INTRODUCTION

The incidence and the mortality attributed to malignant lymphomas have steadily increased over the last four decades and now non-Hodgkin's lymphoma (NHL) accounts for approximately 50,000 new malignancies diagnosed annually **(Jaffe et al., 1999)**.

NHL is often characterized by a higher incidence of specific chromosomal translocations **(Armitage and Weisenburger, 1998)**.

One of the common aberrations seen in NHL-patients is t(14;18)(q32;q21.3) **(Estalilla et al., 2000)**. Several studies were done to know the incidence of t(14;18) among different histologic subtype of NHL **(Rowley, 1988 and Pezella and Mason, 1990)**.

Follicular NHL is frequently associated with t(14;18), leading to overexpression of bcl-2 and subsequent inhibition of apoptosis **(Tsujimoto et al., 1984)**. The t(14;18) chromosomal translocation, although widely recognized as a cytogenetic abnormality associated with follicular lymphoma, has a widely variable frequency in this type of lymphoma **(Pezella et al., 1990)**.

The presence of t(14;18)-positive cells, analyzed by PCR is assumed to correlate with lymphoma activity. However, until now the

clinical significance of the presence or absence of t(14;18)-positive cells in the bone marrow and peripheral blood has not been settled **(Lambrechts et al., 1994)**.

Quantitation of circulating t(14;18)-positive ells, showing variation at diagnosis and over time, may lead to a better understanding of the natural course of the disease and effects of treatment **(Lopez-Guillermo et al., 2000)**.

Olsson and his colleagues (1999) used real-time quantitative PCR to detect t(14;18) positive cells present in human lymphoma tissue, bone marrow aspirates and peripheral blood mononuclear cells.

Aim of the Work

This work **aims** at studying the incidence of the chromosomal abnormality t(14;18) in B-cell NHL and to quantitate t(14;18)-positive cells using Real-time PCR.

QUANTIFICATION OF CHROMOSOMAL t (14;18) IN B – CELL NHL PATIENTS USING REAL – TIME PCR

Thesis

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ABSTRACT

One of the common aberrations seen in non-Hodgkin's lymphoma (NHL) is t(14;18)(q32;21.3). The aim of this work is to study the incidence and quantity of t(14;18) in B-NHL. 33 patients with B-NHL and 10 controls with non-neoplastic lymphoid tissue disorder were tested for quantitation of t(14;18) by real-time quantitative PCR (RQ-PCR). The incidence of t(14;18) was 6.1% among NHLs, 14.3% among follicular lymphomas (FLs), 5.6% among diffuse large cell lymphomas (DLCLs) and 10% among control group. The value of relative quantification for patients was (**1.20 and 0.85**), while for control case was (**0.6**). RQ-PCR is a sensitive and specific procedure. It is an ideal tool for follow-up and quantification of minimal residual disease (MRD).

Keywords:

Real-time PCR, t(14;18).

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LIST OF ABBREVIATIONS

ALK	: Anaplastic lymphoma kinase
ATM locus	: Ataxia telangectasia mutated locus
BL	: Burkitt's lymphoma
bp	: Basepair
CD	: Cluster of differentiation
cDNA	: Complementary DNA
CGH	: Competitive genomic hybridization
CR	: Complete remission
CTL	: Cytotoxic T lymphocyte
DLCL	: Diffuse large cell lymphoma
dNTP	: deoxy nucleoside triphosphate
FCC	: Follicle centre cell
FISH	: Fluorescent in situ hybridization
FL	: Follicular lymphoma
HD	: Hodgkin disease
HIV	: Human immunodeficiency virus
HTLV-I	: Human T lymphotropic virus type I
IHC	: Immunohistochemistry
Kb	: Kilobase
KDa	: Kilo Dalton
LCA	: Leucocyte common antigen
mbr	: Major breakpoint region
MCL	: Mantle cell lymphoma
mM	: Milli mole
MRD	: Minimal residual disease

MZBCL	: Marginal zone B-cell lymphoma
NHL	: Non-Hodgkin's lymphoma
NK	: Natural killer
PBSC	: Peripheral blood stem cells
rpm	: Round per minute
RQ-PCR	: Real-time quantitative PCR
RS	: Read-Sternburg cells
RT-PCR	: Reverse transcriptase PCR
SLL	: Small lymphocytic lymphoma
SLVL	: Splenic lymphoma with villous lymphocytes
SMZL	: Splenic marginal zone lymphoma
TCR	: T cell receptor
TdT	: Terminal deoxynucleotidyl transferase
TMA	: Tissue microarray

CHAPTER I
NON-HODGKIN's LYMPHOMA

Definition:

Lymphomas are a heterogeneous group of malignancies of B cells or T cells that usually originate in the lymph nodes but may originate in any organ of the body **(Brill et al., 1927).**

History:

In **1832, Thomas Hodgkin** published the first article on primary lymphatic malignancy. His work was derived from clinical and gross autopsy findings in seven cases. He was the first to realize that lymphadenopathy could occur as a primary disorder rather than secondary to infection or carcinoma. Since Hodgkin's initial description, there have been four historical phases in the study of NHL: (1) clinical features, 1832 to 1900; (2) histopathology, 1900 to 1972; (3) immunopathology, 1972 to the present; (4) molecular genetics, 1982 to the present. These phases naturally overlap and all contribute to the presentday understanding of NHL.

In 1846, Virchow distinguished lymphoma from leukemia and coined the terms lymphoma and lymphosarcoma. **Billorth, in 1871,** was the first to use the term malignant lymphoma.

The category of follicular lymphomas was initially recognized in

1916 by Ghon and Remaa, who related such neoplasms to normal lymphoid follicles. **Brill and others in 1927 and Symmers in 1927,** failed to appreciate the neoplastic nature of many cases of "giant follicular hyperplasia"; however, this oversight was corrected in 1938 with information gained through long-term follow-up. Thus, the neoplastic, indolent, nature of this category of lymphomas became recognized.

In addition to perceiving the aggressive nature of lymphomas composed of large cells, **Roulet, in 1930,** proposed that the origin of such neoplasms was the sinus lining, and he compared their morphology with that of normal reticulum or syncytial network of nodal sinuses. He used the term reticulum cell sarcoma as another term for malignant lymphoma.

In **1942, Gall and Mallory** developed a classification scheme for NHL that had both clinical and histopathologic significance. The histopathologic phase of NHL culminated in the 1956 classic work of **Rappaport** who developed a morphologic classification that was reproducible and relevant.

In **1974, Lennert and coworkers & Lukes and Collins** classified NHL on the basis of the cell origin within the immune system. Subsequently, monoclonal antibodies to lymphocyte differentiation antigens have been able to detect sequential stages in the development of B & T cells and to identify subtypes of NHL. In the

1980s, the lymphoid origin of NHL was confirmed at the molecular level with the identification of specific Ig gene and T cell receptor (TCR) gene rearrangements **(Korsmeyer, 1981)**.

In **1982**, a Working Formulation (WF) of NHLs separated diseases according to histologic grade (low, intermediate and high) and made correlations with survival **(Rosenberg et al., 1982).** However, classification was based on morphology without utilizing immunophenotyping or molecular genetic techniques.

In **1994**, a Revised European-American Lymphoma (REAL) classification was proposed to identify specific types of lymphomas of B and T cell origin **(Harris et al., 1994).**

In lymphoma, two fundamental features were correlated with differences in survival namely the identification of certain cell types and growth patterns. Neoplasms composed of small, nonreplicating lymphocytes (lymphocytic, well differentiated lymphocytic) were recognized as a favorable group. Those featuring atypical, mitotically active lymphocytes (lymphoblastic, poorly differentiated lymphocytic) were less favorable.