EXPRESSION OF NITRIC OXIDE GENE IN ATHEROSCLEROTIC OCCLUSIVE ARTERIAL DISEASE

Thesis

Submitted for Complete Fulfillment of
The M.D. Degree in

General Surgery

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2010

بسم الله الرحمن الرحيم

"ويسئلونك عن الروح قل الروح من أمر ربي
وما أوتيتم من العلم إلا قليلا"

صدق الله العظيم
(الآية: ٨٥، سورة الإسراء)

ACKNOWLEDGEMENT

Thanks first and last to ALLAH for his great care, support, and guidance in every step in our life

It is my pleasure to express my deepest gratitude and sincere thanks to Prof. Dr. **Ashraf Mohamed Hedayet** *Professor of General & Vascular Surgery, Faculty of Medicine, Cairo University, for his generous concern, sincere supervision, continuous advise and support.*

The credit of bringing this work to light goes to Prof. Dr. **Olfat Gamil Shaker** *Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University. Her continued help and guidance made it possible to bring this work to its final shape. No words can express my feelings towards her.*

My deepest gratitude, appreciation and thanks to Dr. **Ayman Rifaat Abd-Elhassib** *Lecturer of General & Vascular Surgery, Faculty of Medicine, Bani suife University, for his sincere supervision, cooperation, continuous support throughout this work, valuable suggestion and saving no effort or time in reading each word in this work. To him I will always be grateful.*

I wish to thank Dr. **Dr. Hani Abd-Elfatah Zaied** *Lecturer of General & Vascular Surgery, Faculty of Medicine, Bani suife University. His constant encouragement and valuable advises were indispensable. I am definitely indebted to him more than I can express.*

I wish to thank very much and express my deepest gratitude to Prof. Dr. **Ayman Al-Samadoni,** *Professor of General and Vascular Surgery, Faculty of Medicine, Cairo University, for his sincere supervision, valuable suggestions and cooperation, continuous advise and support saving no effort or time in helping me in this work and in reading each word in this work. To him I will always be grateful.*

Finally, I should thank my senior staff, my colleagues at the General Surgery Department, Faculty of Medicine, Cairo University, who helped me a lot throughout this work.

Especial thanks to Prof. Dr. **Mostafa Lotfy Abo El-Nasr,** *Professor and Head of General Surgery Department, Faculty of Medicine, Cairo University, for his generous concern, sincere supervision, continuous advise and support saving no effort to help me. To him I will always be grateful.*

Khaled A. Shawky,
November, 2010

TO MY PARENTS

AND

MY FAMILY

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ABBREVIATIONS

ABI	:	Ankle brachial index
ACE	:	Angiotensin converting enzyme
Ach	:	Acetylcholine
AdiNOS	:	inducible nitric oxide synthase Complementary deoxyribonucleic acid (DNA)
ADMA	:	Asymmetric dimethylarginine
ADP	:	Adenosine diphosphate
AlbSNO	:	Complementary deoxyribonucleic acid (DNA) S-nitrosoalbumin
ApoA	:	Apolipoprotein A_1
ATP	:	Adenosine triphosphate
BH_4	:	Tetrahydrobiopterin
BK	:	Bradykinin
CAD	:	Coronary artery disease
CaM	:	Calcium calmodulin
cAMP	:	Adenylate cyclase
CETF	:	Cholesterol ester transfer protein
cGMP	:	Cyclic guanosine monophosphate
CHD	:	Coronary heart disease
CRP	:	C-reactive protein
CVD	:	Cardiovascular disease
EC	:	Endothelial cell
ECAMs	:	Endothelial cell adhesion molecules
ECM	:	Extracellular matrix
EDCF	:	Endothelium derived contracting factor
EDHF	:	Endothelium derived hyperpolarizing factor
EDRF	:	Endothelial derived relaxing factor
ELAM-1	:	Endothelial leukocyte adhesion molecule
eNOS	:	Endothelial nitric oxide synthase
FCHL	:	Familial combined hyperlipidemia
FGF	:	Fibroblast growth factor
FMD	:	Flow mediated dilatation
GC	:	Guanylate cyclase
GTP	:	Guanosine triphosphate
H_2O_2	:	Hydrogen peroxide
HDL	:	High density lipoprotein
HMG-CoA	:	Hydroxymethylglutaryl coenzyme A
ICAM	:	Intercellular adhesion molecule-1

ICAMs	:	Intercellular adhesion molecules
IFN	:	Interferon
IHD	:	Ischemic heart disease
IL	:	Interleukin
iNOS	:	Inducible nitric oxide synthase
ITP	:	Inositol triphosphate
LCAT	:	Lecithin cholesterol acyl transferase
LDL	:	Low density lipoprotein
LDLC	:	Low density lipoprotein cholesterol
LFA-1	:	Leukocyte function associated antigen
L-NA	:	N^G-nitro-L-arginine
L-NAME	:	N^G-nitro-L-arginine methylester
L-NMMA	:	N^G-monomethyl-L-arginine
LP(a)	:	Lipoprotein(a)
LPS	:	Lipopolysaccharides
Lyso-PC	:	Lysophosphatidylcholine
MI	:	Myocardial infarction
NADPH	:	Nicotine adenine dinucleotide phosphate
NF_KB	:	Nuclear factor Kappa-B
nNOS	:	Neuronal nitric oxide synthase
NO	:	Nitric oxide
NOS	:	Nitric oxide synthase
O_2^-	:	Superoxide
oxLDL	:	Oxidized low density lipoprotein
PAD	:	Peripheral arterial disease
PADGEM	:	Platelet activation dependent granule external membrane protein
PAI-1	:	Plasminogen activator inhibitor
PAOD	:	Peripheral arterial occlusive disease
PDGF	:	Platelet derived growth factor
PET	:	Positron emission tomography
PGI_2	:	Prostacyclin
PKA	:	Protein kinase A
PKC	:	Protein kinase C
PKG	:	Protein kinase G
PLC	:	Phospholipase C
PMCA	:	Plasma membrane pump
QCA	:	Quantitative coronary angiography
ROS	:	Reactive oxygen species
SFA	:	Superficial femoral artery
SIN-1	:	3-morpholino-sydnonimine

SMCs	:	Smooth muscle cells
SNAP	:	S-nitroso-N-acetylpenicillamine
SOD	:	Superoxide dismutase
TC	:	Total cholesterol
TG	:	Triglycerides
TGF-β	:	Transforming growth factor- β
TNF	:	Tumor necrosis factor
t-PA	:	Tissue plasminogen activator
VCAM	:	Vascular cell adhesion molecule
VLDL	:	Very low density lipoprotein
VSMCs	:	Vascular smooth muscle cells
WHHL	:	Watanabe heritable hyperlipidemic
WHO	:	World Health Organization

ABSTRACT

Aim of this work is to detect the expression of eNOS gene in the blood and arterial wall of atherosclerotic occlusive arterial disease patients as well as normal vessels.

This study was carried out on the 40 patients who are suffering from atherosclerotic occlusive arterial disease (23 males and 17 females) and 10 patients (10 male) who are had traumatic arterial injuries as control group. The range of age was 7-95 years for all patients, 42-95 years for the diseased group (POAD), 7-36 years for the control group.

The results of this study showed there is statistical significance in correlation between the two studied groups as there is decrease in expression of eNOS gene in the diseased group than the control group in both tissue (arterial wall) and blood.

Keywords:

Atherosclerotic occlusive arterial disease
Nitric Oxide (NO)
Nitric Oxide Synthase (NOS)
Endothelial Nitric Oxide Synthase (eNOS)

INTRODUCTION

INTRODUCTION

Nitric oxide (NO) is produced by NO synthases (NOS), which oxidize L-arginine to L-citruline NO, the biologically active component of endothelium-derived relaxing factor, has a critical role in the maintenance of vascular homeostasis. Decreased endothelial NO production, as a result of endothelial dysfunction, occurs in the early stage of atherosclerosis and plays a prominent role in endothelium dysfunction. Based on theoretical background, enzymes involved in the metabolism of NO and reactive oxygen species (ROS) play an important role in the development of endothelial dysfunction **(Channon** *et al.,* **2000)**.

There are 3 NOS isoforms. All 3 NOS isoforms have a similar molecular structure and require multiple cofactors, including flavins, Nicotine adenine dinucleotide phosphate (NADPH), and tetrahydrobiopterin, that are required to maintain dimerization and NO production **(Arnal** *et al.,* **1999).**

Neuronal (nNOS, or NOS I) and endothelial (eNOS, or NOS III) isoforms are constitutively expressed and are activated by calcium-calmodulin. The inducible isoforms (iNOS, or NOS II) are regulated primarily at the transcriptional level, independent of agonist stimulation and intracellular calcium levels. eNOS, expressed in endothelial cells, is the predominant NOS isoform in the vessel wall. Under basal conditions, eNOS is inactive and remains membrane bound by virtue of myristoylation, palmitoylation, and an inhibitory

interaction with caveolin, the principal structural protein in caveolae **(Channon** *et al.*, **2000)**.

Shear stress is an important physiological stimulator of eNOS activity, causing rapid membrane release and upregulating eNOS gene expression by transcriptional activation of the eNOS promoter. After vessel injury or in disease states, iNOS expression may be induced in the media, atherosclerotic plaque, or neointima ***(Kubes** et al., **1991).***

The role of endothelial derived NO in the regulation of vascular tone and organ blood flow is well established. Although three isoforms of the human nitric oxide synthase (NOS) gene family are known to exist, it is the endothelial nitric oxide synthase (eNOS) gene that accounts for the synthesis and release of bioactive endothelium derived relaxing factor (EDRF) ***(Wang and Marsden, 1995).***

In addition to relaxing vascular smooth muscle cells, endothelium derived NO inhibits platelet and leukocyte adhesion to vascular endothelium, inhibits vascular smooth muscle cell migration and growth, and limits the oxidation of athergenic low-density lipoprotein. These actions suggest that endothelial NO may have an important atheroprotective role beyond its effect on vessel tone and blood pressure and that an alteration in the activity of the vascular NO system could contribute to the pathogenesis of atherosclerosis ***(Anouk and Marsden, 2000).***

Many investigators have chosen eNOS as a candidate for vascular gene transfer on the basis that eNOS is the predominant isoform present in the normal vessel wall. eNOS gene transfer