بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and scientific Research

Shendi University

Faculty of Graduate Studies and Scientific Research

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

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

By

Elsayed Imam Elsayed Imam

B.Sc. in Medical Laboratory Sciences (Histopathology and Cytology)

Sharq Elneil College-2011

Supervisor

Mohammed AbdelgaderElsheikh Mohammed

Ph.D.,M.Sc.,B.Sc. in Medical Laboratory Sciences

(Histopathology and Cytology)

2018

الايـة

بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُبِمَا تَعْمَلُونَ خَبِيرٌ)

صدق الله العظيم

[المجادلة: ١١]

Dedication

To my father,,,

To my family… always,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,

To my daughter,,,

To all my friends,,,

Sayed………

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.

I am grateful to my supervisor Dr. Mohammed Abdelgader Elsheikh Mohammedfor his help, patience, care, invaluable support and for providing me with the materials that I need to carry out this study.

Special thanks to Dr. Ibrahim Bakheet Dr. Nada Salih and Mr. Mahmoud Ibrahim Osman for their help care and support.

List of contents

Chapter one-Introduction

Chapter two-Literature review

Chapter three-Materials and methods

Chapter four-Results

Chapter five-Discussion, conclusion and recommendations

List of figures

List of tables

List of abbreviations

Ca Cervix…….Cancer of Cervix

CIN.....Cervical Intraepithelial Neoplasia

DPX……....Disterene Plasticizer Xylene

Gyn……...Gynecology

HPVs……...Human papilloma viruses

IHC…….Immunohistochemistry

MLS…….Medical Laboratory Sciences

NCI……..National Cancer Institute

Obs……..Obstetrics

RIKC…….Radiation &Isotopes Khartoum Center

RT…….Room Temperature

STI……...Sexual Transmitted Disease

VIA……..Visual Inspection with Acetic Acid

Abstract

Background; Cervical cancer is the most commonly diagnosed cancer and the fourth leading cause of cancer death in female worldwide. Cervical cancer incidence has decreased over the last decades in many countries, due to the introduction of cervical screening programs. Human papilloma viruses are now recognized as the major cause of cervical cancer .They are called papilloma viruses because certain types may cause wart, or papilloma's, which are benign (non-cancerous) tumors. Human Papilloma Virus type 18 or E7 proteins are major oncoproteins of high-risk human papilloma viruses which play a key role in cervical carcinogenesis.

Materials and methods; This was a descriptive cross sectional study conducted in Khartoum state-Sudan during the period from September 2017 to May 2018. Fifty paraffin-embedded tissue blocks with cervical cancer were subjected in this study to detect Human Papilloma Virus type 18 antigen using immunohistochemistry technique.

Results; Human Papilloma Virus type 18 antigen was detected in 28 cases (56%) with no statistically significant different as the p value was 0.441. Also there was no statistically significant different between age of patients and marker expression as the p value was0.776. There was statistically significant different was found between patient's residence and Human Papilloma Virus type 18 antigen as the p value was 0.019.

Conclusion; Human Papilloma Virus type 18 antigen expression was present in more than 50% of tested samples, also there was no statistically significant different between tumor types and Human Papilloma Virus type 18 antigen expression.

المستخلص

الخلفية؛ يعتبرسرطان عنق الرحم من أكثر السرطنات المشخصة والمسبب الرابع للوفيات من السرطان في النساء في العالم. قل حدوث سرطان عنق الرحم في العقود الأخيرة في معظم البلدان، نتيجة لأدخال برامج الكشف المبكر. تعتبر فيروسات الورم الحلمي الان المسبب الرئيسي لسرطان عنق الرحم. تسمي الفيروسات حلمية نتيجة لأن بعض الأنواع تسبب ثؤلول، أو حلمات، وهي أورام حميدة غير سرطانية. فيروس الورم الحلمي النوع ١٨أو بروتين إي٧ هي البروتينات السرطانية الرئيسية لفيروسات الورم الحلمي عالية الخطورة وهي تلعب دور رئيسى في حدوث سرطان عنق الرحم.

الطرائق والمواد؛ أجريت هذة الدراسة الوصفية المقطعية في ولاية الخرطوم –السودان في الفترة ما بين سبتمبر ٢٠١٧ ألي مايو ٢٠١٨. أشتملت هذة الدراسة علي ٥٠ عينة نسيجية مصابة بسرطان عنق الرحم معمولة بطريقة شمع البرافيين بغرض تحديد فيروس الورم الحلمي النوع ١٨ بأستخدام تقنية الأنسجة المناعية.

النتائج؛ تم تحديد فيروس الورم الحلمي النوع ١٨ في ٢٨ من الحالات (٥٦%) مع عدم وجود فرق ذو دلالة أحصائية لأن القيمة الأحتمالية كانت ٠.٤٤١ . وأيضا لم يكن هنالك فرق ذو دلالة احصائية ما بين عمر المرضي وأفراز الوسم لأن القيمة الأحتمالية كانت ٠.٧٧٦ . كان هنالك فرق ذو دلالة احصائية ما بين مكان أقامة المرضي وأنتجين فيروس الورم الحلمي النوع ١٨ لأن القيمة الاحتمالية كانت ٠.٠١٩ .

الخلاصة؛ وجد أفراز فيروس الورم الحلمي النوع ١٨ في أكثر من ٥٠ % من العينات، أيضا لم يكن هنالك فرق ذو دلالة أحصائية مابين أنواع الورم وأفراز أنتجين الورم الحلمي النوع ١٨.

CHAPTER ONE
INTRODUCTION, RTIONALE AND OBJECTIVES

1.1. Introduction

Cervical cancer is the most commonly diagnosed cancer and the fourth leading Cause of cancer death in female worldwide, accounting for 9% (529,800) of the Total new cancer cases. (Jamal *et al*., 2011) cervical cancer incidence has decreased over the last decades in many countries, due to the introduction of cervical screening programs, a stable or increasing incidence has been reported in low-income countries. (Vaccarella*et a*l., 2013)

Human papilloma viruses (HPVs) are now recognized as the major cause of cervical cancer. They are called papilloma viruses because certain types may cause wart, or papillomas, which are benign (non-cancerous) tumors.

The HPVs that cause the common warts which grow on hands and feet are different from those causes' growths in the throat or genital area. Some types of HPVs are associated with certain types of cancer. These are called "High-risk "oncogenic or carcinogenic HPVs. HPVs types 16 and 18 are responsible for about 70% of cervical cancer. (Munoz, 2006) HPV 18 or E7 proteins are major oncoproteins of high-risk HPVs, which play a key role in cervical carcinogenesis.

These proteins have been shown to immortalize primary human cells. (Barbosa, 1989) Zhai Lu and his colleague have found that; the positive rate of high-risk HPV types of cervical cancer can reach 50%–90%. (Zhai Lu*et al*., 2017).

Also study from Sudan by Nahla Bekri in 2008 using molecular technique showed that; HPV16 and 18 were detected in 14 (35%) of the study specimens. HPV16

Was detected in 57.1% (8/14) and HPV18 was detected in 28.6% (4/14).

Mixed infections were detected in 14.3% (2/14) of positive samples, accordingly, HPV 16 is the most common HPV type associated with cervical

carcinoma in central Sudan. The HPVs were detected in 10 individuals who ages were≥45 and in 4 individuals who age's≤44.

Therefore, cervical cancer is more common in woman older than 45years, suggesting infection at a younger age and slow progressing to cancer. (Burd, 2003)

This study conducted to detect expression of HPV type18 in Sudanese females with cervical cancer using IHC technique, also this study aimed to correlate association of HPV type18 infection with tumor type, grade, age and geographical region of patients included in this study.

1.2. Rationale

Cervical cancer in Sudan is the second most common cancer in females,usually women present late in disease due to lack of efficient screeningprograms and deficient of systemic health care system. Epidemiologicalstudies have shown that human high risks HPVs infection especially type1 6 and 1 8 are the main etiological factors for cervical carcinogenesis. Theprevious studies had shown the relatively higher rate of HPV infection inthe early stage of cervical cancer. This finding reveals the importance ofHPV screening programs for early detection of cervical cancer. In Sudanthe exact role of HPVs infection in the development of cervical cancer isnot fully documented yet. Detection of HPV type 1 8 infection in cervixtissues with cervix cancer by using immunohistochemistry which isspecific and sensitive technique, may clarify the exact role of HPVsinfection with cervical cancer tumorigenesis.

1.3. Objectives

1.3.1. General objective

To detect HPV type 1 8 infection in cervical cancer among Sudanese females

usingIHC technique.

1.3.2. Specific objectives

1. To correlate HPV type 18 infection with cervical cancer subtypes.

2. To correlate existence of HPV type 18 infections with the grade of cancer.

3. To correlate the expression of HPV type 18 with the age of patients.

4.To correlate the expression of HPV type 1 8 with the patients' geographical region.

CHAPTER TWO
LITERATUTRE REVIEW

2.1. Cervical cancer

Is a cancer arising from the cervix. It is due to the abnormal growth of cells that have the ability to invade or spread to other parts of the body.Early on, typically no symptoms are seen, later symptoms may include abnormal vaginal bleeding, pelvic pain, or pain during sexual intercourse. (NCI. 2014)While bleeding after sex may not be serious, it may also indicate the presence of cervical cancer. (Tarney and Han, 2014)Cervical cancer is the third common cancer in the world in females and fourth leading cancer for death in women. Every year 53.0000 cases are diagnosed and 265.653 deaths occur in the world due to cervical cancer. The incidence/prevalence is decreasing in developed countries due to health awareness and regular/systemic cervical cancer screening; some countries using HPV DNA test in addition to Pap tests. This routine screening has reduced the incidence to 70% in developed countries. However, cervical cancer persists in developing countries that constitute 85% of total world cases, despite only 5% global cancer resources in these countries. The incidence in developing countries is 40 per100,000 populations. (Wright *et al*., 2007; Jean, 2012; Marinho and Sousa, 2013; Ononogbu*et al*., 2013; Sinayobye*et al*., 2014)

Cervical cancers yet still the commonest cause of cancer death among women in poor countries. (Lynette *et al*., 2012) Cervical cancer is the second most common cancer found in women with approximately 53.0000 new cases each year resulting in an estimated 275.000 deaths, worldwide. In developed countries, cervical cancer incidences have declined, mostly due to cervical cytology screening campaigns, which requires significant medical resources and laboratory infrastructure. Cervical cancer is on the rise in the developing world, with one-seventh of the world's cervical cancer cases in

China, where no nationwide screening program for the disease currently exists. (Zachary, 2012) Although there have been advances in detection and treatment, cervical cancer is still a major health issue, especially in developing countries. (Ferlay *et al*., 2008)

2.2. HPV

HPV is one of the most common causes of sexually transmitted disease in both men and women worldwide and is thought to be the most common sexually transmitted viral disease in the United States. Genital HPV infection is not a reportable disease, so actual incidence and prevalence figures are not known; however, it is estimated that the incidence of new infections in the United States ranges from 1 million to 5.5 million per year, and the prevalence is estimated to be as high as 20 million. (Eileen, 2003)

HPVs are relatively small, DNA viruses without an envelope; the diameter is 55 nm. They have icosahedra symmetry, and 72 capsomers and a DNA which contains 6800-8400 bases pairs. The virus genome codes 8-l0 proteins in which there are structured(L1andL2) and non-structured (E1; E2; E4; E5; E6 and E7) proteins. They cannot be detected in tissue cultures but only in keratinocyte cultures and xenographs. (de Villiers *et al*., 2005)

The HPV transmission happens with tight contact, which can be genital-genital, manual genital, oral-genital and anal-genital. The viruses get into the body by micro lesions. It causes skin and mucous membrane diseases in a benign or malignant way. On the outer genitals mostly benign diseases are caused by the HPV 6 and 11 types. 70% of the cervix, anus, vulva and penis carcinomas are caused by the HPV 16 and 18 types. (Dillner*et al*., 2000; Diaz, 2008)

Nearly 100 HPV types have been molecularly identified and about 40 of these can

infect the ano-genital tract. (Muñoz *et al*., 2003) On the basis of their oncogenic potential, most of these genital HPV types have been classified as high or low risk for causing cervical cancer. The high-risk types, especially HPV 16 and 18 are implicated in the development of cervical intraepithelial neoplasia and cervical carcinoma.

The low-risk types includes; (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and

CP6108), can cause mild cervical dysplasia but are rarely associated with severe cervical dysplasia or cervical carcinoma. (Bernard, 2005; Iljazović*et al*., 2006) Women found to be positive for high-risk HPV were at increased risk of developing CIN3 than those with a negative HPV test. (Mehal*et al*.,1994)

2.3. Role of HPVS in cervical cancer

Persistent infection by oncogenic types of HPV, such as HPV16, 18, 31, 45 and others, are considered the etiological factor for cervical carcinoma development. Although HPV is not a sufficient factor for developing cervical cancer, several other co-factors were identified, such as; smoking, infection by other sexually transmitted diseases (STI) (Human Immunodeficiency Virus, Chlamydia trachomatis, Cytomegalovirus, etc.), long term use of oral contraceptives, intrauterine device use, multiple full-term pregnancies, young age at full-term pregnancy and poverty. (Missaoui*et al*., 2010; Chattopadhyay, 2011)

2.4. Epidemiology

In developed countries several enhancements have been made in prevention and treatment leading to a decline of both incidence and mortality. (Chattopadhyay, 2011) An estimated 371.000 new cases of invasive cervical cancer are diagnosed worldwide each year, representing nearly 10% of all

cancers in women. In frequency, it is the seventh cancer site over all and third among women, after breast and colorectal cancer. (Parkin*et al*., 1993)

Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease. India has a population of 432.2 million women aged 15 years and older who are at risk of developing cancer. It is the second most common cancer in women aged 15–44 years. India also has the highest age standardized incidence of cervical cancer in South Asia at 22, compared to 19.2 in Bangladesh, 13 in Sri Lanka, and 2.8 in Iran. Therefore, it is vital to understand the epidemiology of cervical cancer in India. (ICO, 2014)

Data from Egyptian studies provide widely varying estimates on the prevalence of pre-invasive cervical lesions ranging from 1% to 8% with an age range from 20–60 years. (El Mosselhy et*al*., 1998) The incidence of cervical cancer in Greenland is one of the highest in the world, and more than five times higher than in the rest of Denmark. (Nielsen *et al.*, 1988), this is in accordance with the well-known risk factors of sexual lifestyle, including early sexual debut, multiple partners, and multiple pregnancies.

In a recent population study, 53% of Greenlandic and only 4% of Danish women reported more than 20 lifetime partners. Also, the average age of sexual debut was found to be significantly lower in Greenland than in Denmark. Cigarette smoking, another cervical cancer risk factor,was reported by 87% of the Greenlandic women and by 54% of the Danish women of the investigation. (Kja~rSK*et al*., 1989)

Despite the presence of a well-established UK screening program for detecting cervicalpre-invasive disease there are approximately 2,800 cases of cervical cancer per annum and1,000 women still die from cervical cancer each year. (Cancer research UK; 2003)

2.5. Cervical cancer in Sudan

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 with the main emphasis on health education programs, teaching women the early symptoms of ca cervix and early detection. Due to lack of awareness the majority of women don't know anything about screening, so there is limited demand. (KamalEldein *et al*., 2017) 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. Data is not yet available on the HPV burden in the general population of Sudan. (ICO/IARC Information Centre on HPV and Cancer.2017).

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 squamous cell carcinoma (SCC) of cervix. High risk HPVs (HR-HPVs) were detected in 41.8% among cases. (Elsheikh *et al*., 2018) Mrudula and Suhasiniin India on 2010, their study concluded that; the study reveals a significant detection of HPV in the South Indian suspected individuals, by the use of advanced techniques such as IHC and PCR. (Mrudula*et al*, 2010) 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. (Shepherd*et al*, 1992)

2.7. Immunohistochemistry (IHC)

IHC is a method to identify specific antigens within tissue sections utilizing an antigen-specific antibody. Detection at the light microscopic level of antigen–antibody interactions can be achieved by labeling the antibody with a substance that can be visualized, either by conjugation to a fluorescent marker or enzyme followed by colorimetric detection. Immunologic detection of antigens dates to the early 20th century when Marrack demonstrated that anti-typhoid and anti-cholera sera-labeled with diazotized benzidine-azo-r-salt imparted a red color to the bacteria. Although groundbreaking for immunological detection of antigens, Coons determined this labeling method to be relatively insensitive when applied to tissues, and subsequently described assays utilizing fluorescent-labeled antibodies in fixed tissues, but interpretation was confounded by the enhanced endogenous fluorescent activity in formalin-fixed tissue. (Clifton *et al*.,2015)

CHAPTER THREE

MATERIALS

&

METHODS

3.1. Study design

This was a descriptive cross-sectional study aimed to detect the immunohistochemical expression of HPV18 in cervical cancer among Sudanese Patients.

3.2. Study duration

This study was carried out during period from September 2017 to May 2018.

3.3. Study area

This study was conducted in Khartoum state. Samples were collected from Omdurman Obstetrics & Gynecology Hospital and then processed in Radiation &Isotopes Khartoum Center (RICK).

3.4. Study populations, samples and samples size

Populations subjected in this study were fifty paraffin tissue blocks diagnosed with cervical cancer during 2016-2018.

3.5. Sampling technique

Convenient sampling technique was used to collected samples in this study.

3.6. Data collection, tools and variables

Master sheets were used to record all patients and sample data; age, residence and grade of cancer. Master sheets were also used to record all IHC results.

3.7. Quality control

Positive and negative control sections were used to evaluate the working solutions and to evaluate the testing slides. All Precautions and quality issues were issued as manufacture instructions (BioGenex-USA).

3.8. Sample processing

One section from each block measures four micrometers was cut using Leica microtome (Leica Microsystems, Nussioch Gmbll, model: RM 2125RT, ser

NO. 8843/04-2005-China) and then stained in haematoxylin and eosin (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 40 C^0. Each floated section was mounted on positive charge immune slide (Thermo Scientific- Italy) to detect immune expression of HPV (type 18).All slides contained sections were dried in dry oven (WTC binder 7200 TUTTLINGEN, B28, NO.88485-USA) at 60C^0 for 30 minutes.

3.9. Methods of detection

3.9.1. H & E

Section of 4 micron thickness was obtained from each formalin-fixed paraffin-embedded tissue block using rotary microtome and then stained using H&E (Mayer's technique) to confirm the histopathological diagnosis. Section was dewaxed in hot plate oven and cleared in two changes of xylene for two minutes, then hydrate through ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then stained in Mayer's haematoxylin for 7 minutes, then washed and running tap water for ten minutes, then stained in eosin for three minutes, then washed in distilled water and hydrated through ascending ethanol, cleared in xylene and mounted in DPX.

3.9.2 Method of immunohistochemistry

The immunohistochemical procedure was done as followed; following deparaffinization in xylene, slides rehydrate through a graded series of alcohol and place in running water. Then Samples steam for antigen retrieval for HPV18 using water bath in coplin jar containing sodium citrate buffer (pH 9.0).

Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 minutes, and then Slides incubate with 100-200 µl of primary antibodies for 20 min at RT, then rinse in Phosphate buffer saline.

The primary antibody for HPV18 (monoclonal) After washing with PBS for 3 min, binding of antibodies detect by incubating for 20 minutes with dextran labeled polymer. Finally, the sections was wash in three changes of PBS, followed (DAB) as a chromogen to produce the characteristic brown stain for up to 5 min. Slides counterstain with haematoxylin.

3.10 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).

3.11 Data analysisand 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 being calculated. P value <0.05 was be considered statically significant.

3.12. Ethical consideration

This study was approved by the board of Medical Laboratory Sciences (MLS) Shendi University. A written agreement was signed 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

4.1 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 group 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 33 cases (66%) followed by cervical mass and vaginal discharge, 11 cases (22%) 5 cases (10%) respectively.

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

Figure 4.5 demonstrates the frequency of HPV18 among study populations. Of the 50 cases 28 cases (56%) were positive and 22 cases (44%) were negative.

Figure 4.6 demonstrates the frequency of HPV18 score. Of 50 cases, the highest frequency was score 4 (16 cases=32%) followed by score 0.00 (13 cases=26%) and score 1 (9 cases=18 %).

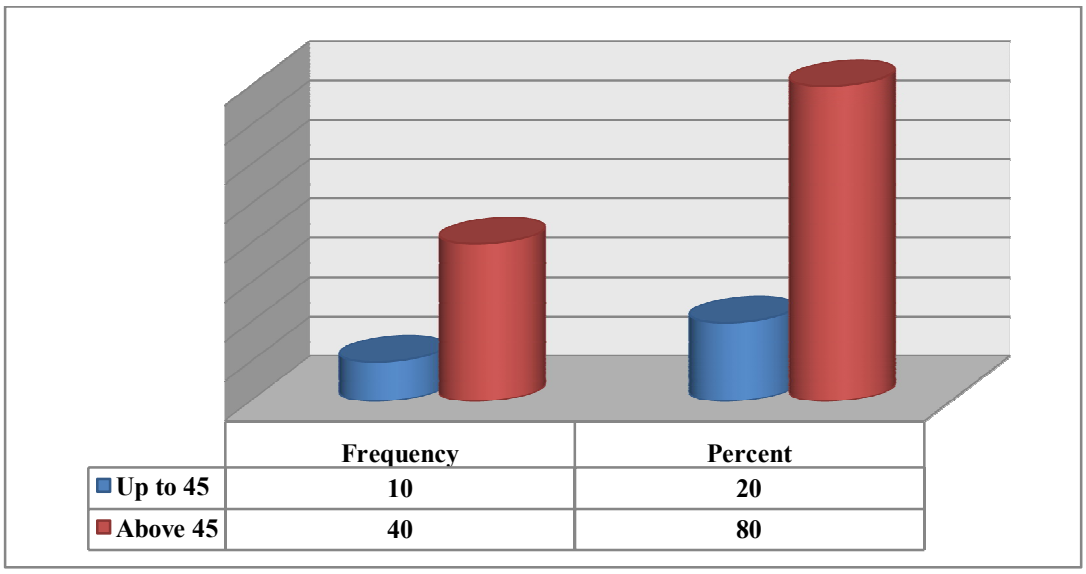
Figure 4.7 demonstrates the frequency of tumors grade among study populations. Of the 50 cases 37 cases (74%) were poorly differentiated, 11 cases (22%) were well differentiated and 2 cases were moderately differentiated.

Table 4.1 illustrates the correlation of HPV18 with tumor types. squamous cell carcinoma was showed the most positive type expression 24/50 (48%), adenocarcinoma 4/50 (8%). With no statistically significant different as the p value was 0.441.

Table 4.2 illustrates the correlation of HPV18 with age groups. Of the 50 investigated immune stained slides, 6/50 (12%) cases were positive in the age group up to45 years and 22/50 (44%) cases were positive in the age group above than 45 years. With no statistically significant different as the p value was 0.776.

Table 4.3 illustrates the correlation of HPV18 with patient's residence. All positive slides for HPV 18 were from group of patients residence in Khartoum 28/50 (56%) with statistically significant different as the p value was 0.019.

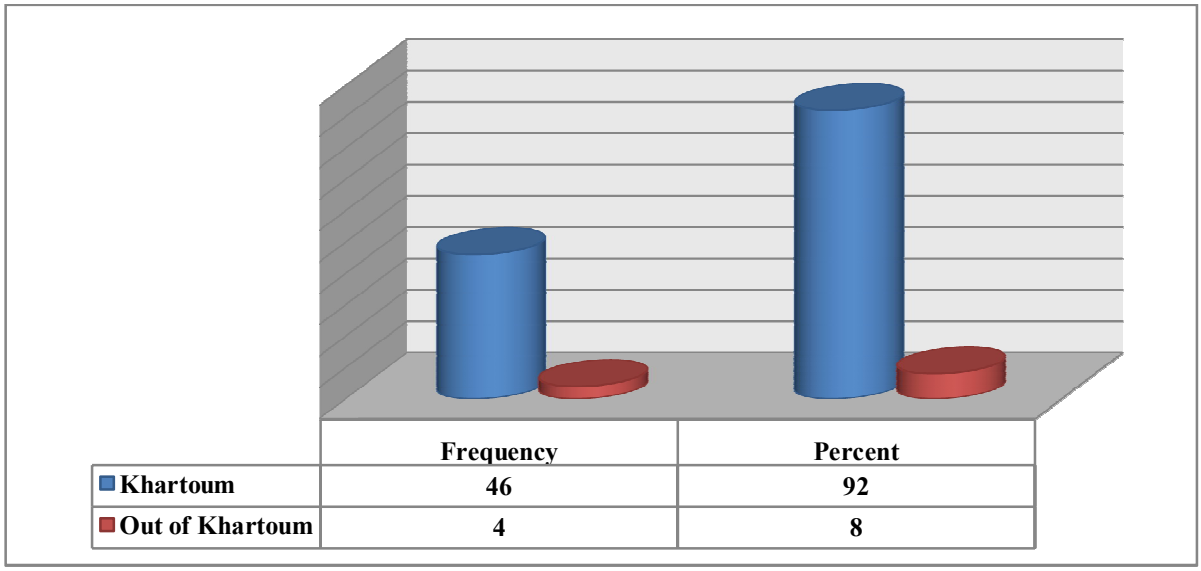
Table 4.4 illustrates the correlation of HPV18 with tumor grade. Of 50 tested slides 18/50 (36%) were positive in poorly differentiated grade,7/50 (14%) were positive in well differentiated grade and 2/50 (4%) were positive in moderately differentiated grade. with no statistically significant different as the p value was 0.163.



	Frequency	Percent
Up to 45	10	20
Above 45	40	80

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

N=50



	Frequency	Percent
Khartoum	46	92
Out of Khartoum	4	8

Fig4. 2: Shows distribution of residence among study populations.

N=50

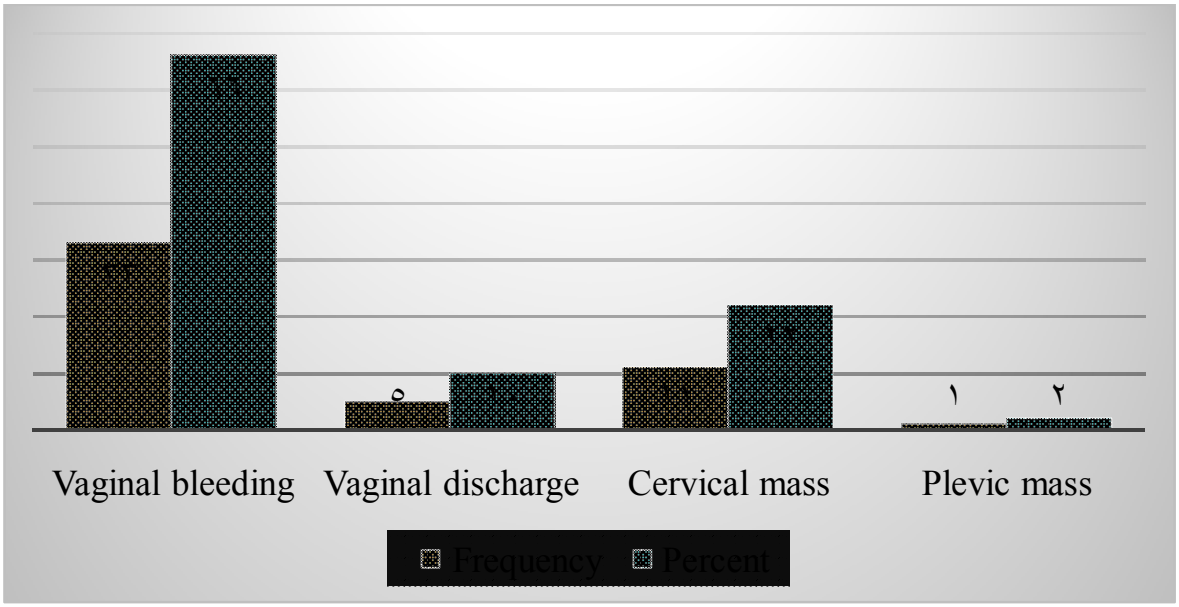
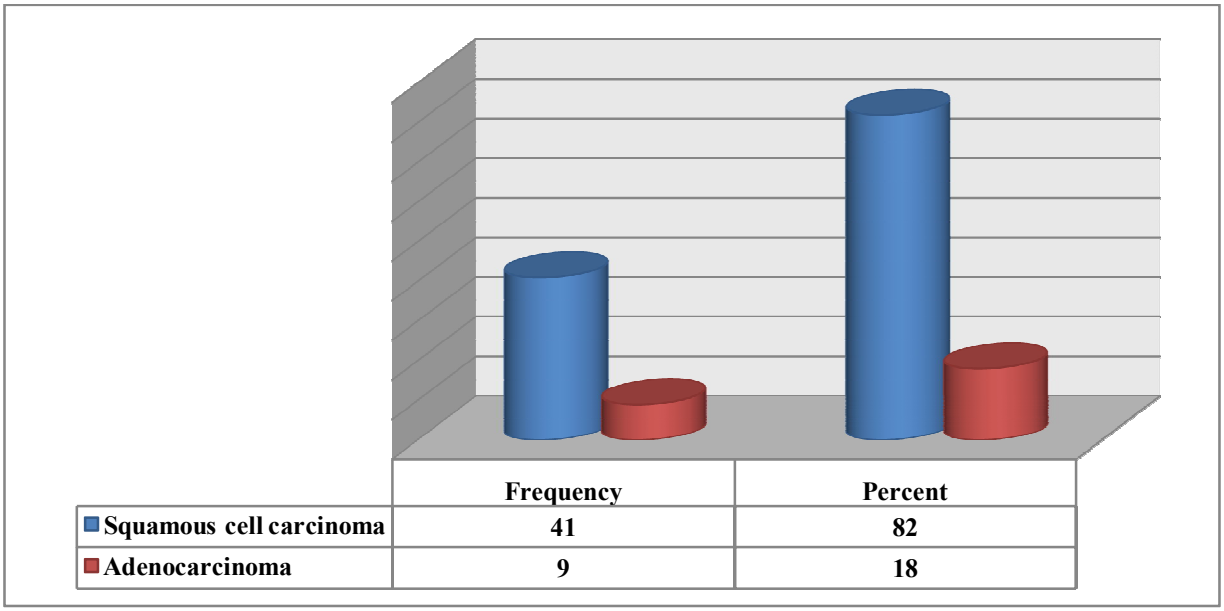


Fig4. 3: Shows distribution of clinical presentation among study populations.

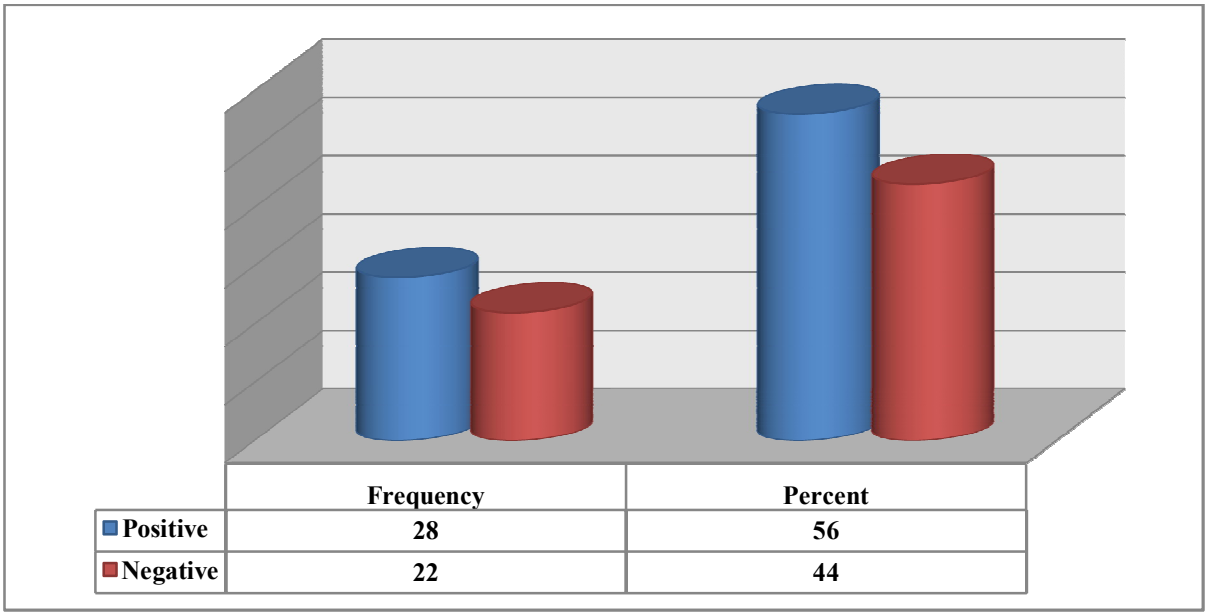
N=50



	Frequency	Percent
Squamous cell carcinoma	41	82
Adenocarcinoma	9	18

Fig 4 .4: Shows distribution of tumor types among study populations.

N=50



	Frequency	Percent
Positive	28	56
Negative	22	44

Fig 4.5: Shows distribution of HPV18 among study populations.

N=50

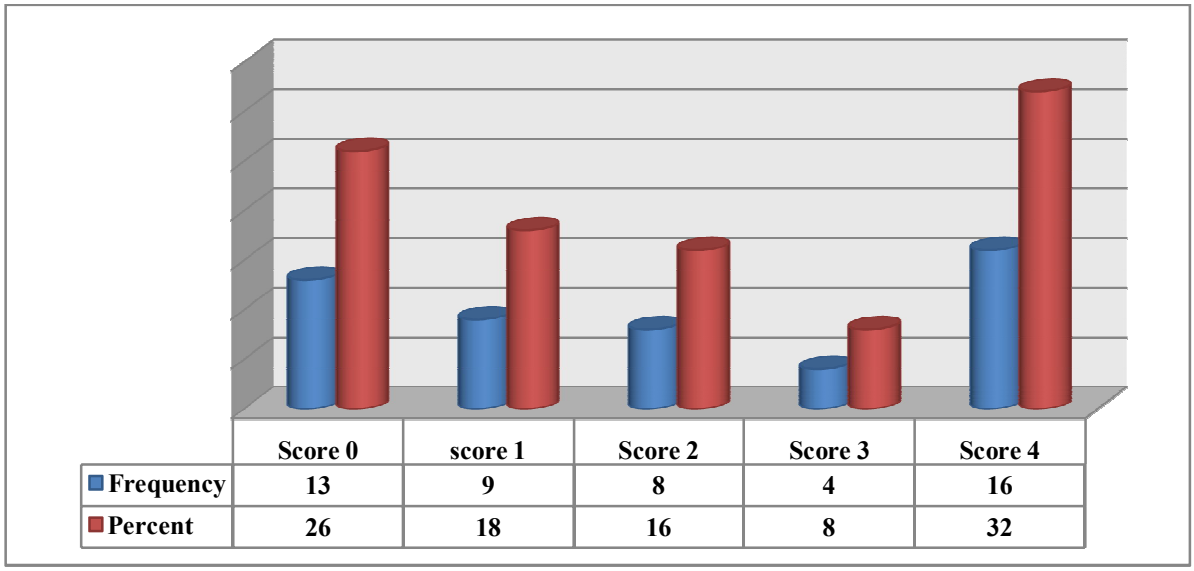
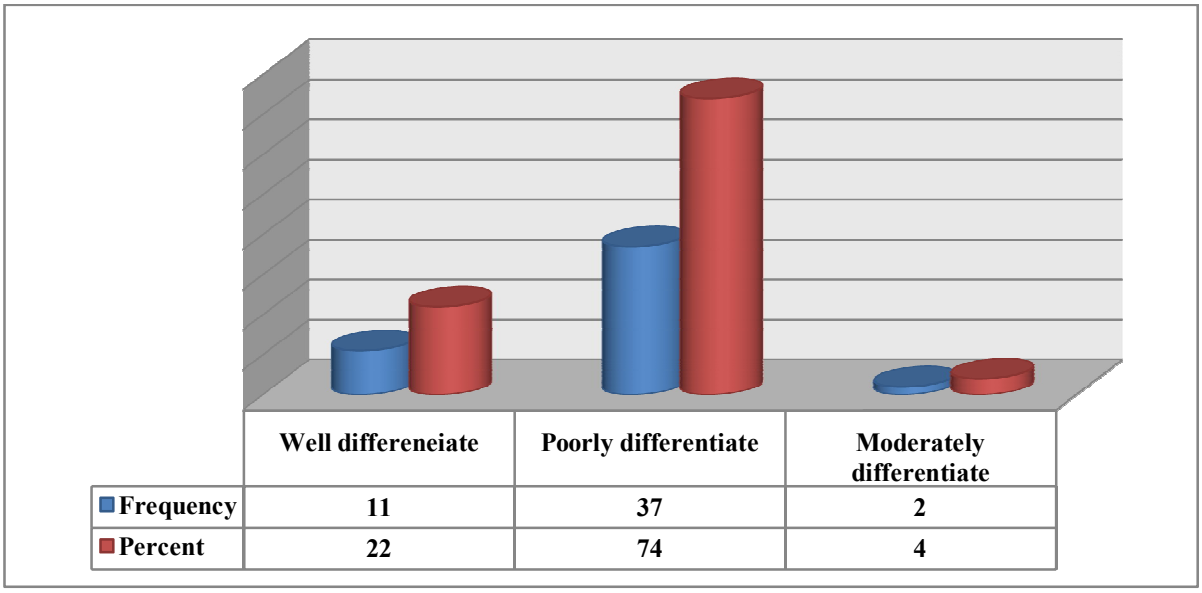


Fig 4.6: Illustrates distribution of HPV18 score among study populations.

N=50



	Well differeneiate	Poorly differentiate	Moderately differentiate
Frequency	11	37	2
Percent	22	74	4

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

N=50

Table 4.1: Correlation of HPV18 with tumor types.

Tumor types	HPV IHC result		Total	P. value
	Positive	Negative		
SCC	24	17	41	
Adenocarcinoma	4	5	9	
Total	28	22	50	0.441

Table 4.2: Correlation of HPV18 with age groups.

Age groups	HPV IHC result		Total	P.value
	Positive	Negative		
Up to 45	6	4	10	
Above 45	22	18	40	0.776
Total	28	22	50	

Table 4.3: Correlation of HPV18 with patient's residence.

Residence	HPV IHC result		Total	P.value
	Positive	Negative		
Khartoum	28	18	46	
Out of Khartoum	0	4	4	0.019
Total	28	22	50	

Table 4.5: Correlation of HPV18 with tumor grade.

Grade	HPV IHC result		Total	P.value
	Positive	Negative		
Well differentiate	7	4	11	
Moderately differentiate	2	0	2	
Poorly differentiate	18	19	37	0.163
Total	28	22	50	

CHAPTER FIVE
DISCUSSION

5.1. Discussion

Cervical cancer is the second-most common type of cancer among women (Soma and Kamaraj, 2010). Human papilloma virus has been considered as the most significant risk factor for cervical cancers. HPV is recognized as a public health problem for its role as a critical factor in the pathogenesis of various cancers (Aggarwal, 2006). Out of 100 HPV genotypes, 30 were shown to infect the uterine cervical epithelium (Nubia *et al,* 2003).The subsets for inducing cervical cancers are low-, intermediate-, and high-risk (Zuna, 2007). The virus is asymptomatic in the benign stage (Kobayashi, 2000; Reichman, 2011), and it clinically manifests as a neoplastic transformation (Tyring, 2000).The primary method for HPV detection is the Pap smear test. Human error is probably the primary threat to accurate interpretation (Burd, 2003).

The purpose of the present study was to detect immunoexpression of HPV type 18 among Sudanese women with cervical cancer. A total of 50 cases of cervix cancer were included in this study, in which the age of patients was ranged between 30 and 80 years with an average age of 58.92, similar results were obtained by Elsheikh *et al*., they concluded most of cervical cancer patients were in the higher age; this is in keeping with the natural history of HPV infection. The mean age at presentation was 55 years (Elsheikh et al., 2018). Our results were higher compared to studies conducted by Badar *et al*., 2007 and Reimers *et al*., 2009, but similar to studies done by Krishnamurthy *et al*., 1997, Herbert *et al.,* 2001 and Patel *et al*., 2009. Also the same age was obtained in Iran by Zarchi and his team, their study concluded that, the average age group incidence of 53.6 years (Zarchi *et al*., 2010). Our findings were consistent with global reports (Pagliusi and Garland, 2007) and national study by (Husain *et al*., 2011) they concluded

that; the commonest age group affected was patients grouped between 41-60 years (52%) followed by 61-80 years (26.3%). According to American Cancer Society cervical cancer tends to occur in midlife and is most frequently diagnosed in women between the ages of 35 and 44. It rarely develops in women younger than 20 (American Cancer Society 2018).

Regarding presenting symptoms, the majority of samples (two thirds) presented with vaginal bleeding and this result consistent with finding of the result conducted in our country done by Elsheikh *et al*., in 2018 which concluded that; abnormal vaginal bleeding was observed in more than two thirds, also consistent result was obtained by (Husain *et al*., 2011) they concluded that vaginal bleeding was the presenting symptom in 88.7% of the studied cases. Also this finding was in agreement with (Shapley *et al*., 2006) they concluded that abnormal vaginal bleeding, such as irregular menstrual bleeding and post coital bleeding, is common.

Mwaka *et al*., on 2015 in Uganda which concluded that; abnormal vaginal bleeding (including post-menopausal and/or post-coital), vaginal discharge and lower abdominal pain were the most common first symptoms reported by participants.

Regarding cancer type, this study indicated that; the commonest cancer type is SCC followed by adenocarcinoma with a ratio 4:1 and this may be due to strong association between HPV infection and squamous epithelium cells of the cervix rather than association with glandular cells of the cervix, this result match with other information published by National Cervical Cancer Coalition in 2018 and Cancer Research UK in 2018 which summarized in most cervical cancers (80 to 90 percent) are squamous cell cancers.

Regarding tumor grade, our study showed that the most common grade was poorly differentiated cancer present in one to quarter of samples and this

may be due to late presentation of females with disease, this finding consistent with other studies conducted by Khenchouche *et al*., in 2013 who concluded that; HR-HPV infection was found in 95% of patients with high grade lesions of SCC and this result disagreement with finding of the study conducted by De Araújo *et al*., in 2013 who concluded that; despite the high prevalence of HPV types 16 and 18, the presence of these virus types did not affect the prognosis of patients.

Concerning geographical residence, our study showed that; most of females with cervical cancer were came from Khartoum Sudan, and this may be due to the reason Khartoum is the capital of the Sudan where most of populations live. Also due to centralization of health services that lead to the discovery of cervical cancer.

High risk HPV type 18 was positive in more than 50% of cases and negative in less than 50% of cases. HPV type 18 prevalence rate in cervical cancer in general 56% and in squamous cell carcinoma is 58.5% (Mrudulaand, 2010), but our results higher than percentage (34.78 %) detected in India (Mrudulaand, 2010), also our results were higher than percentage detected in United Kingdom (0.00%) and South Africa (22%) (Cooper *et al*., 1991), the difference between results it is possible return to a difference in the number of samples, the genomic different between populations, also may be due to variation in geographical distribution of HPV or the cultural limitations.

There is no statistical significant correlation between HPV 18 expression and type of cervix cancer, there was no published data on the same topic.

Investigation of the age classification indicates that; age group above 45 years has been allocated the highest number of HPV positive cases in this study. A similar result showed in Indian study (Mrudulaand, 2010), in

contrast Gita *et al*., reported the highest number of positive cases in the age group of 35–44.

Investigation of the correlation of HPV18 and patients residence showed that; most of patients with HPV18 live in Khartoum state with strong significant association as the p. value was 0.019; this can be traced back to civil civilization, civilizational openness and different lifestyles. There was no published data in the same topic to compare our result with it.

Investigation of the correlation of HPV18 with tumor grade showed that; the poorly differentiated cancer with most HPV18 expression than well differentiated and moderately differentiated samples with no significant different (p. value 0.164), this result in agreement with a study conducted by Robert *et al*., in 1996 which concluded that; HPV type was not associated with established prognostic factors such as stage, grade, lymph node metastasis. Also our result agreement with study conducted by Rossana *et al*., in 2013 which concluded that; the presence of HPV types 16 and 18 virus types did not affect the prognosis of patients with stage I cervical cancer.

5.2. Conclusions

On the base of the obtained results we could conclude that;

- The mean age of patients presenting with cervical tumors is 58.92 years indicate the incidence rate of cervical tumors was more in the age above 45 years.
- The commonest presenting symptom is vaginal bleeding.
- Squamous cell carcinoma was the most frequent type of cervical cancer.
- There was no relationship between tumor types and HPV infection.
- There was no relationship between age groups and HPV infection.
- There was a relationship between civilization residence and HPV infection.
- There was no relationship between tumor grade and HPV infection.

5.3 Recommendations

On the base of the obtained results we recommended that;

- Further study should be conducted using larger sample size.
- For future study, we suggest the use of more advanced techniques like polymerase chine reaction (PCR), in situ hybridization (ISH) to identify the exact prevalence of HPV infection in cervical cancer.
- Introduction of cervical screening program and HPV vaccination may decrease incidence of cervical cancer in Sudan.

References

- **Badar F; Anwar N; Meerza F; *et al*.,** (2007). Cervical Cancer in a Muslim Community. Asian Pac J Cancer Prev. 8(1): 24-6.
- **Barbosa MS; Schlegel R.,** (1989). The E6 and E7 genes of HPV-18 are sufficient for inducing two-stage in vitro transformation ofhuman keratinocytes. Oncogene. 4:1529–1532.
- **Bekri N.,** (2008). Detection of human papilloma virus16 and 18 DNA using type specific polymerase chain reaction in cervical.
- **Bernard HU.,** (2005). The clinical importance of the nomenclature, evolution and taxonomy of human papilloma viruses. J Clin Virol. 32:1-6.
- **Broccolo F; Cassina G; Chiari S; Garcia-Parra R Villa A; Leone BE.,** (2008). Frequency and clinical significance of human beta-herpes viruses in cervical samples from Italian women. J Med Virol.80:147-53.
- **Burd EM.,** (2003). Human papillomavirus and cervical cancer. Clin Microbiol Rev.16:1–17.
- **Cancer research UK**, (2003). UK cervical cancer incidence statistics. London.http://info.cancerresearchuk.org/cancerstats/types/cervix/incidence/.
- **Chattopadhyay K**., (2011). A comprehensive review on host genetic susceptibility to human papilloma virus infection and progression to cervical cancer. Indian J Hum Genet.17:132-44.
- **Claude Fauquet; MA Mayo; J Maniloff; U Desselberger; LA Ball,** (2005). Virus Taxonomy. Academic Press, San Diego. ELSEVIER. 1:145–162.

- **Clifton P. Drew; Wun-JuShieh**, (2015). Current Laboratory Techniques in Rabies Diagnosis. Research and Prevention. Volume 2, ISBN: 978-0-12-801919-1.

- **De AraújoCatãoZampronhaR; Freitas-Junior R., Murta EFC; Michelin M A; BarbarescoAA; Adad SJ; Oton GJB., (**2013). Human papillomavirus types 16 and 18 and the prognosis of patients with stage I cervical cancer. Clinics. 68(6):809–814.
- **de Villiers EM; Fauquet C; Broker TR; Bernard HU; zur Hausen H.,** (2004). Classification of papilloma viruses. Virology. 324(1):17-27.
- **Diaz ML**., (2008). Human papilloma virus: prevention and treatment. ObstetGynecol Clin North Am. (35):199-217.
- **Dillner J; von Krogh G; Horenblas S**., (2000). Etiology of squamous cell carcinoma of the penis. Scand J UrolNephrol Suppl. (205):189-93.
- **Eileen M Burd**, (2003). Human Papillomavirus and Cervical Cancer. Clinical microbiology reviews. (16):1-17.
- **El Mosselhy MH; Moselhy MM; Amin A; Ouda-Pasha E Hamid; Hussein MH**., (1998). Prevalence of reproductive tract infections among married women of reproductive age. National Population Council/El Galaa Teaching Hospital, Egypt.**; Abdel**
- **Mohammed Abdelgader ELSHEIKH; Ibrahim Bakhit YOUSIF; Mahmoud Ibrahim OSMAN; Awad Eljeed Abugooda Alobaid; Ahmed ABDELBADIE; Elsadig Ahmed ADAM,** (2018). Herpes Simplex Virus type 2, Epstein Barr Virus and Human Papilloma Virus Co-infection in Cervical Squamous Cell Carcinoma among Sudanese

Females. International Journal of Current Research in Medical Sciences. 4(2):93-103.

- **Ferlay J; Shin HR; Bray F; Forman D; Mathers C; Parkin DM.,** (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 200*8*. Int J Cancer. 127:2893-917.
- **Gita E; Maryam G; Mohammad R; Hossein G; Afsoun T.,** (2008). PCR detection and high risk typing of human papillomavirus DNA in cervical cancer in Iranian women. Cancer Ther. 6:361–6.
- **Herbert A; Singh N; Smith JA.,** (2001). Adenocarcinoma of the Uterine Cervix Compared with Squamous Cell Carcinoma: a 12-year Study in Southampton and South-west Hampshire. Cytopathology. 12(1): 26-36.
- **Husain N; Helali T; Domi M; Bedri S.,** (2011). Cervical cancer in women diagnosed at the National Health Laboratory. Sudan: A call for screening. 6(3): 183–190.
- **Bruni L-Barrionuevo-Rosas L, Albero G, Serrano B, Mena M, Gomez D, Munoz J, Bosch FX, de Sanjose S.**, (2017). Human Papilloma Virus and Related Disease in the World: Summary Report. ICO/IARC Information Centre on HPV and cancer (HPV information Centre). www.hpvcentre.net/statistics/reports/.
- **Iljazović E; Mustadenagić-Mujanović J; Karasalihović Z; Cickusić E; Avdić S.,** (2006). Presence of high risk human papilloma viruses (HPV) in the low grade cervical lesion. MedArh. 60(1):30-2.
- **Jean Anderson EL; Harshad Sanghvi; Sharon Kibwana; Anjanique Lu.,** (2012). Cervical Cancer Screening and Prevention for HIV-Infected

Women in the Developing World. In Cancer Prevention – From Mechanisms to Translational Benefits. A. Georgakilas, ed. 231-260.

- **Jemal A; Bray F; Center MM; Ferlay J; Ward E; Forman D**., (2011). Global cancer statistics. CA Cancer J Clin. 61:69–90.
- **K Cooper; C S Herrington; A K Graham; M F Evans; J O'D McGee**, (1991). In situ evidence for HPV 16, 18, 33 integration incervical squamous cell cancer in Britain and South Africa. J Clin Pathol. (44):406-409.
- **Kamal Eldein H Mohamed; Ammar Ashmeig Ahmed Ashmeig,** (2017). Cervical cancer: Our experience in Sudan.10.1158/1538-7445.AM2017-771 Published July 2017.
- **Kja~rSK; Teisen C; Haugaard BJ.,** (1989). Risk factors for cervical cancer in Greenland and Denmark. A population-based cross-sectional study. Int J Cancer. 44:40-7.
- **Kobayashi A; Miaskowski C; Wallhagen M; Smith Mucune K**., (2000). Recent developments in understanding the immune response to human papilloma virus infection and cervical neoplasia. Oncol Nurs Forumn. 27:643–51.
- **Krishnamurthy S; Yecole BB; Jussawalla DJ.,** (1997). Uterine Cervical Adenocarcinomas and Squamous Carcinomas in Bombay: 1965-1990. J Obstet Gynaecol Res. 23(6): 521-7.
- **Lu z; Chen H.,** (2016). Expression and clinical significance of high risk human papillomavirus and invasive gene in cervical carcinoma. Asian Pacific Journal of Tropical Medicine. 10(2): 195–200.
- **Lynette Denny; Rose Anorlu,** (2012). Cervical Cancer in Africa. Cancer Epidemiology Biomarkers and Prevention. 21:1434-1438.

- **Marinho-Dias J; Sousa H.,** (2013). Cytomegalovirus infection and cervical cancer: from past doubts to present questions. Actamedica portuguesa. 26(2):154-160.
- **Mehal WZ; Lo YM; Herrington CS; Evans MF; Papadopoulos MC; Odunis K; Ganesan TS; McGee JO; Bell JI; Fleming KA.,** (1994). Role of human papilloma virus in determining the HLA associated risk of cervical carcinogenesis. J Clin Pathol. 47(12):1077-1081.
- **Missaoui N; Hmissa S; Trabelsi A; Frappart L; Mokni M;Korbi S**., (2010). Cervix cancer in Tunisia: clinical and pathological study. Asian Pac J Cancer Prev. 11:235-8.
- **Mrudula Soma; Suhasini Kamaraj**, (2010). Detection of Human Papilloma virus in Cervical Gradings by Immunohistochemistry and Typing of HPV 16 and 18 in High-Grades by Polymerase Chain Reaction. J Lab Physicians. 2(1): 31–36.
- **Muñoz N; Bosch FX; de Sanjosé S; Herrero R; Castellsagué X; Shah KV;Snijders PJ; Meijer CJ.,** (2003). International Agency for Research on Cancer Multicenter Cervical Cancer Study Group: Epidemiologic classification of human papilloma virus types associated with cervical cancer. N Engl J Med. 348(6):518-527.
- **Munoz N; Castellsague X.; de Gonzalez AB; Gissmann L.,** (2006). Chapter 1: HPV in the etiology of human cancer. Vaccine. 24: 1-10.
- **National Cancer Institute,** (2014). Cervical Cancer Treatment. www.ncbi.nlm.nih.gov/pubmedhealth/PMH0032580.
- **National Cancer Institute,** (20014). Defining cancer. www.cancer.gov/publications/dictionaries/cancer-terms.

- **Nielsen NH; Jensen H; Hansen PK.,** (1988). Cervical cytology in Greenland and occurrence of cervical carcinoma, carcinoma in situ, and dysplasia. Extent and impact of uncoordinated screening activity 1976-1985.Arct Med Res.47: 179-88.
- **Nubia M; Xavier BF; Silvia S; Rolando H; Xavier C; Keerti V.,** (2003). Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 348:6.
- **Ononogbu U; Almujtaba M; Modibbo F; Lawal I; Offiong R; Olaniyan O; Dakum P; Spiegelman D; Blattner W; Adebamowo C.,** (2013). Cervical cancer risk factors among HIV-infected Nigerian women. BMC Public Health. 13:582.
- **P Shepherd; D Lunny; R Brookes T; Palmer D; McCance**, (1992). The Detection of Human Papillomaviruses in Cervical Biopsies by Immunohistochemistry and In Situ Hybridization. Scandinavian Journal of Immunology. 36:1-218.
- **Pagliusi SR; Garland SM.,** (2007). International standard reagents for HPV detection. Dis Markers. 23:283-96.
- **Parkin DM; Pisani P; Ferlay J.,** (1993). Estimates of the worldwide incidence of 18 major cancers in 1985. Int J Cancer. 54:594-606.
- **Patel NR; Rollison DE; Barnholtz-Sloan J; *et al*.,** (2009). Racial and Ethnic Disparities in the Incidence of Invasive Cervical Cancer in Florida. Cancer. 115(17): 3991-4000.
- **Reichman RC; Braunwald E; Focci AS; Hauser SL; Longo DL; Jameson JL**., (2011). 15th Ed. 2011.Human papilloma virus. In: Harrison's Principle of Internal Medicine.1118–1120.
- **Reimers LL; Anderson WF; Rosenberg PS; *et al*.,** (2009). Etiologic Heterogeneity for Cervical Carcinoma by Histologic Type, Using

Comparative Age-period-cohort Models. Cancer Epidemiol Biomarkers Prev. 18(3): 792-800.

- **Robert A Burger; Bradley J Monk; Tom Kurosaki; Hoda Anton-Culver; Steven AVasilev; Michael L Berman; Sharon PWilczynski**, (1996). Human Papillomavirus Type 18: Association with Poor Prognosis in Early Stage Cervical Cancer. JNCI: Journal of the National Cancer Institute. 88(19) 1361–1368.
- **Shapley M; Jordan J; Croft PR.,** (2006). A systematic review of postcoital bleeding and risk of cervical cancer. Br J Gen Pract. 56(527):453-60.
- **Sinayobye J; Sklar M; Hoover D; Shi Q; Dusingize J; Cohen M; MutimuraE; Asiimwe-Kateera B; Castle P; Strickler H.,** (2014). Prevalence and risk factors for High-Risk Human Papilloma virus (hrHPV) infection among HIV-infected and Uninfected Rwandan women: implications for hrHPV-based screening in Rwanda. Infect agent cancer. 9: 40.
- **Soma M; Kamaraj S.,** (2010). Detection of Human Papilloma virus in Cervical Gradings by Immunohistochemistry and Typing of HPV 16 and 18 in High-Grades by Polymerase Chain Reaction. Journal of Laboratory Physicians. 2(1):31–36.
- **Tarney CM; Han J.,** (2014). Postcoital bleeding: a review on etiology, diagnosis, and management. Obstetrics and Gynecology International. Volume 2014, Article ID 192087, 8 pages.
- **Tyring SK**., (2000). Human papilloma virus infection: Epidemiology, pathogenesis and host immune response. J Am AcaDermatol.43:18–26

- **Vaccarella S; Lortet-Tieulent J; Plummer M; Franceschi S; Bray F.,** (2013). Worldwide trends in cervical cancer incidence: Impact of screening against changes in disease risk factors. Eur J Cancer. 49(15):3262-73.
- **Wright TC Jr; Massad LS; Dunton CJ; Spitzer M; Wilkinson EJ; Solomon D.,** (2007). 2006 consensus guidelines for the management of women with abnormal cervical screening tests. Journal of lower genital tract disease. (4): 201-222.
- **Younis N; Khattab H; Zurayk H; el-Mouelhy M; Amin MF; Farag AM.,** (1993). A community study of gynecological and related morbidities
  in rural Egypt. Stud FamPlann. 24:175-186.
- **Zachary R.,** (2012). Self-Collection and HPV DNA Testing Could Be an Effective Cervical Cancer Screening Method. J Natl Cancer Inst. doi:10.1093/ jnci/djs029.
- **Zarchi MK; Akhavan A; Fallahzadeh H; *et al*.**, (2010). Outcome of Cervical Cancer in Iranian Patients According to Tumor Histology, Stage of Disease and Therapy. Asian Pacific J Cancer Prev. 11:1289-1291.
- **Zhai Lu; Hua Chen; Xiao-Mei; Zhengn Man-Ling Chen**., (2107). Expression and clinical significance of high risk human papilloma virus and invasive gene in cervical carcinoma.Asian Pacific Journal of Tropical Medicine. 10(2):195-200.
- **Zuna RE; Allen RA; Moore WE; Lu Y; Mattu R; Dunn ST.,** (2007). Distribution of HPV genotypes in women with cervical lesions: evidence for three categories of intra epithelial lesions based on morphology and HPV type. Mod Pathol. 20:167–7

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: ( )

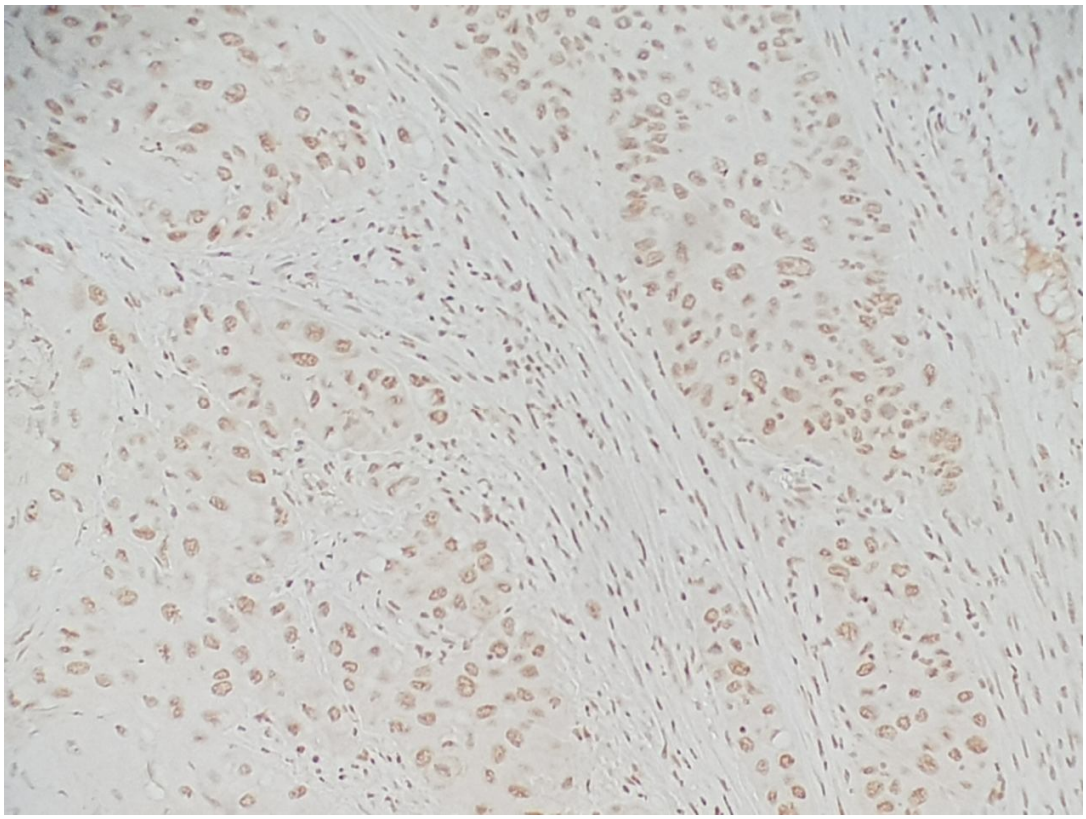
Tumor type: ( )

Tumor grade: ( )

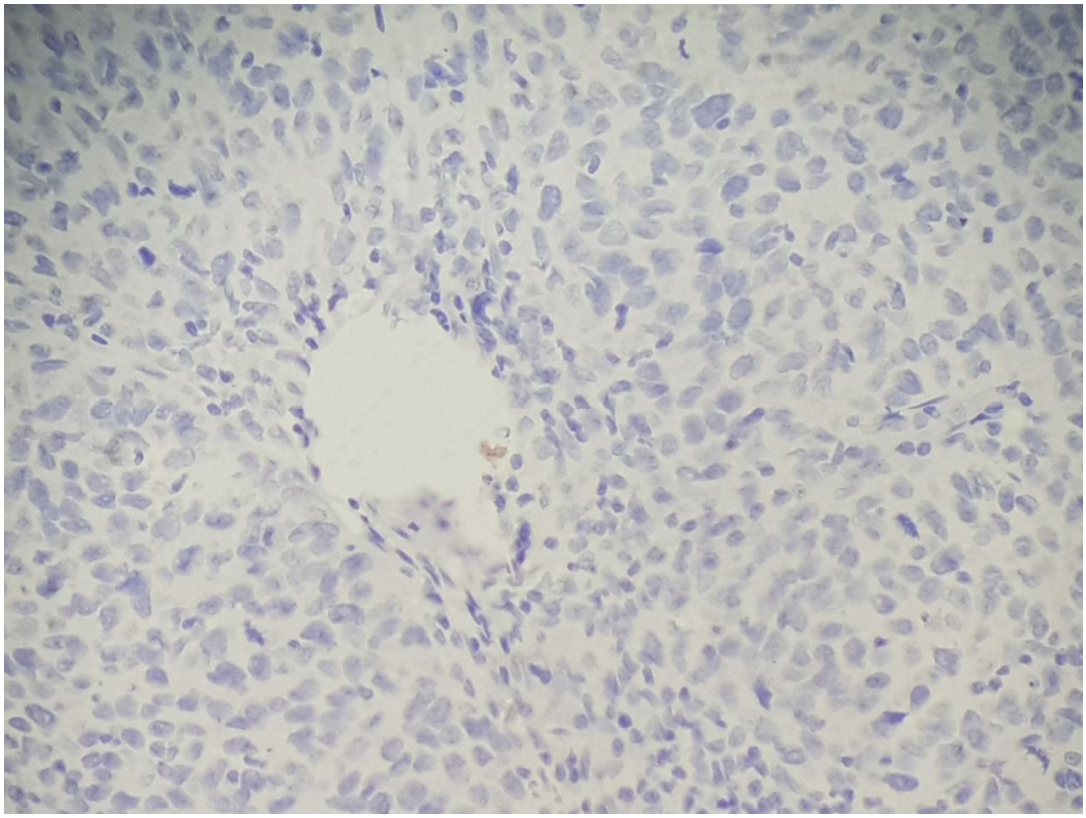
FIGO stage: ( )

IHC result: ( )

Expression score: ( )



SCC showing HPV18 positive immunostaining x40 (score 4) nuclear stains.



SCC showing HPV18 negative immunostaining x40 (score 0.00).

BioGenex
49026 Milmont Drive, Fremont, CA 94538
Tel: +1 (800) 421-4149 Fax: +1 (510) 824-1490
support@biogenex.com

Catalog No.	Description
AM362-5M	6 ml of Prediluted Antibody
MU362-UC	1 ml of Concentrated Antibody

Analyte Specific Reagent. Analytical and performance characteristics are not established.

Doc. No. 932-362M Rev. No. H
Release Date: 06-October, 2011

932-Format-ASR-0808

Clone
Cam Vir-1

Immunoglobulin Class
Mouse IgG2a

Specifications
This antibody stains HPV16 in formalin-fixed, paraffin-embedded tissue sections by immunohistochemical techniques.

Storage
Store at 2-8°; do NOT freeze. Do not use after expiration date on vial.

Source and Format
Mouse Monoclonal Antibody to HPV16 from immunoglobulin fractions, diluted in PBS, pH 7.6, containing 1% BSA and 0.09% sodium azide.

Precautions
For Professional use. This product contains no hazardous material at a reportable concentration according to U.S. 29 CFR 1910.1200, OSHA Hazard Communication Standard and EC Directive 91/155/EC. However, this product contains sodium azide, at concentrations of less than 0.1%. Sodium azide is not classified as a hazardous chemical at the product concentrations. However, toxicity information regarding sodium azide at product concentrations has not been thoroughly investigated. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing (Center for Disease Control, 1976, National Institute for Occupational Safety and Health, 1976). For more information, a Material Safety Data Sheet for sodium azide in pure form is available upon request. Do not pipette reagents by mouth, and avoid contact of reagents and specimens with skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with copious amounts of water. Minimize microbial contamination of reagents or else increase in nonspecific staining may occur.

Quality Control
Each lot of this antibody is tested by immunohistochemistry for Quality Control purposes. Refer to the BioGenex Quality Control Testing Conditions table for additional information.

References
Walboomers J, et al. In: Human Papillomaviruses and Cervical Cancer. Stern P and Stanley M, ed. Oxford Univ Press, 42-63, 1994.
Stanley M. In: Human Papillomaviruses and Cervical Cancer. Stern P and Stanley M, ed. Oxford Univ Press. 116, 1994.
Center for Disease Control. Center for Disease Control Manual Guide- Safety Managements, No. CDC-22, Atlanta, Georgia. April 30,1976.
National Institute for Occupational Safety and Health, (NIOSH). Rockville, MD. Publication No. 78-127, 1976

BioGenex Quality Control Testing Conditions

Parameter	Conditions Used
Control Tissue	N/A
Tissue Type	formalin-fixed, paraffin-embedded tissue sections