Endothelial Nitric Oxide Synthase G894T Gene Polymorphism and Ischemic Heart Disease among Smokers: A Pilot Study

Thesis

Submitted for Partial Fulfillment of Master Degree
in Clinical and Chemical Pathology

By

Hanaa Moustafa El-Saied Mohamed
M.B.B.Ch
Faculty of Medicine - Ain Shams University

Supervised by

Prof. Afaf Abd El Alim Mostafa
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Dr. Rania Ahmed AboShady
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Dr. Dina Aly Mohamed Aly Ragab
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
2017

سورة البقرة الآية: ٣٢

First of all, thanks to Allah whose magnificent help was the main factor in completing this work.

Foremost, I would like to express my sincere gratitude to my advisor Prof. Dr. Afaf Abdelalim Mostafa for the continuous support of throughout my thesis, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor.

My sincere thanks To Asst. Prof. Rania Ahmed Aboshady, for the useful comments, remarks and engagement through the learning process of this master thesis. I'm very grateful for her continuous encouragement and guidance.

My greatest honor was working under supervision of my kindest heart Dr. Dina Aly Ragab. I am so grateful for your precious time and patience at all my hard times.

Lastly, I would like to thank Cardiology unit at Ain Shams University for permitting me to collect subjects and data from.

Contents

List of Abbreviations

AMI	**Acute myocardial infarction.**
ADMA	Asymmetric dimethylarginine.
BH_4	Tetrahydrobiopterin.
Bp	Base pair.
CAD	Coronary artery disease.
CaM	Calmodulin.
CK	Creatine kinase.
CKD	Chronic kidney disease.
CK-MB	Creatine kinase MB fraction.
CNS	Central nervous system.
CRP	C-reactive protein.
CT	Computed tomography.
cTnI	Cardiac troponine I.
cTnT	Cardiac troponine T.
DN	Diabetic nephropathy.
DNA	Deoxyyribonucleicacid.
DDAH	Dimethyl arginine dimethyl amino hydrolase.
ECHO	Electrocardiography.
ECG	Electrocardiogram.
EDTA	Ethylenediaminetetraacetic acid.
EDRF	Endothelium-derived relaxing factor.
eNOS	Endothelial nitric oxide synthase.
FAD	Flavin adenine dinucleotide.
FMN	Flavin mononucleotide.
HLA	Human leukocyte antigen.
IHD	Ischemic heart disease.
IL	Interleukin.

iNOS	Inducible nitric oxide synthase.
IQR	Interquartile range.
LDL	Low density lipoprotein.
L-NMMA	N-monomethyl-L-arginine
MMPs	Metalloproteinases.
MI	Myocardial infarction.
mRNA	Messenger ribonucleic acid.
NADPH	Nicotinamide adenine dinucleotide phosphate.
NOS-1	Nitric oxide synthase 1.
NOS-2	Nitric oxide synthase 2.
NOS-3	Nitric oxide synthase 3.
nNOS	Neuronal nitric oxide synthase.
NO	Nitric oxide.
OX	Oxidative.
PCR	Polymerase chain reaction.
ROS	Reactive oxygen species.
SD	Standard deviation.
SMC	Smooth muscle cells.
SNP	Single nucleotide polymorphism.
SLE	Systemic Lupus Erythematosus.
TIMPs	Tissue inhibitors of Metalloproteinases.
TLR	Toll- like receptors.
TNF	Tumor necrosis factor.
T2DM	Type 2 diabetes mellitus.
UA	Unstable angina.
VCAM-1	Vascular-cell adhesion molecule-1.
VNTR	Variable number tandem repeat.
WHO	World Health Organization.

List of Figures

List of Tables

Abstract

Background: IHD is one of the commonest causes of morbidity and mortality. Atherosclerosis is the basis of CAD; it results in asymmetrical thickening of the arterial wall. Nitric oxide plays an important role in prevention of atherosclerosis. Many studies were interested in studying the association between G894T gene polymorphism and IHD.

Objectives: To determine the association between G894T gene polymorphism and IHD among smokers.

Methods: 80 subjects were included (40 IHD cases and 40 controls). Demographic and clinical data were collected for both groups (age, history of DM, history of HT, family history of IHD). Real time PCR reaction was used to determine the gene polymorphism.

Results: Both descriptive and analytical statistics were done. There was no statistically significant difference between cases and controls regarding the genotype and allelic frequency.

Conclusion: eNOS G894T gene polymorphism doesn't increase risk of IHD among smokers according to our results.

Key words: Ischemic heart disease, endothelial nitric oxide, gene polymorphism, smoking, G894T gene polymorphism .

Introduction

Ischemic heart disease, also known as coronary heart disease is a group of diseases that includes: stable angina, unstable angina, myocardial infarction, and sudden coronary death **(Torpy et al., 2009)**. In 2013, ischemic heart disease was the most common cause of death globally, resulting in 8.14 million deaths **(Mortality and Causes of Death Collaborators, 2015).**

Ischemic heart diseases are the result of complex interactions between a great number of risk factors including high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, and excessive alcohol in addition to the presence of one of several recently identified genetic polymorphisms **(Drenos et al., 2015).**

Endothelial dysfunction is integrally involved in the pathogenesis of ischemic cardiovascular disease, and has been associated with an increased risk of myocardial infarction and stroke **(Lee et al., 2006).**

In the endothelial cell, nitric oxide (NO) is synthesized by the endothelial nitric oxide synthase (eNOS)

encoded by a 26 exon gene located on chromosome 7 **(Dellamea et al., 2014).** 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 atherogenic low-density lipoprotein. These actions suggest that endothelial NO may have an important athero-protective 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 **(Kawashima et al., 2004).** Defects in endothelial cell function and NO production have been described for subjects with atherosclerosis, hypertension, diabetes, as well as obesity **(Bressler et al., 2013).**

Genetic polymorphisms are very common. Studies have shown that at least 30% of structural gene loci of enzymes and proteins are polymorphic, but this usually has no functional significance **(Hopkinson and Whitehouse, 2000).** Approximately 90% of these genetic variations is in the form Single nucleotide polymorphism (SNPs). A SNP is a single base mutation in DNA sequence; they are the simplest type of polymorphism **(Albert et al., 2011).**

The replacement of guanine by thymine at NOS3 nucleotide 894 results in a change of amino acid from glutamate to aspartate at codon 298 of the mature NOS3 protein. Although this is a conservative amino acid substitution, two groups of investigators have shown that NOS3 with aspartate at position 298 is subject to selective proteolytic cleavage which is predicted to result in absence or reduction of NOS activity in homozygous carriers of the 894T allele **(Persu et al., 2002).**

Aim of the Work

The aim of the thesis was to study the G894T Nitric Oxide Synthase gene polymorphism as a risk factor of ischemic heart disease among Egyptian smokers.