

Chapter One

1. Introduction

1.1. Introduction:

Esophageal cancer is one of the cancers associated with a high mortality rate resulting in 10% of the affected people surviving for more than 5 years (Parkin, et al, 2003).

Esophageal cancer has been reported as the sixth most frequent cause of cancer death in the world (Parkin, et al, 2005)

The condition exist in two main forms that differ etiological and pathological characteristics, esophageal squamous cell carcinoma and esophageal adenocarcinoma has been associated with alcohol and tobacco, use the consumption of hot drink, deficiency of certain micronutrient and vitamins, consumption of food contamination with mycotoxins, low consumption of fresh fruit and vegetables, and diet high in spice content (Lambert and Hainaut, 2007). Both Human papilloma virus (HPV) and Epstein Barr virus (EBV) has been linked with esophageal cancer to various degrees (Syrjanen, 2002)

Globally the incidence of esophageal cancer varies significantly by geographic regions, race and gender. High rate of the disease have been reported in south and south-east Africa (Parkin, et al, 2003). According to the radiation and isotope centre of Khartoum record, the esophageal cancer is the fourth most common cancer among Sudanese males and the fifth most common among females (Hamad, 2011)

Among Sudanese the disease is equally prevalent in male and females with some cases diagnosed in people aged less than 40 years. The

observation of equal risks among males and female is supported by the esophageal cancer in Sudan having a relative females preponderance (Malik et al, 1976), these result differ from what is reported in the rest of the world being 4:1 male preponderance (Boyle and Levin, 2008)

The etiology that makes Sudanese to be at higher risk is not known, therefore it need for a well defined study in investigating possible exposures that may be operating increasing susceptibility to esophageal cancer in Sudanese environment. Most of the histopathology examinations of the reported tumors were squamous cell carcinomas, ranging from poorly differentiated to well differentiated type and the majority of patient presented with advanced disease.

During the past 30 years it has become exceedingly apparent that several viruses play significant role in the multistage development of human neoplasm, in fact approximately 15% to 20% of cancer are associated with viral infections (Zurh, 1991; Parkin, 2006)

Epstein Barr virus (EBV) is a lymphotropic human γ -herpes virus consisting of 184Kbp-sized double stranded DNA. It is transmitted primarily through saliva and infects over 95% of the world's population . EBV is the first virus that discovered from human neoplastic cells, Burkitt's lymphoma cell-line, 1964 (Epstein, et al, 1964). Subsequent studies have identified the virus in a variety of malignant neoplasms, including nasopharyngeal carcinoma (NPC), Hodgkin lymphoma (Young and Rickinson, 2004)

The association of EBV with undifferentiated Nasopharyngeal carcinoma has been well documented (Wolf, et al, 1973; Niedobitek, et al, 1991). Recently, the involvement of EBV has been demonstrated in gastric adenocarcinoma of various grades of differentiation with prominent

lymphoid infiltration (Shibata and Weiss, 1992; Tokunaga,1993). Esophageal squamous cell carcinoma thus it is of interest to determine whether EBV is involved in carcinoma of the esophagus, in this study we investigated the presence of EBV in esophageal cancer by immunohistochemical stain. The anatomical and histological association of the esophagus with the Nasopharynx, the possible association of EBV with esophageal cancer has been considered (Joseph, 1999). It strongly suspected that EBV play a role in the carcinogenesis of esophagus among Sudanese patient, so the aim of this study was to search for such aconnection.

Esophageal carcinoma still has a poor prognosis due to late diagnosis, rapid growth and spread, and high rate of recurrence. Most patients present with advanced disease at the time of diagnosis (Katlic, et al, 1990). In comparison to other malignancies of the gastrointestinal tract, there are no suitable biomarkers for esophageal carcinoma. SCC-antigen (Munck-WiklandE, et al, 1988). and CEA have been used as tumor markers for esophageal carcinoma, but their sensitivity has not proven satisfactory (Munck-Wikland ,et al,1988 and Ikedak,1991) .Reflux of acidic gastric contents and bile acids into the lower esophagus has been identified to have a central role in esophageal malignancy and is reported to upregulate caudal-related homologue 2 (CDX2), a regulatory gene involved in embryonic development and axial patterning of the alimentary tract (Reds Saad, et al, 2011). The aim of this study was to characterize the expression of CDX2 in esophageal cancer.

1.2.Rationale:

Esophageal squamous cell carcinoma thus it is of interest to determine whether EBV is involved in carcinoma of the esophagus, in this study we investigated the presence of EBV in esophageal cancer by immunohistochemical stain. The anatomical and histological association of the esophagus with the nasopharynx, the possible association of EBV with esophageal cancer has been considered (Joseph, 1999). It strongly suspected EBV play a role in the carcinogenesis of esophagus among Sudanese patient, so the aim of this study was to search for such a connection.

1.3. Objectives:

1.3.1. General objectives:

Detection of EBV and CDX2 among Sudanese patient with esophageal tumor using Immunohistochemistry.

1.3.2. Specific objectives:

To detect associatation between EBV and esophageal tumor.

To detect associatation between CDX2 and esophageal tumor.

To detect associatation between esophageal tumor and ages of the study population.

Chapter Two

2. Review of literature:

2.1. Anatomy and histology of esophagus:

2.1.1. Esophagus:

The esophagus is a hollow muscular tube that connects the throat to the stomach. It lies behind the trachea (wind pipe) and in front of the spine. Food and liquids that are swallowed travel through the inside of the esophagus (called the lumen) to reach the stomach. In adult, the esophagus is usually between 10 and 13 inches long and is about 1/4 of an inch across at its smallest point. The wall of the esophagus has several layers. These layers are important for understanding where cancer in the esophagus tends to start and how they may grow (American Cancer Society, 2012).

2.2. Gastro-esophageal junction:

The gastro-esophageal junction (also known as the esophagogastric junction) is the junction between the esophagus and the stomach, and it is situated at the lower end of the esophagus (Jonh, Dirckx, 1997). The pink color of the esophageal mucosa contrasts to the deeper red of the gastric mucosa (Anthony Dimarino, et al, 2002 and Kuo, Bradeu ; et al, 2006) and the mucosal transition can be seen as an irregular zig-zag line, which is often called the z-line (Levine, et al, 2010). Histological examination reveals abrupt transition between the nonkeratinized stratified squamous epithelium of the esophagus and the simple columnar epithelium of the stomach (Moore, Keithl, et al, 2002).

2.3 Pathology of the esophagus:

2.3.1 Inflammation and benign condition of the esophagus: 2.3.1.1.

Barrett's esophagus:

Prolonged esophagitis, particularly from gastric reflux, is one factor thought to play a role in the development of Barrett's esophagus. In this condition, there is metaplasia of the lining of the lower esophagus, which changes from stratified squamous epithelia to simple columnar epithelia. Barrett's esophagus is thought to be one of the main contributors to the development of esophageal cancer (Colledge, Nicki; et al, 2010).

2.3.1.2. Esophagitis:

Esophagitis is a general term for any inflammation, irritation, or swelling of the esophagus. Esophagitis is often caused by fluid that contains acid flowing back from the stomach to the esophagus, a condition called gastroesophageal reflux. An autoimmune disorder called eosinophilic esophagitis also causes this condition (U.S National Library of medicine, 2015).

2.3.1.3. Esophageal varices:

Esophageal varices refer to engorged blood vessels present within the esophageal walls. These blood vessels are engorged more than normal, and in the worst cases may partially obstruct the esophagus (Colledge, Nicki; et al, 2010).

2.3.1.4. Motility disorders:

Several disorders affect the motility of food as it travels down the esophagus such as:

2.3.1.4.1. Dysphagia:

is the medical term for the symptom of difficulty in swallowing (Smithard, et al, 2007 and Brady , 2008). Arises from the body of the esophagus, lower esophageal sphincter, or cardia of the stomach, usually due to mechanical causes or motility problems (American Academy of Family Physicians, 2008)

2.3.1.4.2. Achalasia:

Achalasia is a failure of smooth muscle fibers to relax, which can cause a sphincter to remain closed and fail to open when needed (Parkin, et al, 2005)

2.3.1.4.3. A nutcracker esophagus:

Nutcracker esophagus is characterized as a motility disorder of the esophagus, meaning that it is caused by abnormal movement, or peristalsis of the esophagus. Nutcracker esophagus can affect people of any age, but is more common in the sixth and seventh decades of life (Tutuian, Castell, 2006).

2.3.1.5. Esophageal strictures:

Various structures may also constrict the esophagus. These esophageal strictures are usually benign and typically develop after a person has had reflux for many years. Other causes may include congenital esophageal webs and damage to the esophagus by radiotherapy, corrosive ingestion, or eosinophilic esophagitis. A Schatzki ring is fibrosis at the gastro-esophageal junction. Strictures may also develop in chronic anemia, including Paterson-Kelly syndrome and Plummer-Vinson syndrome (Colledge, Nicki; et al, 2010)

2.3.1.6. Esophageal web and ring:

An esophageal ring is defined as a concentric, smooth, and thin (3-5 mm) extension of normal esophageal tissue consisting of 3 anatomic layers of mucosa, submucosa, and muscle. An esophageal ring can be found anywhere along the esophagus, but it usually is found in the distal esophagus. Three types of esophageal rings exist, and they are classified alphabetically as A, B, and C (Hirano, et al, 2000)

2.3.1.6.1. Schatzki ring:

The most famous and common ring in the esophagus is the B ring or Schatzki ring, as depicted below (Muller, et al, 2011) Typically, the Schatzki ring is located at the SQJ, and it marks the proximal margin of a hiatal hernia (Winters, et al, 2003).

2.3.1.7. Congenital malformation:

The esophagus fails to develop or there is an abnormal connection between the trachea and esophagus in about 1 in 3500 births. Conventional classification divides such connections into five types, based on whether the esophagus is a continuous tube or not, and which end of the esophagus (proximal, distal, both or neither) connects to the trachea. About half the time, these abnormalities occurs with additional abnormalities in other parts of the body, especially affecting the heart (Shaw-Smith, 2005)

2.4. Neoplasms:

2.4.1. Cancer of esophagus:

Esophageal carcinoma is one of the most common malignant tumors worldwide. It shows marked geographic variation across the world, with exceptionally high rates in certain areas of China (Li, 1982; Cheng, 1994). A number of environmental factors could be important in the carcinogenesis. Recent evidence has implicated an etiological role of certain microorganism. Such as bacteria, fungi and viruses, in the development of esophageal carcinoma either by producing carcinogens or by interacting directly with the host cell (Chang, et al. 1992; Baehr and Undonald, 1994). Esophageal cancers start in the inner layer (the mucosa) and grow outward (through the sub mucosa and muscle layer). Since two types of cells can line the esophagus. There are two main types of esophageal cancer squamous cell carcinoma and adenocarcinoma. The esophagus is normally lined with squamous cells. The cancer starting in these cells is called squamous cell carcinoma. This type of cancer can occur anywhere along the esophagus. At one time, the squamous cell carcinoma was by far the more common type of esophageal cancer in the United States. This has changed over time, and now it makes up less than half of the esophageal cancer in this country. Cancer that starts in gland cells are called adenocarcinoma. This type of cell is not normally part of the inner lining of the esophagus. Before adenocarcinoma can develop, gland cells must replace an area of squamous cells, which is what happens in Barrett's esophagus. This occurs mainly in the lower esophagus, which is the site of most adenocarcinoma. Cancer that starts at the area where the esophagus joins the stomach the (GE junction), which includes about the first two inches of the stomach (called cardia), tend to behave

like esophagus cancer (and treated like them, as well), so they are grouped with esophagus cancer (American Cancer Society, 2012).

2.4.1.1. The sign and symptoms of esophageal cancer:

Unfortunately, most esophageal cancers do not cause symptoms until they have reached an advanced stage, when they are harder to treat. The most common symptom of esophageal cancer is a problem swallowing, sometimes, people complain of pain or discomfort in the middle part of their chest. About half of people with esophageal cancer lose weight. Other possible symptoms of cancer of the esophagus can include: hoarseness, chronic cough, vomiting, hiccups, pneumonia, bone pain, bleeding in to esophagus. (American cancer society 2015)

2.4.1.2. Type of esophageal cancer:

2.4.1.2.1. Squamous cell carcinoma:

This cancer starts in the cells of the skin like lining of the oesophagus. More than a quarter of the oesophageal cancers diagnosed are squamous cell carcinomas. This type of cancer is found mainly in the upper third and middle of the oesophagus. It develops in the squamous cells that make up the inner lining of the oesophagus. (Cancer research uk.org, 2014)

2.4.1.2.2. Adenocarcinomas:

Adenocarcinoma means a cancer that has started in gland cells. In oesophageal cancer, these are the cells that make mucus in the lining of the oesophagus. The number of adenocarcinomas has increased in the last 30 years. They now make up just over a half of all oesophageal cancers diagnosed. Adenocarcinomas are found mainly in the lower third of the

oesophagus. This is the type of cancer that is most associated with acid reflux and Barrett's oesophagus (Cancer research uk.org, 2014)

2.4.1.2.3. Undifferentiated cancer:

The cells are not mature enough to be at all specialized. So the pathologist cannot say whether the cancer started from gland cells (adenocarcinoma) or skin like cells (squamous cell). (Cancer research uk.org, 2014)

2.4.1.2.4. Rare types of oesophageal cancer:

Rarely, a lump in the oesophagus can be a gastro intestinal stromal tumor (sometimes shortened to GIST). When they grow in the oesophagus, they may be non cancerous (benign) tumors or cancerous (malignant) tumors. (Cancer research Uk.Org, 2014).

2.4.4. Tumor Marker of esophageal cancer:

Despite aggressive attempts at identifying tumor marker specific to esophageal cancer, no marker has been identified that can be used universally to monitor tumor recurrence. Several studies have explored and suggested circulating IgG antibody levels to p16 protein (Jin, et al, 2014) CD25 (Guan, et al, 2013), and FOXP3 (Yel, et al, 2013) to serve as biomarkers for early diagnosis of esophageal cancer. Historically, p53 antibody level, SCC-antigen, CYFRA21-1, and carcinoembryonic antigen (CEA) have been proposed to be potential tumor markers (Bagaria, et al, 2013).

2.4.4.1. Caudal-Related Homologene 2 (CDX2):

CDX2 is a nuclear homeobox transcription factor that belongs to the caudal-related family of CDX homeobox genes. The gene encoding CDX2 is a nonclustered hexapeptide located on chromosome 13q12-13. Homeobox genes play an essential role in the control of normal embryonic development. CDX2 is crucial for axial patterning of the alimentary tract during embryonic development and is involved in the processes of intestinal cell proliferation, differentiation, adhesion, and apoptosis. CDX2 functions within the cell to induce differentiation and inhibit proliferation at the level of gene transcription. It stimulates intestinal epithelium differentiation through activating the transcription of proteins specific to the intestine. CDX2 inhibits epithelial proliferation through upregulating WAF1/p21, a cdk inhibitor that arrests the cell cycle upon DNA damage. CDX2 expression has been reported to be organ specific and is normally expressed throughout embryonic and postnatal life within the nuclei of epithelial cells of the alimentary tract from the proximal duodenum to the distal rectum (Redas Saad, et al, 2011). CDX2 is not expressed in normal esophageal and gastric epithelial cells but is expressed in intestinal metaplasia of the esophagus. In some patients, Barrett's esophagus is complicated by the development of esophageal adenocarcinoma. A recent study has reported CDX2 expression which was significantly weaker or absent in esophageal dysplasia and adenocarcinoma in comparison to metaplastic cells (Redas Saad, et al, 2011).

Streher, et al, 2014 determine CDX2 expression in esophageal mucosa and establish a correlation between this marker and the progression of disease by immunohistochemical method they found that; CDX2 expression was observed among patient with Barrett's esophagus and

adenocarcinoma, but there was no linear correlation between CDX2 expression and metaplasia – adenocarcinoma progression.

Eda, et al, 2003 found that; CDX2 protein is observed in columnar epithelium of Barrett's esophagus, also appear to be a low level of CDX2 protein localized in the cytoplasm of squamous epithelium cells from GERD patients.

Guo, et al, 2007 using semi – quantitative RT – PCR, found that; expression of CDX2 was found in low level in normal esophagus, at higher levels in primary squamous cancers of the esophagus.

2.4.5. Risk factor of Esophagus cancer:

2.4.5.1. Age:

The chance of getting esophageal cancer is low at younger ages and increases with age. Less than 15% of cases are found in people younger than age 55 (American cancer society, 2015).

2.4.5.2. Gender:

Men are more than 3 times as likely as women to get esophageal cancer (American cancer society, 2015).

2.4.5.3. Gastroesophageal reflux disease:

People with GERD have a slightly higher risk of getting adenocarcinoma of the esophagus. This risk seems to be higher in people who have more frequent symptoms. But GERD is very common, and the vast majority of people who have it do not go on to develop esophageal cancer. GERD can also cause Barrett's esophagus, which is linked to an even higher risk (American cancer society, 2015).

2.4.5.4. Barrett's Esophagus:

People with Barrett's esophagus are much more likely than people without this condition to develop adenocarcinoma of the esophagus. Still, most people with Barrett's esophagus do not get esophageal cancer. The risk of cancer is highest if dysplasia is present or if other people in your family also have or have had Barrett's (American cancer society, 2015).

2.4.5.5. Tobacco and alcohol:

The use of tobacco products, including cigarettes, cigars, pipes, and chewing tobacco, is a major risk factor for esophageal cancer. The more a person uses tobacco and the longer it is used, the higher the cancer risk. Someone who smokes a pack of cigarettes a day or more has at least twice the chance of getting adenocarcinoma of the esophagus than a nonsmoker. The link to squamous cell esophageal cancer is even stronger. The risk of esophageal cancer goes down if tobacco use stops. Drinking alcohol also increases the risk of esophageal cancer. The chance of getting esophageal cancer goes up with more consumption of alcohol. Alcohol affects the risk of the squamous cell type more than the risk of adenocarcinoma. Combining smoking and drinking alcohol raises the risk of esophageal cancer much more than using either alone (American cancer society, 2015).

2.4.5.6. Obesity:

People who are overweight or obese (very overweight) have a higher chance of getting adenocarcinoma of the esophagus. This is in part explained by the fact that people who are obese are more likely to have esophageal reflux (American cancer society, 2015).

2.4.5.7. Diet:

Certain substances in the diet may increase esophageal cancer risk. For example, there have been suggestions, as yet not well proven, that a diet high in processed meat may increase the chance of developing esophageal cancer. This may help explain the high rate of this cancer in certain parts of the world. On the other hand, a diet high in fruits and vegetables is linked to a lower risk of esophageal cancer. The exact reasons for this are

not clear, but fruits and vegetables have a number of vitamins and minerals that may help prevent cancer. Drinking very hot liquids frequently may increase the risk for the squamous cell type of esophageal cancer. This might be the result of long-term damage the liquids do to the cells lining the esophagus (American cancer society, 2015).

2.4.5.8. Workplace exposures:

Exposure to chemical fumes in certain workplaces may lead to an increased risk of esophageal cancer. For example, exposure to some of the solvents used for dry cleaning might lead to a greater risk of esophageal cancer. Some studies have found that dry cleaning workers may have a higher rate of esophageal cancer, but not all studies have found this link (American cancer society, 2015).

2.4.5.9. Human papilloma virus (HPV) infection:

Signs of HPV infection have been found in up to one-third of esophagus cancers from patients in parts of Asia and South Africa. But signs of HPV infection have not been found in esophagus cancers from patients in the other areas, including the US (American cancer society, 2015).

2.4.5.10. Epstein Barr virus:

EBV is a member of the herpesvirus family. As with other herpesviruses, EBV is an enveloped virus that contains a DNA core surrounded by an icosahedral nucleocapsid and a tegument. Family members include herpes simplex I and II and varicella-zoster virus (alphavirus subfamily), cytomegalovirus and human herpesvirus 6 and 7 (betaherpesvirus subfamily), and human herpesvirus 8 and EBV (gammaherpesvirus subfamily) (Roizman.B, 1990). Human tumors have been attributed to

both human herpesvirus 8 (Kaposi's sarcoma, primary effusion lymphoma, and Castleman's disease) and to EBV (Burkitt's lymphoma, nasopharyngeal carcinoma, and Hodgkin's and non-Hodgkin's lymphomas). Although herpesviruses are ubiquitous in nature, humans serve as the only natural host for EBV. It is now known that EBV infects >90% of the world's adult population. Upon infection, the individual remains a lifelong carrier of the virus (Henle G, 1979).

2.4.5.10.1. Epstein Barr virus and cancer:

2.4.5.10.1.2. History of Epstein Barr virus infection:

In 1958, Denis Burkitt described a common cancer primarily affecting children in specific regions of Africa. Burkitt believed a virus might be responsible for the cancer, given the climatic and geographic distribution of the cases (Barkitt, 1958). EBV was first identified in 1964 when Anthony Epstein's group discerned virus-like particles by electron microscopy in a cell line that had been established from a Burkitt's lymphoma biopsy (Epstein, et al, 1964). Later, it was found that sera from patients with the lymphoma that Burkitt had described had much higher antibody titers to EBV than did controls without the lymphoma. The subsequent detection of EBV DNA in Burkitt's lymphoma and the experimental production of lymphomas in cotton-top marmosets and owl monkeys established EBV as the first virus clearly implicated in the development of a human tumor (Epstein, et al, 1964).

2.4.5.10.1.3. Epstein Barr virus Infection:

EBV is transmitted from host to host via saliva, and the virus passes through the oropharyngeal epithelium to the B lymphocytes. The virus enters the B cell and causes it to proliferate and spread through the B-cell

compartment. T cells respond and control B-cell proliferation. Resting EBV-infected B cells with limited antigen presentation persist at a frequency of 1 in 1×10^5 - 10^6 cells and constitute the long-term viral reservoir. Intermittently, these resting B cells will enter the lytic cycle and lyse, releasing virions back into the saliva while also infecting more host B lymphocytes (Linde, 1996).

2.4.5.10.1.3.1. Immunity of Epstein Barr virus:

In a primary EBV infection, three antibodies (IgG, IgM and IgA) are produced against EBV viral capsid antigen, two antibodies (IgG and IgA) are produced in response to early antigen D, and one antibody (IgG) is produced in response to early antigen R (Linde, 1996). During a latent infection, EBNA-3A, EBNA-3B, and EBNA-3C all elicit specific CTL responses, which seem to be the dominant latency response to EBV proteins (Murray, et al, 1992 and Khanna, et al, 1992 and Tan, et al, 1999).

2.4.5.10.2. Epstein Barr virus latent protein:

The EBV viral nuclear antigens (EBNAs) were first detected by complementary immunofluorescence in the nuclei of latently infected EBV immortalized B cell, and were subsequently identified as six separate proteins (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, AND leader protein LP, also called EBNA1-6) which translated from a long polycistronic mRNA by alternative splicing (Reedman and Klein, 1973).

2.4.5.10.2.1. EBNA-1:

EBNA1 encodes a DNA – binding nuclear phosphoprotein which is required for the replication and maintenance of the binding of the

episomal EBV genome. Previous work has shown that stable EBNA1 expression in Epithelial cells requires an undifferentiated cellular environment (Knox, et al, 1996).

2.4.5.10.2.2. EBNA-2:

Is an 86 KDa protein which is coded for by BamWYH open reading frame. Expression of EBNA-2 is essential for immortalization and protein plays a pivotal role in this event by Trans activating all the other latent viral genes, including the B cell activation antigens CD21 and CD23 and oncogenes c-myc and c-fgr (Zuckerman, 2004)

2.4.5.10.2.3. EBNA-3A, EBNA-3B, EBNA-3C:

They are families of related protein coded for by the BERF open reading frame, with molecular weights of 140-180KDa. All three proteins inhibit transcription activation of EBNA-2 responsive gene, there by counter balancing the action of EBNA-2, however, only EBNA-3A and EBNA-3C are essential for immortalization of B cell (Zuckerman, 2004).

2.4.5.10.2.4. EBNA-LP:

EBV strains that carry mutation in the gene encoding EBNA-LP are partially defective with respect to their ability to immortalize B cells (Mannick, et al, 1991). EBNA-LP and EBNA-2 are first EBV genes to be expressed after infection of B cells and they act together to active cyclin D2synthesis and hence allow progression of the B cell in to the G1 phase of the cell cycle (Sinclair, et al, 1994)

2.4.5.10.3. EBV latent membrane protein (LMP):

2.4.5.10.3.1. LMP-1:

Is integral membrane protein with molecular weight of approximately 63KDa. It consists of three domains: 1- a 23 amino acid cytoplasmic N-terminus; 2- a 612 amino acid transmembrane domain with six hydrophobic transmembrane segments; 3- a 200 amino acid cytoplasmic C-terminus (Wang, et al, 1995). LMP-1 affects the growth of epithelial cells, including epidermal hyperplasia when produced in the skin of transgenic mice (Wilson, et al, 1990).

2.4.5.10.3.2. LMP-2:

The LMP-1 gene encodes two distinct proteins LMP-2A, LMP-2B, the structure of LMP-2A, LMP-2B are similar; both have 12 transmembrane domains and 27 amino acid cytoplasmic C-terminus; in addition, LMP-2A has 119 amino acid cytoplasmic C-terminus domains; LMP-2A aggregate in patches within the plasma membrane of latently infected B cells (Longnecker and Kieff, 1990), the amino terminus of LMP-2A associated with protein tyrosine kinase, and this interaction is thought to inhibit lytic viral replication and promote cell survival and maintenance of latency. The function of LMP-2B is unknown (Zuckerman, 2004).

2.4.5.10.3.3. EBER:

They are not essential for the transformation of primary B cells by EBV (Swaminathan, et al. 1991). EBER1 and EBER2 are small non polyadenylated RNAs and are the most abundant EBV RNAs in latently infected B cells. Ribosomal protein L22 and the interferon inducible protein kinase (Clarke, et al, 1991; Toczyski, et al, 1994).

2.4.5.10.4. Oncogenic Features of Epstein Barr virus:

To be oncogenic, EBV must maintain its viral genome in the cell, avoid killing the cell, and prevent the cell from becoming a target for destruction by the immune system. Finally, the virus must activate cellular growth control pathways. To maintain viral DNA in the cell, EBV establishes latent infection in B lymphocytes. The EBV genome is maintained in these cells, either as a multicopy circular episome in the host cell or by integrating the viral DNA into the host genome. The virus thus ensures transmission to cell progeny when B lymphocytes replicate. EBV latent genes induce an activated phenotype in the infected B cells. Although these cells are not transformed, if they proceed unchecked or acquire oncogenic mutations, they can become neoplastic. In normal individuals, cytotoxic T-cell responses against latent viral proteins prevent the expansion of these activated B cells. Through normal differentiation of these cells, EBV eventually enters the resting B-cell memory compartment. Only EBNA-1 is expressed in these cells. The EBV growth-promoting latent genes are not expressed, and so the cells are not pathogenic. The limited repertoire of gene products also prevents frequent viral replication. Because cytotoxic responses to EBNA-1 are rare, EBNA-1-expressing lymphocytes escape immune surveillance. This then constitutes the viral reservoir. Intermittently, these cells may enter the lytic cycle during which viral replication occurs and is accompanied by suppression of host protein synthesis with subsequent lysis/death of infected cells, releasing virions to infect more cells. With immune suppression, latently infected cells in the peripheral blood or persistently infected cells on the oropharynx increase in number. The final mandate of the virus in achieving oncogenicity is to activate intracellular signaling involved in control of proliferation. Years after primary EBV infection,

malignancies such as Burkitt's lymphoma, nasopharyngeal carcinoma, and Hodgkin's disease can emerge. These tumors can initiate from a clone of EBV-infected cells. The role of EBV in these late-onset malignancies is complicated. Because EBV is clonal, it clearly sets the stage for progression to frank tumor. However, other factors may be important: specific failure of immune recognition; stimulation of B-cell proliferation by other infections; and/or appearance of secondary genetic aberrations or mutations (MP Thompson, 2004).

Liang-shum, et al, 1999 studied the association between viral infection (HPV and EBV) and the development of esophageal carcinoma in Taiwan, using polymerase chain reaction, Immunohistochemical and in situ hybridization (ISH) they found that; Expression of EBES in ESCC was further confirmed with ISH. None the less, no LMP-1 expression was detected.

Hong, et al, 2000 studied the prevalence of the Epstein-Barr virus in esophageal cancer. Polymerase chain reaction and in situ hybridization were used to detect the Epstein-Barr virus they found that; The Epstein-Barr virus is rarely associated with esophageal cancer.

Yanai, et al, 2003 analyzed the possible EBV association for Japanese squamous cell carcinoma (SCC)-dominant esophageal cancer cases. Using in situ hybridization (ISH) and real-time quantitative polymerase chain reaction (Q-PCR) they found that; EBV is rarely associated with esophageal SCC.

2.4.6. Diagnosis of esophageal cancer:

This is the main test for cancer of the esophagus. An endoscope is a long, thin, flexible tube with a light and camera inside. The endoscope allows them to look at the inside of the esophagus. They will take tissue samples (biopsies) of any abnormal looking areas .also Abarium swallow which is a type X-ray investigation may be used (Cancer research Uk.Org, 2014).

2.4.6.1. Method of esophageal cancer diagnosis:

2.4.6.1.1. Hematoxylin and Eosin:

Hematoxylin and Eosin used for histological classification of esophagus cancer tissue to confirm the histopathological diagnosis and to assess the a dequacy of specimens before being selected for further analysis (sugiura, et al, 1996)

2.4.6.1.2. Immuno histochemistry:

For esophageal cancer antibodies used for Immuno histochemical staining were specific to EBV LMP-1, P53 and CD45RO (UCHL-1) (Dako, Kyoto, Japan) and FasI (Transduction Laboratories, Lexington, KY) . Respectively, immunological staining was performed by an immune peroxidase method.

2.4.6.1.3. Polymerase Chain Reaction:

Is to make a huge number of copies of a gene. The detection of EBV DNA in esophagus carcinoma was based on amplification of the EBV genes by PCR. According to latent gene expression patterns. The expression of viral genes are varied and classified into three patterns, latency1, 11 and 111(Masashi, 2010).

2.4.6.1.4. In situ hybridization:

To determine the localization of EBV in specimens, by performed in situ hybridization using a digoxigenin labeled 30 base probes complementary to EBER1 and EBER2 which are small non-polyadenylated RNAs and are the most abundant EBV RNAs in latently infection. The presence of EBV was identified by the expression of EBERs (Lerner, et al, 1981; Clemens, 1993). Detection of the EBERs by in situ hybridization has become the standard method to detect EBV infection in the routinely processed tumor tissue (Lamrence and Paul 2003).

2.4.7. Stage of esophageal cancer:

The stage of a cancer tells the doctor how far it has spread. It is important because treatment is often decided according to the stage. There are different ways of staging cancers. The 2 main ways are the TNM system and number systems. The TNM stands for Tumour, Node, and Metastasis. The system describes the size of a primary tumour (T), whether there are lymph nodes with cancer cells in them (N) and whether the cancer has spread to a different part of the body (M). The staging system for oesophageal cancer recently changed and now includes cancers of the gastro oesophageal junction (Cancer research Uk.Org, 2014)

2.4.8. Treatment of esophageal cancer:

The main treatments are surgery, radiotherapy and chemotherapy. May have surgery on early cancer. Or surgery and chemotherapy or combined chemotherapy and radiotherapy (chemoradiation). Treatments for advanced cancer if you have an advanced cancer you may have other

treatment, such as laser therapy or stents. If the cancer has spread to other areas of your body you may have chemotherapy to help control its growth. You may also have radiotherapy to shrink the tumors and reduce symptoms (Cancer research U.K., 2014).

Chapter Three

3. Material and Methods

3.1. Study design:

This is retrospective descriptive study aimed to detect the expression of Cdx2 in esophageal tumor association with EBV by using immunohistochemistry techniques.

3.2. Study area:

This study was conducted at Ibn Sena hospital and Sudan University of Science & Technology and college of medical laboratory science during period from April 2014 to October 2014.

3.3. Study sample:

Thirty paraffin blocks (which twenty paraffin blocks that were previously diagnosed as esophageal cancer and ten paraffin blocks that were previously diagnosed as benign esophageal tumor).

3.4. Data collection:

Patient identification data were retrieved from records include Age and sex.

3.5. Materials:

Formalin fixed paraffin embedded tissue blocks, that were previously obtained from patient attending to Ibn Sena hospital were used in this study.

3.6. Sample collection and preparation:

From each paraffin block two section were cut into 4mm thickness ,floated in to preheated floating water path at 40C ,then sections were placed in slides was coated with adhesive salinized glass slide for immunohistochemical stain.

3.6.1. Immuno histochemical Stain procedure:

Rehydration: following deparaffinization in xyelene, slides were rehydrated then placed in running tape water.

Antigen retrieval: antigen retrieval for CDX2 and EBV was performed for 30 minutes.

Blocking: endogenous peroxidase activity blocked with 3% hydrogen peroxide for 10 minute, and then washed in phosphate buffer saline (PBS) for 2 minutes.

Primary anti body : incubate with 100-200 MI of primary antibodies (CDX2 and EBV) for 30 minutes at room temperature in a moisture chamber and then rinsed in phosphate buffer saline for 2 minutes.

Secondary antibody: secondary antibody labeled with horse redish peroxidase for 15 minutes.

DAB chromogen: incubated in diaminobenzidin tetrahydride to procedure the characteristic visualization of the antibody / enzyme complex for 1-3 minutes. Then washed in phosphate buffer saline for 2 minutes. (quartett kit)

Counter stain : slide were counter stained with mayer's hematoxylin and blued in running water for 5 minutes and dehydrated in 50% ,70% ,90% ,100% ethyl alcohol for each , then cleared in xyelene and mounted using DPX media.

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

The negative control slides prepared from the same tissue block , but incubated without primary antibody , each slide was evaluated with the researcher then the results were confirmed by supervisor.

3.7. Statistical analysis:

All patients data as well as the obtained result arranged in standard master sheet then were entered a computer social packed for statistical science (SPSS) program version 11.5 for analysis , frequency, mean and Chi-square correlation test . Proportions were compared with $p>0.05$ was considered statistically not significant.

3.8. Ethical Consideration:

Specimen were taken from Ibn Sena hospital ethically after leader permission from retrieving form consist of patients number , age , and sex.

Chapter Four

4. Result:

This is retrospective descriptive study involved 30 subject twenty out of them were males (66.7%) and ten were females (33.3%) table NO.1.

Sample from 30 patients previously diagnosed as esophageal tumor were included (20 with esophagus cancer and 10 were benign), Their age ranging from 8-98 years with mean age of 59 years old.

The majority of patients >50 years old were 21(70%) with esophageal tumor, and <50 years old were 9 (30%).Table NO.2.

When Epstein Barr virus was tested immunohistochemically, in 30 sample, 5 (16.7%) of them were positive and 25 (83.3%) were negative. Table NO.3.

Also CDX2 was detected immunohistochemically, in 30 samples, 4 (13.3%) samples were positive, and 26 (86.7%) of them were negative. Table NO.4.

The positive detection of EBV in malignant tumor were 5 (16.7%), and no positive result detected in benign tumor, while negative result were detected in 15(50%) of malignant samples, and 10(33.3%) in benign samples, with no significant relation between EBV positivity and type of tumor (p. value: 0.083). Table NO.5

The positive expression of CDX2 in malignant tumor were 4(13.3%), and no positive result detected in benign tumor, while negative result of CDX2 expression were detected in 16 (53.3%) of malignant samples, and

10 (33.3%) in benign samples, with no significant relation between CDX2 expression and type of tumor (p. value: 0.129). table NO.6.

The age group < 50 years were 4 (13.3%) in malignant tumor and 5 (16.7%) in benign, while >50 years were 16 (53.3%) in malignant tumor and 5 (16.7%) in benign, with no significant relation between age group and esophageal tumor (p. value: 0.091). table NO.7.

Table NO.4.1:

Frequency of the study population by Gender:

Gender	Frequency	Percent %
Male	20	66.7%
Female	10	33.3%
Total	30	100%

Table NO.4.2:

Frequency of study population according to ages:

Ages groups	Frequency	Percent
≤ 50 years	9	30%
>50 years	21	70%
Total	30	100%

Table NO.4.3:

Frequency of EBV positivity:

EBV	Frequency	Percent
Negative	25	83.3%
Positive	5	16.7%
Total	30	100%

Table NO.4.4:

Frequency of CDX2 positivity:

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

Table NO.4.5:

The association between EBV positivity and esophageal tumor:

EBV	Diagnosis		Total
	Malignant	Benign	
Negative	15 50%	10 33.3%	25 83.3%
Positive	5 16.7%	0 0%	5 16.7%
Total	20 66.7%	10 33.3%	30 100%

P. value: 0.083

Table NO.4.6:

The association between CDX2 positivity and esophageal tumor:

CDX2	Diagnosis		Total
	Malignant	Benign	
Negative	16 53.3%	10 33.3%	26 86.7%
Positive	4 13.3%	0 0%	4 13.3%
Total	20 66.7%	10 33.3%	30 s100%

P. value: 0.129

Table NO.4.7:

The association between ages groups of the study population and esophageal tumor:

Ages groups	Diagnosis		Total
	Malignant	Benign	
< 50 years	4 13.3%	5 16.7%	9 30%
>50 years	16 53.3%	5 16.7%	21 70%
Total	20 66.7%	10 33.3%	30 100%

P. value: 0.091

Chapter Five

5. Discussion

Cancer continues to be the most serious problem worldwide, which give a reason to be a targeted from researchers to find a new ways of diagnosis, treatment and follow up. Esophageal carcinoma is one of the most common malignant tumors worldwide. It shows marked geographic variation across the world, with exceptionally high rate in certain areas of china (Li, 1982; Cheng, 1994). A number of environmental factors could be important in the carcinogenesis. Resent evidence has implicated on etiological role of esophageal carcinoma either by producing carcinogens or by interacting directly with the host cell (Chang, et al.1992; Baehr and Mcdonald, 1994). There are few literatures which discriminate EBV as risk factor of GIT cancer and possible association EBV with esophageal tumor has been considered (joseph, 1999). Progression from BE to invasive EADC is reflected histologically by barrett metaplasia, dysplasia and adenocarcinoma sequence which is associated with the accumulation of several molecular genetics alterations (Jankwski, et al, 1999 and Fitzgerald, et al, 2006). One recently identified molecule involved in the emergence of the BE phenotype is Caudal- related homologue 2 CDX2 (Guo, et al, 2004). So, the aim of the present study was to detect the expression of CDX2 in esophageal tumor association with Epstein Barr virus among Sudanese patients using immunohistochemistry.

The present study revealed that ; the majority of the esophageal cancer diagnosed patients are over 60 years, this also agree with Cancer Research UK 2009 which state that; over 80% of patients suffering from disease are more than 60 years of age.

Also (Cummings, et al, 2008) found for different type of esophageal cancer, the risk factor increase with age, with a mean age at diagnosis of 67 years.

The present study revealed that; the esophageal tumor is more common in men than women this is supported by American cancer society 2012 which state that men have a 3- to 4- fold higher rate of esophageal tumor compared with women.

This also agree with Cancer Research UK, 2014 found that; the condition is more common in men than women.

The present study revealed that; the CDX2 show there is no significant association between CDX2 expression and type of tumor by immunohistochemistry nuclear localized. This is supported by a study published (Eda, et al, 2003) CDX2 protein is observed in columnar epithelium of BE, also appear to be a low level of CDX2 protein localized in the cytoplasm of squamous epithelial cells from GERD patients.

Also Lord, et al, 2005 found negative CDX2 staining was observed in normal squamous esophageal lining, while strong nuclear staining was seen in all cases of Barrett's intestinal metaplasia, dysplasia and associated adenocarcinoma.

In contrast to our study (Tong liu, et al, 2006) found that CDX2 was expressed in most human esophageal adenocarcinoma cell lines, but not in squamous epithelial cell lines.

Also (Guo, et al, 2007) using semi- quantitative RT- PCR, expression of CDX2 was found in low level in normal esophagus, at higher levels in primary squamous cancers of the esophagus.

The present study revealed that; by immunohistochemistry EBV was show there is no association between EBV infection and type of tumor

Our study agree with (Syrjanen, 2002) found both Human papilloma virus (HPV- 16, HPV- 18) and Epstein Barr virus (EBV) are not generally associated with esophageal carcinogenesis.

Also (Sabine, et al, 2005) studied the presence of EBV DNA by PCR and in situ hybridization; we found Epstein Barr virus is unlikely to play a role in esophageal carcinogenesis.

In contrast to our study (Syrjanen, 2002) found both Human papilloma virus (HPV) and Epstein Barr virus (EBV) has been linked with esophageal cancer to various degrees.

(Hong, et al, 2000) studied the prevalence of Epstein Barr virus in esophageal cancer by polymerase chain reaction and in situ hybridization; we found the Epstein Barr virus is rarely associated with esophageal cancer.

Chapter Six

6.1. Conclusion

On the bases of this study we concluded:

- No association between EBV infection and esophageal tumors.
- The expression of CDX2 not associated with esophageal tumors.

6.2. Recommendations

On the bases of this study we recommended:

- Further studies using large sample size should be done.
- Molecular techniques should be applied to confirm the carcinogenesis of EBV infection in esophageal cancer.