1.1 INTRODUCTION

Esophageal carcinoma affects more than 450000 people worldwide and the incidence is rapidly increasing (Pennathur ,*et al*.2013). Currently, EsC is the eighth most common incident cancer in the world because of its extremely aggressive nature and poor survival rate (Enzinger,*et al*.2003; Mao,*et al*.2011).

EsC exhibits an epidemiologic pattern distinct from all other cancers(Blot,*et al*.1999; Engel ,*et al*.2003). The incidence of esophageal adenocarcinoma has increased sharply over the past few decades, both by period and birth cohort. Etiological studies are required to explain the rapid increase of this lethal cancer (Lepage,*et al*.2008).

According to the radiation and isotope center-Khartoum (RICK) record the esophageal cancer is the fourth most common cancer among sudanese males,and the fifth most common among females (Hamad,2011).

The etiologies of squamous cell esophageal carcinoma seem to differ between low-incidence regions and high-incidence regions. For example, tobacco and alcohol use are important risk factors for esophageal cancer in industrial countries(Castellsague,*et al*.2000), whereas in high-incidence regions other factors, such as nutritional deficiency, have a stronger relation to disease incidence (Sharp,*et al*.2001; Sammon.2007). Northern China is one of the world's highest incidence regions (Li.1982; Munoz and Buiatti.1996), but studies that have been carried out there have not yet identified the basis for the high incidence(Xibib,*et al*.2003;Tran,*et al*.2005).

Involvement of human papillomavirus (HPV) infection in esophageal cancer was first suggested in 1982(Syrjanen.1982), but this studies that have tested this hypothesis have not obtained consistent results

(de Villiers,*et al*.1999; Zhang,*et al*.2011).

Esophageal cancer usually occurs as either squamous cell carcinoma in the middle or upper one-third of the esophagus, or as adenocarcinoma in the lower one-third or junction of the esophagus and stomach (Jemal,*et al*.2010; Fritz,*et al*.2010). In the highest risk area, stretching from northern Iran through the central Asian republics to North-Central China, often referred to as the "esophageal cancer belt," 90% of cases are squamous cell carcinomas(Tran,*et al*.2005; Gholipour,*et al*.2008).

Major risk factors for squamous cell carcinomas in these areas are not well understood, but are thought to include poor nutritional status, low intake of fruits and vegetables, and drinking beverages at high temperatures(Islami,*et al*.2009; Wu, *et al* 2009*).* In low-risk areas such as the United States and several Western countries, smoking and excessive alcohol consumption account for about 90% of the total cases of squamous cell carcinoma of the esophagus(Engel,*et al*.2003). Smoking, overweight and obesity, and chronic gastroesophageal reflux disease, which triggers Barrett's esophagus, are thought to be the major risk factors for adenocarcinoma of the esophagus in the United States and some Western countries(Engel,*et al*.2003; Kamangar,*et al*.2009).

A number of studies also found smokeless tobacco products and betel liquid (with or without tobacco) as risk factors for esophageal cancer in certain parts of Asia(Lee,*et al*.2005; Wu,*et al*.2006).

Infection with human papillomavirus (HPV), especially HPV type 16, has been implicated as a possible risk factor for esophageal cancer in three seroepidemiologic studies(Han,*etal*.1996; Bjorge,*etal*.1997). In two prospective studies(Dillner,*et al*.1995; Bjorge,*et al*.1997), HPV16 seropositivity was associated with a more than sixfold excess risk. Although the number of observed cases did not permit separate analyses

by histologic type, the association appeared to be the strongest in the case of squamous cell carcinoma(Dillner,*et al.*1995; Bjorge,*et al.*1997).

1.2 RATIONAL

Worldwide, esophageal cancer is the eighth most common cancer and the sixth most common cause of death from cancer (Ferlay,*et al*.2010).

Esophagus cancer need further studies and screening program especially in the area of high disease incidence, including viral screening . HPV can not be propagate in culture, its acurately detected by immunohistochemistry and /or PCR.

According to previous study review ,CDX2 tumor marker may include in the diagnostic panel of Barrett's esophagus and esophageal adenocarcinoma.

So other studies aimed to introduce CDX2 and HPV for early detection and screening for high risk patients.

1.3 OBJECTIVES

General objective:

- To detect CDX2 and HPV in esophageal tumors Sudanese patients.

Specific objectives:

- To detect CDX2 in esophagus tumors using immunohistochemistry.
- To detect HPV16 in esophagus tumors using PCR.
- To correlate CDX2 and HPV with type of tumors..

2. REVIEW OF LITERATURE

2.1: Anatomy, histology and physiology of esophagus:

The esophagus is a muscular tube about (25cm) long, extending from the pharynx to the stomach. It begins at the level of the cricoids cartilage in the neck and descends in the midline behind the trachea. in the thorax, it passes downward through the mediastinum and enters the abdominal cavity by piercing the diaphragm at the level of the tenth thoracic vertebra.The esophagus has a short course of about (1.25cm) before it enters the right side of the stomach (Snell ,2007).

The wall of esophagus from the lumen out wards consist of mucosa (stratified squamous epithelium), submucosa (connective tissue) , layer of muscle fiber between layers of fibrous tissue and outer layers of connective tissue. The esophagus has amucosa consisting of atough squamous epithelium without keratin,smooth lamina propria and muscularis mucosa (Kuo,*et al*.2006).

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There are two types of glands with mucus secreeting esophageal gland being found in the submucosa and cardiac gland located in the lamina propria (Takuband Kaiyo .2007).

Functionaly the esophagus conducts food from the pharynx into the stomach .wave-like contractions of the muscular coat,called peristalsis, propel the food onward (Snell ,2007).

2.2: Pathology of esophagus:

2.2.1: Infection and inflammation of esophagus:

Esophagitis is commonly seen in adults and is uncommon in childhood (Prasad, *et al.*2009;Nurko ,*et al.*2009).The most common type of esophagitis is that associated with GERD (ie, reflux esophagitis). *Candida* esophagitis is the most common type of infectious esophagitis. Esophageal reflux symptoms occur monthly in 33-44% of the general population; up to 7-10% of people have daily symptoms, the incidence of symptoms of reflux is up to an order of magnitude higher than the prevalence of esophagitis (Mc Coll .2004).

Infectious esophagitis is most commonly observed in immunosuppressed hosts but has also been reported in healthy adults and children (Rothenberg.2009).

Patients with systemic diseases (eg: diabetes mellitus, adrenal dysfunction, alcoholism) and those of advanced age can be predisposed to infectious esophagitis because of altered immune function. Steroids, cytotoxic agents, radiation, and immune modulators can also contribute to impaired host immune function. Disruption of mucosal protective barriers and antibiotics that suppress the normal bacterial flora may contribute to the invasive ability of commensal organisms(Rothenberg ,2009).

Infectious agents known to cause esophagitis include *Candida* species: *Candida albicans* is the most common offending pathogen, but other *Candida* species, such as *Candida tropicalis, Candida glabrata*, and *Candida parapsilosis,* have also been implicated as rare causes of esophagitis, Noncandidal fungi (eg, *Aspergillus, Histoplasma*, *Cryptococcus, Blastomyces*), Herpes simplex(HSV), Cytomegalo virus

(CMV), Varicella-Zoster virus(VZV), Epstein Barr virus(EBV), Human Papilloma virus(HPV) (Quarto ,*et al.*2008). Bacterial species (eg, normal flora, *Mycobacterium tuberculosis, M. avium-intracellulare*, Staphylococcus, Streptococcus, Lactobacillus, Nocardia). Parasitic infections (eg, Chagas disease, *Trypanosoma cruzi*, *Cryptosporidium*, *Pneumocystis*, *Leishmania donovani*), major predisposing factors for *Candida* esophagitis include antibiotic use, radiation therapy or chemotherapy, hematologic malignancies, and AIDS. Other conditions associated with an increased incidence of *Candida* esophagitis include esophageal stasis, alcoholism, malnutrition, and advanced age. Occasionally, *Candida* esophagitis can occur in otherwise healthy individuals with no underlying esophageal or systemic disease (Levine ,*et al.*1985; Walsh , *et al.*1988; Bianchi Porro ,*et al.*1989; Haron *et al.*1993; Sam ,*et al.*2000; Vidal ,*et al.2007*; Kliemann ,*et al.*2008).

2.2.2: Tumor:

2.2.2.1: Benign tumor of esophagus:

During endoscopy ,various benign esophageal lesions are encountered in the esophagus. most are asymptomatic and have no malignant potential. These various benign lesions can originate from different wall layers in the esophagus .

According to its origin, esophageal tumors can be classified as epithelial tumors(squamous papilloma, adenoma), non epithelial tumors (leiomyoma, granular cell tumor) and cyctic tumors (bronchogenic cyst, duplication cyst, lymphangioma, fibroid polyp, lipoma, hemangioma) (Hoon, *et al.*2014).

2.2.2.2: Malignant tumor of esophagus:

The most important precancerous disease is Barrett's esophagus (McCollum ,*et al*.2006 ; Lordick , *et al*.2006).

Cancer of the esophagus typically occurs in one of two forms, SCCs arising from the stratified squamous epithelial lining of the organ, and adenocarcinomas affecting columnar glandular cells that replace the squamous epithelium (Blot, *et al*.1993). Sarcomas and small cell carcinomas generally represent less than 1%-2% of all esophageal cancers (Young ,*et al*.1973;Kwatra ,*et al* .2003). On rare occasions, other carcinomas, melanomas, leiomyosarcomas, carcinoids, and lymphomas may develop in the esophagus as well (Enzinger, *et al*.2003). SCC is the predominant histologic type of esophageal cancer worldwide (Cook, 2011).

The natural histories of SCCs and adenocarcinomas of esophagus appear to differ substantially. For squamous cell cancers, transition models have described squamous epithelium undergoing inflammatory changes that progress to dysplasia and in situ malignant change (Dawsey ,*et al*.1994).

Most adenocarcinomas, however, tend to arise in the distal esophagus from columnar-lined metaplastic epithelium, commonly known as Barrett's esophagus, which replaces the squamous epithelium during the healing reflux esophagitis and may progress to dysplasia(Huang, *et al*.2011;Kountourakis ,*et al*.2011) .

2.3: Risk factors of esophagus cancer:

Three decades ago the large majority of these cancers were SCCs, but the incidence of esophageal adenocarcinoma has been steadily increasing (Lepage, *et al*.2013). Tobacco and alcohol consumption are the primary causes of SCCs of the esophagus (Blot .1999). One of the strongest emerging risk factors, however, is obesity. Increases in the prevalence of obesity and the incidence of esophageal adenocarcinoma are parallel, and several epidemiologic studies have shown upwards of threefold excess risks among overweight individuals. Further research into the causes of these usually fatal cancers may help identify other potential determinants and provide needed information to help stem their increase (Mao,*et al*.2011)

The incidence of SCC of the esophagus has been found to dramatically increase in the presence of any factor that causes chronic irritation and inflammation, such as excessive alcohol intake, especially in combination with smoking (Guanrei, *et al*.1987; D'Onofrio, *et al*.1997). This does not hold true for adenocarcinoma. This may account for more than 90 percent of all cases of SCC of the esophagus in developed countries (Oze, *et al*.2011).

Chronic esophageal irritation also occurs when food is retained and decomposed by bacteria, releasing various chemical irritants. Frequent consumption of hot beverages also appears to increase the incidence of SCC (Lin, *et al*.2011).

The genetic and molecular changes underlying the development of EsC remain poorly understood. Genetic analysis of these cancers reveals frequent chromosomal losses (4q, 5q, 9p, and 18q), chromosomal gains

(8q, 17q, and 20q), and occasional gene amplifications (7, 8, and 17q) (Enzinger. *et al*.2003).

2.4: Method of diagnosis:

2.4.1: Clinical examination:

Esophagogram showing a malignant esophageal stricture is usually the initial diagnostic study obtained and typically shows a stricture or ulceration of the esophagus. upper endoscopy and endoscopic ultrasonogram showing a transmural adenocarcinoma of the esophagus associated with Barrett's esophagus ,with lymph-node metastases reveals a friable, ulcerated mass. a computed tomographic (CT) scan of the chest, abdomen, and pelvis with intravenous contrast medium should be obtained to detect metastatic disease (Van Dam.1997) .

Patients with esophageal cancer that is thought to be restricted to the esophagus may benefit from further evaluation with the use of endoscopic ultrasonography, This technique can be used to predict the depth of tumor invasion (the tumor stage) in 80 to 90 percent of patients and the extent of lymph-node involvement by metastatic disease (the node stage) in 70 to 80 percent of patients (Van Dam.1997). The ability to detect regional lymph-node involvement may be further enhanced by the use of endoscopic, ultrasonographically guided fine-needle aspiration, which has an accuracy of more than 90 percent at many centers(Vazquez-Sequeiros, *et al*.2001). Positron-emission tomography (PET) with fludeoxyglucose F 18 is increasingly being used to identify disease that has spread to regional lymph nodes or to sites that are undetectable by CT or endoscopic ultrasonography "Cancer of the Distal Esophagus(Panels A and B) with metastasis to a paraesophageal lymph node". Although

thoracoscopic or laparoscopic staging is highly accurate (more than 90 percent) (Krasna,*et al.*1996), These procedures are invasive and have been replaced at many institutions by PET scanning with fludeoxyglucose F 18 (Mealy,*et al.*1996)

2.4.2: Laboratory investigation:

2.4.2.1: CBC.

2.4.2.2: Serological test:

HPV cannot be grown in conventional cell cultures, and serological assays have only limited accuracy (Dillner,1999).

2.4.2.3: In situ hybridisation:

Several studies have been published on the detection of HPV in oesophageal carcinomas using ISH (Chang, *et al.* 1990; Benamouzig, *et al.*1992 ; Ashworth , *et al.*1993; Woo, *et al*1996 ; Takahashi , *et al.* 1998; Chang, *et al.* 2000).

ISH techniques are becoming increasingly sensitive and are currently more often used in routine diagnostics to detect or exclude malignancy (Fiegl, *et al.*2004) .New ISH techniques can be used for the detection of very low copy number of HPV DNA sequences in paraffin-embedded tissue sections. Although the more sensitive methods such as PCR can be performed in formalin-fixed and paraffin-embedded tissues, DNA damage and DNA extraction in these tissues can reduce the sensitivity of PCR. Thus, ISH can detect HPV in cases that may not be identified by PCR (Guo, *et al.*2008).

2.4.2.4: Polymerase chain reaction:

Compared with other techniques, the polymerase chain reaction (PCR) is a simple, rapid, and sensitive method for the detection of HPV DNA in tissue samples. Furthermore, the use of consensus primers is an advantage in PCR based studies because these primers can detect a wide spectrum of HPV types (van den Brule, *et al*.1993).

2.4.2.5: Biopsy:

Histology: histological section prepared from esophagus tissue for H&E stain and/or IHC.

Cytology: dysplastic lesions frequently accompany invasive SCCs, and present with HPV suggestive cellular changes, such as koilocytes (Syrjänen,2000) HPV DNA was recently detected in cytological brushings of the oesophagus in a substantial proportion (17%) of human immunodeficiency virus infected patients without clinically detectable lesions(Trottier , *et al*.1997).

2.5: Treatment of esophagus cancer :

Esophageal tumors usually lead to dysphagia (difficulty swallowing),pain and other symptoms, are diagnosed with biopsy. Small and localized tumors are treated surgically with curative intent. larger tumors tend not to be operable and hence are treated with palliative care, their growth can still be delayed with chemotherapy, radiotherapy or a combination of the two. In some cases chemo-and radiotherapy can render these larger tumors operable. Prognosis depends on the extent of the disease and other medical problems, but is generally fairly poor (Enzinger ,*et al*.2003)

2.6 Tumor markers:

Tumor marker are substances secreted by the neoplastic cells(benign and malignant) in the body, they can be detected in circulation ,urine or body fluids or/and with in tumor cells. The tumor markers are macromolecules whose a ppearace and changes in concentration are related in certain way to the genesis and growth of the tumors in the individuals. They are classified as growth proteins ,growth factors enzyme, oncofetal proteins oncogene antigens glycolipids (Proc Soc, 1997).

2.6.1 CDX2 tumor marker:

CDX2 is present in dysplasia and adenocarcinoma, with some loss of protein primarily in high-grade dysplasia and adenocarcinoma.

The human CDX2 gene is a member of the caudal-type homeobox gene family, whose members are homologs of the caudal gene of *Drosophila melanogaster*. The gene product (CDX2 protein) is important in the early differentiation and maintenance of intestinal epithelium via regulation of intestine-specific gene transcription. 15,22,41,42 .The CDX2 gene is expressed throughout the small and large intestine, with the proximal limit occurring at the gastroduodenal junction. CDX2 has been shown to be present in metaplastic intestinal-type epithelium within the stomach (Phillips, *et al.*2003*)*.

CDX2 protein expression in low-grade and high-grade dysplasia in Barrett's epithelium and in a denocarcinoma (Phillips, *et al*.2003).

CDX2 is not expressed in normal esophageal and gastric epithelial cells but is expressed in intestinal metaplasia of the esophagus(Groisman ,*et al*. 2004; Lord , *et al*. 2005). In some patients, Barrett’s esophagus is

complicated by the development of esophageal adenocarcinoma (Groisman ,*et al*. 2004; Kazumori ,*et al*.2006).

CDX2 plays a critical role in the regulation of cell proliferation and differentiation, especially in the intestine (Suh, *et al*.1996) .CDX2 expression was detected in normal esophagus where there was no methylation, but was more highly expressed in the adenocarcinomas of the esophagus which also lack methylation of the promoter region. Given that many esophageal adenocarcinomas arise from dysplastic lesions in the background of intestinal metaplasia, CDX2 expression might be a significant contributor to the metaplastic and later neoplastic transformation of the human esophagus to adenocarcinoma (Moons, *et al*.2004; Lord, *et al*.2005).

Acid and bile salts induce CDX2 mRNA and protein expression in esophageal squamous cells from patients with Barrett's esophagus, but not from GERD patients without Barrett's esophagus (Xiaofang,*et al*.2010). that exposure to acid and/or bile acids may activate CDX2 expression in human esophageal epithelial cells through promoter demethylation, and ectopic CDX2 expression in esophageal squamous epithelial cells may contribute to intestinal metaplasia of the esophagus(Tong, *et al*.2006).

2.7 Human Papilloma Virus:

HPV was implicated for the first time in the pathogenesis of oesophageal cancer in a study reported in 1982 (Syrjanen.1982). These organisms are small non-enveloped DNA viruses classified as belonging to the Papovaridae family, and more than 70 papillomavirus types have been identified on the basis of sequence divergence (de Roda Husman.1995; Villa.1997). The association of certain types of HPV primarily with

normal tissue or benign lesions, as opposed to the cancer associated types, has led to the concept of low and high oncogenic risk HPVs (Turek.1994; Villa.1997).

HPV16 infection may be a risk factor for esophageal cancer. Further studies of the association between HPV16 infection and the incidence of esophageal cancer are needed (Han ,*et al*.1996).

Human papillomavirus (HPV) has been implicated in ESCC, particularly the sub-types 16 and 18(Sur, *et al*.1998).

According to the studies reviewed, it is likely that HPV infection plays a much more significant role in esophageal carcinogenesis in those areas of the world with a high incidence of ESCC (Poljak ,*et al*.1998).

HPV infection is common in esophageal carcinoma independent of region and ethnic group of origin. Findings in this study raise the possibility that HPV is involved in esophageal carcinogenesis (Xueqian, *et al*.2010)

There are statistically significant associations between esophageal squamous cell carcinoma and seropositivity for E6 for the high-risk mucosal type HPV16 and for the low-risk mucosal type HPV6, but not for any of the other HPV type (Freddy, *et al*.2012).

3. MATERIALS AND METHODS

3.1: Study design:

This is retrospective descriptive study aimed to detect the expression of CDX2 and HPV in esophagus tumor using immunohistochemistry and PCR.

3.2: Study area:

This study was conducted at Ibn Sina Hospital during the period from May to November 2014.

3.3: Materials:

Formalin fixed paraffin embedded tissue blocks, that obtained from patients previously with esophageal tumor were used in this study.

3.4: Sampling:

Thirty paraffin blocks that were previously diagnosed as esophageal tumor (20 were esophageal cancer and 10 were benign) were selected from Ibn Sina Hospital. Patient identification data were retrieved from records include age and sex.

3.5: Sample collection and preparation:

From each paraffin blocks two sections were cut into 4µm thickness, sections were floated into preheated 40*c* using water bath ,and placed in coated slide for immunohistochemistry, also one section was cut and placed in eppendrof tube for DNA extraction.

3.5.1: Immunohistochemical staining procedure:

Rehydration: Following deparaffinization in xylene, slides were rehydrated through agraded series of alcohol and placed in distilled water.

Antigen retrieval: Antigen retrieval for CDX2 and HPV was performed target retrieval solution in 95C for 30 minutes in phosphate buffer saline.

Blocking: Endogenous peroxidase activity blocked with 3% hydrogen peroxidase for 10 minutes ,then washed in phosphate buffer (PBS)for 2 minutes.

Primary antibody: Immune section incubated with primary antibodies (CDX2 and HPV) for 30 minutes at room temperature in a moisture chamber, and then rinsed in phosphate buffer saline for 2 minutes.

Primary antibody enhancer: Immune section incubated with primary antibody enhancer for 15 minutes, then washed in phosphate buffer for 2 minutes.

Secondary antibody: Secondary antibody labeled with horse reddish peroxidase for 15 minutes.

DAB chromogen: Sections were incubated in diaminobenzidin tetrahydrochloride to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for 1-3 minutes, then washed in phosphate buffer for 2 minutes. (quartett kit)

Counter stain: Sections were counter stained with mayer haematoxylin and blued in running tap water for 5 minutes and dehydrated in 50%,70%,90%,100% ethyl alcohol for each ,then cleared in xylene 2 minutes for each, finally mounted using DPX media.

For each run of staining, positive and negative control slides also were prepared.

The negative control slides prepared from the same tissue block, but incubated with PBS instead of primary antibody. Each slide was evaluated with the researcher then the results were confirmed by supervisor.

3.5.2: Polymerase chain reaction:

3.5.2.1: DNA extraction:

DNA was extracted from esophagus according to commercial kits (Sacace Biotechnologies, Italy). Used for DNA extraction according to manufactures instruction.

Used for DNA extraction according to manufactures instruction.

3.5.2.2: PCR amplification:

The tube was closed and transferred to the thermalcycler,then the program was started.

DNA was amplified according to commercial kits(Sacace Biotechnologies, Italy).step one is Denaturation (94-98c) for 20-30 second,Annealing is step two,the reaction temperature is lowerd to (50-65c) for 20-40 second, and step three is Extension , acommonly temperature 72c for 1 min.

3.5.2.3: Analysis of PCR:

The DNA amplifyied by PCR was separated electrophoretically in 3% agarose gel in tris borate EDTA running buffer agarose gel was separated by dissolving 3 gm of agarose in 75ml of TBE buffer, eththedium

bromide was added,10ml of the PCR product were loaded into the wells 4 microliters a 100 bp DNA ladder, was loaded into the first well of the gel.

3.6: Statistical analysis:

The obtained results and variables arranged in standard master sheet, then analyze using social packed for statistical science(SPSS)program frequency, mean and Chi squire tests were calculated.

3.7: Ethical consideration:

Specimens were taken from Ibn Sena Hospital ethically after taken ethical clearance (Appendix 2).

4. RESULTS

The study involved 30 subject twenty out of them were males(66.7%) and ten were females(33.3%) Figure No.(4.1) summarizes this data.

Samples from 30 patients previously diagnosed as esophageal tumors were included (20 with esophagus cancer and 10 were benign) ,their age ranging from 8-98 years with mean age of 59.03 years old.

The majority of involved patients >50 years old were 21(70%) with esophageal tumors, and ≤50 years old were 9(30%).Table No(4.1).

When CDX2 was detected immunohistochemically(Microphotograph 4.1) ,in 30 samples,4samples (13.3%) were positive, and 26 (86.7%) of them were negative . Table (4.2) show the above result.

Also human papilloma virus was tested immunohistochemically (Microphotograph 4. 2).

In 30 samples,7 of them were positive (23.3%) and 23 (76.7%) were negative . Table (4.3) clarify the above mentioned data.

The HPV16 detected by PCR were positive in 3(10%),and 27 (90%) were negative. Table (4.4).

The positive expression of CDX2 in malignant tumor were 4, and no positive result detected in benign tumor. While negative result of CDX2 expression were detected in 16 of malignant samples, and 10 in benign samples as showed in (table 4.5),with no significant relation between CDX2 expression and type of tumor (*p=0.129),*Table (4.5).

In the comparison between the type of tumor and HPV immunohistochemical results the study revealed that,the positive expression of HPV in the malignant tumor samples were 6, and 1 positive result detected in benign tumor .while negative result of HPV expression were detect in 14 of malignant samples, and 9 in benign samples as showed in table (4.6) With no significant relation between CDX2 expression and type of tumor (*P.value 0.222*).

According to result of HPV16 PCR and type of tumor, there are positive in 3 malignant tumor, and no positive result detected in benign tumor, also 17 were negative in malignant tumor and 10 we negative, with no significant relation between HPV16 and type of tumor (*P.0.197*).Table(4.7)

The age group ≤50 years were 4 in malignant tumor and 5 in benign, while >50 years were 16 in malignant tumor and 5 in benign, with no significant relation between age group and esophageal tumor (*P.0.019*).Table (4.8)

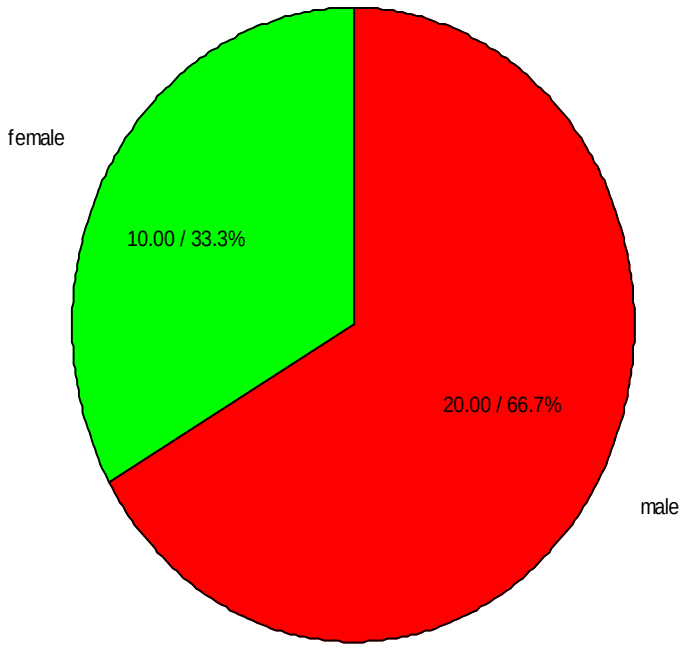


Figure (4.1): Frequency of sex among study population

Table (4.1): Description of study population by age:

Age group	Frequency	Percent
≤50	9	30
>50	21	70
Total	30	100

Table (4.2): Frequency of CDX2 results among study group

CDX2	Frequency	Percent
Positive	4	13.3
Negative	26	86.7
Total	30	100

Table (4.3): Frequency of HPVimmunohistochemical results among study group

HPV	Frequency	Percent
Positive	7	23.3
Negative	23	76.7
Total	30	100

Table (4.4): Frequency of HPV PCR results among study population

HPV(PCR)	Frequency	Percent
Positive	3	10
Negative	27	90
Total	30	100

Table (4.5):Relation between CDX2 expression and Esophagus tumors

Esophagus tumor	CDX2 results		Total
	Positive	Negative	
Malignant	4	16	20
Benign	0	10	10
Total	4	26	30

***P.value* = 0.129**

Table(4.6): Relation between HVP immunohistochemical results and esophagus tumors

Esophagus tumor	HPV immunohistochemical result		Total
	Positive	Negative	
Malignant	6	14	20
Benign	1	9	10
Total	7	23	30

***P.value* = 0.222**

Table(4.7): Relation between HVP molecular results and esophagus tumors

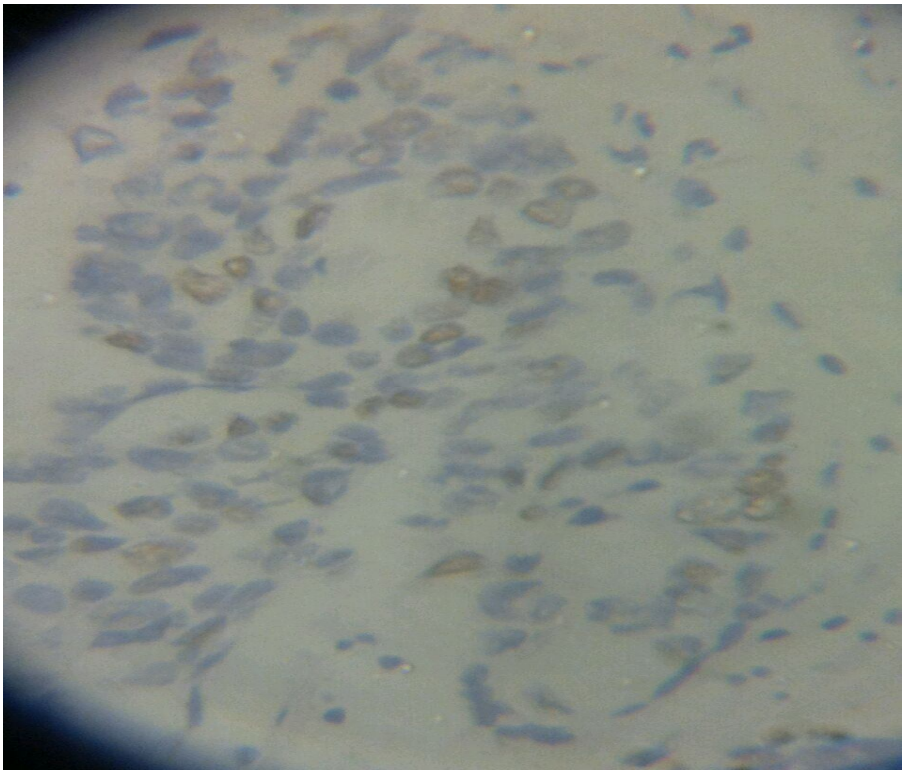
Esophagus tumor	HVP16 PCR		Total
	Positive	Negative	
Malignant	3	17	20
Benign	0	10	10
Total	3	27	30

***P.value* = 0.197**

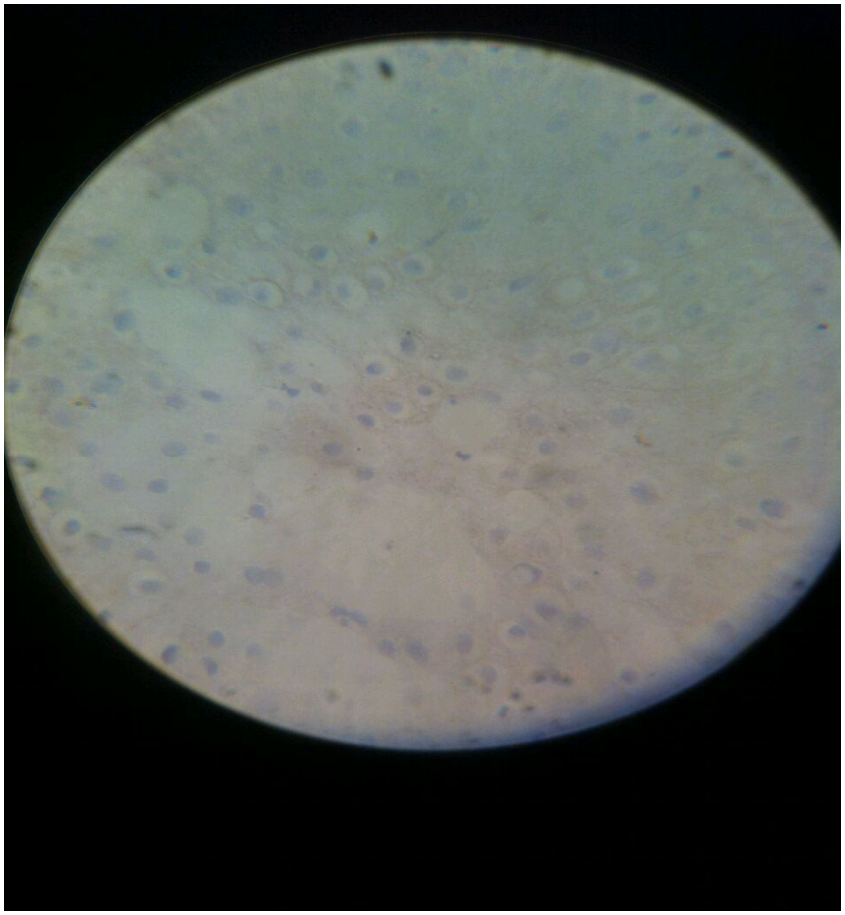
Table (4.8): Relation between age group and esophagus tumors

Esophagus tumor	Age group		Total
	≤50	>50	
Malignant	4	16	20
Benign	5	5	10
Total	9	21	30

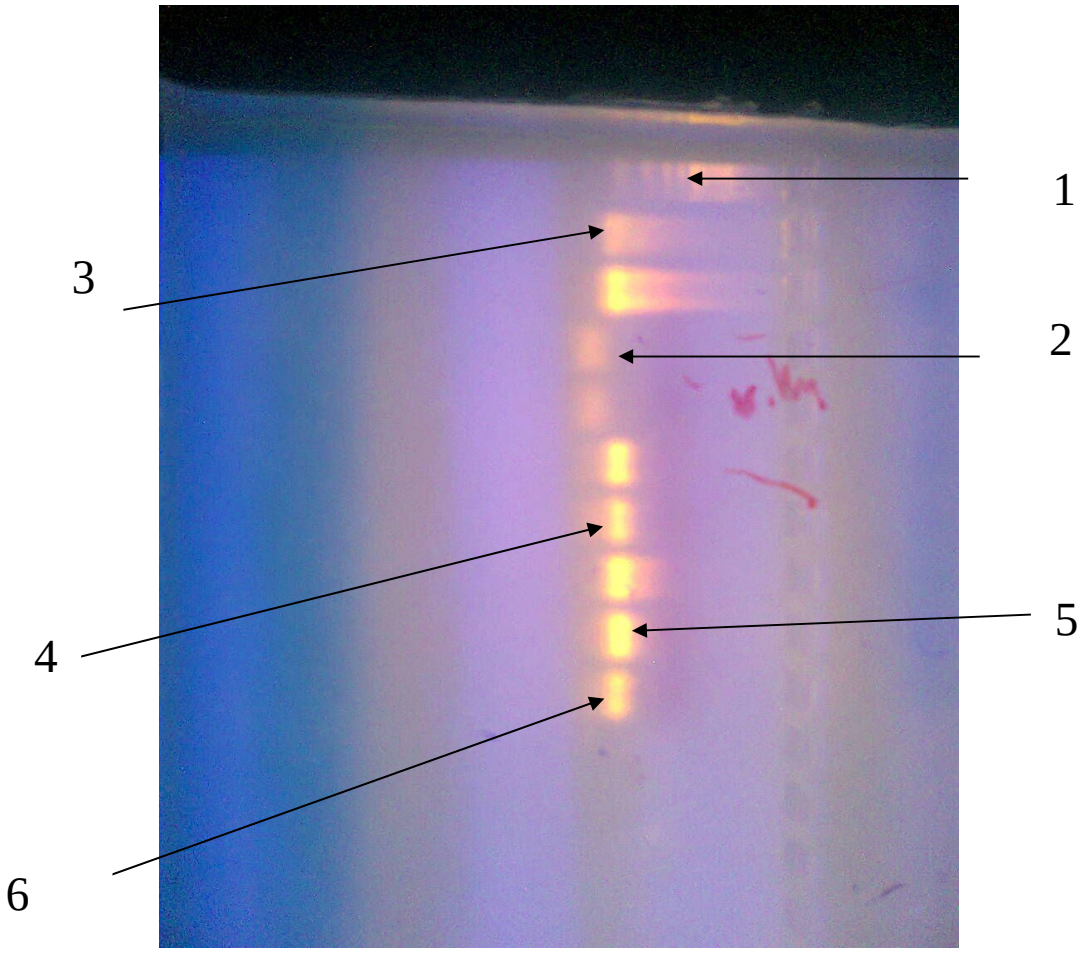
***P.value* = 0.091**



Microphotograph (4.1): Adenocarcinoma of esophagus show positive nuclear Immunohistochemical stain for CDX2, (40X)



Microphotograph (4.2): Squamous cell carcinoma of esophagus show positive cytoplasmic Immunohistochemical stain for HPV, (40X)



Microphotograph (4.3): PCR analysis result for HPV 16

1. Ladder
2. Negative Control
3. Positive Control
4. HPV16
5. HPV16
6. HPV16

5. DISCUSSION

Worldwide, esophageal cancer is the eighth most common cancer and the sixth most common cause of death from cancer, with an estimated incidence of 482000 new cases and 407000 deaths in 2008 (Ferlay,*et al*.2010).

In this study when we compared the esophageal cancer with age we found the most patients are over 50 years, this has been supported by another sutdy conducted in 2008 by descriptive epidemiology of esophageal carcinoma in the Ohio Cancer Registry ,for different types of esophageal cancer, the risk increases with age, with a mean age at diagnosis of 67 years (Cummings ,*et al*.2008).

In 2003 (Enzinger,*et al*) was found the risk factor increase with age- most patients are over 60,and median in United state is 67.

When we compared it with sex we found the disease is more common in men ,this has been supported as in 2008 esophageal cancer is 3 to 4 times more common among males than females (Age-Standardized Esophageal Cancer Incidence Rates by Sex and World Area. Source: GLOBOCAN 2008).

In 2012 (Lubin,*et al*) was found the adenocarcinoma is more common in men.

In our study CDX2 was showed there is no significant association between CDX2 expression and type of tumor by immunohistochemistry nuclear –localized. CDX2 protein is observed in columnar epithelium of BE,also appears to be alow level of CDX2 protein localized in the

cytoplasm of squamous epithelial cells from GERD patients (Eda,*et al*.2003).

Negative CDX2 staining was observed in normal squamous esophageal lining, while strong (3+) nuclear staining was seen in all cases of Barrett's intestinal metaplasia, dysplasia, and associated adenocarcinoma (Lord,*et al*.2005).

In contrast to our study,in 2003 (Guo,*et al*) was found No CDX2 methylation was found in normal tissues. Using semi-quantitative RT-PCR, expression of CDX2 was found in low level in normal esophagus, at higher levels in primary adenocarcinoma of the esophagus, but not in primary squamous cancers of the esophagus.

In 2003 (Phillips,*et al*) was found CDX2 protein expression in low-grade and high-grade dysplasia in Barrett's epithelium and in adenocarcinoma.

In 2004 and 2006 (Groisman ,*et al*) and (Kazumori ,*et al*) were found in some patients, Barrett's esophagus is complicated by the development of esophageal adenocarcinoma.

In 2010 (Xiaofang,*et al*) also was found acid and bile salts induce CDX2 mRNA and protein expression in esophageal squamous cells from patients with Barrett's esophagus, but not from GERD patients without Barrett's esophagus

Our study dis agree with study in 2004 by (Moons ,*et al*) and (Lord,*et al*) in 2005, CDX2 expression was detected in normal esophagus where there was no methylation, but was more highly expressed in the adenocarcinomas of the esophagus which also lack methylation of the promoter region. Given that many esophageal adenocarcinomas arise from dysplastic lesions in the background of intestinal metaplasia, CDX2

expression might be a significant contributor to the metaplastic and later neoplastic transformation of the human esophagus to adenocarcinoma.

Also in 2004 (Groisman ,*et al*) and in 2005(Lord , *et al*) were agree with our study and found CDX2 is not expressed in normal esophageal and gastric epithelial cells but is expressed in intestinal metaplasia of the esophagus.

In our study by immunohistochemistry HPV was showed there is no relation between HPV infection and type of tumor.

Our study agree with study in 2010 (Koshiol,*et al*) was found that HPV is not involved in ESCC carcinogenesis in China. HPV DNA contamination cannot be ruled out as an explanation for high HPV prevalence in ESCC tissue studies with less stringent tissue procurement and processing protocols.

In 2007 (Mohammad, *et al*) was suggest that squamous cell carcinoma of esophagus in Kashmir may arise independent of oncogenic β-catenin mutations and HPV is unlikely to be an etiologic factor for ESCC in this region.

Our results are disagree with HPV studies conducted by (Poljak ,*et al*) in 1998, according to the studies reviewed, it is likely that HPV infection plays a much more significant role in esophageal carcinogenesis with a high incidence of ESCC.

In 2010(Xueqian, *et al*) was found HPV infection is common in esophageal carcinoma independent of region and ethnic group of origin. Findings in the study raise the possibility that HPV is involved in esophageal carcinogenesis.

In 2012 (Freddy,*et al*) was found There are statistically significant associations between esophageal squamous cell carcinoma and seropositivity for E6 for the high-risk mucosal type HPV16 and for the low-risk mucosal type HPV6 ,but not for any of the other HPV type.

In 1998 (Sur ,*et al*) was concluded the infection with oncogenic HPV types may be an integral part in a multistep process that leads to ESCC.

6. CONCLUSSION AND RECOMMENDATION

6.1:CONCLUSSION:

On the basis of this study we conclude that there is no significant relation between HPV & esophageal tumor and HPV 16 is less common.

No relation between CDX2 and esophageal tumor.

6.2:RECOMMENDATION:

On the basis of this study we recommendate:

Further studies should be done to confirm the role of CDX2 and HPV16 in clinical practice.

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