

# **ASSESSMENT OF VITAMIN D LEVEL IN NEWLY DIAGNOSED EGYPTIAN PATIENTS WITH B-CELL LYMPHOPROLIFERATIVE DISORDERS**

*For partial fulfillment of the master degree in Clinical hematology*

*Submitted by*

**Yousra Mohamed Radwan Ali**

*(M.B.B.Ch.)*

*Supervised by*

**Prof. DR. Manal Elhossiny Abo farha**

*Professor of Internal Medicine,*

*Faculty of Medicine- Cairo University*

**DR. Hala Mahmoud Abdelhamid Fahmy**

*Assistant professor of Internal Medicine,*

*Faculty of Medicine- Cairo University*

**DR. Fatma Mohamed Taha**

*Assistant professor of Medical Biochemistry Department,*

*Faculty o Medicine- Cairo University*

**Faculty of Medicine**

**Cairo University**

**2014**



## *Acknowledgment*

First and foremost *“(Thanks to Great Allah)”* the most merciful and kind

It is a pleasure to express my deepest thanks and profound respect to **Dr. Manal El Huseiny Abu Farha** Professor of Internal Medicine, Faculty of Medicine Cairo University for her continuous encouragement and valuable supervision throughout this work,

Very special thanks to **Dr. Hala Mahmud Abd El Hamid**, Assistant professor of Internal Medicine, Faculty of Medicine, Cairo University, for her continuous support and supervision not only in this work but in all my life. No Words can express my deepest gratitude and respect .It is an honor being one of her students and work under her supervision.

I am deeply grateful to **Dr. Fatma Mohamed Taha**, Assistant professor of Medical Biochemistry Department, Faculty of Medicine, Cairo University, for great help in performing this work,

Also I would like to express my gratitude to **Dr. Noha Mohamed El Hussein**, Lecturer of Internal Medicine, Faculty of Medicine, Cairo University, for her help in performance the statistics of this work,

I would like to sincerely thank **Dr. Reham El Shiemy**, Lecturer of Clinical Pathology National Cancer Institute, Cairo University for her help in performance of this work

Words fail to express my deep appreciation to my **Mother and Father** for their continuous help, support and encouragement as without their help, this work never be completed

Special thanks to **DR .Mervat Mohamed Matter** the head of hematology medicine Faculty of Medicine, Cairo University who is the light of my life in hematology medicine.

Last but not least I am deeply grateful to all patients for their participation in this study

*Yousra Mohamed Radwan*

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَأَمَّا الزَّبَدُ فَيَذْهَبُ جُفَاءً وَأَمَّا مَا يَنْفَعُ  
النَّاسَ فَيَمْكُثُ فِي الْأَرْضِ

صدق الله العظيم

الآية السادسة عشر سورة الرعد

## **Abstract**

There is a strong link between vitamin D and cancer playing an important role in reducing incidence and mortality. Concerning hematological malignancies it was found that low level of this vitamin is linked to higher cancer incidence and mortality, so we aim from this study to check vitamin D status and its link to lymphoproliferative disorders. The study group include 60 subjects, 20 patient had CLL and 20 patients had NHL , 20 patients as control group, all groups are subjected to measurement of 25(OH) D by ELISA technique.

Key Words : Vitamin D - Lymphoproliferative disorders - Risk and prognostic factor.

## *List of contents*

| <b>Subject</b>                                                   | <b>page</b> |
|------------------------------------------------------------------|-------------|
| <b>List of Abbreviations</b>                                     | <b>I</b>    |
| <b>List of Tables</b>                                            | <b>VIII</b> |
| <b>List of Figures</b>                                           | <b>IX</b>   |
| <b>Introduction</b>                                              | <b>1</b>    |
| <b>Aim of the work</b>                                           | <b>3</b>    |
| <b>Review of literature</b>                                      |             |
| <b>Chapter (1): Vitamin D</b>                                    | <b>4</b>    |
| <b>Chapter (2): Vitamin D and cancer</b>                         | <b>35</b>   |
| <b>Chapter (3): Vitamin D and hematological malignancies</b>     | <b>46</b>   |
| <b>Chapter (4): B-cell chronic lymphoproliferative disorders</b> | <b>54</b>   |
| <b>Chapter (5): Lymphoma</b>                                     | <b>69</b>   |
| <b>Chapter (6): Chronic lymphocytic leukemia</b>                 | <b>96</b>   |
| <b>Patients and Methods</b>                                      | <b>115</b>  |
| <b>Results</b>                                                   | <b>120</b>  |
| <b>Discussion</b>                                                | <b>129</b>  |
| <b>Recommendations and conclusions</b>                           | <b>138</b>  |
| <b>Summary</b>                                                   | <b>139</b>  |
| <b>References</b>                                                | <b>140</b>  |
| <b>Arabic summary</b>                                            | <b>173</b>  |

---

## *List of Abbreviations*

---

|                       |                                                        |
|-----------------------|--------------------------------------------------------|
| <b>1, 25(OH) 2D3</b>  | <b>1, 25 dihydroxy vitamin D3</b>                      |
| <b>25 OH D3</b>       | <b>25 hydroxyvitamin D3</b>                            |
| <b>AIHA</b>           | <b>Autoimmune hemolytic anemia</b>                     |
| <b>ALCL</b>           | <b>Anaplastic large cell lymphoma</b>                  |
| <b>ALL</b>            | <b>Acute lymphoblastic leukemia</b>                    |
| <b>ALPS</b>           | <b>Autoimmune lymphoproliferative syndrome</b>         |
| <b>ALT</b>            | <b>Alanine transaminase</b>                            |
| <b>AML</b>            | <b>Acute myeloid leukemia</b>                          |
| <b>APC</b>            | <b>Antigen presenting cell</b>                         |
| <b>AST</b>            | <b>Aspartate transaminase</b>                          |
| <b>BAL</b>            | <b>Bcl-2-associated X protein</b>                      |
| <b>BCa</b>            | <b>Breast cancer cell</b>                              |
| <b>Bcl-2</b>          | <b>B-cell leukemia/lymphoma-2genes</b>                 |
| <b>BMT</b>            | <b>Bone marrow transplantation</b>                     |
| <b>BUN</b>            | <b>Blood Urea Nitrogen</b>                             |
| <b>C/EBP</b>          | <b>CCAAT-enhancer-binding proteins</b>                 |
| <b>Ca<sup>2</sup></b> | <b>Calcium ions</b>                                    |
| <b>CBC</b>            | <b>Complete blood count</b>                            |
| <b>CBP</b>            | <b>Calcium- binding proteins</b>                       |
| <b>CD</b>             | <b>Cluster of differentiation</b>                      |
| <b>CDK</b>            | <b>Cyclin-dependent kinase</b>                         |
| <b>CHOP</b>           | <b>Cyclophosphamide, Doxorubicin, Vincristine, and</b> |

*List of Abbreviations (cont.)*

|           |                                                                         |
|-----------|-------------------------------------------------------------------------|
| CHS       | Chédiak-Higashi syndrome                                                |
| CLL       | Chronic lymphocytic leukemia                                            |
| CLL       | Chronic lymphocytic leukemia                                            |
| CMV       | Cytomegalovirus                                                         |
| CNS       | Central nervous system                                                  |
| CODOX-M   | Cyclophosphamide, Vincristine, Doxorubicin,<br>Cytarabine, Methotrexate |
| COX-2     | Cyclooxygenase enzyme                                                   |
| CR        | Complete response                                                       |
| CSF       | Cerebral spinal fluid                                                   |
| CT        | Computed Tomography                                                     |
| CVP       | Cyclophosphamide, Vincristine, and Prednisone                           |
| CXR       | Chest x-ray                                                             |
| CYP24     | 25-hydroxyvitamin D-24-hydroxylase                                      |
| CYP27     | Vitamin D3-25-hydroxylase                                               |
| CYP27b1   | Cytochrome p450 27B1<br>(25-hydroxyvitamin D3 1-alpha-hydroxylase)      |
| DAT       | Direct antiglobulin test                                                |
| DBP       | Vitamin D binding protein                                               |
| DC        | Dendritic cell                                                          |
| del (13q) | Deletions in the long arm of chromosome 13                              |
| del (11q) | Deletions in the long arm of chromosome 11                              |
| del (17p) | Deletion in the short arm of chromosome 17                              |

---

*List of Abbreviations (cont.)*

---

|        |                                                                                                                  |
|--------|------------------------------------------------------------------------------------------------------------------|
| DNA    | Deoxyribonucleic acid                                                                                            |
| EBER   | EBV-encoded RNA                                                                                                  |
| EBV    | Epstein-Barr virus                                                                                               |
| EGFR   | Epidermal growth factor                                                                                          |
| ELISA  | Enzyme-linked immunosorbent assay                                                                                |
| EPOCH  | Etoposide, Vincristine, Doxorubicin, Prednisone<br>Cyclophosphamide                                              |
| ESR    | Erythrocyte sedimentation rate                                                                                   |
| FAS    | The Fas receptor is a death receptor on the surface of<br>cells that leads to programmed cell death (apoptosis). |
| FCM    | Fludarabine, Cyclophosphamide, and Mitoxantrone                                                                  |
| FCR    | Fludarabine, Cyclophosphamide, and Rituximab                                                                     |
| FISH   | Fluorescence in situ-hybridization                                                                               |
| FL     | Follicular lymphoma                                                                                              |
| FLC    | Serum free light chain                                                                                           |
| GCs    | Germinal centers                                                                                                 |
| G-CSF  | Granulocyte colony-stimulating factor                                                                            |
| GIT    | Gastrointestinal tract                                                                                           |
| GM-CSF | Granulocyte macrophage colony stimulating factor                                                                 |
| HAART  | Highly active anti-retroviral therapy                                                                            |
| HCL    | Hairy cell leukemia                                                                                              |
| HCV    | Hepatitis C Virus                                                                                                |
| HHV8   | Human herpes virus-8                                                                                             |



## *List of Abbreviations (cont.)*

|                   |                                                                         |
|-------------------|-------------------------------------------------------------------------|
| <b>Hib</b>        | <b><i>Haemophilus influenza</i> type b</b>                              |
| <b>HIV</b>        | <b>Human immunodeficiency virus</b>                                     |
| <b>HLA</b>        | <b>Human leukocyte antigen</b>                                          |
| <b>HTLV-1</b>     | <b>Human T-cell lymphotropic virus type 1</b>                           |
| <b>Hyper-CVAD</b> | <b>Vincristine, Doxorubicin, Dexamethasone and<br/>Cyclophosphamide</b> |
| <b>IDBP</b>       | <b>Intracellular vitamin D binding protein</b>                          |
| <b>Ig</b>         | <b>Immunoglobulin</b>                                                   |
| <b>IgD</b>        | <b>Immunoglobulin D</b>                                                 |
| <b>IGFBP</b>      | <b>Insulin like growth factor binding protein-3</b>                     |
| <b>IgM</b>        | <b>Immunoglobulin M</b>                                                 |
| <b>IgVH</b>       | <b>Immunoglobulin variable heavy chain</b>                              |
| <b>IL</b>         | <b>Interleukin</b>                                                      |
| <b>IMiD</b>       | <b>Immunomodulatory drug</b>                                            |
| <b>INR</b>        | <b>International normalized ratio</b>                                   |
| <b>ITD</b>        | <b>Internal tandem duplication</b>                                      |
| <b>ITP</b>        | <b>Immune thrombocytopenia</b>                                          |
| <b>IU</b>         | <b>International units</b>                                              |
| <b>IVIG</b>       | <b>Intravenous immunoglobulin</b>                                       |
| <b>LDH</b>        | <b>Lactate dehydrogenase</b>                                            |
| <b>LFTS</b>       | <b>Liver function tests</b>                                             |
| <b>LIP</b>        | <b>Short –length isoform of C/EBP<math>\beta</math></b>                 |
| <b>LN</b>         | <b>Lymph node</b>                                                       |
| <b>LP</b>         | <b>Lumber puncture</b>                                                  |

## *List of Abbreviations (cont.)*

|                   |                                                            |
|-------------------|------------------------------------------------------------|
| <b>MALT</b>       | <b>Mucosa-associated lymphoid tissue</b>                   |
| <b>MBL</b>        | <b>Monoclonal B-lymphocytosis</b>                          |
| <b>MC-26</b>      | <b>Mouse cancer cell</b>                                   |
| <b>MCL</b>        | <b>Mantle cell lymphoma</b>                                |
| <b>MDS</b>        | <b>Myelodysplastic syndromes</b>                           |
| <b>MHC</b>        | <b>Major Histocompatibility complex</b>                    |
| <b>MKP5</b>       | <b>Mitogen-activated protein kinase phosphatase-5</b>      |
| <b>ML</b>         | <b>Milliliter</b>                                          |
| <b>MMP</b>        | <b>Matrix metalloproteinases</b>                           |
| <b>MRD</b>        | <b>Minimal residual disease</b>                            |
| <b>MRI</b>        | <b>Magnetic resonance imaging</b>                          |
| <b>MRNA</b>       | <b>Messenger ribonucleic acid</b>                          |
| <b>NF-KB</b>      | <b>Nuclear factor kappa B</b>                              |
| <b>NHANESIII</b>  | <b>National Health and Nutrition Examination SurveyIII</b> |
| <b><i>NHL</i></b> | <b><i>Non-Hodgkin lymphomas</i></b>                        |
| <b>NK</b>         | <b>Natural killer cell</b>                                 |
| <b>NSAIDs</b>     | <b>Non steroidal anti-inflammatory drugs</b>               |
| <b>OR</b>         | <b>Overall response</b>                                    |
| <b>OS</b>         | <b>Overall survival</b>                                    |
| <b>P21</b>        | <b>Cyclin dependent kinase inhibitor 1</b>                 |
| <b>P27</b>        | <b>Cyclin dependent kinase inhibitor 1B</b>                |
| <b>PA</b>         | <b>Plasminogen activator</b>                               |
| <b>PC</b>         | <b>Prostate cancer cell</b>                                |
| <b>PCNSL</b>      | <b>Primary central nervous system lymphoma</b>             |

## *List of Abbreviations (cont.)*

|                                |                                                                                                                   |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------|
| <b>PCR</b>                     | <b>polymerase chain reaction</b>                                                                                  |
| <b>PCR</b>                     | <b>Pentostatin, Cyclophosphamide, and Rituximab</b>                                                               |
| <b>PET</b>                     | <b>Positron emission tomography</b>                                                                               |
| <b>PFS</b>                     | <b>Progression free survival</b>                                                                                  |
| <b>PGs</b>                     | <b>Prostaglandins</b>                                                                                             |
| <b>PLL</b>                     | <b>Prolymphocytic leukemia</b>                                                                                    |
| <b>PR</b>                      | <b>Partial remission</b>                                                                                          |
| <b>PTLD</b>                    | <b>Post transplant lymphoproliferative disorder</b>                                                               |
| <b>RANK</b>                    | <b>Receptor activator of NF-KB</b>                                                                                |
| <b>RANKL</b>                   | <b>Receptor activator of NF-KB ligand</b>                                                                         |
| <b>RAP</b>                     | <b>Receptor-associated protein</b>                                                                                |
| <b>REAL</b>                    | <b>Revised European American Lymphoma</b>                                                                         |
| <b>SF3B1</b>                   | <b>Splicing factor 3b</b>                                                                                         |
| <b>SLL</b>                     | <b>Small lymphocytic lymphoma</b>                                                                                 |
| <b>Smlg</b>                    | <b>Surface membrane immunoglobulin</b>                                                                            |
| <b>SMZL</b>                    | <b>Splenic marginal zone lymphoma</b>                                                                             |
| <b>Snail A zinc</b>            | <b>finger transcriptional repressor which downregulate the expression of ectodermal genes within the mesoderm</b> |
| <b>TB</b>                      | <b>Tuberculosis</b>                                                                                               |
| <b>TCR</b>                     | <b>T cell receptor</b>                                                                                            |
| <b>TdT</b>                     | <b>Terminal deoxynucleotidyl transferase</b>                                                                      |
| <b>TGF-<math>\alpha</math></b> | <b>Transforming growth factor-<math>\alpha</math></b>                                                             |
| <b>TH-1</b>                    | <b>Thelper 1</b>                                                                                                  |

---

### *List of Abbreviations (cont.)*

---

|              |                                                                                                           |
|--------------|-----------------------------------------------------------------------------------------------------------|
| <b>TH-2</b>  | <b>Thelper 2</b>                                                                                          |
| <b>TLR</b>   | <b>Toll- like receptor</b>                                                                                |
| <b>TNF</b>   | <b>Anti- tumor-necrosis factor</b>                                                                        |
| <b>T-reg</b> | <b>Regulatory T cell</b>                                                                                  |
| <b>TTT</b>   | <b>Time to treatment</b>                                                                                  |
| <b>UA</b>    | <b>Uric acid</b>                                                                                          |
| <b>UAE</b>   | <b>United Arab Emirates</b>                                                                               |
| <b>UV</b>    | <b>Ultra violet</b>                                                                                       |
| <b>VD</b>    | <b>Vitamin D</b>                                                                                          |
| <b>VDAs</b>  | <b>Vitamin D analogues</b>                                                                                |
| <b>VDR</b>   | <b>Vitamin D receptor</b>                                                                                 |
| <b>VDRE</b>  | <b>Vitamin D responsive element</b>                                                                       |
| <b>VEGF</b>  | <b>Vascular endothelial growth factor</b>                                                                 |
| <b>WAS</b>   | <b>Wiskott-Aldrich syndrome</b>                                                                           |
| <b>WASP</b>  | <b>Wiskott-Aldrich syndrome protein</b>                                                                   |
| <b>WHO</b>   | <b>World Health Organization</b>                                                                          |
| <b>ZAP70</b> | <b>Protein tyrosine kinase involved in cell signaling<br/>following antigen recognition on lymphocyte</b> |

---

## *List of Tables*

| Table                                 | Title                                                          | page |
|---------------------------------------|----------------------------------------------------------------|------|
| <b>Tables in review of literature</b> |                                                                |      |
| 1                                     | Dietary sources of vitamin D                                   | 7    |
| 2                                     | Causes of vitamin D deficiency                                 | 32   |
| 3                                     | Cell markers in mature B-cell lymphoproliferative disorders    | 67   |
| 4                                     | Immunohistochemistry profile of common lymphomas               | 95   |
| 5                                     | Scoring system for CLL diagnosis                               | 103  |
| <b>List of Tables in Results</b>      |                                                                |      |
| (I)                                   | Patients demographic data                                      | 120  |
| (II)                                  | Laboratory data of CLL patients                                | 121  |
| (III)                                 | Laboratory data of NHL patients                                | 122  |
| (IV)                                  | Laboratory data of Control group                               | 123  |
| (V)                                   | Comparison between group C &LPD case as regard 25(OH)D level   | 124  |
| (VI)                                  | Comparison between group A and group B as regard 25(OH)D level | 126  |
| (VII)                                 | Comparison between group C & A as regard 25(OH)D level         | 127  |
| (VIII)                                | Comparison between group C & B as regard 25(OH)D level         | 128  |

## *List Of Figures*

| Figure                                 | Title                                                                                                                       | Page  |
|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|-------|
| <b>Figures in review of literature</b> |                                                                                                                             |       |
| Fig 1                                  | Isomers of vitamin D2 and D3                                                                                                | 5     |
| Fig 2 a,b                              | Vitamin D3 synthesis, activation, and catabolism                                                                            | 11,12 |
| Fig 3                                  | Role of vitamin D in mediating certain cellular responses to external signals                                               | 12    |
| Fig 4                                  | Renal and extra renal 1, 25 (OH) 2D3 productions serve endocrine, autocrine , and paracrine functions                       | 13    |
| Fig 5                                  | Roles of megalin and intracellular vitamin D binding protein 3 in the delivery and 1- &-hydroxylation of 25hydroxyvitamin D | 15    |
| Fig 6                                  | Diagrammatic representation of the vitamin D endocrine system                                                               | 18    |
| Fig 7a                                 | Overall Survival of AML and vitamin D level                                                                                 | 51    |
| 7b                                     | Progression Free Survival of AML and vitamin D level                                                                        |       |
| Fig 8                                  | Illustrating the panel of markers useful for the diagnosis of chronic lymphoproliferative disorders                         | 65    |
| Fig 9                                  | Cellular origin of B-cell lymphomas                                                                                         | 70    |
| Fig 10                                 | Incidence of NHL                                                                                                            | 73    |
| Fig 11                                 | Primary Cutaneous CD8+ T-Cell Lymphoma                                                                                      | 78    |
| Fig 12                                 | Mycosis Fungoides picture                                                                                                   | 78    |
| Fig 13                                 | Non-Hodgkin lymphoma: parotid mass                                                                                          | 78    |
| Fig 14                                 | Non-Hodgkin lymphoma: pharyngeal                                                                                            | 78    |
| Fig 15                                 | Non-Hodgkin lymphoma: chest x-ray showing isolated pulmonary nodule in patient with AIDs                                    | 80    |

|                                   |                                                                                                 |     |
|-----------------------------------|-------------------------------------------------------------------------------------------------|-----|
| Fig 16                            | A- Non-Hodgkin lymphoma: CT scan of chest<br>B-Non-Hodgkin lymphoma: CT scan of head            | 81  |
| Fig 17                            | A-Non-Hodgkin lymphoma: CT scan of abdomen<br>B-Non-Hodgkin lymphoma: CT scan show parotid mass | 81  |
| Fig 18                            | <i>Follicular lymphoma histology</i>                                                            | 93  |
| Fig 19                            | Burkitt's lymphoma histology                                                                    | 93  |
| Fig 20                            | Hematoxylin and Eosin (H & E) smear from FL tissue.                                             | 93  |
| Fig 21                            | Mantle cell lymphoma histology                                                                  | 93  |
| Fig 22                            | Infiltrating lymphoma cells express strong positivity with anti-CD20 stain, B-cell origin       | 94  |
| Fig 23                            | Histologic analysis of primary mediastinal large B-cell lymphoma                                | 94  |
| Fig 24                            | Peripheral smear from a patient with chronic lymphocytic leukemia                               | 102 |
| Fig 25                            | Treatment Algorithm for AIHA/ITP                                                                | 114 |
| <b>List of Figures in Results</b> |                                                                                                 |     |
| Fig (I)                           | Sex distribution between study groups                                                           | 120 |
| Fig (II)                          | Comparison between group C &LPD case as regard 25(OH) D level                                   | 124 |
| Fig(III)                          | Distribution of vit D deficiency between patients                                               | 125 |
| Fig (IV)                          | Comparison between group A & B as regard 25(OH)D level                                          | 126 |
| Fig (V)                           | Comparison between group C & A as regard 25(OH)D level                                          | 127 |
| Fig (VI)                          | Comparison between group C & B as regard 25(OH)D level                                          | 128 |