بسم الله الرحمن الرحيم

**Sudan University of Science and Technology**

**College of Graduate Studies**

# Detection of Epithelial Membrane Antigen among Sudanese Patients with Skin Cancers using immunohistochemical

# الكشف عن مستضد الغشاء الظهاري لدى المرضى السودانيين المصابين بسرطان الجلد باستخدام كيمياء الأنسجة المناعية

A dissertation submitted in the partial fulfillment for requirements of Master Degree in Medical Laboratory Science (Histopathology and Cytology)

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# الآية

**قال تعالى :**

**بسم الله الرحمن الرحيم**

(وَقَالُوا۟ لِجُلُودِهِمْ لِمَ شَهِدتُّمْ عَلَيْنَا ۖ قَالُوٓا۟ أَنطَقَنَا ٱللَّهُ ٱلَّذِىٓ أَنطَقَ كُلَّ شَىْءٍ وَهُوَ خَلَقَكُمْ أَوَّلَ مَرَّةٍ وَإِلَيْهِ تُرْجَعُونَ ﴿٢١﴾)

**سورة فصلت الأية (21)**

**Dedication**

*To:*

*My family members, especially*

*My mother and father,My*

*husband,*

*My children*

*Who always encourage and support*

*me.*

*Elresala*

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*All great thanks are firstly to Allah.*

*I would like to express my special thanks to my supervisor Dr. Mohammed Siddig Abdelaziz Dean College of Medical Laboratory Sciences Sudan University of Sciences and Technology, for his advisory effort that made this work come to true. He did not hesitate to devote his knowledge and time for me, and giving his arguments in this field.*

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## Abstract

This is a hospital based descriptive retrospective study conducted at Omdurman Teaching Hospital during the period from January to April 2015, aimed to detect Epithelial Membrane Antigen (EMA) in skin cancer. A total of 38 blocks previously diagnosed as skin cancer were selected for this study. The patient ages ranged between 4 years to 93 years with mean age of 55 years. One section of three micron was cut from each block and stained using immunohistochemical method using modified Dako indirect technique for EMA detection. The data obtained was analyzed using SPSS program version 11.5.

Out of 38 samples with skin cancer histopathological diagnoses revealed 27(71.1%) as squamous cell carcinoma and 11(28.9%) as basal cell carcinoma. The grade of squamous cell carcinoma showed 17(63%) as well differentiated, 7(25.9) as moderately differentiated and 3(11.1) as poorly differentiated.

Positive expression of EMA was detected (16) 95% in squamous cell carcinoma and(1) 5% in basal cell carcinoma, with significant relation between EMA expression and type of cancers.

Positive expression common among well differentiated squamous cell carcinoma while moderate and poor differentiated show commonly negative result with no significant relation between EMA expression and grades of squamous cell cancinoma.

The study concluded that EMA expression may helps in differentiation between squamous cell carcinoma and basal cell carcinoma.

**المستخلص**

أجريت هذه الدراسة الوصفية المستشفوية التراجعية في مستشفي أم درمان التعليمي في الفترة من يناير إلي أبريل 2015 للكشف عن مستضد الغشاء الظهاري في سرطان الجلد.

تم جمع 38 قالب مدعم بالشمع من مرضي مشخصين مسبقا بسرطان الجلد تراوحت أعمارهم بين 4 سنوات الي93 سنة, بمتوسط عمر 55 سنة.

قطعت وصبغت المقاطع النسيجية(3 ميكرون) بطريقة كيمياء الأنسجة المناعية باستخدام طريقة داكو التقنية المعدلة غير المباشرة للكشف عن مستضد الغشاء الظهاري . تم استخدام برنامج الحزم الإحصائية للعلوم الاجتماعية النسخة 11.5 لتحليل النتائج.

من مجموع 38 عينة مشخصة بسرطان الجلد أظهرت الدراسة أن 27 (71.1%) عينة كانت من نوع سرطان الخلايا الحرشفية بينما 11(28.9%) عينة كانت من نوع سرطان الخلايا القاعدية.من مجموع عينات سرطان الخلايا الحرشفية كانت منها 17 (63%) عينة جيدة التمايز و7 (25.9%) عينات متوسطة التمايز و 3(11.1%) عينات ضعيفة التمايز.

أظهرت الدراسة أن سرطان الخلايا الحرشفية ذات تعبير نسيجي عالي لمستضد الغشاء الظهاري (16) 95%‘ بينما سرطان الخلايا القاعدية ليست ذات تعبير عالي للواسمة(1) 5%, مع وجود علاقة ذات دلالة إحصائية بين افراز مستضد الغشاء الظهاري ونوع السرطان.

سرطان الخلايا الحرشفية جيدة التمايز ذات تعبير عالي للوا سمة بينما المتوسطة وضعيفة التمايز ليست ذات تعبيرعالي للواسمة, مع عدم وجود علاقة ذات دلالة إحصائية بين افراز مستضد الغشاء الظهاري ومراحل السرطان.

خلصت الدراسة إلى أن التعبير النسيجي لمستضد الغشاء الظهارى يفيد في التفريق بين سرطان الخلايا الحرشفية و سرطان الخلايا القاعدية.

## List of Abbreviations

| | |
|---|---|
| **NMSCs** | Non Melanoma Skin Cancers |
| **SCC** | Squamous Cell Carcinoma |
| **BCC** | Basal Cell Carcinoma |
| **EMA** | Epithelial Membrane Antigen |
| **MPLSM** | Multi photon Laser Scanning Microscopy |
| **UV** | Ultra Violet |
| **RTRS** | Real Time Raman spectroscopy |
| **CVD** | Cardio Vascular Disease |
| **AK** | Actinic Keratosis |
| **HPV** | Human Papilloma Virus |
| **CMM** | Cutanous Malignant Melanoma |
| **CT** | Computer Tomography Scan |
| **MRI** | Magnetic Resonance Imaging |
| **PET** | Positron Emission Tomography scans |

| | |
|---|---|
| **SBCC** | Superficial Basal Cell Carcinoma |
| **SCCIS** | Squamous Cell Carcinoma In Situ |
| **AJCC** | American Joint Committee on Cancer |
| **TNM** | Tumors, Nodes and Metastases |
| **DAB** | 3.3 Di Amino Benzidine tetrahydrochloide |
| **DPX** | Dextrin Plasticizer Xylene |

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# Chapter One

## 1. Introduction

Skin cancer is normal cell undergo a transformation ,grow and multiply without normal control (Stephanie ,2014). Melanoma and nonmelanom (basal cell carcinoma(BCC) and squamoua cell carcinoma (SCC) skin cancer (NMSCs) are now the most common types of cancer in the white populations (Diepgen and Mahler, 2002).

Skin cancer result in 80, 000 deaths per year , 49,000 of which are due to melanoma and 31,000 of which are due to non-melanoma skin cancers (Lozano, 2012).

BCC and SCC show an average increase in incidence from 3% to 8% per year in developed countries with white populations ( Choudhury *et al*,2012).

In UK the current annual number of basal cell carcinomas (BCCs) is probably closer to 55 000–60 000, surpassing official estimates of 48 000 per year (Geller and Swetter, 2012).

In Europe, increases in melanoma incidence have been documented over the past few decades with wide north/south and east/west variations. The estimated age-standardized rates of new melanoma cases for 2012 were 11·4 per 100 000 for males and 11·0 per 100 000 for females, ranging from approximately six new cases for Central and Eastern Europe, to 10 cases for Southern Europe and up to 19 cases per 100 000 for Northern Europe( Nikolaou and Stratigos,2014).

Skin cancer is commonest cancer in Sudan followed by breast tumors (Saeed *et al*, 2013).

Also in Sudan squamous cell carcinoma was the commonest skin malignancy accounting 42.6% followed by basal cell carcinoma which was seen in 32% of the study groups. The incidence of melanoma is much lower than that of NMSCs (Abdelsamie *et al*, 2012).

There are many risk factors associated with skin cancer, ultraviolet radiation from sun exposure is the primary cause of skin cancer, smoking tobacco, HPV, congenital melanocytic nevi syndrome, radiation and tanning beds (Saladi and Persaud, 2005).

NMSCs diagnosis is done by use biopsy techniques, shave biopsy is usually performed if BCC is suspected. Punch biopsy is preferred if SCC is thought to be present (Juan and Clark, 2001). The use of spectroscopic and imaging techniques, increase diagnostic accuracy and decrease morbidity and mortality (Drakaki *et al*, 2013).

Choice of treatment approach of skin cancer depends on the tumor's location, size, borders, and growth rate. The standard treatment approaches are superficial ablative techniques (electro-desiccation and curettage and cryotherapy) used primarily for low-risk tumors and full-thickness techniques ( micrographic surgery, excisional surgery, and radiotherapy) used to treat high-risk tumors. Removal of the entire tumor is essential to limit and prevent tumor recurrence (Juan and Clark, 2001).

In normal skin, epithelial membrane antigen (EMA) is expressed in sebaceous glands. High-power magnification of EMA-positive areas with more evident sebaceous differentiation pidic microvacuoles, explaining why EMA is one of the most widely used immunohistochemical markers to investigate sebaceous differentiation in cutaneous tumors (Fuertes *et al* 2013).

**Rationale**

Skin cancer is the commonest caner in Sudan ( Saeed, *et al* 2013), so it is the target for researchers to find new ways for early diagnosis, treatment and to decrease morbidity and mortality.

Distinction of basal and squamous cell carcinomas of the skin can be readily achieved with routine immunohistochemistry using Epithelial Membrane Atigen (EMA) (Beer *et al* ,2000).

In Sudan there is no published studies about EMA expression in skin cancer, hence, this study aimed to apply it among Sudanese patients.

**Objectives:**

**General objective:**

To detect the expression of epithelial membrane antigen among Sudanese patients with skin cancers using immunohistochemical.

**Specific objective**:

To correlate between the expression of epithelial membrane antigen and cancer types.

To correlate between the expression of epithelial membrane antigen and cancer grades.

# Chapter Two

## 2. Literature review

### 2.1. Scientific background

The skin is a complex organ covering the whole surface of the body, and is continuous with the mucous membranes lining the body's orifices. It accounts for about 15% of the total adult body weight, and is therefore the largest organ of the body. It exerts multiple vital functions (namely of protection against external physical, chemical and biological aggressions), rendered possible thanks to an elaborate structure, associating tissues(Annee.2002).

### 2.2. Anatomy and physiology of the skin

The skin is the complex structure both anatomically and physiologically. The skin is divided into three layers-the epidermis, the dermis and superficial fascia. As is true with the skin surface ,each of these three layers has a different appearance in different areas of the body (Ira *et al* ,2008).

#### 2.2.1. Epidermis

The epidermis is a stratified, squamous epithelium layer that is composed primarily of two types of cells: keratinocytes and dendritic cells. The keratinocytes differ from the "clear" dendritic cells by possessing intercellular bridges and ample amounts of stainable cytoplasm. The epidermis is a continually renewing layer and gives rise to derivative structures, such as pilosebaceous apparatuses, nails, and sweat glands(Kolarsick *et al,*2011).

#### 2.2.2. Basement membrane

The epidermis and dermis are separated by a thin sheet of fibers called the basement membrane. The basement membrane controls the traffic of the cells and molecules between the dermis and epidermis but also serves, through the binding of a variety of cytokines and growth factors, as a

reservoir for controlled release during physiological remodeling or repair processes (Iozzo , 2005).

### 2.2.3. Dermis

The dermis is an integrated system of fibrous, filamentous, and amorphous connective tissue that accommodates stimulus-induced entry by nerve and vascular networks, epidermally derived appendages, fibroblasts, macrophages, and mast cells. Other blood-borne cells, including lymphocytes, plasma cells, and other leukocytes, enter the dermis in response to various stimuli as well. The dermis comprises the bulk of the skin and provides its pliability, elasticity, and tensile strength. It protects the body from mechanical injury, binds water, aids in thermal regulation, and includes receptors of sensory stimuli (Kolarsick *et al*,2011).

## 2.3. Functions of skin

The primary function of the epidermis is to produce the protective, semi-permeable stratum corneum that permits terrestrial life. The barrier function of the stratum corneum is provided by patterned lipid lamellae localized to the extracellular spaces between corneocytes(Madison, 2003).

The skin serves as a wall-like barrier that separates inside the body from the microbial enemies of the environment and provides a primary defense against infection. The layers of the skin, like the outer wall and secondary inner walls surrounding a medieval city, not only provide protection from external enemies, but also provide niches where normal flora bacteria and fungi can live and conduct busines **(**Brodell and Rosenthal, 2008).

## 2.4. Abnormalities of skin

### 2.4.1. Inflammation of skin

Psoriasis affects more than 2 percent of the world's people. It is characterized by scaly, red cutaneous plaques that contain inflammatory infiltrates and epidermal hyper proliferation (Caroline and Thomas, 1999). Patients with psoriasis, are at higher risk of developing “systemic” co-

morbidities, e.g cardiovascular disease (CVD), metabolic syndrome, and overt diabetes (Batya *et al*, 2010).

Allergic contact dermatitis also known as contact hypersensitivity, is a T-cell–dependent skin disease with the kinetics of a delayed-type hypersensitivity response, this disorder is even more prevalent than psoriasis. Their cytokines (and possibly direct cell-mediated injury of keratinocytes) induce the clinical pattern of cutaneous inflammation that is characteristic of allergic contact dermatitis (Caroline and Thomas, 1999).

Atopic dermatitis can be viewed as an exaggerated cutaneous immune response to environmental antigens, patients with this disorder have a humoral response characterized by IgE antibodies associated with T cells that produce type 2 cytokines (Caroline and Thomas, 1999).

Eczema refers to a broad range of conditions that begin as spongiotic dermatitis and may progress to a lichenified stage (Buxton, 1987).

**2.4.2. Precancerous lesion of the skin**

Actinic keratosis (AK) is a common sun-induced precancerous neoplasm confined to the epidermis. It is the initial manifestation of a continuum of clinical and histologic abnormalities that progresses to invasive squamous cell carcinoma. Like SCCs, the vast majority of AKs are asymptomatic. Although some actinic keratoses may become clinically in apparent, possibly cither due to immune rejection or simply having their external surface unknowingly scraped off, an untreated AK represents a potentially curable fatal cancer. Destructive modalities such as cryosurgery using liquid nitrogen and electro desiccation and curettage are the mainstays of therapy (Robert , 1997).

Approximately 10% of AKs will progress to SCCs. This progression is thought to be due to chronic sun exposure, specifically ultraviolet B sunlight (Aaron and Ellen, 2007).

Precancerous conditions of the skin have the potential to develop into melanoma as, an atypical mole (also called dysplastic nevus) is an unusual mole that looks different from an ordinary mole, characterized by multiple shades of brown or red-brown in color, size is greater than 6 mm, irregular shape, pebbly or centrally raised surface and irregular border . Congenital melanocytic nevi are birthmarks or large moles that are present at birth or may develop during early childhood, characterized by brown or black, even or speckled coloring, raised appearance, presence of coarse dark hair, irregular surface (Traketlli *et al* ,2010).

**2.4.3. Malignant tumors of skin**

**2.4.3.1. Basal cell carcinoma**

Basal-cell carcinomas constitute approximately 80 percent of all non melanoma skin cancers. Australia has the highest rate of basal-cell carcinoma in the world. Exposure to ultraviolet radiation is generally accepted as the major cause of basal-cell carcinoma. Basal-cell carcinoma characteristically arises in body areas exposed to the sun and is most common on the head and neck (80 percent of cases), followed by the trunk (15 percent of cases) and arms and legs. Basal-cell carcinomas have also been reported in unusual sites, including the , breasts, perianal area, genitalia, palms, and soles( Adam *et al* ,2005).

**2.4.3.2. Squamous cell carcinoma**

Squamous cell carcinoma a common cancer in white population, is related to sunshine exposure. All newly diagnosed cases of SCC were in men aged 25 through 79 years .Subjects with pale skin and red hair had an elevated risk of SCC. Subjects whose mother was of southern European ancestry had a reduced risk of SCC. After accounting for pigmentary factors, no association was seen between risk of SCC and cumulative lifetime sun exposure. However, a strong trend toward increasing risk was

seen with increasing chronic occupational sun exposure in the 10 years prior to diagnosis (Richard *et al*,1995)**.**

20 percent of non melanoma skin cancers are squamous-cell carcinomas. In 1994 in the United States, the lifetime risk of squamous-cell carcinoma was 9 to 14 percent among men and 4 to 9 percent among women (Murad and Désirée ,2001).

**2.4.3.3. Malignant melanoma**

A type of skin cancer of melanocytes (pigment-containing cells in the skin).In women, the most common site is the legs, and melanomas in men are most common on the back. It is particularly common among Caucasians. The primary cause, ultraviolet light (UV) exposure(Parkin *et al*, 2005).

Melanoma is less common than other skin cancers. However, it is much more dangerous if it is not found in the early stages. It causes the majority (75%) of deaths related to skin cancer. Globally, in 2012, melanoma occurred in 232,000 people and resulted in 55,000 deaths ( Jerant *et al* ,2000).

**2.5. Sign and symptoms of skin cancer**

Basal cell carcinoma appear as dome-shaped skin growth with visible blood vessels, often pink or skin-colored, can also be brown or black or have flecks of these colors in the growth, grows slowly,may flatten in the center, ooze, and crust over. Tends to bleed easily, or may appear as shiny pink or red, slightly scaly patch, especially when appears on the trunk. It grows slowly and may be mistaken for a patch of eczema, or waxy feeling, hard, pale-white to yellow or skin-colored growth that looks like a scar and can be difficult to see the edges ( Carucci and Leffell ,2008).

Squamous cell carcinoma is commonly a red, scaling, thickened patch on sun-exposed skin, some are firm hard nodules and dome shape like keratoacanthomas, Ulceration and bleeding may occur. When SCC is

not treated, it may develop into a large mass. Merkel cell carcinomas are most often rapidly growing, non-tender red, purple or skin colored bumps that are not painful or itchy (Bickle *et al*,2004). Most melanomas appear as asymmetry, borders (irregular), color (variegated) ,diamet(greater than 6 mm (0.24 inch), about the size of a pencil eraser) and evolving over time(Fiddler , 1995).

**2.6. Risk factors for skin cancer**

The usual associations were observed for sun exposure and pigmentation characteristics, with chronic sun exposure being most strongly associated with SCC risk, and naevi and atypical naevi with CMM risk. Use of ciprofloxacin was associated with a decreased risk of BCC and use of thiazide diuretics was associated with an increased risk of SCC. Ciprofloxacin was also associated with SCC and thiazines with BCC, but these associations lost significance after correction for multiple testing. Consumption of pomegranate, rich in antioxidants, was associated with decreased BCC and SCC risk, also after correcting for multiple testing. Recent experience of stressful events was associated with increased risk, particularly of CMM (Vries *et al*, 2012).

The rising incidence rates of NMSC are probably caused by a combination of increased exposure to ultraviolet (UV) or sun light, increased outdoor activities, changes in clothing style, increased longevity, ozone depletion, genetics and in some cases, immune suppression. An intensive UV exposure in childhood and adolescence was causative for the development of basal cell carcinoma (BCC) whereas for the etiology of SCC a chronic UV exposure in the earlier decades was accused. The frequency of the malignant melanoma occurrence is closely associated with the constitutive color of the skin and the geographical zone. Changes in outdoor activities and exposure to sunlight during the past 50 years are an important factor for the increasing incidence of melanoma (Leiter *et al* ,2014).

Constitutional susceptibility was an independent risk factor for all three types of skin cancer. Sunlamp usage or tanning salon attendance was a risk factor for melanoma after adjusting for potential confounding variables .Higher sun exposure while wearing a bathing suit was an independent risk factor for all three types of skin cancer. There are a significant interaction between constitutional susceptibility and sun exposure while wearing a bathing suit on melanoma risk. This interaction was weaker and non-significant for SCC and BCC (Jiali *et al* ,2006).

sun exposures in both early life and adulthood were predictive of BCC and SCC risks, whereas melanoma risk was predominantly associated with sun exposure in early life (Shaowei *et al* 2009).

exposure to UV nail lights is a risk factor for the development of skin cancer; however, this observation warrants further investigation. In addition, awareness of this possible association may help physicians identify more skin cancers and better educate their patients (Deborah and Carol, 2009).

**7.2. Diagnosis of skin cancer**

**2.7.1. Skin biopsies:**

Skin biopsy is an invaluable tool in the diagnostic armamentarium of a dermatologist. It not only helps in diagnosis in cases of dilemma but also provides an opportunity to find something unusual in routine practice. There are various techniques of performing a skin biopsy and any particular technique chosen is based on the type of lesion, site of lesion and also on the proficiency of the dermatologist. Punch biopsy is the most widely used technique for skin biopsy, it can be used both for diagnostic as well as therapeutic purposes, also can be employed for any solid lesion and small vesicle that can be contained within the punch. Shave biopsy, in which the portion of the lesion that is above the level of the surrounding skin is shaved off using a blade. Superficial lesions such as seborrheic

keratosis can be biopsied in this manner. However, it is better avoided as it does not include deeper tissues(Urmila *et al*,2008).

Incisional and excisional biopsy, in incisional this involves taking a part of the tissue for confirming the diagnosis and is commonly employed when inflammatory dermatosis is suspected. While in excisional biopsy the entire lesion is completely and deeply removed till the subcutis plane. The wound can be closed primarily by sutures. This method is preferred when a neoplasm is suspected (Urmila *et al*,2008).

Sqamous cell carcinoma also diagnosed by using fine –needle aspiration biopsy while for thick melanoma, surgeons may use a method called sentinel lymph node biopsy to remove the lymph node most likely to have cancer cell. Cancer cells may appear first in the sentinel node before spreading to other lymph nodes and other places in the body (Rockville ,2010).

**2.7.2. Spectroscopic and imaging techniques**

Blood tests and imaging test such as a chest x-ray ,a CT scan , MRI ,or a PET may be used to check for the spread of skin cancer(Rockville ,2010).

The importance of dermatological noninvasive imaging techniques has increased over the last decades, aiming at diagnosing non melanoma skin cancer (NMSC). Technological progress has led to the development of various analytical tools, enabling the in vivo/in vitro examination of lesional human skin with the aim to increase diagnostic accuracy and decrease morbidity and mortality. The structure of the skin layers, their chemical composition, and the distribution of their compounds permits the noninvasive photo diagnosis of skin diseases, such as skin cancers, especially for early stages of malignant tumors. An important role in the dermatological diagnosis and disease monitoring has been shown for

promising spectroscopic and imaging techniques, such as fluorescence, diffuse reflectance, Raman and near-infrared spectroscopy, optical coherence tomography, and confocal laser-scanning microscopy (Drakaki *et al* ,2013).

### 2.7.2.1. Multi photon Laser Scanning Microscopy (MPLSM)

Multi photon Laser Scanning Microscopy (MPLSM) could potentially be applied for non-invasive diagnostics of squamous cell carcinoma in situ (SCCIS) and superficial basal cell carcinoma (SBCC) (John *et al*, 2008).

### 2.7.2.2. Real time Raman spectroscopy

Real-time Raman spectroscopy can be used to distinguish malignant from benign skin lesions with good diagnostic accuracy comparable with clinical examination and other optical-based methods *(*Harvey *et al*, 2012).

## 2.8. Staging of skin cancer

Needs to learn the stage of the disease guides the choice of therapy, NMSCs stages run from stage zero (carcinoma in situ) to stage four (metastasis ).Basal cell carcinoma rarely spreads to other parts of the body, but squamous cell carcinoma some time spreads to lymph nodes and other organs .Also melanoma stages run from stage zero (melanoma in situ) to stage four (metastasis) which spread to other organ (Rockville ,2010).

The seventh edition of the American Joint Committee on Cancer *(*AJCC*)* Cancer Staging Manual includes a major revision of the staging protocol for cutaneous carcinomas. There are several significant improvements to the Tumor, Nodes, and Metastases (TNM) staging system, including consideration of high-risk factors within the primary T grade, a decrease in the tumor size threshold from 5 cm to 2 cm, improved stratification of patient lymph node status, as well as exclusion of Merkel cell carcinomas from the staging system for squamous cell carcinoma (SCC) and other cutaneous carcinomas ( Christina *et al* ,2011).

## 2.9. Immunohistochemistry

Immunohistochemistry has become an essential diagnostic tool in dermatopathology. The term covers a group of immunostaining techniques in which labeled antibodies are used to detect the presence of antigens in cells or tissues. The principle of immunohistochemistry lies in the ability of antibodies to bind specifically to their respective antigens. The resulting reaction can only be visualized when the antibody is labeled with a substance that absorbs or emits light or produces color ( Fuertes *et al*, 2013).

### 2.9.1. Tumor markers

Tumor markers are soluble glycoproteins that are found in the blood, urine, or tissues of patients with certain types of cancer. They are typically produced by tumor cells, but in some cases they may be produced by the body in response to malignancy or to certain benign conditions. Tumor markers are not elevated in all cancer patients, particularly patients with early-stage cancer. The various tumor markers differ in their usefulness for screening, diagnosis, prognosis, assessing therapeutic response, and detecting recurrence ( Tchagang *et al* ,2008).

#### 2.9.1.1. EMA (Epithelial Membrane Antigen)

is a glycosylated protein expressed on the surface of various glandular epithelial cells and in tumors derived from these cells. In normal skin, EME expressed in sebaceous glands (in the excretory duct and sebocytes). Histologically, the antibody produces staining of the cytoplasmic (Fuertes *et al*, 2013).

High-power magnification of EMA-positive areas with more evident sebaceous differentiation pidic microvacuoles, explaining why EMA is one of the most widely used immunohistochemical markers to investigate sebaceous differentiation in cutaneous tumors. In most cases, both the secretary portion and the excretory duct of eccrine and apocrine glands

stain for EMA, but there have been reports of negative results for the eccrine duct. EMA is also expressed in perineural cells. It is not expressed in normal squamous epithelium, but is frequently positive in squamous cell carcinoma. It is positive in plasma cells and recognizes neoplastic cells in EMPD, allowing these to be clearly distinguished from neighboring epidermal keratinocytes. Finally, EMA has been observed in neoplastic cells of tumors with diverse lineages of differentiation, such as meningiomas, mesotheliomas, multiple types of mesenchymal tumors, and even certain lymphomas, such as $CD30^+$ anaplastic large-cell T-cell lymphoma (Fuertes *et al*, 2013).

**2.9. Management of skin cancer:**

As the incidence of nonmelanoma skin cancer (NMSC) increases, so does the number of modalities used to treat this condition. Surgery is the most frequent approach used to treat NMSC, and clinicians usually perform Mohs micrographic surgery, conventional excision, electrodesiccation and curettage or cryosurgery ( Julie *et al*, 2007).

regular use of such a sunscreen during the first 18 years of life would reduce the lifetime incidence of basal and squamous cell carcinoma by 78%. Additional benefits of sunscreen use during childhood include reduced risk of sunburn, retarding the pace of skin aging, and possible reduction in melanoma risk (Stern *et al*, 1986).

cryosurgery is an effective treatment that compares favorably with other established methods of therapy for basal cell and squamous cell carcinomas of the skin because the five –year cure rate achieved by it for skin cancer (Emanuel and Andrew ,1991).

# Chapter Three

# 3. Materials and Methods

## 3.1. Materials

Archive tissue blocks of skin cancer were used in this study.

## 3.2. Methods

### 3.2.1. Study design

This is hospital based descriptive retrospective case study aimed to detect epithelial membrane antigen in skin cancer using immunohistochemical method.

### 3.2.2. Study samples

Thirty eight skin tissue blocks were obtained from tissues previously diagnosed as basal and squamous cell carcinoma at Omdurman teaching hospital during the period from January to April 2015.Patient identification data and other information were obtained from patient's file.

### 3.2.3. Sample processing

Section of three micron in thickness was obtained from each formalin fixed paraffin wax embedded tissue using rotary microtome.

### 3.2.4. Sampling staining

**Immunohistochemical staining:**

Paraffin sections were immunostained using modified Daco indirect technique as follows:

**Dewaxing:** Sections were dewaxed in hot plate oven and cleared in two changes of xylene for two minutes.

**Rehydration:** Sections were hydrated through descending concentration of ethanol(100%,90%,70%,50%) and water two minutes for each.

**Antigen retrieval:** Ag retrieval by Daco PT link retrieval technique, then treating with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffer saline (PH 7.4)for five minutes.

**Immunostaining:** Section were treated with epithelial membrane antigen primary antibody for twenty minutes ,then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for thirty minutes, then rinsed in phosphate buffer saline, then treated with substrate and DAB chromogen for seven minutes ,then washed in phosphate buffer saline for 5-10 minutes, then counterstained in Mayer's haematoxylin for one minute, then washed and blued in running tap water for ten minutes ,then dehydrated through ascending concentration of ethanol(50%, 70%,90%,100%),then cleared in xylene and mounted in DPX mountant (Bancroft and Marilyn ,2008).

### 3.2.5. Result interpretation

All quality control measures were adopted during sample processing for immunohistochemical results assessment.

### 3.2.6. Statistical analysis

Data were analyzed using version 11.5 SPSS computer program.

Frequencies, means, and chi-square test were calculated.

### 3.2.7. Ethical considerations

Hospital administration agreements were taken ethically for archive sample and patient data collection.

# Chapter Four

## 4. Results

Thirty eight blocks previously diagnosed as skin cancer were used in this study, 27 (71.1%) of them showed squamous cell carcinoma whilst 11 (28.9%)were basal cell carcinoma (table4.1).The patient ages ranged between 4- 93 years old with mean age of 55 years. Most of them 23 (60.5%) above 50 years and 15 (39.5%) equal or less than 50 years (table 4.3).

The histopathological diagnosis of cancer is most common among age group above 50 years with frequencies 17(44.7) for squamous cell carcinoma and 6(15.7%) for basal cell carcinoma with insignificant relation between Histopathological diagnosis and age groups (P.value 0.722 ) (table 4.7).

EMA expression showed positive result with frequency 17(44.7%) and negative result with frequency 21(55.3%) (table 4.2).

Positive of expression is common among squamous cell carcinomas with frequency 16(42.1%) with significant correlation between EMA expression and cancers type (P.value 0.005) (table 4.5).

Positive expression common among well differentiated squamous cell carcinoma while moderate and poor differentiated show commonly negative result with insignificant relation between EMA expression and cancer grades (P.value 0.442) (table 4.6).

The histopathological diagnosis of cancer is most common among male 28(73.7%) rather than female 10(26.3%), with insignificant relation between histopathological diagnosis and sex of patients (p.value 0.305) (table4.8).

**Table (4.1) Distribution of histopathology diagnosis among study population**

| Histopathology diagnosis | Frequency | % |
|---|---|---|
| Basal cell carcinoma | 11 | 28.9 |
| Squamous cell carcinoma | 27 | 71.1 |
| Total | 38 | 100 |

**Table (4.2) Immunohistochemical result of EMA expression**

| EMA expression | Frequency | % |
|---|---|---|
| Positive | 17 | 44.7 |
| Negative | 21 | 55.3 |
| Total | 38 | 100 |

**Table (4.3) Distribution of age groups among study population**

| Age group(years) | Frequency | % |
|---|---|---|
| ≤ 50 | 15 | 39.5 |
| >51 | 23 | 60.5 |
| Total | 38 | 100 |

**Table( 4.4) Distribution of gender among study population**

| Gender | Frequency | % |
|---|---|---|
| Male | 28 | 73.7 |
| Female | 10 | 26.3 |
| Total | 38 | 100 |

**Table (4.5) Relation between EMA expression and histopathological diagnosis**

| Expression of EMA | Histopathology diagnosis | | Total | P.value |
|---|---|---|---|---|
| | SCC | BCC | | |
| Positive | 16(42.1%) | 1 (2.6%) | 17 (44.7%) | 0.005 |
| Negative | 11(28.9%) | 10(26.3%) | 21(55.3%) | |
| Total | 27(71.1%) | 11(28.9%) | 38(100%) | |

**Table( 4.6) Relation between EMA expression and SCC grades**

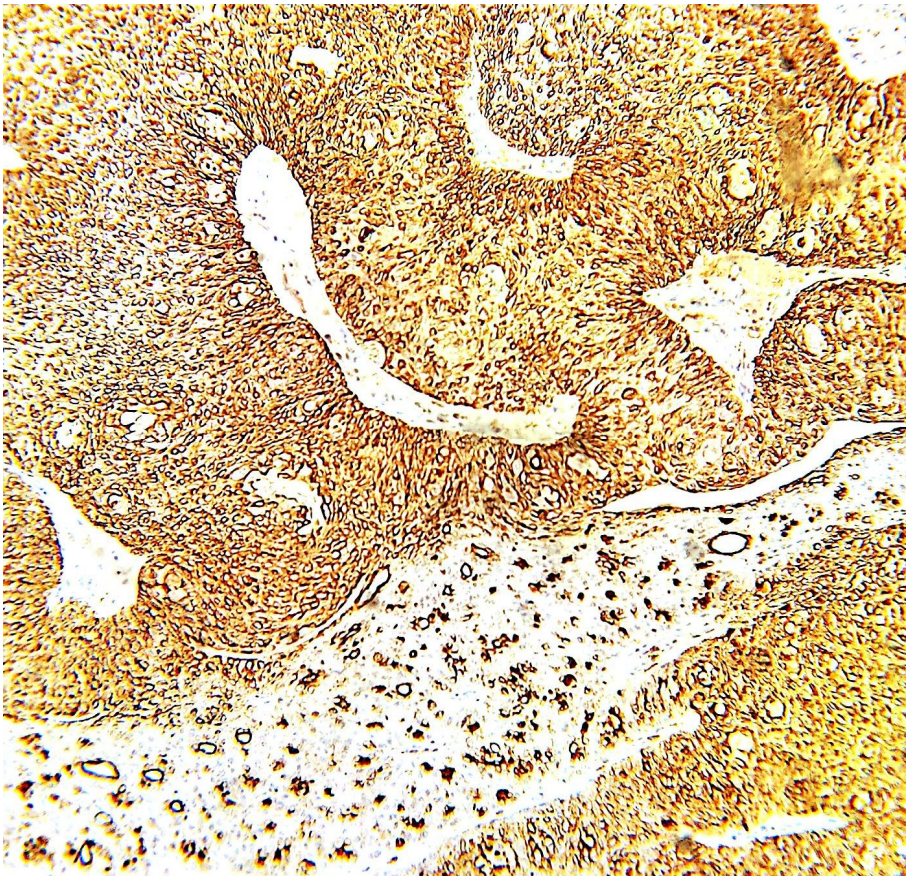
| EMA expression | SCC grades | | | Total | p.value |
|---|---|---|---|---|---|
| | Well differentiation | Moderate differentiation | Poor differentiation | | |
| Positive | 11 ( 40.7%) | 3 ( 11.1%) | 1 (3.7% ) | 15 (55.6%) | 0.442 |
| Negative | 6 (22.2%) | 4 (14.8%) | 2 (7.4%) | 12 (44.4%) | |
| Total | 17 (63%) | 7 (25.9%) | 3 (11.1%) | 27 (100%) | |

**Table (4.7) Relation between histopathological diagnosis and age groups**

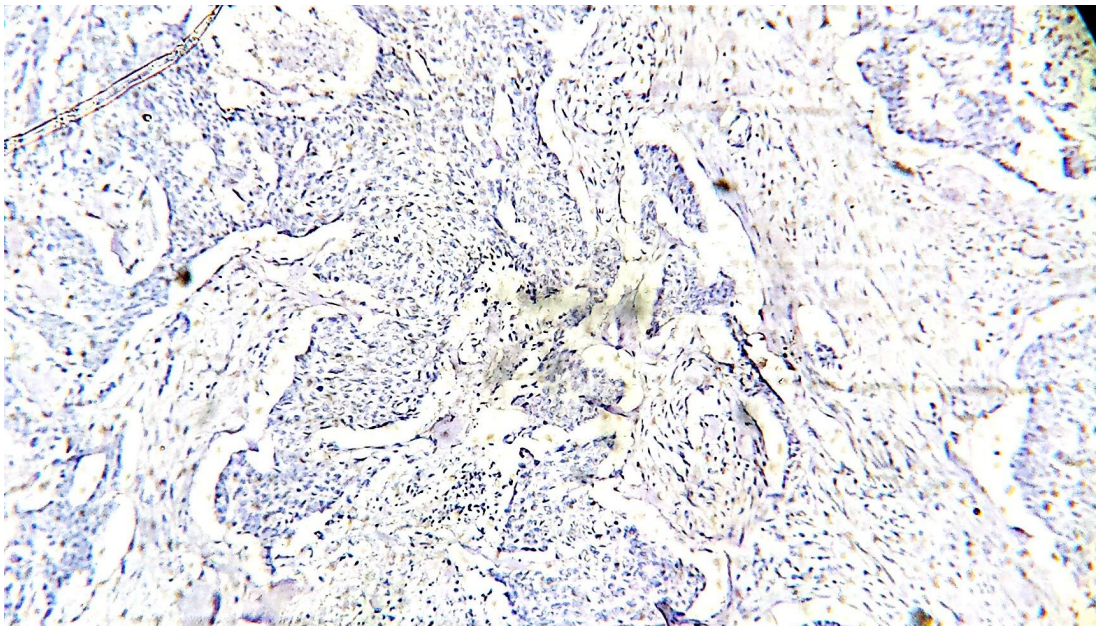
| Histopathology diagnosis | Age Groups | | Total | P.value |
|---|---|---|---|---|
| | 4-50 | 51-93 | | |
| SCC | 10(26.3%) | 17(44.7%) | 27(71.1%) | 0.722 |
| BCC | 5(13.2%) | 6(15.7%) | 11(28.9) | |
| Total | 15(39.5%) | 23(60.5%) | 38(100%) | |

**Table(4.8) Relation between sex and histopathological diagnosis**

| histopathology | male | Female | Total | p.value |
|---|---|---|---|---|
| BCC | 21(53%) | 6(15.8) | 27(100%) | .305 |
| SCC | 7(18.4%) | 4(10.5%) | 11(28.9%) | |
| Total | 28(73.7%) | 10(26.3%) | 38(100%) | |



**Photo(4-1): Section of skin cancer was cut and stained using immunohistochemical method using modified Dako indirect technique for EMA detection, showed positive (EMA) expression in Squamous cell carcinoma (40x).**



**Photo(4-2): Section of skin cancer was cut and stained using immunohistochemical method using modified Dako indirect technique for EMA detection, showed negative EMA expression in basal cell carcinoma (40x)**

# Chapter Five

## 5. Discussion

Skin cancer is the most frequent cancer in white population worldwide. Thirty eight patients previously diagnosed with skin cancer by histopathology was used in this study. The age of patients was aggregate above 50 years (63.5%) .Similar result described by Vries *et al* (2005) , they reported that numbers of patients with SCC will increase overall by 80%, mainly among older males and females. Different study described by Inleslie *et al* (2005), they reported that the incidence of non melanoma skin cancer increase among women and men younger than 40 years. This increase may lead to an exponential increase in the overall occurrence of non melanoma skin cancers over time as this population ages, which emphasizes the need to focus on skin cancer prevention in young adults. Also different study described by Elena *et al* (1995), they reported that a ge does not seem to be a discriminant or permissive factor for a particular histologic pattern of basal cell carcinoma.

The squamous cell carcinoma is commonest skin cancer accounting for 71.1% , while basal cell carcinoma was seen in 28.9% of the patients. Similar finding described by Abdelsamie *et al* ( 2012), they reported that squamous cell carcinoma was the commonest skin malignancy accounting for 42.6% followed by basal cell carcinoma which was seen in 32% of the group. Also the study results were consistent with those of Richard *et al*(1995), they reported that squamous cell carcinoma of the skin (SCC) is a common cancer in white populations. Different study described by Adam and Désirée (2005), they reported that Basal cell carcinomas constitute approximately 80 percent of all non melanoma skin cancers.

In this study there is a significant relation between EMA expression and histopathological diagnosis, the positive expression of EMA was seen in

94% of squamous cell carcinoma, while the expression was 6% in basal cell carcinoma. Because EMA is one of the most widely used immunohistochemical markers to investigate sebaceous differentiation in cutaneous tumors Fuertes et al,( 2013).Similar finding described by heyderman *et al*(1984), they reported that the basal cell carcinomas were negative for EMA and all the squamous cell carcinomas contained EMA.

Also the study results were consistent with those of Beer *et al* (2000),they reported that distinction of basal and squamous cell carcinomas of the skin can be readily achieved with routine immunohistochemistry using EMA. Basal cell carcinoma is negative for EMA while squamous cell carcinoma EMA is positive.

1n this study, the immunohistochemical result show insignificant relation between EMA expression and cancer stages, Positive expression common among well differentiated squamous cell carcinoma while moderate and poor differentiated commonly gave negative result .Similar findings were described by Fernandez *et al* (1987),they reported that well differentiated squamous cell carcinoma stain for EMA more intensely and in greater numbers of cells than less-differentiated squamous cell carcinoma.

# Chapter Six

## 6. Conclusion and Recommendations

### 6.1 Conclusion

On basis of this study ,we conclude that:

EMA expression associated with squamous cell carcinoma rather than basal cell carcinoma, with no relation between EMA expression and SCC grades.

### 6.2 Recommendations

On basis of this study, we recommend that:

EMA should be applied for differential diagnosis between different types of non melanoma skin cancers.

Further study should be done with large sample size.

Molecular technique should be performed to detect the mutation of P53 gene that may cause skin cancer.

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## Appendix I

**Instruments and materials:**

**Instruments:**

Rotary microtome-

Oven-

Coplin jars-

Staining racks-

Electrothermal

Coated slides

Glass slides

Cover glass

Water bath

Dako pen

**Materials:**

Xylene

Ethyl alcohol (absolute, 90%, 70%, 50% )

Mayer's haematoxylin

Distell water

Citrate buffer

Phosphate buffer

Peroxidase blocker

Anti EMA antibodies (primary antibodies)

Dextran polymer conjugate secondary antibodies and HRP

3.3di amino benzidine tetra hydrochloride in substrate buffer

DPX mounting media

**Citrate buffer (PH 6.8)component:**

**Solution A**(0.2 M sodium di-hydrogen orthophosphate,2.83g di- sodium hydrogen orthophosphate,100ml DW)

**Solution B**(2.1g citric acid, 100ml DW) (72.7ml from solution A+22.8ml from solution B)

**Phosphate (PH 7.4) component:**

**Solution A**(0.2 M sodium di- hydrogen orthophosphate, 3.12g di sodium hydrogen orthophosphate,100ml DW)

**Solution B** (0.2 M sodium di- hydrogen orthophosphate,2.83g di sodium hydrogen orthophosphate,100ml DW) (9.5 ml from solution A + 40.5 ml from solution B).

**Mayer's haematoxlin component:**

| | |
|---|---|
| Haemtoxylin powder | 1gm |
| Potassium alum or ammonium alum | 50gm |
| Sodium iodate | 0.2gm |
| Citric acid | 1 gm |
| Chloral hydrate | 50 gm |
| Distilled water | 1000ml |

**Ammoniated water component:**

| | |
|---|---|
| Concentrated ammonia | 0.05ml |
| Tap water | 99.95ml |

# Appendix II

Dako

CE

**FLEX**
**Monoclonal Mouse**
**Anti-Human**
**Epithelial Membrane Antigen**
Clone E29
**Ready-to-Use**
(Dako Autostainer/Autostainer Plus)

**Code IS629**

## ENGLISH

**Intended use**

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human Epithelial Membrane Antigen, Clone E29, Ready-to-Use, (Dako Autostainer/Autostainer Plus) is intended for use in immunohistochemistry together with Dako Autostainer/Autostainer Plus instruments. This antibody is useful for the identification of epithelial cells in a wide variety of tissues and is a useful tool for the identification of neoplastic epithelia (1). EMA is present on the membrane of secretory epithelia. The absence of EMA staining with monoclonal mouse anti-human epithelial membrane antigen, clone E29 is considered to be strongly suggestive of non-epithelial neoplasia (2). 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 heterogenous population of human milk fat globule (HMFG), proteins. HMFG is a complex secretory product of mammary epithelium and EMA can be recovered from the aqueous phase of skimmed milk following extraction in chloroform and methanol. Besides in milk, these proteins are present in a variety of epithelia of both normal and neoplastic types. A number of monoclonal and polyclonal antisera have been raised against these molecules and anti-EMA has been extensively studied in a large number of neoplastic conditions, most often in conjunction with other antibodies (2).

EMA is valuable as a marker in the detection of breast carcinoma metastases in histological sections of liver, lymph node, and bone marrow, and is useful for differentiating anaplastic carcinoma from malignant lymphomas, and for the recognition of spindle cell epithelial malignancies (1, 3).

Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: 1) Principle of Procedure, 2) Materials Required, Not Supplied, 3) Storage, 4) Specimen Preparation, 5) Staining Procedure, 6) Quality Control, 7) Troubleshooting, 8) Interpretation of Staining, 9) General Limitations.

**Reagent provided**

Ready-to-Use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L $NaN_3$.

Clone: E29 Isotype: Ig2a, kappa

**Immunogen**

Human milk fat globule membrane preparation (1, 4).

**Specificity**

In Western blotting of the immunogen the antibody labels bands of 265-400 kDa (1).

**Precautions**

1. For professional users.
2. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage**

Store at 2-8 °C. Do not use after expiration date sta mped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

**Specimen preparation including materials required but not supplied**

The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment with heat-induced epitope retrieval (HIER) is required using Dako PT Link (Code PT100/PT101). For details, please refer to the PT Link User Guide. Optimal results are obtained by pretreating tissues using EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Code K8010/K8004).

Paraffin-embedded sections: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections is recommended using the 3-in-1 specimen preparation procedure for Dako PT Link. Follow the pre-treatment procedure outlined in the package insert for EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Code K8010/K8004). Note: After staining the sections must be dehydrated, cleared and mounted using permanent mounting medium.

Deparaffinized sections: Pre-treatment of deparaffinized formalin-fixed, paraffin-embedded tissue sections is recommended using Dako PT Link and following the same procedure as described for paraffin-embedded sections. After staining the slides should be mounted using aqueous or permanent mounting medium.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8020) is recommended.

**Staining procedure including materials required but not supplied**

The recommended visualization system is EnVision™ FLEX High pH (Dako Autostainer/Autostainer Plus) (Code K8010). The staining steps and incubation times are pre-programmed into the software of Dako Autostainer/Autostainer Plus instruments, using the following protocols:

Template protocol: FLEXRTU2 (200 µL dispense volume) or FLEXRTU3 (300 µL dispense volume)
Autoprogram: EMA (without counterstaining) or EMAH (with counterstaining)

The Auxiliary step should be set to "rinse buffer" in staining runs with ≤10 slides. For staining runs with >10 slides the Auxiliary step should be set to "none". This ascertains comparable wash times.

All incubation steps should be performed at room temperature. For details, please refer to the Operator's Manual for the dedicated instrument. If the protocols are not available on the used Dako Autostainer platform, please contact Dako Technical Services.

Optimal conditions may vary depending on specimen and preparation methods, and should be determined by each individual laboratory. If the evaluating pathologist should desire a different staining intensity, a Dako Application Specialist/Technical Service Specialist can be contacted for information on re-programming of the protocol. Verify that the performance of the adjusted protocol is still valid by evaluating that the staining pattern is identical to the staining pattern described in "Performance characteristics".
Counterstaining in hematoxylin is recommended using EnVision™ FLEX Hematoxylin (Dako Autostainer/Autostainer Plus) (K8018). Non-aqueous, permanent mounting medium is recommended.

Positive and negative controls should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include tonsil and the cells/structures should display reaction patterns as described for this tissue in "Performance characteristics" in all positive specimens. The recommended negative control reagent is FLEX Negative Control, Mouse (Dako Autostainer/Autostainer Plus) (Code IS750).

**Staining interpretation**

The cellular staining pattern is cytoplasmic and membranous.



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positif doit comprendre l'amygdale et les cellules/structures doivent présenter des schémas de réaction tels que décrits pour ces tissus dans les « Caractéristiques de performance » pour tous les échantillons positifs. Le contrôle négatif recommandé est le FLEX Negative Control, Mouse, (Dako Autostainer/Autostainer Plus) (Réf. IS750).

**Interprétation de la coloration**

Le schéma de coloration cellulaire est cytoplasmique et membranaire.

**Caractéristiques de performance**

Tissus sains : L'anticorps marque les cellules épithéliales d'un grand nombre de tissus et de cellules mésothéliales. Figurent notamment : les canaux sudoripares et les glandes sébacées de la peau, l'épithélium de l'appareil digestif, les acini et canaux mammaires, les cellules exocrines du pancréas, l'épithélium de la vessie, les tubules distaux du rein, le col de l'utérus, l'endomètre, l'épithélium respiratoire, la thyroïde et les canaux biliaires. Aucun marquage n'a été observé au niveau de l'épiderme, des cellules endocrines du pancréas, des glomérules et des tubules proximaux du rein, du système nerveux central, du système nerveux périphérique, du tissu conjonctif, des hépatocytes, et du tissu lymphoïde, à l'exception de quelques cellules plasmatiques (1).

Tissus tumoraux : Les adénocarcinomes du rein, du sein, du côlon, de l'estomac, du pancréas, du poumon, de l'endomètre et de l'ovaire étaient tous positifs lors de l'analyse immunocytochimique utilisant l'anti-EMA, E29. Les schémas de coloration étaient principalement cytoplasmiques mais une réactivité apicale et une coloration luminale intracellulaire et membranaire périphérique a également été notée (6). Sont également positifs à l'anticorps : les carcinomes anaplasiques à petites cellules, à cellules squameuses et à cellules transitionnelles, ainsi que les mésothéliomes (6). L'anticorps présentait également une réactivité dans les cas de tumeurs somatiques (7), de carcinomes des cellules rénales (8, 9), de méningiomes (10), de chordome classique (11) et de carcinome neuroendocrine (cellules de Merkel) (12). Les tumeurs et les néoplasmes non réactifs à l'anti-EMA, E29 sont notamment : le carcinome des cellules basales (3), les séminomes (7), les carcinomes embryonnaires (7), les schwannomes (10), les léiomyosarcomes de la vessie (13), les léiomyosarcomes épithélioïdes de la peau et des tissus sous-cutanés (14), les hémangioblastomes capillaires (8), les tumeurs des cellules de la granulosa ovarienne (15) et les néoplasmes angiosarcomateux de la thyroïde (15).