APPLICATION OF ISOELECTRIC FOCUSING FOR DETECTION OF MONOCLONAL IMMUNOGLOBULINS AND ITS CLINICAL USE

Thesis

Submitted for partial fulfillment of
Master Degree in Clinical and Chemical Pathology.

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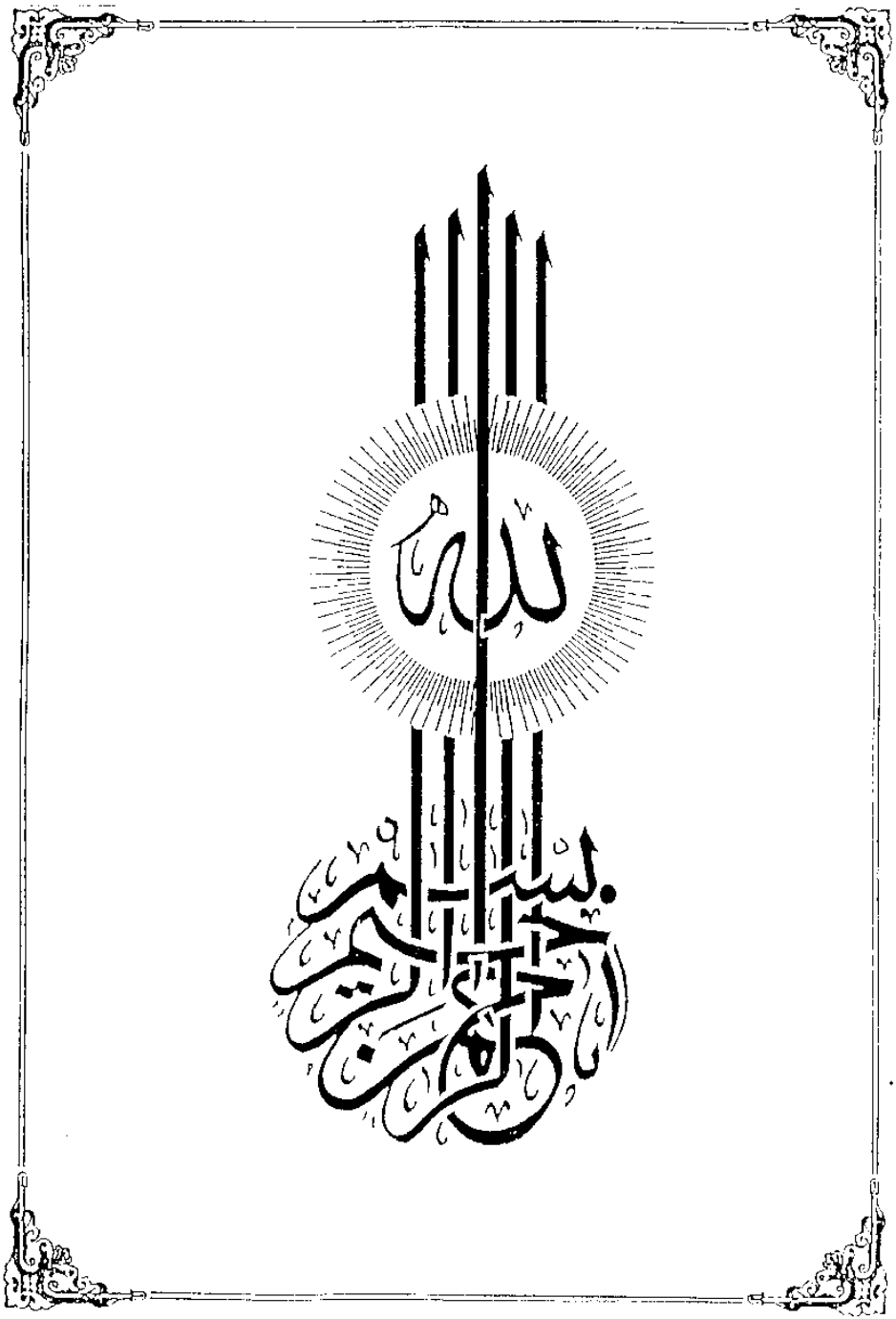
1992

بسم الله الرحمن الرحيم

قالوا سبحانك لا علم لنـــا الا ما علمتنا
أنك أنت العـــليم الحـــكيم

IN THE NAME OF ALLAH, THE BENEFICENT, THE MERCIFUL

They said : ' Be glorified, we have no knowledge except that which you have tought us. Indeed you are the knower, the wise '.



ACKNOWLEDGEMENT

I wish to express my deep appreciation and gratitude to Prof. Dr. **FADILA HASSAN SABRY**, Professor of Clinical Pathology, Ain Shams University, for her close supervision, invaluable support and helpful advice.

I am very grateful to Prof. Dr. **SALWA HOHAMED YOUSSEF**, Professor of Clinical Pathology, Ain Shams University, for her useful advice, great help and valuable directions.

I am also thankful to Dr. **MONA MOHAMED RAFIK**, Assistant Professor of Clinical Pathology, Ain Shams University, for her honest assistance and precious guidance throughout the course of this work.

I am greatly indebted to Dr. **HEBA ADEL SEDKY**, Assistant Professor of Clinical Pathology, Ain Shams University, for her helpful guidance and advice.

Last but not least, these persons can not be forgotten

My Parents

and My Wife.

The Candidate

Mohamed Hassan

LIST OF ABBREVIATIONS

BM	=	Bone marrow
CLL	=	Chronic lymphocytic leukaemia
CSF	=	Cerebrospinal fluid
DW	=	Distilled water
EDTA	=	Ethyline Diamine tetraacetic acid
Hb	=	Haemoglobin
HRAGE	=	High resolution agarose gel electrophoresis
IEF	=	Isoelectric focusing
IEP	=	Immunoelectrophoresis
IFE	=	Immunofixation
Igs	=	Immunoglobulins
IIEF	=	Immunoisoelectric focusing
LN	=	lymph node
MM	=	Multiple myeloma
NHL	=	Non Hodgkin's lymphoma
PAGs	=	Polyacrylamide gels.
PEP	=	Protein electrophoresis
PI	=	Isoelectric point
PLT	=	Platelets
TLC	=	Total leukocytic count
TP	=	Total protein
WM	=	Waldenstrom's macroglobulinaemia

TABLE OF CONTENTS

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

Detection of serum paraprotein is normally carried out either by single radial immunodiffusion or by scanning of a zonal electrophoretic strips. The former technique suffer from disadvantages, since it does not discriminate between paraproteins and polyclonal immunoglobulins, introducing large errors when paraproteins concentration is low relative to the polyclonal immunoglobulins (Sinclair et al., 1983 and 1984).

The initial detection and correct identification of paraproteins is of great importance in the diagnosis of certain B-cell neoplasia, e.g., multiple myeloma, Waldenstrom's macroglobulinemia.

Moreover, certain paraproteins can be detected by isoelectric focusing but not by immunoelectrophoresis in cases of chronic lymphocytic leukemia (Sinclair et al., 1984).

AIM OF THE WORK:

The aim of this work is to detect paraproteins in the sera of patients with lymphoproliferative disorders using isoelectric focusing and immunoelectrophoresis in order to compare both techniques.

REVIEW OF LITERATURE

CHAPTER I

DISORDERS ASSOCIATED WITH PARAPROTEINEMIA

Several diseases of man are characterized by abnormal proliferation of cells that are normally the mediators of specific immunity, lymphocytes and plasma cells.

Disorders associated with paraproteinemia include multiple myeloma, Waldenstrom's macroglobulinemia, non-Hodgkin's lymphoma, chronic lymphatic leukemia, benign monoclonal gammopathy, heavy chain disease and primary amyloidosis (Firkin et al., 1989).

Multiple Myeloma (MM):

Neoplasm of plasma cells are considered neoplasms of the B lymphocyte system, since they appear to arise from plasmacytoid B lymphocytes. Because these cells are responsible for secreting immmunoglobulins, malfunctions of this system result in the excessive production of immunoglobulins or portions of immunoglobulin molecules. The abnormal immunoglobulin is a product of a single clone of lymphoid cells (plasmacytoid lymphocytes or plasma cells) and is called a paraprotein or myeloma protein; the disease is referred to as a monoclonal gammopathy. These abnormal proteins have typical serum electrophoretic and

immunoelectrophoretic patterns and may be associated with neoplastic plasma cells or be secondary to other conditions, such as non-hematopoietic neoplasms, rheumatoid disorders; and chronic inflammatory states. Plasma cell dyscrasias, therefore, encompass a somewhat confusing spectrum of disease in that there is evidence that patients may have paraproteins many years before the onset of clinical disease with no progression to a neoplasm and no clinical evidence associated with the paraprotein. This period may be short in multiple myeloma (2.3 years) or quite long in benign monoclonal gammopathy (25-30 years) (Blattner et al., 1981).

Many specific immunologic tests were performed. The most important of these are serum protein electrophoresis, immunoelectrophoresis and immunofixation electrophoresis, which demonstrate diagnostic, quantifiable patterns of paraproteins. Because some patients may produce cryoglobulins, which will precipitate at low temperatures, serum should be separated at 37°C. In immunoelectrophoresis, antibodies against the major heavy and light chains are used. One heavy chain class and one light chain type are detected. In almost all cases there will be an immunoelectrophoretic precipitin arc for Kappa and Lambda light chains similar in electrophoretic mobility to the heavy chain, except in "heavy; chain disease", in which Kappa and Lambda light chains are not present. Monoclonal Kappa or Lambda light chains are excreted in the urine of

some patients with multiple myeloma and are designated Bence Jones protein. They may be detected by immunoelectrophoresis or immunofixation electrophoresis of concentrated urine (Harris and Bohn, 1985).

Waldenstrom's Macroglobulinemia (WM):

Waldenstrom's macroglobulinemia (WM) is a monoclonal proliferation of B lymphocytes at a more advanced stage of immunological maturation than those of CLL. In WM, a range of cell types is found, including lymphocytes bearing surface immunoglobulin M and D, or M only, plasmacytoid lymphocytes containing cytoplasmic IgM and IgM-secretory plasma cells. In rare cases resembling WM clinically, cytologically and histologically, the paraprotein found is IgG or IgA. Although in classical WM the clinical manifestation are the result of the continuous production over a period of years of large amounts of monoclonal macroglobulin, there is a continuous spectrum of clinical presentations between those with the classical manifestations and those with frank malignant lymphoma (Deutcher and Fahey) 1959).

Non-Hodgkin's Lymphoma (NHL):

Non Hodgkin's lymphomas (NHL) are heterogenous diseases with respect to their morphological appearance, clinical presentation and response to therapy. With the increasing use of immunological markers the great majority of NHL has

proven to be neoplasms of B cell lineage derivation (Pascali and Pezzoli, 1986).

B cell neoplasms are thought to represent monoclonal proliferation of B lymphocytes "Frozen at different stages along the normal pathway of B cell maturation and differentiation (Salmon and Seligmenn, 1977). There are neoplasms such as myeloma and Waldenstrom's macroglobulinemia which involve terminally differentiated B cells actively producing and secreting large amounts of monoclonal immunoglobulins (M components or paraproteins). Most NHL appear, however, to be proliferations of less mature B cells as they have only occasionally been described to spontaneously secrete monoclonal immunoglobulins at level sufficient to be detected in the serum by routine electrophoretic techniques.

Although the association of serum M components with NHL has long been recognized (Heller, 1973; Krauss and Sokal, 1974; Kim and Alexanian, 1975 and Ko and Pruzanski, 1976), yet the actual incidence of such monoclonal immunoglobulin abnormalities in the different histopathological subtypes of NHL is difficult to evaluate from the available data at present. However, there have been relatively few systematic studies of the frequency with which monoclonal free immunoglobulins light chains, i.e., Bence Jones protein,

occur in the urine of patients with NHL (Mc Laughlin and Hobbs, 1973 and Pierson et al., 1980)).

In a survey performed by means of a high resolution agarose gel electrophoretic technique combined with immunofixation, (Pascali and Pezzoli, 1986)) studied 62 consecutive unselected patients with newly diagnosed NHL for the frequency, types, and amount of serum and urine M components. The overall incidence of monoclonal gammopathy was 81%.

Table (1) shows the different classifications of Non-Hodgkin's lymphoma used in NCI-sponsored study.

Table (1): Classification of NHLs as used in the NCI-sponsored study:

British National Lymphoma Investigation Classification
Follicular lymphoma
Follicle cells, predominantly small
Follicle cells, mixed small and large
Follicle cells, predominantly large
Diffuse lymphoma
Lymphocytic, well differentiated (Small round lymphocyte)
Lymphocytic, intermediately differentiated (Small follicle lymphocyte)
Lymphocytic, poorly differentiated (Lymphoblast)
(a) Non-Burkitt
(b) Burkitt;s tumours
(c) Convoluted cell mediastinal lymphoma
Lymphocytic/mixed small lymphoid and large cell (Mixed follicle cells)
Undifferentiated large cell (Large Lymphoid Cell)
Histiocytic cell (Mononuclear Phagocytic Cell)
Plasma cell (Extramedullary Plasma Cell)
Malignant lymphoma unclassified
Plasmacytoid differentiation
Sclerosis, banded
Sclerosis, fine

Lukes and Collins Classification
Undefined cell type
T cell type, small lymphocytic
T cell type, sezary-mycosis fungoides (Cerebriform)
T cell type, convoluted lymphocytic
T cell type, immunoblastic sarcoma (T Cell)
B cell type, small lymphocytic
B cell type, plasmacytoid lymphocytic
Follicular center cell, small cleaved
Follicular center cell, large cleaved
Follicular center cell, small non-cleaved
Follicular center cell, large non-cleaved
Immunoblastic sarcoma (B Cell)
Subtypes of follicular center cell lymphomas
1- Follicular
2- Follicular and diffuse
3- Diffuse
4- Sclerotic with follicles
5- sclerotic without follicles
Histiocytic
Malignant lymphoma, unclassified