Biological Analysis of Exon 5 in P53 Gene among Non-Hodgkin Lymphoma Patient's using DNA Sequencing Technique, National Cancer Institute, Gezira State, Sudan (2017)

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B.Sc. University of Gezira Haematology and Immunhematology 2013

A Dissertation

Submitted to University of Gezira in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Haematology and Immunhaematology

Department of Haematology and Immunhematology

Faculty of Medical Laboratory Sciences

November, 2017
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Date of Examination: 18 / 12 / 2017
DECLARATION

This thesis is a presentation of my original research work, wherever contributions of others are involved, and every effort is made to indicate this clearly, with due reference to the literature, and acknowledgment of collaborative research and discussion.

The work was done under the guidance of Dr. Ozaz Yagoub and Dr. Ahmed Mohammed Ahmed Ali EL-kathir at University of Gezira, Faculty of Medical Laboratory sciences.

Reham Mobark Abbas Ahmed

Place: Wad-Medani

Date: ............
Dedication

To

My Mother

A strong and gentle soul who taught me to trust in Allah, believe
in hard work and that so much could done with little

To

Those who believe in me... That lightens my way and
encouraged me and gave me every piece of help

My brother & my sister
I thank all who in one way or another contribute in a completion of this research. Praise to Allah who gave me strength, courage and patience to bear the burden of this research. I am highly indebted to Dr. Ahmed ELkathir for his support and great help. I deeply thanks to my supervisor Dr. Ozaz Yagoub for her guidance and constant supervision .I am so grateful to the Africa city for science and technology for making possible for me to work there, and extend my thanks to Mohammed Abdu El-rahman and the staff work there. Also, I thank Dr. Salah ALzaki for his advices and help .Finally I thank Dr. Ahmed Mohamed Salih, Dr. Hisham, and Youssef Abdu ALhameed.
Biological Analysis of Exon 5 in P53 Gene among Non-Hodgkin Lymphoma Patient's using DNA Sequencing Technique, National Cancer Institute, Gezira state, Sudan (2017)

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Abstract

Non-Hodgkin lymphoma (NHL) is the diverse group of blood cancers that share a single characteristic. Generally develops in the lymph nodes or in the lymphatic tissue found in organs, which arises from an injury to the DNA of a lymphocyte. The altered DNA in one lymphocyte produces a malignant transformation. Inactivation of the tumor suppressor gene (TP53) by various genetic alterations is a major event in human tumorigenesis. Epidemiological studies of the hematological malignancies indicate that the prognosis of patients with a mutation in the p53 gene is inconsistent. The aim of this study was to detect mutations in p53 (exon5) gene in Sudanese NHL patients. This is a cross-section study was conducted in National Cancer Institute in Gezira state from April-September 2017. Thirty patients (17 males and 13 females) were examined which include different subtypes (4 Burkitt's lymphoma, 16 Diffuse large cell lymphoma, 2 Follicular lymphoma, 3 lymphoblastic lymphoma, 1 Mantle cell lymphoma, 1 marginal zone lymphoma, 1 mixed small & large lymphoma and 2 small lymphocytic lymphoma). The age group from 4-70 years old with mean (37 years). The demographic data of each patient and control were collected using questionnaire. 2-3 ml of blood was collected in EDTA container for DNA extraction, the purified DNA was introduced for amplification by using polymerase chain reaction (PCR), in order to replicate the P53 gene (exon5), then the products about 252 bp in size were evaluated by gel electrophoresis. The products of genomic DNA were sent for sequencing to detect mutations using Sanger technique. The results were analyzed by using Finch TV software and Bioedit to compare the multiple sequence alignment of the patient's and control's sequence to find the differences, also s no differences were found when used the BLAST software. There was no mutation detected in p53 (exon5) in NHL Sudanese patients. The use of other bioinformatics tools is recommended.
التحليل البيولوجي لإكسون (5) لморوث بروتين 53 وسط المرضى المصابين بسرطان الغدد الليمفاوية غير الهودجكين باستخدام تقنية تسلسل الحمض النووي، المعهد القومي للسرطان، ولاية الجزيرة، السودان (2017)

رهام مبارك عباس احمد

ملخص الدراسة

سرطان الغدد الليمفاوية غير هودجكين هو مجموعة متنوعة من سرطانات الدم التي تنقسم سمة واحدة. يتطور بشكل عام في الغدد الليمفاوية أو في الأنسجة الليمفاوية الموجودة في الأعضاء، والتي تنشأ من الخل الجيني للخلية الليمفاوية. إن الحمض النووي المتغير في الليمفاويات الواحدة ينتج تحولًا خبيثًا. تطيل الجينات الكابحة للورم ( מורث بروتين 53) من خلال مختلف التغييرات الجينية هو حدث كبير في الأورام البشرية. وتشير الدراسات الوابئية للأورام الخبيثة الدموية إلى أن تشخيص المرضى الذين يعانون من طفرة في مورث بروتين 53 غير متاسفة. وكان الهدف من هذه الدراسة الكشف عن الطفرات في مورث بروتين 53 (اكسون 5) في مرضى سرطان الغدد الليمفاوية السودانيين غير الهودجكين. تم إجراء دراسة شاملة في المعهد الوطني للسرطان في ولاية الجزيرة من أبريل إلى سبتمبر 2017. تم فحص ثلاثين مريضاً (17 ذكور و13 إناث) تشمل أنواع فرعية مختلفة (4 سرطان الغدد الليمفاوية بيركلي, 16 سرطان الغدد الليمفاوية كبيرة الخلايا المنتشرة, 5 سرطان الغدد الليمفاوية الخلايا الصغيرة, 3 سرطان الغدد الليمفاوية الأورومية, 1 سرطان الغدد الليمفاوية المنطقة الهامشية, 1 سرطان الغدد الليمفاوية الخلايا الردارية, 1 سرطان الغدد الليمفاوية الخلايا المختلطة الصغيرة والكبرية). الفترة العمرية من 4-70 سنة بمتوسط عمر 37 سنة. تم جمع البيانات الديموغرافية لكل مريض والسيطرة باستخدام الإسحاق. تم جمع 2-3 مل من الدم في حاوية إنتا لإستخراج الحمض النووي. وقدم الحمض النووي المنقي إلى التصميم باستخدام فصل البوليمرات المتسلسل من أجل تكرار مورث بروتين 53 (اكسون 5). تم تقييم المنتج حوالي 252 في الحجم من قبل هلام الكحالي. تم إرسال منتجات الحمض النووي الجيني للتكشف عن الطفرات باستخدام تقنية سنجار. تم تحليل النتائج باستخدام برنامج فيتش وبيولوجيا لمقارنة تسلسل متبقي من تسلسل المرضى والسيطرة على الاختلافات. كما لم يتم العثور على أي اختلافات عند استخدام برنامج بلاست. لم يكن هناك أي طفرة اكتشفت في مورث بروتين 53 (اكسون 5) في سرطان الغدد الليمفاوية غير هودجكين للمرضى السودانيين. ووصى باستخدام أدوات المعلوماتية الحيوية الأخرى.
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<td>Hodgkin lymphoma</td>
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<td>DNA</td>
<td>Deoxyribo nucleic acid</td>
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<td>HTLV</td>
<td>Human T-cell lymph cytotropic virus</td>
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<td>HIV</td>
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<td>SPSS</td>
<td>Statistical package of social science</td>
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<td>BLAST</td>
<td>Basic local alignment tool</td>
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Chapter one
Introduction

1.1 Background:
Lymphoma is a general term for a group of blood cancers that start in the lymphatic system, and it is the most common blood cancer in adults and the third most common cancer overall in children. Malignant lymphoma comprises 3.37% of all malignancy worldwide, in Korea malignant lymphoma accounted for 3.69% of all malignancies in 2008 (Jooryung Huh, 2012). The incidence of malignant lymphoma exhibits a marked geographical variation higher in North America, Australia/New Zealand, and Europe, and lower throughout Asia and Africa, except where Burkitt lymphoma is endemic. The age-standardized incidence rates for East Asia reveals lower incidences of Non-Hodgkin lymphoma and Hodgkin lymphoma in East Asia compared with Western countries (E, Roman A G Smith, 2011).

Lymphoma develops when a genetic error or mutation occurs in the way a lymphocyte is produced, causing the abnormal cell to duplicate faster than a normal cell or live longer than a normal lymphocyte. In which a lymphocyte undergoes a malignant change and multiplies, eventually crowding out healthy cells and creating tumors, these tumors enlarge the lymph nodes and or grow in other sites that are part of the immune system (for example the skin, spleen, lymph nodes and other organ). Neoplasms of lymphoid origin characterized by the abnormal proliferation B or T cells in lymphoid tissue, typically causing lymphadenopathy (John Walter 2013). Lymphoma comprises more than 67 subtypes of two related cancers that affect the lymphatic system, Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL).

There are six primary types of Hodgkin lymphoma and at least 61 types of non-Hodgkin lymphoma. Hodgkin lymphomas are different from non-Hodgkin lymphomas in spread and treatment.
1.2 Justification:
The prognostic significance of TP53 mutation for NHL is inconsistent in several studies, and it is secondary events in different types of B & T cell lymphoma. Confirmation of the NHL beside detection of mutation in this gene can improve the therapeutic plan.

1.3 Objectives:

1.3.1 General objective:
To screen P53gene (exon5) for possible mutation in Non Hodgkin lymphoma Sudanese patients using DNA sequencing technique.

1.3.2 Specific objectives:
1. To correlate the relationship between NHL and TP53 mutation.
2. To find out if there any relation between the mutation and some factor like family history, sex and ethnicity.
Chapter two
Literature review

2.1 The Lymphatic System:
The lymphatic system is one of the most important parts of the immune system and protects the body from disease and infection. The lymphatic system is a circulatory system that is made up of a series of thin tubes called lymph vessels that branch like blood vessels into all tissues of the body.

Lymph vessels carry lymph, a fluid that contains white blood cells called lymphocytes. Within this vast network of vessels are groups of small, bean-shaped organs called lymph nodes. Thousands of lymph nodes are found in almost all places in the body, including the elbows, neck, under the arms and in the groin (Kaushansky K, et al 2010). Lymph flows through lymph nodes and specialized lymph tissues such as the spleen, tonsils bone marrow and thymus gland. Lymph nodes filter lymph fluid, removing bacteria, viruses and other foreign substances from the body (Michallet AS, et al 2012). If a large number of bacteria are filtered through a node or series of nodes, swelling may occur and the nodes may become tender to the touch.

Lymphoid neoplasms are a group of distinct entities with widely varying clinical features, immunophenotypes and genetic abnormalities. The WHO classification of lymphoid neoplasm encompasses not only Hodgkin lymphoma (HL) and Non-Hodgkin lymphoma (NHL), but also plasma cell neoplasm and lymphoid leukemia. With the underlying belief that lymphoma and lymphoid leukemia represent solid and circulating phases respectively (Hjalgrim H, et al 2008).

The lymphoma can be divided into two major groups; Hodgkin lymphoma and Non-Hodgkin lymphoma:

2.2 Hodgkin lymphoma:
Hodgkin lymphoma (HL) one of the most curable forms of cancer, was named after Thomas Hodgkin, a British physician who first identified the disease in 1832 (John Walter 2013). Hodgkin lymphoma is a malignant disease of the lymphoid tissue which originates from B lymphocyte and less common T lymphocyte, a characterized
by the presence of multinucleated giant cell known as Red-Sternberg cells (Franziska C, et al 2009).

2.2.1 Epidemiology of Hodgkin Lymphoma:
Hodgkin lymphoma is a rare cancer, accounting for less than one percent of all cases of cancer in the United States. According to the American Cancer Society, approximately 8,500 new cases of Hodgkin lymphoma are projected each year. Although the cancer can occur in both children and adults, it is most commonly diagnosed in young adults between the ages of 15 and 35 and in older adults over age 50 (Caporaso, N.E, et al 2009). Nearly 10 to 15 percent of all Hodgkin lymphomas are diagnosed in children and teenagers. The disease is more common in men than in women. According to the American Cancer Society over the last 30 years incidence rates have decreased in men and slightly increased in women. There are marked geographic variations in Hodgkin’s lymphoma incidence, but they are different for various age groups, there is also an interesting association between Hodgkin’s lymphoma and markers of social class. A higher incidence occurs in western countries and in westernized populations including those who emigrate from low incidence sites to the United States, but HL is less common in Asian countries, especially in the young adult (Franziska C, et al 2009).

2.2.2 Causes and risk factor:
Most cases of HL occur in people who do not have identifiable risk factors; most people with identifiable risk factors do not develop HL (John Walter, 2009), the following are examples of risk factors:

- Patients who have a history of a blood test confirming mononucleosis have increased risk of HL compared to the general population.
- People infected with human T-cell lymphocytotropic virus (HTLV) or human-immunodeficiency virus (HIV) also have increased probability of developing HL.
- A weakened immune system caused by either an inherited condition or the use of immunosuppressants to prevent organ transplant rejection has also been linked to the development of HL.
- A family history of the disease, especially among first-degree relatives of a person with Hodgkin lymphoma, such as parents and siblings.
2.2.3 Development of Hodgkin lymphoma:

Hodgkin lymphoma develops when a lymphocyte (usually a B-cell) becomes abnormal (cancerous). These abnormal cells are called Reed-Sternberg cell (R-S); named after two scientists Dorothy Reed and Carl Sternberg who discovered the cells. Most people with HL have R-S cells, although other abnormal cell types may be present as well (Caporaso, N.E, et al 2009). Reed-Sternberg continually divide making more abnormal cells, which do not die and forming mass of tissue called a tumour. However the presence of R-S cells alone is not conclusive that an individual has HL, in addition to R-S cells the lymphatic tissue sample must also include others cells and features that are characteristic for Hodgkin disease (Thomas, et al 2002). Hodgkin lymphoma usually starts in the lymph nodes and may be first noticed in areas such as (the neck, above the collarbone, under the arms or in the groin). And because lymph tissue all over the body are connected; abnormal lymphocytes can circulates in lymphatic vessels causing the lymphoma to spread from one lymph node to another throughout the body. However, HL can also spread to other areas and organs outside the lymph system.

2.2.4 Types of Hodgkin lymphoma:

Hodgkin lymphoma has been divided into two main classifications; classical Hodgkin lymphoma—which accounts for about 95 percent of all cases of Hodgkin lymphoma—and lymphocyte predominant Hodgkin lymphoma. There are four main subtypes of classical Hodgkin lymphoma and two subtypes of lymphocyte predominant Hodgkin lymphoma, which are determined by tumor type. The type of tumor a patient has may affect treatment choices. In each type of Hodgkin lymphoma, the tumor cells and the R-S cells are mixed with many normal cells. The abnormal cells are usually in the minority (John Walter, 2013).

2.2.4.1 Classical Hodgkin lymphoma:

Subdivided into four major subtypes:

1. **Nodular sclerosis:**

This is the most common subtype of Hodgkin lymphoma, accounting between 60 percent and 80 percent of all cases of the disease. In nodular sclerosis, the involved lymph nodes contain areas composed of R-S cells mixed with normal white blood cells. The lymph nodes often contain prominent scar tissue, hence the name nodular...
sclerosis (scarring). The disease is more common in women than men, and it usually affects adolescents and adults under 50. The great majority of patients are cure with current treatments.

2. **Mixed cellularity:**

This is the second most common type: accounts for about 15 to 30 percent of all cases of Hodgkin lymphoma and is found more commonly in men than women. The disease is characterized by the involved lymph nodes containing many R-S cells in addition to several other cell types, scarring is not apparent and the development of mixed cellularity appears to be associated with HIV and the Epstein-Barr virus. Mixed cellularity primarily affects older adults. More extensive disease is usually present by the time this subtype is diagnosed.

3. **Lymphocyte depletion:**

This is the least common form of Hodgkin lymphoma, accounting for fewer than five percent of all cases, and is characterized by few normal lymphocytes but abundant R-S cells. Lymphocyte depletion is aggressive and usually not diagnosed until the disease is widespread (Hjalgrim H, et al 2008).

4. **Lymphocyte-rich:**

This recently identified form of Hodgkin lymphoma is rare, accounting for less than five percent of all cases. The disease may be diffuse or nodular in form, and is characterized by the presence of numerous normal lymphocytes and very few abnormal cells and classical R-S cells. It is usually diagnosed at an early stage in adults and has a lower lapse rate.

2.2.4.2 **Lymphocyte predominant Hodgkin lymphoma:**

1. **Nodular lymphocyte predominant:**

This type of Hodgkin lymphoma is rare, accounting for five percent to ten percent of all cases. It primarily affects more men than women and is usually diagnosed in people under 35. In nodular lymphocyte predominant, most of the lymphocytes found in the lymph nodes are normal (not cancerous). Typical R-S cells are usually not found in this subtype, but large, abnormal B cells with multi-lobulated nuclei, sometimes referred to as popcorn cells, can be seen, as well as reactive small B-cells, which may be distributed in a nodular pattern within the tissues. This subtype is usually diagnosed at an early stage and is not very aggressive. In many ways this form

2. **Diffuse lymphocyte predominant:**
This type of Hodgkin lymphoma is extremely rare, most cases are in fact nodular lymphocyte predominant Hodgkin lymphoma (described above) with an ill-defined nodular pattern, unlike nodular lymphocyte predominant Hodgkin lymphoma, less small benign B-cell are found. The lymphatic tissue is dominated instead by reactive non-malignant T-cell, disease is recurrence is common in this type.

2.2.5 **Signs and Symptoms:**
The most common warning sign of disease presence is a swelling of lymph nodes that may or may not be painful, most often occurring in the neck. Some people may also experience a swelling of lymph nodes in other parts of the body, such as the armpits and groin. Other symptoms of Hodgkin lymphoma may include unexplained intermittent fevers, weight loss (usually by more than ten percent of a person's normal weight), sweating (usually at night) or lack of energy. A less common symptom is persistent and unexplained itching that grows steadily more severe over time and usually affects the trunk, arms and legs, but it can also be more localized. Coughing, shortness of breath or chest discomfort may be signs of Hodgkin lymphoma in the chest. Usually there is no pain involved in Hodgkin lymphoma, especially in the early stages of the disease, although about five percent of patients may experience pain at the tumor site after drinking alcohol; this is an uncommon but specific symptom (Goldin, L, et al 2005).

2.2.6 **Diagnosis:**
An accurate assessment of Hodgkin lymphoma after physical examination; requires a number of diagnostic test, first examine the tissue under the microscope for the characteristic features of R-S cells in the surrounding tissue and then confirm the diagnosis by analyzing the antigens or markers on the surface of the cells. In classical Hodgkin lymphoma, the cell markers (antigens) areCD30 positive and CD15 positive. In nodular lymphocyte predominate Hodgkin lymphoma, the immunophenotype, or diagnostic proteins are CD30 negative, CD15 negative and CD20 positive (as seen with B lymphocyte) (John Walter, 2013).
Common tests used to make a diagnosis:

i. Biopsies:
A biopsy is a procedure in which a piece of tissue from an area of suspected cancer is removed from the body and examined under a microscope.

ii. Blood tests:
Patients have blood cell counts and other blood tests done to check indicators of disease severity such as blood protein levels, uric acid levels, erythrocyte sedimentation rate (ESR) and liver function.

iii. Bone Marrow examination:
Hodgkin lymphoma can spread to the B.M.

iv. Imaging test:
Imaging tests that help to provide best evaluation of cancer, and are useful in determining how many nodes are involved, how large they are and whether internal organs are affected by the disease; include:

- Chest X-ray.
- CT (computerized tomography) scan.
- PET (positron emission tomography) scan.
- Callium scan.

v. Cardiac function test.

vi. Pulmonary function test.

2.2.7 Staging of Hodgkin lymphoma:
A physical examination and the findings from imaging tests, computed tomography (CT) scans (also called diagnostic radiology), tissue biopsies and blood tests are used to determine the extent of the patient’s NHL. This process is called “staging,” and the information is used to determine appropriate treatments (chemotherapy, radiation);

**Stage I:** Apparent involvement of a single lymph node region or a single organ, such as bone.

**Stage II:** Involvement of two or more lymph node regions that are close to each other; for example, all in the neck and chest, or all in the abdomen and on the same side of the diaphragm (a thin muscle below the lungs).

**Stage III:** Involvement of several lymph node regions in the neck, chest and abdomen (on both sides of the diaphragm).
Stage IV: Widespread involvement of lymph nodes on both sides of the diaphragm and in other organs, such as the lungs, liver and bones.

The four stages of HL can be divided into categories A, B, X and E:

- The A category indicates the absence of fever, exaggerated sweating and weight loss.
- The B category indicates that patients have fever, excessive sweating and weight loss.
- The X category indicates bulky disease (large masses of lymphocytes).
- The E category indicates organs involved outside of the lymph system.

2.2.8 Treatment:

Some Treatment Approaches for Hodgkin Lymphoma:

1) Combination chemotherapy with or without involved field radiation:

Radiation therapy with chemotherapy is the most common treatment approach for HL, involved field radiation therapy targets the evident HL cell masses, and then chemotherapy is used to kill neighboring lymphoma cells. Radiation therapy consists of the use of special machines that produce high-energy rays capable of killing the HL cells (Goldin, L, et al 2005).

Chemotherapy may be given without radiation therapy to patients with widespread disease, fever, drenching night sweats and/or weight loss. It is usually involves at least four drugs given in combination, the drugs are dissolved in fluid and usually administered to the patient by vein through a peripheral intravenous (IV) line (Goldin, L, et al 2005). Chemotherapy combinations include:

- **ABVD** (Adriamycin [doxorubicin], bleomycin, vinblastine and Dacarbazine).
- **BEACOPP** (bleomycin, etoposide, Adriamycin [doxorubicin] cyclophosphamide, Oncovin [vincristine], procarbazine and prednisone)
- **StanfordV** (mechlorethamine[Mustargen], doxorubicin, vinblastine, vincristine, bleomycin, etoposide and prednisone).

2) High-dose chemotherapy with stem cell transplantation.

3) **Brentuximabvedotin (Adcetris)**: given intravenously (IV), has been
approved for the treatment of HL after failure of autologous stem cell transplant and in HL patients who are not autologous stem cell transplant candidates after failure of at least two multi-agent chemotherapy regimens.

2.3 Non-Hodgkin lymphoma:
Non-Hodgkin lymphoma (NHL) is the term used for a diverse group of blood cancers that share a single characteristic they arise from an injury to the DNA of a lymphocyte parent cell. The damage to the DNA is acquired (occurs after birth) rather than inherited. The altered DNA in one lymphocyte produces a malignant transformation. These lymphocytes and the formed cells have a better than normal chance to survive, the accumulation of those cells result in tumor masses found in lymph nodes and other sites in the body (John Walter, 2013).

NHL generally develops in the lymph nodes or in lymphatic tissue found in organs such as the stomach, intestines or skin. In some cases NHL involves marrow (spongy tissue in the hollow central cavity of the bones that is the site of blood cell formation) and blood. Lymphoma cells may develop in one or many sites in the body.

2.3.1 Epidemiology of Hodgkin lymphoma:
NHL is more common in the developed world, with the highest incidence rates in the USA, Australia and New Zealand, and Europe, and the lowest in Eastern and South Central Asia. Around the year 2000 the age standardized (world standard) incidence of NHL was estimated at approximately 14 per 100,000 person-years in the USA and Canada, 10 per 100,000 in Denmark and Sweden, and 3 per 100,000 in South Central Asia. However, the rare T-cell neoplasms are more common in Asia than in other regions (P. Boffetta, 2011).

Worldwide, NHL constitutes the tenth most commonly diagnosed malignancy; whereas in the developed world it ranks seven. In Sweden in 2003, malignant lymphomas (NHL) were the eighth most common new cancer diagnoses among males and the tenth most common in females. In the USA, NHL has climbed to the fifth most frequently diagnosed malignancy in recent years. The most common NHL subtypes by far in developed countries (disregarding CLL and plasma cell entities) are diffuse large B-cell lymphoma (about 30%) and follicular lymphoma (about 20%) . All other NHL subtypes have a frequency of less than 10%. Many subtypes are characterized by a slight preponderance of males, most striking in mantle cell
lymphoma (70% males), whereas females predominate in follicular lymphoma (Dominik D, et al 2007).

2.3.2 Types of Non-Hodgkin Lymphoma:
Lymphomas may be grouped by how quickly they are likely to grow:

- Indolent (also called low-grade) lymphomas grow slowly. They tend to cause few symptoms.
- Aggressive (also called intermediate-grade and high-grade) lymphomas grow and spread more quickly. They tend to cause severe symptoms. Overtime, many indolent lymphomas become aggressive lymphomas.

➔ NHL Subtypes and Frequency:

❖ B-Cell Lymphoma:

1. Diffuse Large B-Cell Lymphoma (31%).
2. Follicular Lymphoma (22%)
3. Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma (7.5%)
4. Small Cell Lymphocytic Lymphoma–Chronic Lymphocytic Leukemia (7%).
5. Mantle Cell Lymphoma (6%).
6. Mediastinal (Thymic) Large B-Cell Lymphoma (2.4%).
7. Lymphoplasmacytic Lymphoma–Waldenström Macroglobulinemia (less than 2%).
8. Nodal Marginal Zone B-Cell Lymphoma (less than 2%).
9. Splenic Marginal Zone Lymphoma (less than 1%).
10. Extranodal Marginal Zone B-Cell Lymphoma (less than 1%).
11. Intravascular Large B-Cell Lymphoma (less than 1%).
12. Primary Effusion Lymphoma (less than 1%).
13. Burkitt Lymphoma-Burkitt Leukemia (2.5%).
14. Lymphomatoid Granulomatosis (less than 1%).

❖ T-Cell and Natural Killer (NK)-Cell Lymphoma (about 12%):

1. Peripheral T-Cell Lymphoma, not otherwise specified.
2. Cutaneous T-Cell Lymphoma (Sézary Syndrome and Mycosis Fungoid).
3. Anaplastic Large Cell Lymphoma.
5. NK-Cell Lymphoma.
2.3.3 Causes and risk factor of NHL:
In general, the risk factors for non-Hodgkin lymphoma include the following:

1. Weakened immune system: The risk of developing lymphoma may be increased by having a weakened immune system (such as from an inherited condition or certain drugs used after an organ transplant).

2. Certain infections: Having certain types of infections increases the risk of developing lymphoma:
   - HIV virus.
   - EBV virus.
   - HTLV-1 virus.
   - H.pylori bacteria.
   - Hepatitis C virus.

3. Age: Although non-Hodgkin lymphoma can occur in young people; the chance of developing this disease goes up with age, most people with non-Hodgkin lymphoma are older than 60.

2.3.4 Signs and Symptoms:
   - Swollen, painless lymph nodes in the neck, armpits or groin.
   - Unexplained weight loss.
   - Fever.
   - Soaking night sweats.
   - Coughing, trouble breathing, or chest pain.
   - Weakness and tiredness that don’t go away.
   - Pain, swelling, or a feeling of fullness in the abdomen.

2.3.5 Diagnosis:

1) Physical examination.

2) Blood test: a complete blood count to check the number of white blood cells. The lab also checks for other cells and substances, such as lactate dehydrogenase (LDH). Lymphoma may cause a high level of LDH.

3) Chest X-ray.

4) Biopsy: A biopsy is the only sure way to diagnose a lymphoma, several method may be used to examining lymph node biopsy include:
   - Immunophenotyping: a process that determine the lymphoma cell (T cell, B cell or NK cell).
● Cytogenetic analysis: Chromosomal abnormalities can be important in identifying specific subtypes of NHL and choosing the most effective treatment approach.
● Gene expression profiling and microarray analysis identify cancer subtypes and risk factors; these tests help predict how patients will respond to treatment and which patients may be at increased risk to relapse.

2.3.6 Staging:
Physical examination and imaging tests help to evaluate the location and distribution of lymph node enlargement, whether organs other than lymph nodes are involved, and the large masses of tumors in one site or another.
NHL has the same stages and categories mentioned previously in HL (Franziska C, et al 2009).

2.3.7 Treatment:
- Chemotherapy
- Radiation.
- Stem cell transplantation.

2.4 Tumor suppressor gene (p53):

2.4.1 Basic information on p53 gene:
Tumor suppressor gene defined as genes which encode proteins that normally inhibit the formation of tumors. Then normal function is to inhibit cell proliferation, or act as the "brakes" for the cell cycle. Mutations in tumor suppressor genes contribute to the development of cancer by inactivating the inhibitory function (Magda Pinyol, et al 2000). P53 gene is mutated in about half of all human cancers; gene mutations are found in only 10–20% of cancers, and p53 mutations are present in 40-45% of cancers, more than 35% of the lung, skin, ovary, pancreas, liver carcinomas and 20% of gliomas, breast carcinomas, cervical carcinoma and breast cancers have p53 mutations (Nigro JM, et al 1989). B.Vogelstein and J.Minna in 1989 were the first to report the presence of p53 mutations in colorectal and lung cancer cells. Indeed, p53 mutation is the most frequent genetic event demonstrated to date.

P53 gene is a tetramer, it was initially identified as a tumor specific nuclear antigen of molecular weight 53KDa, it's spans about 20 kb and is located on the "p" arm of the chromosome 17 (17p13.1). The gene p53 was first discovered in 1979, a protein was identified in simian virus 40-transformed mouse cells (SV40) by immune-
precipitation with anti-T serum; this protein was called protein p53 (Nigro JM, et al 1989). It is now clearly established that p53 belongs to the category of tumor suppressor genes.

2.4.2 Structure of p53 gene:
The protein p53 gene contains three major domains; N-terminal domain (NTD), DNA-binding domain (DBD), and C-terminal domain (CTD). NTD consists of the acidicN-terminus transcription activation region and a proline-rich region. CTD consists of a tetramerization domain (TD) and a regulatory domain (RD) (Patricia A, et al 2012).
The N-terminal region can recruit some transcription factors like TBP, TFIID, RP-A and MDM2; which interacts with RP-A that can bind to single strand DNA. MDM2 binds this region and negatively regulates p53 in two ways. The first one is MDM2 bind to p53 and inhibits the transcription of p53 directly. Secondly, MDM2 would stimulate p53 degradation rapidly. The central region contains four conserved regions, I, II, III, IV shown in the third line. Zn also exists in this domain. About 80% of p53 mutations related to cancer are within this region. The C-terminal region contains TD and RD (Patricia A, et al 2012).

2.4.3 Regulation of p53:
The activation and stabilization of p53 is regulated by multiple proteins in response to diverse stress conditions. These include proteins which can modify p53 for both stabilization and increased transcriptional activity. Include:
- E3 ligase.
- Post-translational modifications.
- Co-factors.

2.4.4 The function of p53 protein:
1) The protein p53 is related to cell senescence:
Senescence in humans is caused by shorter telomeres. Compared with apoptosis, senescence just inhibits the proliferation of tumor cells, and cannot eliminate them from tissues. Cells during senescence undergo morphological changes, like cellular enlargement, and increased synthesis of lysosomes (Nigro JM, 1989). P53 was related to cell senescence, because they found that the level of p53 is elevated transiently and then dropped to the normal level during some cell senescence. They also found that the DNA binding activity and transcription activity of p53 are increased.
2) **The protein p53 can regulate the cell cycle:**

The protein p53 can inhibit transformations of cells because it cannot tolerate any abnormal situations and stimuli. When DNA is damaged, cell division arrest at phase G1/S, G2/M in the majority of organisms. G1/S can inhibit the replication of damaged template DNA, offer the opportunity for damaged DNA to repair, extend survival time of damaged cell and inhibit the proliferation of cells that contain damaged DNA (Wolfgang C, et al 1993).

3) **The protein p53 can trigger cell apoptosis:**

Apoptosis is another cell response for DNA damage. P53 regulates some apoptosis-related genes, which has a master role in many apoptotic pathways. For example; death receptor and mitochondria pathway death ligands bind to extracellular death receptors, forming death-inducible Signaling complex (DISC), then DISC activates caspase 8 with an adaptor protein, such as FADD and pro-caspase 8. The activated caspase 8 then cleaves the inactive precursor enzyme pro-caspase3, forming activated caspase 3 that induces apoptosis.
P53 causes the outer membrane of mitochondria to permeabilise, resulting in the release of cytochrome c, thus forming an apotosome with Apaf 1 and pro-caspase 9. Then the apotosome will activate caspase 9 and in turn activates caspase 3.
P53 activates pro-apoptotic genes and inactivates anti-apoptotic genes during this process (Young KH, et al 2008).

4) **The protein p53 can regulate angiogenesis:**

Angiogenesis is essential for the process of normal cells transformed to tumors. Thrombospondin-1 (TSP-1) is a potent inhibitor for angiogenesis. P53 stimulates the endogenous TSP-1 gene and positively regulates the promoter sequence of TSP-1.

2.4.5 The mechanism of mutant p53 function:

Molecular mechanisms by which mutant p53 may function divided into four main categories; these mechanisms reflect either:

- Mutant p53 binds to DNA to alter gene expression.
- Mutant p53 binds to transcription factors to enhance their function.
- Mutant p53 forms a complex with transcription factors to prevent their function.
- Mutant p53 interacts with proteins to change their function directly.
2.4.6 Analysis of p53 mutation:

Four analytical methods may be used to investigate p53 gene status:

i. **Molecular analysis:**
   Can be used to identify the type of mutation in the p53 gene.

ii. **Immunohistochemical analysis:**
   Can be used to demonstrate accumulation of mutant p53 protein in tumor cells.

iii. **Serological analysis:**
   Can be used to detect anti-p53 antibodies in the serum of the patients.

iv. **Functional analysis:**
   Can be used to measure the transactivating activity of p53.

2.4.7 Therapeutic course to target mutant p53:

Mutant p53 proteins are highly expressed in many cancers, making them extremely attractive targets for therapy. Strategies have focused on destabilization or inactivation of mutant p53 or reactivation of wild-type function in the mutant p53 protein which lead to efficient tumor regression (Young KH, et al 2008).

Destabilization of mutant p53 has been addressed mainly by targeting heat shock k proteins through histone deacetylases to rescue MDM2-dependent degradation of mutant p53, whereas disruption of mutant p53 function may be achieved by preventing its interaction with other transcription factors. A number of compounds or peptides that result in the reactivation of wild-type function in mutant p53, some of these compounds bind to grooves in the mutant p53 proteins and readjust the folding into a wild-type conformation, the reactivating compound appears to be specific for a certain mutation (Y220C) or for a group of mutations (conformational mutants) (Patricia A, et al 2013).

2.4.8 Expression of p53 protein in lymphatic malignancies:

P53 is a tumor suppressor gene mutations have deletion and/or point mutation of which have been found in a wide variety of human tumor such as lung, colon, breast carcinomas, and also been found in various sarcomas and in malignant melanomas (Patricia A, et al 2013). P53 gene /9mutations or accumulation of thep53 protein have also been found in several types of leukemia's and lymphomas, in Burkett's lymphoma cell lines p53 mutations have been found in up to 60% of cases, in B-cell chronic
lymphatic leukemia's p53 mutations are found, also p53 mutations has been found in diffuse large cell lymphomas (Magda Pinyo, et al 2000).

P53 is located in the" p"13 strand of the chromosome17, it encodes a nuclear phosphorprotein which help regulate cell proliferation, because p53 protein is associated with p34 kinase; which functions at the cell cycle control points, p53 protein may also be involved in the transition of the cell cycle from GO/GI phase to S phase, and entry to mitosis. Mutations of the p53 gene often lead to accumulation of a mutated protein in the cells, the mutated protein has an increased half-life and can bind to the wild type p53protein and inactivate it (Patricia A, et al 2013).

P53 is a tumor-suppressor gene, whose inactivation is mainly caused by point mutations in the coding sequence of exons 5, 6, 7, 8 and 9 in one allele with or without loss of the other allele.

- Study in medical journal involved, examined 12 cases of childhood non-Hodgkin lymphoma for alteration of p53 gene using polymerase chain reaction (PCR), upon polymerase chain reaction-single strand conformational polymorphism analysis four p53 gene alterations were demonstrated. These four alterations consisted of one on exon 6 and three on exon 7.

- The alteration of p53 tumor suppressor gene was studied in48patients with 8-cell lymphoma. A sequential combined technique of polymerase chain reaction-mediated single-strand conformational polymorphism (PCR-SSCP) or reverse transcription (RT)-PCR-SSCP and direct sequencing were used as a simple and sensitive approach to analyze nucleotide changes, these mutations were located in highly conserved regions of the p53 gene. Alteration in 9 patients include, two in exon 5, two in exon 6 and six in exon 7.

- 29 patients with NHL were studied by silver staining PCR-SSCP technique. Three cases of P53 gene point mutation was found in 29 cases of NHL. Mutation developed in exon 5 in two cases, and in exon 6 in one case.

- This report details a rapid method for screening the entire p53 coding region (exons 2–11)This method, based on the non-isotopic RNase cleavage assay, uses novel primer sequences Alterations to the wild-type p53 mRNA sequence were found in nine of the 130 patients with low grade lymphoproliferative disorders screened.

- CHEN Yan, XUANG Zhifu, LI Huiyu, YANG Nianlan, and ZHANG Hongyu in Institute of Haematology ,Xiehe University ,Tongji Medical University and Wuhan respectively at 1999 were studied the exons 5-7 of 29 patient with NHL
and 9 kind of human malignant lymphoma cell lines, were studied by silver staining PCR-SSCP technique. Three cases of p53 point mutation were found in 29 cases of NHL. Mutation developed in exon 5 in 2 cases, exon 6 in 1 case. They were all diffuse lymphoma, B cell lymphoma accounted for 2 cases and the other one was T cell lymphoma.
Chapter three

Materials and Methods:

3.1 Study Design:

Cross-section study.

3.2 Study area:

The Study was conducted at the National Cancer Institute in Wad-Medani locality in AL Gezira State during the period of April to September 2017.

3.3 Study population:

- Patients with NHL (from National Cancer Institute).
- Both sexes with different ages were included.

3.4 Study Duration:

The Study was done from April to September 2017.

3.5 Sample size:

- 30 patients were included according to inclusion and exclusion criteria.
- 5 controls.

3.6 Inclusion criteria:

Patients have Non Hodgkin lymphoma were included in this study.

3.7 Exclusion criteria:

- Patients with other malignancy were excluded.
- Patients with records not containing basic information were excluded.
- Patients were transfused blood less than three month.

3.8 Data collection:

Structured tested questionnaire was used to collected data from study population.
3.9 **Statistical analysis:**

This study was analyzed by use statistical package for social sciences (SPSS) software.

3.10 **Ethical consideration:**

- ✓ The permission to conduct this study was obtained from State Ministry of health AL Gezira state.
- ✓ All patients were informed about the study and consent was obtained.
- ✓ Information was collected from the patient under privacy and will be used for research study only.
- ✓ Research approval was obtained from research board faculty of medical laboratory sciences, University of Gezira.

3.11 **Method:**

3.11.1 **Sample collection:**

Blood sample was collected by clean vein puncture technique and about 2-3ml of venous blood was withdrawn and dispensed in EDTA container, then the containers were labeled. And the samples stored in -20°C.

3.11.2 **DNA extraction procedure:**

Use iNtRON’s extraction kit by following these steps.

1. 200ul of whole blood was pipetted into 1.5ml centrifuge tube (Eppendorf tube).
2. 20ul of proteinase K and 5ul of RNase solution was added into sample tube and gently mix.
3. 200ul of buffer BL was added and thoroughly was mixed (avoid vortexing).
4. The lysate was incubated at 56º C for 10 min. (note: for complete lysis, the lysate was mixed 3 or 4 times during incubation by inverting tube. If it lysis perfectly, the red colour of lysate become dark green).
5. Briefly the 1.5ml tube was centrifuged to remove drop from inside the rid. Tube was inverted upside down to see if there is clot. When the
clot was founded the supernatant was removed into new 1.5ml tube and leave the clot.

6. 200ul of absolute ethanol was added into lysate and was mixed well by gently inverting 5-6 times or by pipetting (don’t vortex).

7. Carefully the mixture from step6 was applied to the spin column and centrifuge at 13000rpm for 1min. The filtrate was discarded and the spin column was placed in a 2ml collection tube (reuse).

8. 700ul of buffer WA was added to the spin column and centrifuged for 1min at 13000rpm. The flow- through was discarded and the collecting tube was reused.

9. 700ul of buffer wB was added to the spin column and centrifuged for 1min at 13000rpm. The flow- through was discarded and the collecting tube was reused.

10. Spin column was centrifuged for 1min at 13000rpm to sure all elements discarding.

11. The flow- through was discarded and the spin column was removed into Eppendorf tube.

12. The CE was puted in 70°c, then 50ul of CE was added in filter and centrifuged for 1min at 13000rpm, and then 30ul of CE was added and centrifuged for 1min at 13000rpm to complete volume 80ul.

13. The spin column was removed and eppendrof tube was closed and then was stored the DNA at -20°c.

3.12.3 Quantification of DNA:

The concentration, purity, ratio and protein of the genomic DNA were obtained by using a GeneQuant spectrophotometer. Furthermore, the quality of the purified DNA was evaluated by electrophoresis in 0.25 g agarose gel, stained using 5µl RedSafe™ nucleic acid staining solutionand visualized by UV light.

Table1: Primer used in the PCR for exon 5:
Primer ordered from Humanizing Genomics, Marcogencompany (Seoul, Korea)

<table>
<thead>
<tr>
<th>Exon</th>
<th>Product Size</th>
<th>Sequence</th>
<th>GC%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex5</td>
<td>252</td>
<td>*F (20) 5’-TTTCAACTCTGTCCTTCC-3’</td>
<td>47%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>**R 5’-AACCAGCCCTGTCCTCTC-3’</td>
<td>60%</td>
</tr>
</tbody>
</table>

*Forward sequence  **reverse sequence

### 3.12.4 Preparation of master mix:

Maxime PCR PreMix (i- Taq, for 20ul rnx). 96 (480) tubes.

Maxime PCR PreMix (i- Taq, for 50ul rnx). 96 tubes.

<table>
<thead>
<tr>
<th>Component in</th>
<th>20 ul reaction</th>
<th>50 ul reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>i- Taq\textsuperscript{TM}DNA polymerase (5U/ul).</td>
<td>2.5 U</td>
<td>5 U</td>
</tr>
<tr>
<td>dNTPs.</td>
<td>2.5 mM each</td>
<td>2.5 Mm each</td>
</tr>
<tr>
<td>Reaction buffer (10X).</td>
<td>1 X</td>
<td>1 X</td>
</tr>
<tr>
<td>Gel loading buffer.</td>
<td>1 X</td>
<td>1 X</td>
</tr>
</tbody>
</table>

### 3.12.5 DNA amplification using polymerase chain reaction (PCR):
The PCR reactions were performed to replicate exon 5 of the P53 gene using purified genomic DNA as template. This PCR reaction was done by using MyTaq™ Red Mix Kit. The PCR reaction is consisting of three steps: denaturation, annealing and extension, with different temperature for each one. The first step of PCR is the denaturation in which the DNA sample is heated in 95° c to separates the double-strands. The high temperature break down the hydrogen bond between the nucleotides that form the DNA code. The second step is annealing in which the two primer (forward and reverse) bind to appropriate complementary strand. The temperature of this step various depending on the size of the primer and its homology to target DNA (the appropriate temperature for p53 exon 5 is 69.9° c). Finally, DNA polymerase extend the primers by its polymerase activity, this is done in a temperature optimal for the Taq polymerase which is 72°c. These steps are repeated for 40 times. In sterile 0.2 ml microcentrifuge tubes the PCR ingredients were added in the ratio shown in the (Table).

**Table2:** PCR ingredients and concentration used in the reactions

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>1X</th>
<th>Percentage on each reaction tube (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Primer</td>
<td>1 µl</td>
<td></td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>1 µl</td>
<td></td>
</tr>
<tr>
<td>Ready Master Mix</td>
<td>5 µl</td>
<td></td>
</tr>
<tr>
<td>DMSO*</td>
<td>1 µl</td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td>14 µl</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>3 µl</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25 µl</td>
<td></td>
</tr>
</tbody>
</table>

*DMSO*: Dimethyl sulfoxide.
The conditions for the PCR were as follows:

**Table 3:** Stages, temperature and time used for PCR for exon 5.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation denaturation</td>
<td>95 °C</td>
<td>2 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94 °C</td>
<td>45 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>69.9°C</td>
<td>1 min</td>
</tr>
<tr>
<td>Elongation</td>
<td>72 °C</td>
<td>1 min</td>
</tr>
<tr>
<td>Final elongation</td>
<td>72 °C</td>
<td>7 min</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>4 °C</td>
<td>Infinity</td>
</tr>
</tbody>
</table>

This step was followed by 40 cycles of the three stages.

**3.12.6 Electrophoresis of DNA:**

**3.12.5.1 Preparation of agarose gel:**
1. 0.25g of agarose powder was measured (1%) by sensitive balance.
2. Agarose powder was mixed with 25ml TBE buffer 10X (22.5ml of DW to 2.5ml TBE) in a microwavable flask.
3. Then was microwaved for 1-3min until the agarose is completely dissolved.
4. Agarose solution was lifted to cool down to about 50°C.
5. 1.25ul of the save stain 20X was added to final concentration.
6. The agarose was poured into a gel tray with the well comb in place.
7. Newly poured gel was placed at room temperature for 20-30mins until it has completely solidified.

**3.12.5.2 Loading samples and running an agarose gel:**
1. The running buffer was prepared by add 400ml of DW to 4ml of TBE (10X) buffer to prepare TBE 1X buffer.
2. Once solidified, the agarose gel was placed into the gel box (electrophoreses unit).
3. Gel box was filled with 1X TBE until the gel is covered.
4. 5ul of each PCR product carefully was loaded into the additional wells of gel.
5. The gel was ruined at 80 voltages until the dye line is approximately 75-80% of the way down the gel.
6. The power was turned off, the electrodes were disconnected from the power source and then the gel was removed carefully from the gel box.
7. By using UV transilluminator the DNA fragments was visualized.

3.12.7 DNA Sequencing:
Normal sequencing is a process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases – adenine, guanine, cytosine and thymine – in a strand of DNA. In this study, the DNA sequencing was used for scanning exon5 of p53 as mutation detection method. Normal sequencing was carried out for six samples and four control samples by Macrogen Company (Seoul, Korea) using Sanger technique.

3.12.8 Data analysis:
Data analyzed by using Microsoft excel sheet (2010) and statistical package of social science (SPSS).

3.12.9 Bioinformatics tools:
3.12.9.1 Finch TV:
Bioinformatics programs are use to view and edit DNA sequence chromatogram data. Also, it displays quality values, when available, and can adjust the scale in both vertical and horizontal directions in both single and multipane views. In a chromatogram file, the signal intensities are presented in a graph with the four bases, each is identified by different color. Like many sequence analysis programs, Finch TV uses green for adenine, red for thymine, black for guanine, and blue for cytosine

3.12.9.2 Bioedit:
It is most common program used in molecular biology studies. It was developed initially as biological sequence alignment editor written for windows only. It contains many features for sequence alignments modes of easy hand alignment, split window view, user defined color, and information based shading and auto integration with other programs such as ClustalW and Blast. (Tom Hall et al., 2011).

3.12.9.3 BLAST:
Blast is an abbreviation for Basic Local Alignment Tool which is an online bioinformatics program. The online bioinformatics program is an algorithm for
comparing primary biological sequence information such as the amino–acid sequence of proteins or the nucleotides of DNA sequences.
Chapter Four

Results and Discussion

4.1 Results:

Table (4.1): The frequency of male and female among the study group:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>13</td>
<td>43.3</td>
<td>43.3</td>
<td>43.3</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>56.7</td>
<td>56.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Figure (4.1): Distribution of the age among the study group.
Table (4.3): Distribution of the study group according to the residence:

<table>
<thead>
<tr>
<th>residence</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elghadarif</td>
<td>2</td>
<td>6.7</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Gezira</td>
<td>24</td>
<td>80.0</td>
<td>80.0</td>
<td>86.7</td>
</tr>
<tr>
<td>Kassala</td>
<td>2</td>
<td>6.7</td>
<td>6.7</td>
<td>93.3</td>
</tr>
<tr>
<td>Nahr-Elneel</td>
<td>1</td>
<td>3.3</td>
<td>3.3</td>
<td>96.7</td>
</tr>
<tr>
<td>White Nile</td>
<td>1</td>
<td>3.3</td>
<td>3.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Figure (4.2): Distribution of the study group according to Sudanese tribal stocks:
**Figure (4.5):** Distribution of the study group according to the types of non Hodgkin’s lymphoma:

![Pie chart showing distribution of study group types](image)

**Table (4.6):** Distribution of the study group according to the subtypes of non Hodgkin’s lymphoma:

<table>
<thead>
<tr>
<th>Subtype of non Hodgkin’s lymphoma</th>
<th>Frequency</th>
<th>Percent</th>
<th>Valid Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>burkitt’s lymphoma</td>
<td>4</td>
<td>13.3</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Diffuse large cell lymphoma</td>
<td>16</td>
<td>53.3</td>
<td>53.3</td>
<td>66.7</td>
</tr>
<tr>
<td>folicullar lymphoma</td>
<td>2</td>
<td>6.7</td>
<td>6.7</td>
<td>73.3</td>
</tr>
<tr>
<td>Lymphoma Type</td>
<td>Cases</td>
<td>% Total</td>
<td>% BcR</td>
<td>% BcR3N</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------</td>
<td>---------</td>
<td>-------</td>
<td>---------</td>
</tr>
<tr>
<td>Lymphoblastic lymphoma</td>
<td>3</td>
<td>10.0</td>
<td>10.0</td>
<td>83.3</td>
</tr>
<tr>
<td>Mantle cell lymphoma</td>
<td>1</td>
<td>3.3</td>
<td>3.3</td>
<td>86.7</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>1</td>
<td>3.3</td>
<td>3.3</td>
<td>90.0</td>
</tr>
<tr>
<td>Mixed small &amp; large lymphoma</td>
<td>1</td>
<td>3.3</td>
<td>3.3</td>
<td>93.3</td>
</tr>
<tr>
<td>Small lymphocytic lymphoma</td>
<td>2</td>
<td>6.7</td>
<td>6.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

**Figure (4.7):** Electro-photogram of 5μl genomic DNA from 13Non hodgkin’s lymphoma samples in 0.25% agarose gel, stained with using MyTaq™ Red Mix kit and visualized with UV light.
Figure (4.8): Alignments by Bioedit tool.

Figure (4.9): Alignments by Bioedit tool.
Figure (4.10): The sequencing result by Finch TV.

Figure (4.11): BLAST result.
4.2 Discussion:

Lymphomas constitute a heterogeneous group of malignant disorders with different clinical behaviours, pathological features and epidemiological characteristic. Which arise from an acquired injury to the DNA of a lymphocyte, it include two major type Hodgkin and Non-Hodgkin lymphoma. (Shankland KR et al., 2012). The mutation of p53 gene a secondary genetic event in different types of B-cell and T-cell lymphomas. Aggressive high grade B-cell Non-Hodgkin lymphoma has about a 30% incidence of p53 mutations.

In this study we have analyzed a total of 30 samples of Non-Hodgkin lymphoma for a mutation of p53gene (exon5) using normal sequencing. The data of this study revealed that the disease is common in males than in females and the age of the study group ranging between (4-70) years mostly >17 years old. Most of the study population from Gezira state (80%) and the most of them from Johayna tribe of (43.3%). The most common type of NHL was B cell and the most common subtype was found to be diffuse B cell NHL (53.3%), in contrast to the less common types are lymphoplastic lymphomas (10%).

DNA of p53gene (exon5) was extracted for six samples and four control subject, and then amplified by PCR. The samples were sequenced using Sanger sequencing in which the multiple sequence alignment of the patient sequence with similar nucleotide sequences that obtained from control samples were carried out to find the differences.

The results of this study demonstrated that there was no mutation detected in p53 gene(exon5) and this was in agree with a study reported by CHEN Yan et al., (1999). In contras this study disagreed with a study reported by DJA Adamson et al., (199), Prasad R.K. Koduru et al., (1997) and Klumb CE et al., (2001).
Chapter five

Conclusion and Recommendations

5.1 Conclusion:
Biological screening of p53 (exon5) in Non-Hodgkin's lymphoma Sudanese patients confirm that there is no mutation in this exon.

5.2 Recommendations:
1. The uses of sufficient samples are recommended to confirm mutation in NHL.
2. To apply another alignment of bioinformatics tools other than Bioedit.
Reference:


Appendices:

Questionnaire:

University of Gezira
Faculty of Medical Laboratory Sciences
Hematology And Immune hematology Department

Clinical Evaluation Form (Questionnaire)

Name: .............................................

Age: .............................................

Sex: Male.......................... Female..........................

Residence: .....................................................

Type of non Hodgkin's lymphoma............................................................

Family history..................................................................................

Any history of blood transfusion.

Yes......................... No.............

If the answer yes what the last time of blood transfusion

...............day...............month. .........................year

Other disease..................................................................................

Treatment..................................................................................

Requirement:
Gel electrophoresis (MS major science, Taywan).

UV transilluminator (MS major science, Taywan).

PCR machine (ESCO Micro Pte Ltd, china).

Safety cabin.

Centrifuge.

Vortex.

Pipettes.

Hot plate.

Sensitive balance.

Eppendorf tube.

Cylinders and flasks.

Gloves

Tips (blue-yellow-white)

Cotton

Oven

Incubator

Ultraviolet crosslink

Lab coats and Microwave.
PCR machine

Gel electrophoresis machine
UV transilluminator

Eppendorf tube.