RENAL AMYLOIDOSIS IN EGYPTIANS

Thesis Submitted for Partial fulfillment of Master degree in Pathology

By **Amal Mohamed Labib Ali**

M.B.B.Ch., faculty of Medicine
Demonstrator at Pathology Department,
Faculty of Medicine, Ain Shams University
Supervised by

Prof. Elham Ibrahim Seif

Emeritus Professor of Pathology Faculty of Medicine, Ain Shams University

Prof. Manal Ibrahim Salman

Professor of Pathology
Faculty of Medicine, Ain Shams University
Assistant director of ASUSH- Electron microscope laboratory

Dr. Marwa Mosaad Shakweer

Lecturer of pathology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
2016

ACKNOWLEDGEMENT

First and foremost, I would like to thank *God* for blessing this work until it reached its goals.

My profound thanks and deep appreciation to **Prof.** Elham Ibrahim Seif, who suggested the point, for her support and advice, valuable remarks. She gave me the confidence and courage to fulfill this work.

I am also greatly indebted to **Prof**. **Manal Ibrahim Salman**, for her kind supervision, meticulous remarks and direction throughout this work.

I would like to direct my special thanks to **Dr.** *Marwa Mosaad Shakweer*, for her continuous support throughout this time.

My sincere thanks to **Prof. Nadia Galal**, all members of EM unit & pathology lab for their kind help

Finally, my deep thanks to my family for their support.

TABLE OF CONTENTS

Title	Page No
Introduction	1
Aim of the work	3
Review of literature:	
Chapter 1: amyloidosis overview	4-19
Historical review	4
Amyloidosis: Definition, Nomenclature of Fibril Protein and classification	10
Amyloid Fibrils: Characteristics & Structure	15
Chapter 2: diagnosis of amyloidosis	20-39
Introduction	20
Clinical diagnosis	21
Imaging & SAP scanning	21
Tissue diagnosis	23
Subtyping and Identification of Amyloid Protein	30
I. Immunohistochemistry	31
II. Recent techniques	32
Evaluation of organ involvement	35
Staging system for AL amyloidosis	37
Chapter 3: Renal amyloidosis	40-66
Introduction	40
Incidence.	41
I. Amyloid light chain (AL)	42
II. Amyloid associated amyloidosis (AA)	47
III. Amyloid leucocyte chemotactic factor 2	50
IV. Hereditary Amyloidoses	51
Histopathological changes in renal amyloidosis	56
Differential diagnosis of renal amyloidosis	59
Scoring and grading of renal amyloidosis	63

Materials and Methods	67
Results	73
Discussion	94
Conclusion & Recommendations	107
Summary	109
References	112
Arabic Summary	_

LIST OF TABLES

Table No.	Title	Page
		No.
Table 1:	The 31 amyloid fibril proteins and their precursors in human	12
Table 2:	Updated non-invasive diagnostic criteria for amyloid–related organ involvement	36
Table 3:	Required diagnostic evaluation after amyloidosis is established	44
Table 4:	Conventional systemic chemotherapy options for al amyloidosis	46
Table 5:	Renal diseases with organized deposits	60
Table 6:	Histopathologic Classification of Renal Amyloidosis Based on Glomerular Involvement	64
Table 7:	Scoring of Histopathologic Findings, Numeric Codes	65
Table 8:	Renal Amyloid Prognostic Score (RAPS) and Grade	66
Table 9:	Amyloidosis cases among renal biopsies during the period from 1990 to 2015	73
Table 10:	Demographic characteristics of the studied cases	75
Table 11:	Clinical characteristics of the studied cases	77
Table 12:	Proposed causes for secondary amyloidosis among the studied cases	78
Table 13:	Compartmental affection among the studied samples	80

Table 14:	Glomerular affection among the studied samples	82
Table 15:	Vascular affection of the studied samples	83
Table 16:	Interstitial amyloid deposition among the studied samples	84
Table 17:	LM findings among the studied samples	85
Table 18:	Tubular changes among the studied samples	87
Table 19:	Interstitial fibrosis among the studied samples	88
Table 20:	Interstitial inflammatory infiltrate among the studied samples	89
Table 21:	Fibril site and size among the studied samples	90
Table 22:	Amyloidosis types among the studied samples	91
Table 23:	Comparison between amyloidosis types regarding demographic and Clinical characteristics	92
Table 24:	Comparison between amyloidosis types regarding compartmental affection and LM findings	93

LIST OF FIGURES

Figure No	Title	Page No.
Figure 1:	Mechanisms of amyloid formation and toxicity.	19
Figure 2:	Glomerular (G) & vascular (V) amyloid deposits appears as extracellular amorphous eosinophilic material (H&E X400).	94
Figure 3:	Amyloid deposits show pale magenta in comparison to dark magenta tubular basement membrane (PAS X400)	94
Figure 4:	Amyloid deposits appears pinkish gray in Masson Trichrome (MT X400).	95
Figure 5:	A: Glomerular amyloid deposits(G) showing salmon red staining. (Congo red ×400) B: amyloid deposits show apple green birefringence (Congo red / polarized microscopy×400)	96
Figure 6:	A: Glomerular(G) & vascular amyloid deposits(V) showing salmon red staining. (Congo red ×400) B: Amyloid deposits in the vessel wall show apple green birefringence (Congo red/polarized light ×400)	97
Figure 7:	A: Congo red positive interstitial amyloid deposits (Congo red ×400) B: apple green birefringence under polarized light (Congo red ×400)	98
Figure 8:	A: Tubular amyloid deposits showing	99

	salmon red staining. (Congo red ×400) B: Amyloid deposits show apple green birefringence under polarized light (Congo red ×400)	
Figure 9:	A: Concomitant glomerular, vascular, interstitial and tubular amyloid deposits showing salmon red staining. (Congo red x200) B. amyloid deposits show apple green birefringence (Congo red / polarized light x200)	100
Figure 10:	Glomerular(G) and vascular(V) amyloid deposits showing green fluorescence by examination under fluorescent microscope (Thioflavin T x200)	101
Figure 11:	Congo red stained glomerular amyloid deposits giving red fluorescent with examination under fluorescent microscopy (Congo Red x200)	101
Figure 12:	An electron micrograph of part of glomerulus showing amyloid deposition(A) in relation to glomerular basement membrane (GBM) and mesangium; BC: Bowman's Capsule; PEP: parital epithelial cells; VEP: Visceral epithelial cells; ENDO: endothelial cells; MES: mesangial cells; arrow: amyloid spicule (negative mag: 2800)	102

Figure 13:	An electron micrograph of part of glomerulus showing amyloid deposition A in relation to glomerular basement membrane (GBM) and mesangium. BC: Bowman's Capsule; PEP: parital epithelial cells; END: endothelial cells; MES: mesangial cells; CL: capillary lumen. (negative mag: 2800)	103
Figure 14:	An electron micrograph showing amyloid deposition A in relation to tubular basement membranes (TBM1, TBM2) and interstitial tissue; L: tubular lumen; C; collagen fibers. (negative mag. of I: 8000; negative mag. of II: 28000)	104
Figure 15:	An electron micrograph of blood vessel wall showing amyloid deposition A in subendothelial space (L: lumen; ENDO: endothelial cells; SMC: smooth muscle cells of the media (negative mag:2800)	105
Figure 16:	Amyloid fibrils, randomly arranged non branching fibrils measured 12 nm. (negative mag: 28000)	105
Figure 17:	A glomerulus showing nodular (*) deposition of homogenous eosinophilic material (H&E ×400)	105
Figure 18:	A glomerulus showing diffuse mesangial expansion by homogenous eosinophilic material (H&E ×400)	106
Figure 19:	A glomerulus showing mild mesangial expansion by homogenous eosinophilic	106

Figure 20.	material (H&E ×400) A glomerulus showing moderate	106
Figure 20:	mesangial expansion by homogenous	100
	eosinophilic amyloid material 🖈, also an	
	arteriole showing moderate deposition by the same material is seen (arrow) (H&E ×400)	
Figure 21:	A glomerulus (G) showing marked mesangial expansion by homogenous eosinophilic amyloid material (H&E ×400)	108
Figure 22:	an arteriole showing mild deposition of amorphous eosinophilic amyloid material $*(H\&E \times 400)$	108
Figure 23:	V1 & V2 are mostly occluded small arteries by Congo red positive material, V3 is an arteriole at the vascular pole of a free glomerulus (CR X200)	109
Figure 24:	Interstitial amyloid (IA) deposition with tubular atrophy and thyroidization (H&E x 200)	109
Figure 25:	Glomerulosclerosis (G), tubular atrophy together with interstitial fibrosis and moderate interstitial inflammatory infiltrate (MT X200)	110
Figure 26:	Vascular(V) & peritubular deposition of amyloid material along with marked interstitial inflammatory infiltrate(T: tubules) (H&E x400).	110
Figure 27:	A glomerulus showing positive extracellular homogenous brown staining (*) for serum amyloid A (SAA) (IHC x400)	111

Figure 28	A case of AA amyloidosis showing glomerular and vascular amyloid deposits stained positive for anti SAA (IHC X400)	111
Figure 29:	A case of AA amyloidosis showing glomerular amyloid deposits stained positive for anti SAA (IHC X200)	112
Figure 30:	A case of AA amyloidosis showing vascular and tubular basement membrane(TBM) amyloid deposits stained positive for anti SAA (IHC X200)	112
Figure 31:	A case of AA amyloidosis showing interstitial amyloid deposits stained positive for anti SAA (IHC X200)	113
Figure 32:	Human tonsil showing selective cytoplasmic staining of plasma cells to lambda light chain (IHC x400)	114
Figure 33:	Human tonsil showing selective cytoplasmic staining of plasma cells to lambda light chain (IHC x400)	114
Figure 34:	Human tonsil showing selective cytoplasmic staining of plasma cells to kappa light chain (IHC x400)	115
Figure 35:	A glomerulus showing moderate staining of capillary walls for Kappa light chain in a case of AL amyloidosis- Kappa. (IHC x400)	115

LIST OF GRAPHS

Graph no	Title	Page no
Graph 1:	Amyloidosis cases among renal biopsies during the period from 1990 to 2015	74
Graph 2:	Sex distribution among the studied cases	75
Graph 3:	Clinical characteristics of the studied cases	77
Graph 4:	Causes of secondary amyloidosis among the studied cases	79
Graph 5:	Pattern of Compartment affection among the studied samples	81
Graph 6:	Glomerular affection among the studied samples	82
Graph 7:	Vascular affection among the studied samples	83
Graph 8:	Interstitial amyloid deposition among the studied	84
Graph 9:	LM findings among the studied samples	86
Graph 10:	Tubular changes among the studied sample	87
Graph 11:	Interstitial fibrosis among the studied samples	88
Graph 12:	Interstitial inflammatory infiltrate deposition among the studied samples	89
Graph 13:	Fibril site among the studied samples	90
Graph 14:	Amyloidosis types among the studied samples	91

LIST OF ABBREVIATIONS

AA: Amyloid associated amyloidosis

AD: Alzheimer Disease

AFib: Fibrinogen A α chain amyloidosis

AGel: Gelsolin amyloidosis **AH**: Heavy chain amyloidosis

AHL: Amyloid Heavy & Light chain

AL: Light chain amyloidosis

ALECT2: Leukocyte chemotactic factor 2 amyloidosis

ALys: Lysozyme amyloidosis **ANS**: Autonomic nervous system

Apo AI: Apolipoprotein **A1** amyloidosis **Apo AII**: Apolipoprotein **A2** amyloidosis **Apo AIV**: Apolipoprotein **A4** amyloidosis

APP: β -amyloid precursor protein

ASUSH- EM lab: Ain Shams University Specialized Hospital

- Electron Microscopy laboratory **ATTR**: transthyretin amyloidosis

β2M: Beta 2 Microglobulin

CJD: Creutzfeldt-Jakob disease

CMR: Cardiac Magnetic Resonance Imaging

CNS: Central nervous system

COPD: Chronic obstructive pulmonary disease

CR: Congo red

CRPM: Congo red under polarized microscopy

CST: Cystatin C

CT: computed tomography

EM: electron microscopy

ESRD: End-stage renal disease

FAP: Familial Portuguese Amyloid Polyneuropathy

FGN: fibrillary glomerulonephritis

FLC: free light chain

FMF: familial Mediterranean fever **GA**: glomerular amyloid deposition

GAGs: glycosaminoglycans

GBM: glomerular basement membrane

GFR: glomerular filtration rate

GI: gastrointestinal

 $\textbf{GN:}\ glomerul on ephritis$

GP: glomerulopathy

GS: Glomerular sclerosis

H&E: Hematoxylin and eosin **HDL**: high-density lipoprotein

IA: Interstitial amyloid deposition

IBM/ SSPS: International Business Machines/ Statistical

Package for Social Sciences **IF**: immunofluorescence

Ifib: Interstitial fibrosis and tubular atrophy

Ig: immunoglobulin

IHC: *immunohistochemistry*

Iinf: Interstitial inflammatory infiltration

IL6: interleukin-6

ISA: International Society of Amyloidosis

JMS: Jones methenamine silver

LM: light microscopy

LMD/MS: laser microdissection/mass spectrometry **MPGN**: membranoproliferative glomerulonephritis

MSGB: Minor salivary gland biopsy

NT-proBNP: N-terminal pro-brain natriuretic peptide

PAS: periodic acid Schiff

PNS: Peripheral nervous system

RAPS: Renal Amyloid Prognostic Score

SAA: serum amyloid A

SAP: Serum Amyloid P component **SCA**: Senile Cardiac Amyloidosis

SCT: Stem cell Transplant

SLE: systemic lupus erythematosus **SSA**: senile systemic amyloidosis

TB: tuberculosis

TBM: tubular basement membrane

TEM: Transmission electron microscopy

TNF: Tumor Necrosis Factor

TnT: Troponin T

VA: Vascular amyloid deposition

WHO-IUIS: World Health Organization/International Union

of Immunological Studies