Chapter one

Introduction

1.1 Introduction:

Cervical cancer is a malignant neoplasm that develops in the squamous or glandular cells of the female uterine cervix (Bultaro, et al. 2012).

Cancer of the cervix is the second most common cancer in women worldwide after cancer of the breast. Based up on projection from the American cancer society, more than 555,000 new cases are diagnosed annually and nearly 310,000 women die from the disease. More than 473,000 of the new cases diagnosed in women living in developing countries where cervical cancer is the leading cause of the cancer death (more than 270,000 deaths per year). Cervical cancer is much less common in developed countries, affecting 87,500 women and accounting for approximately 40,000 deaths per year (Harris, 2013).

The cervix cancer is the second most common cancer in Sudan estimated from 12-15.5%. Expected cancer cases 8,000-10,000 new cases every year (RICK and NCI-UG, 2012).

The main risk factors for cervical cancer include exposure to human papilloma virus, smoking, parity and immune suppression. Other factors that have been linked with cervical cancer are race, socioeconomic status and sexually transmitted infections (Hurt, et al. 2012).

Fortunately cervical cancer can be detected even before it become cancer by the papanicolaue test which is evaluated for the presence of precancerous cervical lesion and cervical cancer can be identified, also other test include colposcopy, biopsy , large loop excision of the transformation zone (LLETZ), cone biopsy, chest X-ray, computerized temoghraphy(CT) scan (Dizon,et al.2009).

The surgery and combination of chemotherapy and radiotherapy are the most common treatment for cervical cancer (O'Callaghan, 2011).

Immunohistochemistry is a technique for identifying cellular or tissue constituents (Antigen) by mean of antigen-antibody interaction. The recent introduction of prognostic and predictive markers in immunohistochemistry has made a tremendous impact on patient treatment and management this has been made possible by the gradual development of immunohistochemical methodologies over the past 60 years which allow the identification of specific or highly selective cellular epiptoes in formalin fixed paraffin processed tissue with an antibody and appropriate labeling system (Bancroft, 2008).

Ki67, is a proliferation marker known as predictive factor for tumor development , is define as a nuclear antigen (associated with hetro and euchromatin) expressed during all active phases of all cell cycle (G1,S,G2,M) except G0, the level of Ki67 expression is used to determine the cell proliferation status (Ancuta, et al. 2009).

Her2, (also called cerb2 or neu) is a receptor tyrosine kinase involved in normal cell growth. Her2 over expression is correlated with shortened disease-free and overall survival and it is adverse prognostic factor associated with poorly differentiated, high grade tumor; high rates of cell proliferation; and lymph node involvement (Pollock, et al . 2008).

Cervical cancer is second most common cause of cancer –related death in woman in worldwide , therefore this study done to detect the expression of Ki67 and Her2 marker as prognostic or predictive value to help the management of treatment and useful for helping and facilitating the follow up of this disease.

1-2 Objectives:

1-2-1General objective

To detect the expression of Ki67 and Her2 in cervical tumor among Sudanese women using immunohistochemical method.

1-2-2 specific objectives

To detect the expression of Ki67 and Her2 in cervical tumor using indirect method.

To correlate the expression of Ki67 and Her2 with histological diagnosis and grade of tumor.

Chapter two

Literature review

2. Literature review:

2.1 Scientific background:

The role of the cervix in healthy woman is principally concerned with reproduction; it helps to keep the developing fetus in the uterus and has a part to play in initiation and progression of habour. The mucus produced by cervix is considered important in female fertility. The cervix is also thought to have a function in the female sexual response (Dunleavy, 2009).

2.1.1 Anatomy of cervix:

The cervix is cylindrical and measures 3 to 4cm in height and 2cm in diameter. Its posterior extent is covered with peritoneum, which is reflected from the back of the vagina. The anterior portion of the supravaginal cervix is separated from the bladder by endopelvic fascia. Laterally the cervix is bounded by the structures lying within the broad ligament (Baggish, et al. 2007).

The cervix anatomy is subdivided into the endocervix and ectocervix. The endocervix is named for the upper two thirds of the cervix and the ectocervix the lower two thirds. The ectocervix and endocervix are lined with two different type of epithelium. The endocervix is lined with columnar glandular epithelium and the ectocervix with squamous epithelium (Dunleavy, 2009).

2.2 Inflammatory disorder:

2.2.1 Atypia of repair:

It is occur in cases of severe, acute or long stranding chronic inflammatory or infection, e.g. erosion (ectopion). The squamous and endocervical epithelium undergo reactive change i.e., epithelial

disorganization and nuclear atypia. The pathological change includes nuclear enlargement and mitotic figure (Salhan, et al. 2011).

2.2.2 Cervicitis:

The term chronic cervicitis often conveys differing images to clinician, colposcopies, and pathologist. It describes the clinical appearance of cervix that is red, inflamed and irregular on the surface. The organisms most commonly responsible for active inflammation in the cervix include Candida Albicans, Trichomonas vaginalis and herpes simplex virus (HSV). To this list should be added Chlamydia Trachomatis, Human papilloma virus (HPV) and Cytomegalovirus (Robboy's, et al. 2009).

2.2.3 Endometrosis:

It refers lesions composed of ectopic endometrial glands and stroma. It may occur on the portio vagainalis of the cervix or in the endocervical canal. They may appear as one or more small blue area. The nodules measuring few millimeters in diameters (Salhan, et al. 2011).

2.3 Tumors of cervix:

2.3.1 Benign tumors:

2.3.1.1 Endocervical polyps:

It constitutes the most common growth of uterine cervix; they are focal, hyperplastic protrusion of endocervical fold, including epithelium and substantia propria. It is found most commonly in fourth to sixth decades of life (Salhan, et al. 2011).

2.3.1.2 Inclusion cyst:

Traumatic inclusion cysts are form of epidermal inclusion cyst that commonly occurs in vaginal intrapartum laceration. They are thought to develop from variable fragment of epithelium that may become entrapped within the stroma at the time of obstetric trauma or subsequent surgical

repair. They are uncommon on cervix; they are unilocular cystic structure 1-2 cm in diameter beneath (Salhan, et al. 2011).

2.3.1.3 Other tumor like lesion:

Decidual pseudo polyp and decidualization occur due to decidual changes in cervix in pregnancy. On exocervix it may appear as a raised plaque or pseudo polyp that can be mistaken for carcinoma. Other tumor like lesion may appear as nodules on raised lesion over exocervix may pathologically be a postoperative spindle cell nodule or inflammatory pseudo tumor or lymphoma like lesion, biopsy will settle the tissue (Salhan, et al. 2011).

2.3.2 Cervical intraepithelial neoplasia (CIN):

Cervical intraepithelial neoplasia may be define as " a spectrum of intraepithelial change which begins as a generally well differentiated neoplasm which has traditionally been classified as mild dysplasia and ends with invasive carcinoma. In CIN the full thickness of epithelium is said to be occupied by the neoplastic cells: in many cases however, there is cytoplasmic differentiation in the upper part of the epithelium and hence CIN is usually graded in terms of the proportion of the epithelial thickness occupied by undifferentiated neoplastic cells of basaloid type. The CIN terminology divides cervical cancer precursors into three groups distinguished by the extent to which the full thickness of the epithelium is composed of undifferentiated neoplastic cells: CIN1 corresponds to lesion previously diagnosed as mild dysplasia, CIN2 correspond to moderate dysplasia and CIN3 to both severe dysplasia and carcinoma insitu (Rajaram, et al. 2012).

2.3.3 Invasive carcinoma of cervix:

Cancer is put in categories according to the cell from which they originally formed. Cervical cancer that develops from squamous

epithelial cells is called squamous cell carcinoma. Cervical cancer that develops from columnar epithelial cells is called adenocarcinoma. Most cervical cancers are squamous cell carcinoma. Squamous cell carcinoma is responsible for about 80 to 90 percent of all cervical cancer. The tumors of squamous cell carcinoma look like small lumps or ulcer of different sizes. Adenocarcinoma occurs in the cell that make up the glands in the cervix. The glands of the cervix excrete mucus. About 10 to 20 percent of cervical cancers are adenocarcinoma. There are four types of cervical adenocarcinoma: clear cell adenocarcinoma, papillary adenocarcinoma, mucinous adenocarcinoma, and adenosquamous carcinoma. Cervical adenocarcinoma is most common in women over the age of forty five, but it can occur at any age, even during the teenage years (Hasan, 2009).

2.4 Risk factors:

2.4.1 Human papilloma virus (HPV):

It is well established that HPV definitive pathogenic factors of cervical carcinoma as advocated by Walboomers, who has stated that " HPV is found in nearly all cervical cancer pathological samples highlighting that HPV is the main cause of cervical carcinoma and thus, cervical carcinoma becomes the only human cancer with the definite pathogenic factor (Lai, 2009).

2.4.2 Smoking:

There are a few different ways that smoking can increase the risk of cervical cancer in women which can be by direct and indirect methods of inducing cervical cancer. A direct way of contracting this cancer is a female smoker has a higher chance of CIN3 occurring which has the potential of forming cervical cancer (Walker, et al. 2013).

2.4.3 Parity:

High parity and large number of pregnancies have been correlated with development of cervical cancer for a long time. Pregnancy could also induce a hormonal effect on the cervix which further increases the risk of oncogenic progression (Dunleavy, 2009).

2.4.4 Immunosuppression:

A recent showed that women with HIV/AIDS have a six-fold increased risk of cervical cancer and women who have undergone organ transplant have more than double the risk, strongly suggesting that immunosuppression plays a role (Grulish, et al. 2007). The International Agency for Research on Cancer (IARC) states that HIV is a cause of cervical cancer (Cogliano, et al. 2011).

2.4.5 Other factors:

2.4.5.1 Socioeconomic status:

Cervical cancer is a main problem of the developing countries and the burden of the disease is borne by the poor women in these countries. Inadequate resources in the developing world result in the inequitable burden of cervical cancer in the developing countries. Cervical cancer is synonymous to poverty and disease of poor women and poverty is endemic and a big problem in Africa. Poverty alone is a very important barrier for education, prevention, treatment and care (Zeferino and Derchain, 2006).

2.4.5.2 Sexually transmitted infection:

Infection with two sexually transmitted infections can increase the risk of cervical cancer: Chlamydia Trachomatis and Herpes Simplex Virus-2 (HSV-2). Both have been associated with twofold increase risk but exact mechanism has not been identified (Zenilman and Shahmansh, 2012).

2.4.5.3 Oral contraceptive use:

The duration of oral contraceptive use and risk of cervical cancer have been shown to have a positive correlation in a number of studies (Dunleavy, 2009).

2.5 Diagnosis:

2.5.1 Pap smear:

The main role of the Pap smear (also called a Pap test)is to help prevent cancer. It shows whether women have abnormal pre-cancerous cells in their cervix. All women under 70 years of age who are or who have ever been sexually active should have a Pap smear every two years. Women who have had abnormal cell changes should be tested more often. During a Pap smear, a doctor uses an instrument such as a brush or spatula to take some cells from the surface of the cervix (O'callaghan, 2011).

2.5.2 Colposcopy:

A colposcopy can help identify where abnormal or changed cells are located and what they look like. In this procedure, the instrument called a speculum into vagina to hold the walls slightly apart. Using an instrument called a colposcope, which looks like a pair of binoculars sitting on a large stand, can see a magnified picture of cervix, vagina and vulva. The colposcope is not put into body. The doctor will probably take a tissue sample (biopsy) during the colposcopy (O'callaghan, 2011).

2.5.3 Biopsy:

A biopsy is removes some tissue and sends it to the laboratory for examination under a microscope. Biopsies are typically done in a clinic and the results are usually available in about a week (O'callaghan, 2011).

2.5.4 Large loop excision of the transformation zone (LLETZ):

LLETZ is another type of procedure to remove some cervical tissue for examination. A loop of wire carrying an electric current is used to cut out

the abnormal tissue from the cervix. Sometimes the doctor can remove all visible abnormal cells. The procedure takes about 10 minutes. It may be done under a local anaesthetic in the doctor's clinic or under a general anaesthetic at hospital. In some cases, the doctor may do it at the same time as a colposcopy (O'callaghan, 2011).

2.5.5 Cone biopsy:

A cone biopsy is used to determine how deeply cancer cells have spread into tissue beneath the surface of the cervix. A cone biopsy is also used to treat very early and very small tumors. Further treatment is needed for cancers that are larger. This procedure removes a cone shaped piece of tissue containing the abnormal cells from the cervix. It is performed under a general anaesthetic and involves a day or overnight stay in hospital. Cone biopsy results are usually available within a week (O'callaghan, 2011).

2.6 Staging of cervical cancer:

There are a number of staging systems for cervical cancers but the one which is most widely employed was determined by the international federation of gynecology and obstetrics (FIGO) in the late 1950s, and is known as the FIGO system. Basically stage I tumor are confined to the cervix, whereas stage II to IV extend beyond the cervix. According to the FIGO report 2006, globally 42% of cervical cancer cases are diagnosed at stage I, 30% at stage II, 21% at stage III and 6% at stage IV. The report includes: Stage 0: This is more commonly referred to as carcinoma insitu.

Stage I: describes an invasive cancer which is confined to the cervix.

Stage II: The cancer has begun to spread beyond the cervix but not to the pelvic side wall. It may involve the upper part of the vagina but not the lower third.

Stage III: The cancer has extended through the cervix on to the pelvic wall. On rectal examination there is no cancer free space between tumor and pelvic side wall. The tumor involves the lower third of the vagina.

Stage IV: the carcinoma extends beyond the true pelvic or clinically involved mucosa of bladder or rectum (Dunleavy, 2009).

2.7 Prognostic factors in cervical cancer:

As with most cancers, stage at diagnosis is the most important prognosis factors in cervical cancer women diagnosed with early stage disease have survival rates of 85% to 100%. Stage II a tumor also has a good prognosis, with a five year survival of approximately 90%. However, once the tumor spread further than this the prognosis is poorer. The estimated five-year disease-free survival rate after therapy for stage IB2 and IIB disease is 50-70%, for stage III disease is 30-60% and for stage IV disease is 5-10%. Amongst patients with early stage disease who are treated surgically, a number of other factors have been shown to influence the prognosis of patient diagnosed with cervical cancer including: regional lymph node involvement, tumor size and parametrical invasion (Dunleavy, 2009).

2.8 Treatment of cervical cancer:

2.8.1 Surgery

Surgery is common for women who have small tumors found only within the cervix. The type of surgery will depend on the extent of the cancer such as hysterectomy. A hysterectomy is the surgical removal of the uterus and cervix. These are performed under a general anaesthetic (O'callaghan, 2011).

2.8.2 Radiotherapy

Radiotherapy uses x-rays to kill cancer cells or injure them so they cannot multiply. The radiation is targeted at cancer sites and treatment is

carefully planned to do as little harm as possible to healthy body tissues. Radiotherapy is usually given if not well enough for a major operation or if the cancer has spread into the tissues or lymph nodes surrounding the cervix. It may also be used after surgery or in combination with chemotherapy (O'callaghan, 2011).

2.8.3 Chemotherapy

Chemotherapy uses drugs to kill or slow the growth of cancer cells. The aim is to destroy fast growing cancer cells while causing the least possible damage to healthy cells. However, some healthy, fast growing cells, such as hair and bone marrow cells, may be affected (O'callaghan, 2011).

2.8.4 Chemoradiation:

A combination of radiotherapy and chemotherapy (chemoradiation) is usually used to treat advanced cervical cancer. Research in the US has found that women with invasive cervical cancer have better survival rates when they are given both treatments (O'callaghan, 2011).

2.9 Epidemiology of cervical cancer:

Cervical cancer is a leading cause of morbidity, mortality and premature death among middle-age women in many developing countries. Cervical cancer accounted for an estimated 530,000 newly diagnosed cases, and 275 deaths worldwide in the year 2008, four fifth of which occurred in the low and medium resource countries, the estimated new cervical cancer cases and deaths in different region of the world, it is quite likely that the burden of disease in several countries is under estimated given the inadequately of diagnostic and treatment services and cancer information systems (Rajaram, et al. 2012).

In the Sudan 8000 –10, 000 New Cancer Cases are treated in the two oncology centers. Expected cancer cases 39 000 –40 000 new cases every

year. Cases seen far less than expected cases cervical cancer is second most common cancers in females in Sudan. The Breast Cancer 29 –34.5% and Cervical Cancer 12 –15.5% (RICK, 2012) and(NCI-UG, 2012). Although sufficient knowledge and experience now exist to initiate, expand or reorganize programs to reduce the burden of cervical cancer, prevention and early detection initiatives have unfortunately not received due attention and continue to be neglected in most developing countries (Rajaram, et al. 2012).

2.10 Immunohistochemistry technique:

Immunohistochemical staining can be used in a number of ways. It may be used to identify the tissue of origin of metastatic tumors or to identify specific tumor markers or tumor types.

The basis of immunohistochemistry is an antigen-antibody reaction, revealed in the tissue by a chromogen complexed with, or activated by, antibody, which is visualized by the pathologist down the microscope.

It requires the unmasking of some tissue antigen, by heat treatment or enzyme digestion, in formalin-fixed tissue, prior to application of the primary antibody. Antibodies have been specifically developed against particular tissue antigen. Antigen –antibody complex are visualizing by attaching a secondary antibody to the complexed tissue aggregate which, in turn, is converted into a colored end product by activation of chromogen or by the incorporation of marking chromogen to the antibody (Payne, et al. 2012).

Many antibodies are now available to identify epitopes that survive the rigors of formalin fixation and paraffin-embedding. In cases where morphology and clinical data alone do not allow firm diagnosis. Then immunohistochemistry is invaluable and, with the ever – increasing use of prognostic and predictive markers the pathologist is faced with a

decision that could profoundly affect patient therapy and management (Bancroft, 2008).

2.11 Her2 marker:

Her2 (human epidermal growth factor receptor type 2) also called Cerb2 or neu. It is a receptor tyrosine kinase involved in normal cell growth. Her2 over expression is correlated with shortened disease-free and overall survival and it's an adverse prognostic factor associated with poorly differentiated, high grade tumors; high rates of cell proliferation; and lymph node involvement. However, tumors are expressing. High level of her2 expression can be used to identify patients for whom trastuzumab, a monoclonal antibody that target the extracellular domain of the her2 protein, may be of clinical benefit. Her2 may be assessed by using immunohistochemical analysis to detect her2\neu protein overexpression or by using fluorescence insitu hybridization (FISH) to detect her2\neu gene amplification. Both of these methodologies have been validated to have clinical utility. However, demonstration of her2 amplification by FISH is thought to be better predictor of response to trastuzumab-based therapy than her2 over expression by immunohistochemical analysis. The predictive values for gene amplification are best for, cases that score 3+ on immunohistochemical analysis on a scale of 0 to 3+ immunohistochemical analysis is often used as a screening tool, and FISH is used to confirm a positive result on immunohistochemical analysis (Pollock, et al. 2008).

Carrerase , et al (2007) who concluded that the c-erb-B2 expression changed in relation to severity of the lesion and that it could be helpful in making a differential diagnosis.

Fuchs, et al (2007) who concluded the her2 was over expression in the carcinoma was significantly associated with poor prognosis.

Gupta, et al (2009) who concluded the higher expression of HER-2/neu was noted in malignant lesions as compared to benign lesions

2.12 Ki67 marker:

The antibody Ki67 react with the nuclear Ki67 antigen, a protein encoded by Ki67, on 10q25, Ki67 is only present in proliferating cells. In normal exocervical epithelia, its only expressed in the suprabasal layer, and in CIN cases, its expressed throughout the different epithelial layers. The Ki67 labeling index increases, according to degree of squamouse cervical neoplasia (carreras, et al.2007).

The nuclear antigen Ki67, measured by immunohistochemical analysis, is a used marker of cell proliferation. Its level correlate well with other markers of proliferation such as S-phase fraction measured by flow cytometry and mitotic index. Ki67 also correlate with histologic grade. High level of Ki67 is associated with poorly differentiated tumors. Overall, Ki67 appears to be correlated with decreased disease-free survival, but independent significance is thought to be modest. At this time, the clinical utility of Ki67 as a prognostic marker furthermore, there is interest in evaluating Ki67 as a pharmacodynamic marker of response to endocrine and, potentially, other targeted therapies (Pollock, et al 2008).

Carrerase, et al (2007) who concluded that ki67 expression changed in relation to severity of the lesion and that it could be helpful in making a differential diagnosis.

Brown, et al (1988) who concluded that there was no significant relationship between the percentage of positive cell and tumor grade or cell type.

Davidson , et al (2000) who concluded that Ki-67counts does not appear to have a significant role in the prediction of survival in cervical squamous cell carcinoma.

Chapter three

Materials and Methods

3.1 Materials:

Formalin fixed tissue previously diagnosed as cervical tumors were used in this study, patient's identification data obtained from patient's files records.

3.2 Methodology:

3.2.1 Study design:

This is a descriptive retrospective study aimed to determine the distribution of expression of Her2 and Ki67 in cervical tumor using immunohistochemical method.

3.2.2 Study samples:

Forty five cervical tumor biopsy formalin fixed paraffin embedded archival blocks were taken and previous diagnosed by haematoxylin and eosin, they have been selected as a ten benign and thirty five malignant cervical tissue.

3-2.3 Study area:

This study conducted in Ribat university hospital, Soba university hospital and the Medical military hospital during the period from January to June 2014.

3.2.4 Tissue processing:

3.2.4.1 Immunohistochemical staining:

The 3 micrometer section for IHC incubated for overnight in an oven at 65^0 C, then brought to water using two change of xylene for 3 minutes each; two changes of absolute ethanol 3minutes each; two minutes descending grades of ethanol (90%, 70%, 50%) and finally section rehydrates in distilled water. Then sections retrieved according to Biogenex protocol and Quarteett protocol for Her2 and ki67 respectively

,antigen retrieval continued for 40minutes in water bath at 97c then equilibrated in phosphate buffer saline (P.B.S PH 7.4) at room temperature for 5-10 minutes, then section stained as mentioned in Biogenex protocol or Quarteett protocol following : endogenous peroxidase blocking for 10 minutes, wash in P.B.S for 3 minutes; then primary antibody the Her2 applied code (EP1045Y) and ki67 applied Quarteett kits for 40 min then section washed in P.B.S for 3 minutes, secondary antibody was added for another 40 min, then section washed in P.B.S for 3 minutes and finally color developed by incubation in DAB for 10 minutes then slides rinsed in tap water for 10 minutes, nuclei counter stain briefly in mayer's haematoxylin for 2-3 minutes and then washed in water for 5 minutes, section dehydrated and mounted.

3.2.5 Ethical consideration:

All samples were taken from authorized center for these samples research purposes.

3.2.6 Result interpretation:

All quality control positive and negative control measures were adopted during specimen processing for assessment of immunohistochemical results.

3.2.7 Statistical analysis:

All information about the study samples were entered a computer as well as obtained result. The data was analyzed using version 16 SPSS computer program, frequencies, chi square and means were calculated.

Chapter Four

Results

4- Results:

A total of 45 samples with cervical tumor were investigated by conventional histopathology, immunohistochemical methods. Histopatological diagnosis revealed benign tumors 10(22%) samples and the other 35 (78%) samples malignant tumors. The malignant tumors included 29 (83%) samples squamous cell carcinoma and 6 (17%) samples adenocarcinoma as indicated in Table (4.1).

Out of 45 patients the age ranged between 27 to 85 years old with a mean age of 57 years. Most patients were aggregated in less than the age of 50 years representing 18 (40%) patients and the remaining 27 (60%) patients were older than 50 years as indicated in Table (4.2).

Among the study subject, Ki67 showed positive expression in 14 (31%) samples and negative in 31 (69%) samples. Her2 showed positive expression in15 (33%) samples and negative expression in 30 (67%) samples as indicated in Table (4.3).

Malignant tumors and Ki67 expression and Her2 expression were positive in14 (31%) samples and negative in 21(69%) for Ki67 expression while the Her2 expression positive in 15(33%) samples and negative in 20 (67%) samples. Benign tumors and Ki67 and Her2 there were no positive expression all samples while negative in 10 (22%) samples, this result is the same in Ki67 and Her2. This result showed significant statistical association indicated in Table (4.4)

The number of positive samples for Ki67 and Her2 in squamous cell carcinoma was 14 (40%) samples and 10 (29%) samples respectively. 15 (43%) samples and 19 (54%) samples was negative respectively in squamous cell carcinoma. The positive expression of Ki67 and Her2 in

adenocarcinoma were no positive expression samples and 5.0 (14%) samples respectively and negative expression showed in 6.0 (17%) samples and 1.0 (3.0%) samples in Ki67 and Her2 respectively. This result showed significant statistical association indicated in Table (4.5).

Histopathological grade and Ki67 and Her2 expression were positive in 3.0 (10%) samples and 1.0 (3.0%) samples and negative in 2.0 (7.0%) samples and 4.0 (13%) this result showed in well differentiation in Ki67 and Her2 respectively. Also showed 4.0 (13%) samples and 8.0 (27%) samples were positive and 12 (40%) samples and 8.0 (27%) samples were negative in moderate differentiation in Ki67 and Her2 expression respectively. 7.0 (23%) samples and 1.0 (3.0%) samples were positive expression and negative in 2.0 (7.0%) samples and 8.0 (27%) samples showed in poorly differentiation in Ki67 and Her2 expression respectively. This result showed significant statistical association with Ki67 expression and no significant statistical association with Her2 expression.

Table (4.1): Distribution of the study samples by histopathological diagnosis.

Histopathological diagnosis	Frequency	Percent (%)
Malignant	35	78
Benign	10	22
Total	45	100

Table (4.2): Distribution of age among the study samples.

Age group (year)	Frequency	Percent (%)
Less than 50	18	40
More than50	27	60
Total	45	100

Table (4.3): Expression of Ki67 and Her2 among the study samples.

Variable	Positive		Negative		Total	
	N	%	N	%	N	%
Ki67	14	31	31	69	45	100
Her2	15	33	30	67	45	100

Table (4.4): Correlation between Ki67 and Her2 expression and study samples.

Variable	Malignant				Benign				P.value
	positive		Negative		Positive		Negative		
	N	%	N	%	N	%	N	%	
Ki67	14	31	21	69	0.0	0.0	10	22	0.016
Her2	15	33	20	67	0.0	0.0	10	22	0.011

Table (4.5): Correlation between Ki67 and Her2 expression and type of malignant tumors.

Type of malignant tumors	Ki67 expression				Her2 expression			
	Positive		Negative		Positive		Negative	
	N	%	N	%	N	%	N	%
Squamous cell carcinoma	14	40	15	43	10	29	19	54
Adenocarcinoma	0.0	0.0	6.0	17	5.0	14	1.0	3.0
P.value	0.028				0.028			

Table (4.6): Correlation between expression of Ki67 and Her2 and histopathological grade.

Histopathological grade	Ki67 expression				Her2 expression			
	Positive		Negative		Positive		Negative	
	N	%	N	%	N	%	N	%
Well differentiation	3.0	10	2.0	7.0	1.0	3.0	4.0	13
Moderate differentiation	4.0	13	12	40	8.0	27	8.0	27
Poor differentiation	7.0	23	2.0	7.0	1.0	3.0	8.0	27
P.value	0.032				0.111			

Chapter five

Discussion

5- Discussion:

Cancer of the cervix is one of the most common cancer in women, especially in developing countries (Olsen, et al. 2012), and is the second most common cancer in Sudan, and is a major cause of premature death in middle-aged and older women (RICK,2012).

Our study aimed to detect Ki67 and Her2 expression patterns related to the cervical tumors, the diagnosis of cervical tumors from 45 samples were taken 35 (78%) samples were malignant tumors and 10 (22%) samples were benign tumors.

The age group among study samples 18 (40%) patients were less than 50 year and 27 (60%) were found more than 50 year. This indicates that the risk of developing cervical tumor is increased with increasing age. This result is agree with Rositch, et al (2014) who reported that the higher age-specific cervical cancer incidence rates, in the peak incidence to older women given high rate of cervical cancer in women over the age of 60 to 65 years.

The expression of Ki67 and Her2 among the study group, 14 (31%) samples were Ki67 positive and 31 (69%) from total samples were Ki67 negative, and 15 (33%) from total samples were Her2 positive and 30 (67%) were Her2 negative.

The relation between Ki67 and Her2 and histological diagnosis, there were 14 (31%) samples of malignant tumors were Ki67 positive and 21 (69%) samples were Ki67 negative, and there were 10 (22%) samples from total samples were benign tumors and were Ki67 negative while there were 15 (33%) samples of malignant tumors were Her2 positive and

20 (67%) samples were Her2 negative and there were 10 (22%) samples from total samples were benign tumors were Her2 negative. This result showed that there is significant different between Ki67 and Her2 expression and histological diagnosis (P.value 0.016 and 0.011 respectively) for Ki67 and Her2. This result agrees with Son, et al (2012) who reported that the expression of Ki67 was significant higher in malignant lesion (squamous cell carcinoma than benign lesion). Also these results agree with Gupta, et al (2009) who reported that the higher expression of Her2 was noted in malignant lesion as compared to benign lesion.

The relation between Ki67 and Her2 expression and type of malignant tumors, there were 35 (78%) samples were malignant tumors, 29 (83%) samples were squamous cell carcinoma and 6.0 (17%) samples were adenocarcinoma. Showed 14 (40%) samples were Ki67 positive in squamous cell carcinoma and 15 (43%) samples were Ki67 negative and also were no appearance of Ki67 positive result in adenocarcinoma while there were 6.0 (17%) samples were found Ki67 negative .This result showed significant different between Ki67 and type of malignant tumors (P.value 0.028). This result agree with son, et al (2012) who reported that the specimen containing squamous cell carcinoma was found to have higher frequency of Ki67 expression.

There also the results showed that 10 (29%) samples were squamous cell carcinoma were Her2 positive and 19 (54%) samples were squamous cell carcinoma were Her2 negative, while 5.0 (14%) samples were adenocarcinoma that were Her2 positive and 1.0 (3.0%) samples were Her2 negative in adenocarcinoma. From this result that is significant different between her2 expression and malignant tumors (P.value 0.028).

This result agrees with Lee, et al (2003) who reported that there was significant increased expression of Her2neu with adenocarcinoma.

The relation between expression of Ki67 and Her2 and histopathological grade, there were 5.0 (17%) samples were well differentiation and 16 (53%) samples were moderately differentiation and there were 9 (30%) samples were poorly differentiation , there were 3.0 (10%) samples were Ki67 positive in well differentiation, while there were 2.0 (7.0%) samples Ki67 negative, 40 (13%) samples were Ki67 positive in moderately differentiation and 12 (40%) samples were Ki67 negative in moderately differentiation, while there were 7.0 (23%) samples were Ki67 positive and 2.0 (7.0%) samples were Ki67 negative this result showed in poorly differentiation. From this result that there is significant different between Ki67 expression and histopathology grade (P.value 0.032). These results agree with Carreras, et al (2007) who reported that there was increased expression of Ki67, according to the degree of squamous neoplasia and useful in distinguishing the different grades of neoplasia.

Also showed there were 1.0 (3.0%) samples Her2 positive and 4.0 (13%) samples were Her2 negative in well differentiation and there were 8.0 (27%) samples were Her2 positive and 8.0 (27%) samples were Her2 negative in moderately differentiation while there 1.0 (3.0) samples were Her2 positive and 8.0 (27%) samples were Her2 negative in poorly differentiation. From this result there is no significant different between Her2 expression and histopathological grade (P. value 0.111). These results disagree and discrepant with Gupta, et al (2009) who reported there was expressed of Her2 related with high grade and more aggressive tumors and having poor prognosis in cervix carcinoma.

Chapter six

Conclusion and Recommendations

6-1 Conclusion

From this study we conclude that:

The Ki67 and Her2 expression associated with malignant cervical tumors rather than benign tumors.

The expression of Ki67 correlated significant in squamous cell carcinoma while the expression of Her2 correlated significant with adenocarcinoma.

There is significant relation between the histopathological grade and Ki67expression while there is no significant relation between the histopathological grade and Her2 expression.

6-2 Recommendations:

From this study we recommend that:

Ki67 and Her2 should be examined to confirm the diagnosis.

Molecular biology techniques should be used to detect of genome or amplification and mutation of ki67 and Her2 in history of cervical cancer patients.

Further studies should be done using large sample size.

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Appendix I:

 # Immunohistochemical solution:

- EZ-AR (Citra-base solution PH 6.0):

***** Citra base 10ml

***** Distilled water 90ml

- Hydrogen peroxidase

- Phosphate buffer saline (PH7.4):

*** Stock (A)**

- 0.2M Sodium dihydrogen Orthrophosphate:

Sodium dihydrogen orthrophosphate 3.2g

Distilled water 100ml

*** Stock (B)**

Disodium hydrogen orthrophosphate 2.83g

Distilled water 100ml

- Take 9.5 ml from stock (A) and 40.5ml from stock (B) and made up to 100ml with distilled water.

-DAB chromogen kits:

3.3 Diamino benzidine tetra hydrochloride.

Substrate buffer.

Substrate (hydrogen peroxide).

Other material and reagents:

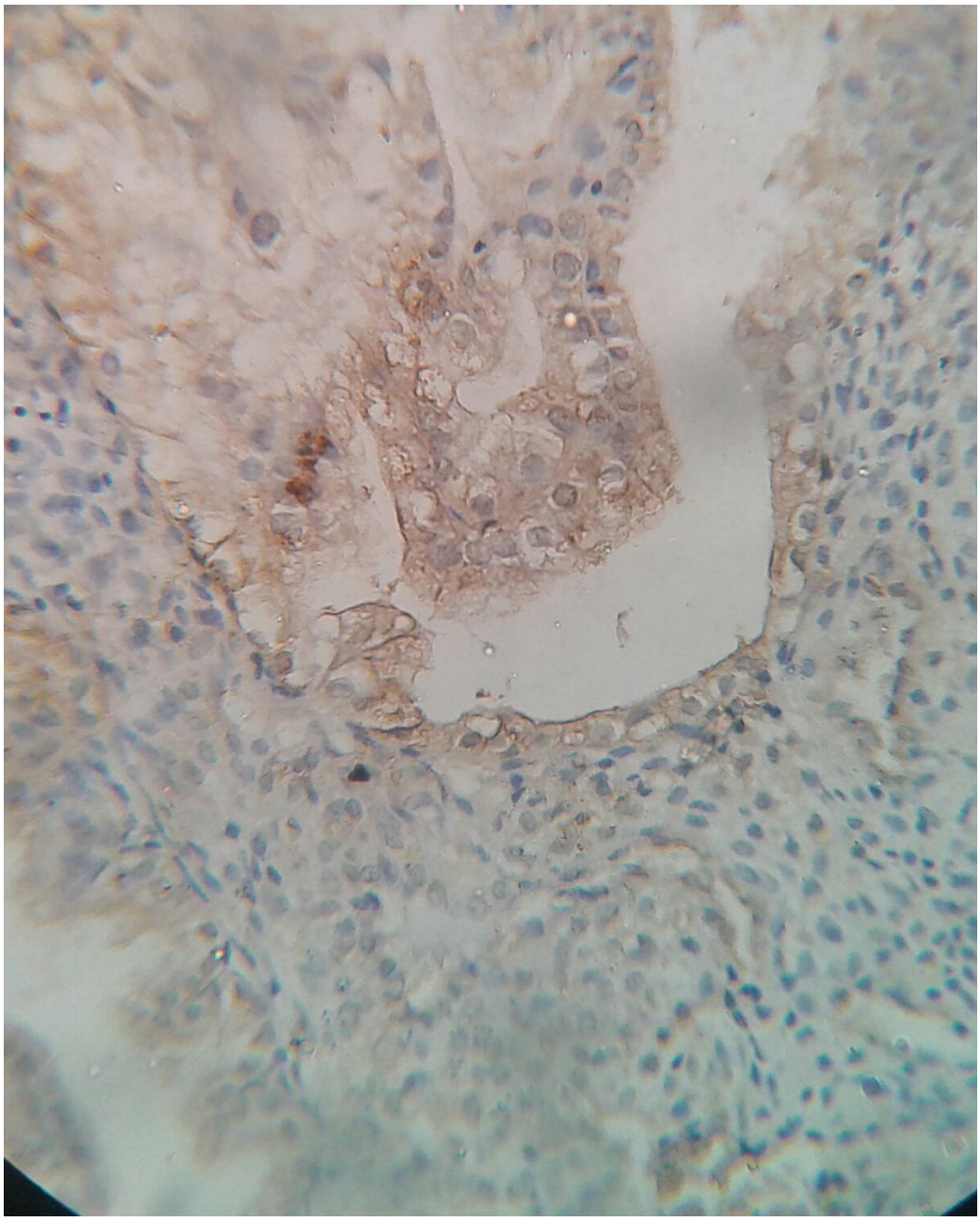
- Water bath.
- Microtome.
- Standard light microscope.
- Coblinjar.
- Measuring cylinder.
- Plastic pasture pipettes.
- Timer.

- Sterile gloves.
-Humid plate.
- Staining jars.
- Slides.
- Cover glass.
- Positive control tissue blocks.
- Xylene.
- Absolute alcohol**.**
- Mounting media. (DPX).

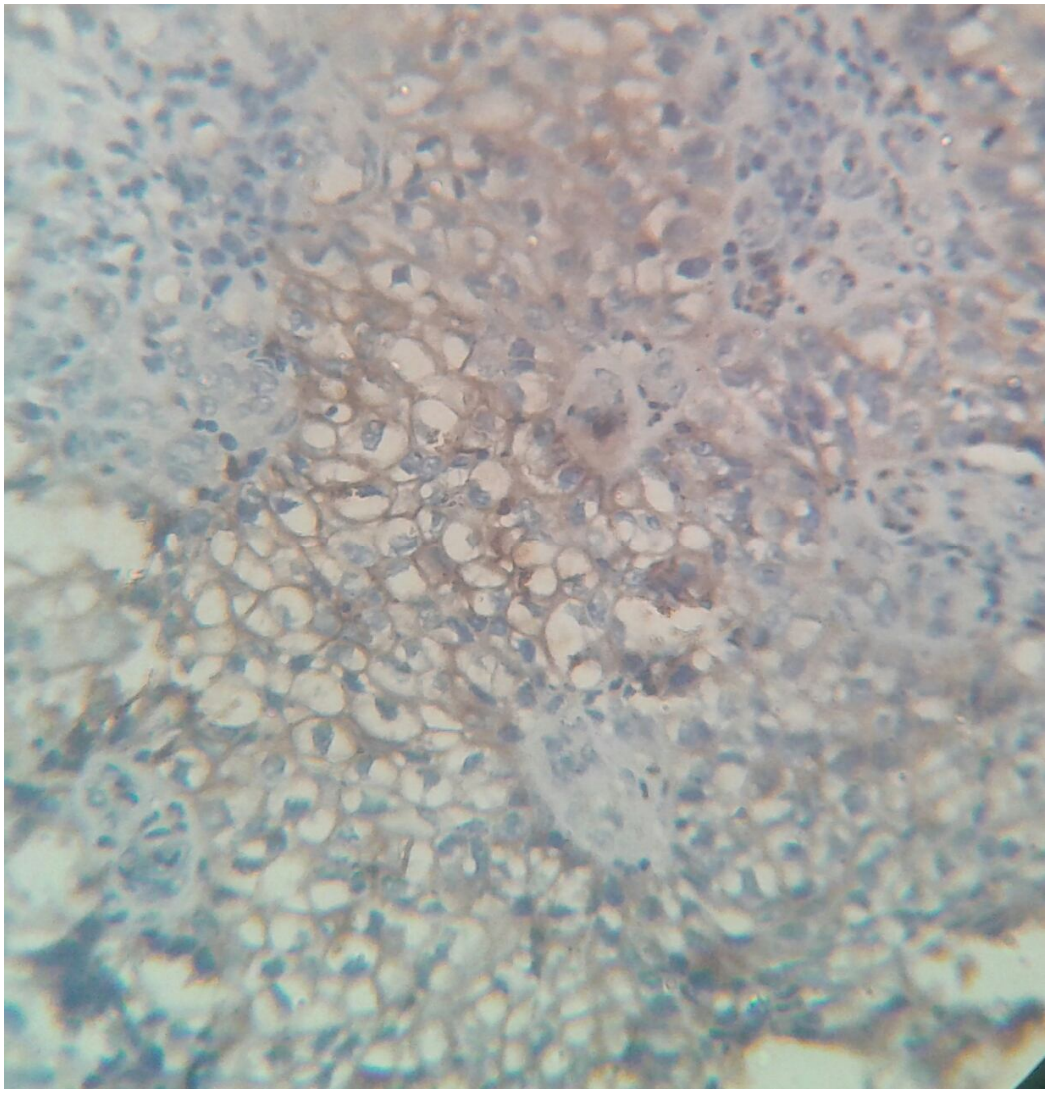
Appendix II:

Kits Leaflets

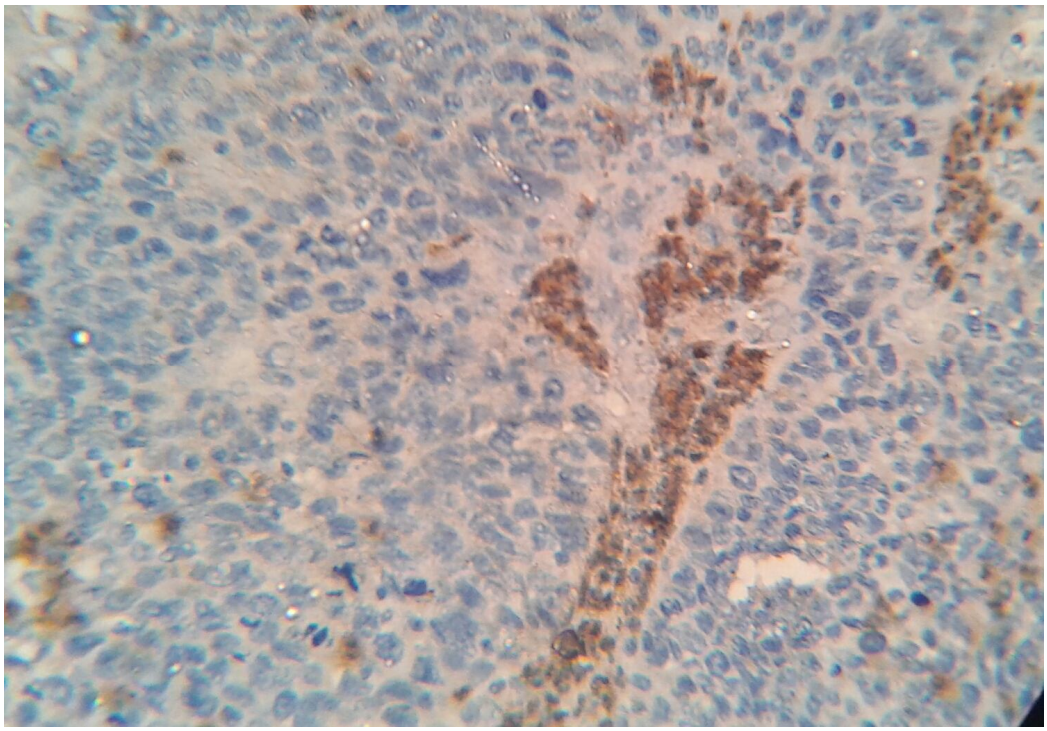
Appendix III



Microfigure1: moderately differentiation squamous cell carcinoma in cervix. Show positive membranous expression of Her2 marker, X40.



Microfigure2: Adenocarcinoma in cervix. Show in positive membranous expression of Her2 marker, X 40.



Microfigure3: Poorly differentiation squamous cell carcinoma in cervix. Show positive nuclear expression of Ki67 marker, X40.