Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Breast Cancer Antigen I and P63 in Prostate Tumors among Sudanese Patients

الكشـف المنـاعي النسـيجي الكيميـائي عـن الجـين المسـبب لسـرطان الثـدي مـن النوع الأول وب63 في أورام البروستاتا لدى المرضى السودانيين

A dissertation submitted for partial fulfillment for the requirement of master degree in medical laboratory science (Histopathological and Cytology)

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بسم الله الرحمن الرحيم

قال تعالى : (وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا) .

صدق الله العظيم

سورة النساء الآية (113)

Dedication

To soul of my grandmother, to my father,

To my mother,

TO my sisters and colleagues…

I delicate this study.

Acknowledgements

All great thanks are firstly to Allah.

I would like to express my gratitude and thanks to my supervisor Dr.Abu Elgasim Abass, for his guidance, helpful suggestion, solving problems and his precious advices as well as continuous assistance through the whole process of the research.

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Thanks for staff of histopathology and cytology in Omdurman teaching hospital and thank for Nada Saleh.

Finally thanks to everyone helped me who was not mentioned.

Abstract

This is analytical retrospective case control hospital based study conducted in Omdurman teaching hospital during the period from October 2018 to October 2019. The study aimed to detect BRCA1 and P63 expression in prostate tumors among Sudanese patients using immunohistochemistry.

Forty paraffin embedded blocks previously diagnosed as prostate tumors were selected for this study. Samples included 20(50%) benign tumors samples and 20(50%) malignant tumors, the malignant samples grade were moderately and poorly differentiated tumors, with frequencies of 9(45%) and 11(55%) respectively.

Sections of 3µm were cut from each paraffin block then stained by immunohistochemical method for detection of BRCA1 and P63. The data obtained was analyzed using SPSS program version 20, mean, frequency, and chi square test were calculated.

The patient's age ranged between 36 and 100 years with a mean age of 73 years. Most patients were more than 50 representing 38(95%) patients and the remaining 2(5%) patients were less than or equal 50 years.

BRCA1 showed negative expression in all samples. P63 positive expression was found in (4/20) in malignant samples, and (16/20) samples showed negative expression, while in benign samples (20/20) showed positive expression, this result showed significant association between p63 expression and prostate tumors (P.value =0.000)

The study concluded that expression BRCA1 is negative in all samples. P63 expression is associated with benign type of prostate tumors.

المستخلص

أجريت هذه الدراسة المستشفوية التحليلة الحالة والحالة الضابطة فى مستشفي أمدرمان التعليمي، فى الفترة ما بين أكتوبر 2018 إلى أكتوبر 2019 هدفت للكشف عن الجين المسبب لسرطان الثدي من النوع الأول و ال ب63 في أورام البروستاتا بإستخدام كيمياء الأنسجة المناعية فى المرضى السودانيين.

تتضمن أربعون قالب شمعي لهذه الدراسة من عينات مرضى كانوا مشخصين مسبقا على أنهم مصابون بأورام البروستاتا. 20 (50%) منها كانوا مشخصين بأورام بروستاتا خبيثة ، و 20 (50%) منها أورام بروستاتا حميدة, كان تمايز الأورام الخبيثة يشتمل على النوع متوسط التفريق9 (45%) والنوع متباين التفريق 11(55%).

من أى قالب شمعى قطع جزء صغير بمقدار3 ميكرون للكشف عن الجين المسبب لسرطان الثدي من النوع الأول و ال ب63 في أورام البروستاتا بإستخدام كيمياء الأنسجة المناعية . وأستخدم برنامج الحزم الإحصائية للعلوم الإجتماعية النسخة 20 لتحليل البيانات حسب المتوسط والتردد وإختبار مربع كاي.

تراوحت أعمار المرضي بين 36 و100 سنة بمتوسط عمر 73 سنة. أظهرت الدراسة أن معظم المرضي كانت أعمارهم أكبر من 50 وكان عددهم 38 مريض (95%) و2 مريضآ (5%) كانت أعمارهم أقل من أو تساوى 50.

لم يظهر الجين المسبب لسرطان الثدى من النوع الأول فى جميع عينات الأورام الخبيثة منها والحميدة. وأظهرت الدراسة أن ال ب63 موجب الظهور في (20/4) عينة من أورام البروستاتا الخبيثة و سالب الظهور فى (20/16) عينة من أورام البروستاتا الخبيثة، بينما موجب الظهور فى (20/20) عينة من أورام البروستاتا الحميدة . هذه النتائج أظهرت علاقة بين ال ب63 وأورام البروستاتا الحميدة (P=0.000).

الدراسة خلصت إلى أن الجين المسبب لسرطان الثدي من النوع الأول كان سالب الظهور فى هذه العينات . توجد علاقة بين ال ب63 وأورام البروستاتا الحميدة .

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CHAPTER ONE

INTRODUCTION

Chapter one

Introduction

1.1 Introduction:

Prostate cancer, also known as carcinoma of the prostate, is the development of cancer in the prostate. Prostate is a gland in the male reproductive system. Most prostate cancers are slow growing; however, some grow relatively quickly (Galani, 2015).

The cancers cells may spread from the prostate to other parts of the body, particularly to the bones and lymph nodes. It may initially cause no symptoms. In later stages it can lead to difficulty urination, blood in the urine, or pain in pelvis, back or when urinating (Ruddon, *et al*.2007).

Prostate cancer is the sixth most common cancer in the world and accounts for 9.7% of cancer in men. It is the leading causes of new in men and is second only to lung cancer as a cause of cancer related deaths in men (Baig, *et al*.2012).

Prostate cancer is the most common cancer in Sudanese men. The age standardized rate is 10.3 and mortality is 8.7 per 100,000 population .It ranked second among all cancers in both sexes after breast cancer (Elamin, *et al*.2015). In Sudan prostate cancer is ranked first among Sudanese men , according to latest WHO data published in 2017,death in Sudan reached 803 or 0.30% of total death , the age adjust death rate in 11.24 per 100,000 of population ranks Sudan (WHO,2017) .

Risk factors associated with prostate cancer are age, race/ethnicity, family history, diet, anthropometric factors, hormones profiles, concomitant medical conditions and genetic factors (Gann, 2002).

Diagnosis of prostate cancer is done by medical history, physical examination .prostate specific antigen blood test, transrectal ultrasound (TRUS), MRI fusion, digital exam rectal and prostate biopsy (Bardan, *et al*. 2007, James, 2014).

There are different treatments for prostate cancer including active surveillance, radical prostatectomy, laparoscopic surgery, radiation therapy, chemotherapy, hormone therapy, proton beam therapy, biological therapy, coping and support patient (Picard, *et al*.2009, Chen and Zhao, 2013).

BRCA1 is are a human gene and its protein product, the human BRCA1 gene is located on the long (q) arm of chromosome 17 at region 2 band 1, from base pair 41,196,312 to base pair 41,277,500 and found in all humans normally expressed in the cells of breast and

other tissue, also known by the synonym breast cancer type 1 susceptibility protein, is responsible for repairing DNA (Check,2006) .

It is a multifunctional tumor suppressor protein implicated in regulating the maintenance of genome integrity through the activation of DNA repair genes, heterochromatin formation, double-strand-break repair, homologous recombination events, and ubiquitination (Chen, *et al.*1998).

Mutations in BRCA1 have been associated with increased risk of breast, ovarian, and more recently, prostate cancer – particularly high grade disease (Agalliu, *et al*.2009). Schayek *et al.* showed that BRCA1 protein expression in prostate differentially regulates *IGF-IR* gene expression in an androgen-dependent manner and found significantly elevated BRCA1 levels in prostate cancer in comparison with normal prostate tissue (Schayek, *et al*.2009).

P63 is a nuclear protein encoded by a gene on chromosome 3q27-29 with homology to p53 regulate growth and development in the epithelium, specific isotopes are expressed in the basal cells of pseduostratifited epithelia, reserve cells of simple columnar epithelia, myoepithelial cells, urotheliuum and squamous epithelium (Paner,*et al*.2008). The p63 gene encodes six protein isoforms. The transactivating isoforms has similar actions with p53, while the N-isoforms inhibit transcription activation by p53 and Trans activating isoforms. P63 is expressed in stratified epithelia and basal cells of the prostate and salivary glands. In mammary epithelium p63 has been show to express only in the myoepithelial layer (Stefanou, *et al*.2004).

Normal prostate epithelium consists of three different types of cells: secretory, basal, and neuroendocrine (Verhagen, *et al*.1998).

P63 shows nuclear staining in basal cells of benign prostate lesions and no staining in prostate adenocarcinoma (Gunia, *et al*.2009).

1.2 Objectives:

1.2.1 General objective:

To study the expression of BRCA1 gene mutation and p63 in prostate tumors among Sudanese patients.

1.2.2 Specific objective:

1- To detect BRCA1 mutant gene and P63 in prostate tumor tissues using immunohistochemical method.
2- To correlate the expression of BRCA1and P63 with histopathological diagnosis of prostate tumors.

CHAPTER TWO

LITERATURE REVIEW

Chapter two

2. Literature review

2.1 Scientific background:

The prostate is male gland found below the bladder and in front of the rectum, and size of the prostate varies with age. In younger men, it is about the size of a walnut, but it can be much larger in older men. It can stay at the same size or grow slowly in adults, as long as male hormones are present. The prostate contains cells that make some of the fluid (semen) that protects and nourishes the sperm. The prostate begins to develop before birth and keeps on growing until a man becomes an adult under influence of hormones called androgens such as testosterone (Snell, 1995).

The main function of prostate is the production of fluid for semen; one part of the semen is produced in the prostate. Together with sperm cells from the testicles, fluid from the seminal vesicle and the secretions released by another pea-sized gland below the prostate (the bulb urethral gland), the prostate fluid makes up the semen. All of these fluids are mixed together in the urethra. The prostatic secretion is important for the proper functioning of the sperm cells, and therefore also for fertility in men. The thin, milky liquid contains many enzymes such as the prostate specific antigen (PSA). This enzyme makes the semen thinner. The hormone-like substance spermine mostly ensures sperm cell motility (Franklin, *et al*.2005).

The prostate also plays apart in controlling the flow of urine. The urethra runs from the bladder, through the prostate, and out through the penis. The muscle fibers of the prostate are wrapped around the urethra and are under involuntary nervous system control, these fibers contract to slow and stop the flow of urine (Martinin, *et al*.2012).

2.2 Abnormalities of prostate:

2.2.1 Inflammation of prostate:

Prostatitis is an infection or inflammation of the prostate gland that present as several syndromes with varying clinical features, it is a complex condition compasses four disorder of prostate, chronic pelvic pain syndrome (CPPS), acute bacterial prostatitis, chronic bacterial prostatitis and asymptomatic prostatitis (Bartoletti, *et al*. 2007).

2.2.2 Pre-cancerous changes of the prostate:

Prostate cancer starts out with very small changes in size and shape of the prostate gland cells; it involves cellular proliferation within prostatic ducts (Zynger and Yang, 2009).

2.2.2.1 Prostatic intraepithelial neoplasia (PIN):

It is the most established precursor of prostatic carcinomas. The presence of prominent nucleoli within an existing duct structure is an easy way to identify the disorder. Four main patterns of high –grade PIN (HGPIN) have been described; tufting, micro papillary, cribrifrom and flat (Majumder, *et al*.2008).

2.2.2.2 Proliferative inflammatory atrophy (PIA):

It belongs to the atrophic lesions that frequently occur in the prostate. PIA is characterized by imbalance between proliferation and apoptosis, and is considered as a benign lesion with certain genetic instability (Woenckhaus and Fenic, 2008).

2.2.2.3 Prostatic nodular hyperplasia (PNH):

It is a stormal hyperplasia, which is an extremely common abnormality of the prostate. It is present in a significant number of men up to the age of 40 years, and its frequency rises regressively with age. It is characterized by proliferation of both epithelial and stromal elements including collagen and elastic fibers; with resultant enlargement of the gland and it some cases urinary obstruction as seen in benign prostate hyperplasia (Maccsween and Whaley, 2001).

2.3 Malignant tumors of prostate:

2.3.1 Prostatic carcinoma:

The pathogenesis of the disease is complex, involving a combination of constitutional and exposure risk factors. The lesion was subsequently named prostatic carcinoma and it was initially categorized into three grades from low to high (Sfanos and De Marzo, 2012).

2.3.2 Prostate adenocarcinoma:

It account for 95% of all cases of prostate cancers, and becomes more common in men over the age of 50. It is characterized by changes in size, shape, or texture of the prostate which are detected by the digital rectal exam (DRE), or by estimation of prostate specific antigen (PSA) level (Stoyanova, *et al*.2013).It is divided microscopically into carcinoma of peripheral duct and acini which includes carcinoma with neuroendocrine feature, and carcinoma of a large duct which is characterized by malignant changes in the large dilated duct (Rosai, 2002).

2.4 Epidemiology of prostate cancer:

It is the second commonest tumor in men worldwide and fourth most commonly occurring cancer overall, these were 1.3 million new cases in 2018 (American institute caner, 2018).

The incidence of prostate cancer varies among various geographical regions of the world with the highest rates reported in France and Norway. The lowest rates have been reported from Asia (Ferlay, *et al*.2012).

In Sudan, according to reports derived in 2009-2010 form the National Cancer Registry for Khartoum state alone, prostate cancer ranked fourth among all cancer sites in Khartoum 7.3 per 100.000. However, by gender it is ranked first among Sudanese men, with an estimated incidence rate of 8.7 per 100.000 populations, and an age standardized rate (ASR) of 10.3 per population. It had the highest age specific rate in seniors aged 65 years and older .More than 80% of men will develop prostate cancer by the age of 80 (Saeed ,*et al*.2014).

2.5 Risk factors for prostate cancer:

The risk factor of prostate cancer includes:

2.5.1 Age:

It is an essential factor in prostate cancer, but in male under 45 years prostate cancer in unusual. As males get older, the prostate cancer incidence progressively increases, with a peak of around 65- 70 years (Bardan, *et al.*2007).

2.5.2 Race:

Incidence rates of prostate cancer among African-American men are 1.6 times higher than Caucasian men. African-Americans are also more than twice more likely to die of prostate cancer than Caucasian men. In black men, prostate cancer is also more likely to be aggressive or advanced. (Stanford and Ostrander, 2001).

2.5.3 Body size:

Certain metabolic alternations sustained in obese men, such as increased levels of insulin, insulin-like growth factor-1 and leptin may increase the risk of prostate cancer (Chang, *et al*.2001).

2.5.4 Diabetes:

Hyperinsulinemia of diabetes may enhance the risk of prostate cancer through the promotion of tumor cell growth (Kasper and Giovannucci, 2006).

2.5.5 Physical activity:

The ability of exercise to modulate hormone levels, prevent obesity, enhance immune function and reduce oxidative stress has all been postulated as mechanisms that may underlie the protective effect of exercise (Travis, *et al*.2016).

2.5.6 Genetic factor:

Men who have a relative with prostate cancer are twice as likely to develop the disease, while those with 2 or more relatives are nearly 4 times as likely to be diagnosed. The risk is even higher if the affected family members were diagnosed before age 65 (Arshad, *et al*. 2013).

2.5.7 Family history:

The risk of developing prostate cancer doubles for men who have a father or brother affected by prostate cancer and risk increase further when multiple first-degree relatives are affected (Crawford, 2003).

2.5.8 Infection:

Infection or inflammation of the prostate (prostatitis) may increase the chance for prostate cancer while another study shows infection may help prevent prostate cancer by increasing blood flow to the area. In particular, infection with the sexually transmitted infections chlamydia, gonorrhea, or syphilis seems to increase risk (Caini, *et al*.2014).

2.5.9 Smoking:

It has an effect on sex steroid hormones levels, mutations in tumor suppressor genes such as P53, and contained exposure to carcinogens such as polycyclic aromatic hydrocarbons contained in cigarette smoke, which has been thought to be associated with prostate cancer (Fowke, *et al*.2015).

2.5.10 Diet:

Diet rich in fats (especially saturated) associated with high calcium and alcohol intake leads to a higher risk of prostate cancer (Bardan, *et al*.2007).

2.5.11 Aspirin and nonsteroidal anti-inflammatory drugs:

It is plays a role in the prevention of prostate cancer by inhibiting the activity of cycio-oxygenese-key enzymes involved in prostaglandin synthesis (Mahmud, *et al*.2010).

2.5.12 Sexual behavior and sexual transmitted disease:

Males which are beginning their sexual activity earlier, having more sex partners, may have an increased risk of prostate cancer. Some possible causes are the sexually transmitted infections, or excess testosterone (Bardan, *et al*.2007).

2.6 Diagnosis of prostate cancer:

2.6.1 Prostate biopsy:

It is a procedure in which small samples are removed from a man’s prostate gland to be tested for the presence of cancer. It is typically performed when the scores from a PSA

blood test rise to level that associated with the possible presence of prostate cancer (Bot, *et al*.2007).

2.6.2 Digital rectal examination (DRE):

It is the simplest and cheapest diagnostic method, considering that the tumors are large enough as indurate nodules on the posterior plane of the prostate (Graif, *et al.*2007).

2.6.3 PSA blood test:

The PSA test measures the blood level of PSA, a protein that is produced by the prostate gland. The higher a man's PSA level (more than 4 ng /ml); the more likely is that he has prostate cancer (Barry, 2001), was reported in both men benign hyperplasia and adenocarcinoma .Therefore, the cutoff point for total PSA was lowered to 0.2-2.1ng/ml for screening Sudanese men for prostate cancer (Elamin, *et al*.2015).

2.6.4 Imaging tests:

Not all men with prostate cancer need to have more tests, but for those who do, these tests are X-rays, magnetic felids, sound waves, or radioactive substances to create pictures of the inside of the body (Bardan, *et al.*2007).

2.6.5 Ultrasound:

It uses transrectal ultrasound to further evaluate prostate. A small probe, about the size and shape of a cigar, is inserted into rectum. The probe uses sound waves to create a picture of prostate gland (Prostate Cancer Foundation, 2017).

2.6.6 Molecular tests:

PCA3 mRNA is expressed almost exclusively by prostate cells and has been shown to be highly over-expressed in prostate cancer cells (Bourdoumis, *et al*.2010).

2.6.7 Gleason score (GS):

The GS is the sum of the primary and secondary patterns with the range of 2 to 10. Biopsies are graded from 1-5 and then an aggregate score incorporating the principal and major secondary score is produced (e.g., 3 + 4 = 7). Scores conventionally tend to be grouped into the following border risk categories: 1-5: low grade prostate cancer, 6-7: intermediate-grade cancer (most prostate cancers fall into this group), 8-10: high grade cancer. However, some studies have shown that the prognosis of GS 7 cancer varies considerably (Stark, *et al.*2009, James, 2014).

2.6.8 Bone scan:

A bone scan show whether any cancer cells have spread from prostate to bone, by using small amount of a safe radioactive dye via arm vein, 2 to 3 hours later , scan is used to find if prostate cancer cells have spread to bone (Galani, 2015).

2.7 Treatment of prostate cancer:

2.7.1 Surgery:

Surgery is mainly suggested for high-risk locally advanced prostate carcinoma. Radical prostatectomy and pelvic lymhpadenectomy (PLDN) are mostly applicable surgery types in prostate cancer (Chen and Zhao, 2013).

2.7.2 Chemotherapy and biological therapy:

Chemotherapy uses drugs to kill rapidly growing cells, including cancer cells with hormone refractory prostate cancer (HRPC) has shown significant improvement in pain and quality of life. May be a treatment option for men with prostate cancer that has spread to remote body locations and also be an option for cancers that don't respond to hormone therapy. The common chemotherapeutic drugs used as the treatment of advanced prostate cancer include mitoxantrone, doxorubicin, vinblastine, paclitaxel, docetaxel, and some others (Chen and Zhao, 2013).

2.7.3 Hormonal therapy:

Both normal prostate tissue and prostate cancers depend on male sex hormones, called androgens, to grow and replicate. Testosterone is a very important androgen of the prostate gland. Men make androgens in their testicles. One of the ways to treat cancer is to remove androgens from the body, thus making the cancer shrink and then grow more slowly (Underwood, *et al*.1996).

2.7.4 Cryosurgery:

Experimental approach of treating prostate cancer whereby probes with liquid nitrogen are implanted into the prostate and then the tissue is frozen. This freezing kills the cancer cells, and it can repeat multiple times if needed (Rubin and Williams, 2001, Galani, 2015).

2.7.5 Radiation therapy:

It uses high-powered energy to kill cancer cells. Prostate cancer radiation therapy can be delivered in two ways: radiation that comes from outside of body (external beam radiation), radiation placed inside body (brachytherapy) (Krambeck, *et al*.2008).

2.7.6 Follow up-testing:

Once patient has been treated from prostate cancer, they need to be closely followed for a recurrence. Regular follow –up and checkups, to determine serum PSA level (Rubin and Williams, 2001).

2.8 Breast Cancer Type 1gene and relation with prostate tumor:

BRCA1 is are a human gene and its protein product, the human *BRCA1* gene is located on the long (q) arm of chromosome 17 at region 2 band 1, from base pair 41,196,312 to base pair 41,277,500 and found in all humans normally expressed in the cells of breast ,ovaries, prostate and other tissue, also known by the synonym breast cancer type 1 susceptibility protein, is responsible for repairing DNA (Check,2006). BRCA genes are tumor suppressors in prostate cancer that play a pivotal role in cellular damage response, regulate cell proliferation, cell cycle progression, transcription, and induction of apoptosis. Mutation results in defective DNA repair by HR, which fosters an ideal environment for carcinogenesis. BRCA mutations confer an increased risk of multiple cancers including breast, ovary, prostate, and other visceral tumors. A BRCA mutation is the only known genetic factor to be associated with prostate cancer. Studies across multiple ethnicities consistently demonstrate that BRCA1 and BRCA2 mutations not only increase the risk of prostate cancer, but also predispose patients to early onset and aggressive, potentially lethal disease. BRCA mutational status may be an important consideration in exploring newer therapeutics such as PARP inhibitors and DNA-damaging agents in prostate cancer (Mani, *et al*. 2009).

BRCA1 mutations are more sensitive to DNA damaging treatments, cancer cells with BRCA1 mutation have defective DNA repair (Davis, *et al*.2001). Normal prostate tissue did not stain for BRCA, mRNA levels increased in BRCA1 protein positive tumors (Schayek, *et al*.2009).

2.9 P63 and its relation with prostate cancer:

P63 is an also named (KET, p51A, p51B, p40 or p73L) is a homologous of the p53 tumor suppressor gene (Yang, *et al*.1998). P63 is a nuclear protein in basal cells of benign prostate encoded by a gene on chromosome 3q27-29 with homology to p53 (Paner, *et al*. 2008).

P63 is essential for development of squamous epithelia 2 major luminal and basal cells (Mills, *et al*.1999).

In prostate p63 expressed in basal cells essential for maintaing normal ductal integrity and proper differtional of luminal layers and duct structure (Yang, *et al*.1999). Expression of p63 regulated by the microRNA, all BPH immuno reactive for p63 is more than 75% of basal cells (Park, *et al.* 2010).

The p63 transcription factor belongs to a family that includes two structurally related proteins, p53 and p73. Whereas p53 plays as well-established role in tumor suppression, p63 and p73 play unique roles in morphogenesis. In particular, p632/2 mice have major factor defects in their limb and craniofacial development, as well as a striking absence of stratified epithelia. The phenotype could be explained by either inability of the p32/2 ectoderm to develop into epithelial lineages, or by lack of stem cell character necessary to sustain epithelial morphogenesis and renewal (Pellegrini, *et al*.2001).

Immunohistochemical analyses of p63 expression in benign and malignant human prostate tissue specimens were preformed. Strong p63 reactivity was observed in benign section stained with monoclonal 4A4 antibody against p63.Nuclear staining in prostate tissue was present in basal cells of the epithelium of benign areas within the pathogenic prostate glands. No expression of p63 was observed in malignant areas of the prostate cancer specimens (Rathore, 2010).

CHAPTER THREE

MATERIALS AND METHODs

Chapter three

3. Materials and methods

3.1 Materials:

Archived tissue blocks of prostate tumors were used in this study.

3.2 Methods:

3.2.1 Study design:

This is hospital analytical retrospective case control study aimed to detect protein expression BRCA1 and p63 in prostate tumors by using immunohistochemistry.

3.2.2 Study samples:

Forty tissue blocks obtained from prostate tumors samples, twenty samples previously diagnosed as prostate cancer and twenty samples which previously diagnosed as benign tumors were selected for this study. Patient's identification data (age, histopathological diagnosis, and grade) were obtained from the patients files.

3.2.3 Study area:

This study was conducted in Omdurman teaching hospital laboratory during the period from October 2018 to October 2019.

3.2.4 Sample processing:

Sections of 3μm thickness were cut by rotary microtome, mounted in positively charged glass slides and put in a 60℃ oven

3.2.5 Immunohistochemical staining:

Immunohistochemical staining was carried out using monoclonal mouse anti human BRCA 1, clone MS110, isotype; IgG1 and p63 clone 4A4, isotype; 1gG2a/Kappa. Tissue sections (3μm) were deparaffinized in xylene and rehydrated in graded alcohol (100%, 90%, 70%, 50%) and water two minutes for each. Antigen retrieval was performed by using PT link water bath with citrate buffer (pH6.8), and then slides section were circulated by Dako pen, were incubated for 10 minutes in 3% hydrogen peroxide to block endogenous peroxidase activity. The slides were treated with anti p63 primary antibody for p63 expression and by anti BRCA1 primary antibody for 20 minutes and washed in phosphate buffer saline (pH7.4). Then treated with secondary biotinylated antibody for 30 minutes, and washed in phosphate buffer saline (pH7.4). After that the avidin peroxidase complex was added for 15 minutes. Then slides were incubated in 3, 3 diamminobenzidine tetra hydrochloride (DAB)−H2O2 mixture for 7 minutes to visualize the reaction and

washed in water. Finely slides were counterstained in Mayer's hematoxylin stain for 1minute, dehydred, cleared and mounted in DPX mounting media (Bancroft, *et al* .2013).

3.2.6 Data analysis:

Data was analyzed by using 20 SPSS computer program. Frequencies, means and chi-square test values were calculated.

3.2.7 Quality control:

For each batch of staining, positive and negative control slides were prepared. The positive controls contained the antigen under investigation and negative controls slides were prepared form same tissue block, but they were incubated with TBS instead of the primary antibodies. Each slide was evaluated with an investigator then the results were confirmed by a consultant histopathologist. Detection of more than 5 cells with brown nuclear per one field was considered as a positive result.

3.2.8 Ethical consideration:

Hospital administration agreements were taken ethically for archived sample and patient's data collection.

Chapter four

Results

Chapter four

4. Results

The study included forty samples, 20(50%) of them were benign prostate hyperplasia while 20(50%) were prostatic adenocarcinoma as indicated in table (4.1).

The patient age ranged between 36 and 100 years with a mean age of 73 years, and standard deviation 12.04, patients age equal or less than 50 years representing 2(5%) and the remaining 38(95%) were more than 50 years as indicated in table (4.2).

The malignant samples grades were moderately and poorly differentiated tumors, with frequencies of 9(45%) and 11(55%), respectively as indicated in table (4.3). No expression in all benign and malignant samples stained with BRCA1 nuclear gene (4.4)

P63 positive nuclear expression was found in 4/20 malignant samples, while 16/20 samples showed negative result. All benign samples 20/20 showed positive nuclear expression. This result showed significant association (P. value =0.000) as indicated in table (4.5).

Table (4.1): Distribution of histopathology diagnosis among study samples:

Histopathology diagnosis	Frequency	Percentage (%)
Benign	20	50
Malignant	20	50
Total	40	100

Table (4.2): Distribution of age group among the study population:

Age group(Years)	Frequency	Percentage (%)
Equal or less 50	2	5
More than 50	38	95
Total	40	100

Table (4.3): Frequency of cancer grade:

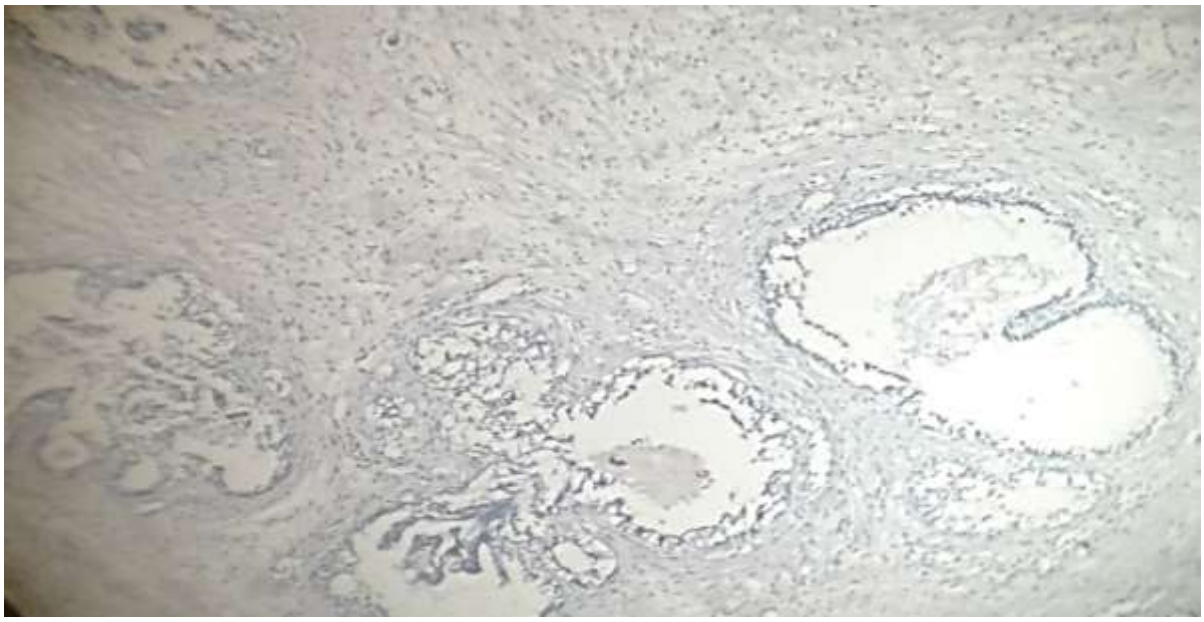
Cancer grade	Frequency	Percentage (%)
Moderate differentiated tumor	9	45
Poor differentiated tumor	11	55
Total	20	100

Table (4.4): Frequency of BRCA1 expression:

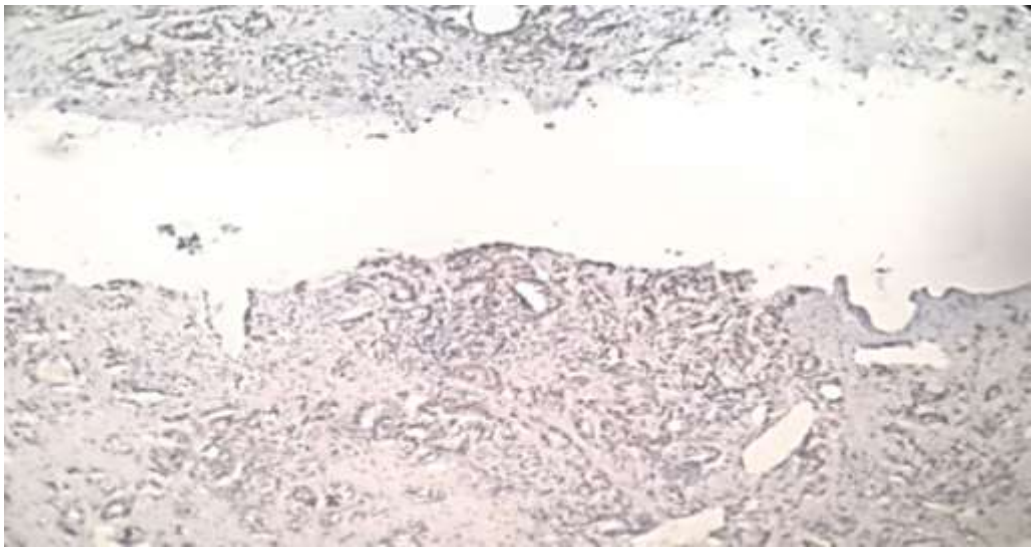
BRCA 1 expression	Frequency	Percentage (%)
Negative	**40**	**100**
Positive	**0**	**-**
Total	**40**	**100**

Table (4.5): Relation between p63 expression and histopathological diagnosis of prostate tumors:

Expression of p63	Histopathological diagnosis				Total	P.value
	Benign		Malignant			
	N	(%)	N	P (%)		
Positive	20	50	4	10	24	0.00
Negative	0	0	16	40	16	
Total	20	50	20	50	40	



Microphotograph (4.1): prostatic adenocarcinoma showed negative expression of p63 (40x)



Microphotograph (4.2): Benign prostatic hyperplasia showed nuclear positive expression of p63 (40x)

Microphotograph (4.3): Benign prostatic hyperplasia showed nuclear negative expression of BRCA-1 (40x)

Chapter five

Discussion, Conclusion and Recommendations

Chapter five

5.1 Discussion

Prostate cancer is one of most significant problems occurring worldwide. The present study involves 40 cases of prostate lesions applied for immunohistochemical stains for BRCA1 and p63. Concerning the age group of study population, the patient's age ranged between 36 and 100years with a mean age of 73years, which explain that the risk of prostate cancer increases with the age. These results agree with Bardan, *et al*. (2007), who stated that the prostate cancer increases with the age, with peak of around 65 – 70 years. Similar findings were described by Smith, (2000), who reported that the risk increases significantly after age of 50. The study results were also consistent with Bostwick, *et al*. (2004) ,who reported that risk of developing prostate cancer increases quickly over the age of 50 in white men and over the age 40 in black men. Agree also with Galani,(2015), who reported that prostate cancer is predominantly a disease of older men (aged 65 – 79 years).Also agree with Elamin, *et al*. *(*2015), who reported that mean age of prostate patients was 72.2 +- 9.25.

The histopathological diagnosis of patients revealed that all types of prostate cancer were adenocarcinomas; this result is compatible with Gillessen, *et al*. (2015), who reported that the most common type of prostate cancer is adenocarcinomas. It was also found to be compatible with Xu,*et a*l. (2000), who reported that nearly everyone with prostate cancer has adenocarcinoma because the glandular cells are the most common types of cells in prostate. All benign samples showed negative expression in BRCA1, that agree with the observation by Schayek *et al*. (2009), found that, BRCA1 was not expressed in normal prostate tissue. Localization of BRCA1 only to the most aggressive tumors may reflect an inefficient attempt to up regulate DNA repair mechanisms in prostate epithelial cells. All malignant samples showed negative expression, agreed with BRCA1 negative prognostic factor in PCa, independent to tumor grade, stage, formalin fixation interval and long term fixation which may result in Ag deficiency and need large sample size and new samples (Castro, *et al*.2013).Disagreed with studies reported *BRCA1* mutation carriers to have a significant increased relative risk of developing prostate cancer (Ford,*et al* .1994)

The expression of p63 revealed that there was significant association between marker expression in benign prostate tumors and this may be due to shedding of secretory cells leaving basal cells.

This study agreed with Signoretti, *et al*. (2000), who reported that p63, is a reliable prostate basal cell marker and that the Np63 isotype is the most abundantly represented in normal prostate basal (PrEC) cells. Because p63 protein is consistently undetectable in prostate cancers. Also agreed with Baig,*etal*.(2012),in which their study concluded that prostatic adenocarcinoma were p63 negative and the most of the benign ambiguous lesions of prostate were p63 positive.

Agreed with (Signoretti, et *al* .2000), reported that all basal cells express p63; therefore, this marker can be useful in distinguishing benign lesions from prostate malignancy.

5.2. Conclusion:

From this study we concluded that:

- The prostatic cancer in this study is commonly among patients more than 50.
- Most histological type of prostate cancer in this study samples is adenocarcinomas.
- There is association between p63 and histopathological diagnosis of prostate cancer.
- No expression in all benign and malignant samples stained with BRCA1 nuclear gene.

5.3. Recommendations:

From this study we recommended that:

- Further research should be done on expression of BRCA-1 and p63 in prostate tumors tissues with large sample size.

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Appendices

APPENDIX1:

Material and instrument for processing and staining of the specimens include:

- Disposable gloves.
- Rotary microtome.
- Positively charged slides (thermo).
- Cover glasses.
- Dry oven.
- Water path (PT LINK).
- Coplin jars.
- Humidity chamber.
- Ethanol (100%, 90%, 70%, 50%).
- Xylene.
- Mayer's haematoxylin (Haematoxylin, DW, potassium or ammonium alum, sodium iodate, citric acid and charcoal hydrate).
- Tris EDTA buffer saline.
- Phosphate buffer saline (pH7.4).
- Peroxidaes blocker (0.3% hydrogen peroxide in methanol).
- Primary antibody (anti-humanp63 and BRCA1).
- Secondary antibody (dextran polymer conjugated secondary antibody + horse reddish peroxidase).
- DAB (3, 3 diaminbenzaldhdeterahydrochloride) substrate solution.
- DPX.

APPENDIX2:

BIOCARE MEDICAL

BRCA-1
SKU: 345 Categories: B, Primary Antibodies

Description

The BRCA-1 antibody gene codes for a nuclear phosphoprotein that plays a role in maintaining genomic stability and acts as a tumor suppressor. Findings suggest that BRCA-1 antibody plays a protective role in epithelial cells undergoing high levels of proliferation in association with differentiation. Additional studies have shown that the complete loss of BRCA-1 nuclear expression and the correlation with poor prognostic markers in breast cancer imply that the altered BRCA-1 phenotype may provide an added prognostic parameter for breast cancer and could be applied as a potential rapid screening technique for BRCA-1 mutations.

Specifications

Weight	**N/A**
Dimensions	**N/A**
Intended Use	**IVD**
Species Reactivity	**Human**
Source	**Mouse Monoclonal**
Clone	**MS110**
Isotype	**IgG1**
Antigen	**BRCA-1**
Localization	**Nuclear**
Positive Control	**Breast cancer**

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SKU: 163 Categories: Cervical, Head and Neck, intelliPATH Antibodies, Oncore Antibodies, P, Primary Antibodies, Prostate, VALENT Antibodies, VP Antibodies

Description

p53 homologue p63 encodes for different isotypes able to either transactivate p53 reporter genes (TAp63) or act as p53-dominant-negatives. p63 is detected in prostatic basal cells in normal prostate; however, it is negative in malignant tumors of the prostate gland. Thus p63 antibody may be a valuable tool in the differential diagnosis of benign and malignant tumors of prostate gland and can be used in a panel of antibodies such as HMW CK [34ßE12], PSA and PSAP. p63 may play a significant role in prostate development by maintaining a prostate stem cell population. Striated muscle staining may be observed with p63.

Specifications

Weight	**N/A**
Dimensions	**N/A**
Intended Use	**IVD**
Species Reactivity	**Human, Mouse, Rat**
Source	**Mouse Monoclonal**
Clone	**4A4**
Isotype	**IgG2a/kappa**
Antigen	**p63**
Localization	**Nuclear**
Positive Control	**Normal prostate**

Datasheets & SDS

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Regulatory Notice: Biocare's IVD-labeled products comply with US-FDA and European IVDD regulation. Other regions may have additional requirements for such labeling, please contact your local distributor.

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