Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemical Detection of Epstein Barr Viruse among Lymphoma Sudanese Patients.

الكشف عن فيروس إبشتاين بار في المرضي السودانيين المصابين بالأورام الليمفاوية

A Dissertation submitted in partial fulfillment of the requirements of M.Sc degree in medical laboratory science (Histopathology and cytology)

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الآيـــــــــة

بسم الله الرحمن الرحيم

قال تعالى:

(لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۖ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا ۚ أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ(286))

صدق الله العظيم

سورة البقرة الاية 286

Dedication

To my father

To my mother

To my son and my daughter

To all my family

To all my teachers

To all my colleagues and friends

With love and respect.

Acknowledgement

I'm grateful to Allah for the care, insight, peaceful and pity in my life. I would like to express my profound thanks to my supervisor, Dr. Abu Elgasim Abass, for his patience, guidance, unlimited assistance, encouragement and sustained interest throughout the course of this work.

I wish to extend my warmest thanks to the staff of the histopathology and cytology department. Sudan University of science and technology for their continuous support and encouragement.

Finally, I would like to thanks everybody who important to the successful realization of this research, as well as expressing my apology to these who I could not mention personally one by one.

Abstract

This is retrospective analytical case control study conducted at Radiation Isotope Center Khartoum (RICK), during the period from April 2014 and August 2015. The study was aimed to detect the presence of Epstein Barr virus in lymphoma using immunohistochemical method.

Sixty paraffin embedded blocks previously diagnosed as lymph node lesions were collected. Samples include 50(83.3%),(malignant tumors 11 hodgkin lymphoma ,39 non Hodgkin lymphoma) and 10(16.7%) samples were benign tumors.

The patient's age ranged between 7month and 80 years with mean 40 years, most patients 33 (55%) were more than 40 years and the remaining 27were less than40 years representing (45%) patients were more than 40 years.

The majority of patients were males and the male: female ratio was 2.3:1 representing 42(70%) and the remaining 18(30%) were females.

One section of 3µm thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (modified new indirect method) for detection of EBV. Data was collected from patients files and the obtained results were analyzed using SPSS computer program.

Immunohistochemical detection of EBV was revealed positive result in 3/50 samples and negative result in 47/50 in malignant samples while all benign tumors gave negative result for EBV, with insignificant statistical association between EBV expression and histopathology diagnosis (P=0.427).

This study concludes that there is no association between EBV detection and lymphoma.

المستخلص

أجريت هذه الدراسة الوصفية الاسترجالية في المركز القومي للعلاج بالأشعة والطب النووي _ الخرطوم خلال الفترة من أبريل 2014 إلى أغسطس2015 هدفت الدراسة للكشف عن فيروس إبشتاين بار في الأورام الليمفاوية بإستخدام كيمياء الأنسجة المناعية .

جمعت ستون عينة مطمورة بشمع البرافين من عينات مرضى تم تشخيصهم مسبقا بأورام الغدد الليمفاوية .تتكون العينات من 50 عينة لأورام خبيثة (11 من نوع هودجكن و39 من نوع غير هودجكن)و10 عينات لأورام حميدة.

قطع من كل عينة واحد مقطع بسمك 3 مايكرون بواسطة المشراح ومن ثم صبغت العينات بواسطة كيمياء الأنسجة المناعية بإستخدام الطريقة المعدلة غير المباشرة الجديدة للكشف عن فيروس إبشتاين بارثم تحليل البيانات المجموعة من ملفات المرضى والنتائج المتحصلة من ملفات المرضى بإستخدام الحزمة الإحصائية للعلوم الإجتماعية لتحليل البيانات .

تراوحت أعمار المرضى بين7 أشهر إلى80 سنة ومتوسط العمر40 سنة ،أغلب المرضى(33) كانت أعمارهم أكثر من40 سنة بنسبة55% وبقية المرضى 27(45%) كانت أعمارهم أقل من 40 سنة.

كان معدل الإصابة عند الذكور أعلى من الإناث ممثلا42 مريضا (70%) و18(30%) مريضا من الإناث

أظهرت الدراسة أن الكشف عن فيروس إبشتاين بار موجب الظهور في3عينات وسالب الظهور في 47عينة من عينات الأورام الخبيثة بينما كل عينات الأورام الحميدة أظهرت نتائج سالبة للفيروس مع عدم وجود علاقة ذات دلالة احصائية بين الاصابةبالفيروس والليمفوما.

List of contents

List of tables

List of Micrographs

CHAPTER ONE

INTRODUCTION

Chapter One

INTRODUCTION

1.1 Introduction

Lymphoma is the cancer of the lymph system (or lymphatic system). It is characterized by the formation of solid tumors in the immune system (Shankland, *et al*.2012).

Lymphoma is the tenth most common cancer worldwide, with around 452.000 new cases diagnosed in 2012 with percentage 3.2 % of total cases of the diagnosed cancers. In Sudan lymphoma is third type of cancers in terms of occurrence with (rate = 8.2 per 100,000) (Saeed, *et al.*2014).

Based on the world health organization (WHO) classification lymphoma was classified into Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL)(Russell, *et al*.2008).The WHO classified lymphoid neoplasm to main categories which comprised mature B-cell neoplasms, mature T-cell and NK-cell neoplasms, Hodgkin lymphoma, post transplantation lymph proliferative disorders (PTLDs). (Elias, *et al.*2011).

Risk factor for lymphoma include, age (Casey, *et al*.2012), infections (Lindsay, *et al*.2014), diseases of immunity and heredity factors (Harsh, 2010).

Diagnosis of lymphoma is done via endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) (Yasuda, *et al*. 2010).Tissue biopsies (Bruce, *et al*. 2009).Molecular diagnosis, PCR-based clonality testing (Vankrieken, *et al*. 2007), Flourescence in situ hybridization (Tsieh, *et al*. 2003). Clinical diagnosis, morphological diagnosis, flowcytometer, cytodiagnosis and radiology (Baba and Catoi, 2007).

Many lymphoma treatment options are available for lymphoma including chemotherapy (Czuczman, *et al*. 1999), Radiotherapy (Peter, *et al*. 2003), Biologic therapy, and radioimmunotherapy (Berinstein, *et al.*1998).

Epstein-Barr virus (EBV), a human lymphotropic herpes virus, it is associated with a number of malignancies including Hodgkin's disease, B cell lymphomas, and nasopharyngeal carcinoma (Jones and Straus.1987).Also causes infectious mononucleosis and Burkitt lymphoma (BL) (Epstein, *et al*.1964). EBV is a ubiquitous virus that infects at least 95% of the population. Most persons are infected during infancy and early childhood and are asymptomatic or have nonspecific symptoms (Cohen, 2000). Infection of adolescents and young adults with EBV often result with fever, lymphadenopathy, sore throat, and splenomegaly, fatigue and myalgias (Ebell, 2004). Males with the X-linked lymph proliferative disease often develop fatal infectious mononucleosis during primary EBV infection (Cohen, 2009). Chronic active EBV disease (CAEBV) is a lymphoproliferative disorder characterized by markedly elevated levels of antibody to EBV or EBV DNA in the blood and EBV RNA or protein in lymphocytes in tissues (Jeffrey, *et al*.2011).

In vitro EBV can transform human B lymphocytes to a state of continuous proliferation, known as cell "immortalization," generating permanent lymphoblastoid cell lines (LCL) that contain multiple copies of the viral genome in an episomal form(Henle, *et al.* 1967).The process of cell immortalization has been suggested to occur via EBV mediated stimulation of a physiological B-cell activation pathway(Thorley and Mann,1985).

1.2 Rationale

Worldwide, lymphoma cancer is the tenth most common worldwide (World Cancer Research Foundation. 2012). In Sudan, few studies have been done on lymphoma.

Lymphoma cancer needs heavy studies and viral screening program especially in the area of high disease incidence, including viral screening. EBV cannot be propagate in culture, it's accurately detected by immunohistochemistry and PCR.

According to previous study review EBV tumor marker may include in the diagnostic and prognostic panel of lymphoma. So other studies aimed to introduce EBV for early detection and screening for high risk patients.

1.3 Objectives

1.2.1General objective:

To study the detection of EBV among lymphoma Sudanese patients.

1.2.2Specific objectives:

1-To detect the detection of EBV on lymphoma tissues using immunohistochemical method.

2-To correlate the detectionion of EBV with histopathological diagnosis.

CHAPTER TWO

LITERATURE REVIEW

Chapter Two

LITERATURE REVIEW

2.1 Anatomy, histology and physiology of lymph node:

Lymph nodes are kidney or oval shaped and range in size from a few millimeters to about 1–2 cm long (Warwick, *et al.*1973).The human body has about 600 lymph nodes (Ferrer, 1998).Each lymph node is surrounded by a fibrous capsule, and inside the lymph node the fibrous capsule extends to form trabeculae. The substance of the lymph node is divided into the outer cortex and the inner medulla (Warwick, *et al.*1973).

The cortex is continuous around the medulla except at the hilum, where the medulla comes in direct contact with the hilum (Warwick, *et al.* 1973). Thin reticular fibers and elastin form a supporting meshwork inside the node (Kaldjian, *et al.* 2001).White blood cells (leukocytes), the most prominent ones being lymphocytes, are tightly packed in the follicles (B cells) and the cortex (T cells) (Kaldjian, *et al.* 2001). Elsewhere in the node, there are only occasional leucocytes. As part of the reticular network there are follicular dendritic cells in the B cell follicle and fibroblasticreticular cells in the T cell cortex (Kaldjian, *et al.*2001).

The reticular network not only provides the structural support, but also the surface for adhesion of the dendritic cells, macrophages and lymphocytes (Kaldjian, *et al.* 2001). It allows exchange of material with blood through the high endothelial venules and provides the growth and regulatory factors necessary for activation and maturation of immune cells (Kaldjian, *et al.* 2001).Lymph is derived from interstitial fluid and originates in the interstitial spaces of most of the body's tissues. A vast system of converging lymphatic vessels funnels lymph to the thorax where it is returned to the circulation via the thoracic duct (Von Andrian and Mempel 2003). The system of lymphatic vessels has been called an "information superhighway" because lymph contains a wealth of information about local

inflammatory conditions in upstream drainage fields (von Andrian and Mempel 2003). Lymph nodes consist of multiple lymphoid lobules surrounded by lymph-filled sinuses and enclosed by a capsule (Sainte-Marie, *et al.*1990).

The complex three dimensional lobules and their surrounding sinuses present a variety of appearances in tissue sections depending on the plane of section (Sainte-Marie, *et al.*1990). The lymphoid lobule is the basic anatomical and functional unit of the lymph node (Kelly, 1975). By common convention, usually the term cortex applied to the superficial cortex and refer to the deep cortex as the paracortex (Sainte-Marie, *et al.*1990).

The superficial cortex contains spherical follicles that are surrounded and separated by interfollicular (or diffuse) cortex. The paracortex consists of deep cortical units (DCUs). Each lobule has a single DCU that can be anatomically and functionally divided into a central DCU and a surrounding peripheral DCU (Sainte-Marie, *et al.*1990).

2.2 Pathology of lymph nodes:

2.2.1Lymphadenopathy:

It is disease of the lymph nodes, in which they are abnormal in size, number, or consistency (King, *et al*.2014).

2.2.2 Tumors of lymph node:

2.2.2.1 Benign tumor of lymph node:

A normal sized lymph node is usually less than one cm in diameter. Of course, there are exceptions in lymph nodes in different regions and at different ages have different sizes, it might be a usual self-limited infection in younger adults or a malignancy in older patients (Shahrzad, *et al*. 2014).

Based on different geographical areas, the etiology varies for example; tuberculosis (TB) is the most common cause of cervical lymph node adenopathy in endemic areas such as Africa. Nonetheless, in a large number of studies, the most common

benign etiologies are non-specific reactive changes in lymph nodes (Shahrzad, *et al*. 2014).

2.2.2.2: Malignant tumor of lymph node:

Lymphoid neoplasms are a group of distinct entities with widely varying clinical features, histology, immunophenotypes, and genetic abnormalities. The WHO classification of lymphoid neoplasm encompasses not only Hodgkin lymphoma and non-Hodgkin lymphoma (NHL), but also plasma cell neoplasm and lymphoid leukemia, with the underlying tenet that lymphoma and lymphoid leukemia represent solid and circulating phases, respectively, of the same disease(Elias , *et al*.2011).

2.2.2.2.1: Malignant lymphoma subtypes:

According to World Health Organization (WHO) 2008 classification lymphoma classified into, mature B-cell neoplasm which comprises many subtypes e.g. chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, splenic marginal zone lymphoma, Hairy cell leukemia, Follicular lymphoma, Mantle cell lymphoma and Burkitt lymphoma (Elias, *et al*. 2011).

And Hodgkin lymphoma which comprises many subtypes e.g. nodular lymphocyte-predominant Hodgkin lymphoma, classical Hodgkin lymphoma, nodular sclerosis classical Hodgkin lymphoma, lymphocyte-rich classical Hodgkin lymphoma, mixed cellularity classical Hodgkin lymphoma and lymphocyte-depleted classical Hodgkin lymphoma (Elias, *et al*.2011).

2.3 Epidemiology of lymphoma:

Lymphoma is the most common form of hematological malignancy represents 5.3% of all cancers in the United States and 55.6% of all blood cancers. (Horner, *et al*. 2009)

The most commonly diagnosed cancer in Sudan among women was breast followed by leukemia, cervix, and ovary, and among men it was prostate cancer followed by leukemia, lymphoma, oral, colorectal, and liver. In children less than 15 years of age, leukemia was the most common cancer followed lymphoma, and cancer of the eye, bone, kidney, and the brain (Saeed, *et al*.2014)

2.4 Risk factors of lymph node cancer:

2.4.1 Age:

The incidence of Hodgkin lymphoma has increased among adolescents and young adults (Casey, *et al*.2012).

2.4.2 Infections:

Human T cell leukeamia- lymphoma virus 1(HTLV-1) implicated in etiology of adult T cell lymphoma. Epstein Barr Virus implicated in etiology of Hodghkin disease ,burkitt lymphoma, post transplant lymphoma. Hepatitis C virus in lymphoplasmacytic lymphoma. Human herpes virus 8 in primary effusion lymphoma (Harsh, 2010). Helicobactor pylori in MALT lymphoma (Lydia, *et* a*l*. 2010).

2.4.3 Environmental risk factors:

Ionizing radiation due to radiation exposure. Chemical carcinogens benzene, tobacco smoking, alcohol, uses of certain hair dye, and exposure to agricultural chemicals. Certain drugs long term exposure to certain drugs such as phenytoin, alkylating agents, and other chemotherapeutic agents (Harsh, 2010).

2.4.4Diseases of immunity

Immunodeficiency diseases and auto immune diseases such as SLE and rheumatoid arthritis (Harsh, 2010).

2.4.5Heredity

An increased risk of developing certain cancers can be inherited in the genetic material passed from generation to generation, accounting for up to 4 percent of all cancers worldwide (Stewart and Kleihues, 2003).

2.5 Methods of diagnosis:

2.5.1 Tissue biopsy:

Patients with enlarged superficial lymph nodes sometimes require surgical biopsy for diagnosis, and there have been many case series describing the pathology found at biopsy (Karadeniz, 1999).

2.5.2Endoscopic ultrasound guided-fine needle aspiration cytology:

These techniques are a minimally invasive technique widely used for the evaluation of deep-seated benign and malignant lesions (Antonio, *et al*.2012).

2.5.3PCR-based clonality testing:

The diagnosis of malignant lymphoma is a recognized difficult area in histopathology. Therefore, detection of clonality in a suspected lymphoproliferation is a valuable diagnostic criterion (Van Krieken, *et al*.2007).

Many studies have concentrated on the specificity of molecular genetics. However, conventional cytogenetic and polymerase chain reaction are of relatively low sensitivity, while the detection of RNA transcripts by reverse transcription PCR and by in situ hybridization is of low specificity (Tsieh, *et al*.2003).

2.5.4 Fluorescence in situ hybridization:

The fluorescence in situ hybridization technique (FISH) seems to be most promising in terms of sensitivity and specificity (Tsieh, *et al*. 2003).

2.6 Treatment of lymph node cancer:

2.6.1Monoclonal anibody:

Anti-CD20 monoclonal antibodies are currently in development with the aim of improving the treatment of B cell malignancies (Christian, *et al*.2013).

2.6.2Chemo therapy:

It is used for those uncommon patients with disseminated disease at presentation or lack of response to local treatment rituximab (Martinelli, *et al*. 2005).

2.6.3Stem-cell transplantation:

Hematopoetic stem cell transplantation is not generally considered as first-line therapy for patients with lymphoid neoplasia. Its place is seen to be in advanced or relapsing disease (Buser, *et al*.2004)

2.6.4Radioimmunotherapy:

Advanced Hodgkin disease requires systemic chemotherapy, sometimes combined with radiotherapy (Kuruvilla, 2009).

2.7 Epstein-Barr Virus:

Epstein-Barr virus (EBV) is a ubiquitous virus that infects at least 95% of the population. Most persons are infected during infancy and early childhood and are asymptomatic or have nonspecific symptoms (Cohen, 2000). Infection of adolescents and young adults with EBV often results in infectious mononucleosis with fever, lymphadenopathy, sore throat, and splenomegaly (Jeffrey, 2009). Additional signs and symptoms can include fatigue, headache, hepatomegaly, and rash. EBV is also associated with a number of malignancies including Hodgkin's disease, B cell lymphomas, and nasopharyngeal carcinoma (Jeffrey, 2009). With the exception of the latter disease, EBV is present in B cells where it can result in lytic infection, with production of virus particles, or a latent infection with various patterns of viral gene expression. EBV can result in fatal infections in some hosts. Males with the X-linked lymph proliferative disease often develop fatal infectious mononucleosis during primary EBV infection. Those who survive the disease often have hypogammaglobulinemia and are at increased risk for developing B cell lymphomas (Jeffrey, 2009). Chronic active EBV (CAEBV) disease is a very rare

disease in the United States and Europe, but occurs more frequently in Asia and South America. Due to EBV present in either T cells or NK cells or B cells (Jeffrey, 2009).

2.8 EBVand lymphoma:

In vitro EBV can transform human B lymphocytes to a state of continuous proliferation, known as cell "immortalization," generating permanent lymphoblastoid cell lines (LCL) that contain multiple copies of the viral genome in an episomal form (Henle, *et al*. 1967).The process of cell immortalization has been suggested to occur via EBV mediated stimulation of a physiological B-cell activation pathway(Thorley and Mann,1985).Basic mechanism of malignant transformation is genetic damage to the DNA of the target white cells followed by proliferation, disrupting normal growth and differentiation (Harsh, 2010).

CHAPTER THREE

MATERIALS AND METHODS

Chapter Three

MATERIALS AND METHODS

3.1 Materials

Archived tissue blocks obtained from lymph nodes samples previously diagnosed as lymphoma and hyperplasia were selected for this study.

3.2 Methods

3.2.1 Study design

This is analytical retrospective case control study aimed to detect the expression of EBV in lymphoma among Sudanese patients using immunohistochemistry.

3.2.2 Study samples

Fifty paraffin blocks previously diagnosed as lymphoma and 10 lymph nodes of benign were selected from Radiation and Isotope Center Khartoum (RICK). Patient identification data, were obtained from patient's records.

3.2.3 Study area

This study was conducted at Radiation and Isotope Center Khartoum (RICK) (Khartoum state) during the period from April 2013 to August 2014.

3.2.4 Immunohistochemical staining

One section of 3µm thickness was obtained from each paraffin block using a SLEE CUT 5062 rotary microtome, then was placed on a positively charged slide and dried overnight at 58° and immunostained using monoclonal primary antibody by biotinylated secondary antibody indirect technique as follows:

Sections were loaded into Ventana Bench Mark GX autostaine, they were deparaffinized in EZ prep solution, and then they covered with cell conditioning 1 (CC1) to unmask the antigenicity by selecting mild CC1, standard CC1 for sixty minutes. The activity of endogenous peroxidase was blocked by the Inhibitor (3% hydrogen peroxide (H_2O_2)), and the endogenous biotin was blocked by biotin blocking solution. Then the sections were treated with the primary antibody (Anti-

Epstein-Barr virus (LMP- 1)) for sixteen minutes, and then slides were incubated in secondary antibody (biotinylated antibody). Then the slides were incubated in Horse Radish Peroxidase (HRP) of concentration less than 300µg/mL in the presence of Copper (5g/L $CuSO_4$) as a co-factor, and then diaminobenzidene (DAB) substrate (2g/L) was added to the sections to visualize the reaction producing dark brown color. Between each two steps slides were washed with the reaction buffer. The sections counterstained with Hematoxylin II (60%) and finally were treated with bluing reagent (0.1 M Li_2CO_3, 0.5 M Na_2CO_3). Slides were moved from instrument, dehydrated in alcohol, cleared in xylene and mounted with DPX.

3.2.5 Result interpretation

All quality control measures were adopted, positive and negative control slides were used during histopathological and immunohistochemical staining.

Detection of more than 5 cells with brown cytoplasm per one field considered as positive result.

3.2.6 Data analysis

The obtained results and variables arranged in standard master sheet, then analyzed using statistical package for social science (SPSS) program. Frequencies, means and Chi squire tests were calculated.

3.2.7 Ethical consideration

Specimens were taken from Radiation Isotope Center Khartoum (RICK) hospital ethically after taken ethical clearance.

CHAPTER FOUR

RESULTS

Chapter four

RESULTS

A total of 50 samples of patients with lymph node disorders were investigated, 40 of them were lymphoma representing 83.3%, and the remaining 10 (16.7%) were benign as indicated in table (4.1).

The age of study population ranged between 7 month and 80 years with mean of age 40 years. Patients less than 40 years were 27 (45%) and older than 40 years were 33 (55%) as indicated in table (4.2)

The sex of study population revealed that 42(70%) patients were males and 18 (30%) patients were females, as indicated in table (4.3).

The description of lymphoma revealed that non Hodghkin lymphoma in 39 (65%) samples, Hodghkin lymphoma in 11(18.3%) samples as indicated in table (4.4).

EBV revealed positive expression in 3(5%) of lymphoma samples and negative expression in 47(78.3%) samples ,while all hyperplasia samples showed negative expression of EBV, as indicated in table (4.4).

Table (4.1): Histopathology diagnosis among the study samples

Sample	Histopathology diagnosis	Frequency	Percent
Malignant	Hodghkin lymphoma	11	18%
	Non-Hodghkin lymphoma	39	65%
Benign	Hyperplasia	10	16.7%
Total		60	100%

Table (4.2): Distribution of age groups among the study population

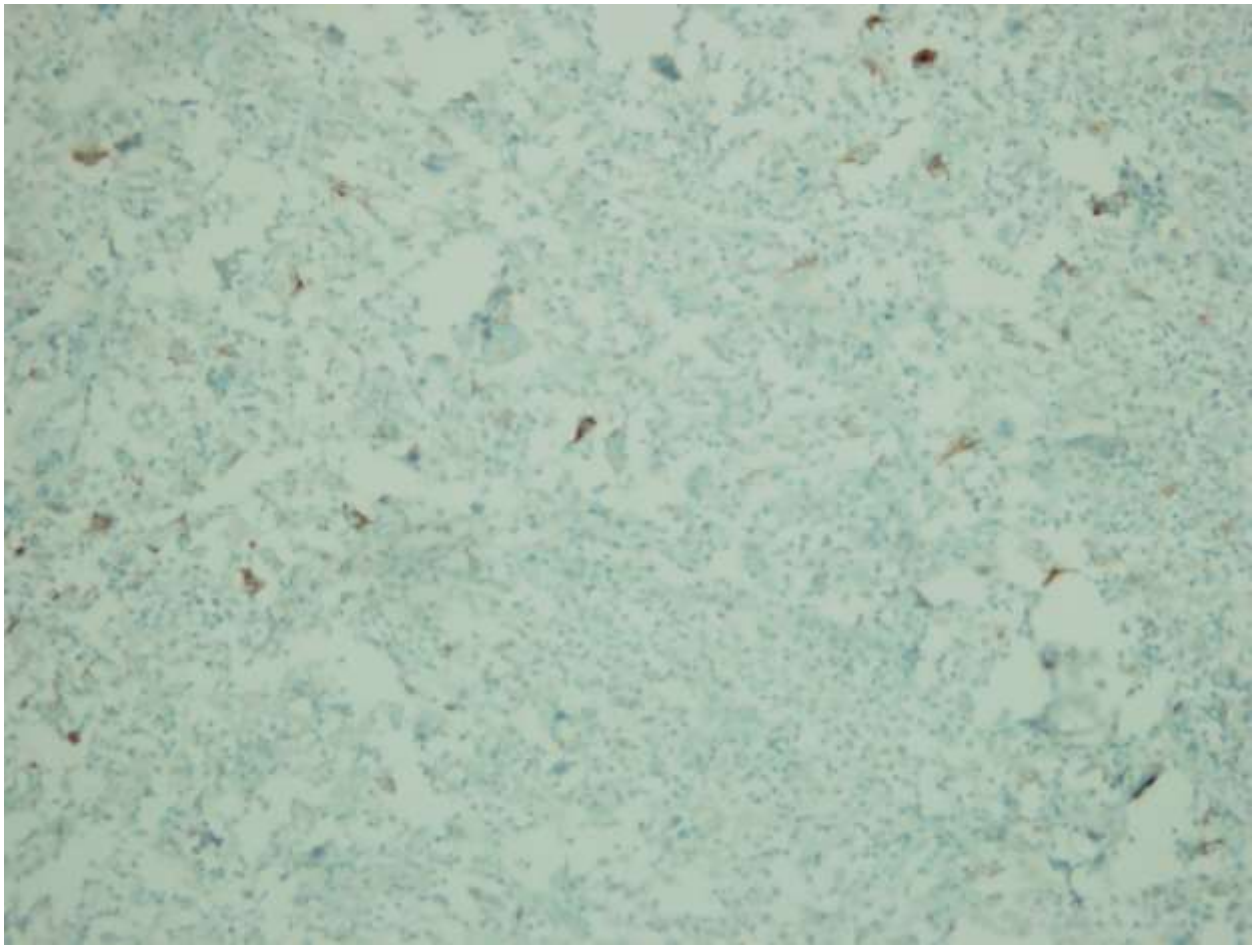
Age	Frequency	Percent
40 years and less	27	45%
More than 40 years	33	55%
Total	60	100%

Table (4.3): The distribution of sex among study population

Sex	Frequency	Percent
Male	42	70%
Female	18	30%
Total	60	100%

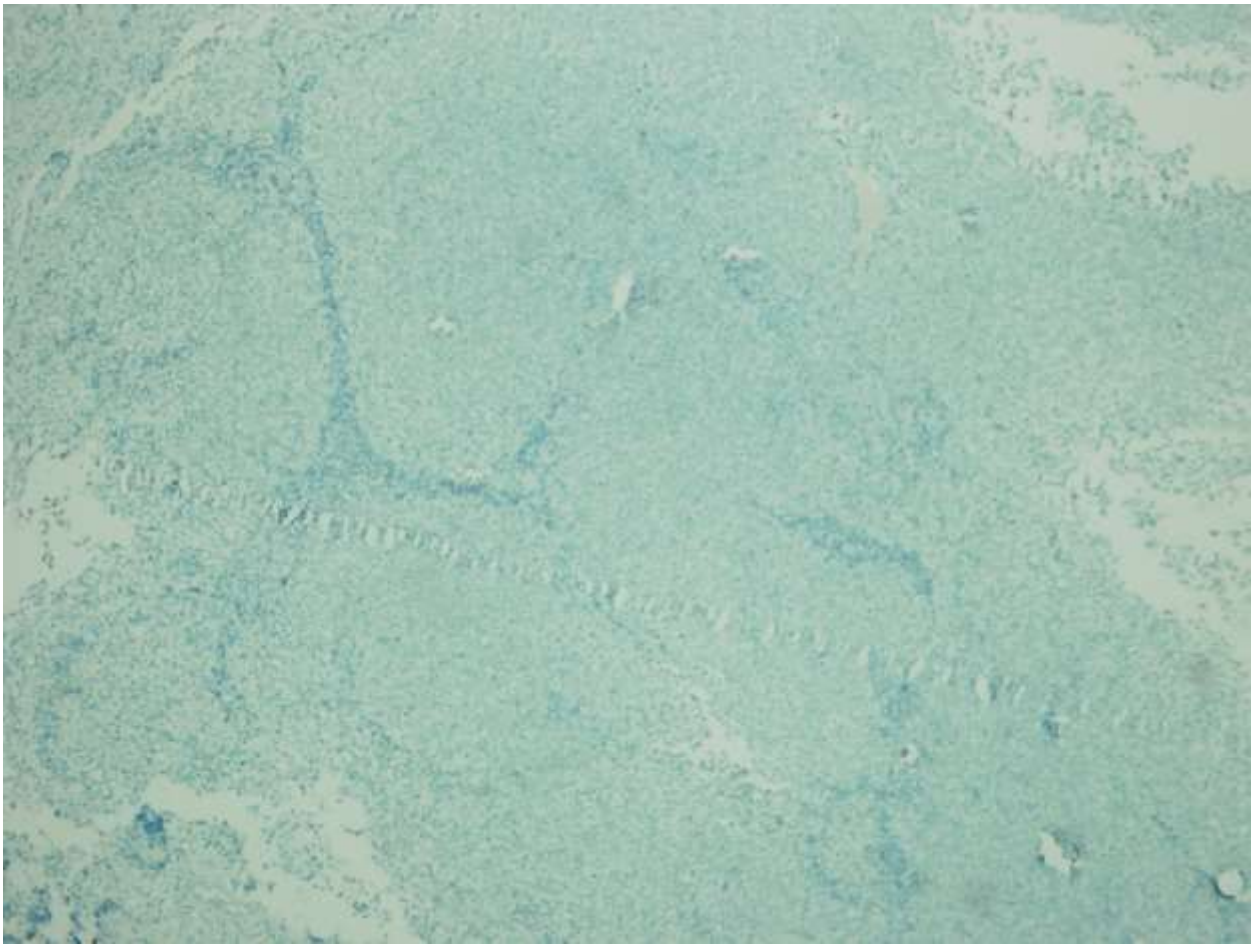
Table (4.4): Relation between the expression of EBV and histopathological diagnosis.

Histopathology diagnosis	EBV expression		Total	P.value
	Positive	Negative		
	N (%)	N (%)	N(%)	
Malignant	3(5%)	47(78.3%)	50 (83.3%)	0.427
Benign	0 (0%)	10 (16.7%)	10 (16.7%)	
Total	3 (5%)	57 (95%)	60 (100%)	



Micrograph (4.1)

Lymphoma showed negative results of EBV (40x)



Micrograph (4.2)

Lymphoma showed negative results of EBV (40x)

CHAPTER FIVE

DISCUSSION

Chapter Five
DISCUSSION

The top most common cancers in both sexes are breast, non-Hodgkin lymphoma, leukemia, esophagus (Elamin, 2015).

In this study out of sixty samples of patients with lymphoproliferative disorder were investigated by immunohistochemical method, 50 of them were lymphoma representing 83.3%, and the remaining 10(16.7%) were benign.

The age of the study population ranged between 7 month to 80 years with mean of age 40 years. Patients less than 40 years were 27(45%) and older than 40 years were 33 (55%). This mean that lymphproliferative disorder occurs in older age.This result incompatible with Abuelhassan, (1993), who reported that lymphoma more marked in children.

Regarding sex that males are more affected with lymphproliferative disorder than female representing (70%) and (30%) respectively. This result supported by Decaudin, *et al.*(2000), who reported that mantle cell lymphomas are characterized by a male predominance these observations suggest a possible relation between the chromosome X and mantle cell lymphomas which has to be explored. Also supported by Grundy, *et al*.(1973), who reported that non-Hodgkin's lymphoma was 2½ times more common among males than females. Also Yakubu, *et al.(*2015), reported lymphoma is common in male. Also Abuelhassan, (1993), reported males were commonly affected.The result also supported by Abuidris, (2008), who reported male: female ratio of 1.6:1.

Lymphoma revealed positive expression of EBV in 3(5%) patients, while all benign lymphnode showed negative expression of EBV in10 (16.7%) patients, this result show insignificant statistical association (P value 0.427).This result supported by Salah, *et al*. (2014), who concluded that there are no association

between EBV and malignant lymphoma and therefore, cannot be used as significant prognostic factor. Also the result supported by Ishtiaq, *et al*.(2013), who reported NHL cases were 38 and only one was positive for LMP 1 (3%). Also the result compatible with Mohammad, *et al*. (2013), who reported that nodal and extra nodal lymphoma are negative for EBV in IHC method.This study incompatible with Ibrahim, *et al*.(2015), who reported that there is sufficient evidence for the carcinogenicity of EBV in the causation of lymphoma. Also incompatible with Mori and Katano, *(*1997), who reported that several subtypes of human malignant lymphomas are known to be highly associated with the EBV. All hyperplasia specimens are EBV negative compatible with Huh, (1998), who examined hyperplasia samples for the presence of the genome of Epstein-Barr virus, he concluded that there is no evidence that EBV plays any role in the pathogenesis of lymphadenitis. Also the result compatible with Jing, (2013), who reported that all hyperplasia specimens were negative for EBV. This result also incompatible with Stefan, *et al*. (2011), who reported that EBV-driven B-cell lymphoproliferative disorders (LPDs) occurs in immunosuppressed patients with primary immune deficiency, or post transplantation immunosuppression or who have received other treatments. McGuire, (1988), also reported that benign lymphoepithelial lesions were positive for EBV genome.

CHAPTER Six

Conclusions and Recommendations

Chapter Six

Conclusion and Recommendations

Conclusion:

On the basis of this study we concluded that:

Most lymphoma patients in Sudan appear to be above 40 years old, the male were affected more than female.

EBV infection not associated with lymphoma

Recommendations:

On the basis of this study we recommended that:

Similar studies should be carried out with larger sample size and combined with additional method like PCR and in situ hybridization or EBNA1 immune stain to detect EBV genome.

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APPENDICES

APPENDICES

MATERIALs AND INSTRUMENTS:

Materials and instruments used for processing and staining of the specimens include:-

Disposable gloves

Microtome knife

Microtome SLEE CUT 5062

Positively charged slide

Cover glass

Oven

Water bath

Embedding center

Bench Mark GX autostainer

EZ prep

Cell Conditioning 1 (CC1)

Reaction buffer

Primary antibody (Anti-Epstein-Barr virus (LMP- 1)).

IVIEW DAB Detection Kit:

- (1) 25 mL IVIEW Inhibitor (3% H_2O_2)
- (1) 25 mL IVIEW Biotinylated Ig Secondary Antibody (< 200µg/mL)

- (1) 25 mL IVIEW SA- HRP (< 300 µg/mL)
- (1) 25 mL IVIEW DAB Substrate (2g/L)
- (1) 25 mL IVIEW H2O2 (< 0.08% H_2O_2)
- (1) 25 mL IVIEW Copper (5g/L $CuSO_4$)

Hematoxylin II (≤ 60%)

Bluing Reagent (0.1 M Li_2CO_3, 0.5 M Na_2CO_3)

Ethanol (70%, 90% and 100%).

Xylene.

DPX mounting media.