Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Epithelial Membrane Antigen in Prostate Tumors among Sudanese Patients

الكشف النسيجي الكيميائي المناعي عن المستضد الغشائي الطلائي لدى المرضى السودانيين المصابين بأورام البروستاتا

A Dissertation Submitted for Partial Fulfillment of the Requirements of M.Sc Degree in Medical Laboratory Sciences

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By:

Mohamed Mohieldin Khalil Hassan

B.Sc in Medical Laboratory Sciences

(Histopathology and Cytology)

(Sharq EL Niel College 2015)

Supervisor

Dr. Abu Elgasim Abass Awad Elkareem

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بسم الله الرحمن الرحيم

قال تعالي :

(وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا (113))

صدق الله العظيم

سورة النساء الآية (113)

Dedication

To soul of my father,

To my mother,

To my brothers, sisters and colleagues…

I dedicate this study.

Acknowledgements

Abstract

This is an analytical retrospective case control hospital based study conducted in Omdurman hospital during the period from October 2016 to February 2017. The study aimed to detect EMA expression in prostate tumors using immunohistochemistry.

Forty paraffin embedded blocks previously diagnosed as prostate tumors were collected. Samples included 30(75%) malignant tumors (Adenocarcinomas) and 10 (25%) benign tumors samples.

The patient's age ranged between 50 and 90 years with a mean age of 69 years, most patients were younger than 60 years representing 30 (75%) and the remaining 10 (25%) were elder than 60 years.

One section of 4 micrometer thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (avidin- biotin technique) for detection of EMA. Data collected from patients files and results obtained were analyzed using SPSS computer program.

Immunohistochemical expression of EMA was revealed positive expression in 21/30 samples and negative results in 9/30 samples in malignant tumors, while most of the benign tumors (8/10) showed negative results and 2/10 showed positive expression, with significant statistical association between EMA cytoplasmic expression and histopathological diagnosis of prostate tumors (P. value =0.017).

Positive EMA cytoplasmic expression was found to be common among poorly differentiated cancers which represent 17 (81%) cases and less common in well and moderately differentiated cancers with frequencies of 1 (4.5%) case and 3 (14.5%) cases respectively. Moderately and poorly differentiated cancers showed negative expression with frequencies of 6 (66.6%) and 3 (33.4%) cases respectively, while no expression was found in well differentiated cancers, hence,

there was a significant statistical relation between EMA cytoplasmic expression and cancer grade *(* P. value =0.015).

This study concludes that EMA cytoplasmic expression is help in differentiation between benign and malignant prostate tumors and also associated with cancer grade.

المستخلص

أجريت هذه الدراسة التحليلية المستشفوية التراجعية الحالة والحالة الضابطة في مستشفي أم درمان في الفترة بين أكتوبر 2016 إلى فبراير 2017. هدفت هذه الدراسة للكشف عن واسمة المستضد الغشائي الطلائي في أورام البروستاتا باستخدام كيمياء مناعة الأنسجة.

جمعت أربعون عينة مطمورة بشمع البرافين مشخصنه مسبقا بأورام البروستاتا، تكونت العينات من 30 (75%) عينة لأورام خبيثة (سرطانات غددية) و 10(25%) عينات لأورام حميدة.

تراوحت أعمار المرضى بين 50-90 سنة بمتوسط أعمار 69 سنة, أغلب المرضى 30 (75%) كانت أعمارهم أقل من 60 سنة بينما بقية المرضى 10(25%) كانت اعمارهم أكثر من 60 سنة.

قطع مقطع واحد من كل عينة بسمك 4 مايكرون بواسطة جهاز المشراح الدوار وصبغت العينات بواسطة كيمياء مناعة الانسجة (تقنية الأفيدين بيوتين) للكشف عن واسمة المستضد الغشائي الطلائي .أخذت البيانات من ملفات المرضى وحللت النتائج باستخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية SPSS.

أظهرت الدراسة أن واسمة المستضد الغشائي الطلائي تعطي نتيجة موجبة في 30/21 عينة، و 30/9 عينة منها سالبة الظهور في الأورام الخبيثة ، بينما الأورام الحميدة أظهر أكثرها 10/8 نتيجة سالبة و10/2 فقط اظهرت نتيجة موجبة مع وجود علاقة ذات دلالة إحصائية بين ظهور واسمة المستضد الغشائي الطلائي و التشخيص النسيجي لاورام البروستاتا (القيمة الاحتمالية = 0.017).

وجد أن الظهور الموجب في السيتوبلازم لواسمة المستضد الغشائي الطلائي منتشر في الأورام الخبيثة ضعيفة التمايز بتردد 17(81%) حاله وبنسبة أقل في الأورام الخبيثة جيدة ومتوسطة التمايز بترددات 1(4.5%) و 3(14.5%) حالات على التوالي. كما وجد أن الأورام الخبيثة متوسطة و ضعيفة التمايز أظهرت نتائج سالبة لظهور الواسمة بترددات 6(66.6%) و 3(33.4%) على التوالي بينما الأورام الخبيثة جيدة التمايز لم تظهر نتائج موجبة إطلاقا وعليه ، هنالك علاقة ذات دلالة إحصائية بين ظهور واسمة المستضد الغشائي الطلائي ودرجة الورم الخبيث (القيمة الاحتمالية = 0.015).

خلصت هذه الدراسة إلى أن واسمة المستضد الغشائي الطلائي تساعد في التفريق بين الأورام الحميدة والخبيثة كما يوجد ارتباط بين ظهور الواسمة ودرجة الورم.

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CHAPTER ONE

INTRODUCTION

Introduction

1.1 Introduction:

Prostate cancer occurs when abnormal cells develop in the prostate. These abnormal cells can continue to multiply in uncontrolled way and sometimes spread outside the prostate into nearby or distant parts of the body (Visakorpi, *et al*. 1995). More than 1.1 million cases of prostate cancer were recorded in 2012, accounting for around 8% of all new cancer cases and 15% in men. About 68% of prostate cases occurred in more developed countries (Ferlay, *et al*. 2015).

In Sudan, prostate cancer is ranked first among Sudanese men, with an estimated incidence rate of 8.7 per 100,000 populations during 2009-2010 (Saeed, *et al*. 2014).

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

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

There are different treatments for prostate cancer including active surveillance, radical prostatectomy, laparoscopic surgery, radiation therapy, chemotherapy and hormone therapy (Picard, *et al*. 2009).

Epithelial membrane antigen (EMA) also called CD227, MUC1 and episialin, is a large cell surface mucin glycoprotien expressed by most glandular and ductal epithelial cells. The expression of EMA normally found in surfaces, maintains lumen formation (Sloane, 1981). The intracytoplasmic expression of EMA might be associated with poorly differentiated areas (Nassar, *et al*. 2004). The intracytoplasmic expression of EMA in poorly differentiated malignant lesions was

also observed by De Roos, *et al*. (2007), who found that 75% of 45 adenocarcinomas prostate tumor were EMA positive cytoplasmic expression, there was an association between EMA cytoplasmic expression and grade of differentiation.

1.2 Objectives:

General objective:

To detect epithelial membrane antigen in prostate tumors among Sudanese patients by immnohistechemistry and it is correlation with histopathological diagnosis and tumor grade.

CHAPTER TWO

LITERATURE REVIEW

Chapter two
2-Literature review

2.1 Scientific background:

The prostate is a 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 stayat 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 bulbourethral 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 theprostatespecificantigen (PSA). This enzyme makes the semen thinner. The hormone-like substance spermine mostly ensures sperm cell motility (ability to move) (Franklin,*et al*. 2005).The prostate also plays a part 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 (Martini,*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 the 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, micropapillary, cribriform and flat (Majumder, *etal*. 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 andFenic,2008).

2.2.2.3Prostatic nodular hyperplasia (PNH):

It is a stromal 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 in some cases urinary obstruction as seen in benign prostatic 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 accounts 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 whicharedetectedby 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 features, 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:

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 from the NationalCancer 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 population, 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 (Saeed, *etal*. 2014).

2.5 Risk factors for prostate cancer:

The risk factors of prostate cancer include:

2.5.1 Age:

It is an essential factor in prostate cancer, but in males under 45 years prostate cancer is 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 Caucasianmen (Stanford and Ostrander, 2001).

2.5.3 Body size:

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

2.5.4Diabetes:

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 have all been postulated as mechanisms that may underlie the protective effect of exercise (Travis, *et al*. 2016).

2.5.6 Aspirin and nonsteroidal anti-inflammatory drugs:

It isplays a role in the prevention of prostate cancer by inhibiting the activity of cyclo-oxygenases-key enzymes involved in prostaglandin synthesis (Mahmud, *et al*. 2010).

2.5.7 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, orexcess testosterone (Bardan, *et al*. 2007).

2.5.8 Smoking:

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

2.5.9 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).

2.6. Diagnosis of prostate cancer:

2.6.1 Prostate biopsy:

Prostate biopsy 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 a level that is associated with the possible presence of prostate cancer (Bot,*et al*. 2007).

2.6.2Digital 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).

2.6.3PSA 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).

2.6.4Imaging tests:

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

2.7Treatment of prostate cancer:

2.7.1 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 be repeated multiple times if needed (Rubin and Williams, 2001).

2.7.2Hormonal 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 prostate cancer is to remove androgens from the body, thus making the cancer shrink and then grow more slowly (Underwood, 1996).

2.7.3 Follow up-testing:

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

2.8 Epithelial membrane antigen EMA and it's relation with prostatetumor:

EMA is typically expressed in the apical surface of normal epithelial cells responsible for maintaining lumen formation. In conventional carcinomas, the labeling was apical in areas with lumen formation;intracytoplasmic and intercellular in the poorly differentiated areas. This provides support for the reversal of cell orientation as an important factor of the morphogenesis and possibly the pathogenesis of invasive carcinomas. SinceEMA is known to have a role in lumen formation and has an inhibitory role in the cell to stroma interaction, it is conceivable that it is a key factor in the detachment of cells from stroma allowing for the dissection of the connective tissue and easing the spread of the cell (Nassar, *et al*. 2004).

EMA is expressed on the membrane of normal cells and benign prostatic tumors, while intracytoplasmic expression is found on malignant prostatic cancers (Wuker and Muller, 1986).

Heyderman*et al*. (1984), reported that in prostatic adenocarcinomas, EMA were present in the luminal membranes of malignant cells with cytoplasmic staining, particularly in poor differentiated carcinomas. Thiswas consolidated by the finding ofKim*et al*. (2014), who mentioned that EMA in the tumor cells (intracytoplasmic) confers to the tumor a worse prognosis and possibly a high propensity to disseminate.

Similarly, DeRoos,*et al*. (2007), reported that poorly differentiated malignant lesions showed positive cytoplasmic expression. Theymentioned that focal and diffused cytoplasmic expression was associated with high grade prostatic cancer.

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 a hospital based analytical retrospective case control study aimed to detect epithelial membrane antigen in prostate tumors using immunohistochemistry.

3.2.2 Study samples:

Tissue blocks obtained from thirty samples were previously diagnosed as prostate cancer and ten samples which were previously diagnosed as benign tumors. Patient's data (age, histopathological diagnosis) was obtained from the patient's files.

3.2.3 Study area:

This study was conducted in Omdurman hospital laboratory during the period from October 2016 to February 2017.

3.2.4 Sample processing:

Sections of 4µm thickness were cut by rotary microtome, mounted in positively charged glass slides and put in a 60^0C oven.

3.2.5 Immunohistochemical staining

Immunohistochemical staining was carried out using monoclonal mouse anti human EMA, clone 100/D5, isotype: IgG1/Kappa (BIO CARE). Tissue sections (4 µm) 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 (pH 6.8), then slides were incubated for 10 minutes in 3% hydrogen peroxide to block endogenous peroxidase activity.

The slides were treated with anti EMA 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 diaminobenzidine tetra hydrochloride (DAB) $-H_2O_2$ mixture for 7 minutes to visualize the reaction and washed in running water. Finely slides were counterstained in Mayer's hematoxylin stain for 1 minute, dehydrated, cleared and mounted in DPX mounting media (Bancroft, *et al*. 2013).

3.2.6 Data analysis:

Data was analyzed using version 11.5 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 control slides were prepared from the 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 cytoplasm 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, 10 (25%) of them with benign prostate hyperplasia while 30 (75%) were prostatic adenocarcinoma as indicated in table (4.1).

The patient's age ranged between 50 and 90 years with a mean age of 69 years, and standard deviation 13.7, most patients were younger than 60 years representing 30 (75%) and the remaining 10 (25%) were elder than 60 years as indicated in table (4.2).

Most of the malignant samples were moderately and poorly differentiated tumors, with frequencies of 9 (30%) and 20 (66.7%) respectively, while only one (3.3%) sample was well differentiated tumors as indicated in table (4.3).

EMA positive cytoplasmic expression was found in 21/30 malignant samples, while 9/30 samples showed negative cytoplasmic expression. Most of the benign samples 8/10 showed negative EMA cytoplasmic expression, while 2/10 revealed positive EMA cytoplasmic expression. This result showed significant association (P. value =0.017) as indicated in table (4.4).

Positive EMA cytoplasmic expression was found to be common among poorly differentiated cancers which represent 17 (81%) and less common in well and moderately differentiated cancers with frequencies of 1 (4.5%) and 3 (14.5%) respectively. Moderately and poorly differentiated cancers showed negative expression frequencies of 6 (66.6) and 3 (33.4) respectively, while no expression was found in well differentiated cancers, hence, there was a significant statistical relation between EMA cytoplasmic expression and cancer grade (P. value =0.015) (table 4.5).

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

Histopathology diagnosis	Frequency	Percentage (%)
Benign	10	25
Malignant	30	75
Total	40	100

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

Age group	Frequency	Percentage (%)
Less than 60 years	30	75
More than 60 years	10	25
Total	40	100

Table (4.3): Frequency of cancer grade:

Cancer grade	Frequency	Percentage (%)
Well differentiated tumor	1	3.3
Moderate differentiated tumor	9	30.0
Poor differentiated tumor	20	66.7
Total	30	100

Table (4.4): Relation between EMA expression and histopathological diagnosis:

Expression of EMA	Histopatholoical diagnosis				Total	P.value
	Benign		Malignant			
	Number	Percentage (%)	Number	Percentage (%)		
Positive	2	20	21	70	23	
Negative	8	80	9	30	17	0.017
Total	10	100	30	100	40	

Table (4.5): Relation between EMA expression and cancer grade:

Cancer grade	EMA expression				Total	P.value
	Positive		Negative			
	Number	Percentage (%)	Number	Percentage (%)		
Well differentiated tumor	1	4.5	0	0	1	
Moderately differentiated tumor	3	14.5	6	66.6	9	0.015
Poorly differentiated tumor	17	81	3	33.4	20	
Total	21	100	9	100	30	
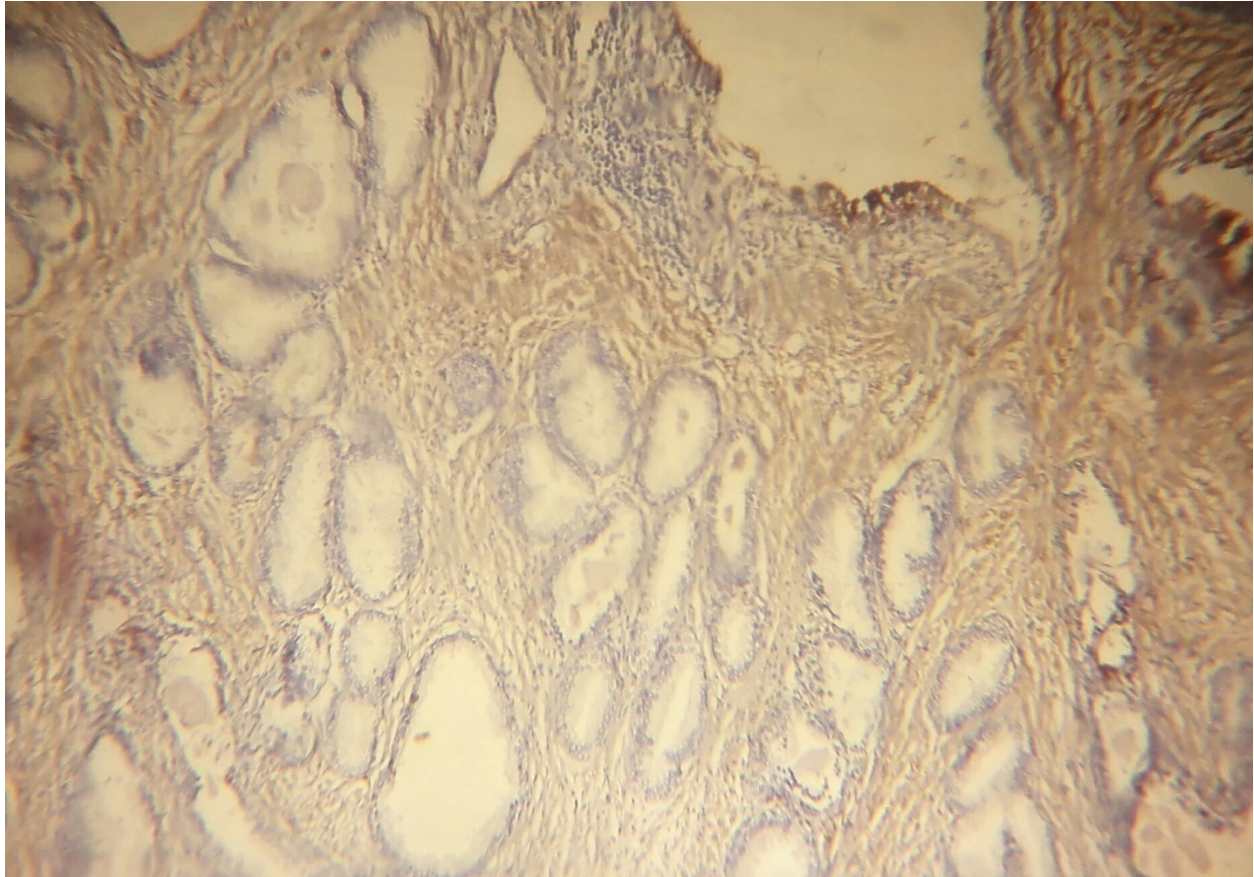
Photograph (4.1): Prostatic adenocarcinoma showing positive cytoplasmic expression of EMA (40X)
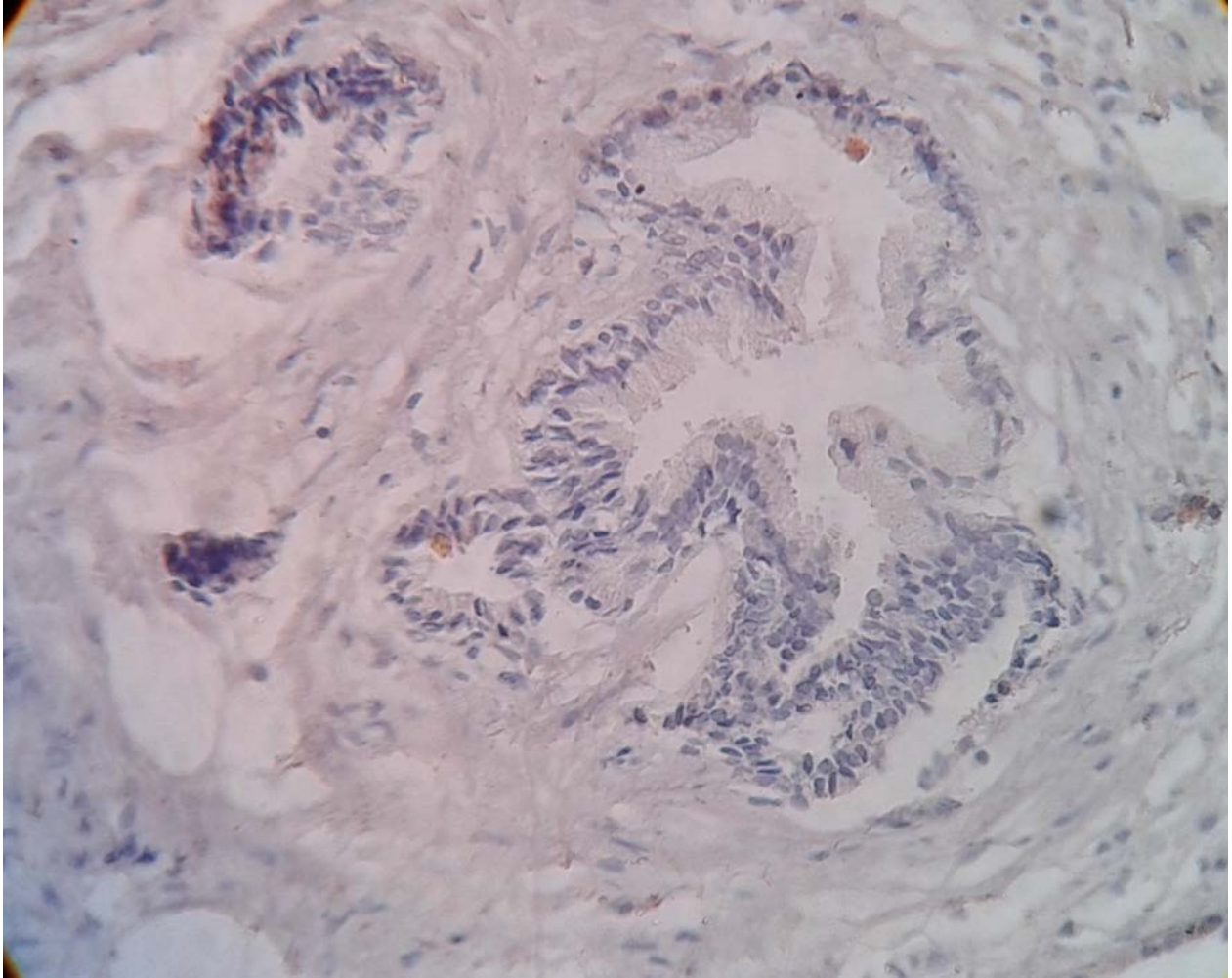
Photograph (4.2): Prostatic hyperplasia showing negative cytoplasmic expression of EMA (40X)

CHAPTER FIVE

DISCUSION

Chapter five

5. Discussion

Prostate cancer is one of the most significant problems occurring worldwide. The present study involves 40 cases of prostatic lesions applied for immunohistochemical staining for epithelial membrane antigen EMA. The patient's age ranged between 50 and 90 years with a mean age of 69years, which explainsthat the risk of prostate cancer increases with age.This result agrees with that of Bardan, *et al.*(2007),whostated that the prostate cancer increases with age,with a peak of around 65 - 70 years. Similar findings were described by Smith, (2000), who reported that the risk increases significantly after the age of 50. The study results were also consistent with those ofBostwick,*et al.* (2004), whoreported that the risk of developing prostate cancer increases quickly over the age of 50 in white men and over the age of 40 in black men.

The histopathological diagnosis of patients revealed thatall types of prostate cancer wereadenocarcinomas; 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 al.* (2000),who reported that nearly everyone with prostate cancer has adenocarcinoma because the glandular cells are the most common types of cells in prostate.

There was a significant association between EMA cytoplasmic expressionand prostatic adenocarcinoma.Thisresult was consolidated by the finding of Heyderman, *et al.* (1984), who found that 16/20 prostate cancers were positive to EMA. On the other hand,Pinkusand Kurtin, (1985)concluded that there was an association between EMA cytoplasmic expression and prostate adenocarcinomas; their results agreed this result.

The current study revealed statistical association between EMA cytoplasmic expression and cancer grade; hence the expression of EMA increased in poorly differentiated tumorsmore than the other grades.This result is consistentwith DeRoos,*et al*.(2007), who reported that 75% (34/45) of poorly differentiated malignant lesions showed positive cytoplasmic expression, and also mentioned that focal and diffuse cytoplasmic expression was associated with high grade prostatic cancers. The results were also found to agree withAbdul-Halim,*et al*.(2011), whoreported that 66.7% (12/18) of poorly differentiated malignant lesions showed positive cytoplasmic expression.

The current study revealed statistical association between EMA cytoplasmic expression and cancer grade; hence the expression of EMA increased in poorly differentiated tumors more than the other grades. This result is consistent with De Roos, *et al*. (2007), who reported that 75% (34/45) of poorly differentiated malignant lesions showed positive cytoplasmic expression. He mentioned that focal and diffused cytoplasmic expression was associated with high grade prostatic cancers. The results were also found to agree with Abdul-Halim, *et al*. (2011), who reported that 66.7% (12/18) of poorly differentiated malignant lesions showed positive cytoplasmic expression.

CHAPTER SIX

CONCULSIONS AND RECOMMENDATIONS

Chapter six

6. Conclusions and recommendations

6.1. Conclusions:

From this study we concluded that:

- The prostatic cancer in this study is commonly among patients under 60.
- Most histological type of prostate cancer in this study samples is adenocarcinomas.
- EMA cytoplasmic expression is associated with prostate cancer and cancer grade.
- **6.2. Recommendations:**

  From this study we recommended that:

  Further researches should be done on expression of EMA in prostate tumors tissues with large sample size.

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APPENDICES

Appendix 1:

Materials and instruments used for processing and staining of the specimens include:

Disposable gloves.

Rotary microtome.

Microtome Knives.

Positively charged slides (Thermo).

Cover glasses.

Dry oven.

Water path (Dako water path).

Coplin jars.

Humidity chamber.

Ethanol (100%, 90%, 70%, 50%).

Xylene.

Mayer's haematoxylin.

Citrate buffer (pH6.8).

0.3 Hydrogen peroxidase.

Primary antibody (EMA).

Secondary antibody (biotinylated secondary antibody).

Streptavidin-HRP.

Substrate chromogen (DAB).

DPX.

Epithelial Membrane Antigen (EMA) [E29]
Concentrated and Prediluted Monoclonal Antibody
Control Number: 901-3038-082614

Catalog Number:	ACI 3038 A, C	API 3038 AA
Description:	0.1, 1.0 ml, concentrated	6.0 ml, prediluted
Dilution:	1:100-1:200	Ready-to-use
Diluent:	Da Vinci Green	N/A

Intended Use:

For In Vitro Diagnostic Use.
Epithelial Membrane Antigen (EMA) [E29] is intended for laboratory use in the qualitative identification of epithelial membrane antigen protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

Epithelial membrane antigen (EMA) belongs to a heterogeneous family of highly glycosylated transmembrane proteins known as human milk fat globule (HMFG) membrane proteins. This family of antigens is not restricted to breast but may also be found in secretory epithelial cells, to a lesser degree, in non-secretory epithelium (e.g., squamous epithelium) and rarely in non-epithelial cells. EMA is best considered a broad-spectrum antibody that is reactive against many types of adenocarcinoma. Breast and skin adnexal tumors are strongly positive. A lesser degree of staining is seen in carcinomas of the endometrium, kidney, thyroid, stomach, pancreas, lung, colon, ovary, prostate and cervix. Embryonal carcinomas, medullary carcinomas of thyroid, squamous carcinomas, sarcomas, lymphomas, and melanomas all tend to be nonreactive or show rare positive cells. Transitional cell carcinomas may show weak reactivity and anaplastic large cell lymphomas can be positive for EMA.

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: E29
Isotype: IgG
Total Protein Concentration: ~10 mg/ml. Call for lot specific Ig concentration
Cellular Localization: Membrane and cytoplasmic
Positive Tissue Control: Colon and breast cancer
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues).
Supplied As: Buffer with protein carrier and preservative.
Storage and Stability:
Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.
Protocol Recommendations:
Deparaffinization and rehydration: Perform deparaffinization of tissues with xylenes or xylenes substitute, followed by rehydration through graded alcohols.
Peroxide Block: Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment: Perform heat retrieval using Biocare's Reveal Decloaker. Refer to the Reveal Decloaker product datasheet for specific instructions.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a probe.
Polymer: Incubate for 10 minutes at RT with a polymer.

Protocol Recommendations cont'd:
Chromogen: Incubate for 5 minutes at RT when using Biocare's DAB – OR – Incubate for 5-7 minutes at RT when using Biocare's Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:

1. Do not allow tissue sections to dry during the staining procedure. Dried tissue sectons may display increased non-specific or uneven staining.
2. This antibody has been standardized with Biocare's MACH 4 detection system. It can also be used on an automated staining system. Use TBS buffer for washing steps.

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3), used as a preservative, is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water.
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The MSDS is available upon request and is located at http://biocare.net/support/msds/.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Biocare Medical
4040 Pike Lane
Concord, CA 94520
USA

IVD CE

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EC REP EMERGO EUROPE
Molenstraat 15
2513 BH, The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080