

Growth of testes plus pathological and biochemical changes in patients with azoospermia syndrome

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Abstract

Infertility is a common medical and social concern. Azoospermia is a syndrome with different histopathological changes, hormonal disturbances and altered size of testes. The aim of this work is to assess testicular histopathological patterns, measure the volume of testis and the size of the seminiferous tubules in the spectrum of the azoospermic patients along with examining hormonal changes. Seventy-eight (78) azoospermic cases were included in this study within the reproductive age group (18-50) years old. Proper history was taken through a detailed questionnaire; physical examination was performed. Testicular volume was calculated using ultrasonography and Lamberts' formula (L (length) x W (width) x H (height) x 0.71). The level of LH, FSH and Testosterone hormones was determined. The mean testicular volume (mL) in obstructive azoospermia (OA) was (16.90), in M.A was (15.17), in SCOS was (9.13), in HS was (13.1) and in end-stage was (7.59). The mean diameter of seminiferous tubules (micron), in OA was (171.55), in M.A was (154.53), in SCOS was (121.55), in HS was (165.47) and in end-stage was (116.92). The hormonal profile of FSH (mlu/ml) in OA was (5.33), in M.A was (8.22), in SCOS was (22.59), in HS was (6.74), and in end-stage was (25.4). The level of LH (mlu/ml) in OA was (5.78), MA; (7.1), SCOS; (14.44), HS; (5.57), and end-stage (13.12) while the hormonal profile of testosterone (ng/ml) in OA was (5.64), M.A; (5.19), SCOS; (3.57), HS; (4.91) and end stage (2.08). In conclusion; non obstructive pathologies are still the commonest causes of azoospermia that are associated with variable level of size change and disturbance in hormonal level. Histopathology is the corner stone for proper diagnosis.

Keywords: Azoospermia; Testes; Seminiferous tubules

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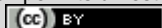
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Introduction

Infertility is a common problem in marital relations with social and medical scope. It is either primary with no previous pregnancy including this or previous relations or it is secondary when there is preceding pregnancy irrespective to the outcome. Male factors are responsible for about



one third of the cases [1]. Male factors include oligoastheno- and azoospermia and other pathologies [2]. Fertility depends on normal growth and maturation of testis under the influence of growth and gonadotrophin hormones (LH & FSH) [3, 4]. Testis cords remain solid until puberty, when they acquire a lumen, thus forming the seminiferous tubules and start function [3]. Normally, the testes reach the inguinal region by approximately 12 weeks' gestation, migrate through the inguinal canal by 28 weeks, and reach the scrotum by 33 weeks [3] azoospermia is defined as complete absence of sperms in the ejaculate and is present in about 1% of all men and in about 10%-15% of infertile men [2]. Azoospermia is one of the few causes of male factor infertility that can be detected by seminal analysis (at least two separate centrifuged semen samples, centrifuged for 15 minutes at 3000 x gravity or greater) [5]. Azoospermia is broadly classified in to obstructive and non-obstructive types. Men with non-obstructive azoospermia (NOA) may be classified into several categories based upon testicular histology; hypospematogenesis (HS) when there is a decreased number of cells of spermatogenic series; the normal number is about 2 million. In the histological examination, germ cells layers will be thinner within the seminiferous tubule ®.

Maturation arrest (MA), a histological description of the interruption of normal germ cell maturation at the level of a specific cell type leading from spermatogonia to spermatids. Sertoli cell only syndrome (SCOS) also called germ cell aplasia or Del Castillo syndrome which describes a condition of the testes in which only Sertoli cells line the seminiferous tubules and the patients have very low or absent spermatogenesis ®.

End stage testes in which tubular and peritubular sclerosis (Hyalinization) is the characteristic feature. Germ cells will be absent from the sclerotic seminiferous tubules and Sertoli cells may or may not be present plus absence or decreased number of Leydig cells ® [6, 7].

Furthermore, there may be mixed patterns of histology with varying degrees of tubular fibrosis to document these categories, it is important to have a testicular biopsy for evaluation [7]. The aim of the study is to measure the range of growth of testis (volume) and clarify the changes (histological and histopathological) in seminiferous tubules (in differentiation). Also, the study analyzes the changes in the hormonal profile. To our best our knowledge no study was done in our locality to investigate the above topic.

Patients and Method

This study was carried out on infertile patients who complained from infertility and attended the infertility unit at Azadi teaching hospital, the International center of In-vitro fertilization (IVF) and infertility management and private clinics in Kirkuk city, the study extended from January 2016 to November 2018, and all the laboratory and imaging studies were done at the above settings. All infertile men with azoospermia were included (78) within the age group (18-50) years old. Azoospermia was defined on the basis of total absence of sperms in at least two standard semen analyses and after examination of sperm pallet obtained by high speed centrifugation (at least



two separate centrifuged semen samples, centrifuged for 15 minutes at 3000 x gravity or greater) [7]. Patients with malignancy or receiving chemo- or radio therapy were excluded.

Our work up included proper history taking, physical examination, testicular volume calculation and testicular histopathological examination, hormonal levels assessment (FSH, LH and testosterone). In the study, the OA group was considered as a control for comparison.

All studied subjects were fully assessed by using a detailed questionnaire, history was obtained from the subjects included the age, type of occupation, family history of infertility, and the duration of infertility.

Medical history such as systemic illnesses (febrile illnesses, diabetes mellitus, renal failure and hepatic failure), past history of malignancy that required chemotherapy and radiotherapy had been sought. Surgical history included inquiry about orchidopexy, herniorrhaphy, retroperitoneal, pelvic surgery; inguinal, scrotal or urethral surgery, testicular trauma, torsion and mal-descended, venereal infections, orchitis and epididymitis. History of drug intake and the duration. General examination was done for gynecomastia, general build and hair distribution with more concentration on examination of the external genitalia (penis, scrotum). Consistency and size of testes, consistency and size of epididymis and the presence of the vas on each side were assessed. Groins were examined for surgical scars, and inguinal hernia hydrocele, presence of undescended testis, varicocele, nodularity, and thickening of epididymis.

Semen samples were collected in a collector (dry, clean and sterile container labeled with the name and age of the patient, period of abstinence and the time of collection) by masturbation after 3 to 5 days of sexual abstinence [5]. The specimen analysis was done and reported according to the WHO 2010 guidelines [5]. Hormonal assay was done through measuring Serum concentration of FSH, LH, and total testosterone were measured using mini-VIDAS apparatus, through an enzyme linked fluorescent assay (ELFA) technique. Ultrasonography for testicular dimensions have used the three dimensions of testes are obtained through ultrasonography and physical examination. The Lambert's equation = L (length) x W (width) x H (height) x 0.71 was used to estimate the testicular volume [8].

Histological evaluation was done for the prepared specimens. Germ cell elements were quantitatively assessed. At least 15 transverse sections of seminiferous tubules were examined in each biopsy to see the size and proportion of tubules in the biopsy. The seminiferous tubules with or without germ cells were observed; number, type, distribution, localization; and morphology of germinal epithelium within the seminiferous tubules were looked for. Sertoli cells, Leydig cells, degenerating cells, tubular diameter; and thickness of tubular basement membrane, all were checked. The diameter of seminiferous tubules was measured by using ocular microscope (provided with reticule eyepiece and stage micrometer). At least the diameter of 12 sections of seminiferous tubules was measured for each slide and the actual diameter was obtained for each section in micrometer.

Statistical analysis

Computerized statistical analyses were performed using statistical package of social science (SPSS), version 16 computer software. Frequency distribution percentage and mean \pm standard error of the mean (SEM) for selected variables were taken. Paired sample t-test and chi-square test were used to assess the statistical significant of difference in the mean. P value less than the point 0.05 was considered statistically significance.

Results

Seventy-eight infertile cases with azoospermia were included in the study, (10,12.82%) of the cases found to have obstructive azoospermia (OA) and (68, 87.18%) cases with non-obstructive azoospermia (NOA). The distribution of cases according to diagnostic testicular biopsy in the NOA category included SCOS which represented the highest percentage among azoospermic cases (35.90%), MA (30.76%), and end-stage testis (12.82%) while HS represented the lowest percentage (7.70%). A histopathological example of each condition is shown in Figure 1.

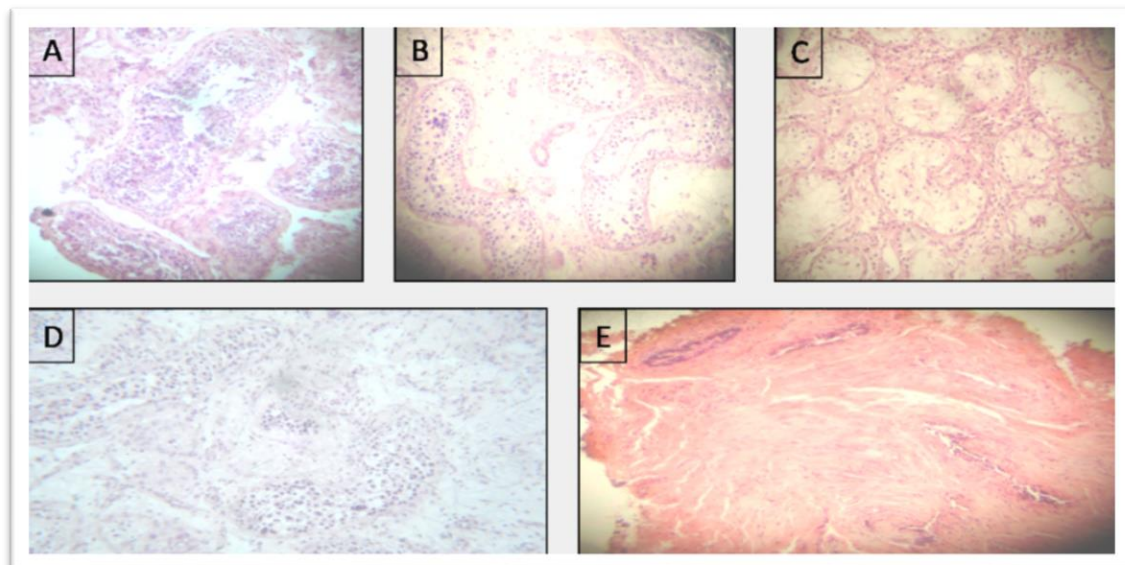


Figure 1.

A histopathological example of each condition is shown ((A=OA, B=MA, C=SCOS, D=HS, E= End Stage))

The mean testicular volume in OA was (16.9) and in NOA as follow MA (14.1), HS (13.1), SCOS (9.13), and end-stage testis (7.59). There was a significant ($P<0.05$) difference between OA and MA; and OA and HS and highly significant ($P<0.001$) difference between OA and SCOS; and OA and end-stage testis, Figure 2.

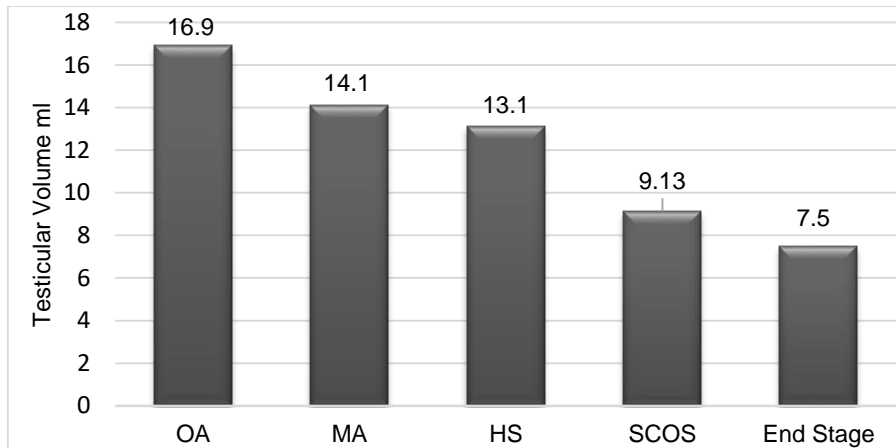


Figure 2.

The mean Testicular Volume in different types of azoospermia.

The mean diameter of seminiferous tubules (microns) in azoospermic cases with OA (171.45), MA (154.55), SCOS (118.73), HS (165.71) and end-stage testis (115.92), shown in Figure (3). Significant ($P < 0.05$) difference was observed between OA and MA; and OA and HS and highly significant ($P < 0.001$) difference between OA and SCOS; and OA and end-stage testis.

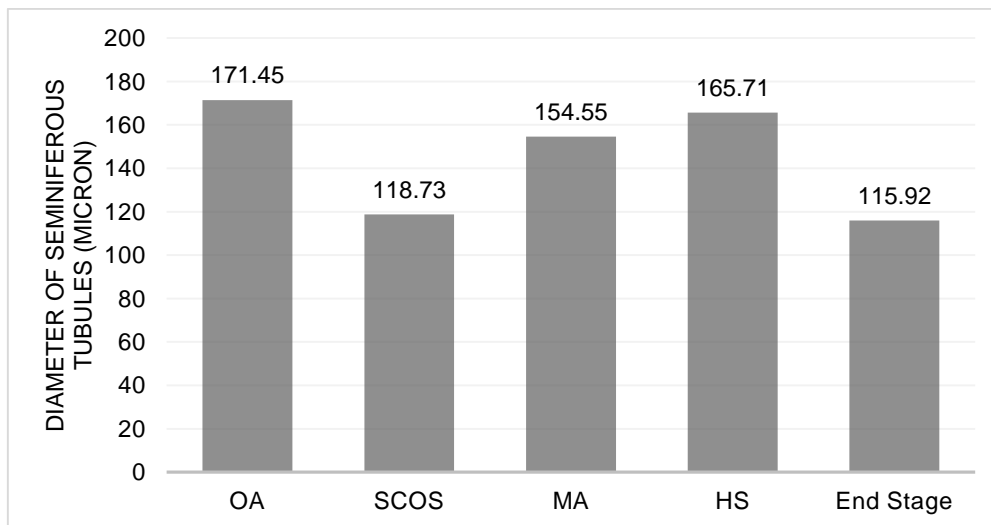


Figure 3.

Diameter of seminiferous tubule

Regarding the mean hormonal levels, follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone level in different categories of azoospermia is shown in figure (4) and were as follow:

- FSH level (mlu/ml) in OA (5.33), MA (8.22), SCOS (22.59), HS (6.74) and end-stage testis (25.40).

- LH level (mlu/ml) in OA (5.78), MA (7.1), SCOS (14.44), HS (5.57) and end-stage testis (13.12).
- The mean testosterone level (ng/ml) in OA (5.64), MA (5.19), SCOS (3.57), HS (4.91) and end-stage testis (2.08).

No significant ($P > 0.05$) difference in FSH, LH and testosterone levels between OA and MA; and OA and HS were noticed; and significant ($P < 0.05$) difference found between OA and SCOS; and OA and End Stage testis.

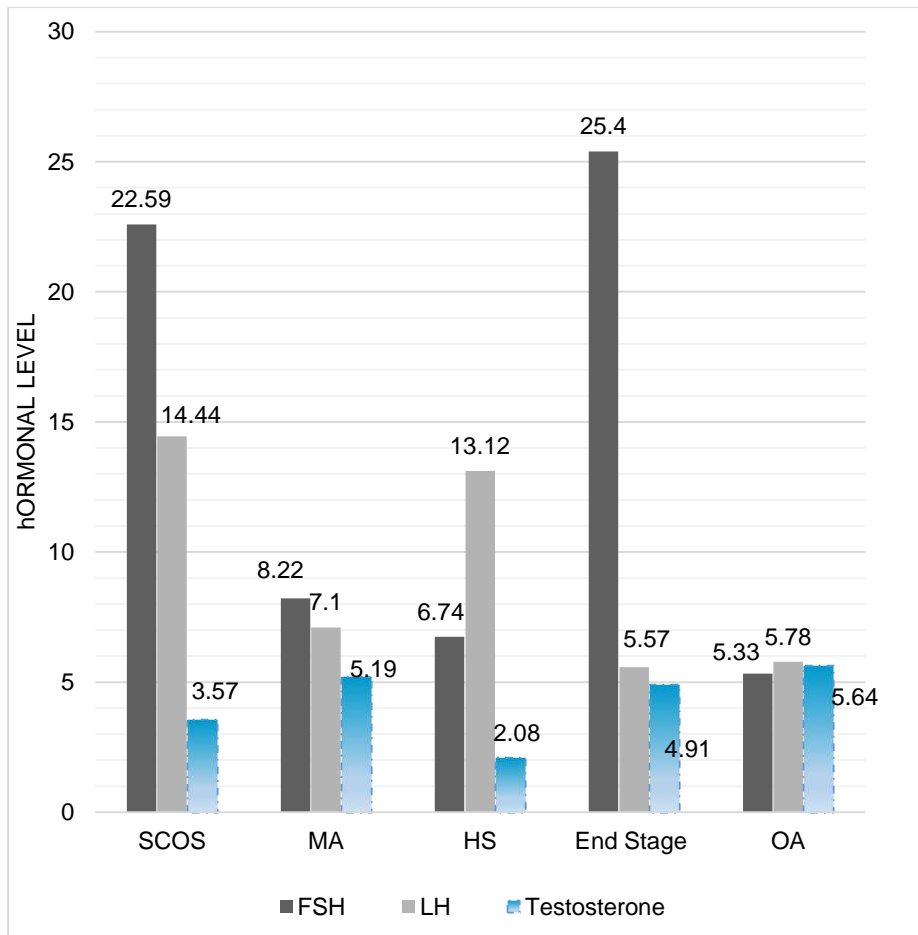


Figure 4.
 Mean hormonal Level

Regarding the risk factors that possibly cause azoospermia are reported in Table (1). Within a total of (78) cases, varicocele has been presented in (13) cases (16.66%); (1) cases with OA, (5) cases with MA, (4) cases with SCOS, (2) cases with HS and (1) case with end-stage testis. Cases presented with medical history of orchitis were (6) cases (7.35%); (4) cases with MA and (2) cases with SCOS. No significant difference reported regarding the effect of varicocele and orchitis on the occurrence of azoospermia. Cases presented with history of cryptorchidism/orchidopexy were (5) of a total of (78) (5.12%); (2) cases with MA, (2) with SCOS and (1) case with end-stage testis. Cases presented with medical history of head

exposure/Febrile illness were (3) cases (3.84%); (1) cases with MA and (2) cases with SCOS. (2) Cases gave a history of testicular trauma (2.56%); (1) case with OA, and (1) case with end-stage testis.

Table 1.

Number and percentage of risk factors associated with azoospermic cases

Risk Factors	OA	MA	SCOS	HS	End Stage
Varicocele	1, 10%	5, 16.6%	4, 14.3%	2,33.2%	1, 10%
Orchitis	0	4, 16.6%	2, 7.2%	0	0
Cryptorchidism/Orchidopexy	0	2, 8.3%	2, 7.14	0	1, 10%
Head /Febrile illness	0	1, 4.16%	2, 7.14%	0	0
Testicular trauma	1, 10%	0	0	0	1, 10%
Pelvic surgery	1, 10%	0	1, 3.57%	0	0

Discussion

The present study was conducted on azoospermic patients; obstructive (OA) and non-obstructive (NOA) which includes: maturation arrest (MA), Sertoli cell only syndrome (SCOS), hypo-spermatogenesis (HS) and end stage testis to compare the growth of testis (volume), histopathological changes, changing in hormonal profile and the contributed risk factors of azoospermia.

In our work, the testicular volumes were calculated using the ultra-sonographic and Lambert's equation formula [8] which provides provide the best estimate of actual volume [8]. It was not unexpected to see that non obstructive testes were larger than those of the patients with obstructive group, since the seminiferous tubules comprise 70% - 80% of the testicular mass, testicular volume reflects spermatogenesis and accurate testicular volume measurement is one way to assess testicular function, pubertal development and it's related to semen profile in infertile men [9]. Other studies [10, 11, 12] reported similar results. It seems that racial and geographical variables influence etiology of azoospermia weather obstructive or non-obstructive and even affects the prevalence of each category within the non-obstructive group. For example, studies of Hirsh A [13], Mubark [14], Rashed et al [15] and Colgan et al [16] reported higher incidence of OA. However, other studies reported low incidence; for example, Meinhard et al [17] reported (5%) for OA while Jordanian study [18]; reported similar results (11.2%). The differences with other studies which reported low incidence may be due to that azoospermic cases with small testes and high FSH were not considered for testicular biopsies in many centers

while our cases were infertile patients candidate for intracytoplasmic sperm insemination (ICSI) and urologist still try to get biopsy from these patients in order to get at least a few sperms to do the insemination.

The level of FSH is a good indicator to assess the amount of testicular damage [19] that explains the very high level we found in our work in the SCOS and end-stage patients which is similar to results obtained by other data [20, 21]. Since FSH is essential for spermatogenesis in man, elevation of serum FSH might indicate a compensatory mechanism secondary to primary testicular failure [22]. The elevated follicle stimulating hormone (FSH) levels may indicate decreased germinal cell mass, diminished Sertoli cell function and consequently primary testicular failure [20]. The concentration of FSH rises with increasing testicular destruction because FSH is under the negative feedback control of inhibin B secreted by seminiferous tubules and when the testis is severely damaged, it will result in decreased inhibin B production and elevated FSH level [23].

LH level showed similar pattern to FSH, as it is dependent on the presence or absence of germ cells in the seminiferous tubules and was elevated only when germ cells were absent from the seminiferous tubules [22], other researchers [20, 24] showed similar finding. The serum level of testosterone, in our data, was within normal range in OA and NOA which is compatible with other results [20, 24, 25], this indicates normal Leydig cell function.

Risk factor or co-morbidities were identified in less than half of the cases, Varicocele was the commonest finding a cross all categories. Various mechanisms have been proposed to explain testicular damage in infertile men, including testicular hypoxia, venous hypertension, elevated temperature, increase in spermatic vein catecholamines, and increased oxidative stress. The influence of varicoceles on testicular function is variable, leaving it apparently unaltered in some cases, and causing partial or total arrest of spermatogenesis in others. As a result, infertile men with varicoceles can exhibit abnormal semen quality ranging from oligospermia to complete azoospermia. Varicocele may be a cause of azoospermia or contributed with other causes. Pasqualotto et al 2003 observed a common association between azoospermia and varicocele as a result of generalized impairment in sperm production. No significant difference found regarding the effect of varicocele on the occurrence of NOA, which is similar to what has been reported by WHO [5]. Orchitis and history of it (as mumps) ranked second after varicocele similar to observed by other researcher [12]. Degenerative changes in the seminiferous tubules and a local activation of the immune system followed in sperm production (oligospermia or azoospermia) may occur following mumps/orchitis [26].

Concerning the history of cryptorchidism/orchidopexy, we reported (5.12%) with NOA, and other reported (22.4%) [12]. It was reported that cryptorchidism could have a direct cause of infertility in azoospermic patients. Cryptorchidism of more than two years of age causes irreversible damage to the spermatogonia cells and massive collagen deposition and fibrosis between the tubules [27].

Competing interests

The author declare that he has no competing interests.

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