بسم الله الرحمن الرحيم

Sudan University of Science and Technology
College of Graduate Studies

Immunohistochemical Detection of Survivin in Non-Hodgkin's Lymphoma among Sudanese Patients

الكشف النسيجي الكيميائي المناعى عن السيرفيفين فى الليمفوما اللاهودجكينية عند المرضى السودانين

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (Histopathology and Cytology)

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قال تعالى:

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَاۖ إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ (32))

صدق الله العظيم

(سورة البقرة الأية 32)

Dedication

To my mother

To my father soul

To my husband

To my brothers

To my family

To all my teachers

To all my colleagues and friends

with love and respect.

Acknowledgment

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, college of medical laboratory science, Sudan university of science and technology for their continuous support and encouragement.

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Abstract

This is an analytical retrospective case control hospital based study was conducted in Radiation and Isotope Center Khartoum (RICK), during the period from August 2016 to February 2017. The study aimed to detect Survivin expression in non-Hodgkin's lymphoma among Sudanese patients using immunohistochemistry.

Forty paraffin embedded blocks from patient's samples previously diagnosed as non-Hodgkin's lymphoma were collected. Samples include 26 (65%) malignant tumors, (including diffuse large B.cell lymphoma 17 (42.5%) samples, Burkhits lymphoma 2 (5.0%) samples, anablastic large cell lymphoma in 1 (2.5%) samples, B.cell non-Hodgkin's lymphoma 2 (5%) samples, follicular lymphoma 2 (5%) samples, small lymphocytic lymphoma 2 (5%) samples) and 14 (35%) reactive hyperplasia benign samples. The patient's age ranged between 22 and 65 years with mean age of 45 years, most patients were above 40 years representing 28 (70%) and the remaining 12 (30%) patients were under 40 years.

One section of 3 micrometer thickness was cut from each paraffin block by rotary microtome and stained by immunohistochemical method (indirect streptoavidin-biotin immunoperoxidase technique) for detection of Survivin. Data collected from patients files and results obtained were analyzed using SPSS computer program. Immunohistochemical expression of survivin was revealed positive expression in 20/26 samples and negative expression in 6/26 samples in malignant, while benign samples revealed positive expression in 4/14 samples and negative expression in 10/14 samples for survivin with significant statistical association between survivin expression and histopathology diagnosis of non-Hodgkin's lymphoma (P.value = 0.006).

This study concludes that Survivin expression is associated with malignant forms of non-Hodgkin's lymphoma.

المستخلص

أجريت هذه الدراسة التحليلية التراجعية المستشفوية الحالة و الحالة الضابطة فى المركز القومى للعلاج بالاشعة و الطب النووي فى ولاية الخرطوم خلال الفتره من أغسطس 2016 الى فبراير 2017. هدفت الدراسة للكشف عن السيرفيفين فى الليمفوما اللا هودجكينية باستخدام كيمياء الانسجه المناعية. جمعت أربعون عينة مطمورة بشمع البارفين, من عينات مرضى تم تشخيصهم مسبقا الليمفوما اللا هودجكينية. تتكون العينات من 26 (65%) عينة لاورام خبيثة، تضمنت الليمفوما اللا هودجكينية المنتشرة الكبيرة (42.5%) من العينات، و الليمفاوية بوركيت فى عينة (5%) ، ورم الخلاية الكبيرة المتحولة فى عينة (2.5%) ، ليمفوما بائية الخلاية فى عينة (5%) ، سرطان الغدد الليمفاوية الجريبى فى عينة (5%) ، ليمفوما لبمفاوية صغيرة فى عينة (5%)، و14 (35%) عينة للأورام حميدة تضمنت فرط التنسيج التفاعلى.

تراوحت أعمار المرضى بين 22 ـ 65 سنة متوسط العمر 45 سنة، أغلب المرضى 28 (70%) كانت أعمارهم أكثر من 40 سنة و بقية المرضى 12 (30%) كانت أعمارهم اقل من 40 سنة.

تم قطع مقطع واحد من كل عينة بسمك 3µm بواسطة جهاز المشراح الدوار. تم صبغ العينات بواسطة كيمياء الانسجة المناعية للكشف عن سيرفيفين. تم جمع البيانات من ملفات المرضى. تم أستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية SPSS لتحليل البيانات.

أظهرت الدراسة أن التعبير المناعى للواسمة سيرفيفين انها موجبة الظهور فى 26/20 عينة وسالبة الظهور فى 26/6 عينة من عينات الاورام الخبيثة بينما عينات الأورام الحميدة أظهرت أنها موجبة الظهور فى 14/4 عينة و سالبة الظهور فى 14/10 عينة للسيرفيفين مع وجود علاقة زات دلالة إحصائية بين السيرفيفين ونوع الورم (القيمةالاحتماليه=0.006).

خلصت الدراسة الى ان هناك علاقة بين افراز السيرفيفين و لليمفوما اللاهودجكينية.

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Chapter One

Introduction

1.1 Introduction:

Lymphoma is a cancer of the lymphocyte occurs when cells grow abnormally and out of control. Lymphoma is heterogeneous group of malignances of lymphoid system. The two major types of lymphomas are Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) (Sathiya and Muthuchelian, 2009).

Worldwide, non-Hodgkin's lymphoma are comprise 85% of all lymphomas, account for 3–4% of all cancer (Basem and Ahmed, 2015). NHLs are further classified into B-cell lymphomas which account for about 90% and T-cell lymphomas which is about 10% (Chuan, *et al.* 2015).

In Sudan, NHL is the second most common cancer in men after prostate cancer (Gasmelseed, *et al.* 2014).

Risk factors of non-Hodgkin's lymohoma are age, gender, body weight, diet, exposure to certain chemical, radiation exposure, immune system deficiency, autoimmune disease and relation to infections (e.g.EBV, HIV) (Van Krieken, 2008).

The diagnosis of lymphoma is done by biopsy, histological examination of neoplastic lymphocytes, physical examination, blood test, molecular technique cytogenetic, flow cytometry, immunohistochemical analysis, computed tomography (CT) scan, ultrasound, magnetic resonance imaging (MRI) and positron emission tomography (PET) (Sathiya and Muthuchelian, 2009).

Lymphoma is treated by chemotherapy, biological therapy, radiation therapy and stem cell transplantation (Stephen and James, 2005).

Survivin is a member of apoptosis inhibiting protein (IAPs) family. It inhibits both caspase–dependent and caspase-independent apoptosis. play a critical role in regulating the cell cycle and mitosis. In malignant cell survivin is over expressed and has been shown to be an almost universal tumor antigen being expressed in most human neoplasms. In tumor cell survivin is critical for cell division and inhabitation of apoptosis (Chuan, *et al.* 2015).

Ellen *et al.* reported that survivin expression in malignant tumor (55%) of non-Hodgkin's lymphoma (Ellen, *et al.* 2004).

The expression of survivin has been demonstrated to be a promising prognostic indicator, associated with a worse overall survival (OS) in a number of cancers (Chuan, *et al*, 2015).

1.2 Objectives:

1.2.1 General objective:

To study the expression of survivin in non-Hodgkin's lymphoma among Sudanese patients.

1.2.2 Specific objectives:

1- To detect survivin expression in lymphoma sample using immunohistochemistry.

2- To correlate survivin expression with non-Hodgkin lymphoma subtypes and tumor grade.

Chapter Two

Literature Review

2.1 Scientific background:

Lymphoma is a cancer of the immune system and include more than 20 malignant diseases that originate from B and T lymphocyte. About 90 percent of people with lymphoma have non-Hodgkin lymphoma (Andrew, *et al*.2007).

2.2 Structure of lymph node:

Lymph node is a large accumulation of lymphatic tissue organized as a definite lympatic organ. Such nodes are located along the course of lymphatic vessels. Lymph node are scattered in large number, usually in group. They are fat, well defined bodies' varing from 1mm to 25mm in diameter. There form is rounded or kidney-shape, there surface is somewhat rough. Usually there is a slight indentation,the hilus on one side of the node where blood vessels enter and leave the organ. Lymphatic vessels enter the node at many places over its convex surface but leave it only at the hilus. The lymph node is covered by a capsule of dense collagen fiber. Trabeculae of dense collagenous connective tissue arise from the capsule and pentetrate the organe. Some loosely meshed areas occur under the capsule and along the trabecular, where they are called subcapsular and trabecular sinuses respectively. Developmentally, lymph sacs exist till the end of embryonic period. During early foetal period these sacs are transformed into groups of lumph nodes. Mesenchymal cell invade lymph sacs and form capsule and connective tissue framework of lymph node primordia. (Aksh, *et al*. 2012).

2.3 Disorders of the non-Hodgkin lymphoma:

2.3.1 Indolent disorder:

2.3.1.1 Follicular lymphoma:

Follicular lymphoma is generally an indolent B-cell lymphoproliferative disorder of transformed follicular center B-cells. FL is characterized by diffuse lymphoadenopathy, bone marrow involvement, splenomegaly and less commonly other extranodal sites of involvement. In general cytopenia can occur but constitutional symptoms of fever, night sweats and weight loss are uncommon (Arnold, 2014).

2.3.1.2 Cutaneous T.cell lymphoma (CTCL):

CTCL are a heterogenous group of non-Hodgkin lymphoma. They are a clinically and histologically diverse group of T lymphocyte malignancies that manifest in the skin. Mycosis fungoides (MF) is the most common subtybe of CTCL and typically runs an indolent course. Sézary syndrome (SS) is a rare subtybe of CTCL that is traditionally defined as the triad of erythroderma, lymphadenopathy and the presence of circulating monoclonal T lymphocytes with distinctive cerebriform nuclei (sézary cells) (Arulogun, *etal*. 2015).

2.3.1.3 Lymphoplasmacytic lymphoma:

Lymphoplasmacytic lymphoma is an indolent malignancy of B-cell and plasma cell. The disease present in the adult with bone marrow and lymph node involvement. Extranodal involvement is rare but has been reported in spleen and liver (Albawardi, *et al*. 2013).

2.3.1.4 Marginal zone lymphoma (MZL):

In the world health organization (WHO) classification of tumor of hematopoietic and lymphoid tissues the group of marginal zone lymphomas (MZL) comprises three different entities, namely the

extranodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue currently named MALT lymphoma and previously defined as low grade B-cell lymphoma of MALT type. Nodal marginal zone B-cell lymphoma previously known as monocytoid lymphoma. Splenic marginal zone B-cell lymphoma. The term MZL means that extranodal MZL, nodal MZL, splenic MZL are believed to drive from B cell normally present in the marginal zone, which is outer part of the mantle zone of B-cell follicles. While splenic and nodal MZL are quite rare, each comprising approximately 2% of lymphomas, the extranodal MZL of MALT type is not uncommon, representing approximately 8% of the total number of non-Hodgkin lymphoma cases in western countries. The overall survival (OS) rates range between 80% to 95% at 5 years. Splenic MZL mainly occurring in the elderly, commonly pursue a truly indolent course with approximately 70% of patients alive at 10 years from the diagnosis and nearly 30% of patients eventually dying of causes unrelated with the lymphoma (Franco, 2013).

2.3.1.5 Small lymphocytic lymphoma/Chronic lymphocytic leukemia (SLL/CLL):

SLL/CLL is relatively indolent, small B-cell neoplasm and is the counterpart of the peripheral blood bone marrow disorder. In the current world health organization (WHO) classification these entities are unified as SLL/CLL and considered to be functionally equivalent in terms of treatment and prognosis. SLL/CLL has no pathognomonic molecular or cytogenetic event (Dennis, *et al*. 2005).

2.3.1.6 Waldenstrom macroglobulinemia:

It is an indolent lymphoma characterized by bone marrow infiltration with lymphoplasmacytic cell associated with a monoclonal immunoglobulin (IgM) protein. It's considered incurable (Pashtoon, *et al*. 2015).

2.3.2 Aggressive disorder:

2.3.2.1 Diffuse large B-cell lymphoma:

It is the most common type of lymphoma with an incidence of three to five cases per 100.000 inhabitants and is increasing with age (Verhoef, *et al*. 2013).

2.3.2.2 AIDS associated lymphoma:

Acquired immunodeficiency syndrome related lymphoma is usually a late manifestation of infection by the human immunodeficiency virus (HIV) with a predilection for widespread, extra nodal disease, involvement of the CNS and poor prognosis. The prevalence of AIDS has increase among minority populations in women, those who acquired HIV by heterosexual (Levine, *et al.* 2000).

2.3.2.3 Anaplastic large cell lymphoma:

It is most common in children and young adult′s but has a bimodal age distribution and can occur in older adults. Anaplastic large cell lymphoma represents approximately 10% to 15% of pediatric/adolescent non-Hodgkin lymphoma, as compared to 2% of adult non-Hodgkin lymphoma and 30% to 40% of pediatric large cell lymphoma (Kinney, *et al*. 2011).

2.3.2.4 Burkitt lymphoma:

It has had an important role in the understanding of tumorigenesis. It was the first human tumor to be associated with a virus, one of the first tumors shown to have a chromosomal translocation that activates an oncogene and the first lymphoma reported to be associated with HIV infection. Burkitt lymphoma is the fastest growing human tumor, with a cell doubling time of 24-84h and was the first childhood tumour to respond to chemotherapy alone (Molynevx, *et al*. 2012).

2.3.2.5 Central nervous system lymphoma:

Malignant lymphoma can occur in the central nervous system in the absence of involvement elsewhere at the time of diagnosis or as secondary involvement in the setting of systemic lymphoma. The most common morphology in both primary and secondary CNS lymphoma is that of a diffuse large B-cell lymphoma (DLBCL), but avariety of lymphomas including low-grade B-cell lymphomas and T-cell lymphomas can affect the CNS (Giannini, *et al*. 2014).

2.3.2.6 Lymphoblastic lymphoma:

It is a highly aggressive neoplasm of lymphoblasts of either B-cell (B.LBL) or T-cell origin (T.LBL). LBL is a rare disease for which specific incidence data are missing (Bassan, *et al*.2015).

2.3.2.7 Mantle cell lymphoma (MCL):

It is a biological heterogeneous disease, account for approximately 6% of all NHL, although MCL may occur in younger patients and the majority of patients are males (Alan and Andre, 2015).

2.3.2.8 Peripheral T-cell lymphoma (PTCL):

PTCL are drived from post-thymic mature T-cell, They are rare and accounting for only a small portion of all lymphomas (Kerry and Tony, 2012).

2.4 Epidemiology of NHL:

Worldwide, non-Hodgkin lymphoma are comprise 85% of all lymphomas, account for 3-4% of all cancer. NHL has numerous histological subgroup, entails various biological behavior, clinical properties and epidemiological differences (Basem and Ahmed, 2015). According to different types of lymphoid cells, NHL is further classified into B-cell lymphomas which account for about 90% and T-cell lymphomas which is about 10 % (Chuan, *et al*. 2015).

The incidence of non-Hodgkin lymphoma is steadily during the last several decades. NHL is ranked as the 12^{th} most common cancer worldwide and the 4^{th} most common cancer in Sudan with an age standardized rate (ASR) of 5.9 per 100,000 in both sexes. In Sudan NHL is the second most common cancer in men after prostate cancer with an (ASR) of 8.2 per 100.000, while in females rank the fifth with ASR of 3.7 per 100.000 (Gasmelseed, *et al*. 2014).

2.5 Risk factor of NHL:

2.5.1 Age:

The risk of NHL increases with age. The most common type occur most often in people in their 60s and 80s (Bairey, *et al. 2006).*

2.5.2 Gender:

NHL incidence among males is significantly higher than in female. In addition to gender itself, gravidity has a protective role against NHL occurrence (Horesh and Horowitz, 2014).

2.5.3 Exposure to certain chemicals:

Exposure to certain chemicals such as pesticides and petro chemicals may increase the risk of NHL (Eriksson, *et al*. 2008).

2.5.4 Radiation exposure:

It is suggested that increasing exposure to ultraviolet radiation may be at least in part responsible for observed increasing in incidence of NHL (Yawei, *et al*. 2011).

2.5.5 Immune system deficiency:

The Immune deficiency is one of the best characterized and strongest known risk factors for NHL. Incidence of NHL in people with congenital or acquired immune deficiency is 50 or more times higher than population rates. Risk of NHL increases with degree of immune deficiency (Andrew, *et al*. 2007).

2.5.6 Autoimmune disease:

Autoimmune disease comprise a heterogeneous group of condition associated with failure of the immune system to recognize self and consequent inflammatory disease. It's clear that certain specific autoimmune disorders markedly increase the risk of NHL (Andrew, *et al*. 2007).

2.5.7 Autoimmune disease and relation to infection (e.g. EBV, HIV):

The risk of EBV-associated NHL in HIV-1 infected patients compared with the general population is mainly increased by impaired immunosurveillance against EBV and B-cell chronic immune activation (Petrara, *et al* 2013).

2.5.8 Body weight:

Over weight and obesity may be associated with an elevated risk of NHL and particularly of diffuse large B-cell lymphoma (Larsson and wolk, 2007).

2.5.9 Diet:

High caloric and protein intakes were found to be associated with elevated risk of NHL, also found to be associated with increased risk of NHL, whereas a significantly reduced risk was observed with higher consumption of vegetables (Amanat, *et al* 2013).

2.6 Diagnosis of NHL:

2.6.1 Biopsy:

The diagnosis is made by removing the enlarged lymph node or part of it and examining under microscope. Biopsy is made on the basis excisional lymph node or extranodal tissue biopsy providing enough material for formalin-fixed sample. Core biopsy should only be performed in patient without easily accessible lymph node or in patients requiring emergency treatment. Fine needle aspiration (FNA) alone is not acceptable as a reliable for initial diagnosis of NHL (Fadilah, 2009).

2.6.2 Histological examination of neoplastic lymphocyte:

It is encompass both nodal and extranodal lymphomas, provides distinction of individual lymphoid neoplasms based upon morphologic, immunophenotyping, cytogenetic and clinical features. Histologic examination typically is the gold standard and the majority of the lymphoid neoplasms will require the utilization of one or more other anacillary techniques (Paul and Avery, 2004).

2.6.3 Physical examination:

This exam includes checking for enlarged lymph node in the neck, under arms, groin and also checks for a swollen spleen or liver (Paul and Avery, 2004).

2.6.4 Blood test:

Blood test measure the amount of certain type of cell and chemicals in the blood. They are not used to diagnose lymphoma. Patient with known or suspected lymphoma will have a complete blood count (CBC). This test measures the different cell in the blood. In patient already known to have lymphoma, low blood cell count can mean that the lymphoma is growing in the bone marrow and affecting new blood cell formation (Candelaria, 2016)

2.6.5 Molecular technique cytogenetic:

This technique allows doctors to evaluate the chromosome (long strands of DNA) in the lymphoma cell. The cell are looked at under a microscope to see if chromosomes have any abnormalities,some lymphoma cell may have too many chromosomes, too few chromosomes or other change such as a translocation. These changes can help identify the type of lymphoma and to identify the specific chromosomal translocations that are more commonly seen in particular NHL subtypes may be necessary in case of diagnostic difficulties (Paul and Avery, 2004).

2.6.6 Flow cytometral:

This test looks for certain substance on the outside surface of cell that help identify what types of cell they are. Flow cytometry can help determine whether the lymph node is swollen because of lymphoma or some other cancer or a non-cancerous disease. It has

also become very useful in helping doctors determine the exact type of lymphoma so that they can select the best treatment (Zahid, 2006).

2.6.7 Immunohistochemical analysis:

In this test a part of the biopsy sample is treated with special antibodies that attach only to specific molecular on the cell surface. These antibodies causes color changes which can be seen under a microscope. This test may be helpful in distinguishing different type of lymphoma from one another and from other diseases. (Fadilah, 2009).

2.6.8 Computed tomography (CT) scan:

The CT scan is an x-ray tests that produces detailed, cross-sectional images of the body. CT scan can show the detail in soft tissue such as internal organs. This scan can help tell if any lymph nodes or organs in the body are enlarged and are useful for looking for lymphoma in the abdomen, pelvis, chest, head and neck (Raanani, *et al*. 2005).

2.6.9 Ultrasound:

Ultrasound can be used to look at lymph nodes near the surface of the body or to look inside abdomen for enlarged lymph nodes and also able to characterize the abnormal shape, appearance and had limited role in follow up and it use no radiation (Khaleel and Mohammed, 2015).

2.6.10 Magnetic resonance imaging (MRI):

This test is not used as often as CT scan for lymphoma, but if doctor is concerned about spread to the spinal cord or brain, MRI is very useful for looking at these areas. MRI scans provide detailed images of soft tissues in the body (Thomas, *et al*. 2011).

2.6.11 Positron emission tomography (PET):

PET scans are very useful for showing up cancerous cells. They use a radioactive form of a sugar called (fluorodeoxyglucose or FDG) to show up the most active cell in the body and lymphoma cell are usually very active. PET scans can help tell if an enlarged lymph node contains lymphoma and can also be used after treatment in helping decide whether an enlarged lymph node still contain lymphoma or is merely scar tissue (Markus and Hinrich, 2005).

2.7 Treatment of NHL:

2.7.1 Chemotherapy:

Chemotherapy for lymphoma uses drugs to kill lymphoma cells. It is called systemic therapy because the drugs travel through the blood stream. The drugs can reach lymphoma cells in almost all part of the body (Kimby, *et al.* 2001).

2.7.2 Biological therapy:

This type of treatment helps the immune system fight cancer. Monoclonal antibodies are the type of biological therapy used for lymphoma. They are protein made in the lab that can bind to cancer cells and help the immune system kill lymphoma cell (Tim, *et al.* 2014).

2.7.3 Radiation therapy:

Radiation therapy also called (radiotherapy) uses high energy rays to kill lymphoma cells. It can shrink tumor and help control pain (Tim, *et al.* 2014).

2.7.4 Stem cell transplantation:

If lymphoma return after treatment may receive stem cell transplantation. A transplant of blood-forming stem cells allows to receive high dose of chemotherapy, radiation therapy or both (Gribben, 2017).

2.8 Survivin and it's relation with non-Hodgkin's lymphoma:

Survivin is a member of the inhibitor of apoptosis protein (IAP) family and functions both as an apoptosis inhibitor and as a regulator of cell division. Survivin overexpression is common in many human tumors and correlates with survival in non-Hodgkin's lymphoma (Ansel, *et al.* 2004).

Survivin is expressed during the mitotic phase of cell cycle and localizes to multiple components of the mitotic apparatus and centrosome. Survivin overexpression has been shown to counteract apoptosis in vitro and also in transgenic animals (Altieri, 2001). A recent study has also shown that survivin is overexpressed in diffuse large B-cell lymphoma and this is negative prognostic factor for survival in these patients (Adida, *et al.* 2000).

Overexpression of survivin has been demonstrated in a variety of human cancer. Among hematologic malignancies, survivin is expressed in high grade non Hodgkin's lymphomas. Moreover, in one study survivin expression predicted clinical outcome in patients with diffuse large B-cell lymphoma. Anaplastic large-cell lymphoma (ALCL) account for approximately 3% of adult and 10% to 30% of childhood non-Hodgkin's lymphomas (Altieri, 2001).

An increased level of survivin expression has been reported in the malignant lymphocytes from patients with large-cell non-Hodgkin's lymphoma and was found to be associated with a poor clinical outcome. This would suggest that survivin may be an important molecule in aggressive lymphomas and may be a potential therapeutic target in this disease (Adida, *et al.* 2000).

Li and Huanming reported that expression of survivin is significantly higher in aggressive NHL (P.value 0.000). It has been suggested that survivin may contribute to the progression of NHL by playing an important role in promoting cell proliferation, inhibiting cell apoptosis and enlisting angiogenesis. Survivin expression is closely related to malignant grade and therefore may be considered an important prognostic factor of NHL (Li and Huanming. 2006).

Chapter Three

Material and Methods

3.1 Materials:

Archived block of non-Hodgkin's lymphoma tumor were selected for this study.

3.2 Methods:

3.2.1 Study design:

This is hospital based analytical retrospective case control study aimed to study the expression of survivin in non-Hodge ken lymphoma tissues.

3.2.2 Study samples:

Tissue blocks obtained from twenty six samples previously diagnosed as malignant (NHL) tumors and fourteen samples diagnosed as benign tumors. Patient's data (age, histopathological diagnosis, malignant tumor grade) were obtained from patient files.

3.2.3 Study area:

This study was held in Radiation and Isotope Center (Khartoum State) during period from August 2016 to February 2017.

3.2.4 Sample processing:

Section to be stained were cut at 3µm thickness by rotary microtome, mounted in positively charged glass slides and put at 60 °C oven for 30 minutes.

3.2.5 Immunohistochemical staining:

Immunohistochemical staining was carried out using indirect steroavidin biotin immune peroxidase technique. Tissue sections (3µm) were deparaffinized in xylene and rehydrated in graded alcohol (100%, 90%, 70%, 50% and water) Antigen retrieval was

performed by using Dako water path with citrate buffer (pH6.8). slides were then incubated for 10 minutes in 0.3% hydrogen peroxide to block endogenous peroxidase activity.

The slides then treated with anti survivin primary antibody for 30 minutes. Then sections were incubated in biotinylated secondary antibody for 15 minutes, then washed in phosphate buffer saline (pH7.4), then incubated in streptavidin-HRP (horseradish peroxidase) for 15 minutes, washed in phosphate buffer saline (pH7.4), incubated in diaminobenzidine tetra hydrochloride (DAB) substrate solution washed in running tap water. Then counterstained in Mayer's hematoxylin stain for 1 minute. Dehydrated, cleared and mounted in DPX mounting media (Thermo scientific, 2014).

3.2.6 Data analysis:

Data analysis was done using SPSS 20 computer program. Frequencies mean and chi-square test values were calculated.

3.2.7 Results analysis:

All quality control measure were adopted, positive and negative control slides were used during immunohistochemical staining. Detection of more than 5 cells with cytoplasm brown color per one field considered as positive result.

3.2.8 Ethical consideration:

Samples were collected after taking ethical acceptance from hospital administration.

Chapter Four

4. Results

The age of study population range between 22 and 65 years with mean age of 45 years and standard deviation 12.6.

Most patients were more than 40 years representing 28 (70%) and the remaining 12 (30%) were less than 40 years as indicated in table (4.1). The study includes forty samples, 26 (65%) samples were malignant and 14 (35%) samples were benign. The diagnosis of malignant samples include diffuse large B-cell lymphoma 17 (42.5%), Burkhits lymphoma 2 (5%) and anablastic large cell lymphoma 1 (2.5%), B.cell non-Hodgkin's lymphoma 2 (5%), Follicular lymphoma 2 (5%) and small lymphocytic lymphoma 2 (5%). And the diagnosis of benign sample include reactive hyperplasia in 14 (35%). as indicate in Table (4.2).

Survivin positive expression was found (20/26) in malignant samples, while (6/26) samples showed negative expression, benign samples showed positive expression (4/14) samples, while (10/14) samples showed negative expression for survivin. This result showed significant association (P.value=0.006) as indicated in table (4.3).

Table (4.1): Distribution of age group among the study population:

Age group	Frequency	Percentage
Less than or equal 40 years	12	30%
More than or equal 40 years	28	70%
Total	40	100%

Table (4.2): Distribution of histopathological diagnosis among the study samples:

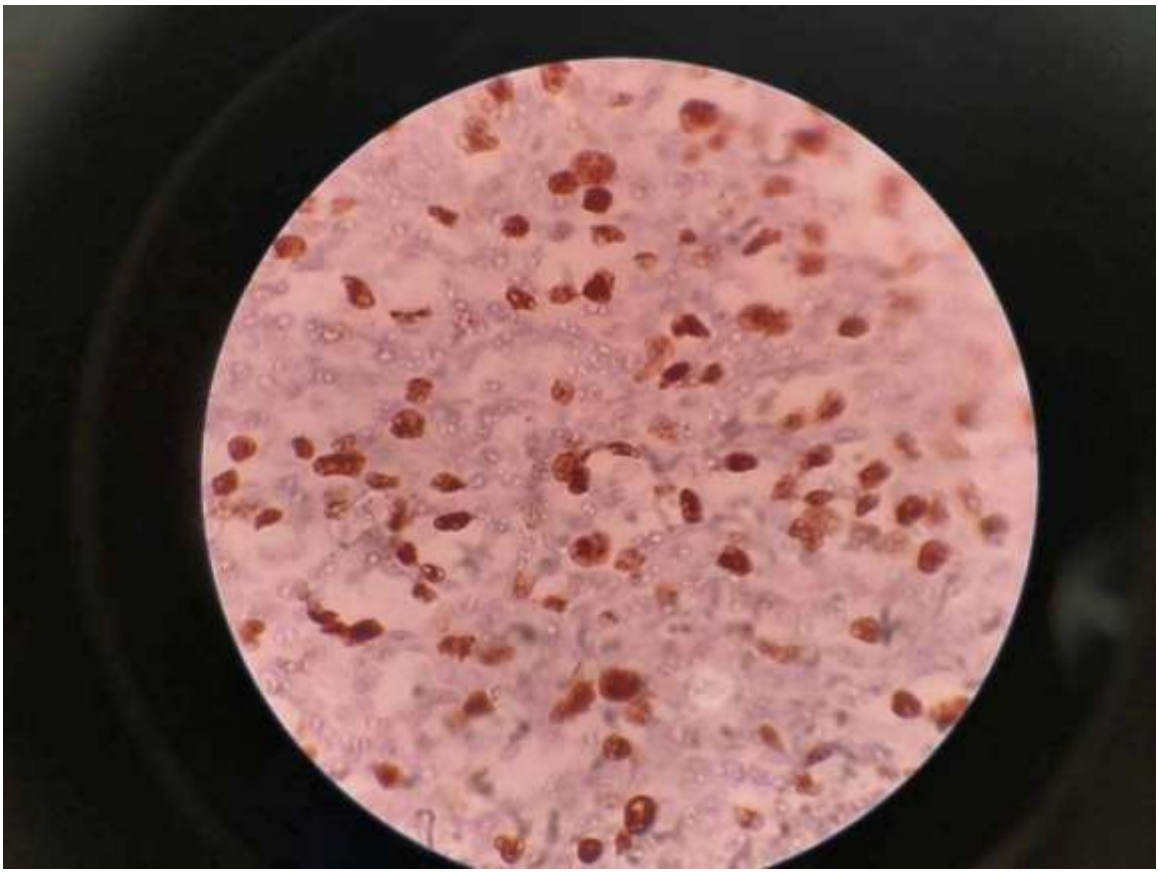
Histopathological diagnosis	Type	Frequency	Percentage
Benign	Reactive hyperplasia	14	35%
Malignant	B.cell NHL	2	5%
	Follicular hyperplasia	2	5%
	Small lymphocytic lymphoma	2	5%
	Diffuse large NHL	17	42.5%
	Burkhits lymphoma	2	5%
	Anablastic NHL	1	2.5%
Total		40	100%

Table (4.3): Relation between histopathological diagnosis and Survivin expression:

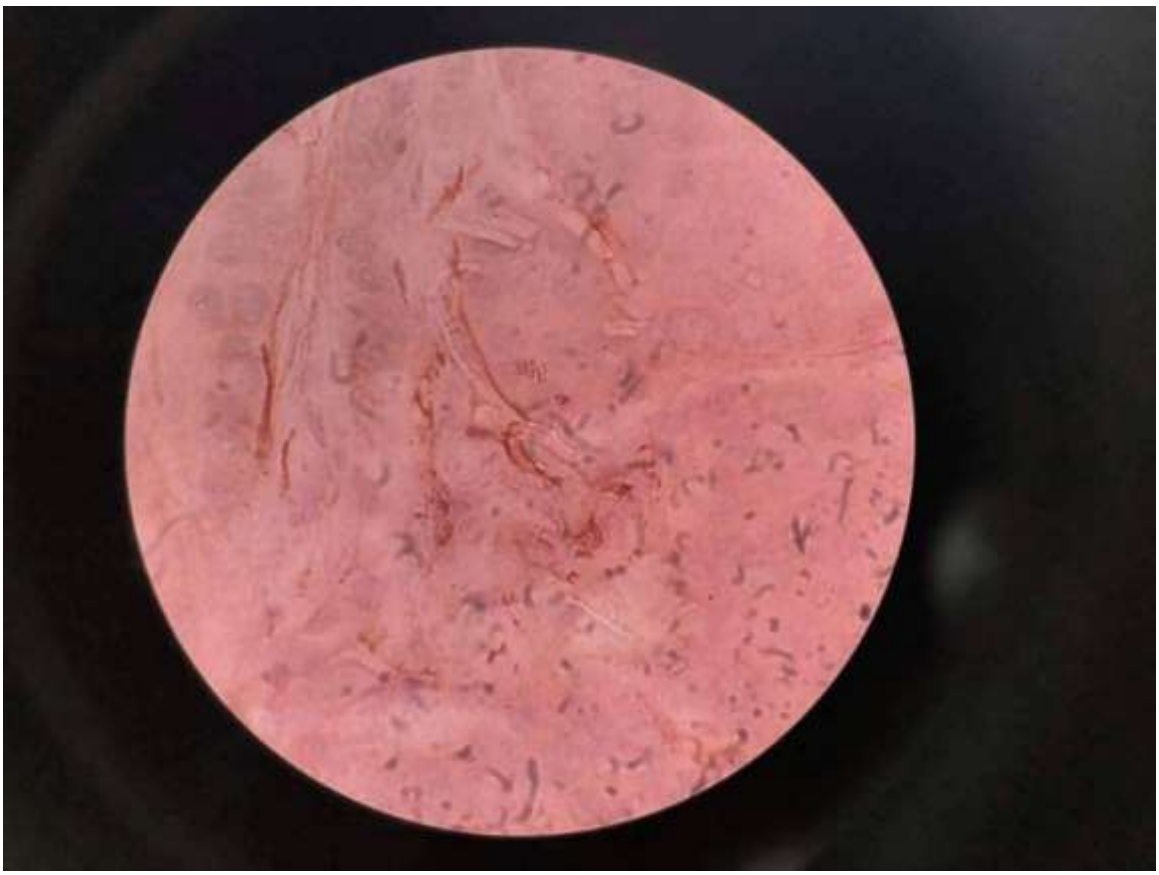
Histopathological diagnosis	Survivin expression		Total	P.value
	Positive	Negative		
Reactive hyperplasia	4(29.0%)	10(71%)	14	0.006
Lymphoma	20(77%)	6 (23%)	26	
			40	

Table (4.4): Relation between histopathological diagnoses of non-Hodgkin's subtypes and Survivin expression:

Type Histopathological diagnosis	Survivin expression	
	Positive	Negative
B.cell NHL	2	0
Diffuse large NHL	14	3
Follicular hyperplasia	0	2
Burkhits lymphoma	1	1
Anablastic NHL	1	0
Small lymphocytic lymphoma	2	0



Photograph (4.1) Diffuse large non-Hodgkin's lymphoma show positive expression of survivin in cytoplasm (40x).



Photograph (4.2) Reactive hyperplasia show negative expression of survivin in cytoplasm (40x).

Chapter Five

5.1 Discussion

The present study involved 40 cases of non-Hodgkin lymphoma tumors, Immunohistochemical stained for survivin. Regarding the age group of patient's the study revealed the most patients were more than 40 years, indicating that older patients are more susceptible to NHL. This result is compatible with Kenneth *et al*. (1984), who proved that there was a direct correlation between the age and Non-Hodgkin's Lymphoma. Also agree with Akyurek *et al*. (2006), who reported that the incidence rate of Non-Hodgkin's lymphoma increased greatly in older peoples. The histopathological diagnosis of patients revealed that more frequent type of non-Hodgkin's lymphoma was diffuse large B-cell lymphoma, this result is compatible with Martinez, (2015). Who reported that 30-40% cases of malignant tumors of non-Hodgkin's lymphoma were diagnosed as diffuse large B-cell lymphoma. Its compatible with Maurizio *et al.* (2013), who reported that 37% cases of malignant lesions of non-Hodgkin's lymphoma were diagnosed as diffuse large B-cell lymphoma.

Regarding Survivin expression, the study found that (20/26) 77% of malignant lesions showed positive expression and (6/26) 23% showed negative expression, while (4/14) 29% of benign lesions showed positive expression and (10/14) 71% showed negative expression. This relation showed significant association (P.value=0.006). This result is compatible with Adida *et al.* (2000), who reported that 61% of malignant lesion showed positive expression. Also agree with Ellen *et al.* (2004), who reported that survivin was expressed in 34(55%) tumors also showed significant association (P.value=0.007).

5.2 Conclusion:

From this study we conclude that:

The age of the non-Hodgkin's lymphoma in our study is commonly more than 40 years.

Most histological type of non-Hodgkin's lymphoma in our study is the diffuse large non-Hodgkin lymphoma.

Survivin expression is associated with non-Hodgkin's lymophoma comparent to benign and also associated with diffuse large non-Hodgkin's lymphoma type.

5.3 Recommendations:

From this study we recommended that:

Further research should be done on expression of survivin in non-Hodgkin's lymphoma with small sample size.

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Appendix 1:

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

Disposable gloves.

Rotary microtome.

Microtome knives.

Positively changed slides (Thermo).

Cover glasses.

Dry oven.

Water path (Dako water path).

Coplin jars.

Humidity chamber.

Ethanol (100%, 90%, 70%, 50%).

Xylene.

Mayer's haematoxylin.

Phosphate buffer (pH 7.4).

Citrate buffer (pH 6.8).

0.3 Hydrogen peroxidase.

Primary antibody (survivin).

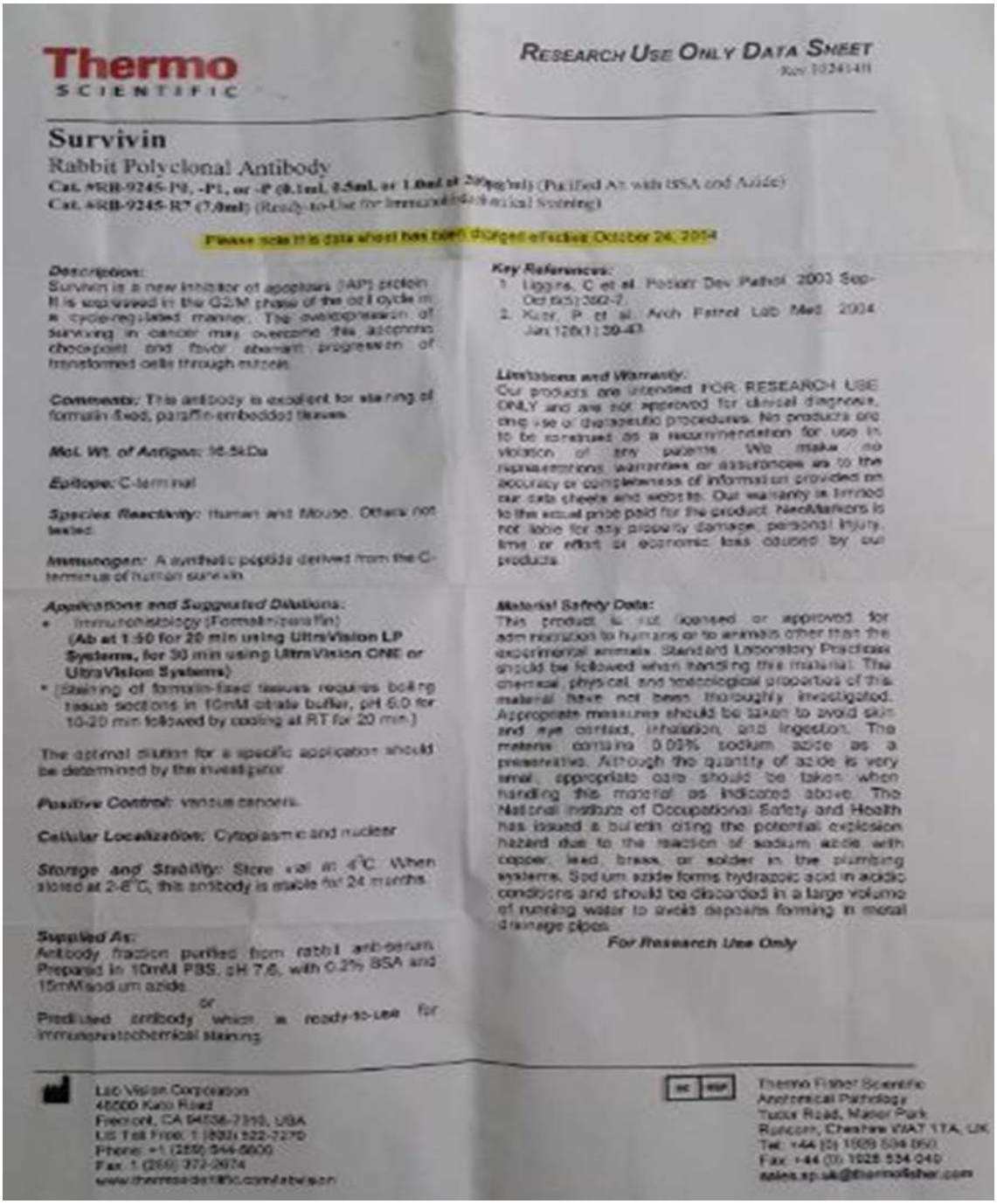
Secondary antibody (biotinylated secondary antibody).

Streptavidin-HRP.

Substrate chromogen (DAB).

DPX.

Appendix 2:



Thermo SCIENTIFIC

RESEARCH USE ONLY DATA SHEET

Survivin

Rabbit Polyclonal Antibody

Cat. #RB-9245-P0, -P1, or -P (0.1mL, 0.5mL or 1.0mL at 200µg/mL) (Purified Ab with BSA and Azide)

Cat. #RB-9245-R7 (7.0mL) (Ready-to-Use for Immunohistochemical Staining)

Please note this data sheet has been changed effective October 24, 2014

Description:
Survivin is a new inhibitor of apoptosis (IAP) protein. It is expressed in the G2/M phase of the cell cycle in a cycle-regulated manner. The overexpression of survivin in cancer may overcome the apoptosis checkpoint and favor aberrant progression of transformed cells through mitosis.

Comments: This antibody is excellent for staining of formalin fixed, paraffin-embedded tissues.

Mol. Wt. of Antigen: 16.5kDa

Epitope: C-terminal

Species Reactivity: Human and Mouse. Others not tested.

Immunogen: A synthetic peptide derived from the C-terminus of human survivin.

Applications and Suggested Dilutions:

- Immunohistology (Formalin/paraffin)
  (Ab at 1:50 for 20 min using UltraVision LP Systems, for 30 min using UltraVision ONE or UltraVision Systems)
- (Staining of formalin-fixed tissues requires boiling tissue sections in 10mM citrate buffer, pH 6.0 for 10-20 min followed by cooling at RT for 20 min.)

The optimal dilution for a specific application should be determined by the investigator.

Positive Control: various cancers.

Cellular Localization: Cytoplasmic and nuclear

Storage and Stability: Store vial at 4°C. When stored at 2-8°C, this antibody is stable for 24 months.

Supplied As:
Antibody fraction purified from rabbit anti-serum. Prepared in 10mM PBS, pH 7.6, with 0.2% BSA and 15mM sodium azide.
or
Predilued antibody which is ready-to-use for immunohistochemical staining.

Key References:
1. Liggins, C et al. Pediatr Dev Pathol. 2003 Sep-Oct;6(5):392-7.
2. Kuter, P et al. Arch Pathol Lab Med. 2004 Jun;128(6):639-43.

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