining is checked against a molecular weight ladder, it is impossible to show in IHC that the staining corresponds with the protein of interest. For this reason, primary antibodies must be well-validated in a Western Blot or similar procedure. The technique is even more widely used in diagnostic surgical pathology for immunophenotyping tumors (e.g. immunostaining for e-cadherin to differentiate between DCIS (ductal carcinoma in situ: stains positive) and LCIS (lobular carcinoma in situ: does not stain positive)( SirAlkhatim *et al*.,2015) More recently, Immunohistochemical techniques have been useful in differential diagnoses of multiple forms of salivary gland, head, and neck carcinomas(Zhu *et al*.,2015).

The diversity of IHC markers used in diagnostic surgical pathology is substantial. Many clinical laboratories in tertiary hospitals will have menus of over 200 antibodies used as diagnostic, prognostic and predictive biomarkers.

**KI-67 TUMOUR MARKER**

The Ki67 antigen was originally identified in the 1980s, as a proliferation-associated nuclear antigen, which is only detected in dividing cells (G1-, S-, G2- and M-phase) and not in quiescent cells (G0 phase) (Gerlach *et al*.,1999). Ki67 levels are low in the G1 and S phases and peak early in mitosis. In later phases of mitosis, a sharp decrease in Ki67 levels occurs . The gene encoding Ki67 is a continuous sequence of 29,965-bp length located on chromosome 10q25-ter and is comprised of 15 exons with sizes ranging from 67 to 6845 bp and 14 introns with sizes ranging from 87 to 3569 bp. Exon 13 contains 16 homologous segments of 366 bp (Ki67 repeats) located at the center of this gene. The complete gene is comprised of a 74 bp 5′ region and a 264 bp 3′ region in the Ki67 protein(Halm *et al*.,2000).

The quantity of pKi67 present at any time during the cell cycle is regulated by a precise balance between synthesis and degradation, as indicated by its short half life of 1–1.5 h . Ki67 protein expression coincides with the transit of cells through mitosis and undergoes phosphorylation and dephosphorylation during mitosis *in vivo*, rendering it susceptible to protease degradation. Furthermore, its structure indicates that its expression is regulated by proteolytic pathways, such as those controlled by the key regulatory complex cyclin B/cyclin-dependent kinase 2 . pKi67 is known to share structural similarities (including a so-called fork head-associated domain) with other proteins, such as DUN1 and RAD, which are involved in cell cycle regulation(Panteva *et al.,*2011).

The characterization of the Ki67 promoter region is essential for understanding gene transcription, and it is therefore important to investigate this in order to develop targeted interventions aimed at modulating gene expression. In a previous study, deletion analysis and a dual luciferase reporter assay were used to locate the Ki-67 core promoter from −223 to +12 nt relative to the transcriptional initiation site, which is a TATA less, GC rich region comprised of several putative Sp1 binding sites. It was demonstrated that the region from −223 to +12 nt could drive the transcription of the Ki-67 gene, and that the Sp1 binding site is essential for the transcriptional regulation of the Ki-67 gene . An electrophoretic mobility shift assay revealed three Sp1-binding sites in the Ki67 promoter that are essential for its basal transcriptional activity(Chen *et al*.,2012).

It was found that expression of p53 is correlated with that of Ki67 in several types of cancer, including oral squamous cell cancer and breast cancer . How p53 may affect Ki67 gene expression is not yet clear. As there are three Sp1-binding sites in the Ki67 promoter and as p53 represses the transcription of genes at Sp1-binding sites of promoters, it is likely that p53 inhibits Ki67 promoter activity via p53- and Sp1-dependent pathways. It is hypothesized that there are at least two transcriptional regulatory mechanisms. One is that the p53-binding motifs affect the transcriptional repression of the Ki67 promoter. The other is a possible interaction between p53 and Sp1 at Sp1-binding sites on the Ki67 promoter(Kim *et al*.,2013).

Ki67 is frequently used as an indicator of cell proliferation. A number of diagnostic applications for pKi67 have been described, where Ki67 was significantly more highly expressed in malignant than in normal tissue(Zhang *et al*.,2012). pKi67 also tended to increase with decreasing tissue differentiation, and it was correlated with the presence of occult metastasis and the clinical stage of tumors (Fernandez *et al*.,1994). Proliferative activity in tumors can be determined by mitotic counting, flow-cytometric determination of synthesis-phase fraction and immunohistochemistry using antibodies reactive against various proliferating cellular antigens. The Ki67/MIB-1 monoclonal antibody is commonly used, and is reactive against the nuclear antigen Ki67 that is expressed during cell cycle phases G1, S, G2 and M, but is not found during G0 (Prayson.,2005). The percentage of immunoreactive tumor cell nuclei is expressed as a labeling index (LI). Studies thus far have all shown a positive correlation between Ki67/MIB-1 LI and tumor grade in human malignancy. Due to the limitations of routine histological examination of tumor tissue in predicting tumor behavior, Ki67/MIB-1 immunostaining has been introduced for its potential to improve the information provided by the grading system (Kim *et al*.,2013). Its presence in a variety of tumors indicates that it may be possible to use Ki67 in routine grading of cancer (Nabi *et al*.,2008). Judicious use of this proliferation marker in combination with established histopathological features of malignancy may serve as a more reliable indicator of the likelihood of tumor recurrence (Morimoto *et al*.,2008).

The data on Ki67 as a diagnostic marker is scarce and based on varying laboratory and statistical methods. Cancer has a complex pathogenesis and reliable early diagnosis is difficult. Symptoms usually do not appear until the disease has progressed to an advanced stage. Therefore, further research into diagnostic and prognostic markers may aid early diagnosis. Notably, the expression of Ki67 reflects the tumor proliferation rate and correlates with initiation, progression, metastasis and prognosis of a number of types of tumors (D'Angelo et al.,2010). Certain regulators of these processes, such as Smac , minichromosome maintenance 7, p53 Bcl-2, proliferating cell nuclear antigen (PCNA) and CD105 have been investigated(Ben‐Izhak *et al.,*2002). In a number of studies, Ki67 appeared to be closely correlated with pancreatic tumor severity as well as with expression of Smac and thus may be useful as a diagnostic and prognostic marker or, in conjunction with Smac, as an indicator of treatment efficacy . In a further study, Chen *et al*  reported that utilizing Ki67 LI and vascular endothelial growth factor scoring is useful to effectively and accurately predict outcomes and optimize personal therapy in judging the outcomes of non-muscle invasive bladder cancer This novel molecular grading system could enhance the efficiency of the conventional system(Chen *et al.,*2012)

Colorectal carcinoma is the fourth leading cause of cancer-related mortality worldwide . It has been demonstrated that the Ki67 LI was higher in Dukes’ stage B than in Dukes’ stage C carcinoma. They concluded that the positive rate of Ki-67 antibody in poorly differentiated adenocarcinoma and mucinous carcinomas was significantly lower than in well differentiated and moderately differentiated adenocarcinomas, suggesting that proliferative activity is low in cancers with poor differentiation. On the other hand, it has been demonstrated that the Ki-67 LI is high in well to moderately differentiated, non-mucinous adenocarcinomas in an early Dukes’ stage (A or B) as compared with that in poorly differentiated, mucinous adenocarcinomas or signet-ring cell colorectal carcinomas in an advanced Dukes’ stage (C). MIB-1 is a monoclonal antibody that recognizes a fixation resistant epitope of the Ki67 antigen and it is used to estimate the proliferative fraction of neoplasia (Nabi *et al*.,2008). Using MIB-1, it was observed that Ki67 LI was high in Grade I and Grade II as compared with the Grade III carcinomas.

The Ki67 index is a diagnostic and prognostic aid in several fields of pathology and an established predictive tool in others . However, existing Ki67 index estimations are time-consuming and cumbersome, and may be subject to inter-observer variability. To improve the accuracy of the Ki67 index, current research recommends the use of an IHC cocktail, which detects Ki67 and the melanocytic marker, melanoma antigen recognized by T cells (MART1) . In melanocytic pathology, current research favors using Ki67/MART1 double stains to accurately distinguish Ki67-positive melanocytic cells from other proliferating Ki67-positive cells, including lymphocytes, stromal cells and epithelial cells. The usability and cost benefit of automated MART1-verified Ki67 indices in routine settings require investigation in a prospective study with a consecutive inclusion of specimens. When predicting a clinical outcome for the individual patient, automated MART1-verified Ki67 indices may be more reliable as a result of a reduction in false positive results with this assay(Li *et al*., 2015).

**ALPHA‐METHYLACYL COA RACEMASE**

**Alpha-methylacyl-CoA racemase** (AMACR) is an enzyme that in humans is encoded by the *AMACR* gene. AMACR catalyzes the following chemical reaction:

(2*R*)-2-methylacyl-CoA {\displaystyle \rightleftharpoons } (2*S*)-2-methylacyl-CoA

In mammalian cells, the enzyme is responsible for converting (2*R*)-methylacyl-CoA esters to their (2*S*)-methylacyl-CoA epimers and known substrates, including coenzyme A esters of pristanic acid (mostly derived from phytanic acid, a 3-methyl branched-chain fatty acid that is abundant in the diet) and bile acids derived from cholesterol. This transformation is required in order to degrade (2*R*)-methylacyl-CoA esters by β-oxidation, which process requires the (2*S*)-epimer. The enzyme is known to be localised in peroxisomes and mitochondria, both of which are known to β-oxidize 2-methylacyl-CoA esters(Darley *et al*.,2009).

The isomerase α-methylacyl-CoA racemase (AMACR) is most commonly known for its physiologic role in catalyzing the stereoconversion of the α-methyl proton of branched chained fatty acids undergoing β-oxidation in the mitochondria and peroxisomes(Autio *et al.*,2014). Deficiencies in AMACR protein or activity have been associated with several peroxisomal disorders that lead to neurological impairment due to accumulation of branched-chain fatty acids (Dick *et al*.,2011). The effects of such deficiencies can be ameliorated by decreasing the intake of these lipids that come primarily from meat and dairy-based diets (Lloyd, et al.,2008). In the early 2000s, two research groups independently verified AMACR as a prostate cancer (PCa) biomarker based on its specific overexpression in malignant tissue compared to benign prostate tissue by immunohistochemistry (IHC) (Walsh.,2002). Subsequent studies established that AMACR protein was also present in metastatic lesions - not only localized primary PCa - and its expression was independent of the androgen receptor (AR) signaling axis (Zha, *et al*.,2003). Over the years, AMACR has been established as a dependable biomarker of PCa with IHC analysis finding that AMACR expression in needles biopsies had a 97% sensitivity and 100% specificity for PCa detection . Since its initial discovery in PCa, AMACR overexpression has been documented in a number of other cancers including colon, ovarian and breast (Zhou *et al*.,2002).

The near-universal overexpression of AMACR in PCa has made it an attractive target for molecular imaging. Due to its overexpression in PCa compared to normal tissue, an AMACR imaging probe can potentially be used to non-invasively differentiate aggressive disease from indolent disease. A number of factors have hindered the development of imaging probes for AMACR. Ideally, an AMACR imaging probe would be a small-molecule inhibitor of its enzymatic activity. There have been a number of studies that tried to develop assays for AMACR detection for high throughput screens of AMACR inhibitors, but none of the inhibitors identified have moved toward clinical application . Another complicating factor for a small-molecule imaging probe to be successful is that the probe will have to cross the cell membrane and possibly the membrane of an organelle to reach enzymatically active AMACR. A more favorable approach is a molecular-genetic imaging strategy where the transcriptional specificity of the AMACR promoter is harnessed to drive the expression of reporter genes for cancer detection. The DNA construct containing the promoter and reporter gene can be delivered by viral or non-viral means into the cell where transcription and translation of AMACR are occurring. The reporter genes can encode proteins for a number of imaging modalities including positron emission tomography, magnetic resonance, and bioluminescence imaging (Yi *et al*.,2008).

In this study, we detail the development of a molecular-genetic imaging technology for AMACR that can detect PCa in vivo. Initially, truncated versions of the full-length 2,295 base pair (bp) AMACR promoter were cloned and analyzed for transcriptional output using a luciferase assay in AR-negative and AR-positive PCa cell lines. From these experiments, we identified a 565 bp minimal AMACR promoter that was cancer-specific and possessed output equal to or greater than the full-length promoter. An advanced two-step transcriptional activation (A.TSTA) system was then used to enhance the output to the minimal AMACR promoter (Zhou *et al*.,2002).. This system - placed downstream of the minimal AMACR promoter and upstream of luciferase - expresses a GAL4-VP16 fusion protein driven by the minimal promoter. The fusion protein binds GAL4 binding sites upstream of the transcription initiation site that results in an increased transcription of luciferase. Using this system, the output of the minimal promoter was enhanced while still retaining specificity. The enhanced promoter system along with luciferase was then incorporated into a non-replicative adenovirus (Ad) vector. Ad vectors are an efficient natural gene delivery system and are well-researched for cancer gene therapy . The highly efficient delivery of the non-replicative Ad allowed for the imaging of AR-positive and AR-negative PCa xenografts in vivo using bioluminescence. Our data provide proof-of-concept that the tissue-specificity of the AMACR promoter can be exploited for detecting PCa via reporter gene imaging. In the future, this strategy could even be applied to therapy by delivering suicide genes or using conditionally replicative adenoviruses for oncolytic and radioviral therapy(Sharma *et al*.,2009).

**Treatment**

Prostate cancer is a slowly developing disease, and according to the age at diagnosis, patients may die from other causes, such as diabetes, cardiovascular diseases and stroke, among others. Therefore, an individualized assessment is required to determine which therapeutic

modalities are the most suitable in each case.

**Treatment for localized disease**

*a. Active monitoring*

There is broad evidence that cases with low-risk PC and some with intermediate-risk PC with low tumor volume can be monitored. The goal is to detect the 30% of tumors

that are most aggressive and require other treatment modalities, such as radical prostatectomy and radiation. In some very special cases, high-intensity focused ultrasound (HIFU) or cryotherapy is also required. The cancer-specific mortality is minimal (approximately3%) at 10 and 15 years in patients with a 3+3 Gleason grade. Active monitoring is performed with PSA and annual biopsies to determine disease progression; if progression occurs, decisions are made together with the patient concerning the use of other treatment modalities24-26 Statistical models have been developed to predict the progression of a tumor. The Epstein criteria stand out among them and are the origin of the term“insignificant cancer” (tumor volume less than 0.2 cc, Gleason < 7 and organ-confined disease). To date, no tumor marker can classify PC as indolent or insignificant Passive monitoring is an option for patients at low risk and with other comorbidities that do not allow them greater than 10-year survival.25 In this case, a common strategy is to treat symptoms once they appear (e.g., if urinary retention exists, transurethral prostatic resection is applied; if there are pathological fractures, treatment is applied according to the fracture site). The patients who likely benefit most from monitoring are those with low-risk or indolent tumors and those who present other comorbidities with a lower than 10-year survival.

*b. Radical prostatectomy*

Radical prostatectomy (RP) as a treatment for PC has existed for over 100 years. Controversies have arisen regarding this procedure due to the two studies that assessed the survival of a group with RP versus a group with monitoring only. The European study found lower PC mortality in the RP group than the observation group (14.6 vs 20.7%, respectively) and a lower rate of metastasis development after 15 years (21.7% in the RP group vs 33.4% in the observation group). The USA study did not find differences between the two groups.25-27 The most common complications related to RP are urinary incontinence (5-20%) and injury to the neurovascular bundles that regulate erection, which results in erectile dysfunction (ED) (40 and 80%).28-31 The significant impacts of these two complications explain why other more conservative treatments are receiving growing acceptance. The National Comprehensive Cancer Network (NCCN) recommends a geriatric assessment in all patients over 65 with oncological disease to choose the therapy with the least physical impact and thus better quality of life for the patient.32 The last 10 years have seen an increase in the use of new technologies for the surgical treatment of PC, such as laparoscopic and robotic techniques. Although these techniques have not shown improvements in cancer control, urinary incontinence or erectile dysfunction compared to open surgery, they show better results regarding bleeding, hospital stays and cosmetic appearances. Therefore, treatment options should be explained in detail, and the patient should decide which option fits best.

*c. Radiotherapy*

3D-conformal radiotherapy (RT) has shown comparable results to RP in the oncological control of PC without the immediate surgical morbidities. However, it is associated with other previously acknowledged morbidities in the medium and long term, such as ED and urinary incontinence. Studies have shown that among patients treated with RT, those under 72 have an increased overall survival compared with those over 72, regardless of comorbidities(Castillejos-Molina, *et al*.,2016).

**CHAPTER THREE**

**3.0 MATERIALS AND METHODS**

This is a comparative study that is aimed at determining the predictive ability of the immunohistochemical markers; Ki-67 and Alpha‐methylacyl CoA racemase , of the progression of BPH to a Prostatic malignant lesion.

**3.1 TISSUE SAMPLE SELECTION**

A total of 60 formalin fixed, paraffin was embedded tissue blocks are obtained and cut at 5 microns, including; 10 normal prostatic tissue, 20 BPH, 30 precancerous and 60 all together . These tissue blocks were obtained from Federal Teaching Hospital, Ido-Ekiti, Ekiti State.

* 1. **METHODOLOGY**
  2. **PRINCIPLE OF KI- 67**

The genetic information of an organism is found in the nucleus of each cell in the form of DNA organised into chromosomes. The exact structure of those chromosomes changes as the cell moves through the different stages of the cell division cycle. During the stage called mitosis, where the DNA of a cell (which has previously been duplicated) is shared into two daughter cells, the chromosomes become tightly packed structures that can be readily moved through the cytoplasm. Since the late nineteenth century, it has been known that a layer of proteins, called the perichromosomal layer, coats the condensed chromosomes. However, virtually nothing was known about the role this layer performs.

One of the first proteins to join the perichromosomal layer after mitosis begins is called Ki-67. This is only found in the cell nucleus when a cell is actively growing and dividing, and so is widely used as a marker in experiments investigating these processes: for example, Ki-67 is used to detect growing tumour cells amongst the normal cells in tissues of the body, and to measure the effectiveness of drugs designed to stop the growth of tumours. Again, however, little is known about what Ki-67 actually does.

Booth et al. now reveal that when Ki-67 is not present in a cell, chromosomes do not have a perichromosomal layer—or at best, have a small remnant of one. This allowed Booth et al. to investigate the role of the perichromosomal layer as well. When the chromosomes first go through mitosis without a perichromosomal layer, no changes to the shape or the behaviour of the chromosomes are seen. However, the new nuclei are smaller than normal and their contents are arranged differently. This causes problems with the ability of daughter cells to synthesise protein building blocks and leads to an increased rate of spontaneous cell death when daughter cells try to undergo the next mitosis (Booth,*et al.,*2014).

* + 1. **Ki-67** **IMMUNOHISTOCHEMISTRY**

Formalin-fixed paraffin-embedded tissue blocks were sectioned by using Rotary microtome and low profile disposable knives by using 4 microns as the thickness of choice. Sections were then floated on a floating water bath adjusted to 45°C. Finally, clear-coated glass slides were used to pick up the floated sections and slides were left in a 60°C overnight in a hot air oven. Next day after oven drying sections were subjected to xylene and then to decrease graded of alcohols (100%, 90%, 70%, and 50%) for dehydration. The section was boiled in preheated retrieval buffer, performed in citrate buffer with pH 6.0 for 40 min at 95°C in a water bath followed by cooling at a refrigerator for 10 min. After cooling the slides, Phosphate Buffer Saline (PBS) was added to the slides for 5 min. Endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide (Envision FLEX Peroxidase-Blocking reagent K8000/K8010) for 7 min and then washed with PBS for 7 min. The slides were then incubated with primary antibody (monoclonal mouse anti-human Ki67 IS626) for 30 min followed by phosphate buffer saline for 7 min. Then the slides were incubated with secondary antibody (EnVision FLEX/HRP K8000/K8010) for 30 min, and then washed with PBS for 7min, and also sections were incubated with streptavidin peroxidase. Substrate-DAB Chromogen (K8000/K8010 Substrate Working Mix) was added for 10 min. The DAB Chromogen was prepared as described by the manufacturer (Dako) by adding one drop from the chromogen to 1ml of the substrate buffer. Slides were then washed in distilled water for 20 min and counterstained with Mayer's hematoxylin. The slides were evaluated individually in a standard light microscope for immunohistochemical staining( Mohamed *et al*.,2018).

**PRINCIPLE OF ALPHA‐METHYLACYL COA RACEMASE**

AMACR is a well-characterized enzyme that plays a key role in peroxisomal b-oxidation of dietary branched fatty acids and C27-bile acid intermediates. It catalyzes the conversion of (R)-a-methyl-branched-chain fatty acyl-CoA esters to their (S)-stereoisomers. AMACR was identified as being overexpressed in prostate carcinoma cells when compared with benign or normal prostate epithelial cells. The function of AMACR in prostate cancer has not been clarified yet. Several investigators have examined the mechanistic relationships between AMACR expression and hormone status. It has been reported that AMACR expression in hormone-sensitive cell lines and found its expression remained unchanged after exposure to antiandrogen drugs, suggesting that AMACR expression may not be directly regulated by the androgen pathway . a-methylacyl-CoA racemase could not affect the stabilization of androgen receptor or modulate the expression of the androgen receptor–targeted gene, it indicating that the expression of AMACR is independent of androgen receptor–mediated signaling . But  analyzed patients who had received hormonal therapy and found that those with localized prostate carcinoma had significantly diminished levels of AMACR expression. However, the exact mechanism by which hormonal therapy influences the expression level of AMACR remains elusive. Further studies are needed to further explore the mechanisms(Jiang, *et al.,*2013).

The data from the present study suggest that AMACR is also functionally important for the growth of PCa cells. Overexpressed AMACR from both clinical tissues and PCa cell lines is wild type by sequence analysis and functionally active by enzymatic assay. Correspondingly, enzyme activity of AMACR increases approximately 4-fold in PCa in comparison with adjacent normal prostate. Small interference RNA (siRNA) against AMACR, but not the control inverted siRNA, reduced the expression of AMACR and significantly impaired proliferation of the androgen-responsive PCa cell line LAPC-4. No effect was observed in HeLaS3 cells, which express AMACR at a low level. Cell cycle analyses revealed a G(2)-M cell cycle arrest in LAPC-4 cells treated with siRNA compared with mock treatment or control inverted siRNA. Expression of a siRNA-resistant form of AMACR in LAPC-4 cells protects the cells from growth arrest after AMACR-specific siRNA treatment. Data from Western blotting and luciferase-based reporter assays suggest that the function and expression of AMACR are independent of androgen receptor-mediated signaling. Moreover, simultaneous inhibition of both the AMACR pathway by siRNA and androgen signaling by means of androgen withdrawal or antiandrogen suppressed the growth of LAPC-4 cells to a greater extent than either treatment alone. Taken together, these data suggest that AMACR is essential for optimal growth of PCa cells in vitro and that this enzyme has the potential to be a complementary target with androgen ablation in PCa treatment (Zha *et al.,*2003).

**3.1.2 ALPHA‐METHYLACYL COA RACEMASE IMMUNOHISTOCHEMISTRY**

Specimen was all embedded and tissue was routinely processed.10 H and E stained slides were examined thoroughly by light microscopy and a provisional histopathological diagnosis was established. Cases were categorized into inflammatory lesions, benign lesions, prostatic intraepithelial neoplasia and malignancy. The blocks from all suspicious and control cases were cut and mounted on poly L-Lysine coated glass slides. Endogenous peroxidase activity was blocked by freshly prepared 0.3% hydrogen peroxide in methanol for 20 min. Subsequently, heat induced epitope retrieval was performed. IHC was done by using anti AMACR antibody Monoclonal rabbit Anti Human AMACR-clone 13H4) and a monoclonal anti p63 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA: Monoclonal mouse antihuman p63 antibody clone sc‑8431). 3, 3‘-diaminobenzidine (DAB) was used as chromogen and counterstained with hematoxylin or eosin. IHC signals were brown with eosin and dark brown or black with hematoxylin counterstains. (YAKUBU,2016).

**3.1.3 EXPECTED OUTCOME**

Results will be presented in figures and tables, pictures will also be used where necessary.

* **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.

* **Ki-67**

In correlation to pre-existing literatures;

According to the Ki-67 expression among different tumour grades in the current study, the Gleason scoring was: twenty-seven cases of low-grade prostatic cancer were included, 74% cases were scored as score zero, while the remaining cases (16%) were scored as score 1 . On the other hand, Twenty cases of high-grade prostatic cancer were involved in this study; fourteen cases were scored as score 1, only two cases were scored as score 2 whereas the remaining three cases scored as score 3( Mohamed *et al*.,2018).

* **ALPHA‐METHYLACYL COA RACEMASE**

In correlation to pre-existing literatures;

Among 93/120 cases confirmed as carcinoma by H and E, 85 cases (91.3%) showed AMACR positivity. Among 5/120 cases confirmed as atypical glands suspicious for malignancy by H and E, 4 cases (80%) showed AMACR positivity. Out of 3/120 cases confirmed as PIN by H and E, 2 cases (66%) showed AMACR positivity. Out of 19/120 cases confirmed as benign prostatic hyperplasia by H and E, all the 19 cases (100%) showed AMACR negativity. High grade carcinoma (Gleason pattern 5) showed AMACR negativity. Sensitivity and specificity of AMACR in prostatic carcinoma were 90% and 100% respectively. Positive predictive value of AMACR in prostatic carcinoma was 100% and negative predictive value of AMACR in prostatic carcinoma was 65%; 99% of prostatic carcinoma showed high serum PSA value (Kandasamy *et al.,*2017).

**RESULT**

**Ki-67**

Ki-67 expression will be significantly low in benign prostatic hyperplasia (19%) as compared with prostatic carcinoma (81%), (P < 0.05) and ki67 marker highly expressed in prostate carcinoma as compared with benign prostatic hyperplasia.

**AMACR**

Level of AMACR protein expression in malignant epithelia will be greater than in adjacent benign epithelia . both pin and atrophic lesions which are thought to be cancerous will show high level of cytoplasmic staining of AMACR(YAKUBU,2016).

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