



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



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**Immunohistochemical Detection of Human Papilloma Virus Type 16**  
**among Sudanese Females with Cervical cancer**

A thesis submitted for partial fulfillment of the requirements of the M.sc degree in  
Medical Laboratory Sciences (histopathology and cytology)

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# الاية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا )

صدق الله العظيم

[الإسراء: ٨٥]

## Dedication

To my mother.....

To my father.....

To my sister.....

To my brothers.....

To my family... always.....

To all my friends.....

With regard.....Mazin

## **Acknowledgment**

All the thanks to Allah for the gift of life, health and faith and for the strength and desire that he gave me to continue this way, and look always for the best.

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## List of abbreviations

ACC.....	Adenocarcinoma
Ca Cervix.....	Cancer of Cervix
CIN.....	Cervical Intraepithelial Neoplasia
DAB.....	Diaminobenzidine
DPX.....	Distyrene Plasticizer Xylene
FFPE.....	Formalin Fixed Paraffin Embedded
Gyn.....	Gynecology
H & E.....	Heamatoxylin and Eosin
HPVs.....	Human papilloma viruses
HR-HPV.....	High Risk Human Papilloma Virus
IHC.....	Immunohistochemistry
ISH.....	In Situ Hybridization
MLS.....	Medical Laboratory Sciences
Obs.....	Obstetrics
PBS.....	Phosphate Buffer Saline
<i>pRb</i> .....	Retinoblastoma protein
RIKC.....	Radiation &Isotopes Khartoum Center
RT.....	Room Temperature
SCC.....	Squamous Cell Carcinoma
VIA.....	Visual Inspection with Acetic Acid

## **Abstract**

**Background;** Cervical cancer is considered one of most common gynecological cancers worldwide. In some countries that have an economic power and appropriate education cervical screening programs are introduced that help in early detection and decrease the rate of incidence of cervical cancer in last year's. HPVs are considered the most common etiological cause of cervical cancer. According to symptoms that appear in patient the HPVs divided into LR-HPVs and HR-HPVs. HPV16 is the major type of HR-HPVs infection that stimulate cervical cancer.

**Materials and methods;** This was a descriptive cross sectional study conducted in Khartoum state-Sudan during period from September 2017 to July 2018. Fifty formalin fixed paraffin embedded tissue samples with cervical cancer were included in this study subjected to detect of HPV type 16 antigen using IHC technique.

**Results;** HPV16 antigen was detected in 29 cases (58%), with no statistically significant difference as the p value was 0.098. There was no statistically significant correlation between HPV16 and age of patients as the p value was 0.567. There was no relation between HPV16 and patients residence as the p value was 0.473.

**Conclusion;** There was no statistically significant difference between HPV16 and tumor type.

## المستخلص

**الخلفية؛** يعتبر سرطان عنق الرحم من أكثر السرطانات أنتشاراً في النساء في العالم. في بعض البلدان التي لديها قوة اقتصادية وتعليم مناسب تم إدخال برامج مسح عنق الرحم التي تساعد في الكشف المبكر و تقلل من معدل حدوث سرطان عنق الرحم في السنوات الأخيرة. تعتبر فيروسات الورم الحليمي البشري المسبب الرئيسي لسرطان عنق الرحم. قسمت فيروسات الورم الحليمي ألي فيروسات الورم الحليمي البشري قليل الخطورة وعالي الخطورة على حسب الأعراض التي تظهر على المريض. يعتبر فيروس الورم الحليمي البشري عالي الخطورة النوع ١٦ أكثر نوع من فيروسات الورم الحليمي البشري التي تسبب سرطان عنق الرحم .

**الطرائق والمواد؛** أجريت هذه الدراسة الوصفية المقطعية في ولاية الخرطوم بالسودان خلال الفترة من سبتمبر ٢٠١٧ ألي مايو ٢٠١٨. شملت هذه الدراسة خمسون عينة نسيجية مجهزة بشمع البارافين مصابة بسرطان عنق الرحم بغرض تحديد أنتجين فيروس الورم الحليمي البشري النوع ١٦ باستخدام تقنية الأنسجة المناعية.

**النتائج؛** وجد أنتجين فيروس الورم الحليمي رقم ١٦ في ٢٩ عينة (٥٨%)، مع عدم وجود فرق ذو دلالة إحصائية لأن القيمة الاحتمالية كانت ٠.٠٩٨. لم توجد علاقة ذات دلالة إحصائية ما بين فيروس الورم الحليمي البشري رقم ١٦ و عمر المرضى لأن القيمة الاحتمالية كانت ٠.٥٦٧. لم توجد علاقة بين فيروس الورم الحليمي البشري رقم ١٦ و مكان إقامة المرضى لأن القيمة الاحتمالية كانت ٠.٤٧٣ .

**الخلاصة؛** لم يوجد فرق ذو دلالة إحصائية ما بين فيروس الورم الحليمي البشري رقم ١٦ ونوع الورم.

**CHAPTER ONE**  
**INTRODUCTION,**  
**RATIONALE AND**  
**OBJECTIVES**

## **1.1. Introduction**

Cervical cancer is considered the second most common gynecological cancer worldwide. (Fiedler *et al.*, 2014) Cervical cancer is the growth of abnormal cells on the surface of a woman's cervix. Most cervical cancers begin with microscopic changes in the cells located in the outer layer of the cervix. These changes, known as dysplasia may develop into cancer. The most common types of cervical cancer is squamous cell carcinoma. (Bekri, 2016) Nearly ten thousand women were diagnosed with cervical cancer worldwide each year, especially in developing countries and shows younger trend, of which the mortality ranks first in female malignancies. (Abboud *et al.*, 2017) Epidemiological and molecular studies have shown that human papilloma virus (HPV) is the most important etiological agent for cervical carcinogenesis. High-risk long-term infection of HPV can easily increase the incidence of cervical intraepithelial neoplasia (CIN) and cervical cancer. HPV is a non-enveloped DNA virus, and has very strict tissue characteristics, which mainly attacks tissues like the skin and the squamous epithelium. (Lu *et al.*, 2016) The HPVs are relatively small, DNA viruses without an envelope; the diameter is 55 nm. They have icosahedral symmetry, and 72 capsomers and a DNA which contains 6800-8400 bases pairs. The virus genome codes 8-10 proteins in which there are structured (L1 and L2) and non-structured (E1; E2; E4; E5; E6 and E7) proteins. ( de Villiers *et al.*, 2005)

HPVs are composed of a big group of over hundred related viruses. (Braaten and Laufer, 2008) Some of them can cause warts, and some can in worst case lead to cancer. They can be spread through sexual and non-sexual contact, depending on the virus. About 40 HPV types can transmit through direct sexual contact, from the skin and mucous membranes of the infected people to the skin and mucous membranes of their partners. (NHI, Available from: <https://www.cancer.gov/aboutcancer/causes-prevention/risk/infectious-agents/hpv-fact-sheet>, 2017) Most of HPV infections go away on their own, without any signs or symptoms. This means also that infected people can unknowingly pass HPV to their sexual partners. (CDC, Available from: <http://www.cdc.gov/std/hpv/stdfact-hpv.htm>, 2017) HPV type (16,18) and a number of additional HPV types have been found in about 70% of all biopsies derived from cancer of the cervix throughout the world, the remaining 30% being due to other high-risk HPV types (such as HPV-31, -33, -35, -39, -45,-51, -66). (Gissmann and Gross, 1986)

HPV16 is the most frequently found virus type and accounts for 50 to 60% of cancerous samples, the presence of HPV18 vary between 10 and 20%. (zur, 1996)

In this study we tested immunoexpression of HPV type 16 in Sudanese females with cervical cancer and also to study the association of HPV type16 infection with patient age, residence and grade of cancer.

## **1.2. Rationale**

Cervical cancer in Sudan is second commonest cancer. It's the leading cause of cancer death. There is no perfection systemic screening program in Sudan, and this is due to poor economic status, Sudanese traditional and habits. Usually women present late in advance stages of disease. Worldwide, the role of high risk HPV (HR-HPV) in the tumorigenesis of cervical cancer is well documented, but in Sudan, the exact role of HR-HPV in the development of cervical cancer is still debate. The international studies had shown the relatively higher incidence rate of HPV infection in earlier stages of cancer. This finding reveals that; there is need to identify HPV infection earlier in order to minimize incidence of cervical cancer and death cases result from this cancer. HPV type 16 is the major cause of cervical cancer over the world .This study subjected to determine the existence of this HR-HPV type in cervical tissues with squamous and adenocarcinomas by using specific and sensitive immunohistochemistry method. Further we aimed to identify the role of virus in cancer progression, differentiation and proliferation.

## **1.3. Objectives**

### **1.3.1. General objective**

To detect the immunoexpression of HPV type 16 in cervical cancers among Sudanese females.

### **1.3.2. Specific objectives**

1. To correlate the immunoexpression of HPV type 16 with the tumor subtypes.
2. To correlate the expression of HPV type 16 and the grade of cancer.
3. To correlate the expression of HPV type 16 and the patients' residence.
4. To correlate the expression of HPV type 16 with age of patients.



**CHAPTER TWO**  
**LITERATURE REVIEW**

## 2.1. Cervical cancer

The cervix is located in the lower part of the uterus also called uterine cervix, it connects the body of the uterus by the cervix part called endocervix to the birth canal by the part named exocervix. Cells covering the cervix are referred to as squamous cells and the glandular cells. (American Cancer Society, *Available from:*

<http://www.cancer.org/acs/groups/cid/documents/webcontent/003094-pdf.pdf>, 2010) Cervical cancer is the fourth most common type of cancer in women in the world and in some low-income countries it is the most common cancer in women. (Ferlay *et al.*, 2010; IARC, 2012) A cervical cancer is a malignant cancer of the cervix or within the cervical area. It may form in the interior lining of the cervix, junction of the vagina and the uterus. (Saonere, 2010)

Compared with other cancers, screening for cervical cancer is the most effective, as it has the ability to detect through Pap smear pre-cancerous stage and high-risk type HPV that cause 70% cervical cancers. (WHO, *Available from:* <http://www.who.int/mediacentre/factsheets/fs297/en/>, 2012) Cervical cancer can be classified into different histological subtypes, of which squamous cell carcinoma is the most common one (accounting for about 80% of all cervical cancers). The second most common type is adenocarcinoma, accounting for approximately 15% of cervical cancers. Very rarely, other types, such as neuro-endocrine carcinomas and clear-cell carcinomas, are diagnosed. Cervical squamous cell carcinomas develop through premalignant precursor lesions called cervical intraepithelial

neoplasia (CIN). CIN lesions are classified into three groups: mild (CIN1), moderate (CIN2), or severe (CIN3, including carcinoma in situ) lesions, depending on the extent of replacement of the epithelial lining by atypical cells. In CIN1, which represents productive infections, up to one-third of the cells of the lower epithelial layer is replaced by atypical cells. In CIN3, the most advanced precursor stage representing a transforming HPV infection from two-thirds up to the whole epithelial layer consists of atypical cells. Moreover, from CIN1 to CIN3, the cells become more atypical. Lesions are graded as carcinoma when atypical cells pass the basal layer (invasion). Whether cervical cancer develops through consecutive CIN1, 2 and 3 is under debate, since CIN2/3 lesions may develop rapidly following a high-risk HPV (HR-HPV) infection, leaving only a very limited time frame for a preceding CIN1 lesion. (Arbyn *et al.*, 2009; Kok *et al.*, 2011)

## **2.2. HPV**

Several epidemiological studies established the main cause of cervical cancer to be persistent infection with certain genotypes of HPV. (Muñoz *et al.*, 1992; Bosch *et al.*, 2002) The HPV virus infection which is the infection mainly responsible for cervical cancer is transmitted through sexual intercourse. There are about 100 different types of HPV viruses but only about 40 of these types affect the genital areas. Some of the other types infect the skin on other body areas like the hands, or feet. Genital warts known as condylomata acuminata are usually small, flat cauliflower like bumps that carry HPV virus even though at times it is in small percentage therefore not necessarily at risk of causing cervical cancer. Types 6 and 11 are responsible for the causing of the warts which develop in a period of six weeks to eight months. (Liles and Itano, 2003)

HPV virus hardly has any symptoms therefore causing it to be very hard to identify hence the need to go for cervical checkup and HPV testing. (Godfrey, 2007) HPVs are circular double-stranded DNA viruses with close to 8000 base pairs. At present about 130 types of HPV have been identified which infect skin and mucosal epithelial at specific sites of the body. Mucosotropic HPVs can be further divided into high- and low-risk types depending on their carcinogenic potential. (zur, 2002; Muñoz *et al.*, 2003; de Villiers *et al.*, 2004; Stanley, 2010)

HPVs divided into low-risk HPVs and high-risk HPVs. Low-risk HPVs are the types that can cause warts. (CDC, Available from: [https://www.cdc.gov/cancer/hpv/basic\\_info/](https://www.cdc.gov/cancer/hpv/basic_info/), 2017)

On the other hand, high-risk HPVs can cause several types of cancer such as: cervical cancer, anal cancer, oropharyngeal cancers (cancers of the middle part of throat, including the soft palate, the base of the tongue, and the tonsils), vaginal cancer, vulvar cancer and penile cancer. (NCI, Available from: <https://www.cancer.gov/aboutcancer/causes-prevention/risk/infectious-agents/hpv-fact-sheet>, 2017)

### **2.3. Role of HPV in cervical cancer**

The HPV progression into cervical cancer is a slow process, at times taking over 20 years from the time of initial infection. Most HPV infections occur during the teen age years or early twenties, whereas cervical cancer is more common among women over the age of 35. (Hoover *et al.*, 2000) The molecular mechanism of HPV infection which transforms the epithelial cells into dysplasia or cancer cells begins with the integration of the viral genome

to host genome with decreased expression of L1/2 protein and increased expression of E1/2 & E6/7 protein. E2 has a crucial role in a viral life as a transcription and a replication factor. E2 disruption is seen in 62.5% cases of Squamous cell carcinoma (SCC) and 18.1% cases of benign lesions. The affinity of HPV viral proteins for the products of tumor suppressor genes differ depending on the oncogenic potential of HPV. E6 and E7 of HR-HPV bind pRb and p53 respectively with high affinity. The E6 protein caused the inactivation of P53 protein and increased expression of P14 protein (tumour suppressor genes). E7 protein caused the inactivation of Rb protein and increased expression of P16 protein (tumour suppressor genes). E7 protein of HR-HPV has 10 folds higher efficiency to bind Rb protein than E7 of low-risk HPV. Hence, E6 and E7 together causes early immortalization of epithelial cells, deregulation of cell cycle, chromosomal instability and cervical carcinogenesis. Inactivation of P53 protein causes decreased apoptosis, and that of Rb protein causes increased proliferation of cells. In addition, chronic cervicitis by HPV causes production reactive oxygen species and free radicals that cause oxidative stress followed by the negative impact on genetic and cellular processes. (Jones, 1995; Prayitno, 2006; Jean, 2012; Kahla et al., 2012)

## **2.4. Epidemiology**

According to the world health organization, cervical cancer is said to be the world's second deadly cancer with an estimate of about 493,243 women diagnosed with it and 273,505 dying from it per year. Cervical cancer is also the world's second most frequent among women between 15 and 44 years of age. In Finland about 2.23 million women aged 15 years and over are at risk of getting cervical cancer, current estimation states that out of 164 diagnosed

with cervical cancer per year about 81 of them die as a result of the disease. It is the 15th most common cancer in Finland and the 4th most common cancer among the women in Finland. (WHO, Available from <http://whqlibdoc.who.int/publications/002/9241545720.pdf>, 2002) Cervical cancer was once known as the most deadly cancer in America until the years 1955 to 1992 when its rates decreased by 70% due to increase in pap smear screening and more awareness among society, it is said to decline by 3% each year but the numbers still remain high. According to the American Cancer Society recent estimate states that in the year 2011 about 12,710 new cases of invasive cancer will be diagnosed and of these about 4,290 deaths will be recorded. (American Cancer Society, Available from: <http://www.cancer.org/acs/groups/cid/documents/webcontent/003094-pdf.pdf>, 2010) Cervical cancer is one of the most important diseases affecting women worldwide, with approximately 500,000 new cases and 230,000 deaths per year. The Brazilian National Cancer Institute estimated the incidence of new cases of cervical cancer within Brazil to be 17,450 in 2012. (Ministério, 2012)

In America within an estimate of every six minutes a gynecological cancer is diagnosed with the majority being cervical cancer among women of the ages 40-55 years of age. In 2007 an average of about 12,000 to 16,000 females were diagnosed with cervical cancer. (Godfrey, 2007)

In India, it ranks as second common cancer in females after oral cancer especially in rural and semi-urban population constituting 17% of total female cancers.

However in metropolitan cities breast cancer is taking the lead. Every year 122,844 cases are diagnosed, and 64,477 deaths occur in India due to

cervical cancer. (Kalyani *et al.*, 2010) Worldwide, about 500,000 new cases of cervical cancer were reported in 2006 and most of these cases have been reported from developing countries in Africa, Central America, and South America where there is lack of screening and early detection programs. (Mojgan *et al.*, 2009) In Sudan, cervical cancer is the second most common cancer type among women. (Hamad, 2006)

## **2.5 Cervical cancer in Sudan**

Sudan has a population of 12.02 million women ages 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 833 women are diagnosed with cervical cancer and 534 die from the disease. Cervical cancer ranks as the 2nd most frequent cancer among women in Sudan and the 5th most frequent cancer among women between 15 and 44 years of age. (ICO/IARC Information Centre on HPV and Cancer, Available from: [www.hpvcentre.net/statistics/reports/SDN\\_FS.pdf](http://www.hpvcentre.net/statistics/reports/SDN_FS.pdf), 2017)

Cervical cancer is the second most prevalent cancer in Sudanese Women; breast cancer forms between 29 - 35% of female cancers while Ca Cervix forms 12 - 16%. There are limited screening activities in the country using VIA, and Pap smears in Obs. and Gyn. clinics. (Kamal Eldein *et al.*, 2017)

Cervical cancer is the second cancer among women in Sudan, with more than two-thirds of all women with invasive cervical cancer being diagnosed at an advanced stage. (Ahmed *et al.*, 2011) Other data published by Husain *et al.*, on 2011 said that cancer of the cervix is the second most prevalent cancer of women to date in the Sudan, in a concerted review of the records of the hospital-based cancer registry of the Radiation & Isotope Centre of Khartoum RICK. (Husain *et al.*, 2011)

## **2.6 Previous studies**

There are many studies were done in this area, one of these study done in our country by Elsheikh *et al.*, conducted on 2016, which included 180 samples in which 98 out of them with SCC of cervix. High risk HPVs (HR-HPVs) were detected in 41.8% among cases. (Elsheikh *et al.*, 2018)

Another study conducted by Shepherd *et al.*, on 1992 to detect of HPVS 16 and 18 by IHC and ISH the outcome of the study, the HPVs were positive in 22% of all CIN samples whereas 25% of the tumors were positive for HPV 16 by in situ hybridization. Sections of cervical warts and CIN positive for HPV types by in situ hybridization were also positive by antibody staining which suggests that both techniques are detecting replicating virus.(P. Shepherd *et al.* , 1992)

## **2.7. Immunohistochemistry (IHC)**

IHC is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of antibody binding being identified either by direct labeling of the antibody or by use a secondary labeling method. The recent introduction of prognostic and predictive markers in IHC has made a tremendous impact on patient treatment and management.

Immunohistochemistry is widely employed in establishing diagnosis, predicting prognosis and response to therapy and in the study of disease pathogenesis. (Bancroft and Gamble, 2008)



**CHAPTER THREE**  
**MATERIALS**  
**&**  
**METHODS**

### **3.1. Study design**

This was a descriptive cross sectional study aimed to assess the immunohistochemical expression of HPV16 in cervical cancer tissues from Sudanese Patients.

### **3.2. Study duration and area**

This study was carried out during period from September 2017 to July 2018. This study was done at Khartoum state. Samples subjected in this study were collected from Omdurman Obstetrics & Gynecology Hospital and processed in Radiation & Isotopes Khartoum Center (RICK). The diagnosis and interpretation of the stained slides were done by well expertise histopathologist.

### **3.3. Study populations, and samples size**

Populations recruited in this study were formalin fixed paraffin embedded tissue blocks (FFPE), previously diagnosed with cervical cancer during period from 2016-2018 were used as case group. Fifty FFPE samples with cervical cancer were included in this study.

### **3.4. Sampling technique, tools of Data collection and variables**

Convenient sampling technique was used to collect samples in this study. Master sheet were used to record all patients, samples data and results of immunostained sections.

### **3.5. Quality controls**

All precautions instructed by the IHC materials manufacture were issued, control sections were used to evaluate the working solutions, to avoid false results and to evaluate the testing slides. The procedure of the IHC technique was carried out after quality controls.

### **3.6. Sample processing**

One section from each block measured four micrometers was cut using Leica microtome (Leica Microsystems, Nussioch Gmbll, model: RM 2125RT, ser NO. 8843/04-2005-China) and then stained in H&E to confirm diagnosis of each block.

Then one section was cut from each recruited block using the same microtome and floated in 70% ethanol and water bath (Electrothermal ser NO.18861434-China) at 40c0, . Each floated section was mounted on positive charge immune slide (Thermo Scientific- Italy) to detect immune expression of HPV (type 16).

All slides contained sections were dried in dry oven (WTC binder 7200 TUTTLINGEN, B28, NO.88485-USA) at 60c0 for 30 minutes.

### **3.7. Staining techniques**

#### **3.7.1. Haematoxylin and Eosin (H &E)**

Section of 4 micron thickness was stained using haematoxylin and eosin (Mayer's technique).section was cleared in to changes of xyelene for two minutes in each changes , then each slide was hydrate through ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then stained in

Mayer's haematoxylin for 7 minutes, then blued in running tap water for ten minutes, then each slide was counter stained in eosin for three minutes, then washed in distilled water and dehydrated through ascending ethanol, cleared in xylene and mounted in Disterene a plasticizer and xylene (DPX).

### **3.7.2. IHC**

The IHC procedure was done as followed; following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and place in running water. Then slides were steamed for antigen retrieval for using water bath in plastic coplin jar containing sodium citrate buffer (pH 9.0) at 95<sup>0</sup>C endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 minutes, and then Slides incubate with 100-200 µl of specific primary antibodies (Anti-Papilloma virus type 16-Cam Vir-1) for HPV16 for 20 min at RT, then rinsed in Phosphate buffer saline (PBS). After washing with PBS for 3 min, binding of antibodies was detected by incubating slides for 20 minutes with dextran labelled polymer. Finally, the slided were washed in three changes of PBS, followed by applying diaminobenzidine (DAB) as a chromogen to produce the characteristic brown stain for up to 5 min. Slides were then counterstained with Mayer's haematoxylin for 3-5 min, slides were then dehydrated, cleared and mounted, this method and reagents used were obtained from (BioGenex-USA).

### **3.8. Interpretation of IHC results**

Interpretation of IHC results was achieved according to manufacture instructions (BioGenex-USA) and the scoring of IHC staining achieved according to (DAKO-USA). Negative result achieved when no crisp brown

staining were observed in the nucleus of the target malignant cell (score 0.00) or when a faint/barely or incomplete nuclear staining detected in more than 10% regarded as (score 1). A weak to moderate complete nuclear staining is observed in more than 10% of tumor cells regarded as a weakly positive (score 2). Strong complete nuclear staining is observed in up to 50% of tumor cells regarded as a moderate positive (score 3). Strong complete nuclear staining is observed in more than 50% of tumor cells regarded as a strong positive (score 4).

### **3.9. Data analysis and presentation**

All obtained results were analyzed by Statistical Package for the Social Sciences (SPSS) version 22.0, with Pearson's chi-square test used to assess intergroup significance. Other variables, frequencies, mean values were calculated and presented in form of figures and tables. P value <0.05 was considered statically significant.

### **3.10. Ethical consideration**

This study was approved from the board of Medical Laboratory Sciences (MLS) and College of Higher Studies at Shendi University. A written agreement was assigned prior to sample collection with each hospital and laboratory administration. The aims and the benefits of this study were explained well with assurance on confidentiality.

# **CHAPTER FOUR**

## **RESULTS**

## 4. Results

A total of 50 cases (patients with histopathologically confirmed with cervical cancer) were included in this study. The age of patients was ranged from 30-80 years with average mean of 58.92 years. The ages were divided into two age groups the first category of up to 45 years represented 10 cases (20%) the second group was older than 45 years represented 40 cases (80%) as indicated in figure 4.1.

Figure 4.2 summarizes the frequency of residence among study populations, most of patients were from Khartoum state 46 cases (92%) the other patients were from outside the state of Khartoum 4 cases (8%).

Figure 4.3 demonstrates the frequency of clinical presentation of patients, the most frequent clinical presentation was vaginal bleeding present in 33 cases(66%) followed by cervical mass and vaginal discharge, 11 cases (22%) 5 cases (10%) respectively, then pelvic mass revealed in 1 case (2%).

Figure 4.4 summarizes the frequency of types of cervical cancer; squamous cell carcinoma was most frequent type was found in 41 cases (82%), adenocarcinoma was found in 9 cases (18%).

Figure 4.5 demonstrates the frequency of HPV results among study populations. Of the 50 cases 29 cases (58%) were positive and 21 cases (42%) were negative.

Figure 4.6 demonstrates the frequency of marker expression score. Of 50 cases, the highest frequency was score 2 and score 1 observed in 15 cases (30%) for each followed by score 3, score 0.00 and score 4 observed in 9 cases (18 %), 6 cases (12 %), and 5 cases (10%) respectively.

Figure 4.7 demonstrates the frequency of tumors grade among study populations. Of the 50 cases 37 cases were poorly differentiated (74%), 11 cases were well differentiated (22%) and moderately differentiated observed in 2 cases (4%).

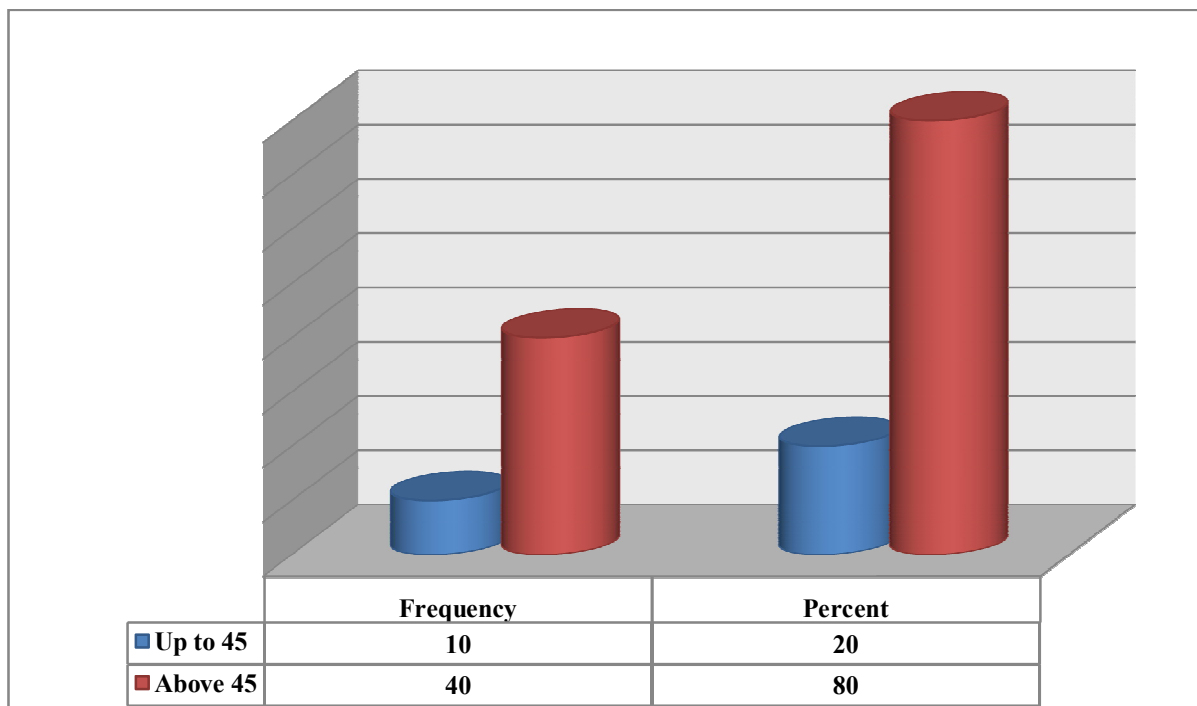
Table 4.1 illustrates the association of tumor types and marker expression. squamous cell carcinoma was most positive type expression 26/41 (63.4%), adenocarcinoma 3/9 (33.%). With no statistically significant different, p value was 0.098.

Table 4.2 illustrates the correlation between age groups and HPV IHC result. Among 50 tested samples , 5/50(10%) cases were positive in the age group up to 45 years and 24/50 (48%) cases were positive in the age group above than 45 years. With no statistically significant different, p value was 0.567.

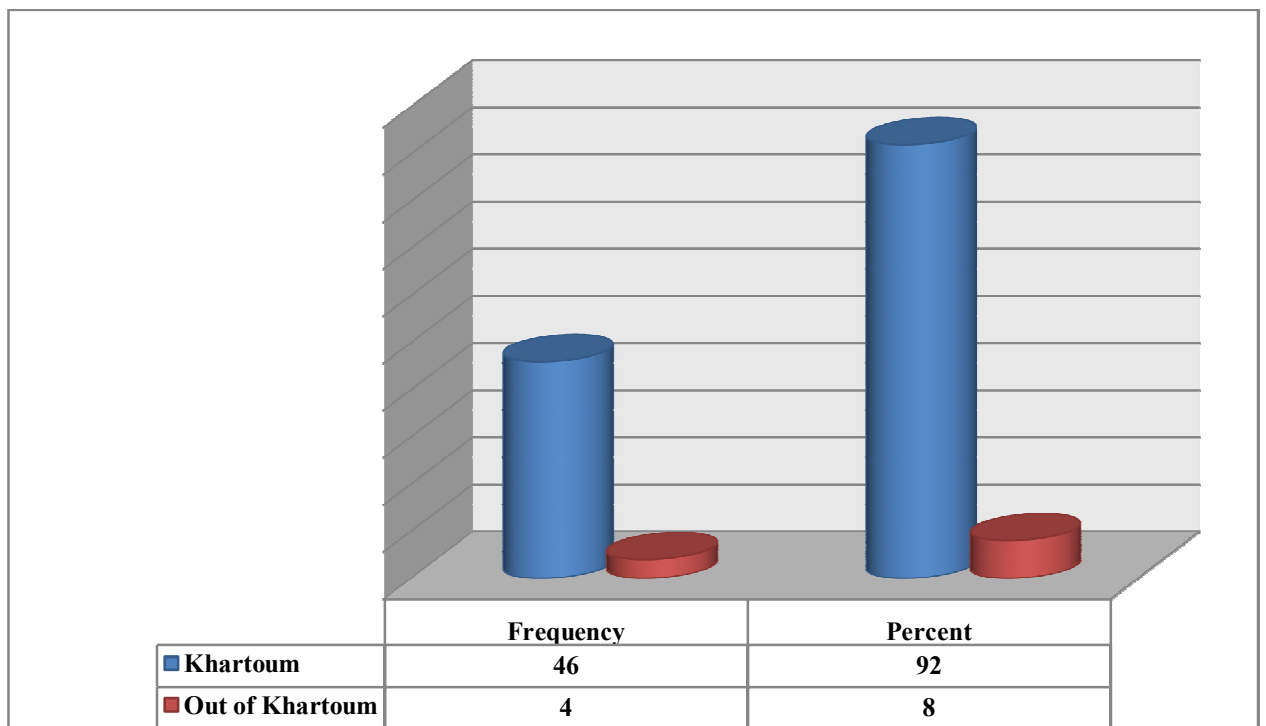
Table 4.3 illustrates the correlation between residence and HPV IHC results. Of 50 study samples 26/50 (52%) were positive in residence in group of Khartoum and 3/50 cases (6%) were positive in residence group out of Khartoum. With no statistically significant different, p value was 0.473.

Table 4.4 illustrates the correlation between tumor grade and HPV IHC results. Of 50 tested samples 20/50 (40%) were positive in poorly differentiated , 8/50 (16%) were positive in well differentiated and 2/50 (4%) were positive in moderately differentiated tumors.

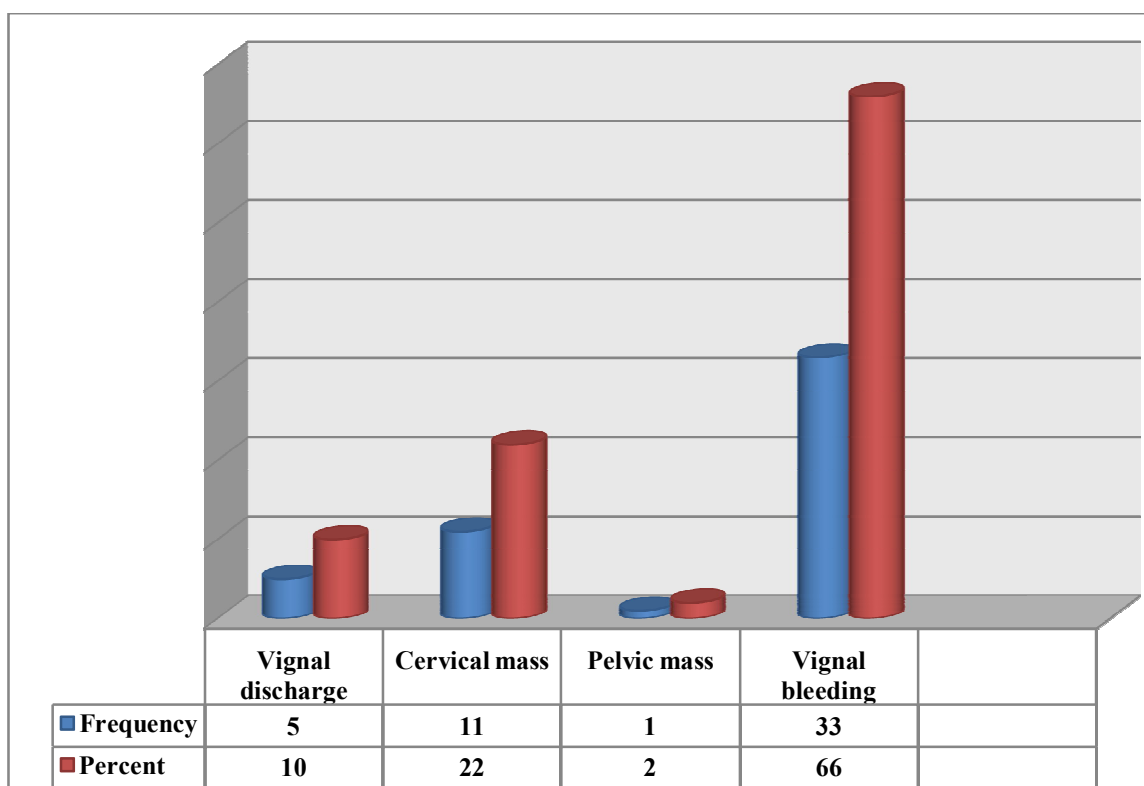




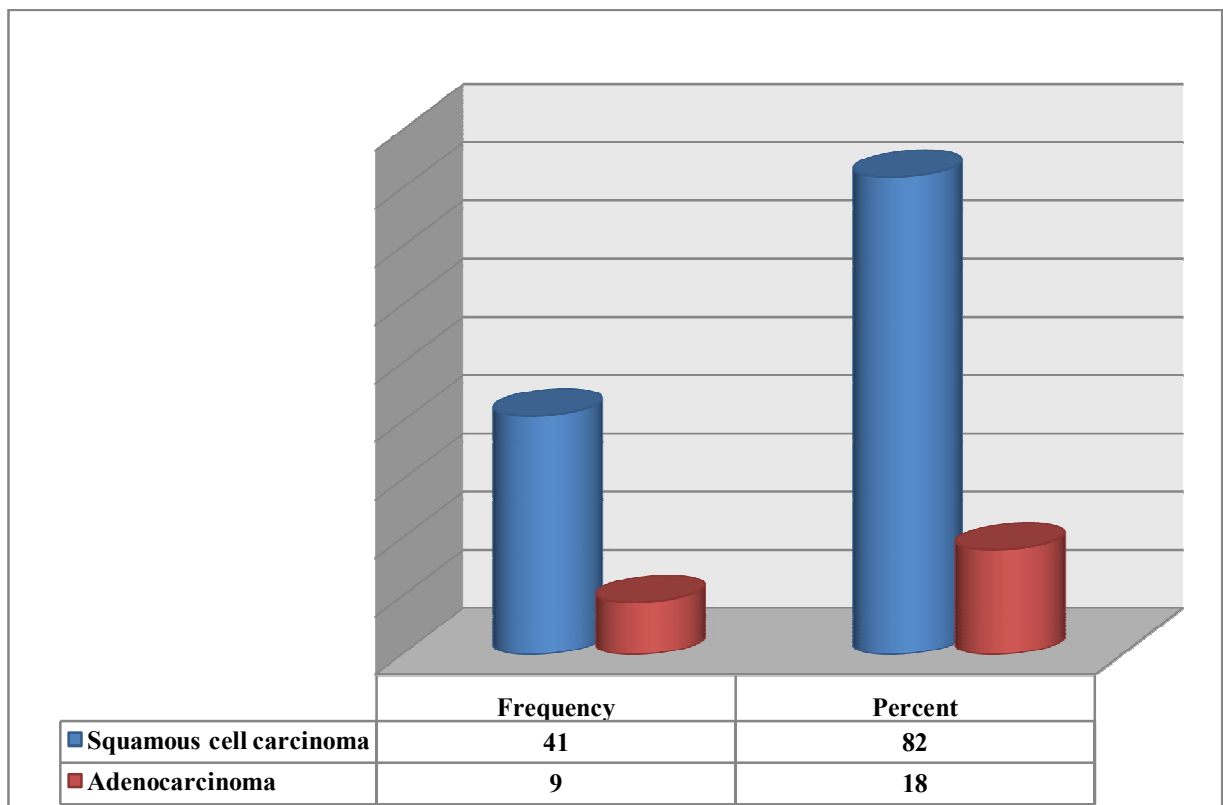
**Fig 4.1: Shows distribution of age groups among study populations.**



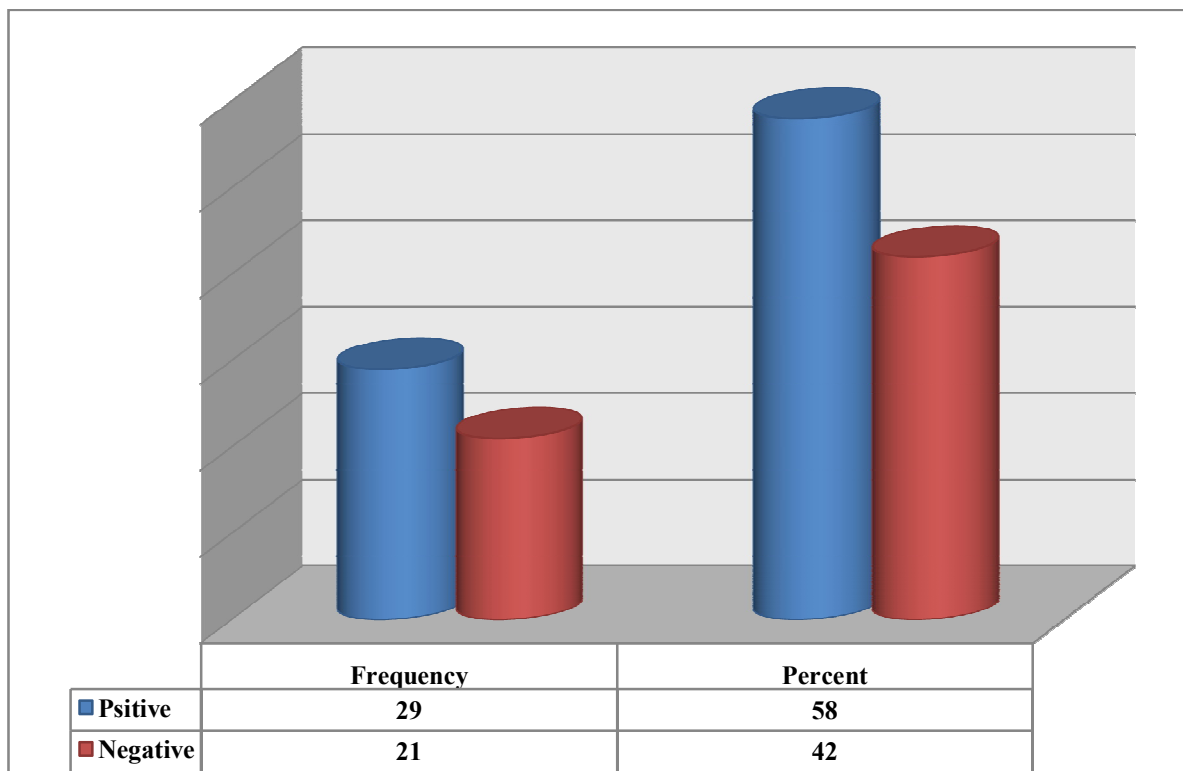
**Fig 4.2: Illustrates distribution of residence among study populations.**



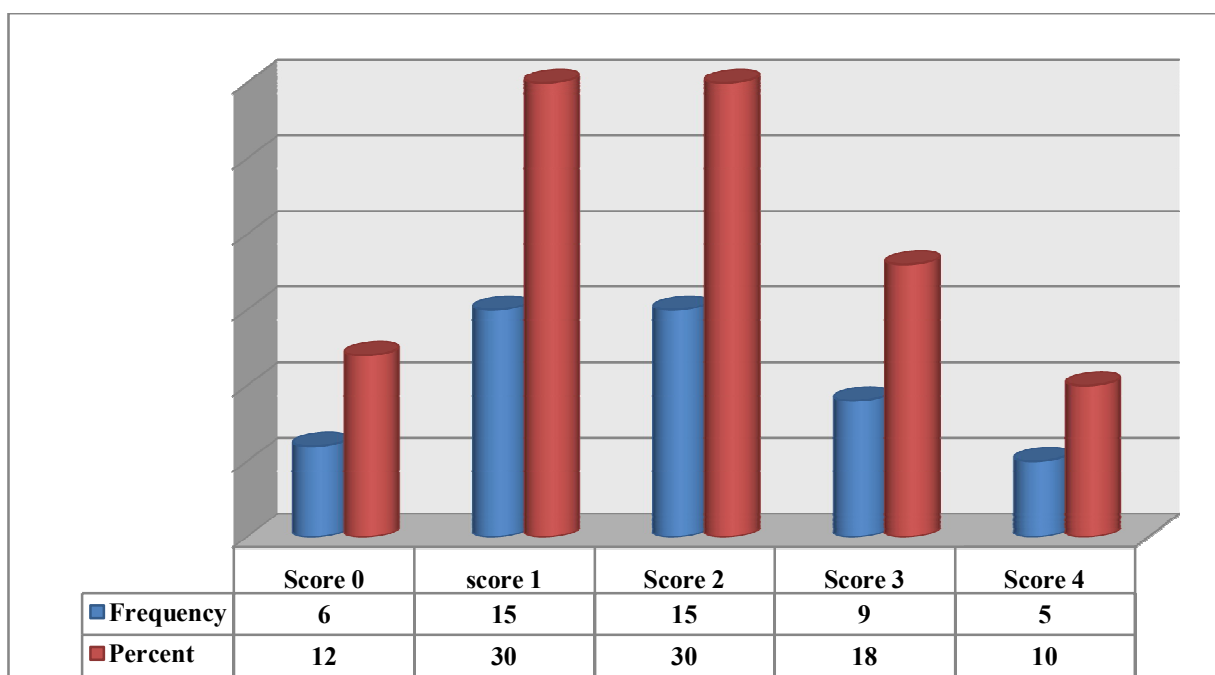
**Fig 4.3: Shows distribution of clinical presentation among study populations.**



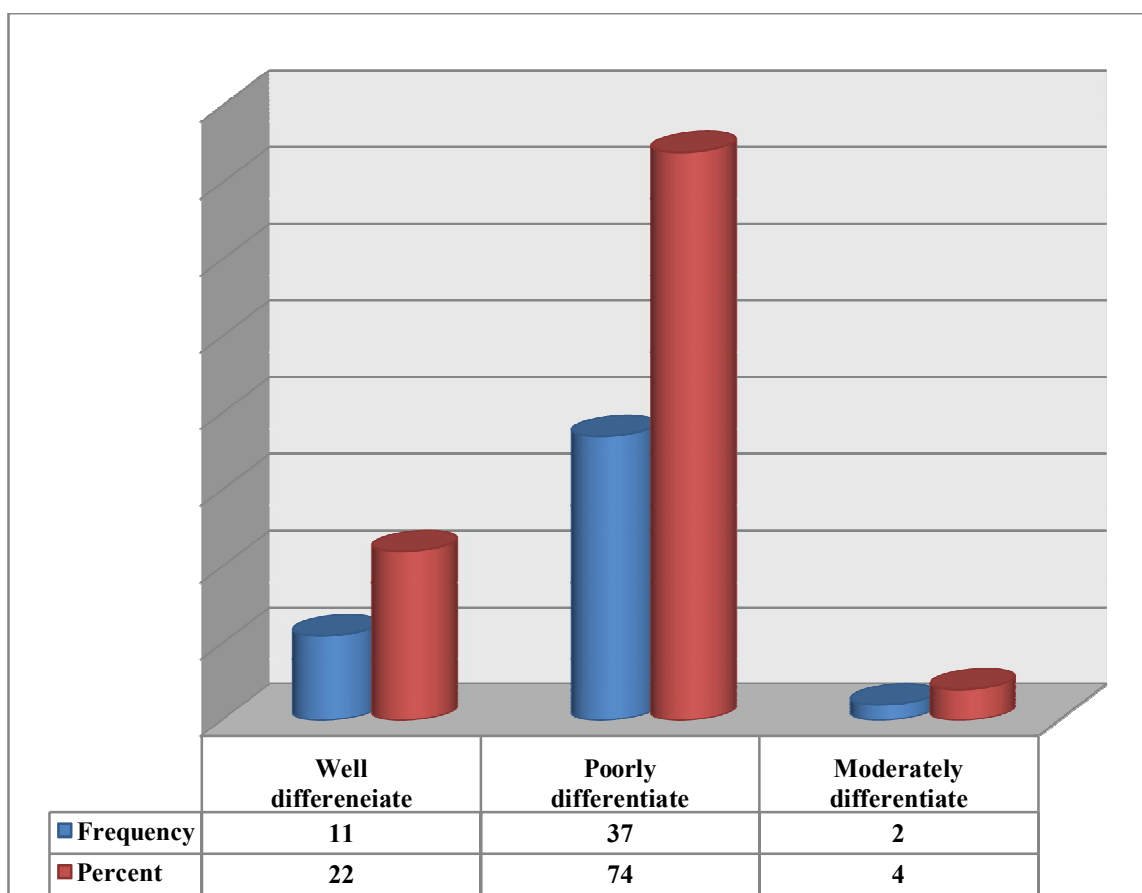
**Fig 4.4: Shows distribution of tumor types among study populations.**



**Fig 4.5: Illustrates distribution of HPV IHC result among study populations.**



**Fig 4.6: Shows distribution of HPV 16 expression score among study populations.**



**Fig 4.7: Shows distribution of tumor grades among study populations.**

**Table 4.1: Correlation of HPV IHC result with tumor types.**

Tumor types	HPV IHC result		Total	p value
	Positive	Negative		
Squamous cell carcinoma	26	15	41	0.098
Adenocarcinoma	3	6	9	
Total	29	21	50	



**Table 4.2: Correlation of HPV IHC result with age groups.**

Age groups	HPV IHC result		Total	p value
	Positive	Negative		
Up to 45	5	5	10	0.567
Above 45	24	17	40	
Total	29	21	50	

**Table 4.3: Correlation of HPV IHC result with patient's residence.**

Residence	HPV IHC result		Total	p value
	Positive	Negative		
Khartoum	26	20	46	0.473
Out of Khartoum	3	1	4	
Total	29	21	50	

**Table 4.4: Correlation of HPV IHC result with tumor grade.**

Grade	HPV IHC result		Total	p value
	Positive	Negative		
Well differentiate	8	3	11	0.401
Moderately differentiate	2	0	2	
Poorly differentiate	20	17	37	
Total	28	22	50	

# **CHAPTER FIVE**

## **DISCUSSION**

## 5.1. Discussion

Cervical cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death in female's worldwide, accounting for 9% (529,800) of the total new cancer cases (Jemal *et al.*, 2011). Epidemiological and molecular studies have shown that human papilloma virus (HPV) is the most important etiological agent for cervical carcinogenesis. Cervical cancer incidence associated with the increasing level of E2F transcription factor which is releases from pRB after binding to HPV E7 oncoprotein (Khleif, *et al.*, 1996).

The purpose of the present study was to detect immunoexpression of HPV type 16 among Sudanese women with cervical cancer.

The age of patients ranged in age between 30 and 80 years with an average age of 58.92, 80% of patients were elderly (post menopausal), similar result was obtained in Iran study by Zarchi and his team, their study concluded that, the average age group incidence of 53.6 years (Zarchi *et al.*, 2010).

92% of patients were from Khartoum capital of the Sudan and this may be due to concentration of populations in Khartoum where the economic, health, education and civilization services are available.

Regarding clinical presentation, our study showed that; the commonest presentation symptom was vaginal bleeding (66%) followed by cervical mass (22%) followed by vaginal discharge (10%) followed by pelvic mass (2%). This finding was similar to that result obtained by Rubina *et al.*, they summarized the commonest presenting symptom was vaginal bleeding present in 78% patients (Rubina *et al.*, 2015).

Concerning tumor types, our result indicated that; SCC was the most common type (82%) followed by ACC (18%), this result was consistent with other studies demonstrated that; squamous cell carcinoma present in about 80-85% and adenocarcinoma present in about 15% of cervical cancers in the UK (Cancer Research UK website, 2009), also similar results revealed in WHO report on 2014 (WHO, 2014).

HPV type 16 prevalence rate in cervical cancer tissues was 58%, this result might be indicated a positive association between HPV 16 and cervical cancer development, this percentage is in agreement with the percentage of (55%) obtained by Mona *et al.*, in 2011 in the study conducted in Ethiopian women.

Regarding HPV16 IHC score, the higher scores were score 1 and 2 followed by 3, 0.00 and 4 respectively, this result indicated that; the concentration of HPV16 infection not correlated to the cancer progression. I did not found published data that correlated concentration rate of HPV 16 with cervical cancer.

Concerning the tumor grade, the current study showed that; commonest grade was poorly differentiated (74%), this finding indicated that; usually women present late with cancer in Sudan, there was no published data found regarding this topic.

The correlation of HPV16 IHC results with tumor subtypes showed insignificant statistical correlation as the p value was 0.098 this result is in agreement with the result obtained by Hossein Ayatollahi *et al.*, which concluded that no significant differences between cervix cancer type and immunoexpression of HPV 16 (p value 0.56), our result was in

disagreement with the result obtained by Chaloob *et al.*, in study conducted on 2016 in Iran which concluded that the study established a highly significant increase in IHC of expression of HPV16 (p value<0.001).

The correlation of HPV 16 with patients' age showed insignificant statistical association as the p value was 0.567 this result is in agreement with the result obtained by Chaloob *et al.*, which concluded that there was non-significant association between IHC expression of HPV 16 and age of patients (p value>0.05).

Investigation of the relationship between residence and HPV IHC results showed that there was no statistical significant correlation of HPV 16 and patient's residence as the p value was 0.473, I did not found published data in the same topic to compare our result with it.

Concerning investigation of the relation between tumor grade and HPV IHC results, our study showed that the poorly differentiated cancer samples was showed high rates of positive expression of HPV16 than well differentiated and moderately differentiated samples, but with no statistically significant different as the p value was 0.401, I did not found published data found regarding this topic.

## 5.2. Conclusion

From the obtained results we concluded:

- Cervical cancer occurred in elderly females.
- 92% of patients with cervical cancer came from Khartoum.
- 66% of patients present with vaginal bleeding.
- The most common type of cervical cancer is squamous cell carcinoma.
- 58% of tested samples present with HPV 16.
- 74% of tested samples with poorly differentiated cancer.
- Existence of HPV 16 infection not correlated with tumor type.
- Existence of HPV 16 infection not correlated with patient's age.
- Existence of HPV 16 infection not correlated with patient's residence.
- Existence of HPV 16 infection and it's invalid to grade tumors.



### **5.3. Recommendations**

This study recommended:

- Further studies should be conducted in Sudan in order to detect the exact role and existence of HPV16 in cervical cancer.
- Future studies should included large sample size with different grades of CIN and cervical cancer using IHC and other advanced techniques such as ISH and PCR to obtain clear picture about HPV16 prevalence in cervical lesions.
- Future studies should study all HR-HPVs in cervical lesions.
- Screening, educational and awareness centers of cervical cancer should be established in Sudan to prevent and minimize incidence of cervical cancer in Sudan.

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## Appendix

### Preparation of Mayer's hematoxylin

Hematoxylin .....	1 g
Distilled water.....	1000 ml
Sodium iodate.....	0.2 g
Potassium alum.....	50 g
Citric acid.....	1 g
Chloral hydrate.....	50 g

### Preparation of eosin Y

Eosin Y.....	1 g
Distilled water.....	100 ml
Glacial acetic acid.....	0.05 ml
Crystal thymol.....	small amount

# Shendi University

## Faculty of Graduate Studies and Scientific Research

### Immunohistochemical Detection of Human Papilloma Virus Type 18 among Sudanese Females with Cervical Cancer

#### Questionnaire sheet

##### Demographic data:

**P.T No.:** ( )

**Hospital No.:** ( )

**Age:** ( )

**Residence:** ( )

##### Analytical data:

**Clinical presentation:** ( )

**Diagnosis:** ( )

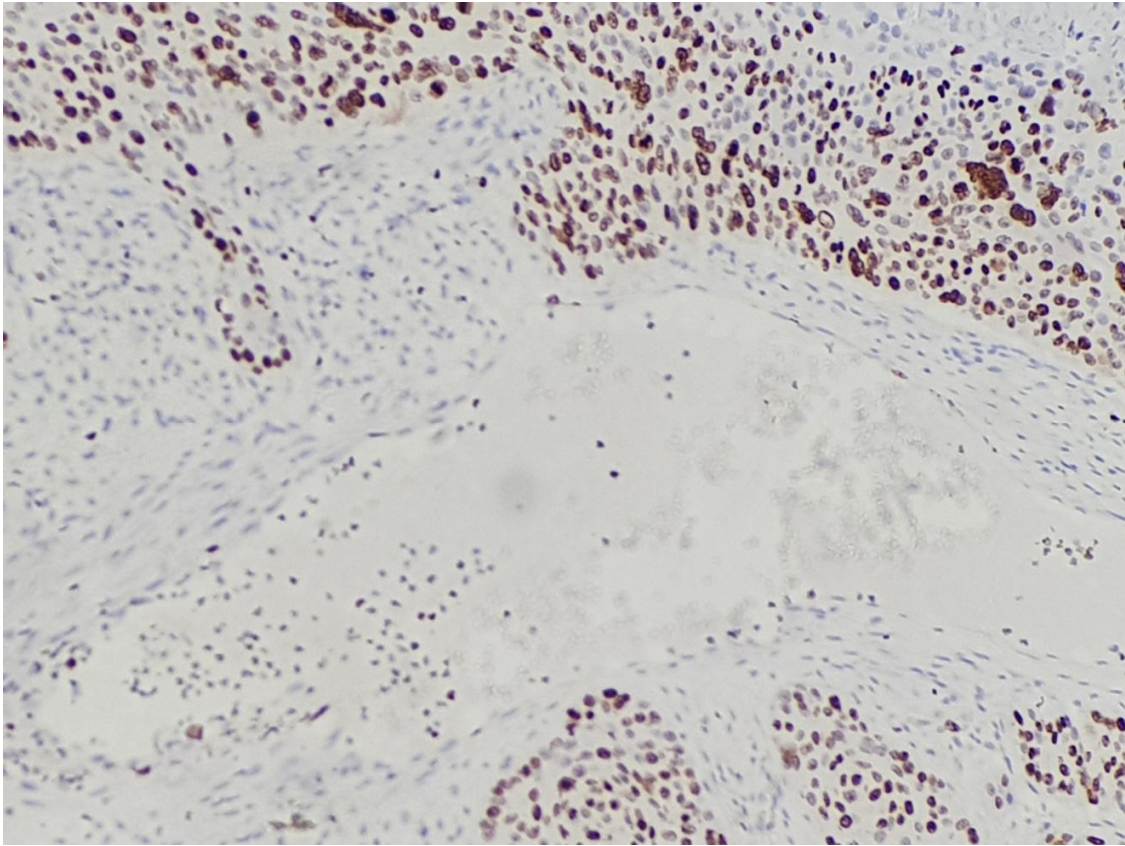
**Tumor type:** ( )

**Tumor grade:** ( )

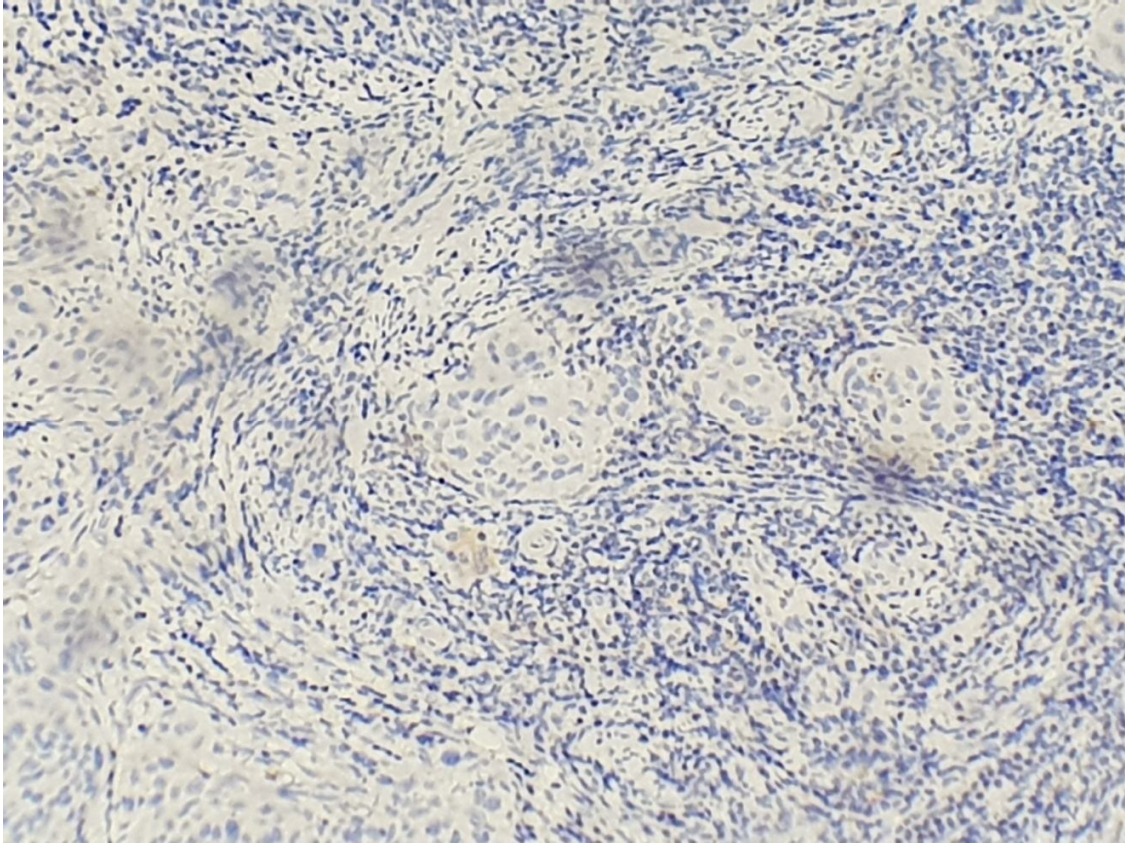
**FIGO stage:** ( )

**IHC result:** ( )

**Expression score:** ( )



**SCC showing HPV 16 positive immunostaining x40 (score4) nuclear stains.**



**SCC showing HPV 16 negative immunostaining x40 (score0.00).**