The effect of cigarette smoking on expression of transforming growth factor beta-1 and its receptor in cavernous tissue of adult rats

Thesis

Submitted for fulfillment of Master Degree
In Dermatology and Andrology

By

Bryar Taib Ahmed
(M.B.Ch.B)

Supervised by

Dr. Mohamed Mohamed Farid
Professor of Andrology & STDs
Cairo University

Dr. Ihab Ismail Kamel
Assistant Professor of Andrology & STDs
Cairo University

Dr.Laila Ahmed Rashed
Assistant Professor of Biochemistry
Cairo University

Faculty of Medicine
Cairo University
2010

بسم الله الرحمن الرحيم

يرفَعِ الله الذين آمنوا منكُم والذين أُوتوا الْعلْم درجات والله بِما تعملُونَ خبِير

صدق الله العظيم

(سورة المجادلة: الآية ١١)

Acknowledgment

Thanks to God first and foremost. I feel always indebted to God, the most kind and the most merciful.

I would like to express my gratefulness and respect to
Dr. Mohamed Mohamed Farid
Professor of Andrology, Cairo University.
For his moral and scientific support and for giving me the honor of working under his supervision and valuable guidance.

My special thanks and deepest gratitude to
Dr. Ihab Ismail Kamel
Assistant professor of Andrology, Cairo University.
For his constructive and instructive comments and valuable suggestions. He encouraged me all the time for a better performance, without his generous help, this work would not have been accomplished in its present picture.

I would like to express my gratitude and appreciation to
Dr. Laila Ahmed Rashed
Assistant professor of Biochemistry, Cairo University.
For her efforts in laboratory work and moral help.

Words cannot describe my gratefulness and gratitude for my greatest ***father***, ***mother*** *and* ***wife*** *who provided me with every means of support throughout my life.*

TABLE OF CONTENTS

Page number

LIST OF ABBREVIATIONS..... **i-iv**
LIST OF TABLES..... **v**
LIST OF FIGURES..... **vi-vii**

INTRODUCTION..... **1-2**

AIM OF THE WORK..... **3**

REVIEW OF LITERATURE..... **4-64**

Chapter I: Anatomy of the penis..... 4-16
Compartments of the penis..... 4-5
Coverings of the penis..... 5-6
Ligaments of the penis..... 7
Muscles of the penis..... 8-9
Arterial supply of the penis..... 10-11
Venous drainage of the penis..... 12-13
Lymphatic drainage of the penis..... 14
Nerve supply of the penis..... 14-16

Chapter II: Physiology of erection..... **17-23**
Phases of erection..... 17
Classification of erection..... 18-19
Mechanism of penile erection..... 19-23

Chapter III: Erectile dysfunction..... **24-41**
Psychogenic ED..... 25-26
Organic ED..... 26-34
- Arteriogenic ED..... 26-27
- Venogenic ED..... 27-28
- Neurogenic ED..... 28-29
- Endocrinologic ED..... 29-30
- ED due to structural abnormalities of the penis..... 30-31
- Traumatic ED..... 31
- Drug induced ED..... 32-33
- ED due to systemic diseases..... 33-34

Table of contents

Risk factors of erectile dysfunction..... **35-41**
- Aging..... 35
- Diabetes mellitus..... 36
- Hyperlipidemia..... 37
- Hypertension..... 38
- Smoking..... 38-41

Chapter IV: Growth factors..... **42-64**
Nomenclature of growth factors..... 43
Classification of growth factors..... 43-53
Transforming growth factor-beta..... 54-55
Isoforms of Transforming growth factor -beta..... 55-56
Structure of Transforming growth factor -beta..... 56-58
Transforming growth factor -beta receptor..... 58-59
Transforming growth factor -beta signaling..... 60-61
Role of transforming growth factor -beta1 in erectile dysfunction..... 62-64

SUBJECTS AND METHODS..... **65-78**

RESULTS..... **79-89**

DISCUSSION..... **90-95**

SUMMARY..... **96-97**

REFERENCES..... **98-113**

ARABIC SUMMARY

List of Abbreviations

ALK	Activin receptor-like kinase
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BMPs	Bone morphogenic proteins
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
CC	Corpora cavernosa
cDNA	Cyclic deoxyribonucleic acid
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
Co-Smad	Common-partner Smad
CS	Corpus spongiosum
CSF	Colony-stimulating growth factors
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclase
dNTPs	Deoxynucleotide triphosphate
DPP-C	Decapentapllegic transcript
EB	Ethidium Bromide
ED	Erectile dysfunction
EGF	Epidermal growth factor
ELISA	Enzyme Linked Immunosorbent Assay
eNOS	Endothelial NO synthase
EPO	Erythropoietin

FGF	Fibroblast growth Factor
FGFs	Fibroblast growth factors
GFs	Growth factors
GM-CSF	Granulocyte-macrophage colony-stimulating growth factors
GTC	Guanidine thiocyanate
GTP	Guanosine triphosphate
HDL-C	High density lipoprotein -C
HPRI	Human Placental Ribonuclease Inhibitor
HTN	Hypertension
IFNs	Interferons
IFN-α	Interferon Alpha
IFN-β	Interferon Beta
IFN-γ	Interferon Gamma
IGF	Insulin growth factor
IGF-I	Insulin growth factor I
IGF-II	Insulin growth factor II
I-Smads	Inhibitory Smads
KGF	Keratinocyte growth factor
L	Lumber nerve
LAP	Latency associated peptide
LTBP	Latent TGF-ß–binding protein
M6P/IGFll-R	Mannose-6-phosphate / type II insulin like growth factor receptor
MIS	Mullerian inhibiting substance
MMAS	Massachusetts Male Aging Study
MMLV	Moloney murine leukemia virus
mRNA	Messenger ribonucleic acid
NANC	Non-adrenergic non-cholinergic

NGF	Nerve growth factor
nNOS	Neuronal NO synthase
NO	Nitric oxide
p-value	Probability value
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factors
PLGF	Placental growth factor
PMSF	Phenylmethylsulfonyl fluoride
PO2	Partial oxygen tension
RNA	Ribonucleic acid
RNase	Ribonuclease
R-Smads	Receptor-activated smads
RT-PCR	Reverse transcription polymerase chain reaction
S	Sacral nerve
SCF	Stem cell growth factor
SD	Standard deviation
SPSS	Statistical Package for the Social Science
T	Thoracic nerve
TA	Tunica albuginea
TAE	Tris-Acetate EDTA buffer
Taq	Thermus aquaticus
TGFs	Transforming growth factors
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor-beta
TGF-β R-I	Transforming growth factor-beta type I receptor
TGF-β R-II	Transforming growth factor-beta type II receptor

TGF-β R-III	Transforming growth factor-beta type III receptor
TGF-β R-IV	Transforming growth factor-beta type IV receptor
TGF-β_1	Transforming growth factor-beta one
TGF-β_1 R-II	Transforming growth factor-beta one type II receptor
TMB	3,3´,5,5´-tetramentylbenzidine
TNF	Tumor necrosis factor
UPA-R	Urokinase plasminogen activator receptors
VEGF	Vascular endothelial growth factor

LIST OF TABLES

Table number	Title	Page number
(1)	Drugs causing erectile dysfunction.	**33**
(2)	Mean, standard deviation, minimum and maximum values of TGF-β_1 level (pg/mg) in cavernous tissue of all experimental groups.	**79**
(3)	Mean, standard deviation, minimum and maximum values of TGF-β_1 R-II level (µg/dl) in cavernous tissue of all experimental groups.	**80**
(4)	Comparison of TGF-β_1 level in cavernous tissue between group I and group II.	**81**
(5)	Comparison of TGF-β_1 level in cavernous tissue between group I and group III.	**82**
(6)	Comparison of TGF-β_1 level in cavernous tissue between group II and group III.	**83**
(7)	Comparison of TGF-β_1 R-II level in cavernous tissue between group I and group II.	**84**
(8)	Comparison of TGF-β_1 R-II level in cavernous tissue between group I and group III.	**85**
(9)	Comparison of TGF-β_1 R-II level in cavernous tissue between group II and group III.	**86**

LIST OF FIGURES

Figure number	Title	Page number
(1)	Axial cross section of the penis.	**7**
(2)	Schematic illustration of three dimensional structure of the human penis; ventral aspect, showing muscle of the penis.	**9**
(3)	Schematic illustration of branches of penile artery.	**11**
(4)	Schematic illustration of venous drainage of the human penis.	**13**
(5)	Schematic illustration of nerve supply of penis.	**16**
(6)	Molecular mechanism of penile smooth muscle contraction.	**20**
(7)	Molecular mechanism of penile smooth muscle relaxation.	**22**
(8)	Mechanisms of erection and flaccidity.	**23**
(9)	Two dimensional structure of TGF-beta.	**57**
(10)	Structure of TGF-ß complex.	**58**
(11)	Mechanism of TGF signaling pathway.	**61**
(12)	Effect of repeated tissue injury, leading to the continuous production of TGF-β and extracellular matrix, resulting in tissue fibrosis	**64**
(13)	Mean values of TGF-β_1 level in cavernous tissue of all experimental groups.	**80**

(14)	Mean values of TGF-β_1 R-II level in cavernous tissue of all experimental groups.	**81**
(15)	Mean values of TGF-β_1 level in cavernous tissue between group I and group II.	**82**
(16)	Mean values of TGF-β_1 level in cavernous tissue between group I and group III.	**83**
(17)	Mean values of TGF-β_1 level in cavernous tissue between group II and group III.	**84**
(18)	Mean values of TGF-β_1 R-II level in cavernous tissue between group I and group II.	**85**
(19)	Mean values of TGF-β_1 R-II level in cavernous tissue between group I and group III.	**86**
(20)	Mean values of TGF-β_1 R-II level in cavernous tissue between group II and group III.	**87**
(21)	Correlation between level of TGF-β_1 and TGF-β_1 R-II in group I.	**88**
(22)	Relation between level of TGF-β_1 and TGF-β_1 R-II in group II.	**88**
(23)	Correlation between level of TGF-β_1 and TGF-β_1 R-II in group III.	**89**

Introduction

Introduction

National Institutes of Health defined erectile dysfunction (ED) as the consistent inability to achieve or to maintain an erection sufficient for satisfactory sexual activity **(Golijanin et al., 2007)**. Erectile dysfunction is classified into either primary ED or secondary ED. Primary ED is rather unusual, and denotes that the man has never been able to achieve and/or sustain an erection. This is usually due to physical causes such as endocrinopathies, nervous system disorders, or maldevelopment of the penis and its incompetent veins **(Sehgal and Srivastava, 2003)**. ED is secondary if previously existing potency has been lost which may be psychogenic, organic or combined **(McKendry et al., 1983).** Previously, psychogenic ED was believed to be the most common type with 90% prevalence. This belief has given way to the realization that most men with ED have a mixed condition that may be either predominantly psychogenic or predominantly organic **(Dean and Lue, 2005).** Organic ED can be arteriogenic, venogenic, neurogenic, endocrinologic, structural abnormality induced, traumatic, drug induced or due to other causes by systemic illnesses **(Kandeel et al., 2000).** There are several risk factors of ED, the vascular risk factors are most common **(Donatucci & Lue, 1993)**. These factors include hypertension, hypercholesterolaemia, smoking and diabetes, often cause atherosclerosis **(Rose, 1991)** and finally result in ED.

Despite the health hazards caused by tobacco use, cigarette smoking is still highly prevalent. It is believed that approximately 36% of men worldwide smoke **(World Health Organization, 2008)**. The link between cigarette smoking and ED has been supported from invitro and animal studies and epidemiological evidence from volunteers and patients. These studies have indicated that cigarette smoking is an independent risk factor for inducing vasculogenic impotence **(Safarinejad, 2003 and Chew et al, 2009)**.

The cytokine transforming growth factor-beta (TGF-β) has been implicated in the development and function of a diverse array of reproductive processes in both males and females **(Ingman et al, 2002)**. The Transforming growth factor-β_1 (TGF-β_1) is a pleiotropic cytokine that induces connective tissue expression and inhibits the growth of vascular smooth muscle cells. Much attention has been focused on transforming growth factor-β_1 as a fibrogenic cytokine. This protein has been shown to increase collagen synthesis in human corpus cavernosal smooth muscle cell by 2.5–4.5-fold **(Moreland et al., 1998)**. It alters the composition of the extracellular matrix by inducing expression of collagens, fibronectin and proteoglycans while inhibiting the activity and expression of collagenase and other proteases. Under continuous ischaemic conditions, TGF-β_1 induces synthesis of its own mRNA, leading to a further increase in TGF-β_1 synthesis that reinforces the development of severe fibrosis **(Border et al, 1994)**.