Deanship of Graduate Studies
Al-Quds University

Determinants of Hodgkin Lymphoma among Palestinians

Nurah Khalil Mohammad Ayesh

M.Sc. Thesis

Jerusalem-Palestine

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Thesis Approval

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Dedication

To

Everyone stood by my side and never left

Everyone believed in me and never gave up on me

Family, friends, and best friends

I dedicate this work…

Nurah Khalil Mohammad Ayesh

Declaration:

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.

Signed .....

Nurah Khalil Mohammad Ayesh

Date: 02.12.2017

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Abstract

Background: Hodgkin lymphoma accounts for less than 1% of all neoplasms worldwide, yet it is considered to be one of the most common malignancies in young adults with an annual incidence of 3 cases per 100,000 persons. Several factors have been reported to contribute to the risk of Hodgkin lymphoma; but this disease has never been assessed among Palestinians. Therefore, the current study aims to focus on investigating different etiological factors associated with Hodgkin lymphoma including lifestyle factors, medical history, family history and proxies of infection. Moreover, it aims to examine Epstein-Barr virus (EBV) positivity among Palestinian HL cases.

Methods: Data were collected from 162 HL pathology reports from the medical files of three Palestinian hospitals; Augusta Victoria Hospital in Jerusalem, Beit-Jala Hospital in Bethlehem and Rafidia Hospital in Nablus. **Part I**: A retrospective-cohort study was conducted to describe the disease characteristics of HL in Palestine. Furthermore, we obtained 30 paraffin-embedded blocks for immunohistochemical testing to confirm HL diagnosis and to detect EBV positivity in HL cases. **Part II**: A case-control study was conducted including 63 pathologically confirmed incident HL cases and 85 cancer-free controls, in which a questionnaire was used for data collection and blood samples were collected for future genetic and serologic purposes of the study.

Results: Mean age at diagnosis for Hodgkin lymphoma cases was 23 years with a male to female ratio of 1.25:1. Nodular sclerosis was the most common subtype with 51.1%, followed by mixed cellularity with 39.1%. EBV was found in about 33% of the paraffin-embedded blocks of Hodgkin lymphoma cases. Family history of cancer in first-degree relatives was associated with 4.6 folds increase in the risk of HL. Furthermore, tonsillectomy has been found to be associated with 4.2 folds increased risk of HL. Physical activity was found to play a protective role in the etiology of HL.

Conclusions: In conclusion, this is the first study to describe the main characteristics of Hodgkin lymphoma and examine its etiology among Palestinians.

Keywords: Hodgkin lymphoma, case-control study, retrospective-cohort study, risk factors, exposure, environment, infection, EBV, immunohistochemistry, Palestine.

محددات سرطان الغدد الليمفاوية – الهودجكن في فلسطين

إعداد: نورا خليل محمد عايش

إشراف: د. رانية أبو سير

ملخص:

خلفية الدراسة: يمثل ورم الغدد الليمفاوية الهودجكن أقل من 1% من جميع الأورام في جميع أنحاء العالم، لكنه على الرغم من ذلك يعتبر واحداً من أكثر أورام الدم الخبيثة شيوعاً بين الشباب البالغين حيث أن معدل الإصابة السنوي به يعادل 3 حالات بين كل 100,000 من الأفراد. على الرغم من أن هنالك العديد من العوامل المرتبطة بخطر الإصابة بورم الغدد الليمفاوية الهودجكن، الا أن هذا المرض لم يتم تقييمه بين الفلسطينين بعد، ولذلك فإن الدراسة الحالية تهدف الى تحرّي العوامل المختلفة المرتبطة بخطر الإصابة بورم الغدد الليمفاوية الهودجكن بما فيها أسلوب الحياة والتاريخ الطبي والعائلي وعوامل التعرّض للعدوى. بالإضافة الى ذلك فإن هذه الدراسة تهدف أيضا لتحرّي وجود فيروس ابشتاين بار في الحالات المصابة بورم الغدد الليمفاوية–الهودجكن بين الفلسطينين.

منهجية البحث: تم جمع المعلومات من 162 تقرير أنسجة من ملفات المرضى المصابين بورم الغدد الليمفاوية الهودجكن من ثلاثة مستشفيات فلسطينية وهي: مستشفى المطلع في القدس ومستشفى بيت جالا في بيت لحم ومستشفى رفيديا في نابلس. الجزء الأول: تم فيه إجراء دراسة التعرض بأثر رجعي لوصف معالم المرض في فلسطين والتي تضمنت الحصول على 30 كتلة بارافين شمعية لأنسجة مشخصة بسرطان الغدد الليمفاوية الهودجكن لعمل اختبار الأنسجة المناعية لها لتأكيد التشخيص الأولي للورم ولتحرّي وجود فيروس ابشتاين بار في هذه الحالات. الجزء الثاني: تم فيه اجراء دراسة الحالات والشواهد ل 63 حالة مرضية مثبتة التشخيص بسرطان الغدد الليمفاوية عن طريق فحص

الأنسجة بالإضافة الى 85 من الشواهد لأشخاص سليمين وغير مصابين بأي نوع من أنواع السرطان، حيث تم استخدام استبانة لجمع البيانات من هذه الحالات و سحب عينات دم لإجراء الاختبارات الجينية والمصلية لاستخدامها في البحوث المستقبلية للدراسة.

النتائج: معدل العمر عند التشخيص لحالات سرطان الغدد الليمفاوية الهودجكن كان 23 عاماً بتوزيعٍ متقاربٍ بين الذكور والإناث حيث أن نسبة الذكور إلى الإناث كانت 1:1.25. وكان النوع الفرعي التصلب العقدي الأكثر شيوعا بنسبة 51.1%، تليها الخلوية المختلطة بنسبة 39.1٪. كما وتم العثور على فيروس ابشتاين بار في حوالي 33٪ من كتل البارافين الشمعية التابعة لحالات سرطان الغدد الليمفاوية الهودجكن مؤكدة التشخيص. وقد ارتبط التاريخ العائلي للسرطان في الأقارب من الدرجة الأولى مع زيادة خطر الإصابة بسرطان الغدد الليمفاوية الهودجكن بحوالي 4.6 ضعف. وأفادت نتائج الدراسة أيضا بأن استئصال اللوزتين يزيد من خطر الإصابة بسرطان الغدد الليمفاوية الهودجكن بحوالي 4.2 ضعف. وقد تبين بأن النشاط الرياضي والجسدي يؤدي الى الحماية من الإصابة بسرطان الغدد الليمفاوية الهودجكن الى حد ما.

الاستنتاج والتوصيات: هذه الدراسة تعتبر الدراسة الأولى التي تصف الخصائص الرئيسية لسرطان الغدد الليمفاوية الهودجكن وتفحص مسبباتها بين الفلسطينيين.

الكلمات المفتاحية: سرطان الغدد الليمفاوية الهودجكن، دراسة الحالات والشواهد، دراسة التعرّض بأثر رجعي، عوامل الخطر، التعرّض، البيئة، العدوى، فيروس ابشتاين بار، فحوصات الأنسجة المناعية، فلسطين.

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List of abbreviations

Abbreviation	Term
ABVD	Adriamycin, Bleomycin, Vinblastine, Dacarbazine
ADC	Antibody drug conjugate
AIDS	Acquired immune deficiency syndrome
AVH	Augusta Victoria Hospital
BCL	B cell lymphoma
BCR	B cell receptor
BEACOPP	Bleomycin, Etoposide, Adriamycin, Cyclophosphamide, Oncovin, Procarbazine, Prednisone
CCL	C-C motif chemokine ligand
CD	Cluster of differentiation
CHL	Classical Hodgkin lymphoma
CI	Confidence interval
DLBCL	Diffuse large B cell lymphoma
EBER	Epstein Barr encoding region
EBNA	Epstein Barr nuclear antigen
EBV	Epstein Barr virus
GC	Germinal center
GWAS	Genome wide association studies
H&E	Hematoxylin and eosin
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HHV	Human herpes virus
HIS	Hospital information system
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
HRS	Hodgkin Reed Sternberg
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL	Interleukin
IM	Infectious mononucleosis
IRB	Institutional review board
JAK/STAT	Janus kinase/signal transducer and activator of transcription
KSHV	Kaposi sarcoma herpes virus
L&H	Lymphocytic and histocytic
LCA	Leukocyte common antigen
LMP	Latent membrane protein
LP	Lymphocyte predominant
MHC	Major histocompatibility complex
MOH	Ministry of health
MOPP	Mustargen, Oncovin, Procarbazine, Prednisone
NF-kb	Necrosis factor kappa B
NHL	Non Hodgkin lymphoma
NK	Natural killer

NLPHL	Nodular lymphocyte predominant Hodgkin lymphoma
OR	Odds ratio
PD	Program death
PI3K	Phosphoinositide 3-kinase
PTLD	Post-transplant lymphoproliferative disease
REAL	Revised European American lymphoma
sIg	Surface immunoglobulin
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for the Social Sciences
TARC	Thymus and activation-regulated chemokine
UV	Ultraviolet
WHO	World Health Organization

Chapter One

Introduction

In this chapter, we provide research background, research problem and study justification. We also provide the main goal of the study and its objectives. Furthermore, at the end of this chapter we provide a summary of the thesis chapters.

1.1 Background

Hodgkin lymphoma (HL) accounts for less than 1% of all neoplasms worldwide, yet it is considered to be one of the most common malignancies in young adults (Gobbi *et al.*, 2013; Sickinger *et al.*, 2015). In the Western world, HL is considered to be one of the most common lymphomas with an annual incidence of about 3 cases per 100,000 persons (Kuppers *et al.*, 2012). HL is one of the most curable malignancies among young adults worldwide with cure rates of 90% (Gobbi *et al.*, 2013; Sickinger *et al.*, 2015). It is a disease of the lymphatic system, which most often develops in the lymph nodes with the presentation of painless lymphadenopathy in affected areas, primarily in the cervical region (Hjalgrim, 2012). Lymphomas can also affect organs such as liver, lung and bone marrow (Hjalgrim, 2012; Kuppers *et al.*, 2012). HL is a B-cell lymphoma representing 10% to 15% of all lymphomas in developed countries (Sickinger *et al.*, 2015). Moreover, it is well characterized by the rarity of the neoplastic elements in the cell population of about 1%, where the majority of cells are non-neoplastic mostly consisting of T-lymphocytes, in addition to plasma cells, macrophages, mast cells, dendritic cells, neutrophils, eosinophils, and fibroblasts (Gobbi *et al.*, 2013; Jona *et al.*, 2013; Kuppers, 2009; Kuppers *et al.*, 2012; Matsuki & Younes, 2015).

Based on differences in the morphology, the histological picture and the phenotype of the tumor cells; Hodgkin lymphoma was classified into classical and nodular lymphocyte–predominant form (Gobbi *et al.*, 2013; Hjalgrim, 2012; Jona *et al.*, 2013; Kuppers, 2012; Kuppers *et al.*, 2012). Classical Hodgkin lymphoma (CHL) accounts for 95% of all cases, while nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) represents the

remaining 5% (Hjalgrim, 2012; Kuppers, 2009). Tumor cells are called Hodgkin and Reed Sternberg (HRS) cells which are seen in CHL, whereas lymphocyte predominant (LP) cells previously known as lymphocytic and histocytic (L&H) are found in NLPHL (Jona *et al.*, 2013; Kuppers, 2009; Kuppers *et al.*, 2012). A clonal B-cell origin of both lymphocyte predominant and CHL was recently established, therefore enabling the term 'Hodgkin disease' to be changed to 'Hodgkin lymphoma' (Gobbi *et al.*, 2013).

Several risk factors have been associated with the risk of HL including infection, which is considered to be one of the main risk factors for cancer. However, Epstein Barr virus (EBV) infection is well known to be associated with the risk of HL (Oh & Weiderpass, 2014). Several studies worldwide described the relationship between HL and EBV infection (Hjalgrim, 2012; Kuppers, 2009). The aim of this study was to examine the association between different risk factors, including lifestyle factors, medical history, family history, proxies of infection and the risk of developing HL among Palestinians. In addition it aimed to examine EBV positivity among HL cases.

1.2 Problem Statement

Hodgkin lymphoma is considered to be one of the most common hematological malignancies among young adults, though etiological factors have not been defined yet. Several factors have been reported to contribute to the risk of HL; but this disease has never been assessed among Palestinians. Therefore, the current study aims to focus on investigating different etiological factors affecting HL in Palestine. In addition, it aims to examine EBV positivity among HL cases.

1.3 Study Justification

Hodgkin lymphoma represents 10-15% of all lymphomas in developed countries (Sickinger *et al.*, 2015). Although HL accounts for less than 1% of all neoplasms worldwide and in Palestine, it is considered to be one of the most common malignancies in young adults (Gobbi *et al.*, 2013; Sickinger *et al.*, 2015). Despite the huge worldwide research efforts, risk factors of this disease have never been examined among Palestinians. Thus, this study will formulate the basis for studying HL among Palestinians focusing on different risk factors associated with the risk of developing HL.

1.4 Research Hypothesis

H_0: There is no association between lifestyle factors, medical history, family history, proxies of infection and the risk of Hodgkin lymphoma among Palestinians.

H_1: There is an association between lifestyle factors, medical history, family history, proxies of infection and the risk of Hodgkin lymphoma among Palestinians.

1.5 Study Goal

The ultimate goal of this study is to participate in health care improvement of Palestinians by conducting a study for Hodgkin lymphoma as a common type of hematological malignancy among young adults and examining its association with variable risk factors and to examine EBV positivity among HL cases.

1.6 Study Objectives

- To mount a platform to study HL in Palestine.
- To describe HL characteristics including demographic, clinical and pathological aspects among Palestinians.
- To examine the association between lifestyle factors, medical history, family history, proxies of infection and the risk of HL among Palestinians.
- To examine EBV positivity among HL cases by detecting the presence of LMP1 in HRS cells.

1.7 Study Expected Outcomes

This study describes the main characteristics of Hodgkin lymphoma and examines its risk factors among Palestinians, and examines EBV positivity among HL cases.

1.8 Summary of Thesis Chapters

This thesis is divided into five chapters. Chapter one, includes a brief description of the problem we examined, the importance of this research and its objectives. Chapter two, emphasized on literature review related to the research problems, whereas chapter three demonstrates the methodology of the study. Results were reported in chapter four. Lastly,

chapter five discusses the results, and provides recommendations in addition to the main limitations faced this study.

Chapter Two

Literature Review

In this chapter, we highlight many aspects concerning Hodgkin lymphoma viewed in the literature including epidemiology, pathogenesis, classification, etiology (a special insight on the relationship between Hodgkin lymphoma development and EBV infection) and treatment.

2.1 Epidemiology of Hodgkin lymphoma

Hodgkin lymphoma accounts for less than 1% of all neoplasms worldwide, yet it is considered to be one of the most common malignancies in young adults (Gobbi *et al.*, 2013; Sickinger *et al.*, 2015). About 65,950 new cases are diagnosed with HL annually worldwide (Salati *et al.*, 2014). In USA, HL represents 0.6% of all new cancer cases and 10% of all lymphomas, leading to an approximately 9,000 new cases per year (Batlevi & Younes, 2013; Matsuki & Younes, 2015). In the UK, 1,845 people were diagnosed with HL in 2011 (Gobbi *et al.*, 2013). Worldwide prevalence rates vary from more than 5.5 per 100,000 in Yemen and Lebanon to less than 1 per 100,000 in China and Japan (Hoffbrand AV, 2011). Moreover, HL is one of the most common lymphomas in the Western world, with an annual incidence of about 3 cases per 100,000 individuals (Kuppers, 2009; Kuppers *et al.*, 2012). High risk areas of HL include Northern America, Western Europe, Australia/New Zealand with an incidence rate of 2.4 per 100,000, Southern Europe with 2.3 per 100,000, and Northern Europe with 2.2 per 100,000 incidence rate (Oh & Weiderpass, 2014). In Israel, in the period between 1960 and 1969, the age standardized rate of HL was 2.27 per 100,000, and it was elevated to 3.61 in the period between 1997 and 2005 (Salati *et al.*, 2014).

Incidence rates of HL vary depending on regional, racial, ethnic background, and socioeconomic differences as well as disease subtype, age and gender (Huang *et al.*, 2011; Maggioncalda *et al.*, 2011). Caucasians showed the highest incidence rates, followed by African Americans and Hispanics, where much lower incidence was found among

Orientals (Salati *et al.*, 2014). In economically underdeveloped countries, the overall incidence of HL was lower than in developed countries, with the exception of children under the age of 15, where a higher incidence was seen. There was only a mild increase in incidence throughout adolescence and young adulthood (Gobbi *et al.*, 2013; Pahwa *et al.*, 2003). In addition, the incidence of HL in men was slightly higher than in women among all subtypes, except for the nodular sclerosis subtype that affects young females slightly more often than males (Thomas *et al.*, 2002).

Hodgkin lymphoma has a bimodal age distribution with one peak around the age of 25 years and another after the age of 60 years (Sickinger *et al.*, 2015). MacMahon first recognized this bimodal age distribution of HL in incidence data for the white population of Brooklyn, New York, USA, 1942-1953. Sooner after, similar distributions were reported in German Hodgkin lymphoma mortality data for 1952-1957 (Hjalgrim, 2012). This two-peak was strongly present in Western populations. In Orientals, the first peak of 25 years was present in children with predominance of nodular sclerosis subtype, while the second peak of 60 years was clearly observed among elderly with predominance of mixed cellularity subtype. Meanwhile, the first peak was almost absent in Japanese HL patients (Salati *et al.*, 2014). Similar to that, in Pakistan the bimodal age distribution was absent and most cases were less than 30 years old. In Egypt the average age at presentation was 31, and in India it was 30 years (Sultan *et al.*, 2016).

In Palestine, the only source of data about HL incidence was the annual report of the Palestinian Ministry of Health (MOH), which reported that the incidence rate of HL in the year 2010 was 0.8 (MOH, 2011) , and in 2011 it was 0.6 (MOH, 2012), whereas in 2012 it increased up to 1.2 (MOH, 2013) . Another increase in the incidence was noted in 2013 where it reached 1.4 (MOH, 2014). However, in 2014 the incidence increased to be 1.7 (MOH, 2015), and 2.7 in the year 2015 (MOH, 2016). The latest annual report of the Palestinian MOH reported that incidence of HL was 2.1 per 100,000 in the year 2016 (MOH, 2017). There was a trend in the number of reported cases of HL from 2011 to 2015. Furthermore, frequencies among males and females from 2010 to 2016 were almost equal with a total of 140 female cases and 143 male cases (Figure 2.1).

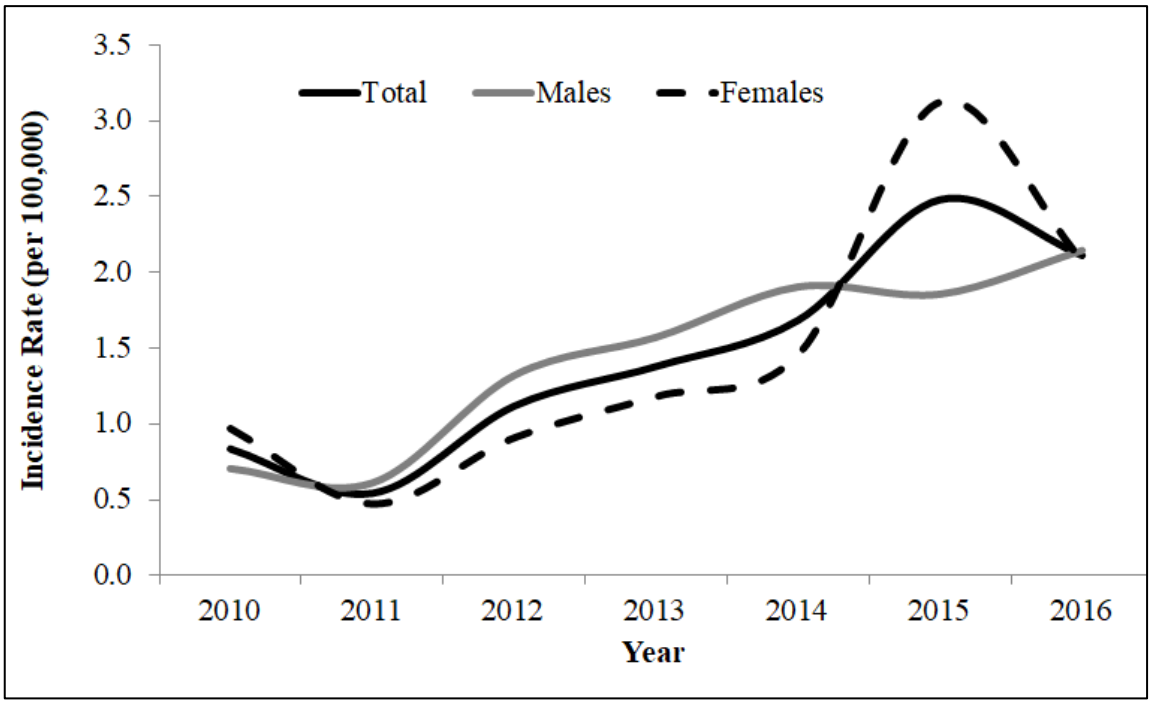

Figure 2.1: Incidence rates of Hodgkin lymphoma among Palestinians by gender (2010-2016).

2.2 Pathogenesis of Hodgkin lymphoma

The main feature of this lymphoma is the rarity of neoplastic elements in the cell population of about 1%, where the majority of cells are non-neoplastic (Gobbi *et al.*, 2013; Jona *et al.*, 2013; Matsuki & Younes, 2015). This is considered to be one of the reasons that the systematic analysis of these cells has been frustrating effort for a long period of time (Kuppers, 2009; Thomas *et al.*, 2002). The neoplastic elements present with various inflammatory cells including B-cells, T-cells, mast cells, macrophages, eosinophils, neutrophils, and plasma cells comprising the tumor microenvironment (Matsuki & Younes, 2015).

The characteristic multinucleated cells that are the hallmark of the disease were discovered by Dorothy Reed and Carl Sternberg (Jona *et al.*, 2013). Tumor cells are called Hodgkin and Reed Sternberg (HRS) cells which are seen in classical Hodgkin lymphoma, whereas lymphocyte predominant (LP) cells previously known as lymphocytic and histocytic (L&H) are found in Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) (Jona *et al.*, 2013; Kuppers, 2009; Kuppers *et al.*, 2012).

Additionally, HRS cells are expected to be derived from germinal center (GC) B-cells that had developed unfavorable immunoglobulin V gene mutations and normally would have undergo apoptosis, whereas LP cells are thought to be derived from antigen-selected GC B-cells (Kuppers, 2009). Yet, a minority of HL cases show T-cell features, implying that they are derived from T-cells (Jona *et al.*, 2013). The GC B-cells are antigen activated mature B-cells involved in T-dependent immune responses that undergo extensive proliferation in the histological construction of the germinal cells (Kuppers, 2012).

2.2.1 The role of HRS and LP cells in the pathogenesis of Hodgkin lymphoma

Hodgkin lymphoma is well characterized by the rarity of the neoplastic cells, which usually account for 0.1% to 10% of the cells in the affected tissues. Both HRS cells in classical HL and LP cells in NLPHL, constitute the neoplastic population of Hodgkin lymphoma and their role in HL pathogenesis is well known (Kuppers, 2009).

2.2.1.1 Hodgkin and Reed Sternberg cells

Hodgkin and Reed Sternberg cells are derived from germinal center or post germinal center B-cells in the majority of cases of CHL (Thomas *et al.*, 2002). They have lost their B-cell phenotype and they express markers and transcriptional regulators of other hematolymphoid cell types (Schmitz *et al.*, 2009). Loss of the B-cell phenotype is due to the down regulation of several transcription factors (Nakatsuka & Aozasa, 2006; Schmitz *et al.*, 2009), including receptor tyrosine kinases, nuclear factor-kappa B (NF-kB), and Janus kinase/signal transducer and activator of transcription (JAK/STAT), phosphoinositide 3-kinase (PI3K) (Matsuki & Younes, 2015; Schmitz *et al.*, 2009). The dysregulation of these transcription factors may explain the acquisition of survival and anti-apoptotic features of HRS cells (Matsuki & Younes, 2015).

Hodgkin and Reed Sternberg cells in nearly all cases carry rearranged and somatically mutated immunoglobulin heavy and light chain genes (Kuppers, 2009, 2012; Kuppers *et al.*, 2012; Kuppers & Hansmann, 2005; Thomas *et al.*, 2002). Normally GC B-cells acquiring such mutations rapidly undergo apoptosis, but the pathogenesis of HL in GC B-cells enables the crippled HRS cell precursors to escape apoptosis (Jona *et al.*, 2013; Kuppers *et al.*, 2012). Several key transcription factors that regulate the expression of many B-cell specific genes are either at strongly reduced levels in HRS cells or only not

expressed at all (Kuppers, 2009). For example, HRS cells lack most typical B-lineage markers such as CD20, sIg or CD79a. Conversely, HRS cells express markers that are typical for other cell types, including CD15 (granulocytes), CD30 (monocytes and T-cells), Perforin (T-cells), Syndecan (Memory B-cells), Fascin and TARC (dendritic cells) (Thomas *et al.*, 2002).

2.2.1.2 Lymphocyte predominant cells

Lymphocyte predominant cells are derived from antigen-selected GC B-cells (Kuppers, 2012). The detection of clonal immunoglobulin heavy and light chain variable (V) gene rearrangements of the LP cells proved their B-cell derivation and their monoclonality as well. The immunoglobulin (Ig) V gene of LP cells carries somatic mutations, which are introduced during the GC reaction. Further validation of the GC B-cell origin of the LP cells is the presence of several cases that showed intraclonal diversity as a sign of ongoing hypermutation during clonal expansion (Kuppers *et al.*, 2012). LP cells express typical GC B-cell markers, including B-cell lymphoma 6 (BCL6), which is considered to be the key regulator of the GC B-cell program, CD20, surface immunoglobulin (sIg) or the J-chain (Kuppers, 2009; Kuppers *et al.*, 2012; Thomas *et al.*, 2002). Furthermore, there is a positive expression of CD79a, and CD45, and negative expression of CD30 and CD15 (Amini, 2002).

2.2.2 Postulated mechanisms in Hodgkin lymphoma pathogenesis

2.2.2.1 Apoptosis resistance

A high load of somatic mutations is acquired in Ig genes of the B-cells during the process of hypermutation in the germinal center reaction. This procedure gives rise to a big collection of diversified Ig genes and may result in B-cell receptor (BCR) with high affinity to the corresponding antigen. B-cells undergo a process termed as affinity maturation in which they are selected for the expression of high affinity surface Ig (sIg) in the germinal center and leave it as memory B-cells or plasma cells. Germinal center B-cells that don't fulfil this criteria are normally eliminated by apoptosis mediated by Fas receptor. HRS cells are characterized by the absence of sIg expression, and as a result of that such a cell should be negatively selected in the germinal center and should undergo apoptosis. The HRS cells escape apoptosis and expand clonally to cause systemic disease (Thomas *et al.*, 2002).

2.2.2.2 Microenvironment interactions

Malignant cells of Hodgkin lymphoma are surrounded by CD4+ and CD8+ T-cells, in addition to B-cells, plasma cells, macrophages, mast cells, dendritic cells, neutrophils, eosinophils, and fibroblasts forming their non-malignant microenvironment. HRS cells attract these cells through the secretion of cytokines and chemokines (Kuppers, 2009; Kuppers *et al.*, 2012). For example, HRS cells attract eosinophils by the secretion of Interleukin 5 (IL5), Interleukin 9 (IL9), chemokine ligand 5 (CCL5), chemokine ligand 28 (CCL28) and granulocyte-macrophage colony stimulating factor. Moreover, they attract T-helper cells and T-regulatory (T-reg) cells by the secretion of CCL5, chemokine ligand 17 (CCL17), and chemokine ligand 22 (CCL22) (Kuppers, 2009). The attraction of these cells and the interaction with the HRS cells is very necessary for the survival and proliferation of the HRS cells through helping them to escape an attack from cytotoxic T or natural killer cells, thus, escaping apoptosis (Kuppers, 2009; Kuppers *et al.*, 2012) and preventing an effective immune response to take place in this microenvironment (Jona *et al.*, 2013).

T-cells represent the largest population of the infiltrating cells and are predominant in the contact with HRS and LP cells (Kuppers, 2012; Thomas *et al.*, 2002). Elevated levels of Interleukin 6 (IL-6), Interleukin 7 (IL-7), Interleukin 8 (IL-8), Interleukin 10 (IL-10), soluble CD30, B-cell-activating factor of the tumor necrosis factor are secreted by the HRS cells and their microenvironment. These substances are used as prognostic factors since they are decreased with continuous treatment and diminished tumor (Jona *et al.*, 2013).

2.3 Classification of Hodgkin lymphoma

The evolution of several clinical and pathologic classification systems over the past 50 years changed the understanding of lymphoid neoplasms. These systems started with Rappaport classification in 1956, which was based on morphology. After that, in 1974, Kiel classification depended on cell lineage and differentiation. Working Formulation used morphology and clinical prognosis in 1982. These schemes were mostly substituted in 1994 by the Revised European-American Lymphoma (REAL) classification, which merged morphologic, immunophenotypic, genotypic, and clinical features into disease subtype definitions. In 2001, the World Health Organization (WHO) introduced a new classification built on the REAL and other classifications. Within the lymphoid neoplasms,

the WHO system distinguishes HL from NHL based on morphologic and immunologic characteristics (Morton *et al.*, 2007).

According to the WHO classification, which was published in 2001, and updated in 2008, Hodgkin lymphoma was sub classified into two classes "based on differences in the histological picture and the phenotype of the tumor cells". 1) Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), and 2) Classical Hodgkin lymphoma containing: nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted (Gobbi *et al.*, 2013; Hjalgrim, 2012; Jona *et al.*, 2013; Kuppers, 2012; Kuppers *et al.*, 2012). CHL accounts for 95% of all cases while NLPHL represents the remaining 5% (Hjalgrim, 2012; Kuppers, 2009). The most common subtype among young adults is nodular sclerosis. The frequency of mixed cellularity increases with age, while both lymphocyte predominant and lymphocyte depleted occur at lower frequencies (Hjalgrim, 2012; Thomas *et al.*, 2002).

2.3.1 Nodular lymphocyte-predominant Hodgkin lymphoma

This class represents only 4-5% of all HL cases (Agostinelli & Pileri, 2014). It generally shows a nodular growth pattern that may or may not be accompanied by diffuse areas. This class is distinguished from CHL by the absence of HRS cells and the presence of neoplastic cells named lymphocytic and histocytic (L&H cells) or "popcorn" cells which are large, irregular containing polylobulated nuclei with small nucleoli and abundant cytoplasm (Townsend & Linch, 2012). There is a non-neoplastic background in NLPHL represented by small lymphocytes and variable amounts of histocytes. Eosinophiles, neutrophils, and plasma cells are hardly seen (Mathas *et al.*, 2016).

This lymphoma may occur at any time of age, but it is more common in adult males. At diagnosis, it is usually localized, and bone marrow is rarely involved. Peripheral lymph nodes mainly cervical or inguinal nodes are commonly involved, and the mediastinum is usually spread (Gobbi *et al.*, 2013). Progression of this type to a diffuse large B-cell lymphoma has been reported in about 3-5% of the cases (Agostinelli & Pileri, 2014). This subtype looks like lymphocyte rich HL, and the most important difference between them is the lymphocyte predominant tumor cells which are usually CD30 and CD15 negative (Mathas *et al.*, 2016).

2.3.2 Classical Hodgkin Lymphoma

This major class is subdivided into four entities including: nodular sclerosis, mixed cellularity, lymphocyte depletion, and lymphocyte rich. Below, is a brief idea about each one of them.

2.3.2.1 Nodular sclerosis

It is the most common subtype of CHL, representing 75-80% of all subtypes. The occurrence of nodules of variable size separated by dense collagenous fibrous bands is the main feature of this unit. An important feature which enables the distinction of nodular sclerosis from lymphocyte depletion subtype, is that these bands show typical green birefringence in polarized light. This subtype usually affects adolescence and young adults, and it has a slightly higher incidence among females (Gobbi *et al.*, 2013). Nodular sclerosis is characterized by the presence of nodular compartments containing HRS cells and non-malignant microenvironment. In Western countries, nodular sclerosis is considered to be the most frequent subtypes, where it consists about 80% of cases and is usually EBV negative (Mathas *et al.*, 2016).

2.3.2.2 Mixed cellularity

Here, the infiltrate is diffuse or unclearly nodular, without band forming sclerosis. Reed-Sternberg cells are more represented than nodular sclerosis. Mixed cellularity affects usually adults, males more than females, with lymph nodes, spleen, liver, or bone marrow involvement (Gobbi *et al.*, 2013). This subtype is more frequent in poor countries while nodular sclerosis is more frequent in rich countries. Moreover, unlike nodular sclerosis, HRS cells in mixed cellularity subtype are usually infected with EBV (Myriam *et al.*, 2017).

2.3.2.3 Lymphocyte depletion

The design of lymph nodes in this subtype is mainly represented and is completely affected by diffuse and dense fibrosis, necrosis may be found as well. Reed-Sternberg cells exclusively represent most of the residual cells, while the non-neoplastic background is rare. Lymphocyte depletion is the least common variant of all classical Hodgkin lymphoma subtypes. It occurs preferentially in elderly patients, and in non-industrialized countries. There is a presentation of abdominal lymphadenopathy, or extranodal disease with the involvement of spleen, liver, and bone marrow. The response to treatment is usually worse

than other subtypes where the stage of diagnosis is generally advanced (Gobbi *et al.*, 2013). Usually, it is associated with HIV positive patients and HRS cells are usually EBV infected (Mathas *et al.*, 2016).

2.3.2.4 Lymphocyte-rich

There is a diffuse or focal, and occasionally interfollicular involvement with reactive cellular non-neoplastic background composed of small lymphocytes, and rare neutrophils, eosinophils, and plasma cells. Reed-Sternberg cells and lacunar cells, both present infrequently and display the immunophenotypic/molecular properties of CHL. This subtype was recently introduced into CHL classification. It occurs with low aggressiveness, early stage diagnosis, most frequently in patients older than 50 years of age, and with the involvement of subdiaphragmatic sites (Gobbi *et al.*, 2013). This subtype is rare and almost 30-50% of its cases are EBV positive. HRS cells usually express B-cell transcription factors and CD20 more frequently than other HL subtypes (Mathas *et al.*, 2016).

2.4 Etiology of Hodgkin lymphoma

Hodgkin lymphoma is one of the most common malignancies among adolescence and young adults. However, its etiology has far evaded clarification, since many factors are thought to be associated with the risk of HL in different degrees. Below, are some of these factors.

2.4.1 Immune modulation

2.4.1.1 Immunosuppression

Immunosuppressive diseases including acquired immunodeficiency syndrome (AIDS) and post-transplant lymphoproliferative disease (PTLD) are characterized by uncontrolled and dysfunctional lymphocyte proliferation, leading to well-known increased risks of lymphoma, including HL (Landgren *et al.*, 2006).

Following the onset of AIDS, it was noted that the incidence of lymphoma in patients with HIV infection was increased compared to general population, whereas the risk of HL in HIV infected individuals was increased from 5 to 20 folds (Riedel *et al.*, 2015; Uldrick & Little, 2015). CHL is considered to be the fifth commonest tumor in HIV infected

individuals in USA, following NHL, Kaposi sarcoma, lung cancer and anal cancer (Uldrick & Little, 2015). The risk is elevated due to the immunosuppression, cytokine dysregulation, transforming properties of the virus itself, and from the opportunistic infections with EBV, human herpes virus 8 (HHV8) and other lymphotrophic herpesviruses (Grogg *et al.*, 2007). It is also noted that there is a strong relationship between HIV infection and EBV presence in the HRS cells, since EBV is found in about 95% of HIV positive HL, whereas in HIV negative HL, EBV is positive in only about 30% of cases (Taylor *et al.*, 2015; Uldrick & Little, 2015). HIV associated HL is most likely to be in two major subtypes of HL which are; lymphocyte depleted and mixed cellularity (Riedel *et al.*, 2015).

Moreover, organ transplantation is a lifesaving option for individuals with end-stage organ disease. However, patients undergoing solid organ transplant must receive intensive long-term immunosuppressive therapy in order to prevent rejection of the transplant, exposing them to a decreased control of oncogenic viral infections and to a high risk of developing PTLDs including Hodgkin and Non Hodgkin lymphomas. The development of HL in transplant recipients, comprises less than 6% of all PTLDs and the risk for its development was lower than that for NHL, where HL risk was reported as 2 to 3.6 folds higher than that in the general population (Clarke *et al.*, 2013; Hall *et al.*, 2013; Mihaljevic *et al.*, 2016).

2.4.1.2 Autoimmune diseases

Autoimmune diseases are characterized by dysregulated lymphocyte reactivity against self-antigens and the production of autoantibodies, leading to a damage of the targeted tissues, such as joints or skin (Landgren *et al.*, 2006). There have been many efforts to describe the role of autoimmunity in different subtypes of lymphomas including HL (Landgren *et al.*, 2006). Furthermore, HL has been associated with several autoimmune conditions including rheumatoid arthritis, systemic lupus erythematosus (SLE), sarcoidosis, Behcet's disease, Sjögren's syndrome and immune thrombocytopenic purpura (Anderson *et al.*, 2009; Bernatsky *et al.*, 2014; Fallah *et al.*, 2014; Hollander *et al.*, 2015). A pooled analysis of cohort studies of SLE reported 3 folds increased risk of HL, due to the use of immunosuppressive SLE treatments (Bernatsky *et al.*, 2007). In consistent with this, Landgren et al. found that a personal history of systemic autoimmune conditions was strongly associated with increased risk of HL, with the most elevated risk estimates seen for rheumatoid arthritis and SLE (Landgren *et al.*, 2006). This increased risk can also be

explained with the autoimmune conditions, dysregulation and hyperactivity of B-cells, impairment of T-cell control, and the treatment with different immunosuppressive drugs (Fallah *et al.*, 2014).

2.4.1.3 Atopy

Immune dysregulation, such as atopic diseases including asthma, allergy, and eczema are also related to lymphoma, where they have been described to be associated with reduced risk in the development of hematological malignancies. These results can be explained by the immune surveillance hypothesis, which suggested that allergy and other atopic diseases can enhance the immune system to detect and eliminate malignant cells, thus protect against malignancies (Soderberg *et al.*, 2006).

2.4.2 Genetic predisposition

It has been suggested that patients with cancer show a high sensitivity to mutational agents as a result of genetic predisposition (Sud *et al.*, 2017). However, lymphomas have been only recently considered to have an important inherited genetic etiology. There have been many evidences to verify the association of genetic predisposition in developing different types of lymphomas including HL. These evidences can be summarized with familial predisposition and genetic risk factors (Cerhan & Slager, 2015).

2.4.2.1 Familial predisposition

Familial risk factors have been reported to be associated with the risk of lymphomas including HL (Cerhan & Slager, 2015). A large case-control study reported a 3.3 folds increase in HL risk in a family with a history of HL, whereas, others reported even higher risks of HL (Cerhan & Slager, 2015; Thomas *et al.*, 2002). Studies in France (Rudant *et al.*, 2007) and England (Pang *et al.*, 2008), reported a 5.4 to 5.8 folds increase in the risk of HL in first and second-degree relatives respectively (Linabery *et al.*, 2015). Furthermore, a positive family history of cancer in first-degree relatives was associated with an increased risk of HL, where about 40% of HL patients had a first degree relative with cancer (Gobbi *et al.*, 2013).

In Canada and USA, the risk in siblings of HL cases was reported to be 5.9 folds increased (Linabery *et al.*, 2015). Moreover, the risk of HL in siblings is higher than in parents and in

other non-sibling brothers and sisters. In addition, the risk is higher in sisters than in bothers (Cerhan & Slager, 2015; Kharazmi *et al.*, 2015). In contrast, a Swedish study suggested that HL risk in parents was higher than that of siblings of HL cases of less than 36 years old, whereas risks for parents and siblings were 8.8 and 7.2 folds respectively (Linabery *et al.*, 2015). Nevertheless, in a twin study, the risk of HL was 100 folds higher in monozygotic twin compared to dizygotic twin where no excess risk was found (Cerhan & Slager, 2015).

2.4.2.2 Single nucleotide polymorphisms

Several genes and pathways have been reported to be associated with the risk of HL, which is characterized by high genomic instability, including numerous mutations in genes related to B-cell function and specific signaling pathways. These pathways include NF-kappa-B, JAK/STAT and more recently B2M pathway. Specific mutations in genes have been identified to be associated with HL such as mutated (*NFKBIA*, *TNFRSF14)* genes in HL and in diffuse large B-cell lymphomas (*CARD11*, *STAT6*, *CREBBP*, *CMYB*), as well as single nucleotide variants (SNVs) in genes (*BTK*, *NFKB2*) (Mata *et al.*, 2015).

One of the major genes associated with HL is the Human leukocyte antigen (HLA) complex, where HL was reported as the first disease to be associated with HLA in a study fifty years ago. HLA is a gene complex encoding the major histocompatibility complex (MHC) in humans (McAulay & Jarrett, 2015).

Single nucleotide polymorphism (SNP) is considered to be the most common type of genetic variation, where a single base pair is changed in the DNA sequence. Other variations may include insertions, deletions, translocations, inversions. Five genome wide association studies (GWAS) reported SNPs in the MHC region and outside the MHC region that were HL associated. These genome wide association studies (GWAS) reported SNPs mapping to HLA class II, in close proximity to *HLA-DRA* and *HLA-DRB1*. The 6p21.32 locus marked by rs6903608 (near *HLA-DRA*) was associated with EBV negative CHL. Additional GWAS signals at 6p21 have been identified in HLA class I (Cerhan & Slager, 2015). Outside of the MHC region, GWAS-discovered loci for HL include 2p16.1 (near *REL*), 10p14 (near *GATA3*), 8q24.21 (telomeric to *PVT1* and near *MYC*), 5q31 (a nonsynonymous SNP in *IL13*), 3p24.1 (5′ to *EOMES*), 6q23.3 (intergenic

to *HBS1L* and *MYB*), and 19p13.3 (in intron 2 of *TCF3*), with only the 2p16.1 and 5q31 loci showing stronger associations with EBV (negative) status (Cerhan & Slager, 2015).

Moreover, it was reported that interaction of genetic factors with infectious exposures including EBV infection may also attribute to the etiology of HL (Cerhan & Slager, 2015). Where it has been found that HLA polymorphism is linked to EBV status of lymphoma, where EBV positive HL is linked to HLA class I (including *HLA-A*01 and *02*) and EBV negative HL has a stronger association with HLA class II (including *HLA-DRB1*) (Cerhan & Slager, 2015; Martin *et al.*, 2015; McAulay & Jarrett, 2015).

2.4.3 Lifestyle factors

Modifiable lifestyle factors, including dietary intake, smoking, and physical activity were reported to account for two thirds of all non-genetic cancer causes (Gorini *et al.*, 2007). Furthermore, they have been also reported to play an important role in the etiology of HL (Nieters *et al.*, 2006).

Little is known about the association of dietary factors and the development of Hodgkin lymphoma. Evidences have indicated that high intake of dietary fat can alter the immune response in humans and increase the progression of lymphomas in mice. Saturated fat may also induce anti-apoptotic response in T-cells. Gender, age and tumor EBV status determined the type of association between dietary fat and the risk of Hodgkin lymphoma, which was positive with saturated fat, and negative with monounsaturated fat in young women. The positive association was particularly in EBV negative patients (Gao *et al.*, 2013). Moreover, 25% decrease in total fat intake has been shown to improve some measures in human immune response, thus, the risk of HL is also decreased with high fruit intake and low fat products. Nevertheless, several studies reported positive associations between HL risk and a diet rich in desserts and sweets. Others reported positive associations between HL risk and high meat diets. A study in Italy reported inverse associations with diets rich in whole grains. No association with vegetables, fruits, or red meat, whereas increased intake of liver and ham was reported to be associated with increased risk of HL (Epstein *et al.*, 2015; Gao *et al.*, 2013).

Tobacco smoking is considered to be one of the risk factors for HL. Smoking is thought to downregulate natural killer cells and macrophages activity, increasing lymphocyte counts, and altering their function thus promoting the pathogenesis of lymphomas (Taborelli *et al.*, 2017). A positive association between HL and tobacco smoking has been supported through previous investigations including a Greek case-control study (Sergentanis *et al.*, 2013), which reported that both the intensity of smoking and the years of smoking were related to an increase in the risk of HL. When most studies reported no association between smoking and HL risk (Fernberg *et al.*, 2006), some suggested positive (Besson *et al.*, 2006) and others suggested negative associations (Bernard *et al.*, 1987). Hjalgrim *et al.* reported that the risk of HL is increased among current smokers compared with never-smokers (Hjalgrim *et al.*, 2007; Taborelli *et al.*, 2017). Other European multi-center case-control study suggested that smokers above 35 years had about 2.5 folds increase in the risk of HL, particularly in nodular sclerosis subtype compared to patients who never smoked (Taborelli *et al.*, 2017).

In a pooled analysis of 12 case-control studies regarding cigarette smoking (Kamper-Jorgensen *et al.*, 2013) and in consistent with large cohort study among United Kingdom women (Kroll *et al.*, 2012), and two meta-analysis (Castillo *et al.*, 2011; Sergentanis *et al.*, 2013); Kamper *et al.* concluded that self-reported history of cigarette smoking was associated with slightly increased risk of HL overall (Kamper-Jorgensen *et al.*, 2013). Karunanayake et al. found that developing HL in patients with smoking history of more than 25 years, is 1.9 times more than that in nonsmokers (Karunanayake *et al.*, 2009).

Physical activity may play a role in the prevention of hematologic cancers, including HL, through its positive association with the immune function. This prevention can be related to antioxidant defense systems, insulin sensitivity, and anti-inflammatory mechanisms (Jochem *et al.*, 2014). Physical activity may also increase the production of antioxidant enzymes, thereby improving DNA repair capacity (Gomez-Cabrera *et al.*, 2008). On the other hand, several studies showed that there is no significant association between physical activity and the risk of HL (Jochem *et al.*, 2014).

2.4.4 Occupational exposures

Many studies have investigated the association between farming and the main types of lymph node malignancies (Orsi *et al.*, 2009). A French meta-analysis examined this relationship where it was positively but weakly associated with HL, and that the associations were more noticeable in the USA (Khuder *et al.*, 1999). Meanwhile, Orsi and his colleagues supported the hypothesis that occupational pesticide exposures may be involved in the development of HL. In addition, they also found significant associations between HL and the use of fungicides especially triazole and urea herbicides in a case-control study in France (Orsi *et al.*, 2009). Nevertheless, Karunanayake *et al.* found that risk for HL was increased among Canadian men who used chlorpyrifos (Karunanayake *et al.*, 2012).

Risk of hematological cancers is increased in hairdressers and cosmetologists since hair coloring products include more than 5000 chemical substance, which have been described to be carcinogenic, mutagenic and associated with lymphoma etiology (de Sanjose *et al.*, 2006; Takkouche *et al.*, 2009). Hair dyes in the past contained some animal carcinogens and some In-vitro mutagenic substances. After oxidation, these substances can cause DNA damaging in human peripheral lymphocytes (Tavani *et al.*, 2005). In agreement with that, Takkouche *et al.* reported a 15% increase in the risk of hematological cancers, due to personal hair dyes use, describing a slight elevation in HL risk (Takkouche *et al.*, 2005).

2.4.5 Medical history

Tonsillectomy is thought to have a role in the development of HL. This role comes from the hypothesis of the 'lymphoid tissue removal', which assists in the development of the disease (Vestergaard *et al.*, 2010). Different studies reported either an increase in the risk of HL (Vianna *et al.*, 1980) or no association at all following tonsillectomy (Becker *et al.*, 2005). A Swedish cohort study reported a 4 folds increase in the risk of HL for people who underwent tonsillectomy before the age of 12 years (Liaw *et al.*, 1997). Two other American studies found similar results also (Johnson & Johnson, 1972; Vianna *et al.*, 1980). In a national wide cohort study in Denmark, Vestergaard *et al.* found that the risk for HL has been increased in people tonsillectomized before the age of 15 years (Vestergaard *et al.*, 2010).

Drugs may also play a role in the pathogenesis of HL. In the United Kingdom, a case-control study was conducted to find the association between different types of drugs given to pregnant women and the effect of these drugs on the children born. It was found that analgesic use of NO2B subgroup of drugs during pregnancy was significantly associated with a 5-folds increase in the risk of HL in the born children (Bonaventure *et al.*, 2015).

Ultraviolet radiation has been inversely associated with HL risk. Monnereau *et al.* described statistically significant associations with HL risk for UV radiation exposures during childhood and adulthood. Risks were significant only for EBV-positive HL, where the UV radiation exposure may protect against HL. This inverse association could be explained by the UV radiation induction of vitamin D3 synthesis in the skin, which plays a role in the inhibition of the proliferation of cancer cells (Monnereau *et al.*, 2013). Furthermore, significantly increased risk of HL was found after exposure to ionizing radiation from uranium (Karunanayake *et al.*, 2009).

2.4.6 Proxies of infection

Number of siblings can be considered as one of the infectious etiologies of HL, the larger the family the greater the chance to develop infection (Chang *et al.*, 2004; Westergaard *et al.*, 1997). One hypothesis of infection assumes that, the risk for leukemia or other hematopoietic malignancy can be increased as a result of increased number of family members, whereas the level of exposure is increased (Altieri *et al.*, 2006). In agreement with this hypothesis, there have been two small studies, one from Portugal (Sobrinho-Simoes & Areias, 1978) and the other from Israel (Bogger-Goren *et al.*, 1983); that investigated the importance of family structure in developing HL in children, and they found that HL cases originated from big families. A study in Brazil (Kirchhoff *et al.*, 1980), and another hospital-based case-control study in Italy (Serraino *et al.*, 1991); both suggested a positive association between HL risk and high educational level or economic level which are both related to a small family size. On the other hand, an American study of young women suggested that the risk for HL is no longer related to birth order or family size, due to the demographic variations in recent years (Chang *et al.*, 2004).

According to the other infection hypothesis, late or delayed exposure to common bacterial or viral infections in childhood is considered to be a risk factor for both leukemia and HL

(Altieri *et al.*, 2006). In addition, a later birth order may reflect an earlier exposure to infections from older siblings whom can get infections easily from their classmates at school (Chang *et al.*, 2004; Westergaard *et al.*, 1997). In contrast, Westergaard *et al.* suggested that early exposure to infections might increase the risk of HL in children, while in adults, the late exposure may increase the risk (Westergaard *et al.*, 1997).

Contact to pets has been also associated with risk of HL. In a case-control study in Sardinia, Italy, the authors suggested that early-life exposure to pets, birds and mainly with chickens might be associated with a reduction in the risk of lymphoma (Bellizzi *et al.*, 2011). However, according to a Canadian study of the effect of exposure to farm animals, the risk for HL was minimal (Pahwa *et al.*, 2003).

2.4.7 Infectious exposures

Infection is one of the main risk factors for cancer. The International Agency for Research on Cancer Monograph has identified eleven biological agents as group 1 carcinogens. This group includes EBV, which is mostly associated with the risk of HL. In 1990, it has been estimated that 15.6% of the worldwide cancer incidence was attributed to infection with either EBV, HBV, HCV, or HIV-1, and different types of viral infections (Pisani *et al.*, 1997). Therefore, in developed countries, 21% of cancer cases and 9% in more developed countries could have been eliminated by the prevention of these infectious diseases (Oh & Weiderpass, 2014).

2.4.7.1 HBV

HBV infection is reported to be associated with HL (Makvandi *et al.*, 2015). In non-Hodgkin lymphoma (NHL), 30% of the cases have been reported to be HBsAg positive among Romanian patients, which was significantly higher than that of normal population (Cucuianu *et al.*, 1999). This ratio imports the major role played by HBV infection in the lymphogenesis of NHL, while in HL the HBV infection ratios have been reported to be less. In a Chinese study done by Miao-Zhen Qiu *et al.*, HBsAg was positive in only 15% of HL cases, which is the same for the healthy population (Qiu *et al.*, 2010).

2.4.7.2 HCV

In the general population, the prevalence of HCV is 1.5% while in lymphoma patients this ratio raises up to 15% (Mazzaro *et al.*, 1996). The role of HCV in lymphogenesis can be described by triggering of this virus to the autoimmune response, activating lymphocytes, increasing cytokine production, thus modifying the expression of the host epitopes through liver necrosis (Keresztes *et al.*, 2003). In addition to that, Radmehr *et al.* reported that a long persistence of HCV in B-cells can end up with lymphoma either Hodgkin or non-Hodgkin lymphoma (Radmehr *et al.*, 2016). It has been also suggested that chemotherapy induces HCV infection, resulting in hepatic dysfunction or liver damage (Guarino *et al.*, 2017). Synchronized HCV infection and HL is extremely rare according to Pejsa *et al.* (Pejsa *et al.*, 2004).

In Hungarian blood donors, HCV infection was found in 0.73% of the population. Moreover, in HL cases this ratio is increased to 9.0%, being 12 times higher than in normal population (Gasztonyi *et al.*, 2000). In an Italian study, there was no difference of HCV prevalence between HL cases and normal population (Keresztes *et al.*, 2003).

2.4.7.3 Human Herpes Virus 6 (HHV6)

Human Herpes Virus 6 (HHV6) is a lymphotropic virus, which replicates in T-lymphocytes. It was first discovered in 1986 and is classified into two major subgroups, HHV-6A and HHV-6B. This virus is associated with several malignancies including NHL, ALL, carcinoma, and HL (Kiani *et al.*, 2016).

2.4.7.4 EBV

Many diseases are associated with EBV infection including, gastric carcinoma, nasopharyngeal carcinoma, NK-T cell lymphoma, Burkett lymphoma, diffuse large B-cell lymphomas (DLBCL), post-transplant B-cell lymphomas, infectious mononucleosis, multiple sclerosis, rheumatoid arthritis and HL (Correia *et al.*, 2017; Mandage *et al.*, 2017). The association between EBV and HL is suggested to be causal. In developed countries, 30-50% of HL cases are attributed to EBV infection, while in developing countries the attributable percentage was up to 70-100% of HL cases. In Taiwan and Japan, it is 60-65%. In Pakistan, the incidence of EBV association with HL is increased up to 87% in children, while in adults it is only 49%. The increased association in children may be explained by the lowered immune status and different environmental factors (Fatima *et al.*, 2011).

2.4.8 EBV infection and the risk of Hodgkin lymphoma

2.4.8.1 EBV infection

Epstein Barr Virus (EBV) is a human herpes virus that belongs to the *Herpesviridae* family, *Gammaherpesvirinae* subfamily, and *Lymphocryptovirus* genus. This family contains more than 100 diverse viruses, but only the following are considered to be pathogenic: herpes complex virus 1, herpes complex virus 2, cytomegalovirus, human herpes virus 6, human herpes virus 7, Kaposi sarcoma associated herpes virus, varicella zoster virus, and finally EBV (Grywalska & Rolinski, 2015).

Infection with EBV is common, and has been detected throughout the world. About 90% of adults and before the age of thirty become EBV positive (Dunmire *et al.*, 2015). In Indian children, the EBV positive reported cases were 96.6%, and 90.3% in Brazilian children (Qi *et al.*, 2013). In less developed countries, EBV infection tends to happen in children aged 3 or 4 years, while it is delayed until adolescence in more developed countries. In a recent study (Grywalska & Rolinski, 2015), 90% of 1,148 subjects participating in the US National Health and Nutrition Examination Surveys, had IgG antibodies against EBV, indicating an old infection with EBV (Balfour *et al.*, 2013a). There are many factors contributing to the early acquisition of EBV including race, socioeconomic status, geographical distribution, maternal education, daycare attendance, school catchment area, crowding bedroom, *etc.* (Dunmire *et al.*, 2015).

Moreover, recently different studies have reported that the primary age of EBV infection is increased in many countries (Mandage *et al.*, 2017). In a USA study for 6-19 years old individuals, the prevalence of EBV infection has been decreased from 72% in the years 2003-2004, to 65% in the years 2009-2010 (Balfour *et al.*, 2013b). Another Japanese study for 5-7 years old children reported that the seropositivity has declined from 80% in early 1990s, to 59% in the years 1995-1999 (Takeuchi *et al.*, 2006). Nevertheless, a study in England and Wales also suggested an increase in the EBV infection age (Morris *et al.*, 2002). In France, Fourcade *et al.* reported a 15 years old increase in the seroprevalence of EBV (Fourcade *et al.*, 2017).

EBV mainly causes infectious mononucleosis (IM), which is involved with cervical lymph node enlargement, sore throat, fatigue and fever. Infectious mononucleosis is considered to be a significant health concern and burden due to its acute infection and because of the

long term consequences ending up with different types of autoimmune diseases and cancers (Balfour *et al.*, 2015). The main way of transmission of primary EBV infection among young adults is kissing, in addition to blood transfusion, hematopoietic cell transplantation and solid organ transplantation (Dunmire *et al.*, 2015). Infectious mononucleosis is a risk factor for HL, where an incidence peak of HL is observed 2 to 3 years after IM. This observation can be explained by the fact that EBV is an oncovirus for B-lymphocytes (Fourcade *et al.*, 2017).

EBV has two major targets of infection, epithelial cells and B-lymphocytes which are thought to contribute to EBV associated tumors (Chesnokova & Hutt-Fletcher, 2014). EBV accounts for 0.5 to 2.0% of cancers (Khan & Hashim, 2014). In a recent analysis done at 2010, it was found that 1.8% of all cancer deaths were linked with EBV infection (Jha *et al.*, 2016). Infection with EBV may contribute in the development of several malignancies, including gastric carcinoma, nasopharyngeal carcinoma, Burkitt lymphoma, diffuse large B-cell lymphomas, and HL (Li *et al.*, 2016; Mohamed *et al.*, 2014).

EBV can establish two forms of infection in the host, the latent and the lytic infection (Li *et al.*, 2016). Once EBV infects B-lymphocyte, establishment of latency can follow the promotion of a lytic infection (De Paoli & Carbone, 2015). EBV expresses several genes during the latent infection, including six genes for EBV nuclear antigens, (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C and EBNA-LP), three EBV latent membrane proteins (LMP-1, LMP-2A, and LMP-2B), BamHI-A rightward transcripts, and two short noncoding RNAs (EBER-1, and EBER-2) (Grywalska & Rolinski, 2015).

Depending on different patterns of expression of latent genes, EBV shows three types of latency. EBNA-1 dominates in latency type I, which is detected in Burkitt lymphoma and stomach cancer. In HL and nasopharyngeal carcinoma, type II latency is well characterized by the expression of EBNA-1, LMP-1, and LMP-2, while all latent genes are expressed in type III latency (Cesarman, 2014; Grywalska & Rolinski, 2015). EBV can establish latent infection, where the expressed proteins and noncoding RNAs may affect the cell cycle by inhibiting apoptosis, and promoting cell proliferation (Cesarman, 2014).

2.4.8.2 EBV association with Hodgkin lymphoma

Hodgkin lymphoma is a complex of related conditions that are facilitated by infectious disease, immune incapability and genetic susceptibilities. The descriptive epidemiology of the disease puts forward an infectious disease process underlying its etiology in children and young adults (MacMahon, 1966). Since the discovery of EBV in 1964, the association between EBV infection and HL has been clearly observed (Swerdlow, 2003). EBV plays an essential pathologic role in HL, whereas patients who developed IM in adulthood had a threefold greater risk of developing HL than those who did not (Grywalska & Rolinski, 2015; Jona *et al.*, 2013; Salati *et al.*, 2014).

The association between EBV infection and the risk of HL varies depending on gender, age, ethnicity and histological subtype (Kennedy-Nasser *et al.*, 2011). EBV positivity in HL patients is about 17% in African American children, 46% of Caucasian, 86% of Hispanic, 93% of Asian, in Peru and Mexico it ranges between 50 to 95%, 92% in Kenya, 65% in China, 90% in Greece, 61.5% in Turkey, 50% in Egypt, 48% in Italy, and 30% in Israel (Adelusola *et al.*, 2009; Kennedy-Nasser *et al.*, 2011; Salati *et al.*, 2014). A Vietnamese study revealed that in children, almost all cases of HL were EBV positive (Chang *et al.*, 2005). Another study in Iran by Katebi *et al.* also showed that about 93% of HL cases were EBV positive as well (Adelusola *et al.*, 2009; Kennedy-Nasser *et al.*, 2011; Salati *et al.*, 2014). Moreover, EBV positive rates are commonly higher in South Asian children than in non- South Asian children in United Kingdom, and in males than in females (Mohamed *et al.*, 2014). EBV is infrequently found in nodular sclerosis HL (15%-20%), rarely found in NLPHL, never found in lymphocyte rich HL, and is more commonly associated with mixed cellularity HL and lymphocyte depleted HL (75% and 95% respectively) (Grywalska & Rolinski, 2015).

Furthermore, economic level has been also reported to be linked to the association between HL and EBV infection, whereas in developing countries, EBV is found in 90% of childhood HL and 60% of adulthood HL, and in developed countries EBV infection is found in 40% of cases (Oh & Weiderpass, 2014). In Saudi Arabia, Al-Kurava *et al.* found that EBV infection was not associated with the risk of HL, unlike developing countries, where EBV infection has been suspected to be associated with high HL incidence (Al-Kuraya *et al.*, 2006).

2.4.8.3 EBV pathogenesis in Hodgkin lymphoma

EBV expresses type II latency in HRS cells, which is considered as the hallmark of EBV positive HL, where EBNA-1, LMP-1, and LMP-2A are expressed (Grywalska & Rolinski, 2015; Kennedy-Nasser *et al.*, 2011). EBNA-1 is responsible for the replication of the viral genome, and through the production of chemokine ligand 20 (CCL20), it helps in attracting regulatory T-cells (T regs), therefore resulting in tumor progression by inhibiting EBV specific immune responses (Jona *et al.*, 2013). Both LMP-1 and LMP-2A are EBV oncogenes that mimic proliferative and survival signals of B-cells, suppress apoptosis, resulting in establishing lifelong persistence by allowing genome replication while remaining immune silent and latent in these B-cells (Jha *et al.*, 2016). To be specific, LMP-1 contributes to the pathogenesis of EBV in HL by mimicking CD40, and it also can constitutively activate NF kappa B. Another major role of LMP-1 lies in its ability to turn off the expression of BCL-6, and to turn on the expression of BCL-2 driving the differentiation of memory B-cells from the germinal center cells. BCL-2 is an oncoprotein involved in apoptosis inhibition, whereas increased expression of BCL-2 causes resistance to chemotherapy and radiation therapy, while decreased BCL-2 expression may stimulate apoptotic responses to anticancer drugs. Moreover, BCL-2 is widely related to relapses and non-responders (Flangea *et al.*, 2008). In HL, measuring the expression of BCL-2 can be a useful, independent prognostic marker and can be used in association with clinical parameters to identify newly diagnosed patients with a good, intermediate, or poor prognosis (Sup *et al.*, 2005).

On the other hand, LMP-2A by its tyrosine based activation motif can mimic B-cell receptor (BCR) leading to EBV lytic reactivation. Meanwhile, BCR is responsible for two signals, one is to ensure the survival of the resting B-cells, and the other is to activate, proliferate and differentiate B-cells into plasma cells (Cesarman, 2014; Jha *et al.*, 2016; Thorley-Lawson, 2015). Since both CD40 and BCR signaling are the main regulators of survival and selection of GC B-cells, it was speculated that LMP1 and LMP2 can save BCR deficient cells from apoptosis by replacing these signals (Kuppers *et al.*, 2012).

Regarding the pathogenesis of the virus, total number of latently infected cells may increase by EBV lytic infection when the transmission of the virus from cell to cell is enhanced (Li *et al.*, 2016). In EBV positive HL children, it is thought that the primary infection plays a major role in the development of the tumor, while in older patients a weak

immune response toward EBV latent genes may be the main cause (Jha *et al.*, 2016). Thus, EBV is thought to provide anti-apoptotic functions in HL, facilitating the survival of HRS cells and their precursors (Mohamed *et al.*, 2014). The virus regulates its own cycle and redirect the fate of the infected B-cells towards long-term persistence in the memory pool by switching between different forms of latency in the B-cell system (Vockerodt *et al.*, 2014). The unusual microenvironment of the non-neoplastic cells also contributes to the pathogenesis of EBV associated HL by enhancing tumor cell growth, survival and by redirecting the functions of EBV latent genes expressed by HRS cells (Mohamed *et al.*, 2014).

2.5 Treatment of Hodgkin lymphoma

Hodgkin lymphoma is characterized by the rarity of neoplastic HRS cells and plethora of the reactive inflammatory cells, including T-cells, eosinophils, plasma cells, histocytes, macrophages, fibroblasts, dendritic cells, *etc.* (Ansell, 2015; Lin & Diefenbach, 2016). This microenvironment contributes to tumor evasion from the host's immune surveillance and growth control via autocrine and paracrine production of many inflammatory cytokines and chemokines. This inflammatory microenvironment has been a great target for novel targeted therapies (Lin & Diefenbach, 2016).

Initial treatment of HL patients is based primarily on the stage of the tumor. According to that, patients with early stages, are usually treated with doxorubicin, bleomycin, vinblastine, and dacarbazine regimen (ABVD), followed by field limited radiation therapy. Patients with advanced stage are usually treated with more intense and prolonged chemotherapy courses composing of ABVD or bleomycin, etoposide, doxoruicin, cyclophosphamide, vincristine, procarbazine, and prednisone regimen (BEACOPP), in addition to radiation therapy. For patients with refractory or relapsed HL, autologous stem transplant is considered to be the standard care besides initial and high dose chemotherapy. The majority of HL patients are cured by these standard therapies (Lin & Diefenbach, 2016).

A significant minority of patients who don't respond to these therapies including stem cell transplantation, end up with a refractory or relapsed disease with bad prognosis (Lin & Diefenbach, 2016); to meet the needs of these patients, new strategies and therapies are

being developed, summed up with two strategies, antibody drug conjugate (ADCs) and checkpoint inhibition. In ADCs, specific targets on the cell surface are used to deliver cytotoxic chemotherapy to the inside of the tumor cells. In checkpoint inhibition, the immune system of the host is induced to execute the tumor cells (Villasboas & Ansell, 2016). The most recently tested ADCs are trastuzumab emtansine and brentuximab vedotin (BV). BV recognizes CD30 on the surface of the tumor cells and neutralizes it. BV can perform its task since CD30 is expressed on tumor cells and not on most of normal cells. Examples on checkpoint inhibitors are program dealth-1 (PD-1) inhibitors: nivolumab and pembrolizumab. Once PD-1 ligands attach their receptors, they start a set of events leading to a decreased function and survival of the immune cells, allowing tumor cells to progress and escape death (Villasboas & Ansell, 2016). PD-1 ligands (PD-L1 and PD-L2) are highly expressed in HRS cells and are also induced by EBV infection (Ansell, 2015).

In 1970, Devita *et al.* introduced the first combined chemotherapy for HL, named Mustargen, Oncovin, Procarbazine, Prednisone (MOPP) regimen. About 60% to 80% complete remission was achieved in HL patients after only 6 to 8 cycles of MOPP. In 1982, a comparison between ABVD and MOPP lead to the introduction of ABVD as the standard regimen for the treatment of HL up to nowadays (Witkowska *et al.*, 2015).

Radiotherapy has been found to be associated with acute ad long-term toxicity. It may induce the impairment of thyroid or pulmonary function, cardiovascular disease, and may also lead to other cancers. Thus, recent studies and trials suggested the reduction of the field exposed to radiation rather than extended field, to reduce toxicity. The dose of X-ray and the exact place of radiation can determine how toxic the radiation is. Skin changes, fatigue, dry mouth, diarrhea, nausea and change of taste, are the major side-effects of radiotherapy (Witkowska *et al.*, 2015).

Chapter Three

Methodology

This multicenter study is considered to be the first study in Palestine to focus on Hodgkin lymphoma and investigate the familial predisposition, lifestyle, environmental and medical risk factors associated with the risk of Hodgkin lymphoma. This chapter describes the details of the study and the tools that were used.

3.1 General Study Design

The study was started by assigning HL cases. This was achieved by going through the medical records in three Palestinian hospitals, Beit-Jala Hospital in the South, Rafidia Hospital in the North, and Augusta Victoria Hospital (AVH) in the middle, and identified a total of 168 HL cases. For the confirmation of HL diagnosis, a pathology report was required. Pathology reports were retrieved from either the medical files or the patients themselves and the final study included a total of 162 histopathologically confirmed HL cases. The medical files alongside the pathology reports were used to complete the study pathology report for the description of the disease characteristics among the study population. The general study scheme is shown in figure 3.1.

Further, from each of the three study centers we tried to retrieve paraffin-embedded blocks of the cases to be used in a retrospective-cohort study to perform IHC staining and detect EBV positivity in the malignant tissue. A total of 30 paraffin-embedded blocks were retrieved and used in this part of the study.

Moreover, using the pathology reports we identified the incident cases (diagnosed within <24 months of recruitment) and recruited them to participate in a case-control study to examine the risk factors of HL. A total of 63 incident HL cases participated in this part of the study through completing an interview-based questionnaire and giving blood samples for further genetic and serologic analysis. In addition, 85 cancer-free controls were recruited to participate in the study from the participating hospitals, thirteen MOH primary

healthcare centers and Al-Makassed Blood Bank. Cases and controls were administered the same interview-based questionnaire and blood samples were collected from them.

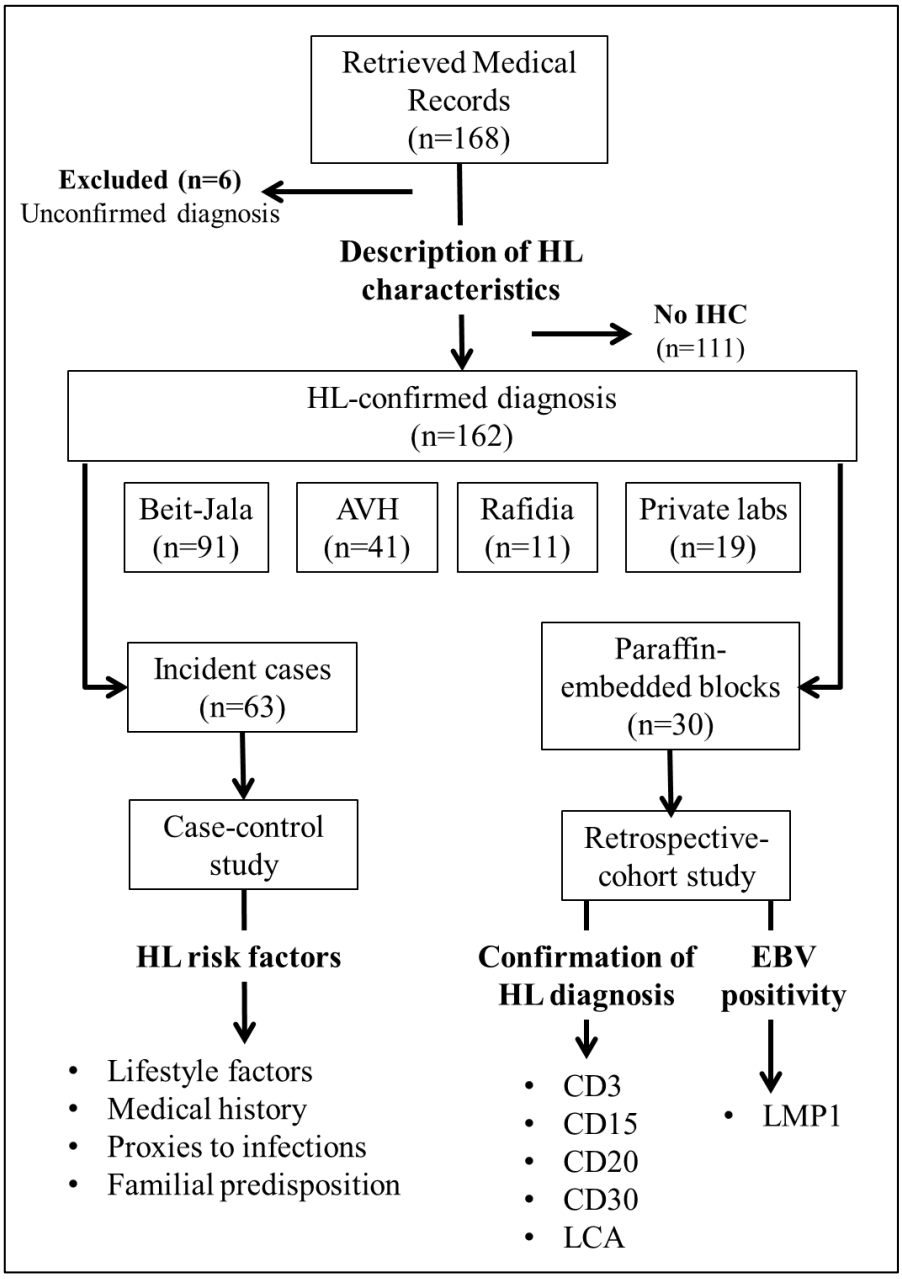

Figure 3.1: General study scheme.

3.2 Part One: Retrospective-Cohort Study

3.2.1 Study Design

In a retrospective-cohort study, disease characteristics were determined by examining 162 HL histopathologically-confirmed cases in the medical files. In addition,

immunohistochemistry profile was also extracted from their pathology reports. We were able to retrieve paraffin-embedded blocks of thirty HL cases from the participating hospitals which were used to confirm HL diagnosis and to detect EBV positivity among HL patients by immunohistochemistry.

3.2.2 Study Centers

HL cases were recognized through medical records in three major Palestinian hospitals providing cancer-care for patients and the Palestinian Cancer Registry. Participating hospitals included Beit-Jala Governmental Hospital, AVH and Rafidia Hospital.

3.2.3 Study Population

Eligible HL cases were included based on the presence of confirmatory pathology report. A total of 162 confirmed HL cases were included in the study. The majority of pathology reports were released by Beit-Jala Hospital (56.2%) and AVH (25.3%). The rest were released from Rafidia Hospital or other referral diagnostic laboratories and hospitals (Table 3.1). Furthermore, from the 162 pathologically confirmed cases we were only able to retrieve 30 paraffin-embedded blocks for IHC staining and testing for EBV. The remaining 113 paraffin-embedded blocks were excluded due to either bad quality tissue preservation, bad archiving conditions, missing blocks in old archives or due to the retrieval of blocks by patients' families (Figure 3.2).

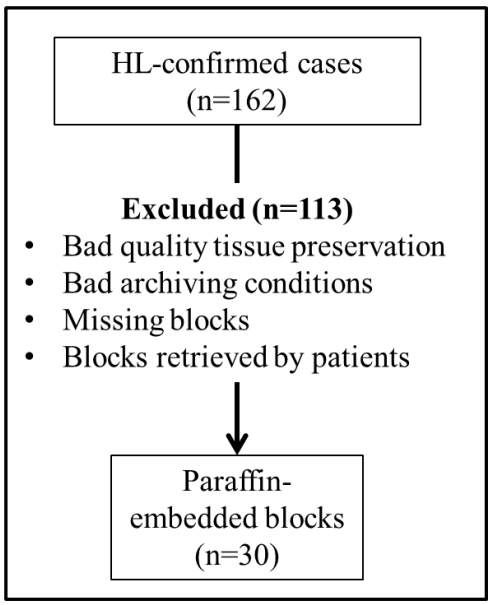

Figure 3.2: Exclusion of cases from the retrospective-cohort study .

Table 3.1: Source of pathology reports and paraffin-embedded blocks.

Hospital	No. of pathology reports	No. of paraffin-embedded blocks
Beit-Jala	91	14
AVH	41	12
Rafidia	11	4
Private and others	19	0

3.2.4 Study Tools

i. **Pathology report**: The confirmation of the diagnosis of HL cases was achieved by obtaining pathology reports from the patients' files. The medical reports were used to complete the pathology report which contains the disease characteristics of each subject. Data obtained included age at diagnosis, HL subtype and IHC profile performed for each case and other clinical characteristics (appendix 3.1). Out of the 162 cases, IHC staining was only performed (either partial or complete IHC panel) for 61 cases. The remaining 101 cases were diagnosed without IHC staining.

ii. **Immunohistochemistry**: Immunohistochemistry (IHC) staining profile was performed for 30 paraffin-embedded blocks. Seven slices, 4 μm thick each, were obtained from each one of the thirty paraffin-embedded blocks. These slices were immediately adhered to the IHC glass slides (Superfrost Plus slides from Thermoscientific Company) which are made to bind fresh frozen or paraffin-embedded tissue sections. The procedure of slicing was done at all three hospitals' pathology laboratories, in which the paraffin-embedded blocks were retrieved, including pathology laboratories of Beit-Jala Governmental Hospital, Rafidia Hospital and AVH. After slicing, slides were delivered to AVH pathology laboratory where the staining procedure was conducted using an automated staining machine (Ventana, Benchmark Company) with the applying of the immunohistochemistry panel described in table 3.2. Immunostaining evaluation and reporting was performed at AVH by the attending pathologist as the following:

   a) To confirm the diagnosis of HL cases for the thirty paraffin-embedded blocks, the IHC panel performed included CD3, CD15, CD20, CD30 and LCA. In addition, hematoxylin & eosin (H&E) stain was also performed.

   b) To detect EBV positivity among HL cases, LMP1 antibody was added to the conventional panel. LMP1 is EBV latent membrane protein that is expressed by EBV during the latent infection, and is used to detect the presence of EBV among HL cases.

Table 3.2: Antibodies used in IHC staining of HL cases.

Antigen	Company of the antibody	Site of expression
CD3	Leica, Germany	T-cells
CD15	Scytek, USA	Granulocytes
CD20	Leica, Germany	T&B-cells
CD30	Scytek, USA	T-cells & monocytes
LCA	Leica, Germany	Hematopiotic cells
LMP1	Leica, Germany	EBV- associated cells

3.3 Part Two: Case-Control Study

3.3.1 Study Design

A multicenter case-control study of histopathologically confirmed incident HL cases and cancer-free controls was conducted to investigate which factors were associated with the risk of HL. All cases and controls answered a self-reported interview-based questionnaire and gave blood samples both in EDTA and plain tubes of 10 ml each for further genetic and serologic analysis for other studies.

3.3.2 Study Centers

Cases were recognized through medical records and the Palestinian Cancer Registry. Participating hospitals included Beit-Jala Governmental Hospital (south), August Victoria Hospital (AVH) (middle) and Rafidia Governmental Hospital (north).

Controls were recruited from Beit-Jala Governmental Hospital, AVH, Al-Makassed Blood Bank, and thirteen Ministry of Health primary health-care centers distributed all over the West Bank.

3.3.3 Study Population

Cases: From the 162 originally confirmed HL cases, incident cases were defined as diagnosis of HL within <24 months of recruitment. A total of 63 cases participated in the study. Inclusion and exclusion criteria for HL patients in the case-control study are listed below.

Inclusion criteria:

- Pathologically confirmed HL cases.
- Palestinian individuals of all ages.
- Incident cases (< 24 months of diagnosis).
- Consent on participation.

Exclusion criteria:

- Missing contact information.
- Refusal to participate.
- >24 months of diagnosis.

Controls: 85 hospital and clinic-based controls were recruited for this study. For each case, at least one control who met the eligibility criteria of controls was recruited from the participating centers. The controls were frequency-matched to the cases by gender, age, and region.

Inclusion criteria:

- Cancer-free at time of recruitment as stated by controls themselves.
- Palestinian individuals, frequency-matched to cases in terms of age, gender and region.
- Consent on participation.
- Not related by blood to cases or other controls.

3.3.4 Study Tools

Questionnaire: An extensive interview-based questionnaire (Appendices 3.2 and 3.3) was used for data collection. This questionnaire was initially developed and validated by the International Lymphoma Epidemiology Consortium (InterLymph). The questionnaire was translated from English to Arabic by forward and backward translation to be used in previous study. It was composed of six sections including demographics (age, gender, marital status, number of children, religion, and educational level), job history (current or previous work and occupational exposures), residential history (place, period and circumstances of housing), lifestyle and hobbies (personal measurements, smoking, hair dyeing, sunlight exposure, dietary style, physical activity, gardening and certain hobbies). The last

part of the questionnaire was the health section, which included medical history (history of hospitalization for serious infections, history of diseases, vaccinations and medications received, and blood transfusion), and family history of cancer and immune diseases among first and second degree relatives.

3.4 Ethical Considerations

The participants signed a consent expressing their willingness to participate in the project and further it confirmed the confidentiality of the participants' information and their freedom to quit the study at any moment without intimidations (Appendix 3.4). Furthermore, a coded system was adopted to ensure the confidentiality of the data. Moreover, ethical approval by the institutional review board (IRB) committee of Al-Quds University was obtained earlier (Appendix 3.5). Regarding recruitment centers, approval from the Ministry of Health was obtained to access whatever needed to facilitate our research work in Beit-Jala Governmental Hospital and Rafidia Hospital (including data, patients' files, phone numbers, paraffin-embedded blocks and blood samples) (Appendix 3.6).

3.5 Statistical Analysis

Data were coded, then entered and analyzed using the Statistical Package for Social Sciences (SPSS V20.0.0). Descriptive statistical analysis was used to calculate frequencies and percentages for categorical variables, and means, medians, ranges and standard deviations (SD) for continuous variables. The principle measure of association was the odds ratio (ORs). ORs and 95% confidence intervals (95% CI) were computed using unconditional logistic regression to evaluate the association between different risk factors and the development of Hodgkin lymphoma. Multivariate models were designed to adjust for expected confounders like age, gender, region and family history of hematological malignancies.

Chapter Four

Results

In this chapter, we provide an overview of the disease characteristics among Palestinians. In addition, we demonstrate the findings regarding risk factors of Hodgkin lymphoma and the prevalence of EBV among HL cases.

4.1 Disease characteristics

In this study, the mean age at diagnosis for HL cases was 23 years (Figure 4.1). The age distribution among HL cases at diagnosis is described in figure 4.1. The age shows a bimodal distribution with one peak between the ages of 5 and 10 and another between 20 and 24. Furthermore, the male to female ratio was 1.25:1 (Figure 4.2). Regarding histological subtypes of HL as determined by the pathology reports; nodular sclerosis was the most common subtype with 51.1% (Figure 4.3 and 4.4), followed by mixed cellularity with 39.1% as the second common subtype (Figure 4.5), where lymphocyte rich and lymphocyte depleted were found in low frequencies with 4.5% and 1.5% respectively (Figure 4.6 and 4.7).

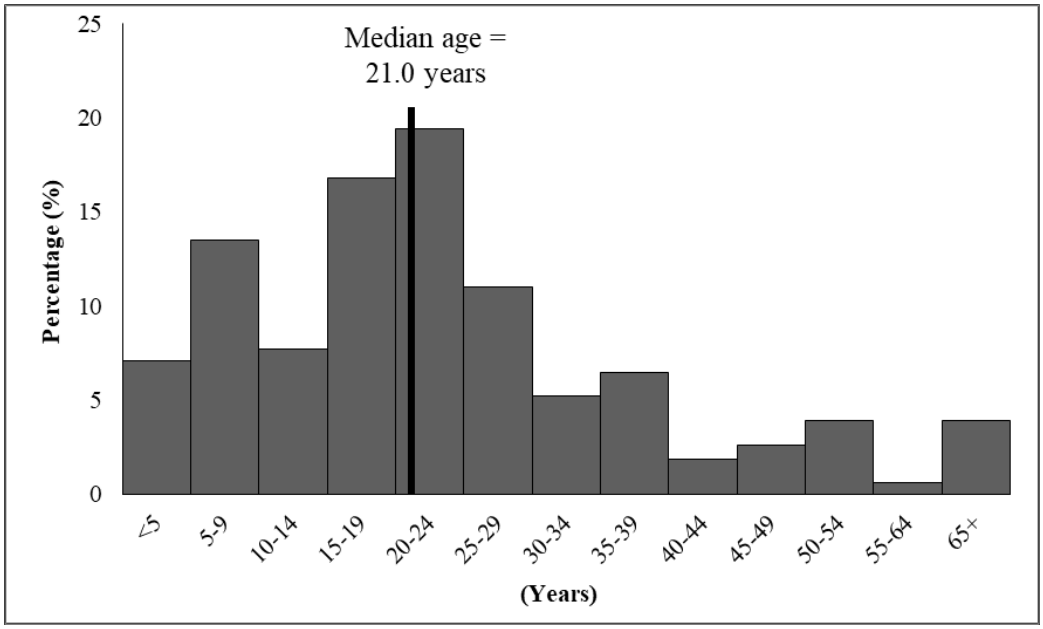

Figure 4.1: Age distribution among HL cases at diagnosis.

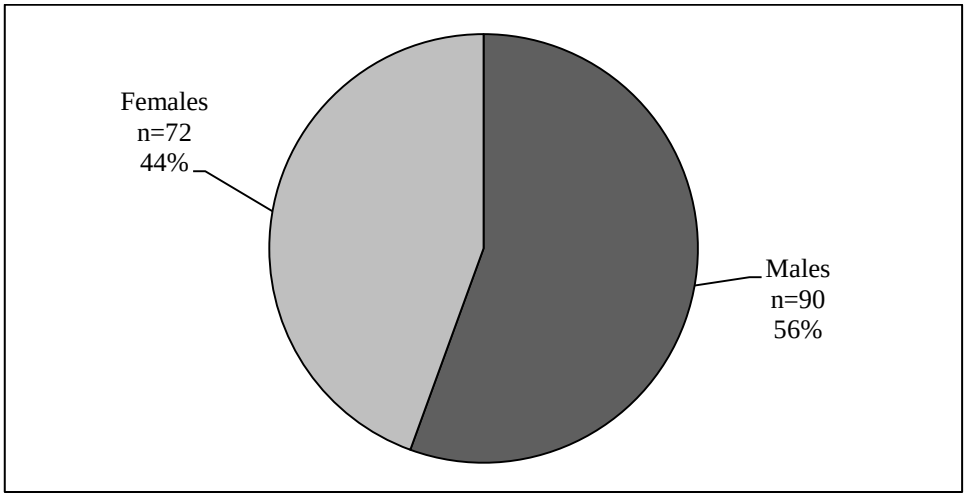

Figure 4.2: Distribution of HL cases by gender.

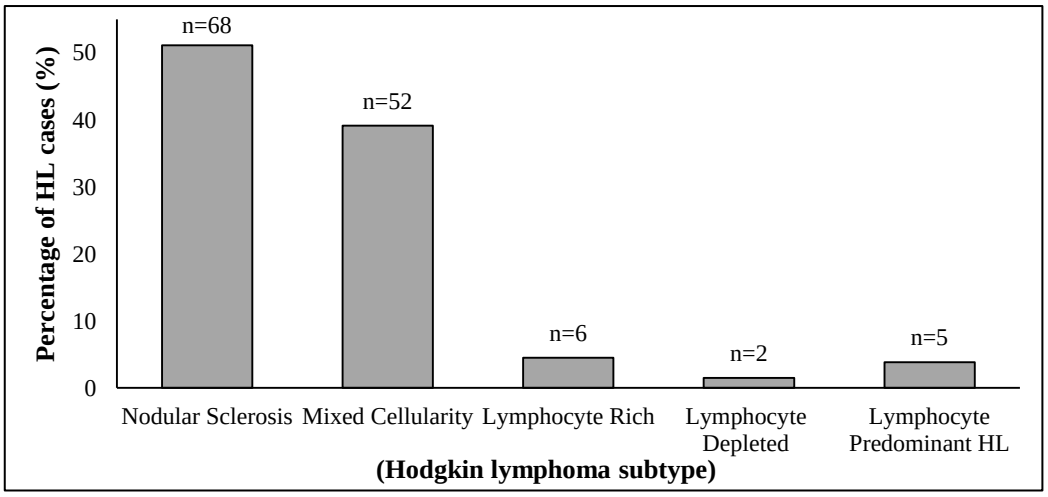

Figure 4.3: Distribution of HL cases by histologic subtype.

After analyzing all data obtained from the available pathology reports, it was found that the common profile for confirming the diagnosis of HL was CD3 negative (Figure 4.8), CD15 positive (Figure 4.9), CD20 negative (Figure 4.10), CD30 positive (Figure 4.11), LCA negative (Figure 4.12) (Table 4.1).

Table 4.1: IHC of HL cases as found in the pathology reports.

CD marker	CD3	CD15	CD20	CD30	LCA
Positive n (%)	1 (0.6)	48 (29.6)	13(8.0)	61 (37.7)	0 (0.0)
Negative n (%)	46(28.4)	7 (4.3)	38 (23.5)	0 (0.0)	36 (22.2)
Not performed n (%)	115 (71.0)	107 (66.0)	111 (68.5)	101 (62.3)	126 (77.8)
Total n (%)	**162 (100%)**	**162 (100%)**	**162 (100%)**	**162 (100%)**	**162 (100%)**

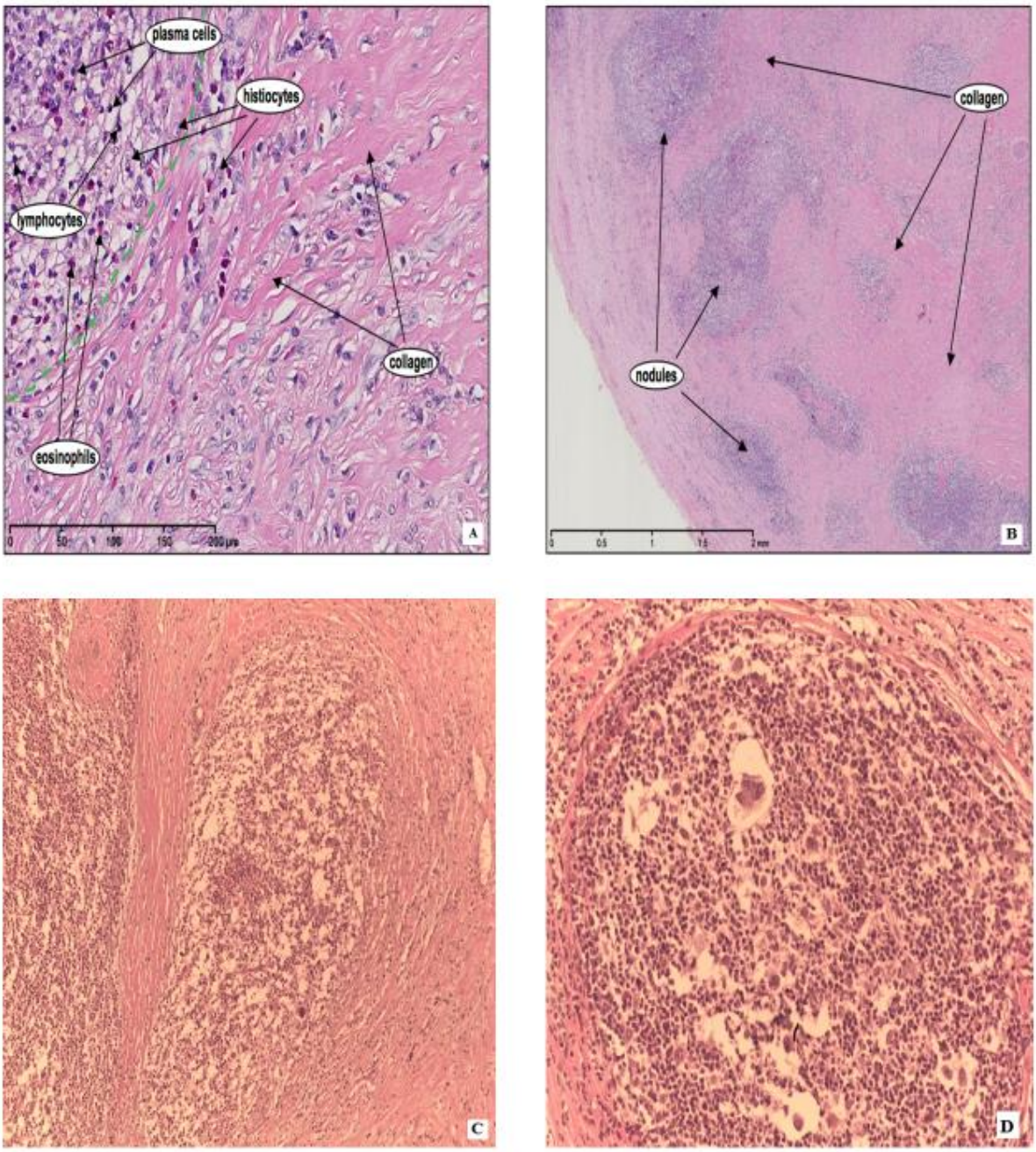

Figure 4.4: Hodgkin lymphoma, nodular sclerosis subtype. **A.** A low magnification H&E showing dense bands of collagen causing effacement of lymph node architecture, and formation of distinct nodules. **B.** High magnification H&E showing dense collagen bands and the mixture of cells within the nodules (Pathopedia, 2017). **C.** and **D.** A nodular sclerosis case from our study.

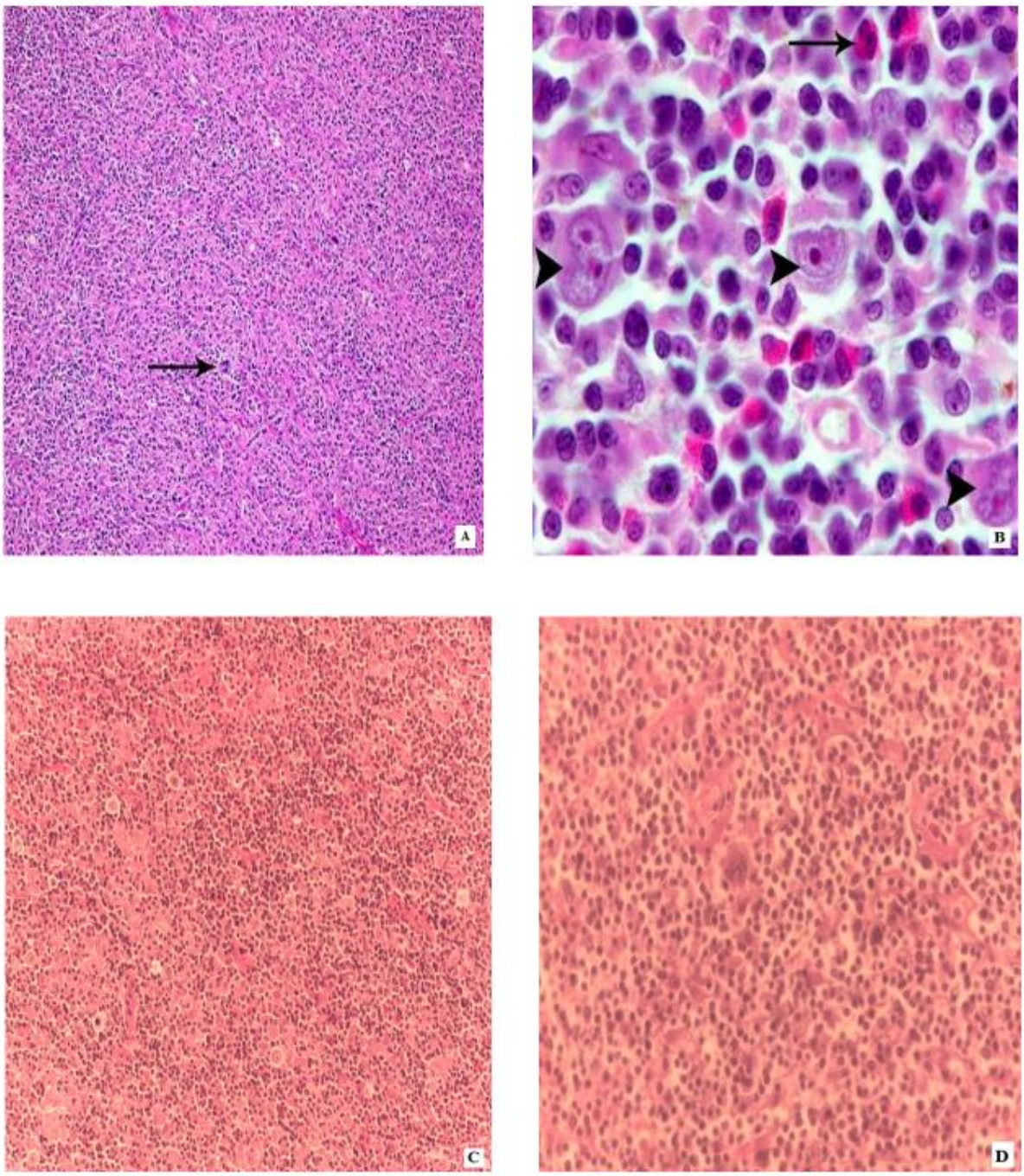

Figure 4.5: Hodgkin lymphoma, mixed cellularity subtype. **A.** A mixed inflammatory cell infiltrate population of small lymphocytes, histocytes, plasma cells, and eosinophils in varying proportions, in addition to mononucleated and multinucleated large HRS cells (arrow). **B.** A mixed inflammatory background composed of small lymphocytes, eosinophils (long arrow), histocytes, and scattered HRS cells (arrowheads) (Pathopedia, 2017). **C.** and **D.** A mixed cellularity case from our study.

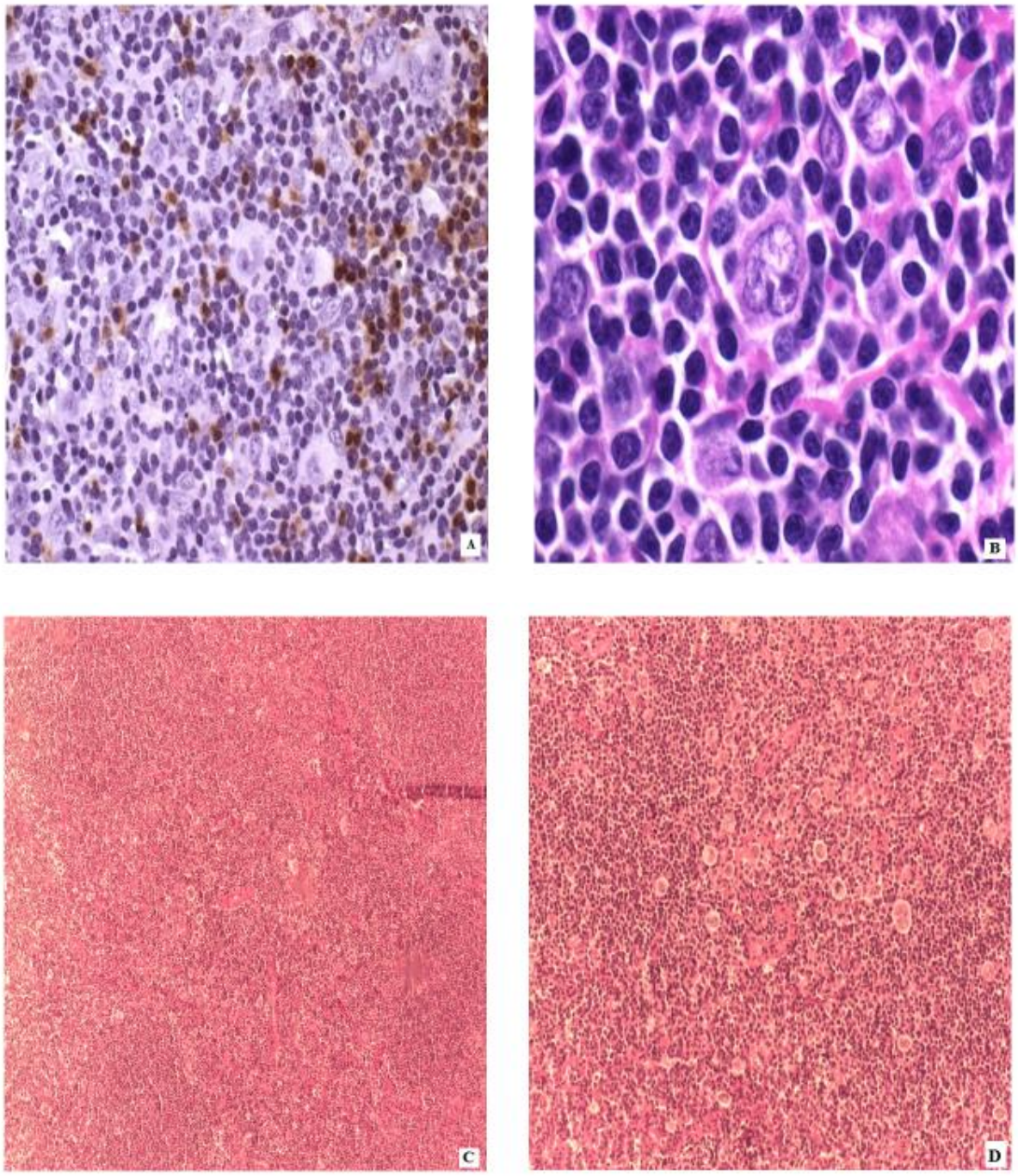

Figure 4.6: Hodgkin lymphoma, lymphocyte rich subtype. **A.** Numerous mature-looking lymphocytes surround scattered, large, pale-staining lymphocytic and histocytes cells. **B.** In this tissue, it is difficult to find typical HRS cells and instead lymphohistiocytic popcorn cells with large folded nucleus may be seen (Pathopedia, 2017). **C.** and **D.** A lymphocyte rich subtype case from our study.

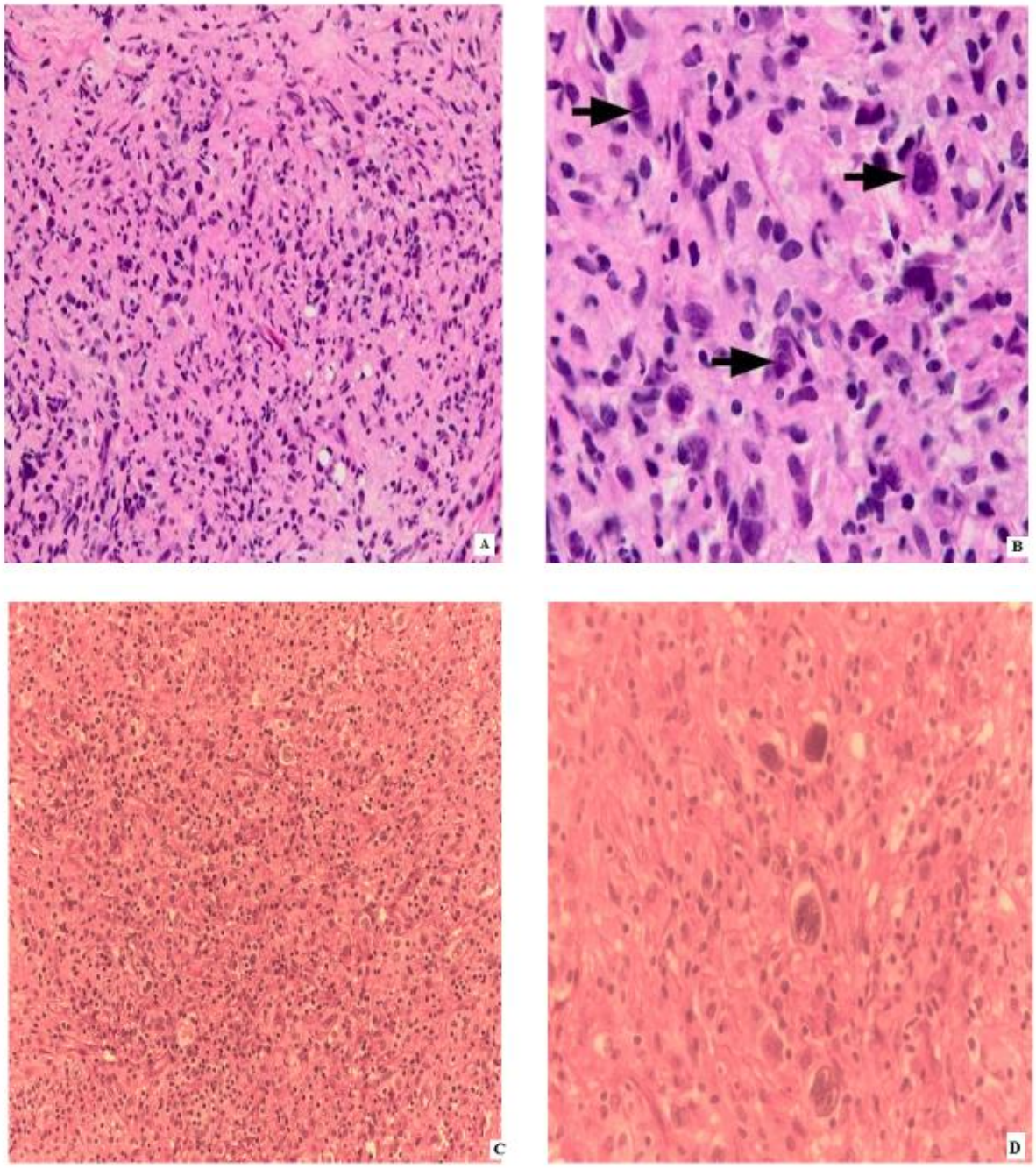

Figure 4.7: Hodgkin lymphoma, lymphocyte depleted subtype. **A.** Depletion of lymphocytes and abundance of HRS cells. **B.** Multiple large HRS cells in a sparse background of small lymphocytes (Pathopedia, 2017). **C.** and **D.** A lymphocyte depleted subtype case from our study.

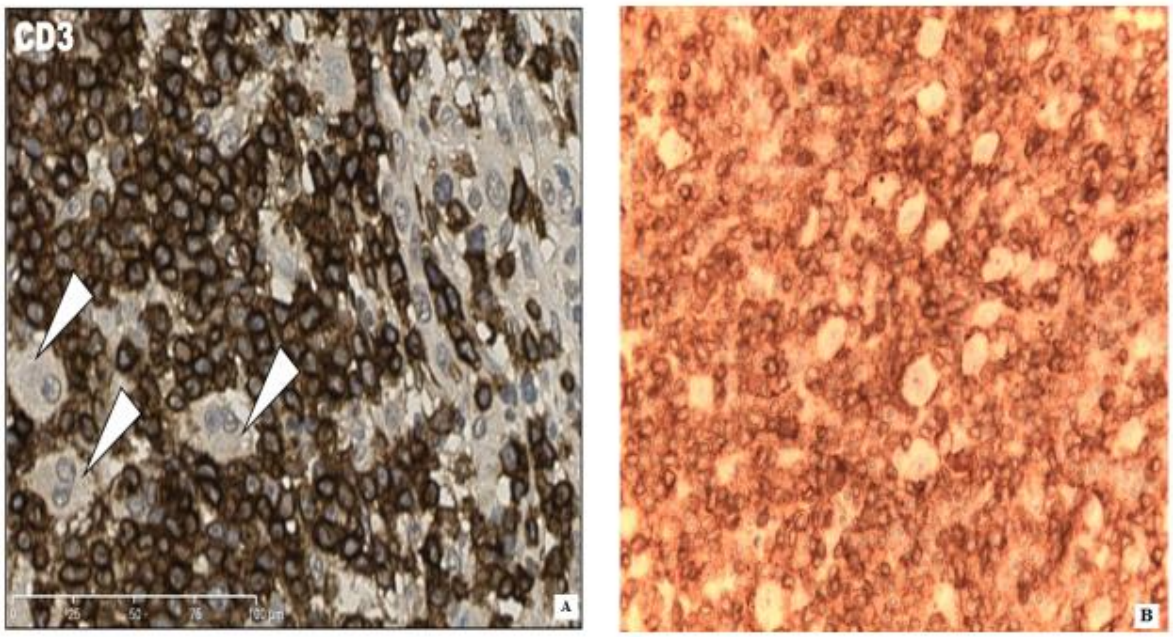

Figure 4.8: IHC staining for CD3 **A.** High magnification IHC CD3 showing that hugging "rosette" pattern of T-cells around the large irregular CD3-negative cells (arrowheads) (Pathopedia, 2017). **B.** CD3 negative in our study.

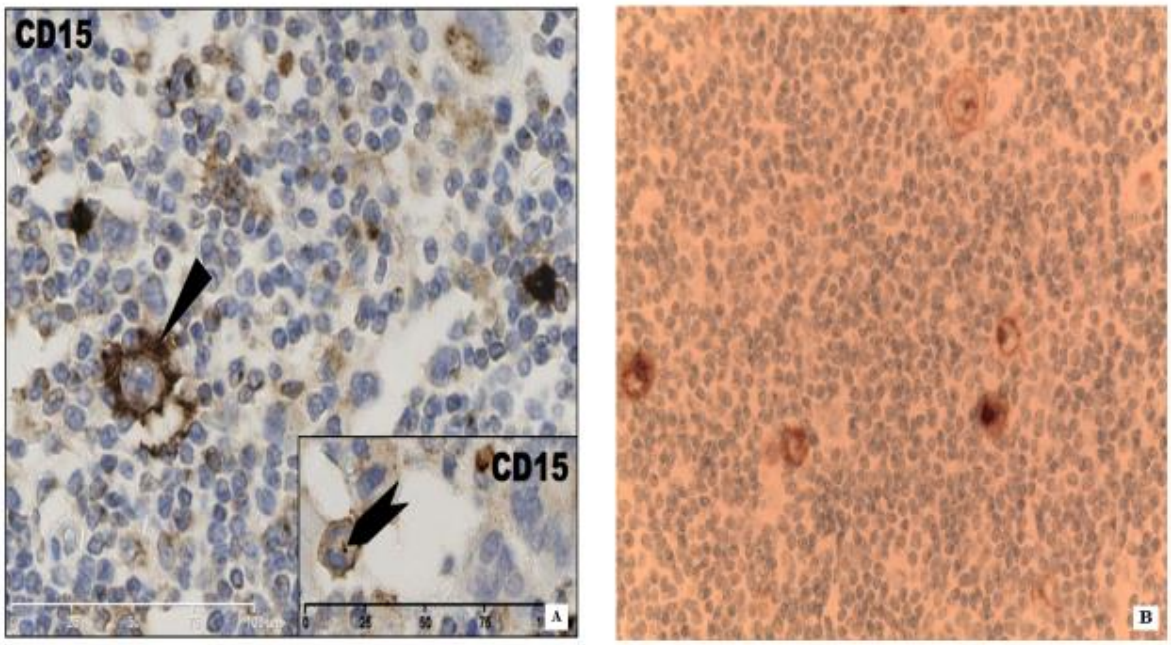

Figure 4.9: IHC staining for CD15 **A.** High magnification IHC CD15 demonstrating the cell membrane positivity in the large cells, and the dark dot-staining of the paranuclear Golgi apparatus (Pathopedia, 2017). **B.** CD15 positive in our study.

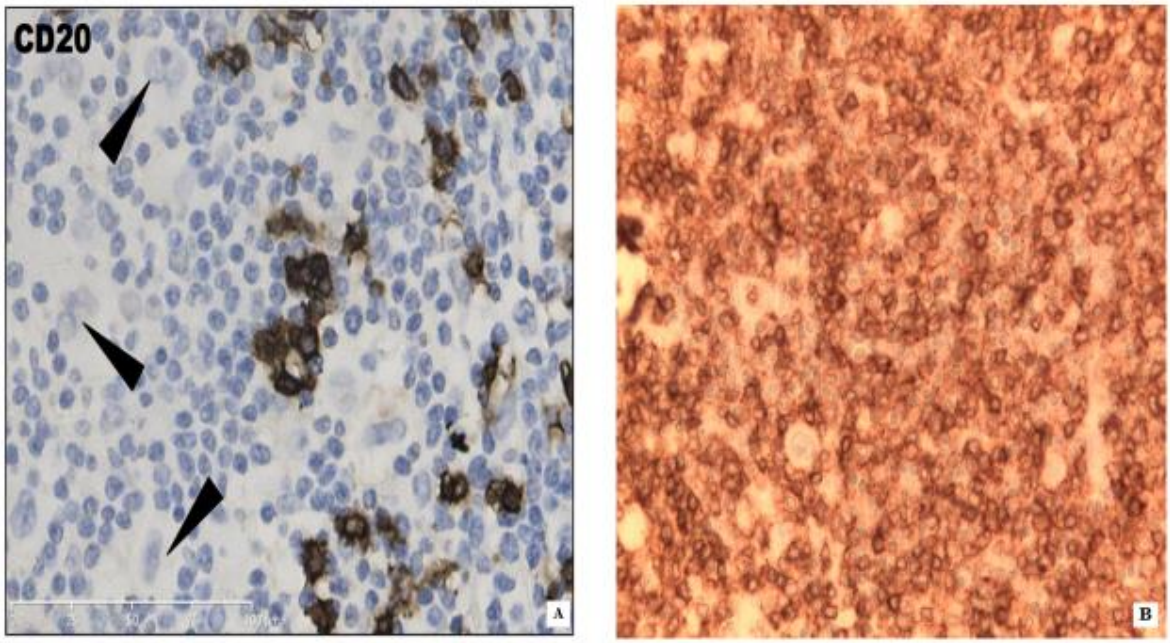

Figure 4.10: IHC staining for CD20 **A.** High magnification IHC CD20 demonstrating CD20-negative large irregular cells (arrowheads) (Pathopedia, 2017). **B.** CD20 negative in our study.

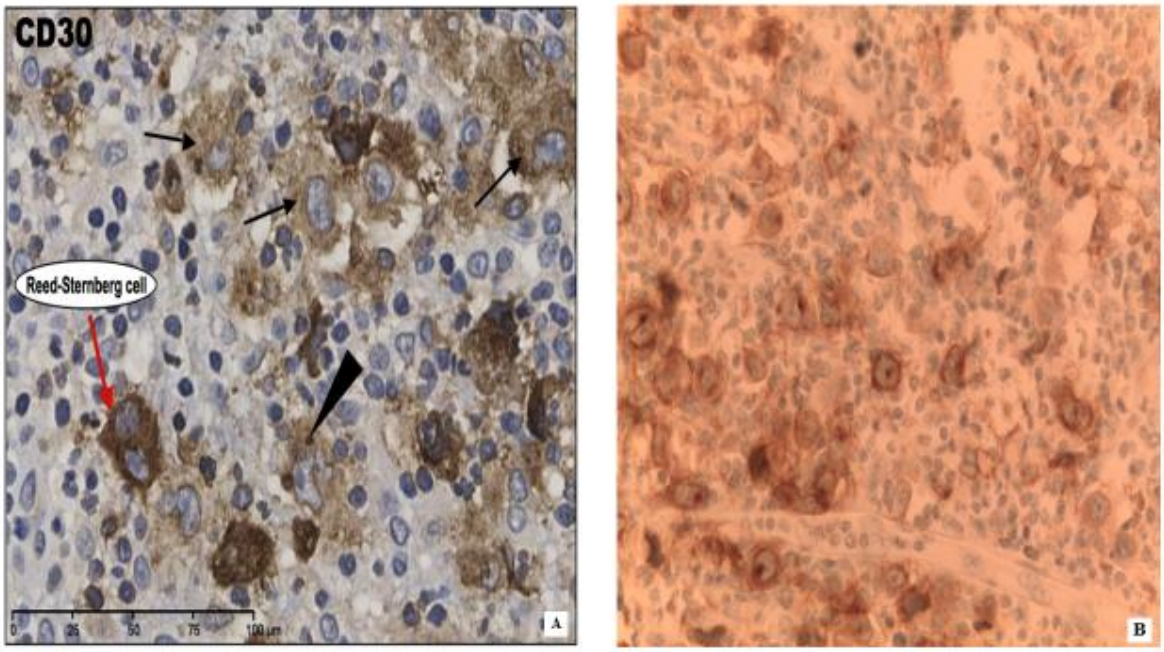

Figure 4.11: IHC staining for CD30 **A.** High power IHC CD30 demonstrates positivity in the Reed-Sternberg cells and mononuclear Hodgkin cells (arrows). CD30 has a characteristic staining pattern in large-cell membranes, and the Golgi apparatus – the dark paranuclear dot (arrowhead) (Pathopedia, 2017). **B.** CD30 positive in our study.

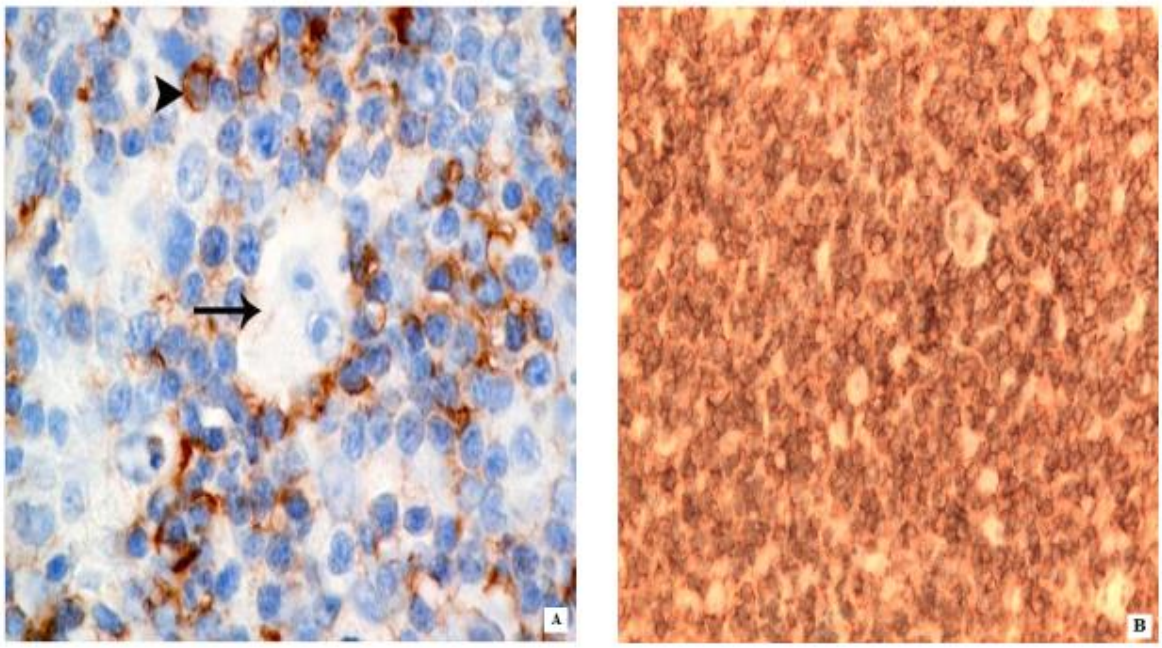

Figure 4.12: IHC staining for LCA **A.** LCA is negative. The surrounding reactive small lymphocytes are positive for LCA and may result in a false impression of HRS cells being positive for LCA (Pathopedia, 2017). **B.** LCA negative in our study.

4.2 Results of the retrospective-cohort study

This part was performed to confirm HL diagnosis in the 30 blocks by IHC using the following antibody panel: H&E, CD3, CD15, CD20, CD30, and LCA. Results obtained are described in table 4.2. Regarding subtype distribution, it is detailed in table 4.3.

Table 4.2: IHC results for the 30 paraffin-embedded blocks retrieved in our study (n=30).

CD marker	**CD3**	**CD15**	**CD20**	**CD30**	**LCA**
Positive n (%)	0 (0.0)	26 (86.7)	2 (6.7)	29 (96.7)	0 (0.0)
Negative n (%)	30 (100)	4 (13.3)	28 (93.3)	1 (3.3)	30 (100.0)
Total n (%)	**30 (100)**	**30 (100)**	**30 (100)**	**30 (100)**	**30 (100)**

Table 4.3: Subtype distribution among paraffin-embedded blocks retrieved in our study (n=30).

Subtype	**N**	**%**
Nodular sclerosis	15	50.0
Mixed cellularity	7	23.3
Lymphocyte rich	1	3.3
Lymphocyte depleted	1	3.3
Missing	6	20.0
Total	**30**	**100**

Moreover, in our specific part for investigating the prevalence of EBV among these cases, we used LMP1 antibody in immunohistochemistry to detect EBV positivity, where EBV was found to be positive in 33.3% of cases (Figure 4.13). Positive cases were distributed between mixed cellularity and nodular sclerosis subtypes, whereas 50% (n=5) of the positive results were mixed cellularity and 40% (n=4) were of the nodular sclerosis subtype.

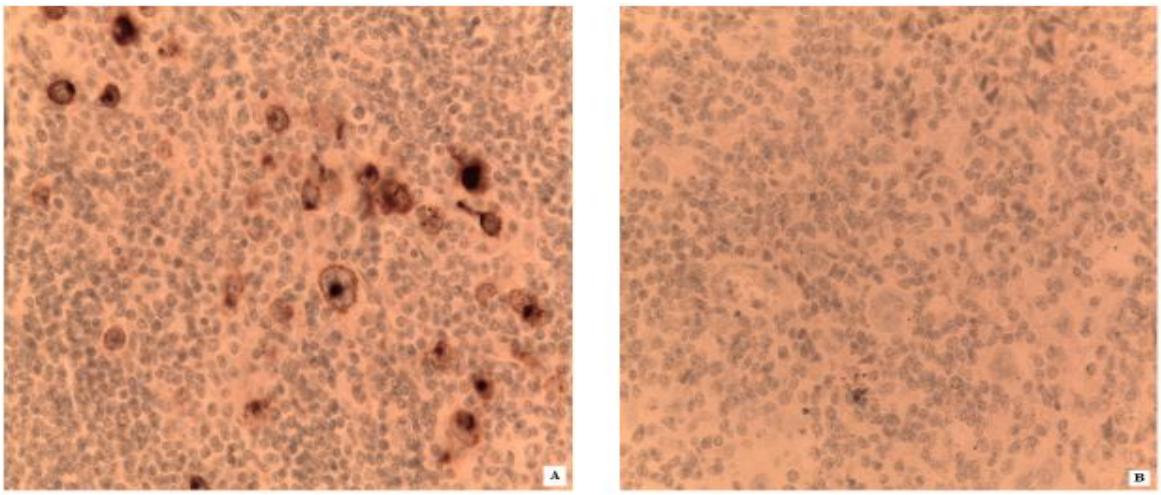

Figure 4.13: IHC staining for LMP1 **A.** Positive LMP1 immunohistochemical staining in HL case. **B.** Negative LMP1 immunohistochemical staining in HL case.

4.3 Results of the case-control study

In this part of the study the risk factors of HL were investigated through a case-control study of 63 incident HL cases and 85 cancer-free controls. Cases and controls were distributed by recruitment center as shown in table 4.4. The majority of cases (66.7%) were recruited from Beit-Jala Hospital, 31.7% from AVH, and 1.6% from Rafidia Hospital. As for the controls, 89.0% were recruited through MOH primary healthcare centers distributed all over the West Bank, the rest were recruited through the participating hospitals (AVH, and Beit-Jala Hospital) and Al-Makassed Blood Bank (Table 4.4).

Regarding the sociodemographic characteristics of the study population, the median age at recruitment of cases was 24 years (range: 4–73) and that of controls was 34 years (range: 2–74) years, while the mean age of the cases was almost 26±14 years and for controls it was about 37±19 years. The majority of cases were around 25 years old. Furthermore, most of the cases were from the south of the West Bank (66.1%) and 27.4% were from the middle. As for the educational level, the majority of cases (about 62%) have completed

their primary school. Regarding marital status, most of cases were single (52.4%) (Table 4.4).

Table 4.4: Distribution of HL cases and controls according to the center of recruitment and demographic characteristics.

Variable	Category	Controls (n=85) n (%)	Cases (n=63) n (%)
Recruitment center	AVH	4 (4.7)	20 (31.7)
	Rafidia	0 (0.0)	1 (1.6)
	Beit-Jala	3 (3.5)	42 (66.7)
	Al-Makassed Blood Bank	2 (2.4)	0 (0.0)
	MOH centers	76 (89.4)	0 (0.0)
Age at interview (years)	Mean ± SD	36.72 (± 18.69)	25.90 (± 13.78)
	Median (min – max)	34.0 (2.0 – 74.0)	24.0 (4.0 – 73.0)
	< 5	2 (2.4)	2 (3.2)
	5 – 9	2 (2.4)	4 (6.3)
	10 – 14	6 (7.1)	5 (7.9)
	15 – 19	8 (9.4)	10 (15.9)
	20 – 24	8 (9.4)	11 (17.5)
	25 – 29	11 (12.9)	12 (19.0)
	30 – 34	7 (8.2)	5 (7.9)
	35 – 39	8 (9.4)	6 (9.5)
	40 – 44	4 (4.7)	2 (3.2)
	45 – 49	1 (1.2)	1 (1.6)
	50 – 54	5 (5.9)	2 (3.2)
	55 – 64	13 (15.3)	2 (3.2)
	65+	10 (11.8)	1 (1.6)
Gender	Male	42 (49.4)	32 (50.8)
	Female	43 (50.6)	31 (49.2)
Marital status	Single	30 (35.3)	33 (52.4)
	Married/divorced/widowed	55 (64.7)	30 (47.6)
Regional distribution	North	7 (8.2)	2 (3.2)
	Middle	21 (24.7)	17 (27.4)
	South	54 (63.5)	41 (66.1)
	Gaza	0 (0.0)	1 (1.6)
	Other	3 (3.5)	1 (1.6)
Educational level (years of schooling)	Illiterate	5 (6.1)	3 (4.9)
	1 to ≤ 6	23 (28.0)	20 (32.8)
	>6 to ≤ 12	23 (28.0)	26 (42.6)
	Diploma/Bachelor	28 (34.1)	12 (19.7)
	Higher studies	3 (3.7)	0 (0.0)

4.3.1 Lifestyle factors and Hodgkin lymphoma

Breast feeding had almost no association with HL. In addition, performing regular physical activity was associated with lower risk of HL. The frequency and intensity of physical activity have been found to alter the association. Practicing physical activity once a week showed a protective association, whereas higher and lower frequencies were associated in

elevated risk. In addition, increasing the intensity of physical activity increased the risk of HL (Table 4.5).

Table 4.5: HL association with lifestyle factors.

Variable	**Category**	**Controls (n=85)**	**Cases (n=63)**	**OR* (95%CI)**
		n (%)	**n (%)**	
Breast feeding	No	6 (7.3)	7 (11.3)	1.00 (-)
	Yes	76 (92.7)	55 (88.7)	0.88 (0.24-3.22)
Practicing regular physical activity during the last 10 years	No	55 (64.7)	46 (73.0)	1.00 (-)
	Yes	30 (35.3)	17 (27.0)	0.73 (0.34-1.58)
Intensity of physical activity	None	57 (69.5)	49 (79.0)	1.00 (-)
	Intermediate	19 (23.2)	9 (14.5)	1.47 (0.38-5.77)
	Strenuous	6 (7.3)	7 (6.5)	1.26 (0.26-6.09)
Frequency of practice	None	54 (66.7)	46 (73.0)	1.00 (-)
	< 1 / week	3 (3.7)	1 (1.6)	1.37 (0.48-3.87)
	Once / week	2 (2.5)	3 (4.8)	0.55 (0.04-7.98)
	Twice / week	6 (7.4)	6 (9.5)	2.24 (0.29-17.21)
	≥ 3 / week	16 (19.8)	7 (11.1)	1.90 (0.42-8.64)

* OR is adjusted for age, gender, education, familial history, and region.

4.3.2 Medical history and Hodgkin lymphoma

Regarding health status in childhood, it was found that children who were more frequently sick were almost at the same risk of developing HL compared to healthy children who had a good health status. Furthermore, people who underwent tonsillectomy showed a 4.2 folds increased risk for developing HL. In addition, Atopy was associated with a slight lower risk of HL (Table 4.6).

Table 4.6: HL association with medical history.

Variable	**Category**	**Controls (n=85)**	**Cases (n=63)**	**OR* (95%CI)**
		n (%)	**n (%)**	
Childhood state of health	Healthy	60 (72.3)	45 (71.4)	1.00 (-)
	Sick	23 (27.7)	18 (28.6)	1.05 (0.48-2.28)
Atopy	No	58 (74.4)	47 (75.8)	1.00 (-)
	Yes	20 (25.6)	15 (24.2)	0.89 (0.39-2.04)
Tonsillectomy	No	82 (97.6)	57 (90.5)	1.00 (-)
	Yes	2 (2.4)	6 (9.5)	4.25 (0.72-25.01)

* OR is adjusted for age, gender, education, familial history, and region.

4.3.3 Proxies of infection and Hodgkin lymphoma

Regarding sibshipsize and birth order of cases, having more siblings was associated with increased risk of HL, and having six or more siblings increased the risk of HL by 3.1 folds compared to those having two or less siblings. Moreover, the later the birth order the lower the risk for HL. Meanwhile, having pets at home had a negative association with HL risk (Table 4.7).

Table 4.7: Proxies of infection and their association with HL.

Variable	**Category**	**Controls (n=85)**	**Cases (n=63)**	**OR* (95%CI)**
		n (%)	**n (%)**	
Number of siblings	≤ 2	7 (8.3)	3 (4.8)	1.00 (-)
	3 – 5	24 (28.6)	20 (31.7)	1.91 (0.37-9.86)
	6+	53 (63.1)	40 (63.5)	3.11 (0.63-15.34)
Birth order	1	10 (12.2)	12 (19.4)	1.00 (-)
	2 – 3	25 (30.5)	21 (33.9)	0.59 (0.19-1.85)
	4+	47 (57.3)	29 (46.8)	0.43 (0.15-1.28)
Contact with pets or large animals	No	56 (65.9)	48 (76.2)	1.00 (-)
	Yes	29 (34.1)	15 (23.8)	0.48 (0.22-1.08)

* OR is adjusted for age, gender, education, familial history, and region.

4.3.4 Family history and Hodgkin lymphoma

Family history of cancer was studied and noted that the risk of HL increased by 4.6 folds when any of first degree relatives had cancer. If any second degree relative had a cancer or any hematopoietic disease, no significant associations were found nor in any first degree relative with hematopoietic disease (Table 4.8).

Table 4.8: Family history of cancer and the risk of HL.

Variable	**Category**	**Controls (n=85)**	**Cases (n=63)**	**OR* (95%CI)**
		n (%)	**n (%)**	
First degree relatives with cancer	No	70 (89.7)	55 (90.2)	1.00 (-)
	Yes	8 (10.3)	6 (9.8)	4.63 (0.70-30.46)
First degree relatives with hematopoietic disease	No	73 (93.6)	59 (96.7)	1.00 (-)
	Yes	5 (6.4)	2 (3.3)	0.72 (0.12-4.23)
Second degree relatives with cancer	No	46 (62.2)	40 (64.5)	1.00 (-)
	Yes	28 (37.8)	22 (35.5)	0.89 (0.36-2.17)
Second degree relatives with hematopoietic disease	No	61 (82.4)	54 (88.5)	1.00 (-)
	Yes	13 (17.6)	7 (11.5)	0.65 (0.19-2.23)

* OR is adjusted for age, gender, education, familial history, and region.

Chapter Five

Discussion, Conclusions, Limitations and Recommendations

This study was designed and conducted to mount and initiate a platform to study Hodgkin lymphoma in Palestine for the first time through assessing the disease characteristics, demographics, clinical and pathological aspects. In order to accomplish these objectives, a case-control study was conducted for pathologically confirmed Hodgkin lymphoma cases and cancer-free controls using a face to face interview-based questionnaire. In addition, this study examined EBV positivity among Hodgkin lymphoma cases and confirmed initial Hodgkin lymphoma diagnosis by conducting a retrospective-cohort study, where paraffin-embedded blocks of Hodgkin lymphoma cases were retrieved and IHC staining was performed. Furthermore, this chapter highlights, discusses and interprets the major findings obtained from the study. Moreover, conclusions, limitations, and recommendations are also included.

5.1 Discussion

5.1.1 Disease characteristics

Our study revealed that the male to female ratio was 1.25:1, which was almost similar to the worldwide distribution which also had a slight increase among males (Thomas *et al.*, 2002). Furthermore, previous studies reported a bimodal age distribution of HL with one peak around the age of 25 years and another after the age of 60 years (Sickinger *et al.*, 2015). On the other hand, Sader-Ghorra *et al.* reported one peak of age of occurrence of HL cases between 15 and 29 years(Sader-Ghorra *et al.*, 2014). Our findings were similar to those of the Western countries with a bimodal age distribution, though the peaks were in young children and young adults. This could be explained by the young age structure of the Palestinian population which shifts the peaks towards younger ages (UNFPA, 2017). On the other hand, most developing countries reported one enlarged peak. In Pakistan and India there was also one peak at the age of 30 ref, and it was at the age of 31 in Egypt (Sultan *et al.*, 2016).

Most of the cases, about two thirds (66.1%), were recruited from the south of the West Bank (including Hebron and Bethlehem), whereas only 27.4% were recruited from the middle (including Ramallah, Jericho, and Jerusalem). This might refer to the higher incidence of cancer in the south compared to the rest of the West Bank. According to the MOH annual report for the year 2016; 1,071 cancer cases were reported in Bethlehem and Hebron from a total of 2,536 cases reported all over the West Bank (MOH, 2017).

Regarding the subtype distribution, nodular sclerosis was the most common subtype (51.1%) among our cases population followed by mixed cellularity (39.1%), where lymphocyte rich and lymphocyte depleted were found in less frequencies, as well as NLPHL which was also found in very low frequencies. These findings are in agreement with the worldwide categorization and classification of HL where nodular sclerosis was found to be the most common subtype followed by mixed cellularity and other subtypes were reported in low frequencies (Hjalgrim, 2012; Thomas *et al.*, 2002). Furthermore, Fatima *et al.* also reported that nodular sclerosis was the most common subtype in the Western countries followed by mixed cellularity, while in Asian countries, mixed cellularity was the most common subtype (Fatima *et al.*, 2011). Moreover, in Kuwait, nodular sclerosis was the predominant subtype of CHL patients (58.9%), whereas mixed cellularity was the second most frequent subtype (25.9%) (Alshemmari *et al.*, 2011).

Immunohistochemistry is known to be an effective tool for the diagnosis and sub-classification of hematolymphoid neoplasms (Ferry, 2017). Moreover, according to the WHO classification in 2008, HRS cells in most cases of HL are positive for CD30 and CD15 (Carbone *et al.*, 1992; Chuang, 2017). In our study we used IHC to confirm the initial diagnosis of HL that was reported in the pathology reports of the patients which were obtained from their files. The IHC panel used was an international HL diagnosis panel including CD3-, CD15+, CD20-, CD30+, and LCA- (Chang *et al.*, 2004; Lynnhtun *et al.*, 2014). Our results of the IHC profile were compatible with the international panel of HL diagnosis, thus HL diagnosis was confirmed as it was matching between initial and subsequent diagnosis of HL cases indicating the validation of the diagnosis retrospectively obtained from HL pathology reports.

5.1.2 EBV positivity among HL cases

Regarding EBV positivity among HL cases which was examined by IHC using LMP1 antibody, our results showed that 33.3% of HL cases were EBV positive. Our results were compatible with the global EBV prevalence among HL cases which was found to be 40% of all HL cases (Weinreb *et al.*, 1996). Furthermore, our findings were also close to that reported in Israel in 1997 (30%) (Benharroch *et al.*, 1997). Meanwhile, lower rates were noticed in African American children (17%) (Weinreb *et al.*, 1996), while higher rates were found among Caucasian (46%), Hispanic (86%) and Asian (93%) (Kennedy-Nasser *et al.*, 2011). Other studies reported variable rates in Kenya (92%), China (65%), Greece (90%), Turkey (61.5%), Egypt (50%), and Italy 48% (Adelusola *et al.*, 2009; Kennedy-Nasser *et al.*, 2011; Salati *et al.*, 2014).

EBV positive HL cases were distributed between nodular sclerosis (40%) and mixed cellularity (50%) with a predominance of mixed cellularity. This was compatible with other studies which reported that EBV is infrequently found in nodular sclerosis HL, rarely found in NLPHL, never found in lymphocyte rich HL, and is more commonly associated with mixed cellularity HL and lymphocyte depleted HL (Grywalska & Rolinski, 2015). In Israel 45% of EBV positive HL cases were of the mixed cellularity subtype where nodular sclerosis formed 21% of the positive cases (Benharroch *et al.*, 1997).

5.1.3 Lifestyle factors and Hodgkin lymphoma

Breastfeeding affects cancer, cardiovascular diseases, diabetes mellitus, and obesity. In addition, it reduces episodes of diarrhea, and decreases the incidence of infections. According to a case-control study conducted in Israel between 2011 and 2013 to examine the association between breastfeeding and the risk of developing childhood leukemia and lymphoma; it was found that the risk for developing either childhood leukemia or lymphoma was 64% decreased in children who had breastfeeding (Amitay *et al.*, 2016). Furthermore, many studies examined the association between breastfeeding and HL development, while some reported protective effects of the mothers' milk against malignancies and HL in particular (Ladomenou *et al.*, 2010), others reported no association between breastfeeding and childhood HL (Wang *et al.*, 2013). Similarly, our findings found no association between HL development and the history of breastfeeding.

Regarding sports and physical activity, few studies examined this association. In our study we found that regular physical activity was associated with a decreased risk of HL, whereas increased intensity and frequency of sports increased the risk. Opposite to our findings, a study investigating the association between physical activity and the risk of HL in women, reported that strenuous physical activity was associated with a decreased risk of HL (Keegan *et al.*, 2006). The protective effect of physical activity could be explained by its role in increasing the activity of natural killer cells, reducing inflammation, and decreasing the insulin and insulin-like growth factors which results in the stimulation of the cell turnover and inhibition of the cell death (Keegan *et al.*, 2006; Vermaete *et al.*, 2013). Controversially, a meta-analysis of 15 cohort studies and 8 case-controls studies showed that the association between physical activity and risk of HL was weak (Jochem *et al.*, 2014).

5.1.4 Medical history and Hodgkin lymphoma

Our study reported a 4.2 folds increased risk for developing HL in people who underwent tonsillectomy. However, tonsillectomy is considered to be an indicator for tonsillitis, and is thought to have a role in the development of HL. This role comes from the hypothesis of the 'lymphoid tissue removal', which assists in the development of the disease. Similar to our results, a Swedish wide cohort study reported a 4 folds increase in the risk of HL for people who underwent tonsillectomy before the age of 12 years (Vestergaard *et al.*, 2010).

Immune dysregulation, such as atopic diseases including asthma, allergy, atopic dermatitis and eczema are also related to lymphoma. Most studies have described asthma to be associated with reduced risk in the development of hematological malignancies. These results can be explained by the immune surveillance hypothesis, which suggested that allergy and other atopic diseases can enhance the immune system to detect and eliminate malignant cells, thus protect against malignancies (Soderberg *et al.*, 2006). In consistent with that, our results showed a slight decrease in the risk of HL among patients developing atopies. This protective association could be attributed to the enhancement of the immune system against atopic reactions which can also fight against cancer-associated antigens (Vajdic *et al.*, 2009).

5.1.5 Proxies of infection and Hodgkin lymphoma

Having pets at home was associated with a decreased risk of HL in this study subjects, which could be related to the early infection hypothesis, which assumes that early exposure to infection and immune system modulation can be a protective tool against childhood leukemia and Hodgkin lymphoma development (Altieri *et al.*, 2006; Rudant *et al.*, 2011). This was also reported in a case-control study in Sardinia, Italy where the authors suggested that early-life exposure to pets, birds and essentially chickens might be associated with a reduction in the risk of lymphoma (Bellizzi *et al.*, 2011).

Number of siblings can be considered as one of the proxies of infectious etiologies of HL, since environment and infections play a major role in the etiology of HL (Chang *et al.*, 2004; Westergaard *et al.*, 1997). In agreement with this, our study cases with 6 or more siblings had almost 3 folds increased risk for HL development compared to patients with 2 or less siblings. When number of siblings is increased, exposure level to infections coming from other siblings is increased (Chang *et al.*, 2004). Moreover, two small studies, one from Portugal (Sobrinho-Simoes & Areias, 1978) and another from Israel (Bogger-Goren *et al.*, 1983); that investigated the importance of family structure in developing HL in children, found that HL cases were mainly originated from big families. Meanwhile, a study in Brazil (Kirchhoff *et al.*, 1980), and another hospital-based case-control study in Italy (Serraino *et al.*, 1991); suggested a positive association between HL risk and high educational level or economic level, which are both related to a small family size (Chang *et al.*, 2004; Westergaard *et al.*, 1997).

Moreover, we found that the later the birth order; the lower the risk for HL. Being the fourth child or more was associated with lower risk of HL. A later birth order may reflect an earlier exposure to infections from older siblings whom can get infections easily from their classmates at school (Chang *et al.*, 2004; Westergaard *et al.*, 1997). Our results were inconsistent with the infection hypothesis, which suggested that late or delayed exposure to common bacterial or viral infections in childhood is considered to be a risk factor for both leukemia and Hodgkin lymphoma (Altieri *et al.*, 2006).

5.1.6 Family history of cancer and Hodgkin lymphoma

In this study, the risk of HL increased 4.6 folds in patients with a first-degree relative diagnosed with cancer. This was clear in studies in France and England, which reported a 5.4 to 5.8 folds increase in the risk of HL in first and second-degree relatives respectively (Linabery *et al.*, 2015). Moreover, Gobbi *et al.* reported an increased risk of HL in patients with a positive family history of cancer in the first-degree relatives, where about 40% of HL patients had a first-degree relative with cancer (Gobbi *et al.*, 2013). According to other studies, the risk for developing HL increased three to nine-folds in a family members of HL affected patients (Cerhan & Slager, 2015; Thomas *et al.*, 2002).

5.2 Conclusions

In conclusion, this study highlighted different factors affecting HL development among the Palestinian population, including lifestyle factors, medical history, family history and proxies of infection. Furthermore, it described main features of HL among Palestinians.

5.3 Limitations

Our study is the first study investigating Hodgkin lymphoma among Palestinians, describing pathological, etiological and different features of Hodgkin lymphoma. We covered several aspects through conducting a face-to-face interview-based questionnaire, obtaining paraffin-embedded blocks, drawing blood samples, for further investigations. Despite that, the recall bias in a case-control study remains a worldwide problem. Nevertheless, self-reported instead of registered medical history has been also a potential source of bias, since the cases are more able to report the history of exposures and correlate it to the disease than controls.

Limited number of reported and registered Hodgkin lymphoma patients made the mission of ascertaining cases challenging. It took us a long period of time to identify, contact and further to convince the patients to participate in our study. Furthermore, we faced a difficulty in ascertaining cases because the information in the cancer registry in the West Bank is incomplete and patient's details may be missing. The computerized hospital information system (HIS) has been introduced into the Palestinian governmental hospitals in 2013. Nevertheless, this system considered only new cases, while data for older cases

haven't been entered, and as a consequence of this, it was hard and almost impossible to get any old data neither from HIS nor from the archived files.

Small sample size is considered to be a major limitation to our study including both, the case-control study and the retrospective-cohort study. A small sample size may reduce the power of the study and increase the margin of error. Moreover, this study was supposed to investigate Hodgkin lymphoma among Palestinians in the West Bank and Gaza Strip. Unfortunately, only the West Bank population was included except for one case from Gaza Strip that was recruited at AVH. Even recruiting cases from the West Bank was challenging due to the political issues and the difficulty to travel freely between the different districts.

Finally, although we limited our study to patients with confirmed histopathological diagnosis of HL, the possibility of misclassification of diagnosis and subtyping of the disease was not eliminated since the diagnosis is not based on the state-of-the-art immunohistochemistry. In this study, more than 60% of the cases were diagnosed without any type of immunostaining and for some of the other cases the panel was incomplete. Accurate diagnosis is not only important in studying HL, it is also crucial in planning the course of treatment. In addition, EBV has a prognostic value in the treatment and the follow-up of the patients, yet, it is not part of the screening tests in medical practice in Palestine.

5.4 Recommendations

- Further investigations with larger sample size are recommended.
- Genetic studies on the samples collected will be useful in highlighting the disease genetics and to know more about its etiology which is still unclear and controversial.
- Other environmental exposures shall be studied as well.
- MOH should improve the hospital information system and include all patients' data in the system.
- HL diagnosis should be based on state-of-the-art immunohistochemistry.

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Appendices

Appendix 3.1

Pathology Report

Patient Name: _____

Patient Code: _____

1. **Date of Diagnosis: ___/___ /___**
2. **Age at Diagnosis (years) : _____**
3. **Date of last follow up:____/____/_____**
4. **Hospital of diagnosis:**
   1. Augusta Victoria **2.** Nablus (National)
   3. Cancer Registry **4.** Beit Jala **5.** other:_____

5. **Histological diagnosis:**

1. DLBCL (large cell)	**6.** SLL	**11.** Mycosis fungoides
2. Follicular	**7.** Lymphoblastic	**12. NHL**
3. MALT	**8.** Low grade lymphoma	**13.** Hodgkin lymphoma
4. MANTLE	**9.** B-cell NHL	**14.** others:_____
5. Burkitt	**10.** T-cell lymphoma	

6. **Immunostain: A. T cell B. Bcell C. unspecified.**

1. IHC (P-Positive N- Negative) **2.**CD20 (P-Positive N- Negative) **3.**CD10 (P-Positive N- Negative) **4.**BCL6 (P-Positive N- Negative) **5.** BCL2 (P-Positive N- Negative) **6.** CD43(P-Positive N- Negative) **7.**CD79A (P-Positive N- Negative) **8.**CD5 (P-Positive N- Negative) **9.**CD23(P-Positive N- Negative) **10**.kappa (P-Positive N- Negative)	**11.**lambda (P-Positive N- Negative) **12.** CD22 (P-Positive N- Negative) **13.** CD19 (P-Positive N- Negative) **14.** CD30 (P-Positive N- Negative) **15.** CLA (P-Positive N- Negative) **16.** ALK (P-Positive N- Negative) **17.** CD3 (P-Positive N- Negative) **18.** CD2 (P-Positive N- Negative)

7. Site of biopsy:

1. Lymph Nodes (LN):	**3. Organs:**
1.1. Cervical LN 1.2. Axillary LN 1.3. Mediastinal & Hylum 1.4. Para aortic LN 1.5. Abdominal LN 1.6. Inguinal LN 1.7. Submandibular LN 1.8. Other LN:_____	3.1. Nasopharynx 3.2. Oropharynx 3.3. Thyroid 3.4. Lungs 3.5. Breast 3.6. Stomach 3.7. Colon 3.8. Small Intestine 3.9. Pancreas 3.10. testes 3.11. Ovaries 3.12. skin 3.13. Brain 3.14. Bone Marrow 3.15. Others organs: _____
2. Lymphoid Organs: 2.1. Tonsils 2.2. Spleen	

8. Spread of disease:

1. Nodal **2.** Extranodal **3.** Undefined

9. Stage :

1. **I** 2. **II** 3. **III** 4. **IV**

10. Presence of B-symptoms (fever, weight loss, night sweat)

1. Yes 2. No **3.** Unknown

11. Treatment received:

1. CHOP
2. Rituximab
3. Other Chemotherapy:_____
4. Radiotherapy
5. Surgery
6. Transplantation: 6.1.Autologus 6.2. Allogenic

12. LDH at diagnosis:_____

Appendix 3.2

English Study Questionnaire

Hodgkin Lymphoma

Interviewer name: _____ Code ☐ ☐

Date of Interview: ____/____/____

Time Started ____: ____ Finished at ____:____

Site of Interview: 1. Home 2. Hospital 3. Clinic 4. Others

Part I: Demographic Information

I would like to ask you about your sociodemographic information including your marital status, education, place of birth, and others

Q1) ID Number ☐☐☐☐☐☐☐☐☐

Q2) Interviewee Name: ☐

Q3) Gender: 1. Male ☐ 2. Female ☐

Q4) Date of Birth	Year				Month		Day	

Q5) Marital status:

1. Single ☐
2. First marriage ☐
3. Second marriage or more ☐
4. Divorced or separated ☐
5. Widowed ☐

Q6) How many births did you have? (including all living and dead) ☐

Q7) How many are alive? ☐

Q8) What were the causes of death

Q9) I would like to ask about the sex and birthdates of your children?

Child Number	Sex	Date of Birth Day/Month/Year
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

1. Male **2**. Female

Q10) How many siblings do you have?

Q11) What is your birth order in the family

Q12) What is your religion?

1. Muslim ☐
2. Jewish ☐
3. Christian ☐
4. others ☐

Q13) How many years did you complete in school?

Q14) Before kindergarten did you go to:

1. Day care
2. Nursery school
3. Baby sitter who takes care of more than one child
4. Baby sitter at home
5. Mother stayed home

Q15) Did you go to kindergarten?

1. Yes ☐ 2. No ☐

Q16) What is your highest diploma?

1. Never went to school☐	2. Partial Primary (< 6th grade)☐	3. Primary school completed ☐
4. Partial Secondary ☐	5. High school completed ☐	6. Diploma ☐
7. Bachelor degree ☐	8. Higher research degrees ☐	

(Primary school: 1st grade-6th grade, **Secondary school:** 7th grade-12th grade**)**

Q17) Did you receive technical training? (If no go to 20)

1. Yes ☐ 2. No ☐

Q18) How long did you train? _____

Q19) What was the profession that you trained for? _____

Q20) Where were you, your parents and grandparents born?

Relative	Country	City
Interviewee		
Mother		
Father		
Grandfather (father side)		
Grandmother (father side)		
Grandfather (mother side)		
Grandmother (mother side)		

Part II: Job Information

I would like to ask you about your previous jobs and about your current ones, what type of exposures did you have in that work? Please report if you switched positions within the same employer. Please report periods of unemployment, military services, and maternity leave *etc*.).

Q21) Are you currently employed?

1. Yes ☐ 2. No ☐

Q22) Before your illness, did you have a regular job?

1. Yes ☐ 2. No ☐

Occupation	Start time	Finish time	Breaks	Place	Exposures
1)What is your current occupation:					
2) What were your former jobs **a.**					
b.					
c.					
3) Were you ever occupied in one of the following?					

Exposures codes:

1. Pesticides **2.** Meat products **3.** Organic solvents **4.** Inorganic Solvents **5**. Gasoline **6**. UV radiation **7**.Cosmic radiation **8.** Ionizing radiation **9**. Electromagnetic radiation **10**. Infectious agents / microorganisms **11**. Animals **12**. Antibiotics **13.** Paints **14**. Hair dyes **15**. Asbestos **16.** Animal skin **17**. Glues **18**. Sunlight **19**. Medicines (pharmaceticals) **20** Flour dust **21** Cleaning materials **22**. Wood dust **23**.Others.

Occupation	Start time	Finish time	Breaks	Place	Exposures
1. Agriculture & gardening					
2. Teacher					
3. Textile					
4. Wood industry					
5. Flour workers					
6. Dry cleaning					
7. Chemical Industry					
8.Gasoline/ petroleum workers					
9. Lab technicians					
10. Health care provider a. doctors b. nurses c. physiotherapist					
11. Photo-imager					
12. Veterinary					
13. Air crew					
14. Butcher					
15. Hair dresser					
16. Asbestos worker					
17. Leather worker					
18. Construction Workers					
19. Cleaners					
20. House wives					
21. Others					

Exposures codes:

1. Pesticides **2.** Meat products **3.** Organic solvents **4.** Inorganic Solvents **5**. Gasoline **6**. UV radiation **7**.Cosmic radiation **8.** Ionizing radiation **9**. Electromagnetic radiation **10**. Infectious agents / microorganisms **11**. Animals **12**. Antibiotics **13.** Paints **14**. Hair dyes **15**. Asbestos **16.** Animal skin **17**. Glues **18**. Sunlight **19**. Medicines (pharmaceticals) **20** Flour dust **21** Cleaning materials **22**. Wood dust **23**.Others.

Part III: Housing

I would like to have some information about your current and previous residences

Q23) What type of residence have you lived in?

(Do not include residence of less than 3 years)

Addresses	**Type of settle - ment**	**House type**	**Which storey did you live on**	**Water source**	**# of persons residing in the house**	**# of rooms**	**Bath-room**	**From what year to what year?**
Current Residence								
Previous 1.								
2.								
3.								
4.								
5.								

- ***Type of settlement***: **1.** City >100000 persons **2.**Town 20000-99999 persons **3.**Small town 5000-19999 persons **4.** Village <5000 persons **5.** Agricultural settlement **6**.Private farm or rural dwelling **7**. Other.
- ***House type:*** **1.** A private house **2.** A multifamily (10 families) house **3.** An apartment building (>10 families) **4.** Tent **5.** Agricultural settlement.
- ***Storey:*** **1.** Ground floor **2.** Second floor **3.**Third floor **4**. Higher floor.
- ***Drinking water source:*** **1.** Pipes **2.** A well **3.** Cisterns **4.** Mineral water **5.** Don't know.
- ***Bathroom:*** **1.** Indoors **2.** Outdoors.

Part IV: Habits

I would like to ask you about some of your personal characteristics as your measurements, and other habits as smoking, hair dying, sun exposure, and your diet

Q24) What are your measurements?

Parameter	Measurement	Measurement (before 10yrs)
Height		
Weight		

1: The same **2**: Much higher **3:** Somewhat higher (<10%) **4**: Much lower **5:** Somewhat lower (<10%)

Q25) Have you ever smoked? (If never, go to Q31)

1. Cigarettes ☐
2. Nargilah ☐
3. Pipes ☐
4. Tobacco ☐
5. Never smoked ☐

Q26) Are you a smoker now?

1. Yes ☐ **2.** No ☐

Q27) Have you stopped smoking?

1. Yes ☐ **2.** No ☐

Q28) How many years did you smoke?

Q29) How many cigarettes do (did) you smoke per day?

Period of time	Number of cigarettes
Average of smoking before illness	
Current level of smoking	

1: less than 10 **2**: 11-20 **3**: 21-40 **4**: More than 40

Q30) What is the average of your smoking, before illness and currently?

Period if time	Nargilah	Pipes	Tobacco
Before illness			
Currently			

1: Everyday **2:** More than once/week **3:** Less than once/week

Q31) Did you ever dye your hair? (If No go to37)

1. Yes ☐ **2.** No ☐

Q32) Do you dye your hair regularly?

1. Yes ☐ **2.** No ☐

Q33) At what age did you begin to dye your hair? ☐

Q34) On average, how many times do you dye your hair?

1. Less than once/yr **2.** One-three times/yr **3.** Four-six times/yr **4**. More than seven times/yr

Q35) What colour do you use in general?

1. Black **2.** Brown **3**. Blonde **4.** Henna colour **5**. Others

Q36) Is the dye that you use artificial?

1. Yes ☐ **2.** No ☐

Q37) Did you ever have a severe sun burn in childhood?

1. Yes ☐ **2.** No ☐

Q38) How many hours per week do (did) you expose to sunlight outdoor not as part of your work but including your leisure time and your travelling to and from the work? ☐

Q39) When you are out of door, is your head covered?

1. Always **2**. Most of the times **3.** Sometimes **4.** Never

Q40) When you are out of door, do you wear long sleeves?

1. Always **2**. Most of the times **3.** Sometimes **4.** Never

Q41) Do you use sun screen when you go out in the sun

1. Always **2**. Most of the times **3.** Sometimes **4.** Never

Q42) Were you breast fed?

1. Yes ☐ **2.** No ☐ **3**. Don't know ☐

Q43) Have you ever been vegetarian? (If No, go to Q45)

1. Yes ☐ **2.** No ☐

Q44) How many years have you been vegetarian? ☐

Q45) Do you eat red meat regularly?

1. Yes ☐ **2.** No ☐

Q46) How many times a week do you eat red meat? ☐

Q47) Do you eat white meat regularly?

1. Yes ☐ **2.** No ☐

Q48) How many times a week do you eat white meat? ☐

Q49) How many fruits per day do you eat on average?

1: Zero **2**:1-3 **3**: 4-7 **4**: More than 7

Q50) How many vegetables do you eat per day on average?

1: Zero **2**:1-3 **3**: 4-7 4: More than 7

Q51) What kind of oil do you mainly cook with?

1: Olive **2:** Soya **3:** Canola **4:** Sunflower **5:** Other

Q52) How often do you usually eat or drink...? ***Please tick one box for each line***

	1) never	2) <1/wk	3) 1/wk	4) 2-4X/ week	5) 5-6X/ week	6) 1/day	7) >1/day	8) Number of servings per day
Fruits								
Vegetables								
Meat								
Chicken								
Fish								
Whole milk (3% fat or more)								
Reduced fat milk (<3% fat)								
Other milk products (like yogurt, cheese, chocolate milk, pudding								
Drink water								**___glasses**
Other non-alcoholic drinks (hot or cold)								**___glasses**
Alcoholic drinks								**___glasses**

Part V: Hobbies

We would like to ask you about your hobbies that you used to practice, like the physical activities, arts, and others.

Q53) During the last 10 years, have you practiced regular physical activity? (If not, go to Q57)

1. Yes ☐ 2. No ☐

Q54) What type of activity have you practiced?

1. Strenuous (like Jogging) ☐
2. Moderate (like walking) ☐
3. Light (like gardening) ☐

Q55) How often did you perform physical activity?

1. Three times a week or more ☐
2. Two times a week ☐
3. Once a week ☐
4. Less than this ☐

Q56) Did you practice any of the following physical activities, and how often?

#	Physical Activities	1. Don't do this activity	2. 2-3 times a month or more seldom	About once a week	2 times a week or more
1	Football, handball, basketball, tennis, hockey or other ball games				
2	Athletics, gymnastics				
3	Aerobics / fitness club exercise/Trade mill at home				
4	Jogging, running				
5	Karate, Judo taekwondo				
6	Wrestling				
7	Boxing/Kick boxing				
8	Weightlifting/Weight-training				
9	Dancing (disco, techno, folkdance, line dance, ballet)				
10	Camping				
11	Swimming				
12	Cycling				
13	Climbing				
14	Skateboarding, roller skating				
15	Hiking, fishing				
16	Water activities (sailing, surfing, water-skiing)				

Q57) Do you keep a garden as a hobby? (If not, go to Q66)

1. Yes ☐ 2. No ☐

Q58) What type of gardening do you perform?

1. Indoor ☐ 2. Outdoor ☐

Q59) How many years have you practiced gardening? ☐

Q60) How many hours per week did you practice gardening?

1. Less than 10 hours/week ☐
2. 10-20 hours/week ☐
3. More than 20 hours/week ☐

Q61) Do (did) you grow fruits and vegetables?

1. For your own use ☐
2. For sale ☐
3. Do not grow fruits and vegetables ☐

Q62) Do (did) you use pesticides? (If not, go to Q64)

1. Yes ☐ 2. No ☐

Q63) Do (did) you wear protective gloves and wearing when you use pesticides?

1. All the time 2. Most of the time 3. Sometime 4. Never

Q64) Do (did) you wash your hands after using pesticides?

1. All the time 2. Most of the time 3. Sometime 4. Never

Q65) Your pesticides are (were) against:

1. Weeds ☐ 2. Insects ☐ 3. Fungus ☐ 4. Don't know ☐

Q66) Do (did) you spray insecticides in your house?

1. ≥1time/week 2. <1 time/week-1time/month 3. Few times/year 4. Never

Q67) Do you remember the name of the pesticide(s) whether being used in the house or in gardening? (If No go to 69)

1. Yes ☐ 2. No ☐

Q68) What is (are) the name of the pesticide(s) did you use?

Name of Pesticide

Q69) When you were a baby or a small child, did you go to the agricultural field with your parents or older siblings?

1. Yes ☐ 2. No ☐

Q70) Do (did) you practice art as a hobby? (If not, go to Q77)

1. Yes ☐ 2. No ☐

Q71) What type of art do (did) you practice?

1. Painting ☐
2. Sculpture ☐
3. Pottery and ceramics ☐
4. Glasswork ☐
5. Lithography and prints ☐
6. Iron work ☐
7. Model making ☐

Q72) In your hobbies were (are) you exposed to any of the following chemicals?

1. Oil paints
2. Acrylic paints
3. Other paints

` 4. Solvents (as turpentine, kerosene, glues, dust, lead) _____

Q73) How many years did you practice this art? ☐

Q74) At what age did you start practicing this art? ☐

Q75) At what age did you stop practicing this art? ☐

Q76) How many hours per week did you practice this art?

1. Less than 10 hours/week ☐
2. 10-20 hours/week ☐
3. More than 20 hours/week ☐

Q77) Do (did) you have other hobbies that involve the use of chemicals? (If not, go to Q82)

1. Yes ☐ 2. No ☐

Q78) What is this hobby? ☐
Q79) What type of chemical is involved in this hobby? ☐
Q80) At what age did you practice this hobby? ☐

Q81) How many hours per week do (did) you practice this hobby?

1. Less than 10 hours/week ☐
2. 10-20 hours/week ☐
3. More than 20 hours/week ☐

Part V: Health

Now I am going to ask you about your health

Q82) Have you ever suffered from diarrhoea lasting more than two days? (If not, go to Q84)

1. Yes ☐ 2. No ☐ 3. Don't remember

Q83) Did you have any serious diarrhoea from any of the following agents:

Causative agent	Number of times	When was your last infection
1. 1. Salmonella ☐		
2. 2. Shigella ☐		
3. 3. Campylobacter ☐		
4. 4. Yersinia ☐		
5. 5. Strongiloidosis ☐		
6. 6. Amebae ☐		
7. 7. Other parasitic infection ☐		
8. 8. E.coli ☐		
9. I was told it was a viral infection ☐		
10. They did not find the causative agent ☐		
11. They didn't check ☐		
12. Other ☐		

Q84) Did you have a serious infection that required hospitalization during infancy (before the first year of age)?

1. Yes 2. No

Q85) Did you ever have any other serious infections that required hospitalization (like pneumonia)? (If no go to Q88)

1. Yes 2. No

Q86) How many times were you hospitalized for infections and at what age?

Age	# of times	Type of infection
1. More than 40yrs		
2. 21-40yrs		
3. 11-20yrs		
4. 1-10yrs		
5. Less than 1yr		

Infection codes

1. Sinusitis **2.** Bronchitis **3.** Enteritis **4.** Gall bladder infection **5.** Urinary tract infection **6.** Prostatitis (men only) **7**.Anal infection **8**. Dermatitis **9.** Gynaecologic infection (women only) **10.** Meningitis **11.** Appendicitis **12**. Other

Q87) Apart from infections requiring hospitalization, did you suffer from any of the following disease(s)? If yes, when?

Disease	Yes	No	Don't remember	Age
1. Hepatitis 0				
2. Hepatitis A				
3. Hepatitis B				
4. Hepatitis C				
5. Herpes: lips, nose, ear, other				
6. Infectious Mononucleosis				
7. Asthma				
8. Eczema				
9. Tonsillitis				
10. Measles				
11. Mumps				
12. Rubella				
13. Rheumatic fever				
14. Arthritis				
15. Tuberculosis				
16. Brucellosis				
17. Sinusitis				
18. Enteritis				
19. Polio				
20. Typhus				
21. Ulcer				
22. Allergy				
23. Other				

Infection time code:

1. More than 40yrs **2**. 21-40yrs **3**. > 11-20yrs **4**. 1-10yrs **5**. Less than

Q88) Did you receive vaccinations to the following microorganisms?

Disease	Yes	No	Don't remember	Age of the first vaccination	Age of the last vaccination
1. Tetanus					
2. Small Pox					
3. Typhoid					
4. Measles					
5. Mumps					
6. Rubella					
7. Whooping cough					
8. Polio injection					
9. Polio drinking					
10. TB/BCG					
11. Yellow Fever					
12. Viral meningitis					
13. Cholera					
14. Hepatitis A					
15. Hepatitis B					
16. Haemophilus					
17. Pneumococcus					
18. Influenza					
19. others					

Q89) Did you undergo tonsillectomy? (If not, go to Q91)

1. Yes ☐ 2. No ☐

Q90) At what age? ☐

Q91) Were (have) you ever administered antibiotics? (If not, go to Q93)

1. Yes ☐ 2. No ☐ 3. Don't know ☐

Q92) On average, how many times per year were you administered antibiotics and at what age?

Age	# of times
1. More than 40yrs	
2. 21-40yrs	
3. 11-20yrs	
4. 1-10yrs	
5. Less than 1yr	

Q93) Did you ever have an X-ray?

1. Yes ☐ 2. No ☐ 3. Don't remember

Q94) Why did you perform an X-ray?

X-ray	# of times	Age
1. Dental x-rays		
2. Chest x-rays		
3. Mammography (women)		
4. Bone x-rays		
5. Other		

1. >40yrs **2**. 21-40yrs **3**. > 11-20yrs **4**. 1-10yrs **5.** Less than 1yr

Q95) Which one of the following sentences describes your childhood the best up to 18?

1. I was sick more often than my friends ☐
2. I was away from school more than my friends ☐
3. I got more medications than my brothers and sisters ☐
4. I was a healthy child other than the normal childhood diseases ☐
5. I was sick much less often than my siblings and friends

Q96) Did you have pets or large animals at home or on the grounds of your home? (If not, go to Q98)

1. Yes ☐ 2. No ☐

Q97) What type of animal (do) did you have?

1. Cat ☐
2. Dog ☐
3. Bird ☐
4. Horse ☐
5. Cow ☐
6. Camel ☐
7. Goat ☐
8. Sheep ☐
9. Donkey ☐
10. Pig ☐
11. Others ☐

Q98) Have (were) you ever prescribed any of the following medications? If yes, at what age and how many times?

1. Yes ☐ 2. No ☐ 3. Don't Know ☐

Medication	Never	Occasional <1/wk	Regular	
			Year started	Year ended
1. Steroids				
2. Contraceptives				
3. Hormone replacement therapy				
4. Other hormones				
5. Antifungal (oral)				
6. Non-steroidal anti-inflammatory				
7. Paracetamols				
8. Antidepressants				
9. Anti-parasitic				
10. Anti-anxiety				
11. Antiviral				
12. Antihistamines				
13. B-Blockers				
14. Diuretics				
15. Anti-hypertensive drugs				
16. Thyroid replacement				
17. Anticoagulants				
18. Aspirin				
19. Chemotherapy				
20. Others				

Q99) Were you ever transfused with blood?

1. Yes ☐ 2. No ☐ 3. Don't know ☐

Q100) Prior to your current illness, did you ever have cancer? (If not, go to Q102)

1. Yes ☐ 2. No ☐

Q101) What was the treatment you received?

1. Chemotherapy ☐
2. Surgery ☐
3. Radiotherapy ☐
4. Don't know ☐

Q102) Did any of your first degree relatives have cancer? If yes, what was the cancer type and who was that?

1. Yes ☐ 2. No ☐ 3. Don't Know ☐

Cancer type	Siblings	Mother	Father	Child 1	Child 2	Child 3
1. Any Cancer						
2. Non Hodgkin's Lymphoma						
3. Hodgkin's Lymphoma						
4. CLL						
5. ALL						
6. Multiple Myeloma						
7. Acute Myeloid Leukaemia (AML)						
8. CML						
9. Blood cancer						
10. Other blood problems						

Q103) Did any of your second degree relatives have cancer? If yes, what was the cancer type and who was that?

1. Yes ☐ 2. No ☐ 3. Don't Know ☐

Cancer type	GM/ m	GF/ m	GM/ f	GF/ f	Uncles	aunts	cousins/ nephew	Nieces
1. Any Cancer								
2. Non Hodgkin's Lymphoma								
3. Hodgkin's Lymphoma								
4. CLL								
5. ALL								
6. Multiple Myeloma								
7. Acute Myeloid Leukaemia (AML)								
8. CML								
9. Blood cancer								
10. Other blood problems								

GM (m): grandmother on mother's side
GM (f): grandmother on father's side
GF (m): grandfather on mother's side
GF (f): grandfather on father's side

Q104) Did any of your first degree relatives suffer from any of the following diseases? (If yes, who was that?)

1. Yes ☐ 2. No ☐ 3. Don't Know ☐

Disease	Siblings	Mother	Father	Child 1	Child 2	Child 3
1. Frequent Infection						
2. Allergy						
3. Rheumatoid Arthritis						
4. Autoimmune diseases						
5. Other immune problems						

Q105) Did any of your second degree relatives suffered from any of the following diseases? (If yes, who was that?)

1. Yes☐ 2. No☐ 3. Don't Know☐

Disease	GM/ m	GF/ m	GM /f	GF/ f	Uncle s	aunts	cousins/ nephew	Nieces
1. Frequent Infection								
2. Allergy								
3. Arthritis								
4. Autoimmune diseases								
5. Other immune problems								

GM (m): grandmother on mother's side
GF (m): grandfather on mother's side
GM (f): grandmother on father's side
GF (f): grandfather on father's side

Q106) How often do you go to the dentist?

1. For regular check-ups (at least once a year) ☐
2. For regular check-ups (less than once a year) ☐
3. Only when I have a toothache or other problem ☐
4. Never ☐

Q107) Do you own a car?

1. Yes ☐ 2. No ☐

Q108) How did you get to the hospital today?

1. Walk ☐ 2. Private car ☐ 3. Taxi ☐ 4. Public Transportation ☐ 5. Other ☐

Q109) When is your next visit? ☐

Thank you very much for you co-operation.

Q110) Interviewer rating of interview

1. Highly reliable ☐
2. Somewhat reliable ☐
3. Somewhat unreliable ☐
4. Unreliable ☐

Appendix 3.3

Arabic Study Questionnaire

الورم الليمفاوي الهودجكن

الشخص الذي أجرى المقابلة: _____

كود الشخص الذي أجرى المقابلة: _____

هل تم؟ **توقيع الموافقة عن علم للمشاركة** ☐

إلصاق رقم الشخص المشارك على الاستبيان ☐

إلصاق رقم الشخص المشارك على أنابيب الدم ☐

إلصاق رقم الشخص المشارك على الاستبيان الباثولوجي ☐

سحب أنبوب أحمر وأنبوب بنفسجي ☐

اسم الشخص المشارك: _____

رقم الشخص المشارك: _____

رقم الهاتف: _____

رقم الخلوي: _____

اسم الطبيب المعالج: _____

معلومات المقابلة:

تاريخ المقابلة: ____/____/____

وقت بداية المقابلة _____:_____

وقت نهاية المقابلة_____:_____

مكان المقابلة

1. **المنزل** ☐
2. **المستشفى** ☐ _____
3. **العيادة** ☐
4. **في مكان أخر** ☐ _____

القسم الأول: المعلومات السكانية

للمجموعة الضابطة فقط:

هل أنت مرافق (لمريض اللمفوما / لمريض أخر)؟ _____

ما هي صلة قرابتك للمريض؟ _____

أود أن أسألك حول معلوماتك الديموغرافية والتي تتضمن الحالة الاجتماعية، التعليم، مكان الولادة و معلومات أخرى.

س 1) رقم الشخص المشارك

س 2) الأحرف الأولى من اسم الشخص المشارك

س 3) الجنس: ☐ **1 .ذكر** ☐**2.أنثى**

س 4) تاريخ الميلاد	اليوم		الشهر		السنة			

س 5) الحالة الاجتماعية:

1. أعزب
2. متزوج لمرة واحدة
3. متزوج لمرتين أو أكثر
4. مطلق أو منفصل
5. أرمل

س 6) كم مولود لديك؟ (يتضمن الأحياء منهم والمتوفون و لا يشمل الإجهاض)

س 7) كم عدد الأحياء؟

س 8) ما هي أسباب الوفاة؟

_____

_____

_____

س 9) أود أن أسألك حول تواريخ ميلاد أطفالك وجنسهم؟

رقم الطفل	الجنس	تاريخ الميلاد		
		يوم	شهر	سنة
1				
2				
3				
4				
5				
6				
7				
8				
9				

*** 1. ذكر 2. أنثى

س 10) كم عدد الأشقاء عندك؟

س 11) ما هو ترتيبك في العائلة؟

س 12) ما هو دينك؟

1. مسلم ☐
2. مسيحي ☐
3. أخر ☐

س 13) كم عدد سنوات الدراسة في المدرسة؟

س 14) قبل الروضة هل ذهبت إلى:

1. مركز الرعاية اليومية
2. الحضانة
3. حاضنة أطفال والتي تعتني بأكثر من طفل واحد
4. حاضنة أطفال في البيت
5. البقاء مع الأم في المنزل

س 15) هل ذهبت إلى الروضة؟

1. نعم **2.** لا

س 16) ما هي أعلى شهادة علمية حصلت عليها؟

1. لم أذهب إلى المدرسة ☐	2. أساسي جزئي (< الصف السادس) ☐	3. أكملت الدراسة الأساسية ☐
4. ثانوي جزئي ☐	5. أكملت الدراسة الثانوية ☐	6. دبلوم ☐
7. درجة البكالوريوس ☐	8. درجات عليا ☐	9. درجة أكاديمية جزئية ☐

المرحلة الأساسية: الصف الأول – الصف السادس **المرحلة الثانوية**: الصف السابع – الصف الثاني عشر

س17) هل تلقيت تدريبا تقنيا؟ (إذا كانت الإجابة " لا "، اذهب إلى س 20)

1. نعم 2. لا

س 18) كم كانت مدة التدريب؟ _____

س 19) ما هي المهنة التي تدربت عليها؟ _____

آباؤك و أجدادك

س 20) أين ولدت و أين ولد آباؤك وأين ولد أجدادك؟

القريب	الدولة	المدينة
الشخص المقابل		
الأم		
الأب		
الجد (من جهة الأب)		
الجدة (من جهة الأب)		
الجد (من جهة الأم)		
الجدة (من جهة الأم)		

القسم الثاني: المعلومات الوظيفية

أود أن أسألك حول عملك السابق وعملك الحالي، ما هي العناصر/ الأشياء التي تعرضت (تتعرض) لها خلال عملك؟ (لا تشمل الوظائف التي عملت فيها لمدة تقل عن ستة أشهر. رجاءً أبلغنا فيما إذا غيرت موقعك داخل العمل نفسه. رجاءً أبلغنا عن فترات البطالة، وفترات الانقطاع عن العمل، وإجازة الأمومة الخ).

س 21) هل لديك وظيفة حاليا؟

1. نعم **2.** لا

س 22) قبل مرضك، هل كان عندك عمل منتظم؟

1.نعم **2.** لا

العمل	تاريخ البداية	تاريخ الانتهاء	فترات الانقطاع	المكان	التعرض لـ
1) ما هو عملك الحالي؟ _____					
2) ما هي وظائفك السابقة؟					
أ.					
ب.					
ج.					
د.					
3) هل سبق لك أن عملت في إحدى المجالات الآتية؟					
1. الزراعة والبستنة					
2. التعليم					
3. النسيج					
4. صناعة الخشب					
5. عمال طحين					
6. التنظيف الجاف					
7. الصناعة الكيماوية					
8. البنزين / عمال نفط					
9. فنيو المختبر					
10. مقدم خدمات الرعاية الصحية 1) الأطباء 2) الممرضين 3) . فنيو العلاج الطبيعي					
11. فنيو الأشعة					
12. طبيب بيطري					
13. الملاحين والاطقم الجوية					
14. الجزار (اللحام)					
15. مزين الشعر (الكوافير/ الكوافيرة)					
16. عمال الأسبست					
17. عمال الجلود					
18. عمال البناء					
19. عمال التنظيفات					
20. ربة البيت					
21. أخرى					

*****رموز التعرض :**

1. المبيدات الحشرية	2. منتجات اللحوم	3. المذيبات العضوية	4. المذيبات غير العضوية
5.البنزين والنفط ومشتقاته	6. الأشعة فوق البنفسجية	7. الإشعاع الكوني	8. الإشعاع الناتج عن التأيين
9. الموجات المغناطيسية	10. الميكروبات / الكائنات الدقيقة	11. الحيوانات	12. المضادات الحيوية
13.الأطلية/الدهان	14.أصباغ الشعر	15. الأسبستوس	16 . جلد الحيوانات
17.الأصماغ	18. ضوء الشمس	19. الأدوية	20 . غبار الطحين
21. مواد التنظيف	22. غبار الخشب	23.أخرى	

القسم الثالث: السكن

أود أن أسألك حول سكنك الحالي والسابق (لا تشمل الإقامة في سكن لمدة تقل عن 3 سنوات)

س 23) ما نوع السكن الذي عشت فيه؟

العنوان	تصنيف المكان	نوع المنزل	الطابق الذي تعيش فيه	مصدر ماء الشرب	عدد الأشخاص المقيمين في المنزل	عدد الغرف	مكان الحمام	الفترة الزمنية
الحالي: الشارع:_____ المدينة (البلدة):_____								
السابق: 1. الشارع:_____ المدينة (البلدة):_____								
2. الشارع:_____ المدينة (البلدة):_____								
3. الشارع:_____ المدينة (البلدة):_____								
4. الشارع:_____ المدينة (البلدة):_____								

***مبنى

- **نوع المنزل:** 1. منزل خاص 2. مبنى سكني (اقل من 10 عائلات) 3. مبنى سكني (أكثر من 10 عائلات) 4. خيمة 5.سكن في مزرعة 6.أخرى
- **الطابق:** 1 طابق أرضي 2. طابق ثاني 3. طابق ثالث 4. طابق أعلى 5.أخرى
- **مصدر الماء:** 1.أنابيب 2. بئر 3. صهاريج 4. مياه معدنية 5. لا أعرف 6. أخرى
- **الحمام:** 1. في الداخل 2. في الخارج 3.أخرى

القسم الرابع: العادات

أود أن أسألك حول بعض خصائصك الشخصية كقياساتك الجسمية، وبعض عاداتك كالتدخين، تزيين الشعر، التعرض للشمس، والحمية الغذائية و أخرى:

المؤشرات	القياس عند المرض	القياس 6 اشهر قبل المرض
الطول		
الوزن		

س 24) ما هي قياساتك الجسمية؟

1. نفس الشيء

2. أعلى بكثير

3. أعلى بقليل (حتى 10%)

4. أقل بكثير

5. أقل بقليل (حتى 10%)

س 25) هل سبق لك أن دخنت (إذا لم تدخن أبدا، اذهب إلى س31)؟

1. السجائر
2. النرجيلة
3. الغليون
4. التبغ
5. لم أدخن أبدا

س 26) هل أنت مدخن حاليا؟

1. نعم 2. لا

س 27) هل أقلعت عن التدخين؟

1. نعم 2. لا

س28) كم سنة دخنت؟

س 29) كم عدد السجائر التي تدخنها (دخنتها) في اليوم؟

الفترة الزمنية	عدد السجائر
معدل التدخين قبل المرض	
المستوى الحالي للتدخين	

1: 10 سجائر أو أقل **2**: 11-20 **3**: 21-40 **4**: أكثر من 40 سيجارة

س 30) ما هو معدل تدخينك (للنرجيلة أو الغليون أو التبغ) ؟، قبل المرض و حاليا؟

الفترة الزمنية	النرجيلة	الغليون	التبغ
معدل التدخين قبل المرض			
المستوى الحالي للتدخين			

1: كل يوم **2:** أكثر من مرة في الأسبوع **3:** أقل من مرة في الأسبوع

س 31) هل سبق لك أن صبغت شعرك؟؟ (إذا كانت الإجابة " لا "، اذهب إلى س 37)

1.نعم 2. لا

س 32) هل تصبغين (تصبغ) شعرك بانتظام؟

1. نعم 2. لا

س 33) في أي عمر بدأت بصباغة شعرك؟ ☐

س 34) بالمعدل، كم مرة تصبغين (تصبغ) شعرك؟

1.أقل من مرة/سنة 2. 1-3 مرات/سنة 3. 4-6 مرات/سنة 4. أكثر من 7 مرات في السنة

س 35) أي لون تستخدمين (تستخدم) في العادة؟

1. الأسود 2. البني 3. الأشقر 4. لون الحناء 5. ألوان أخرى

س 36) هل الصبغة التي تستخدمها اصطناعية؟

1.نعم 2. لا

س 37) هل تعرضت للإصابة بحروق شمس حادة في طفولتك؟

1.نعم 2. لا 3.لا أذكر

س 38) كم ساعة في الأسبوع تتعرض (تعرضت) لضوء الشمس في الخارج، خارج ساعات عملك (اشمل تعرضك خلال أوقات فراغك وذهابك ورجوعك من العمل)

☐

س 39) عندما تكون في الخارج، هل يكون رأسك مغطى؟

1. دائما 2. معظم الوقت 3. أحيانا 4. أبدا

س 40) عندما تكون في الخارج، هل تلبس أكمام طويلة؟

1. دائما 2. معظم الوقت 3. أحيانا 4. أبدا

س 41) هل تستخدم واقي شمس عندما تخرج في الشمس؟

1. دائما 2. معظم الوقت 3. أحيانا 4. أبدا

س 42) هل تلقيت رضاعة طبيعية؟

1. نعم 2. لا 3. لا أدري

س 43) هل أنت نباتي (لا تأكل أي نوع من اللحوم)؟ (إذا كانت الإجابة لا، اذهب إلى س 45)

1. نعم ☐ 2. لا ☐

س 44) كم سنة كنت نباتي؟

س 45) هل تتناول اللحوم الحمراء بانتظام؟ (إذا كانت الإجابة لا، انتقل إلى سؤال 47)

1. نعم 2. لا

س 46) كم مرة في الأسبوع تأكل اللحوم الحمراء؟

س 47) هل تتناول اللحوم البيضاء بانتظام؟ (إذا كانت الإجابة لا، انتقل إلى سؤال 49)

1. نعم 2. لا

س 48) كم مرة في الأسبوع تأكل اللحوم البيضاء؟

س 49) ما هو معدل حبات الفاكهة التي تتناولها يوميا؟

1) ولا مرة 2) 1-3 3) 4-7 4) أكثر من 7

س 50) ما هو معدل حبات الخضار التي تتناولها يوميا؟

2) ولا مرة 2) 1-3 3) 4-7 4) أكثر من 7

س 51) أي نوع من الزيوت تستخدمه/ تستخدمينها في الطهي والقلي بشكل أساسي؟

1) الزيتون 2) الصويا 3) الذرة 4) عباد الشمس 5) أخرى_____

س 52) عادة كم مرة تأكل أو تشرب الأصناف التالية:

	1	2	3	4	5	6	7	8
	ولا مرة	أقل من مرة في الأسبوع	مرة في الأسبوع	4-2 أيام في الأسبوع	6-5 أيام في الأسبوع	مرة واحدة في كل يوم	أكثر من مرة واحدة في اليوم	الكمية في اليوم
1.فواكه	☐	☐	☐	☐	☐	☐	☐	___
2. خُضراوات	☐	☐	☐	☐	☐	☐	☐	___
3.لحوم حمراء	☐	☐	☐	☐	☐	☐	☐	
4.لحوم بيضاء	☐	☐	☐	☐	☐	☐	☐	
5.سمك	☐	☐	☐	☐	☐	☐	☐	
6.حليب كامل الدسم (3% او أكثر)	☐	☐	☐	☐	☐	☐	☐	
7.حليب قليل الدسم (أقل من 3%)	☐	☐	☐	☐	☐	☐	☐	
8.منتجات الحليب (اللبن أو الجبن أو الشكولاتة بالحليب)	☐	☐	☐	☐	☐	☐	☐	
9.شرب الماء فقط								_أكواب
10.مشروبات غير كحولية (ساخنة وباردة)								_أكواب
11.مشروبات كحولية								_أكواب

القسم الخامس: الهوايات

أود أن أسألك حول هواياتك كالجهد البدني الذي تمارسه، الفنون، وأخرى.

س 53) أثناء السنوات العشر الأخيرة، هل مارست أي جهد بدني منتظم؟ (إذا كانت الإجابة " لا "، اذهب إلى س 57)

1.نعم 2. لا

س 54) ما هو نوع الجهد الذي مارسته؟

1. شاق (كالركض)
2. متوسط (كالمشي)
3. خفيف (كالبستنة)

س 55) في أغلب الأحيان، كم مرة مارست الجهد البدني؟

1. ثلاث مرات في الأسبوع أو أكثر
2. مرتين في الأسبوع
3. مرة واحدة أسبوعيا
4. أقل من ذلك

س 56) هل مارست أي من النشاطات البدنية الآتية، وكم مرة عادة؟

#	النشاطات البدنية	1.لا أقوم بهذا النشاط	2. مرتين- ثلاث مرات بالشهر	3.مرة بالأسبوع	4.مرتين بالأسبوع أو أكثر
1	كرة قدم، يد، تنس، سلة، الهوكي، ألعاب كرة أخرى	☐	☐	☐	☐
2	ألعاب رياضية (ألعاب قوى)، جمباز	☐	☐	☐	☐
3	تمارين لياقة بدنية، اشتراك في نادي لياقة بدنية، جهاز ركض بيتي	☐	☐	☐	☐
4	المشي السريع والركض	☐	☐	☐	☐
5	الكاراتيه، جودو، تايكوندو	☐	☐	☐	☐
6	المصارعة	☐	☐	☐	☐
7	الملاكمة	☐	☐	☐	☐
8	رفع الأثقال	☐	☐	☐	☐
9	الرقص والدبكة	☐	☐	☐	☐
10	الكشافة	☐	☐	☐	☐
11	السباحة	☐	☐	☐	☐
12	ركوب الدراجات الهوائية	☐	☐	☐	☐
13	تسلق الجبال	☐	☐	☐	☐
14	التزلج والتزحلق	☐	☐	☐	☐
15	المشي الطويل وصيد الأسماك	☐	☐	☐	☐
16	الأنشطة المائية (الإبحار، ركوب الأمواج، والتزحلق على الماء)	☐	☐	☐	☐

س 57) هل تعتني بالحديقة كهواية؟ (إذا كانت الإجابة " لا "، اذهب إلى س 66)

1.نعم 2. لا

س 58) أي نوع من البستنة تؤدي؟

1. في الداخل 2. في الخارج

س 59) كم سنة مارست البستنة؟

س 60) كم ساعة في الأسبوع مارست البستنة؟

1. أقل من 10 ساعات في الأسبوع
2. 10-20 ساعة في الأسبوع
3. أكثر من 20 ساعة في الأسبوع

س 61) هل تزرع (زرعت) الخضار والفواكه؟

1. لك شخصيا
2. للبيع
3. لا أزرع الخضار والفواكه

س 62) هل تستعمل أو (استعملت) المبيدات الحشرية؟ (إذا كانت الإجابة " لا "، اذهب إلى س 66)

1. نعم 2. لا 3. لا أعرف

س 63) هل ترتدي (ارتديت) قفازات وقائية عندما تستخدم المبيدات الحشرية؟

1. في جميع الأوقات 2. في معظم الأوقات 3. أحيانا 4. أبدا

س 64) هل تغسل (غسلت) يديك بعد استخدام المبيدات؟

1. في جميع الأوقات 2. في معظم الأوقات 3. أحيانا 4. أبدا

س 65) المبيدات الحشرية التي تستخدمها أو استخدمتها هي ضد:

1. الأعشاب 2. الحشرات 3. الفطريات 4. لا أعرف

س 66) هل ترش (رشيت) مبيدات حشرية داخل منزلك؟

1. مرة أو أكثر في الأسبوع
2. أقل من مرة في الأسبوع – مرة في الشهر
3. بعض المرات في السنة
4. أبدا
5.

س 67) هل تذكر اسم المبيد (المبيدات) الحشرية (التي استخدمتها في البستنة أو في منزلك)؟ (إذا كانت الإجابة " لا "، اذهب إلى س 69)

1. نعم 2. لا

س 68) ما هو اسم (أسماء) المبيد (المبيدات) الحشرية التي استعملتها؟

اسم المبيد

س 69) عندما كنت رضيع أو طفل صغير، هل كنت تذهب إلى الحقل الزراعي مع والديك أو أشقاءك الأكبر منك سنا؟

1. نعم 2. لا 3. لا أذكر

س 70) هل الأشغال اليدوية (كانت) وما زالت من أحد هواياتك؟ (إذا كانت الإجابة " لا "، اذهب إلى 77)

1. نعم 2. لا 3. لا أذكر

س 71) أي نوع من الأشغال مارست (أو تمارس حاليا)؟

1. التلوين
2. النحت
3. الفخاريات والسيراميك
4. الزُجَاجِيّات
5. الطباعة والطباعة على الحجر
6. العمل الحديدي
7. فن تشكيلي
8. غيرها _____

س 72) خلال ممارستك للأشغال اليدوية، هل تعرضت (تتعرض) للمواد الكيماوية التالية:

1. ألوان زيتية
2. أطلية سائلة (أكريلية)
3. دهانات أخرى
) 4. مذيبات (التربنتين، الكاز
5. الأصماغ
6. الغبار
7. الرصاص
8. غيرها _____

س 73) كم عدد السنوات التي مارست فيهم الأشغال اليدوية؟ ☐

س 74) كم كان عمرك عندما بدأت بممارسة الأشغال اليدوية؟ ☐

س 75) كم كان عمرك عندما توقفت عن ممارسة الأشغال اليدوية؟ ☐

س 76) كم ساعة في الأسبوع تمارس (مارست) الأشغال اليدوية؟

1. أقل من 10 ساعات في الأسبوع 2. 10-20 ساعة في الأسبوع 3. أكثر من 20 ساعة في الأسبوع

س 77) هل عندك هوايات أخرى والتي تتضمن استخدام الكيماويات؟ (إذا كانت الإجابة " لا "، اذهب إلى س 82)

1. نعم 2. لا

س 78) ما هي الهواية؟ ☐

س 79) ما هو نوع المادة الكيميائية المستخدمة في هذه الهواية؟ ☐

س 80) كم كان عمرك عندما مارست هذه الهواية؟ ☐

س 81) كم ساعة في الأسبوع تمارس(مارست) هذه الهواية؟

1.أقل من 10 ساعات في الأسبوع 2. 10-20 ساعة في الأسبوع 3. أكثر من 20 ساعة في الأسبوع

القسم السادس: الصحة

الآن، أريد أن أسألك حول حالتك الصحية قبل المرض

س 82) قبل المرض، هل سبق لك أن عانيت من إسهال دام لأكثر من يومين؟ (إذا كانت الإجابة " لا "، اذهب إلى س 84)

1. نعم 2. لا 3.لا أذكر

س83) كم مرة عانيت من هذا إسهال خلال السنوات العشر الأخيرة قبل المرض و هل كان الإسهال الحاد نتيجة أحد المسببات الآتية:

المسبب	عدد المرات	متى كانت أخر عدوى
1. Salmonella (السالمونيلا) ☐		
2. Shigella (شيغِيلّا) ☐		
3. Campylobacter (الكامبيلوبكتر) ☐		
4. Yersinia (اليَرْسَنِيَّيا) ☐		
5. Strongiloidosis (الأسْطونِيَّات) ☐		
6.الأميبا ☐		
7. عدوى طفيلية أخرى ☐		
8. E.coli (اي كولاي) ☐		
9. أعلمت بأن المسبب فايروس ☐		
10 . لم يجدوا المسبب ☐		
11. لم يتم الفحص ☐		
12. أخرى ☐		

س 84) هل عانيت من أي مرض والذي تطلب العلاج في المستشفى خلال السنة الأولى من عمرك؟

1. نعم 2. لا 3. لا أعرف

ما هو هذا المرض؟ _____

س 85) هل عانيت من أي التهاب حاد والذي تطلب العلاج في المستشفى؟ (إذا كانت الإجابة " لا "، اذهب إلى س 87)

1. نعم 2. لا 3. لا أعرف

س 86) ما هو هذا الالتهاب، وكم مرة دخلت المستشفى نتيجة الالتهاب وفي أي عمر؟

العمر	عدد المرات	نوع العدوى
1. أكثر من 40 سنة		
2. 21- 40 سنة		
3. 11-20 سنة		
4. 1-10 سنوات		
5. أقل من سنة		

***رموز العدوى:**

1. التهاب الجيوب **2.** التهاب الشعب الهوائية **3.** التهاب معوي **4.** عدوى المرارة
5. عدوى المسالك البولية **6.** التهاب البروستات **7.** العدوى الشرجية **8.** التهاب الجلد
9. عدوى في الجهاز التناسلي الأنثوي (للنساء فقط) **10.** التهاب السحايا **11.** التهاب الزائدة لدودية

س 87) بغض النظر عن الالتهابات التي تطلبت العلاج في المستشفيات، هل عانيت من أي من الأمراض الأتية؟ إذا كان الجواب نعم، متى؟ (استخدم رمز زمن العدوى الموجود تحت الجدول لتحديد العمر)

المرض	نعم	لا	لا أذكر	العمر
1. التهاب الكبد 0				
2. التهاب الكبد A				
3. التهاب الكبد B				
4. التهاب الكبد C				
5. Herpes (القوباء): الشفتين، الأنف ، الأذن ، أخرى				
6. (EBV) Infectious Mononucleosis				
7. Asthma (الربو)				
8. Eczema (الأكزيما)				
9. Tonsillitis (إلتهاب اللوزتين)				
10. Measles (الحصبة)				
11. Mumps (النكاف)				
12. Rubella (الحصبة الألمانية)				
13. حمى الرُّوماتزم Rheumatic fever				
14. التهاب المفاصل Rheumatoid arthritis				
15. السل				
16. Brucellosis (الحمى المالطية)				
17. التهاب الجيوب				
18. التهاب معوي				
19. شلل الأطفال				
20. التيفوس				
21. القرحة				
22. الحساسية				
23. الالتهابات المعوية (مثل حساسية القمح او الجلوتين)				
24. الصدفية				
25. الأمراض المناعية الذاتية				
26. الأمراض المناعية الأخرى				
27. أمراض أخرى				

***رمز الجيل:** 1. أكثر من 40 سنة 2. 21 – 40 سنة 3. 11-20 سنة 4. 1-10 سنوات 5. أقل من سنة

س 88) هل تلقيت التطعيمات ضد الأمراض التالية؟

المرض	نعم	لا	لا أذكر	العمر عند التطعيم الأول	العمر عند اخر تطعيم
1.داء الكزاز					
2.الجدري					
3.التيفوئيد					
4.الحصبة					
5.النكاف					
6.الحصبة الألمانية					
7.السعال الديكي					
8. شلل الأطفال (تطعيم بالحقن)					
9.شلل الأطفال (تطعيم سائل بالفم)					
10. السل					
11. الحمى الصفراء					
12. التهاب السحايا الفيروسي					
13. الكوليرا					
14.التهاب الكبد الحاد (أ)					
15. التهاب الكبد (ب)					
16. بكتيريا الهيموفيلس					
17.نيوموكوكس (البكتيريا المكورة الدورية)					
18.فايروس الانفلونزا					
19.الخناق					
20.أخرى					

س 89) هل خضعت لاستئصال اللوزتين؟ (إذا كانت الإجابة " لا "، اذهب إلى س 91)

1. نعم 2. لا

س 90) كم كان عمرك؟ ☐

س 91) هل سبق لك أن تعاطيت مضادات حيوية؟ (إذا كانت الإجابة " لا "، اذهب إلى س 93)

1. نعم 2. لا

س 92) بالمعدل، كم مرة في السنة تناولت المضادات الحيوية، وفي أي سن؟

العمر	معدل عدد المرات في السنة
1. أكثر من 40 سنة	
2. 21- 40 سنة	
3. 11-20 سنة	
4. 1-10 سنوات	
5. أقل من سنة	

س 93) هل سبق لك أن تعرضت للأشعة قبل مرضك؟

1. نعم 2. لا 3. لا أذكر

س 94) لماذا قمت بعمل الأشعة؟

أشعة X	عدد المرات	السنة
1.أشعة أسنان		
2.أشعة صدر		
3.تصوير الثدي (للنساء)		
4.أشعة عظام		
5.أخرى		

*****رمز الجيل:**

1. أكثر من 40 سنة 2. 21 – 40 سنة 3. 11-20 سنة 4. 1-10 سنوات 5. أقل من سنة

س 95) أي من الجمل التالية تصف طفولتك حتى سن 18؟

1. كنت أمرض في أغلب الأحيان أكثر من أصدقائي
2 تغيبت عن المدرسة أكثر من أصدقائي
3. حصلت على أدوية أكثر من أخوتي وأخواتي
4. كنت طفلا بصحة جيدة فيما عدا تعرضي لأمراض الطفولة العادية
5. كنت أمرض ولكن أقل بكثير من أصدقائي وأشقائي

س 96) هل لديك حيوانات أليفة أو حيوانات كبيرة في منزلك أو في حدائق منزلك؟ (إذا كانت الإجابة " لا "، اذهب إلى س98)

1. نعم 2. لا

س 97) ما نوع الحيوانات عندك (كان عندك)؟

1. قط
2. كلب
3. طيور
4. حصان
5. بقرة
6. جمل
7. ماعز
8. أغنام
9. حمار
10. أخرى

س 98) هل سبق لك أن تناولت أي من الأدوية الآتية بوصفة طبية؟ إذا كانت الإجابة نعم، في أي عمر، وكم مرة؟

الأدوية	أبدا	أحيانا	بشكل منتظم	
			سنة البدء	سنة الانتهاء
1. الستيرويدات (الكورتزون ومشتقاته)				
2. موانع الحمل الهرمونية				
3. علاج بديل هرموني في سن اليأس (استروجين)				
4. الهرمونات الأخرى _____				
5. مضاد الفطريات (فموي)				
6. NSAIDs (الأدوية الغير إستيرودية المُضادة للإلتهاب)				
7. خافضات الحرارة				
8. مضادات الاكتئاب				
9. مضادات الطفيليات				
10. مضادات القلق				
11. مُضادات الفيروسات				
12. مضادات الهيستامين				
13. مثبطات بيتا				
14. مدرات البول				
15. الأدوية الخافضة لضغط الدم				
16. Thyroid replacement (البديل الدرقي)				
17. أدوية تمييع الدم				
18. الأسبرين				
19. العلاج الكيماوي				
20. أخرى _____				

س 99) هل سبق وأن نقل إليك دم قبل مرضك؟

1. نعم 2. لا 3. لا أعرف

س 100) قبل مرضك الحالي، هل سبق لك أن أصبت بالسرطان؟ (إذا كانت الإجابة " لا "، اذهب إلى س102)

1. نعم 2. لا

س 101) ما هو العلاج الذي تلقيته؟

1. العلاج الكيماوي
2. الجراحة
3. العلاج بالأشعة
4. لا أعرف

س 102) هل احد أقربائك من الدرجة الأولى مصاب بالسرطان؟ (إذا كانت الإجابة نعم، فمن هو وما اسمه الثلاثي)

1. نعم 2. لا 3. لا أعرف

نوع السرطان	الأشقاء	الأم	الأب	الطفل 1	الطفل 2	الطفل 3
1.أي سرطان (نوعه)						
2.الأورام الليمفاوية الغير هودجكن Non Hodgkin's Lymphoma						
3.الأورام الليمفاوية الهودجكن Hodgkin's Lymphoma						
4. سرطان الدم اللمفاوي المزمن Chronic lymphocytic leukemia						
5. سرطان الدم اللمفاوي الحاد Acute lymphocytic leukemia						
6. السرطان النخاعي المتعدد Multiple Myeloma						
7. سرطان الدم الحبيبي الحاد Acute Myeloid Leukemia						
8. سرطان الدم الحبيبي المزمن Chronic Myeloid Leukemia						
9. سرطان الدم						
10.أمراض الدم الأخرى						

س 103) هل أحد أقربائك من الدرجة الثانية مصاب بالسرطان؟ (إذا كانت الإجابة نعم، فمن هو)

1. نعم 2. لا 3.لا أعرف

نوع السرطان	الجدة من جهة (الأم)	الجد من جهة (الأم)	الجدة من جهة (الأب)	الجدة من جهة (الأب)	العم أو الخال	العمة أو الخالة	ابن/ة العم أو الخال	ابن الأخ أو الأخت/ ابنة الأخ أو الأخت
1.أي سرطان (نوعه)								
2.الأورام الليمفاوية الغير هودجكن Non Hodgkin's Lymphoma								
3.الأورام الليمفاوية الهودجكن Hodgkin's Lymphoma								
4. سرطان الدم اللمفاوي المزمن Chronic lymphocytic leukemia								
5. سرطان الدم اللمفاوي الحاد Acute lymphocytic leukemia								
6. السرطان النخاعي المتعدد Multiple Myeloma								
7. سرطان الدم الحبيبي الحاد Acute Myeloid Leukemia								
8. سرطان الدم الحبيبي المزمن Chronic Myeloid Leukemia								
9.سرطان الدم								
10.أمراض الدم الأخرى								

س 104) هل أحد أقربائك من الدرجة الأولى كان يعاني أي من الأمراض الآتية؟ إذا كانت الإجابة نعم، فمن هو؟

1.نعم 2. لا 3.لا أعرف

الأمراض	الأشقاء	الأم	الأب	الطفل 1	الطفل 2	الطفل 3
1.العدوى المتكررة						
2.الحساسية						
3.إلتهاب المفاصل(الروماتزم)						
4. الأمراض المناعية الذاتية (Autoimmune Diseases)						
5. الأمراض المناعية الأخرى						

س 105) هل أحد أقربائك من الدرجة الثانية كان يعاني أي من الأمراض الآتية؟ إذا كانت الإجابة نعم، فمن هو؟

1.نعم 2. لا 3.لا أعرف

الامراض	الجدة من جهة (الأم)	الجد من جهة (الأم)	الجدة من جهة (الأب)	الجدة من جهة (الأب)	العم أو الخال	العمة أو الخالة	ابن/ة العم أو الخال	ابن الأخ أو الأخت/ أبنة الأخ أو الأخت
1.العدوى المتكررة								
2.الحساسية								
3.إلتهاب المفاصل(الروماتزم)								
4. الأمراض المناعية الذاتية (Autoimmune Diseases)								
5. الأمراض المناعية الأخرى								

س 106) كم مرة تذهب إلى طبيب الأسنان؟

1.للفحوصات المنتظمة (مرة أو أكثر في السنة)
2. للفحوصات المنتظمة (أقل من مرة كل سنة)
3.فقط عندما يكون عندي وجع أسنان أو مشكلة أخرى
4. أبدا

س 107) هل تمتلك سيارة؟

1.نعم 2. لا

س108) كيف وصلت إلى المستشفى اليوم؟

1.مشيا على الأقدام 2. سيارة خاصة 3.تاكسي 4.النقل العام 5. أخر

س 109) متى زيارتك القادمة للمستشفى أو العيادة؟

شكرا جزيلا لتعاونك

س 110) تقييمات المقابلة

1. معتمد جدا
2. معتمد إلى حدا ما
3. غير معتمد إلى حد ما
4. غير معتمد

Appendix 3.4

Informed Consent Form

الموافقة على المشاركة في دراسة ورم الغدد الليمفاوية (الهودجكن)

لقد طلب مني المشاركة في دراسة تبحث في أسباب الورم الليمفاوي الهودجكن، حيث سأشارك كحالة (مريض مُشخص بهذا المرض)، أو كفرد من المجموعة الضابطة (شخص سليم لا يعاني من هذا المرض، ولكن لديه صديق أو أحد أفراد عائلته يُعالجون في هذا المستشفى). هذه الدراسة سوف تقارن المعطيات الشخصية، والتاريخ الطبي، وسُبل التعرض، وذلك للأشخاص المصابين وغير المصابين بهذا المرض. وهذه الدراسة لديها القدرة على اكتشاف معلومات هامة تتعلق بأسباب هذا المرض.

سوف يُطلب مني الإجابة على أسئلة تتعلق بنفسي وتفاصيلي الطبية والعلاج في المستشفى بالماضي ومعلومات عن عائلتي وأين عشت ووظائفي التي عملت بها. مع العلم ان جميع المعلومات في هذا الاستبيان ستحفظ بسرية تامة. والمقابلة سوف تستمر لمدة 30 دقيقة أو أقل.

كما وسيطلب مني تقديم عينة دم (حوالي 15 سم). الدم سوف يُفحص من أجل بعض الخصائص الجينية التي تتعلق بورم الغدد الليمفاوية كما وسيتم فحصه لاصابات فيروسية سابقة والمعروف بأن لها علاقة بورم الغدد الليمفاوية. المعلومات من هذا الفحص أيضا ستُحفظ بسرية تامة. قد يكون هناك شعور بعدم الراحة نتيجة أخذ عينة الدم ولا يوجد أي آثار جانبية متوقعة من المشاركة في هذه الدراسة.

الاسم: _____

رقم الهوية: _____

أوافق على اجراء المقابلة (التوقيع): _____

أوافق على إعطاء عينة الدم (التوقيع): _____

أوافق على تخرين عينة الدم وأن تستخدم في دراسات لاحقة (التوقيع): _____

التاريخ: _____

Appendix 3.5

Institutional Review Board (IRB) Approval Letter

Al-Quds University
Jerusalem
Deanship of Scientific Research

بسم الله الرحمن الرحيم

جامعة القدس
القدس
عمادة البحث العلمي

Research Ethics Committee
Committee's Decision Letter

Date: May 6th, 2017
Ref No: 15/REC/2017

Dear Dr. Rania Abu Seir,

Thank you for submitting your application for research ethics approval. After reviewing your application entitled

"Hodgkin Lymphoma in Palestine: Environmental and Genetic Determinants"

The Research Ethics Committee confirms that it is in accordance with the research ethics guidelines at Al-Quds University.

Please inform us if there will be any changes in your research methodology, subjects, plan and we would appreciate receiving a copy of your final research report.

Thank you again and wish you productive research that serves the best interest of your subjects.

Dina M. Bitar PhD
Research Ethics Committee Chair

Cc. Prof. Imad Abu Kishek - President
Cc. Members of the committee
Cc. file

Abu-Dies, Jerusalem P.O.Box 20002
Tel-Fax: #970-02-2791293
research@admin.alquds.edu
أبوديس، القدس ص.ب. 20002
تلفاكس: #970-02-2791293

Appendix 3.6

MOH Approval Letter

Al-Quds University
Faculty of Medicine
Abu-Dies, Jerusalem

جامعة القدس
كلية الطب
أبوديس – القدس

التاريخ: 2015/7/1

حضرة الدكتور محمد أبو غالية المحترم،
مدير عام الإدارة العامة للمستشفيات،

تحية طيبة و بعد،

الموضوع: تسهيل الإجراءات البحثية لدراسة سرطان الغدد الليمفاوية

أرجو من حضرتكم تسهيل مهمة الطالبة نورا خليل محمد عايش والطالب بشار جمال دودين – طلبة دراسات عليا والسماح لها الحصول على ما لإنشاء عينة من مرضى سرطان الغدد الليمفاوية في مستشفيات الضفة الغربية للمساعدة في دراسة العوامل الجينية والبيئية والفيروسية التي تلعب دوراً في الإصابة بهذا النوع من السرطان وذلك من خلال تعبئة استبيان والحصول على عينات دم وعينات شرائح من قوالب الشمع للمرضى التابعين للدراسة.

شاكرين لكم تعاونكم

د. هاني عابدين
عميد كلية الطب