

**EFFECT OF INTRAVENOUS IRON THERAPY ON PAPP-
A AND CD40 LIGAND MARKERS IN CHRONIC RENAL
FAILURE PATIENTS**

A Thesis

Submitted to the Medical Research Institute

Alexandria University

For partial fulfillment of the

Requirement of the degree of

Ph.D. Degree

In

Applied Medical Chemistry

By

Saber Hamed Abdel Gawad Mostafa

Master in Applied Medical Chemistry

Medical Research Institute

Alexandria University

2001

Medical Research Institute

Alexandria University

2010

EFFECT OF INTRAVENOUS IRON THERAPY ON PAPP-A AND CD40 LIGAND MARKERS IN CHRONIC RENAL FAILURE PATIENTS

Presented by
Saber Hamed Abdel Gawad Mostafa
M.Sc. in Applied Medical Chemistry 2001

For the Degree of
Ph. Doctor
In
Applied Medical Chemistry

Examiners Committee

Approved

Prof. Dr. Zienat Abdel-Fatah El-Kholy

.....

*Prof. of Applied Medical Chemistry
Applied Medical Chemistry Department
Medical Research Institute
Alexandria University*

Prof. Dr. Usama Abdel-Azim Sharaf El-Din

.....

*Prof. of Internal Medicine
Faculty of Medicine
Cairo University*

Prof. Dr. Abdallah Mahmoud Fouda

.....

*Prof. of Biochemistry
Biochemistry Department
Faculty of Medicine
Tanta University*

Prof. Dr. Nabila Gaber Ali Hussein

.....

*Prof. of Applied Medical Chemistry
Applied Medical Chemistry Department
Medical Research Institute
Alexandria University*

Advisors' Committee:

Prof. Dr. Zienat Abdel-Fatah El-Kholy

*Prof. of Applied Medical Chemistry
Applied Medical Chemistry Department
Medical Research Institute
Alexandria University*

Prof. Dr. Usama Abdel-Azim Sharaf El-Din

*Prof. of Internal Medicine
Faculty of Medicine
Cairo University*

Prof. Dr. Hesham Gamal El-Din Hamed

*Prof. of Clinical Pathology and Immunology
National Research Center*

Prof. Dr. Neveen Abd El Moneim Hussein

*Lecturer of Applied Medical Chemistry
Applied Medical Chemistry Department
Medical Research Institute
Alexandria University*

تأثير محقار الحديد الوريدي على بعض معاملات PAPP-A

و CD40 Ligand في مرضى الفشل الكلوي المزمن

مقدمة إلى معهد البحوث الطبية

جامعة الإسكندرية

إيفاء جزئياً لشروط الحصول على درجة

الكيمياء الطبية التطبيقية

ماجستير الكيمياء الطبية التطبيقية

معهد البحوث الطبية

جامعة الإسكندرية

قسم الكيمياء الطبية التطبيقية

معهد البحوث الطبية

جامعة الإسكندرية

تأثير محقار الحديد الوريدي على بعض معاملات PAPP-A و CD40 Ligand في مرضى الفشل الكلوي المزمن

ماجستير الكيمياء الطبية التطبيقية جامعة الإسكندرية

الكيمياء الطبية التطبيقية

.....

. / زينات عبدالفتاح الخولى
أستاذ الكيمياء الطبية التطبيقية
قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

.....

. / اسامة عبد العظيم شرف الدين

كلية الطب
جامعة القاهرة

.....

. /
الكيمياء الحيوية
قسم الكيمياء الحيوية
كلية الطب

.....

. / نبيلة على حسين
الكيمياء الطبية التطبيقية
قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

.....

. / زينات عبد الفتاح الخولى
أستاذ الكيمياء الطبية التطبيقية
قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

.....

. / أسامة عبد العظيم شرف الدين
كلية الطب
جامعة القاهرة

.....

. / هشام جمال الدين حامد
أستاذ الباثولوجيا الإكلينيكية والمناعة

.....

. / نيفين عبد المنعم حسين
مدرس الكيمياء الطبية التطبيقية
قسم الكيمياء الطبية التطبيقية
معهد البحوث الطبية
جامعة الإسكندرية

Dedicated to

The Soul of My Mother

My Beloved Father

My Sincere Wife

My Beloved Children

Malak & Mena

Acknowledgement

Praise to **ALLAH**, the most gracious and the most merciful who guides us the right way. Without his help nothing could be achieved.

I would like to express sincere gratitude and appreciations to my supervisor **Prof. Dr Zienat Abdel-Fatah El-Kholy** Prof. of Applied Medical Chemistry, Medical Research Institute, Alexandria University, she has generously denoted much of her time to achieve this work, and revision offers, without her ideas, her efforts, guidance and cooperation, this work will not be done.

I would like to express my great respect and gratitude to my supervisor **Prof. Dr Usama Abdel-Azim Sharaf El-Din** Prof. of Internal Medicine, Faculty of Medicine, Cairo University, for his suggestion the points of the study, remarkably valuable guidance and precious instructions during performing this work. His unlimited support was undoubtedly.

I would like to express my deepest gratitude and especially thanks to my supervisor **Prof. Dr Hesham Gamal El-Din Hamed** Prof. of Clinical Pathology and Immunology, National Research Center, for his help and valuable guidance and revision of this work.

Words cannot express my appreciation and respect to my supervisor **Dr. Neveen Abd El Moneim Hussein** Lecturer of Applied Medical Chemistry, Medical Research Institute, Alexandria University, for her help and extraordinary effort in revision of this work.

My great appreciation is extended to all those who shared either practically or morally in the accomplishment of this work.

LIST OF CONTENTS

Chapter	Page
ACKNOWLEDGEMENT.....	i
LIST OF CONTENTS.....	ii
LIST OF TABLES.....	iii
LIST OF FIGURES.....	v
LIST OF ABBREVIATIONS	vii
• Introduction	1
• Pathophysiology of the anemia of chronic renal failure.....	3
• Iron deficiency anemia.....	9
• Iron supplements (therapy).....	13
• Cardiovascular aspects of chronic renal failure.....	14
• Cardiovascular disease Biomarkers.....	26
• Proinflammatory cytokines CD40 ligand.....	28
• Pregnancy associated plasma protein-A.....	30
• Aim of the work.....	31
• Subjects and Methods.....	32
• Results.....	46
• Discussion.....	75
• Summary and Conclusion.....	80
• References.....	84
• Arabic Summary.	
• Protocol.	

LIST OF TABLES

Table	Page
(I) Traditional and nontraditional risk factors for cardiovascular disease in chronic kidney disease.	18
(II) Potential causes of inflammation in patients with CRF.	23
(III) The determination of prothrombin conc. and INR.	36
(IV) The evaluation of fibrinogen concentration level.	38
(1) Age range, mean and the median of the three groups.	46
(2) Results of routine and coagulation parameters of the CRF patients group (I).	47
(3) Results of the pregnancy associated plasma protein-A level in CRF patients group (I).	48
(4) Results of the cytokine CD40 Ligand percent in CRF patients group (I).	48
(5) Results of routine and coagulation parameters of the CRF patients not yet dialyzed group (II).	49
(6) Results of the pregnancy associated plasma protein-A level in CRF patients not yet dialyzed group (II).	50
(7) Results of the cytokine CD40 Ligand percent in CRF patients not yet dialyzed group (II).	50
(8) Results of routine, coagulation and the specific parameters of the control group (III).	51
(9) Statistical analysis of specific parameters of the CRF patients group (I) and group (II) versus the control group (III).	53
(10) Statistical analysis of serum PAPP-A level for CRF patients group (I).	57

Table	Page
(11) Statistical analysis of serum CD40 Ligand percent for CRF patients group (I).	60
(12) Statistical analysis of serum PAPP-A level for CRF patients group (II).	63
(13) Statistical analysis of serum CD40 Ligand percent for CRF patients group (II).	66
(14) Effect of maintenance hemodialysis on the CRF patients group (I) versus the group (II).	69
(15) Effect of intravenous iron therapy on the CRF patients group (I) versus the CRF patients group (II).	73

LIST OF FIGURES

Figure	Page
(1) The normal erythropoiesis process.	4
(2) The mechanism by which anemia can cause heart failure and renal failure.	5
(3) The cardio-renal anemia syndrome.	6
(4) Anemia triggers a multitude of process designed to compensate for reduced oxygen carriage and tissue hypoxia.	8
(5) Peripheral Blood Smear, Iron-deficiency anemia.	10
(6) The iron cycle in hemodialysis patients.	12
(7) Cardiomyopathy and ischemic heart disease in chronic uremia.	14
(8) Left ventricular pressure overload, left ventricular volume overload and myocyte death in chronic kidney disease.	15
(9) Cardiovascular risk factors in chronic kidney disease.	17
(10) The process of oxidative stress in end stage renal dialysis patients.	21
(11) Cytokines involved in atherogenesis. Cytokines are produced by several cell types, including inflammatory and vascular cells.	25
(12) Biomarkers of acute coronary syndromes.	27
(13) The standard curve for determination of pregnancy associated plasma protein –A.	43
(14) Fibrinogen level among CRF patients group (I) & group (II) versus control group (III).	54
(15) Ferritin conc. among CRF patients group (I) & group (II) versus control group (III).	54

Figure		Page
(16)	Highly sensitive C-reactive protein Conc. among CRF patients group (I) & group (II) versus control group (III).	54
(17)	Pregnancy associated plasma protein-A level among CRF patients group (I) & group (II) versus control group (III).	55
(18)	Cytokine CD40 Ligand percent among CRF patients group (I) & group (II) versus control group (III).	55
(19)	Pregnancy associated plasma protein-A level among CRF patients group (I).	58
(20)	Cytokine CD40 Ligand percent among CRF patients group (I).	61
(21)	Pregnancy associated plasma protein-A level among CRF patients group (II).	64
(22)	Cytokine CD40 ligand among CRF patients not yet dialyzed group (II).	67
(23)	Effect of maintenance hemodialysis on fibrinogen level among CRF patients group (I) versus group (II).	70
(24)	Effect of maintenance hemodialysis on fibrinogen level among CRF patients group (I) versus group (II).	70
(25)	Effect of maintenance hemodialysis on highly sensitive C-reactive protein conc. among CRF patients group (I) versus group (II).	70
(26)	Effect of maintenance hemodialysis on pregnancy associated plasma protein-A level among CRF patients group (I) versus group (II).	71
(27)	Effect of maintenance hemodialysis on cytokine CD40 ligand percent among CRF patients group (I) versus group (II).	71
(28)	Effect of intravenous iron therapy on pregnancy associated plasma protein-A level among CRF patients group (I) versus group (II).	74
(29)	Effect of intravenous iron therapy on cytokine CD40 ligand percent among CRF patients group (I) versus group (II).	74

LIST OF ABBREVIATIONS

ACS	Acute coronary syndrome
ADP	Adenosine Diphosphate
AGEs	Advanced glycation end products
ARF	Acute renal failure
ARIC	Atherosclerosis risk in communities
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
CD40 L	Cluster of differentiation 40 ligand
CHF	Congestive heart failure
CKD	Chronic kidney disease
CRF	Chronic renal failure
CRP	C- reactive protein
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
EC	Endothelial cells
EPO	Erythropoietin
ESRD	End stage renal disease
Fe	Iron
FeOOH	Ferric oxyhydroxide
GFR	Glomerular filtration rate
HD	Hemodialysis
HDL	High density lipoprotein
H₂O₂	Hydrogen peroxide
IGFBP-4	Insulin-like growth factor-binding protein 4
IGFBP-5	Insulin-like growth factor-binding protein 5
IGF-I	Insulin-like growth factor I
IL-1	Interleukin 1
IL-6	Interleukin 6
IV	Intravenous
KGD	Protein (Lys – Gly – Asp)

LDL	Low density lipoprotein
LV	Left ventricle
LVH	Left ventricular hypertrophy
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MEIA	Microparticle enzyme immunoassay
NA	Not applicable
ND	Not done
NKT	Natural killer T cells
NO[•]	Nitric oxide
O₂^{•-}	Superoxide
[•]OH	Hydroxyl radical
PAPP-A	Pregnancy associated plasma protein-A
PD	Peritoneal dialysis
PE	Phycoerythrin
proMBP	Proform of eosinophil major basic protein
PWV	Pulse wave velocity
RAS	Renin angiotensin system
RCT	Randomized controlled trial
ROS	Reactive oxygen species
sCD40L	Soluble CD40 ligand
sICAM-1	Soluble intercellular adhesion molecules-1
SNS	Sympathetic nervous system
sVCAM-1	Soluble vascular adhesion molecules-1
TIBC	Total iron binding capacity
TNF	Tumor necrosis factor
UK	United Kingdom