# THE VALUE OF $B_2$-MICROGLOBULIN IN THE DIAGNOSIS OF RENAL DISEASES

A THESIS SUBMITTED AS PARTIAL FULFILLMENT
FOR M.D. DEGREE IN INTERNAL MEDICINE

BY

MOHAMMED ABDEL-HAMID ISMAIL EL-BOKL
Assistant Lecturer in Department of Internal Medicine,
Faculty of Medicine ,Ain Shams University

SUPERVISED
BY

Prof. Dr. MOHAMMAD S. SABBOUR
Prof. of Internal Medicine
Faculty of Medicine
Ain Shams University

Prof. Dr. LEILA OSMAN
Prof. of Clinical Pathology
Faculty of Medicine
Ain Shams University

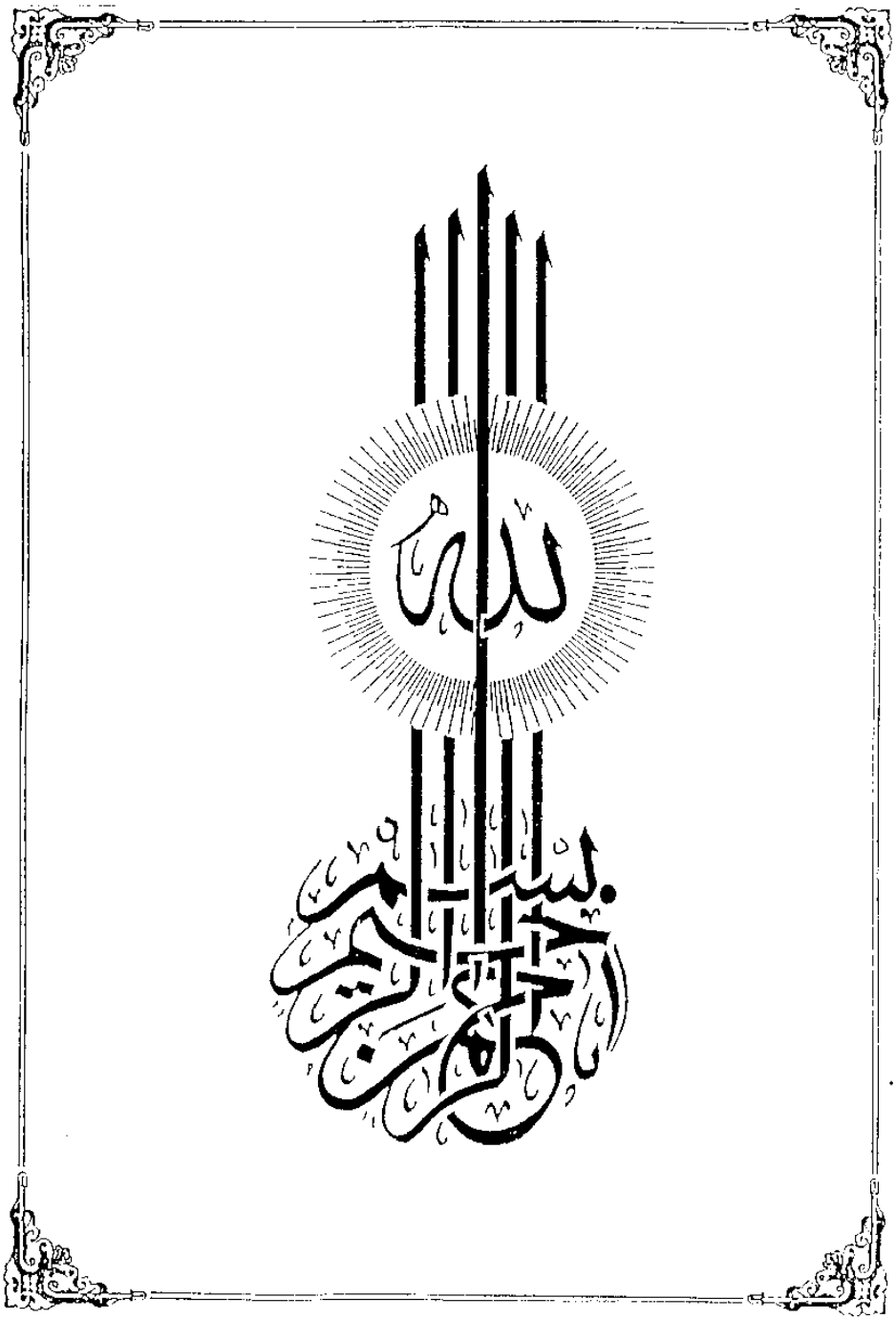
FACULTY OF MEDICINE
AIN SHAMS UNIVERSITY
1985

ACKNOWLEDGMENT

I am, as always, indebted to my Prof. Dr.MOHMMAD S. SABBOUR Prof. of Internal Medicine, Faculty of Medicine, Ain Shams University . I wish to express my highest appreciation for his constructive , valuable critisisms , guidance, and continous encouragement to proceed in this work .

I would like also to express my deepest gratitude to Prof. Dr. LIELA OSMAN Prof. of Clinical Pathology, Faculty of Medicine, Ain Shams University , for her expert assistance and experienced advice she kindly offered me throughout this work .

Finally I extend my gratitude to every one who gave me help and advise .



# CONTENTS


Page

CHAPTER I

FORWARD ---------- 1

INTRODUCTION ---------- 3

- Structure and properties of $B_2$-microglobulin . ---------- 3
- $B_2$-microglobulin synthesis . ---------- 7
- $B_2$-microglobulin in various biological fluids . -------- 10
  - Saliva. ---------- 10
  - Synovial fluid. ---------- 11
  - Cerebro spinal fluid .---------- 11
  - The amniotic fluid. ---------- 15
- The serum level of $B_2$-microglobulin in healthy subjects. 17
- Catabolism of $B_2$-microglobulin. ---------- 18
- The value of $B_2$-microglobulin in renal disorders.-------- 20
  - Estimation of glomerular filtration rate by serum $B_2$-microglobulin. ---------- 20
  - $B_2$-microglobulin in renal tubular disorders.---- 27
  - Differentiation between glomerular and tubular proteinuria .---------- 30
  - $B_2$-microglobulin and kidney transplantation.---- 32
  - $B_2$-microglobulin in obstructive uropathy.-------- 36
  - $B_2$-microglobulin in hepatorenal syndrome.-------- 38
  - $B_2$-microglobulin and nephrotoxicity.---------- 40
    - . Aminoglycoside nephrotoxicity .---------- 40
    - . Heavy metals .---------- 47
- Effects of hyper $B_2$-microglobulinemia.---------- 49
- $B_2$-microglobulin in malignancy .---------- 51
  - $B_2$-microglobulin in hoematological malignancies. 53
    - . Chronic lymphocytic lymphoma.--------- 53
    - . Lymphomas .---------- 55
    - . Multiple myeloma. ---------- 58

Page

CHAPTER II

MATERIAL AND METHODS . ---------- 61

CHAPTER III

RESULTS . ---------- 70

- The diagnostic value of serum $B_2$-microglobulin in assessment of glomerular filtration rate .---------- 70
- $B_2$-microglobulin in various renal diseases .---------- 73
- $B_2$-microglobulin in connective tissue diseases .---------- 79
- The value of urine $B_2$-microglobulin and total protein in differentiation of glomerular, tubular and normal proteinuria .---------- 81
- $B_2$-microglobulin in malignant diseases.---------- 82
- The diagnostic value of serum to urine ratio of $B_2$-microglobulin .---------- 83
- Tables and figures .---------- 84

CHAPTER IV

DISCUSSION .---------- 109

CHAPTER V

SUMMARY .---------- 135

REFERENCES .---------- 140

ARABIC SUMMARY .

| Table No. | | Page |
|---|---|---|
| 13. | $B_2$-microglobulin in patients with obstructive uropathy. | 95 |
| 14. | Urine $B_2$-microglobulin in patients with obstructive uropathy with serum $B_2$-microglobulin $<$ 4500 ug/L. | 96 |
| 15. | $B_2$-microglobulin in patients with polycystic kidneys. | 97 |
| 16. | Urine $B_2$-microglobulin in patients with serum $B_2$-microglobulin $<$ 4500 ug/L. with different renal diseases . | 98 |
| 17. | Urine $B_2$-microglobulin in patients treated with potentially nephrotoxic drugs . | 99 |
| 18. | $B_2$-microglobulin in patients with different connective tissue diseases . | 100 |
| 19. | Distribution of patients with connective tissue diseases according to serum $B_2$-microglobulin and creatinine . | 101 |
| 20 | Urine $B_2$-microglobulin in patients with connective tissue diseases with serum $B_2$-microglobulin $<$ 4500 ug/L. | 102 |
| 21. | Urine protein : Urine $B_2$-microglobulin ratio in patients with different diseases and healthy controls . | 103 |
| 22. | $B_2$-microglobulin in patients with neoplastic disorders. | 104 |
| 23. | Distribution of patients with neoplastic disorders according to serum $B_2$-microglobulin and creatinine . | 105 |
| 24. | Serum to urine ( S/U) ratio of $B_2$-microglobulin in different diseases and controls . | 106 |

# LIST OF TABLES


| Table No. | | Page |
|---|---|---|
| 1. | Serum $B_2$-microglobulin and serum creatinine in patients with creatinine clearance above and below 80 ml/min. | 84 |
| 2. | Serum $B_2$-microglobulin and serum creatinine in patients with creatinine clearance above and below 50 ml/min. | 84 |
| 3. | $B_2$-microglobulin in patients with primary glomerulopathy. | 85 |
| 4. | Distribution of patients with primary glomerulopathy according to serum $B_2$-microglobulin and creatinine . | 86 |
| 5. | Urine $B_2$-microglobulin in patients with primary glomerulopathy with serum $B_2$-microglobulin $<$ 4500 ug/L. | 87 |
| 6. | Urine $B_2$-microglobulin in patients with primary glomerulopathy with serum $B_2$-microglobulin $>$ 4500 ug/L. | 88 |
| 7. | $B_2$-microglobulin and serum creatinine in patients with secondary glomerulopathies . | 89 |
| 8. | Urine $B_2$-microglobulin in patients with secondary glomerulopaties with serum $B_2$-microglobulin $<$ 4500 ug/L. | 90 |
| 9. | Urine $B_2$-microglobulin in patients with secondary glomerulopathies with serum $B_2$-microglobulin $>$ 4500 ug/L. | 91 |
| 10. | $B_2$-microglobulin in relation to the degree of protein-uria in patients with primary and secondary glomerulopathies . | 92 |
| 11. | $B_2$-microglobulin in patients with pyelonephritis . | 93 |
| 12. | $B_2$-microglobulin in patients with pyelonephritis with serum $B_2$-microglobulin $<$ 4500 ug/L. | |

## LIST OF FIGURES


Figure No. — Page

1. Regression lines describing the correlation between creatinine clearance and serum levels of $B_2$-microglobulin ( solid lines ) and creatinine (broken line). 107

2. Urine Protein : Urine $B_2$-microglobulin ratio in patients with different diseases and healthy controls . 108

## FORWARD

$B_2$ - microglobulin was first isolated in 1964 and characterized in 1968 by Berggard and Bearn ( Berggard and Bearn, 1968 ). This protein has been also observed in urine of patients with tubular proteinuria by Piscator, 1966 and by Harrison and Northam,1966. The research that followed the initial discovery of $B_2$- microglobulin has produced a wealth of information concerning structure, synthesis, and turnover of the protein . The determination of this protein has proved to be a valuable research tool in a large number of fields . In clinical medicine , serum and urinary levels in patients with a wide range of disorders have been characterized . In particular, urinary measurments of the protein in conditions with renal tubular dysfunction have proved to be of great value . Serum levels of the protein are dependent on the glomerular filtration rate . Thus, the elevation of a serum value should always take this parameter into consideration . A reduction in the glomerular filtration rate reduces the catabolism of the protein in the kidney and causes high serum levels(Karlsson, et al, 1980 ).

The clinical use of $B_2$-microglobulin serum analysis in inflammatory or neoplastic diseases and in various biological fluid continues to receive attention . In inflammatory disorders

and neoplastic diseases , especially those of a lymphopoetic origin or those accompanied with an activation of the lymphopoetic cells, increased levels of $B_2$-microglobulin are found and reflect an increased rate of synthesis. In such instances, studies of the protein promise to be a diagnostic and prognostic aid ( Karlsson, et al.,1980). When additional information concerning the stimulus for and the regulation of $B_2$-microglobulin production has been obtained, and its detailed function within the immune system has been revealed, it will be possible to explain many of the interesting observations made so far.

# INTRODUCTION

## Structure and properties of $B_2$-microglobulin :

The $B_2$-microglobulin has an almost spherical shape ( Karlsson, 1974) It was reported to consist of a single polypeptide chain composed of 100 aminoacids ( table 1) with 2 half-cystine residues involved in a disulphide bond ( Berggard and Bearn, 1968) . But Parker and Strominger (1982) reported that automated sequence analysis of $B_2$-microglobulin confirms that the puplished sequence contains an error, an additional serine residues at position 67, so human $B_2$-microglobulin has 99 residues like mouse and rabbit homologues. The amino acid composition excludes the possibility of two identical polypeptide chains. It is unlikely that the protein is composed of two unequal chains, since only one $NH_2$ - terminal residue was observed and since the reduced and alkylated protein usually gave only one zone in electrophoresis at both acid and alkaline pH(Berggard and Bearn, 1968). The molecular weight calculated from the amino acid composition ( 11,815) was close to that found by ultra-centrifugation ( 11,600)(Berggard and Bearn,1968 ). The nitrogen content of $B_2$-microglobulin was found to be 16.3% of the dry ash free weight of the protein . This figure accounts for 97% of the theoretical nitrogen content, 16.8%, calculated from

Table (1): Amino acid composition of $B_2$-microglobulin ( Berggard and Bearn, 1968).

| Amino acid | Residues/molecule |
|---|---|
| Lysine | 8 |
| Histidine | 4 |
| Ammonia | 9 |
| Arginine | 5 |
| Aspartic acid | 12 |
| Threonine | 5 |
| Serine | 10 |
| Glutamic acid | 11 |
| Proline | 5 |
| Glycin | 3 |
| Alanine | 2 |
| Half-Cystine | 2 |
| Valine | 7 |
| Methionine | 1 |
| Isoleucine | 5 |
| Leucine | 7 |
| Tyrosine | 6 |
| Phenylalanine | 5 |
| Tryptophan | 2 |
| Total(excluding ammonia ) | 100 |

the amino acid composition . No free sulfhydryl groups were detected . This suggests that the two half-cystine residues observed by amino acid analysis are involved in a disulfide bond( Berggard and Bearn, 1968 ). $B_2$-microglobulin was found to be free from those carbohydrates characteristic of glycoproteins namely, total hexose, hexosamine, and sialic acid .(Berggard and Bearn, 1968 ). The stokes molecular radius of $B_2$-microglobulin is 16 A ( Karlsson, 1974 ).

Table (2): Physical and chemical properties of $B_2$-microglobulin (Berggard and Bearn,1968).

| | |
|---|---|
| Sedimentation Coefficient | 1.6 S |
| Molecular weight | |
| Found | 11,600 |
| Calculated | 11,815 |
| Nitrogen content | |
| Experimental | 16.3 % |
| Calculated | 16.8 % |
| Free sulfhydryl groups | Nil |
| Carbohydrate | Nil |

$B_2$-microglobulin is related to both immune system and the human leucocytic antigen ( HLA ). It exhibits homology with the constant domains of the immunoglobulin G (Ig.G) light and heavy chains and is an integral part of HLA bound to cell surface ( Groves and Greenberg, 1982 ). The HLA molecule is composed of two polypeptide chains; the heavy chain carries the specificity and the light chain is a $B_2$-microglobulin molecule ( Arce-Gomez,et al., 1978). The HLA allo-antigens can be detected by serological methods only if $B_2$-microglobulin is bound to the heavy chain ( Arce-Gomez,et al.,1978)$B_2$-microglobulin seems to control the expression of HLA on the cell surface and possibly their biosynthesis, just like immunoglobulin light chains control the biosynthesis and/or expression of the heavy chain ( Revillard, 1979).

The gene responsible for $B_2$-microglobulin is not in the HLA region ( on chromosome 6 ) but is on chromosome 15 ( Crumpton and Snary, 1977). While the presence of genetic variability in the structural gene for $B_2$-microglobulin cannot be excluded, it is quite unlikely that there is a high degree of variation from individual to individual . No allotype of human $B_2$-microglobulin has so far been identified and no $B_2$-microglobulin allo-antibody has been detected in sera collected after pregnancy, repeated blood transfusions or organ transplantation( Revillard, 1979 ).

$B_2$-microglobulin synthesis :

Studies on the biosynthesis of $B_2$-microglobulin have shown that this molecule is synthesized by almost all human cells ( Revillard, 1979). In normal persons the rate of synthesis is quite constant, about 0.13 mg/h.kg, range 0.11 - 0.18 mg/h.kg. i.e. a mean 24 hours production of about 220 mg $B_2$-microglobulin in a 70 kg person ( Karlsson, 1980 ). It was suggested that the production of $B_2$-microglobulin is reduced in patients with terminal renal insufficiency ( Vincent,et al. 1978). The serum levels of $B_2$-microglobulin in that study were found stable upon sequential measurments over a 6-monthes period. The reduced synthesis of $B_2$-microglobulin might be due to a feed back mechanism controlled by high serum concentration or, alternatively , it could be one consequence of disturbed protein metabolism and/or uraemic toxicity ( Vincent, et al.,1978).

The capacity of normal and malignant human cells to synthesize $B_2$-microglobulin had been studied in vitro by Evrin and Nilsson (1974). Quantitative determination of $B_2$-microglobulin by radioimmunoassay were performed on culture media harvested from freshly explanted lymphoma cells and cell lines of hematopoietic, mesenchymal and epithelial origin . $B_2$-microglobulin was detected in all