Seminal Plasma Clusterin as a Biomarker for Spermatogenesis in Varicocele Patients before and after Varicocelectomy

Thesis

Submitted for Partial Fulfillment of Master Degree in Dermatology, Venereology and Andrology

By

Maged Samir Daruish
M.B.B.Ch. Faculty of Medicine - Ain Shams University - 2014

Under Supervision of

Prof. Dr. Hanan Mohamed Ahmed Saleh
Professor of Dermatology, Venereology and Andrology
Faculty of Medicine - Ain Shams University

Dr. Ahmed Abdelfattah Afify
Lecturer of Dermatology, Venereology and Andrology
Faculty of Medicine - Ain Shams University

Dr. Walid Abdelhady Ahmed
Lecturer of Clinical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine – Ain Shams University
2018

سورة البقرة الآية: ٣٢

Acknowledgments

First and foremost, I feel always indebted to ***Allah*** *the Most Beneficent and Merciful.*

I wish to express my deepest thanks, gratitude and appreciation to ***Prof. Dr. Hanan Mohamed Ahmed Saleh****, Professor of Dermatology, Venereology and Andrology,, Faculty of Medicine, Ain Shams University, for her meticulous supervision, kind guidance, valuable instructions and generous help.*

Special thanks are due to ***Dr. Ahmed Abdelfattah Afify****, Lecturer of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University, for his sincere efforts, fruitful encouragement.*

I am deeply thankful to ***Dr. Walid Abdelhady Ahmed****, Lecturer of Clinical Pathology, Faculty of Medicine, Ain Shams University, for his great help, outstanding support, active participation and guidance.*

Maged Daruish

Dedication

To:

My Mother

for her endless love, support,

and continuous care

&

My Friends

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abb.	Full term
8 -OHdG	8 –hydroxyl -20 deoxyguanosine
ART	Assisted reproductive technhology
ATP	Adenosine tri-phosphate
Clu	Clusterin
DCs	Dendritic cells
DFI	DNA fragmentation index
ELISA	Enzyme-linked immune-sorbent assay
HDL	High density lipoprotein
HSPs	Heat Shock proteins
ICSI	Intra-cytoplasmic sperm injection
IGF-1R	Insluin growth factor-1 receptor
LDL	Low density lipoprotein
MAA	Methoxyacetic acid
MDA	Malondialdehyde
MD-TESE	Microdissection testicular sperm extraction
nClu	Nuclear clusterin
ROS	Reactive oxygen species
SCD	Sperm chromatin dispersion
sClu	Secretory clusterin
SCSA	Sperm chromatin structure assay
SDF	Sperm DNA fragmentation
StAR	Steroidogenic acute regulatory protein
TGF-B	Transforming growth factor
TUNEL	deoxynucleotidyl transferase mediated dTUP nick- end labelling
VEGF	Vascular endothelial growth factor
α -Clu	α -Clusterin
β -Clu	β –Clusterin

INTRODUCTION

A varicocele is an abnormally dilated pampiniform plexus of veins, which is the venous network that drains blood from the testicles. Varicocele prevalence in the general population is estimated to be 15%-20% ***(Chan et al., 2013).***

The prevalence is 35-50% among men with primary infertility and 80% among men with secondary infertility ***(Agarwal et al., 2015).***

Many theories have been postulated to explain the mechanism through which varicocele can affect fertility e.g. elevated temperature, alterations in the hypothalamic-pituitary gonadal axis, and oxidative stress. Apoptosis is reportedly increased in patients with varicocele ***(Chan et al., 2013; Agarwal et al., 2015).***

The seminal plasma is composed of secretions from accessory sex glands and is rich in sugars, lipids, proteins, and other metabolites that interact with spermatozoa ***(Sharma et al., 2013).***

Clusterin (Clu) is a soluble glycoprotein which is found in high concentrations in body fluids. Clu consists of two chains–α -clusterin (α -Clu) and β -clusterin (β -Clu) that are linked by 5 disulphide bonds ***(Matukumalli et al., 2016).***

Clu has chaperone-like activity and is involved in multiple cell activities, including cell-cycle control, proliferation, cell-cell adhesion ***(Cunin et al., 2016).***

Several functions have been attributed to clusterin such as complement and inflammation regulation, lipid transport, apoptosis, cell differentiation. It was found to be involved in the pathogenesis of many disorders such as Alzheimer's disease, cancers, autoimmune disorders and chronic inflammatory disorders ***(Matukumalli et al., 2016).***

It has been proposed that full-length clusterin binds to misfolded proteins through the hydrophobic zones present in its structure and prevents those proteins from accumulating ***(Bailey et al., 2001).***

Most of the functions of Clu are believed to be related to protein homeostasis regulation (proteostasis). Clu is found along with misfolded proteins in serious disorders such as Alzheimer's disease ***(Yerbery et al., 2005).***

This may result from the chaperone activity of CLU being overpowered by an excess of misfolded proteins and Clu becoming entrapped within the insoluble deposits produced ***(Wilson et al., 2016).***

In the male genital tract, Clu is the main protein synthesized by Sertoli cells and is secreted into the fluid of the seminiferous tubules to be deposited into the membranes of

elongating spermatids and mature spermatozoa, giving rise to the possibility that it has a role in sperm development. Another form of Clu is synthesized and exported to the sperm membrane by the sperms ***(Tenniswood et al., 1998).***

So, two main forms of Clu protein exist; Secretory clusterin (sClu) which is anti-apoptotic and cytoprotective and nuclear clusterin (nClu) which is pro-apoptotic ***(Xiu et al., 2015).***

sClu is a powerful anti-apoptotic. It was found to be highly expressed in resistant cancers acting against treatment induced apoptosis. Binding of sClu to surface receptors on stressed cells promotes cell survival, inhibits proapoptotic pathways such as p53 and Bax pathways ***(Wilson et al., 2016).***

Its level in seminal plasma was negatively correlated to DNA fragmentation and sperms abnormal morphology. Thus, human sCLU of seminal plasma has potential to be a biomarker of semen quality in male infertility studies ***(Salhi et al., 2013).***

Fukuda et al. in (2016) identified serum and seminal clusterin level ≥18 ng ml as significant predictor of sperm retrieval in testicular biopsy. Accordingly, it might be worthy to further evaluate the significance of seminal clusterin level as a biomarker for the assessment of spermatogenic status in infertile men.

Aim of the Work

As clusterin was suggested as a biomarker for infertility, we decided to explore clusterin levels in seminal plasma before and after varicocelectomy, and correlate them with sperm parameters.

VARICOCELE AND INFERTILITY

Definition and Incidence:

Primary infertility is defined as the inability to conceive after 1 year of regular unprotected sexual intercourse with no previous pregnancies. While secondary infertility refers to failure to conceive for 1 year since the last live birth. Nearly 15% of couples are affected by infertility with the male factor seen in about half of them ***(Poongothai et al., 2009).***

Varicocele is an abnormally dilated and tortuous testicular pampiniform plexus of veins in the spermatic cord, due to venous reflux of blood into the internal spermatic vein secondary to malfunctioning valves ***(Mcclure et al., 1986).***

It is considered to be the most common treatable cause of male infertility. About 80% of men with varicocele have normal fertility. Varicocele is found in about 35% of primary infertile men and more than 70% of men with secondary infertility ***(Gorelick et al., 1993).***

According to ***Hamada et al. (2016)*** the prevalence of varicocele increases with age by about 10% for each decade of life with 18% of men age 30–39 years having varicocele ***(Levinger et al., 2007).***

This could be due to either an absolute increase in men with varicocele causing secondary infertility or a relative

increase due to decreased incidence of other etiologies of male factor infertility ***(Jarow et al., 2001).***

1.1 Anatomy:

The pampiniform plexus of veins drains into the internal spermatic vein. The right internal spermatic vein drains obliquely into the inferior vena cava, while the left internal spermatic vein drains in a perpendicular manner into the left renal vein ***(Sofikitis et al., 1993)*** (Fig. 1).

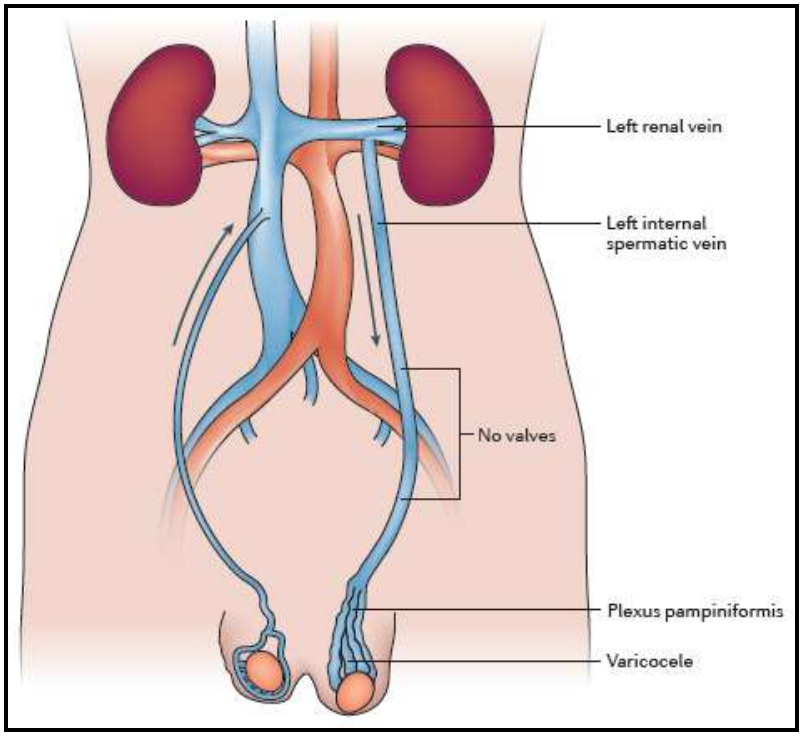

Figure (1): Testicular venous drainage ***(Jensen et al., 2017).***

Alternative drainage of the testicle include the external pudendal vein which drains into the great saphenous vein; the

cremasteric vein, which drains into the inferior epigastric vein and the external iliac vein; and the vasal vein, which drains into the internal iliac vein (Fig. 2) The bilateral internal spermatic veins may communicate at the level of L3 ***(Sofikitis et al., 1993).***

Left-sided varicocele is 10 times more common than on the right and occurs bilaterally in roughly 10% of patients while right-sided varicocele occurs in less than 1% ***(Diamond et al., 2003)***.

The left internal spermatic vein is affected by the increased left renal venous pressures, caused by the compression of the left renal vein between the aorta and superior mesenteric vein "nutcracker syndrome*"**(Little et al., 2002)***.