بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Association of Human Papilloma Virus with Non-melanoma Skin Cancer in Khartoum state

العلاقه بين فيروس الورم الحليمي البشري مع سرطان الجلد غير القتامي في ولاية الخرطوم

A dissertation submitted in partial fulfillment for requirements of M.Sc Degree in Medical Laboratory Science (Histopathology and cytology)

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الآية

بسم الله الرحمن الرحيم

قال تعالي:

﴿ إِنَّ الَّذِينَ كَفَرُوا بِآيَاتِنَا سَوْفَ نُصْلِيهِمْ نَارًا كُلَّمَا نَضِجَتْ جُلُودُهُمْ بَدَّلْنَاهُمْ جُلُودًا غَيْرَهَا لِيَذُوقُوا الْعَذَابَ إِنَّ اللَّهَ كَانَ عَزِيزًا حَكِيمًا ﴿56﴾ ﴾

صدق الله العظيم

سورة النساء , الاية(56).

Dedication

To the soul of my brother, the only hero I ever had–the greatest man

I ever knew

To the my wonderful father and beloved mother

To my siblings Ahmed , Afrah and

friends

Acknowledgment

My great indebtedness should be extended to all those helped me to accomplish this work, as without their great efforted exerted this dessertation would have never seen light.

My especial gratitude dose to my supervisor Dr. Mohamed Siddig for his so generously given time and sincere efforts to fulfill this work.

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Abstract

This descriptive retrospective cross sectional study was carried out in Omdurman teaching hospital, during the period from March to October 2015, to detect the association between human papilloma virus infection and non-melanoma skin cancers.

The study included 40 formalin fixed paraffin embedded blocks previously diagnosed as skin cancers , 20 samples were diagnosed as squamous cell carcinoma and 20 samples were basal cell carcinoma.

From each block only one section with 3µm in diameter were cut, to detect HPV using immunohistochemical method by modified indirect method of Thermo manufacture.

The patient data were collected from patient file`s. Data were analyzed usingSPSS computer program, frequencies, mean, chi square test were calculated.

Among study group 26(65%) were males and 14(35%) were females, their age ranged between 20-90 year with mean age 63year.

The immunohistochemical result of HPV among study group were positive in 14 (35%) cases,11 of them were SCC and the remaining were BCC while negative in 26 (65%) cases,17 of them were BCC the other 9 were SCC and with a significant association between HPV infection and type of skin cancer (P.value 0.008) .

The result of sex in study group showed 12 were male and 8 were female in SCC, while 14 male and 6 female in BCC , with no relation between sex and type of skin cancer.

The study revealed that the patient in age group >60 were 15 patient and remaining 5 were <60 in SCC, while in basal cell carcinoma 10 were <60 and other 10 were >60 years.

The study concludes there is a significant association between HPV infection and type of skin cancers and no relation between age and sex with . type of skin cancer

ملخص الأطروحة

أجريت هذه الدراسة الوصفية بمستشفى أمدرمان التعليمي ، فى الفترة من مارس – اكتوبر2015 ، لتقييم العلاقة بين فيروس الورم الحليمى البشري مع سرطان الجلد غير الميلانومي. شملت الدراسة 40 عينه محفوظة بالفورمالين ومصبوبة فى قوالب البرافين ، والتى تم تشخيصها مسبقا بسرطان الجلد ،20 منها كانوا مصابين بسرطان الخلايا الحرشفيه للجلد و 20 كانوا مصابين بسرطان الخلايا القاعدية . تم قطع 3 مايكرون من جميع العينات للكشف عن فيروس الورم الحليمي فى سرطان الجلد باستخدام الموسمات السرطانية بالطريقة غير المباشرة المعدلة من شركة ثيرمو. جمعت بيانات المرضى من السجلات الخاصة بهم ومن ثم حللت إحصائيا باستخدام البرنامج المحوسب, وتم حساب المتوسط ، التكرار , واختبار مربع كاي .

أظهرت الدراسة ان 26 منهم ذكورا و 14 اناثا وكانت أعمارهم بين 20–90 سنة بمتوسط عمر 63 سنه .

أظهرت نتيجة الموسمات السرطانية للكشف عن الفيروس (فيروس الورم الحليمي) نتيجة إيجابية فى 14 (35%) كان 11 منهم من مرضى سرطان الخلايا الحرشفية و3 من مرضى سرطان الخلاياالقاعديه, بينما كانت سلبية فى 26 (65 %)'17

منهم من مرضى سرطان الخلايا القاعديه و9 من مرضى الخلاياالحرشفية للجلد مع وجود علاقة ذات دلاله إحصائية بين الإصابة بالفيروس وسرطان الجلد.

أظهرت الدراسة أن 12 من المرضى كانت ذكورا و8 إناثا فى سرطان الخلايا الحرشفية بينما كان 14 من المرضى ذكورا و6 إناثا فى سرطان الخلايا القاعدية للجلد.

أظهرت الدراسة أن الفئة العمرية أكبر من 60 سنة كانوا 15 وأقل من 60 سنة كانت 5 فى الخلايا الحرشفية، بينما كانت فى سرطان الخلايا القاعدية 10 منهم أكبر من 60 و10 من المرضى أكبر من 60.

خلصت الدراسة إلى وجود علاقة إحصائية بين الإصابة بفيروس الورم الحليمي البشري ونوع سرطان الجلد غير الميلانومي، بينما لا توجود علاقة بين العمر والجنس مع نوع لسرطان الجلد.

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List of Abbreviation

SCC	**Squamouscell carcinoma**
BCC	**Basal cell carcinoma**
HPV	**Human papilloma virus**
PDT	**Photodynamic therapy**
mTHPC	**metatetrahydroxyphenylchlorin**
AK	**Actinic kertosis**

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Chapter One

1.1: Introduction:

Skin cancer is abnormal growth of the cells, that capable to invade and destroy the underlying tissue structure (de Martel *et al*., 2008). Often sub divided into melanoma and non-melanoma Skin cancer. Non-melanoma originates from the external skin surface , include squamous cell carcinoma and basal cell carcinoma (Cakir *et al*., 2012).

Melanoma and non-melanoma skin cancer are now the most common types of cancer in white population (Gloster *et al*., 1996). Both tumors entities show an increas incidence rate worldwide but are stable or have decreasing mortality rate (Long et al. 2011).Also noted in about 35-45% of all malignancies in Caucasians (Ridky *et al*., 2007).

Squamous cell carcinoma (SCC) more frequent skin malignancy accounting for 42.6% followed by Basal cell carcinoma (BCC) accounting 32% of skin cancer(Abdelsamie *et al*., 2012). In Sudanese patients skin cancer represent 18.5% comparing to all malignancies in the earliest reports (Lynch *et al*., 1963).

Ultraviolet radiation (UVR) is the major environmental factor that influences the induction of skin tumors (Brenner *et al*., 2008). Clinical behavior and epidemiology of SCC suggest a viral etiology such as Human papilloma viruses

(HPV) , also organ transplantation increase risk for SCC (Hartevelt *et al*., 1990). Human papilloma virus are more than 100 different types have been

identified, each is known by a number , that subdivided into low-risk such as (6, 11, 42) ; usually causes benign proliferative lesions (warts), while the other type high risk oncogenic types such (16, 18, 31) have etiological role in epithelial cancer (Rana *et al*., 2007).

Diagnosis of skin cancer started with physical examination, skin biopsy via microscopic examination, the diagnosis confirmed with advance techniques; such as PCR and immunohistochemistry (Schell *et al.*, 2013) .

Advance treatment of skin tumors include systemic therapies such as epidermal growth factor receptor inhibitor , and topical immunomodulating drugs such as imiquimod (Ibrahim *et al*., 2014).

1.2: Objectives

1.2.1: General objective:

To detect the association of HPV with nonmelanoma skin cancers using immunohistochemical techniques .

1.2.2: Specific objectives:

1- To detect HPV in skin cancers among patients in Khartoum State.

2- To correlate between the type of skin cancer with sex and age group

Chapter two

2. Review of Literature

2.1 Anatomy, histology and physiology of the skin:-

2.1.1 Anatomy and Histology

Skin is the largest organ of the body, varies greatly in different regions. Their thickness varies from 0.5mm thick on the eyelids to 4.0mm thick on the heels of feet, it has major barrier between the inside and outside of body (Miller *et al.,* 2006).

Skin is composed of several subunits, the main three layers are:

Epidermis or surface epithelium, which is a self-regenerating stratified squamous epithelium that produces a protective protein layer of keratin.

Dermis an underlying layer of dense collagenous connective tissue that contains hair follicles, sweat glands, blood and lymphatic vessels, sensory receptors and nerves, and connective tissue cells.

Hypodermis another connective tissue layer that is rich in white adipose cells and contains large blood vessels that supply the smaller vessels of the dermis (Goldsmith *et al*., 1983).

2.1.2. Functions of skin

Protection against UV light, mechanical, thermal, chemical stresses, dehydration and invasion by micro-organisms. Sensation skin has receptors that sense touch, pressure, pain and temperature. Also has thermoregulatry function; various features of the skin are involved in regulating temperature of the body, For example sweat glands, hair, and adipose tissue . And also

has metabolic functions subcutaneous adipose tissue is involved in production of vitamin D, and triglycerides (Cotton *et al*.,1992).

2.2 Skin pathology:-

2.2.1.Inflammation of skin:-

Non infected inflammatory condition include

Eczema and contact dermatitis dermatitis can be induced by reactions to number of allergens and drugs . Eczema is commonly in atopic individuals who suffer from asthma or hay fever.

Lichen planus it's chronic process in which purple polygonal plaques develop on the skin mainly seen in oral mucosa and genitalia typically in middle-aged women (RE Cotton *et al*.,1992).

. **2.2. 2.Infections:-**

2.2.2.1. Bacterial infection

Include Erysipelas; is a superficial streptococcal infection of the skin and Necrotizing fasciitis; is a rare infection of the subcutaneous tissues and fascia that eventually leads to necrosis (RE Cotton *et al*., 1992).

2.2.2.2 Fungal and yeast infections

Cutaneous candidiasis is a yeast infection caused primarly by Candida albicans, also Tinea versicolor is a common superficial infection of the skin caused by the ubiquitous yeast.

Malassezia furfur is Dermatophytosis implies infection with fungi, organisms with high affinity for keratinized tissue, such as the skin, nails, and hair (Cotton *et al*., 1992).

2.2.2.3 Viral infections

a painful, self-limited, often recurrent dermatitis, characterized by small grouped vesicles on an erythematous base caused by Herpes simplex virus infection.

Warts are common and benign epithelial growths caused by human papillomavirus low risk type. Also Molluscum contagiosum; is an infectious viral disease caused by the poxvirus (Cotton *et al*., 1992).

2.2.3 Precancerous conditions of the skin

Actinic kertosis (AK) is common sun-induced precancerous neoplasm confined to the epidermis, initial manifestation of continuum of clinical and histological abnormalities which may progress to invasive squamous cell carcinoma (Ibrahim *et al*., 2009).

Bowen's disease also known as carcinoma in situ , represents early SCC confined to the epidermis. Mostly common on trunk , arms or leg , It takes longe time to develop in to an invasive cancer, but the risk of developing into cancer remains until treated (Saini *et a.*, 2015).

Congenital melanocytic nevi are birthmarks or large moles that are present at birth or may develop during early childhood that may have an increased risk for developing melanoma (Brash *et al*., 1998).

2.2.4. Skin cancer:

2.2.4.1. Squamous cell carcinoma

It`s a common form of skin cancer that develop in the thin, flat squamous cells, that make up the outer layer of the skin. Most frequantly seen in sun-exposed area such as head neck and hand, in elderly white people ,

which can spread to other part of the body, most cases of SCC shown relation with P53 mutation. In early diagnosis and treatment SCC highly curable (Cakir *et al*., 2012).

2.2.4.2 Basal cell carcinoma (BCC)

Is a heterogeneous malignant neoplasm with different biological and clinical behaviors, often slow growing, rarly metastasizes and conveying an excellent prognosis. However, BCC is one of the most frequent skin cancer worldwide and can cause great morbidity, mostly occur in highly visible areas of the body, often replace or invade and destroy local tissue(Wong *et al.*,2003).

2.2.4.3 Melanoma

It`s a type of skin cancer which forms from melanocytes pigment-containing cells in the skin, particularly common among Caucasians, especially northern Europeans and those who live in sunny climates. In females, the most common site in the legs, while on back in males (James *et al.* 2006).

Less common skin cancers include Merkel cell carcinoma, Kaposi's sarcoma, keratoacanthoma , spindle cell tumors, sebaceous carcinomas, leiomyosarcoma, and angiosarcoma .

2.3. Symptoms of skin cancer :-

The main symptom of non-melanoma skin cancer is an appearance of a lump or discolored patch on the skin that doesn't heal. Squamous cell carcinoma appears as a firm pink lump and may have a flat, scaly and crusted surface and the lump is often tender to touch, bleeds easily and may develop into ulcer, while in the

Basal cell type usually appears as a small red or pink lump, although it can be pearly-white or 'waxy' looking. It can also look like a red, scaly patch ,

the lump slowly grows and may become crusty, bleed or develop into a painless ulcer (American Society of clinical oncology, 2015).

2.4. Risk factors:-

2.4.1. Ultraviolet (UV) light exposure

Exposure to ultraviolet (UV) rays is the major risk factor for most skin cancers. Sunlight is the main source of UV rays, tanning beds are another source of UV rays (Natafji *et al.* 2015).

2.4.2. Human papilloma virus (HPV) infection

It`s DNA virus of which there are more than 200 subtype, establish productive infections in keratiocyte of the skin or mucous membrane (Stanley *et al*. 2012). The infection attracte a lot of attention because of their possible role in the production of cancers in skin and cervix in human (Forslund *et al*., 2007).

HPV subdivided into low-risk (6, 11, 42–44, 54, 61, 70, 72, and 81) and high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 61, 66, 68, 73, and 82), it`s sexual transmitted infections .

Low-risk HPV (6 and 11) are usually causes benign proliferative lesions such as condyloma acuminate, rarely associated with invasive squamous cancer. In human skin this viruses are responsible for squamous cell Papillomas (warts or verrcuae), predominantly found in sites extensively exposed to the sun light (Forslund *et al*., 2007).

The precise clinical appearance of the warte depends on the particular HPV type concerned and the body site involved. The keratotic, exophytic growth of verrucae vulgaris may occur anywhere on the skin or oral mucosa while the flate verruca plana occurs more commonly on the face and backs of hands (Collan *et al*., 2004).

High-risk type usually associated with lesions that are at a high risk for malignant progression, particularly in the genital tract (Walter *et al*., 1996).

The small HPV genome consists of about 8000 base pairs of circular double-stranded DNA. It codes for only eight genes , which are classified as "early" (E) or "late" (L) depending on the timing of their expression . The E6 and E7 gene products play the most significant part in oncogenesity (Arron *et al*., 2011, Aldabagh *et al*., 2012).

They have a number of cellular targets, with a multitude of effects that lead to malignant transformation. The two most important appear to be the binding of E6 to p53, which results in the blocking of apoptosis, and the second binding of E7 to the retinoblastoma tumor suppression protein pRB, which abolishes cell-cycle arrest and leads to unscheduled cellular proliferation (Marluce *et al*., 2008).

2.4.3. Radiation

People who have had radiation treatment have a higher risk for developing to skin cancer in area that received the treatment (Saladi *et al*., 2005).

2.4.4. Other risk factors

Long-term of sever skin inflammation or injury , p53over expression may play role , basal cell nevus syndrome (also known as nevoid basal cell carcinoma syndrome or Gorlin syndrome) ,weaken immune system such HIV patients and smoking tobacco (Maurer *et al*., 1997).

2.5. Diagnosis of skin cancer:-

2.5.1. Physical examination

look at the skin to determine whether the skin changes are likely to be skin cancer; examine growths, moles, and dry patches. Further testing may be needed to confirm that diagnosis.

2.5.2. Skin biopsy

Remove of biopsy for lab testing .include; Shave (tangential) biopsy, Punch biopsy, Incisional and excisional biopsies . the confirmation of diagnosis through different tests such as immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) (Zuber ,*et al.*, 2002).

2.5.3. Fine Neddle Aspiration Cytology

Role of FNA cytology in diagnosis of skin and superfacial soft tissue lesion when subcutaneous soft tissue present with wide array of a lesion ranging from nonspecific dermatoses and inflammatory lesion to frank neoplasm (Bhowmik , *et al*., 2015).

2.5.4. Computed tomography (CT) scan

Use special x-ray equipment to make 3-dimensional and cross-sectional images of organs and tissues . A computer turns the images into detailed pictures, to shown if the cancer has spread to the lymph nodes or other internal organs (Schell *et al*., 2013).

2.5.5. Magnetic resonance imaging (MRI)

Use powerful magnetic forces and radio-frequency waves to make cross-sectional images of organs and tissues. A computer turns the images into 3-dimensional pictures, to see if cancer spread to other sites of the body (Schell *et al*., 2013).

2.5.6. Immunohistochemistry (IHC)

It`s powerful method for localizing specific antigen in formalin-fixed , paraffin embedded (FFBE)tissue based on antigen-antibody interaction .The

technique is widely used in dermatologic diagnosis and research , and their application continue to be extended because it's easy to use ,reliability and versatility of the result .

An antigen-antibody construct is visualized through light microscopy by mean of colour signal . The result of IHC appear as brown colour , and also visualized the morphology of the tissue around specific antigen by counter staining with hematoxylin (blue). IHC marker are reported semi quantitatively, and has diagnostic and prognostic implications, particulary for skin tumors, lymphoma, and in detection of infectious microorganism (Vivien *et al*., 2015).

2.6. Staging of skin cancer:-

Use TNM staging system which is common for all cancers. That design put information together to give a number of stage from 0 to (Balch *et al*., 2001).

2.6.1.Stage 0 is also called Bowen's disease or carcinoma in situ. Carcinoma means there are cancer cells but they have not yet spread or grown into surrounding areas of the skin. If not treated, may develop into a squamous cell carcinoma, So may describe this stage as pre cancerous or pre malignant (Balch *et al*., 2001).

2.6.2. Stage 1 means the cancer is 2cm across or less and has 1 or no high risk features. High risk features mean ;the cancer more than 2mm thick ,has grown into the lower dermism ,has grown into the space around a nerve (perineural invasion), Started on the ear or lip and their looks very abnormal under the microscope (the cells are poorly differentiated or undifferentiated) (Balch *et al*., 2001).

2.6.3. Stage 2 means the cancer is more than 2cm across, or has 2 or more high risk features (Balch *et al*., 2001).

2. 6.4.Stage 3 means the cancer has grown into the bones in the face, such as the bone around the eye, or has spread to a nearby lymph node on the same side of the body (Balch *et al*., 2001).

2.6.5. Stage 4 means the cancer has grown into the spine, ribs , or has spread to an internal organ, such as the lung (Balch *et al*., 2001)

2.7. Mangment of non-melanoma skin cancer:-

2.7.1. Surgical excision

Surgical excision is typically the treatment of choice, which provide histopathological information , high cure rate, acceptable cosmetic and functional outcome (Scalvenzi *et al* .2008, Kimyai - Asadi *et al*., 2007).

2.7.2. Mohs micrographic surgery

Is method of surgical excision with high intrinsic value with is cost-effectiveness when comparison to traditional surgical excision (Telfer *et al*., 2008).

2.7.3. Chemotherapy

Chemotherapy involves using medicines to kill cancerous cells. It's only recommended when the tumor is contained within the top layer of the skin, involves applying a cream containing cancer-killing medicines to the affected area (Butler *et al*., 2009).

2.7.4. Photodynamic therapy (PDT)

Photodynamic therapy (PDT) using systematic photosensitizer metatetrahydroxyphenylchlorin (mTHPC) which has ability to treat multiple NMSCs up to adepth of 10mm in asingle session (Lucena *et al* .2008).

2.7.5. Radiotherapy

Is reserved for cases where surgery is not preferred choice , or for high risk cases where adjuvant therapy is recommended (Telfer *et al*., 2008).

Chapter Three

3. Materials and Methods

3.1 Study design:

This is a descriptive retrospective cross sectional study, aimed to assess the presence of HPV in Skin diagnosed as squamous cell carcinoma and basal cell carcinoma among Sudanese patients.

3.2. Study area:

Study was conducted in Khartoum state ..

3.3 Study population:

Forty paraffin blocks were selected 20 cases were previously diagnosed as squamous cell carcinoma and 20 cases were basal cell carcinoma using routine Hematoxylin and Eosin stain. Patient's data were collected from patient's files.

3.4 Sample collection and preparation:

40 paraffin blocks were collected, from each one sections of 3µm were cut, sections floated into preheated floating water bath at 40˚C, slides were coated with adhesive salinized glass slide for immunohistochemistry.

3.5 Staining procedures:

Immunohistochemical staining:

Procedure was carried out using monoclonal mouse anti human HPV cloneMIB-1,

the immunohistochemical procedure were done as follow:

Fourty Sections (3μm) from formalin-fixed, paraffin-embedded tumors was cut and mounted on to salinized slides (thermo). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and placed in running water. Samples steamed for antigen retrieval for HPV using PT, slides were placed in coplin jars containing enough sodium citrate buffer (pH 9.0) to cover the sections, then were boiled at high temp for 10 minutes then sections were cooled at RT. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 min, then Slides were incubated with 100μl of primary antibodies for 20 min at room temperature in a moisture chamber, and then rinsed in Phosphate buffer saline.Then , the biotinylated link was added for 10 minutes and washed in three changes of PBS, followed by addition of 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for 5 min. Slides were counterstained with haematoxylin.

3.6.Result interpretation:

Positive HPV staining was identified in form of dark brown colour around the nucleus. The obtained results and variables was arranged in standard master sheet, and were entered in a computer program SPSS and analyzed.

3.7 Statistical analysis:

The obtaining results, as well as all clinical information data were entered a computer program SPSS (version 11.5), frequencies ,mean and chi square tests were calculated.

3.8 Ethical consideration

Samples were collected after permission from hospital administration.

Chapter Four

4. Results

In this descriptive retrospective study, the expression of HPV marker were detected in 40 Skin paraffin wax embedded blocks, of which 20 (50%) were squamous cell carcinoma, 20(50%) were basal cell carcinoma .

As shown in Table (4.1), the age of the involved patients range between 20 to 90 years old; the majority of the patients (more than 60%) were between the age group 60to 90years old, with mean age 63

The majority of study group were male 26 (65%) while female were 14(35%) as shown in table (4.2).

Table (4.3) show the expression of the HPV marker in14(35%) were positive to HPV marker while 25(65%) were negative.

The association between skin cancer (squamous and basal cell carcinoma) and expression of HPV marker, was summarized in Tables (4.4), 11(27.5%) cases positive in SCC were 9 (22.5%)of cases negative. While BCC, 3(7.5%) cases were positive and 17(65%) were negative.

The result statistically significant (P value 0.008) for association of HPV immunohistochemical result and skin cancer type .

In table (4.5 and 4.6) shown 12 cases were male and 8cases were female out of 20 cases of SCC, while15 of them >60 years old and 5<60 years old. In BCC 14 cases male and 6 cases female that 10>60years old, while 10<60 years old.

Table 4.1.: Frequency of the age among study population

Age	Frequency	percent
<60	15	37.5
>60	25	62.5
Total	40	100

Table 4.2.: Frequency of the sex among study population

Sex	Frequency	percent
Male	26	65
Female	14	35
Total	40	100

Table 4.3.Frequency of Immunohistochemical result of HPV

Immunohistochemical result	Frequency	percent
Positive	14	35
Negative	26	65
Total	40	100

Table 4.4.Relation of HPV immunhistochemical result with type skin cancer

Diagnosis of skin cancer	HPV immunohistochemical result		Total
	Positive	Negative	
SCC	11	9	20
BCC	3	17	20
Total	14	26	40

P value (0.008)

Table 4.5.Relation of skin cancer with sex

Diagnosis	Sex		Total
	Male	Female	
SCC	12	8	20
BCC	14	6	20
Total	26	14	40

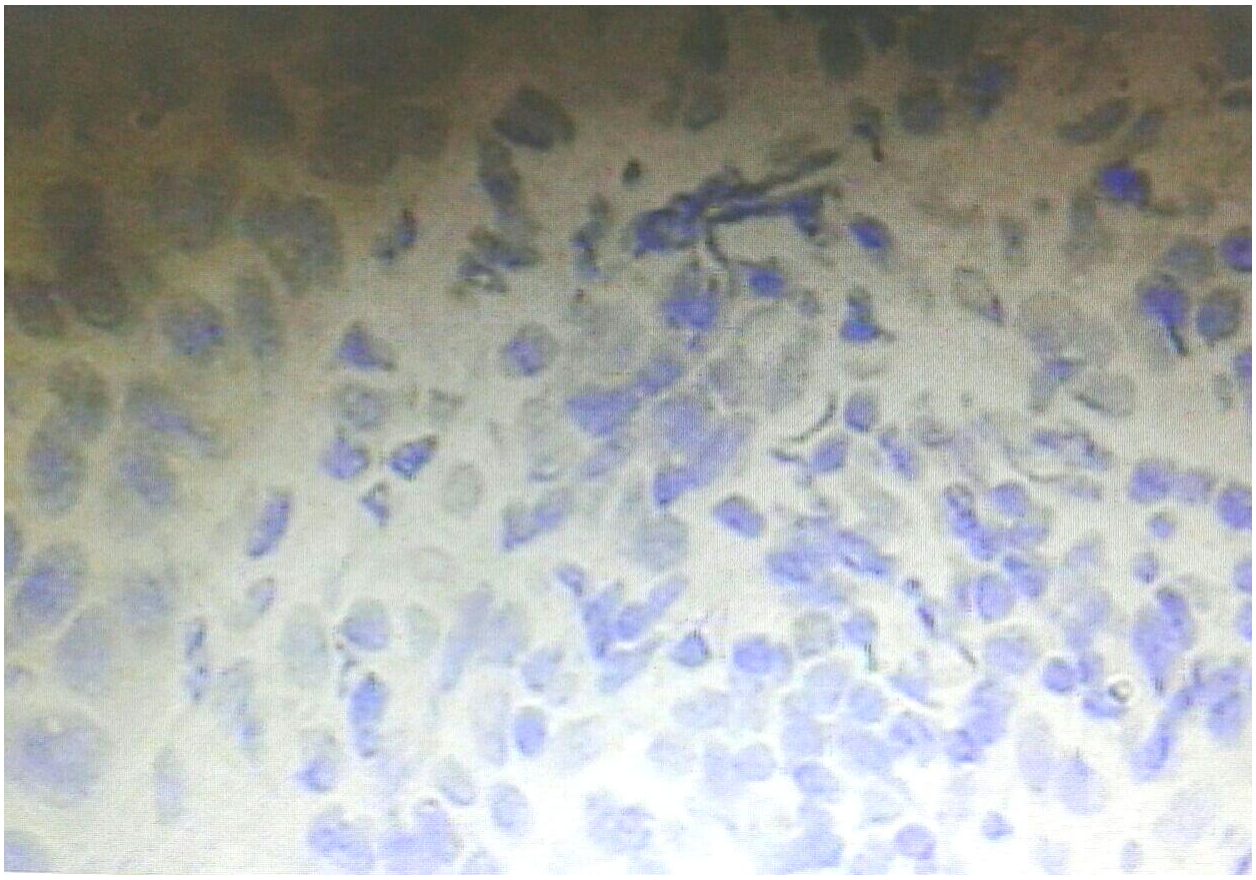
P value (0.741)

Table 4.6.Relation of skin cancer with age group

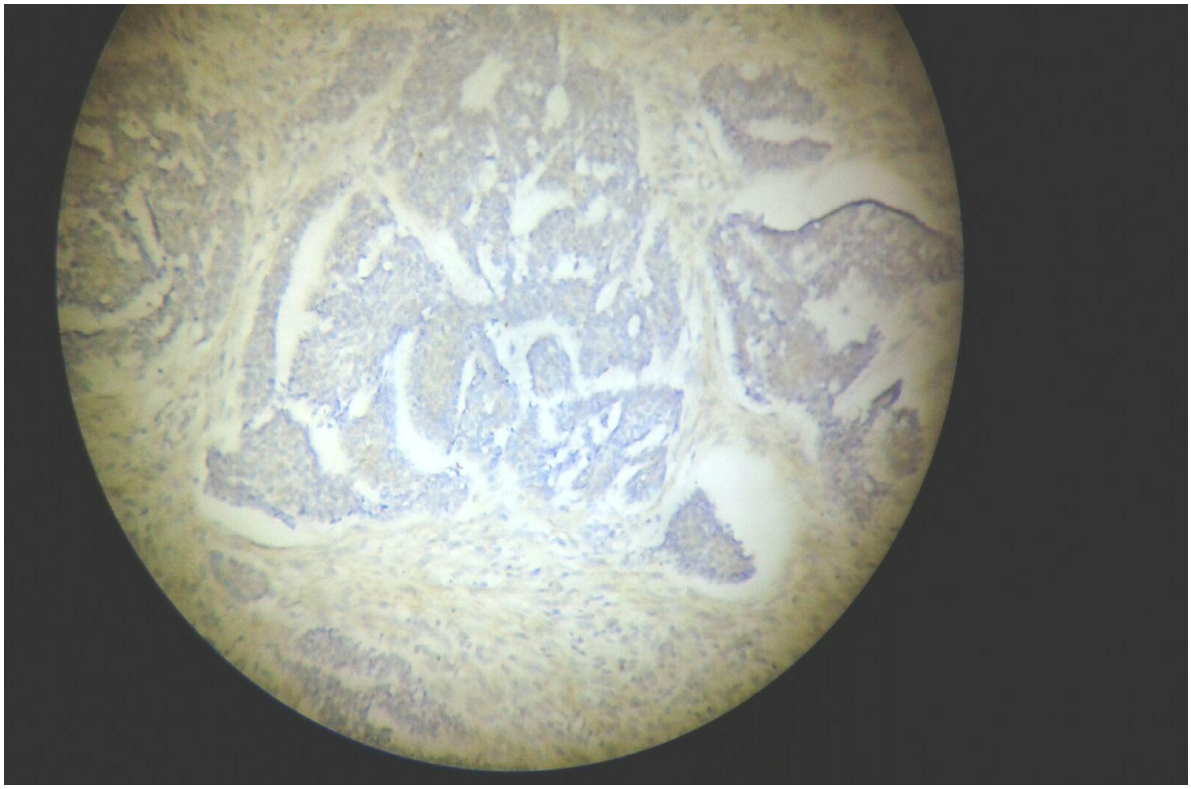
Diagnosis	Age group		Total
	>60	<60	
SCC	15	5	20
BCC	10	10	20
Total	25	15	40

P value (0.740)

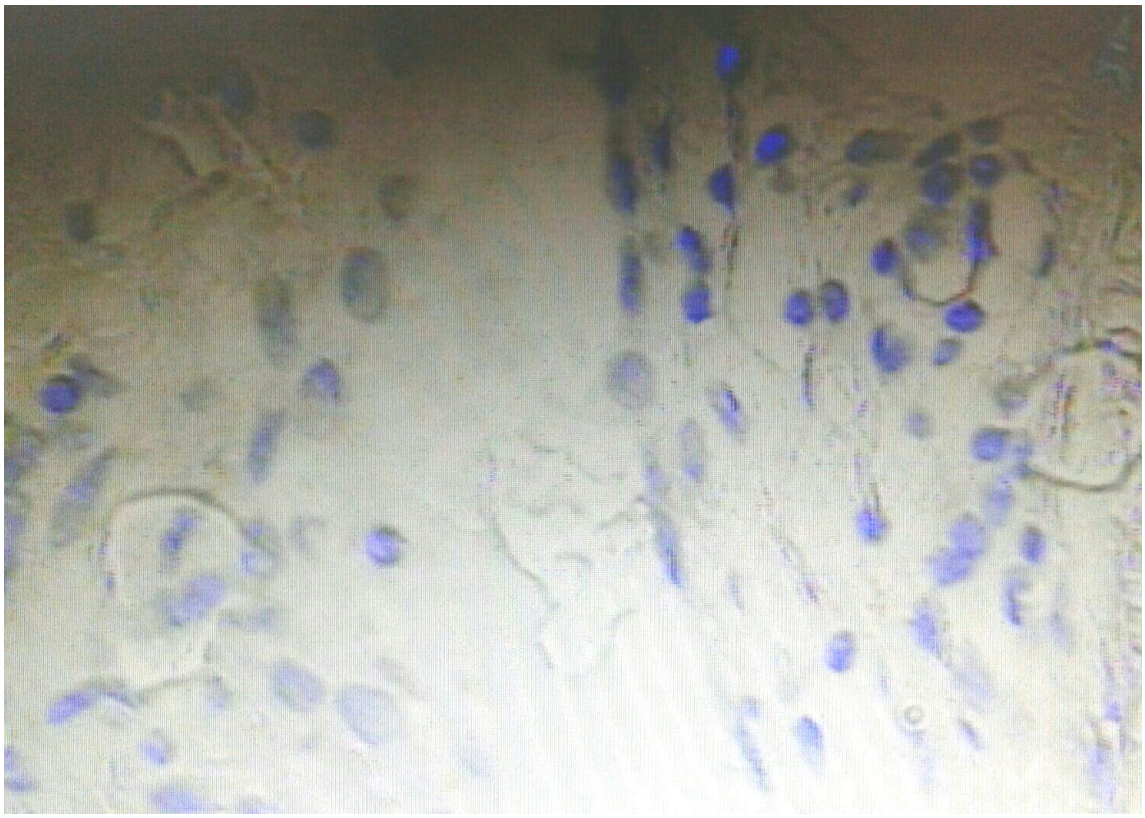
Microphotograph 4.1. Showed positive HPV immunohistochemical result of squamous cell carcinoma(brown colour) by 40x.



Microphotograph 4.2 Showed negative immunohistochemical result of HPV in squamous cell carcinoma of skin by 40x.



Microphotograph 4.3.Showed positive immunohistochemical result for HPV of basal cell carcinoma by 40x.



Microphotograph 4.4 showed negative immunohistochemistry for HPV in Basal cell carcinoma of skin by 40x.

Chapter five

5- Discussion

Skin cancer is a common malignant neoplasm comprising a large heterogeneous group of cancers with variable histological , biological and clinical characteristics in the world (Linares *et al*., 2015).

Skin cancer has been reported to be one of the most frequent malignancy in white populations . Also was noted to be one of common types of malignancy in Sudanese (Lynch *et al*., 1963) .

The present study revealed that; the majority of the diagnosed patients were above 60 years old (elder age) about (62.5%) which decreased in younger age, this findings supported by Abbas., *et al* (2015), they found that; the incidence rate of skin cancer has slowed down and decreased in younger age.

The majority of cases of squamous and basal cell carcinoma were male 26 (65%) out of 40 cases, this result supported by Abbas., *et al* (2015), they found that; Male had higher incidence rate in non melanoma skin cancer than female , also supported by Lither. ,*et al* (2008) ,the highest incidence rate of nonmelanoma skin cancer has been reported in Queensland , Astralia with(56) male and(43) were female out of 100, but didn`t match with Chuang, *et al* (1995) ,they found that: Japanese female had nonmelanoma skin cancer more than male.

The expression of the demonstrated HPV was shown as follow ; 14 (35%) of the study samples were positive for HPV, this result is nerly to Aubin., *et al* (2003) ,they found ; cutanous HPV have been detected in about 60 to 90 %

of patient ; these result support the role of HPV infection in skin carcinogenesise , and also Harwood , *et al.*, (2000), that found m; HPV was detected in 37/40(84%) of squamous cell carcinoma ,18/25(75%) of basal cell carcinoma , but did not match withstudy of Park. , *et al* (2013) low rate detection of high risk HPV in Korean patient with skin cancer.

20(50%) of the samples were squamous cell carcinoma, 11 (27.5%) out of them were positive for the HPV and 9 (22.5%) were negative, while 3(7.5%) out of 20(50%) were positive with HPV in basal cell carcinoma ,and 17(42.5%) were negative, this findings indicate that the presence of HPV in squamous cell carcinoma is higher, this result is match with study of Schmidt., *et al* (2015) , cutanous SCC provide evidence of general susceptibility to oncogenic HPV but didn`t match with study of Accardi *etal.*, (2014) they found that; HPV pervalance and viral load decrease during skin carcinogenesis and being significantly higher in actinic Keratosis than skin cancer. While lesser presence with basal cell carcinoma, which is match with study of Birch-Johansen., *et al* (2012),they found ; HPV tended to be more prevelant in NMSC especially in squamous cell carcinoma compared to basal cell carcinoma ,indicating apotential link between HPV and SCC. While didn`t match with study of Mina., *etal* (2013); there is no association between HPV and BCC).

Regarding the association of HPV expression with non melanoma skin cancer, our findings showed statistical association between HPV and skin cancer which is supported by Aubin., *et al* (2003); the HPV infection have a role in skin carcinogenesis as co-factor with UV rays, while didn`t match withstudy of Ally., *et al* (2013) they found that ; there is no association with presence of HPV and skin cancer type.

Chapter six

6.Conclusion and Recommendation

6.1Conclusion:

On the basis of this study we conclude that:

Skin cancer is more frequent among Sudanese males than females .

The majority of cases were elder age.

There were differences between the skin cancer types regarding their presence of HPV markers;

There is association between the HPV infection and the type of skin cancer.

There is no association between the type of skin cancer with age and sex.

6.2.Recommendations:

On basis of this study we recommend that further study should be done involving large sample size in non- melanoma skin cancer using advance technique such as HPV DNA testing .

In addition further researches should be aimed to understand the natural history of cutaneous HPV including the mechanism by which transforms to cancer which can be prevented through detection and treatment of precancerous (verrcus) lesions (Dysplastic lesions)which may decrease the percentage of skin cancer.

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Appendices(1)

Insterument and materials

Insterument :

Rotary microtome

Oven

Water bath

Electrothermal

Coplin jar

Staning racks

Coated slides

Glass slides

Cover glass

Dako pen

Pencile

Pipette

Material:-

Xylene

Ethyle alcohole (absolute, 90%,70%,50%)

Distell water

Soduim Citrate buffer

Phosphate buffer

Peroxidase blocker

Anti HPV antibody(primary antibody)

3.3 di amino benzidine tetra hydrochloride in substrate buffer.

Mayer's haematoxylin

DPX mounting media

Phosphate buffer(PH7.4) prepration:

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

Solution B (0.2 sodium di hydrogen orthophosphate ,2.83 sodium di hydrogen orthophosphate ,100ml DW)

(9.5ml from solution A+40.5ml solution B) .

Mayer`s Haematoxlyin component:

Haematoxylin powder	1g
Potassium or ammonium alum	50g
Citric acid	1g
Chloral hydrate	50g
Distill water	100ml

Ammoniated water:

Concentrated ammonia	0.05ml
Tap water	99.95ml