المستخلص

# Evaluation of hormonal disturbance and DNA damages in azoospermic patients تقييم الاضطرابات الهرمونية والأضرار الوراثية لدى مرضى اللانطفية

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# Abstract

The present study was designed to evaluate the hormonal disturbance and the extent of DNA damage in blood cells and its association with azoospermia. Total of 68 subjects (48 azoospermic patients and 20 age-matched healthy subjects) participated in this study. Certain semen parameters were investigated according to World Health Organization guidelines [8]. Serum testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and prolactin levels were also determined by enzyme-linked immunosorbent assay (ELISA). DNA damage was determined by using single cell gel electrophoresis (comet assay). It was found that the levels of FSH and prolactin hormones in azoospermic patients were significantly higher than that in healthy subjects (10.30 versus 8.23 m IU/ml for FSH; and 11.02 versus 6.15 ng/ml for prolactin). Comet assay results revealed a significant increase in the length of tail, the mean of comet score(%) and tail lengthening(%) accompanied with a significant decrease in the length of nucleus in azoospermic group versus control group. Data from this study indicate that damage of blood DNA may be important in the etiology of azoospermia and that the comet assay of blood cells may have potential diagnostic value in the evaluation of azoospermia.

صممت الدراسة الحالية لتقييم مدى تأثير الاضطرابات الهرمونية و الأضرار الوراثية في خلايا الدم وعلاقتها باللانطفية (azoospermia). تضمنت الدراسة 68 شخصا 48 منهم يعانون من اللانطفية و 20 شخصا سليما . تم تقدير المعايير التقليدية للسائل المنوي استنادا الى دليل منظمة الصحة العالمية [8] . تم قياس مستويات هرمونات التستستيرون ، FSH ، LH ، الاولاكتين بواسطة الـ FSL . قدرت الأضرار الوراثية باستخدام اختبار التحليل المذنب FSH ، LH والبرولاكتين بواسطة الـ FSL . قدرت الأضرار الأشخاص الذين يعانون من اللانطفية أعلى معنويا مقارنة بمستويات هرمونات HSL والبرولاكتين في مقارنة بـ FSL 8.20 النوراثية باستخدام اختبار التحليل المذنب FSH ، LH والبرولاكتين بواسطة الـ FSL . قدرت الأضرار الأشخاص الذين يعانون من اللانطفية أعلى معنويا مقارنة بمستوياتها لدى الأشخاص السليمين ( 0.30 مقارنة بـ MIU 8.23 من اللانطفية الحلام والتية زيادة معنوية في طول الذيل ( 10.30 ) معدل قيمة المرولاكتين) . أظهرت النتائج المتعلقه بالاضرار الوراثية زيادة معنوية في طول الذيل ( 110 ) ومعدل قيمة المونية باستخدام الذين بمجموعة السيطرة . تشير نتائج هذه الدراسة الى ان الأضرار الوراثية ربما تكون معروبة معنوية في طول النواة في معنوية معنوية معنوية في طول الذيل ( 110 ) ومعدل قيمة الم ذلب comet score والنسبة المنوبة المتطالة الذيل مصحوبة بانخفاض معنوي في طول النواة في مجموعة اللانطفية مقارنة بمجموعة السيطرة . تشير نتائج هذه الدراسة الى ان الأضرار الوراثية ربما تكون من الأسباب المهمة لللانطفية وان إجراء فحص المذنب لخلايا الدم ربما تكون له قيمة تشخيصية في تقييمه .

# Introduction

The level and patterns of infertility varies widely, being found low in developed countries and high in undeveloped countries [1].

Male infertility is a common reproductive disorder and a major cause of conception failure in humans. [2] indicated that male infertility accounts for 40 % of infertility problems. Some evidence suggests a decreasing trend of male infertility in the last 50

years and decreased mean sperm count in fertile men [3]. Although the exact factors and mechanisms causing male infertility are still largely elusive, one of the significant developments in recent years is the discovery that reactive oxygen species (ROS) and oxidative damage are closely associated with impaired sperm function and male infertility [4, 5]. Reactive oxygen species has been shown to possess deleterious effects on sperm functions such as motility [6, 7]. Reactive oxygen species production in infertile subjects was also found to be significantly higher than that in normal subjects [6]. Deoxyribonucleic acid is one of the main molecular targets for ROS. Recently, there is increasing evidence suggesting that DNA damage is closely associated with impaired sperm function and male infertility. The significance of DNA damage in male infertility is relatively less well documented.

The objective of the present study was to understand and delineate the nature and level of DNA damage in association with some reproductive hormones and semen parameters in azoospermic men.

## **Material and Methods**

The study was carried out according to the guidelines and approval of Genetic Engineering and Biotechnology Institute for higher studies, University of Baghdad, Iraq.

### **Collection of Semen Samples**

Semen samples were obtained from 48 men (24-43) years old attending Kamal Alsamaraee Fertility Centre, Baghdad, Iraq, who had a history of infertility for 21 years. Controls consisted of samples obtained from 20 volunteers (21-47) years old who donated semen samples. Semen samples were collected by masturbation after (2-7) days of sexual abstinence and allowed to liquefy at 37°C for 30 minutes before analysis. Then, semen volume (sensitive to 0.1 ml) was measured. The pH values were measured for all semen samples (7.20 versus 7.01 for control and patient groups, respectively). All semen samples were evaluated according to [8] guidelines, patients who had >  $20 \times 10^{6}$ / ml sperm concentration, >50 % motility or > 30 % normal forms, were considered to have normal semen parameters.

#### **Collection of Blood Samples**

Blood samples were drawn on the same day that the semen samples were collected. Each blood sample was divided into two portions; one was centrifuged and serum was stored at  $-20^{\circ}$ C until analysis, and the other was used for evaluation of DNA damage by using comet assay.

## **Levels of Hormones**

Serum testosterone, LH, FSH and prolactin concentrations were determined by ELISA using Enanthos 2020 System and according to the manufacturer's instructions. **Comet Assay** 

The basic protocol for the comet assay was followed as described by [9]. The slides were coded and for each subjects and 50 cells were screened under a standard transmission binocular microscope. Each cell had the appearance of a comet Figure (1) with a brightly florescent head and tail.



Figure (1): Blood processed using single cell gel electrophoresis (COMET) assay showing three classes of comet: (A) Cells with intact and undamaged DNA. (B) Cells with intermediate degree of DNA damage. (C) Cells with high degree of DNA damage.

DNA migration length was measured by scoring the slides under oil immersion using an ocular micrometer, calibrated with help of a stage micrometer. Each division on the micrometer scale of the eyepiece measured 0.94  $\mu$ m DNA migration lengths. Mean DNA migration length in  $\mu$ m was calculated by taking the average of the measurements obtained for all the comets. The percentages of comet score and tail lengthening were calculated [9, 10].

## **Statistical Analysis**

Data were reported as mean  $\pm$  SD.The comparisons between two groups were tested by ANOVA using GLM [11]. p<0.05 was considered as statistically significant.

## Results

The seminal and hormonal profiles are shown in Table (1). Follicle-stimulating hormone and prolactin elevation were detected in significant (p < 0.05) levels (10+1.9, 11.02+1.3 respectively). Non of these elevated levels showed a homogenized results with other hormones. Thirty six (75%) of 48 subjects were found with abnormal hormonal levels and 13 (27%) were with normal levels. Among 36 azoospermic men with abnormal hormonal levels, prolactin elevation was detected in 21 (58.3%) patients. This elevation was detected alone in 13 azoospermic and combined with other hormonal abnormalities levels in 8 patients.

The statistical analysis of the COMET results Table (2) showed that there was a significant (P=0.0001) increase in the length of tail, the mean % of comet score and mean % of tail lengthening and significant decrease in the length of nucleus in azoospermic group when compared with control group. This increase was shown to be higher in the mean % of comet score and the mean % of tail lengthening.

Table (1): The age range, duration of infertility, seminal and hormonal profiles in control and azoospermic groups.

parameters	Azoospermic (No. 48)	Control(No. 20)		
Age range (year)	24-43	21-47		
Duration of infertility (year)	2-21	00		
Seminal profile				
No. of sperms (million / ml)	00	60		
Motility (%)		80		
Normal sperm (%)		91		
Volume (ml)	3.06	3.10		
рН	7.01	7.20		
Time of liquefaction (minute)	30	30		
Hormonal profile (mean ± SD )				
FSH (m IU / ml)	$10.3 \pm 1.9 *$	$\textbf{8.23} \pm \textbf{2.01}$		
LH (m IU / ml)	$9.01 \pm 0.9$	$\textbf{7.86} \pm \textbf{1.92}$		
Prolactin (ng / ml)	$11.02 \pm 1.3 *$	$6.15 \pm 1.65$		
Testosterone (ng / ml)	$3.94 \pm 1.98$	$6.97 \pm 1.64$		

\* significant difference at (p < 0.05).

$(\text{Incall} \pm \text{SD})$			
parameters	Azoospermic	Control	p-value
Length of nucleus (µm)	$2.95 \pm 1.25$	$6.75 \pm 1.31$	0.0001**
Length of tail (µm)	$\textbf{4.82} \pm \textbf{0.94}$	$0.56 \pm 0.07$	0.0001**
The mean of comet score (%)	$62.70 \pm 8.45$	$\textbf{7.87} \pm \textbf{1.60}$	0.0001**
The mean of tail lengthening (%)	$183.78 \pm 58.56$	$\textbf{8.58} \pm \textbf{1.90}$	0.0001**

Table (2): Comparison of comet assay parameters between control and azoospermic groups. (mean  $\pm$  SD)

**\*\*** highly significant difference.

#### Discussion

The reproductive hormones and DNA damages are very important criteria to be considered in male infertility [12].

Hormones like FSH, LH and prolactin have a major role in male spermatogenesis [13]. They stimulate testosterone production.

The current results found that FSH and Prolactin are a reliable indicators for azoospermia since no significant differences was detected in the testosterone levels between azoospermic patients and control which indicate that the azoospermia cases in this study could be due to FSH and Prolactin elevation rather than testosterone deficiency. Thirteen of another azoospermic patients were also found with normal levels of all hormones estimated. This suggests that damages in testosterone receptors could be the reason behind azoospermia as well to FSH and prolactin elevation.

Previous studies in Iraq about azoospermia were found that the elevated FSH is an indicator for germinal epithelial damage, decreased Sertoli cells function and azoospermia [14, 15,16]. Prolactin is generally only thought of as factor in female fertility, but in fact, also play a role in male fertility. Elevation of prolactin in male can cause decreased testosterone level or abnormal sperms [17]. Such elevation also tends to inhibit the production of gonadotropin releasing hormone (GnRH) from hypothalamus which is essential for stimulating the production and release of both FSH and LH from the anterior pituitary [18].

In parallel to the hormonal disturbances, DNA damage was also detected in azoospermic men. A significant increase in DNA damage in lymphocytes of azoospermic men was revealed in comparison to controls. Whether the damages detected in the DNA was correlated to the hormonal disturbance or another factor such as oxidative stress was not clear. However, exogenous factors such as fertility inducers, heavy metals, oxidants, smoking and others have been reported to induce DNA damages in both sperm and lymphocytes [19, 20].

It was concluded from the present study that blood DNA damage may be important in the etiology of azoospermia and may have potential diagnostic value in the evaluation of azoospermia.

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