# DETERMINATION OF INTERLEUKIN 17 IN TESTICULAR BIOPSIES OF AZOOSPERMIC PATIENTS

***Thesis***

***Submitted For Partial Fulfillment For M.D. Degree in Andrology***

By

**Hossam Galal Abdelmaksoud Elenany**

**Supervisors**

**Prof. Dr. Mohamed Mohamed Farid Raiaa**

*Professor of Andrology and STDs*

*Faculty of medicine - Cairo University*

**Prof. Dr. Laila Ahmed Rashed**

*Professor of Biochemistry*

*Faculty of Medecine- Cairo University*

**Prof .DR. Yasser Ibrahim Elkhayat**

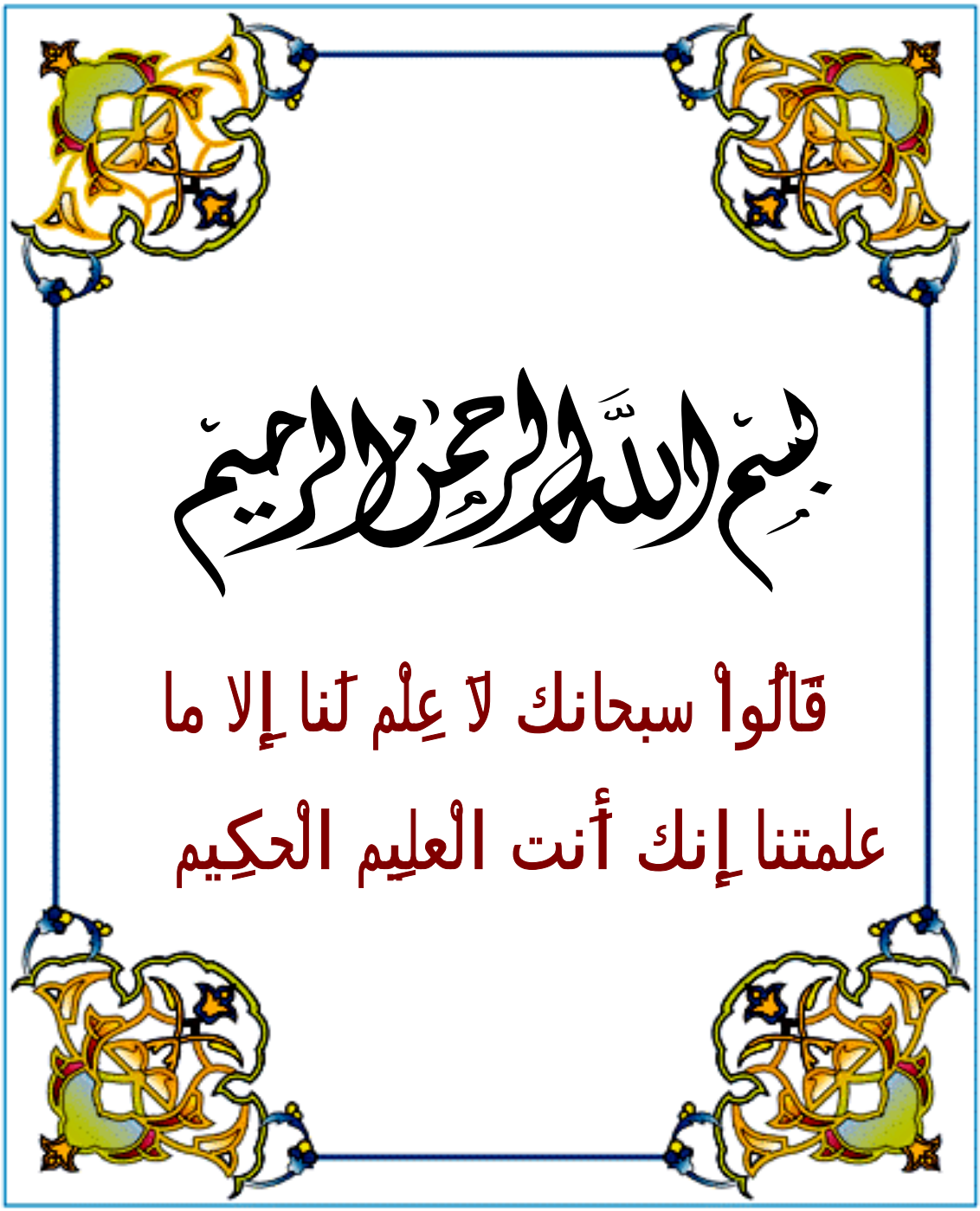
*Assistant Professor of Andrology and STDs*

*Faculty of Medecine-Cairo University*

Faculty of Medecine

Cairo University

(2014)


بسم الله الرحمن الرحيم
قَالُواْ سبحانك لاَ عِلْم لَنا إِلا ما
علمتنا إِنك أَنت الْعلِيم الْحكِيم

# *Abstract*

**Background:** Male factor infertility affects almost half of infertile couples. Infection and inflammation of the male genital tract including the testis are accepted as important etiological factors of infertility in men. Interleukin 17 is a specific cytokine for T helper 17 cells which had been shown to be involved in inflammatory diseases.

**Aim of study:** To add more insight to the role played by Th17 in infertility, by studying the expression of IL-17 in the testicular biopsies of azoospermic patients. It also aimed to analyze the relationship between IL-17 and the testicular pathology.

**Patients and methods:** This study included 62 infertile males, classified in 3 groups. The first group included 25 patients complaining of functional azoospermia whose semen analysis were normozoospermic and deteriorated to azoospermia, the second group included 25 patients complaining of obstructive azoospermia. 12 normozoospermic controls in the third group who failed to provide a semen sample on the day of ICSI examined for IL-17 by PCR & immunohistochemistry.

**Results:** The mean testicular level of IL-17 in the functional group and obstructive group was significantly higher than in controls ($P<0.05$). A weak positive correlation has been found between the testicular level of IL-17 in the functional group and the period since last time the patient showed sperms in his analysis ,also a significant correlation has been detected between IL-17 level and the testicular pathological pattern with the highest level in mixed Sertoli cell only syndrome and lowest level in hypospermatogenesis.

To add on a minimum level of expression was detected in the control normozoospermic group which indicates a role for IL-17 cytokine in the normal testicular function.

**Conclusions:** A basic role is present for the immune system in regulation of testicular function which is obvious to be disturbed with any dysregulation in the testicular milieu. Inflammation is a basic cause in induction of male infertility through imbalance in the testicular microenvironment privilege. IL-17 high level in both functional and obstructive group predicts an inflammatory reaction contributing in the etiopathogenesis of azoospermia.

**Key words:**

**(Azoospermia, T helper 17, Interleukin 17, testicular immune privilege)**

# Acknowledgment

*First, thanks to GOD for blessing this work until it has reached its end, as a part of his generous help throughout our life.*

*It was an honor to work under the supervision of eminent professors, who lent me their whole hearted support and immense facilities as is their usual with their juniors. To them, I owe more than I can record.*

*I would like to express my deepest gratitude and highest appreciation to* **Prof. Dr. Mohamed Farid Raiaa,** *professor of Andrology and STDs, Faculty of Medicine, Cairo University for his continuous encouragement, generous support and unlimited help.*

*I would like to express my deepest gratitude and highest appreciation to* **Prof. Dr. Laila Rashed,** *Professor of Biochemistry, Faculty of Medicine, Cairo University, for her generous support and her great effort in the lab work.*

*I am deeply indebted to* **Prof. Dr. Yasser Alkhyat** *assistant professor of Andrology and STDs, Faculty of Medicine, Cairo University for his unlimited effort, cooperation and useful instructions.*

*Endless thanks to* **Dr. Ahmed Alguingy** *Lecturer of Andrology and STDs, Faculty of Medicine, Cairo University, for his constructive valuable advice and excellent supervision, his time and supreme effort are clear in every part of this work.*

*I am also grateful to* **Prof. Dr. Ibrahim Fahmy, Prof. Dr. Mohamed Abbas** *professors of Andrology and STDs, Faculty of Medicine, Cairo University &* **Dr. Eslam Fathy** *Lecturer of Andrology and STDs, Faculty of Medicine, Cairo University for their valuable help.*

*I would like to offer my deepest gratitude to all my senior staff, and my colleagues in the Department of Andrology, Cairo University for their help during the preparation of this work.*

*Finally, I dedicate this work to my loved ones, my family with special dedication to my wife and to my beloved daughters their love and endless giving and support could never be forgotten or paralleled.*

# *Lists of Contents*

# *List of Abbreviations*

| | |
|---|---|
| **AID** | **Acquired immune deviation** |
| **Aire** | **Autoimmune regulatory** |
| **AMH** | **Anti-Muellerian hormone** |
| **AO** | **Autoimmune orchitis** |
| **APCs** | **Antigen presenting cells** |
| **AZF** | **Azoospermic factor** |
| **BMPs** | **Bone morphogenetic proteins** |
| **BTB** | **blood testicular barrier** |
| **CBAVD** | **Congenital bilateral absence of the vas deferens** |
| **CCR** | **Chemokine receptor** |
| **CF** | **Cystic fibrosis** |
| **CFTR** | **Cystic fibrosis transmembrane regulator gene** |
| **CIS** | **Carcinoma insitu** |
| **COX-2** | **Cyclo-oxygenase 2** |
| **Crry** | **Complement receptor-related protein** |
| **CXCR6** | **Chemokine receptor** |
| **DAMPs** | **Damage-associated molecular patterns** |
| **DAZ** | **Deleted in azoospermia** |
| **DCs** | **Dendritic cells** |
| **DFFRY** | **Drosophila fat-facets related Y** |
| **EAO** | **Experimental autoimmune orchitis** |
| **EDO** | **Ejaculatory duct obstruction** |
| **EV** | **Epidydymo-vasostomy** |
| **FADD** | **Fas-associated death domain protein** |
| **FOXP3** | **Forkhead box P3** |
| **FSH** | **Follicle stimulating hormone** |
| **Gas6/ProS** | **Growth-arrest-specific gene 6 and Protein S** |
| **HCG** | **Human chorionic gonadotropin** |
| **HLA** | **Human leukocytic antigen** |
| **HMGB1** | **High-mobility group box1** |
| **HO- 1** | **Haemoxygenase** |
| **HS** | **Hypospermatogenesis** |
| **HSPs** | **Heat shock proteins** |
| **IDO** | **Indoleamine 2,3-dioxygenase** |
| **IFN** | **Interferon** |
| **IFNAR** | **Interferon Alpha Receptor** |
| **IL** | **Interleukin** |
| **iNOS** | **Inducible nitric oxide synthtase** |
| **iTreg** | **Induced T regulatory cells** |

| | |
|---|---|
| LH | Leutinizing hormone |
| LPS | Lipopolysaccahride |
| lyso-GPCs | Lyso-glycerophosphatidylcholines |
| MA | Maturation arrest |
| MCP-1 | Monocyte chemoattractant protein-1 |
| MHC | Major histocompatibility complex |
| MPCs | Myoid peritubular cells |
| NFκB | Nuclear factor kappa beta |
| NK | Natural killer |
| NO | Nitric oxide |
| NOA | Non obstructive azoospermia |
| OA | Obstructive azoospermia |
| PAMPs | pathogen-associated molecular patterns |
| PAR2 | proteinase-activated receptor-2 |
| PD-1 | Programmed death receptor-1 |
| PDL1 | Programmed death ligand 1 |
| PESA | Percutaneous sperm extraction |
| RBM | RNA-binding motif |
| RORC | Retinoic acid-receptor-related orphan receptor-c |
| ROS | Reactive oxygen species |
| SCO | Sertoli cell only |
| SCs | Sertoli cells |
| Smad | Mothers against decapentaplegic homolog |
| SOCS | Suppressor of cytokine signaling |
| SRY | Sex determining region Y |
| StAR | Steroidogenic acute regulatory protein |
| STAT | Signal transducer and activator of transcription |
| Tbet | T-box expressed in T cells |
| TcR | T cell receptor |
| TESE | Testicular sperm extraction |
| TGF- β | Transforming growth factor beta |
| Th17 | T helper 17 |
| Th3 | T helper 3 |
| TJs | Tight junctions |
| TLR | Toll-like receptor |
| TNFR | Tumor necrosis factor receptor |
| TNF-α | Tumor necrosis factor alpha |
| TRADD | TNFR-associated death domain protein |
| Tregs | Regulatory T cells |
| TRUS | Tran-rectal ultra sound |
| TURED | Transurethral resection of the ejaculatory ducts |

# *List of Tables*

# *List of Figurers*

# *List of Graphs*

# INTRODUCTION

The incidence of azoospermic males in general population is 1% and in infertile men it ranges from 10% to 20% which may result from pre testicular, testicular or post testicular causes. Non obstructive azoospermia represent the testicular causes which detected by testicular biopsy and has prevalence ranged from 40% to 60% **(Jarow et al., 1989)** while obstructive azoospermia accounts for 6.1% to 13.6% of patients presenting for fertility evaluation **(Nieschlag and Lenzi, 2013).**

The testis is an immunologically privileged site where germ cell antigens are protected from autoimmune attack **(Fijak and Meinhardt, 2006)**. However, the testicular environment does not preclude inflammatory reactions and recruitment of tissue-specific T lymphocytes appears to be a crucial component of the inflammation cascade **(Schuppe and Meinhardt, 2005).** In fact, testicular inflammatory disorders leading to impairment of spermatogenesis are thought to be a primary reason for male infertility **(Schuppe et al., 2008).** Histopathological alterations in the damaged testis are manifested by increased numbers of leucocytes in the interstitium, autoantibody production, different degrees of germ cell degeneration and sloughing resulting in aspermatogenesis and atrophy of the seminiferous tubules **(Suescun et al., 2003).**

Furthermore, several studies could show that T lymphocytes play an essential role in the pathogenesis of male infertility because of infection and inflammatory conditions **(Meinhardt et al., 2000) .**

However, the precise immunopathogenic mechanisms responsible for the T lymphocytes in the infertile testis are not completely known.

T helper (Th) 17 cells have recently been identified as a distinct lineage of CD4+ T cells that is characterized by the production of interleukin (IL)-17A, IL-17F, TNF-a, IL-21 and IL-22 . Accumulating data suggest that Th17 cells play a significant role in infectious diseases, autoimmune conditions, adaptive immune response, mucosal immunity and tumour **(Dong, 2008)**.

By reviewing the literature, there was no study which assessed the level of IL-17 in human testicular biopsies using real time PCR.

# AIM OF THE STUDY

The **aim** of the study is to assess the role of IL-17 regarding the etiopathogenesis of azoospermia either functional or obstructive.

# NON-OBSTRUCTIVE AZOOSPERMIA

## Introduction

The incidence of azoospermic males in general population is 1% and in infertile men it ranges from 10% to 20% which may result from pre testicular, testicular or post testicular causes. Non obstructive azoospermia represent the testicular causes which detected by testicular biopsy and has prevalence ranged from 40% to 60% **(Jarow et al., 1989).**

Azoospermia may result from pre-testicular or testicular causes affecting spermatogenesis or post-testicular causes resulting in obstruction of the genital tract where complete spermatogenesis is found at histopathological examination **(Jow et al., 1993).** Pre-testicular causes are including defect in the hypothalamus or pituitary gland resulting in impairment the production of FSH and/or LH resulting in secondary spermatogenic (testicular) failure. Primary spermatogenic failure is defined as any spermatogenic alteration by cases other than hypothalamic-pituitary diseases. The severe forms of primary spermatogenic failure present clinically by azoospermia with different causes. Testicular causes of azoospermia are collectively referred to as non-obstructive azoospermia **(Jarow et al., 1989).**

## Etiology

The causes of non obstructive azoospermia can be classified either congenital or acquired causes, the next table will represent the causes of non obstructive azoospermia.

Table (1): Causes of non obstructive azoospermia

| Congenital causes |
|---|
| **Genetic disorder**<br>• Klinefelter's syndrome<br>• Y chromosome microdeletions<br>• Myotonic dystrophy<br>• Kennedy's syndrome<br>• Androgen insensitivity syndromes<br>• Noonan's syndrome<br>• Sex reversal syndrome (XX male)<br>**Other disorders**<br>• Maldescended testes<br>• Bilateral anorchia (Vanishing Testis Syndrome)<br>• Gonadal dysgenesis<br>• Varicocele |
| **Acquired Causes** |
| **Trauma**<br>**Testicular torsion**<br>**Testicular tumors**<br>**Medications**<br>• Cytotoxic drugs<br>• Hormones (androgens, antiandrogens, oestrogens, progestagens, anabolics)<br>• Hormonally active drugs (cimetidine, spironolactone, digoxin, ketoconazole)<br>• Psychotropic drugs, certain antiepileptics, antiemetics.<br>• Anthelmintics (niridazole)<br>• Salazosulphapyridine<br>**Radiotherapy**<br>**Surgeries that can cause devascularization of the testes**<br>**Infections**<br>• Viral infections (Mumps orchitis, influenza)<br>• Bacterial (brucellosis, typhoid fever)<br>• Specific granulomas (Syphilis, Leprosy)<br>**Environmental factors** (toxins, irradiation, heat)<br>**Systemic diseases** (liver cirrhosis, renal failure) |
| **Idiopathic** |

**(Fahmy, 2010)**