Evaluation of Histopathological Patterns of Testicular Biopsies in Azoospermic Patients: A Study of 3 Years in Al-Najaf Center

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Abstract

Background: Testicular biopsy is still considered the main dependable technique required for the assessment of azoospermic patients. Many studies have been done to explore the main changes that occurred in testicular tissue related to the status of azoospermia. Little has been written and published in our country, so this study has been conducted to look for how much the histopathological pattern plays a role in the assessment of azoospermic testicular biopsy. **Objectives:** To evaluate the most common histopathological patterns of testicular specimens from male patients in whom the chief complaint is azoospermia and to categorize each case according to the Modified Johnson's scoring system. **Materials and Methods:** A cross-sectional study, including a review of 180 male patients with a history of infertility with azoospermia or cryptorchidism, aged 18–60 years, submitted for histopathological examination, in Al-Najaf center for histopathology, during a period of 3 years (2020–2022). **Results:** A total of 180 cases of testicular biopsy from azoospermic patients were evaluated. The most frequent type is germ cell maturation arrest (84 cases) (46.67%) which is further categorized into early and late arrest with a spermatogenesis score 3–7 according to the Modified Johnson's score. The second most frequent type is germ cell aplasia/sertoli cell-only syndrome (54 cases) (30%) with spermatogenesis scored as 2. **Conclusion:** This study has recognized four histopathological patterns of testicular biopsies in the case of azoospermia and identified that germ cell maturation arrest was the most frequent pattern in our population.

Keywords: Azoospermia, histopathology, infertility, Johnson's score, spermatogenesis, testicular biopsy

Introduction

Infertility is a stressful problem affecting approximately 48.5 million people all around the world.[1] For each infertile couples, husband and wife should be investigated at the same time. Since in our community, the male cause of infertility is often ignored due to social factors so many cases are misdiagnosed.[2]

Infertility is affecting an estimated 8%–12% of all couples worldwide, in which the male factor is the primary or contributing cause in 50% of these cases.[3,4]

Infertility in both sexes can be divided into primary and secondary cases. Primary infertility is defined as the inability to get conception after one year of coupling or marriage, whereas secondary infertility is the inability to conceive after one or more previous gestations without treatment (failure to conceive after 6 months to one year of trying).[5]

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_121_23

In male infertility, the histological study will provide information not only on the condition, in our subject, spermatogenesis, but also about the progression of the condition and its causes.[6]

To evaluate azoospermia in infertile men, we need history and physical examination with seminal fluid analysis and hormonal assay. We can use other useful tests such as ultrasonography, vasography, anti-sperm antibodies, chromosomal and genetic studies, along with testicular biopsy which can give valuable information about spermatogenesis in order to assess the treatment and the prognosis.[7,8]

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Submission: 05-Feb-2023 **Accepted:** 31-Mar-2023 **Published:** 09-May-2024

How to cite this article: Allebawi SAH, Al-Janabi AAH. Evaluation of histopathological patterns of testicular biopsies in azoospermic patients: A study of 3 years in Al-Najaf Center. Med J Babylon 2024;21:79-84.

Testicular biopsy in infertility is used for the diagnostic assessment of the adequacy of spermatogenesis and also to exclude the obstructive type of azoospermia and to look for normal spermatozoa if there is a chance for surgery. Also, the concern about germ cell tumors or germ cell neoplasia *in situ* in infertile patients with cryptorchidism.[9,10]

Also, testicular biopsy is used nowadays for therapeutic purposes in non-obstructive azoospermia to extract spermatozoa from men for in vitro fertilization (IVF).[9,10]

A good biopsy depends on the surgical technique, proper fixation of the biopsy, and the processing. The size of the biopsy should be not more than 3 mm and about 0.1% of the entire volume of the testis.[11]

Spermatogenesis is an extraordinarily complex process that produces male haploid germ cells from diploid spermatogonial stem cells, which occur in seminiferous tubules. This process starts at puberty and continues for life. The spermatogonial stem cells that are found close to the basement membrane of the seminiferous tubules undergo mitotic division to produce type A cells and type B cells. The B cells differentiate into primary spermatocytes, which in turn divide meiotically into two secondary spermatocytes (meiosis I), and each secondary spermatocyte divides into two haploid spermatids (meiosis II). By a process known as spermiogenesis, the spermatids transformed into spermatozoa which are found in the lumens of the tubules.[12]

Spermatogenesis in azoospermia can be normal spermatogenesis which means obstructive cause, or it may be abnormal which means a problem in the process itself (non-obstructive cause).[2]

Causes of infertility encountered in azoospermic patients were histologically classified according to the modified Johnson's scoring system into:[13]

- Normal spermatogenesis: Normal progression of spermatogenesis from spermatogonia to spermatozoa with normal-looking seminiferous tubules with a thin basement membrane [Figure 1A].
- Germ cell maturation arrest: It means that germ cells fail to become mature spermatozoa. It could be early (complete) which occurs at the spermatocyte level so the sperm level is zero, or late (incomplete) which may rarely have few spermatids in a few seminiferous tubules [Figure 1B].
- Germ cell aplasia/sertoli cell-only syndrome: There are no germ cells in the tubules-only sertoli cells, which may show tubular basement membrane thickening [Figure 1C].
- Seminiferous tubule hyalinization/interstitial fibrosis: no germ cells, no sertoli cells could be found, with tubular basement membrane thickening and a smaller tube diameter [Figure 1D].

Materials and Methods

This is a cross-sectional study of testicular specimens of 180 men with a history of infertility and their chief

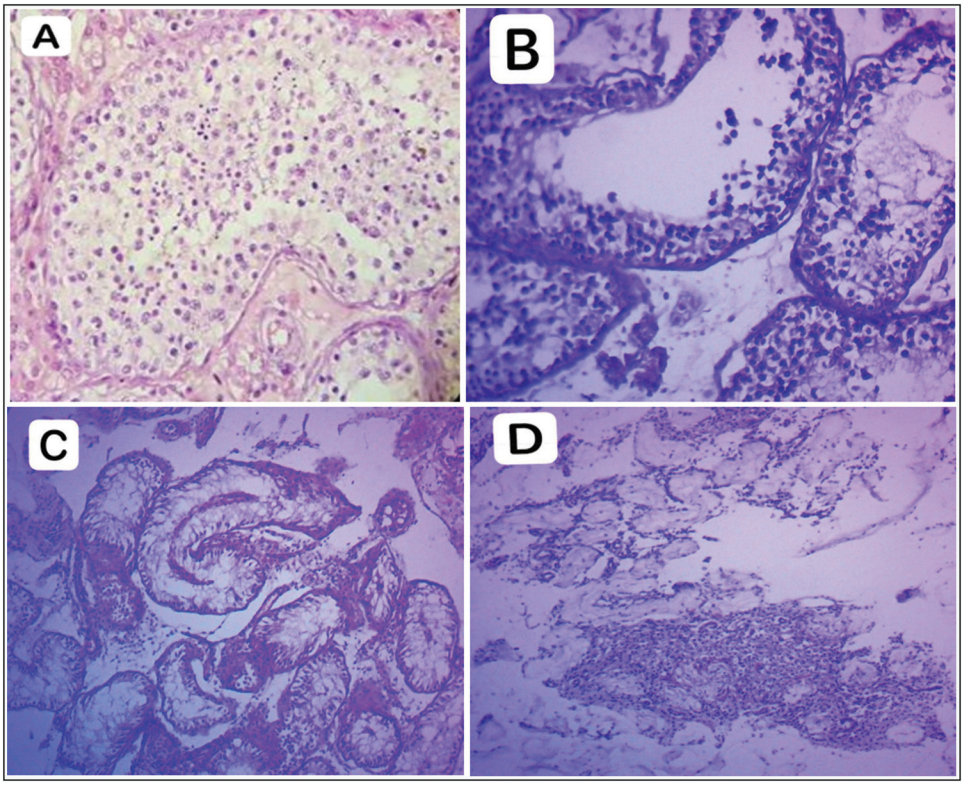

Figure 1: (A) Normal spermatogenesis.[14] (B) Maturation arrest. (C) Germ cell aplasia/sertoli cell-only syndrome. (D) Seminiferous tubule hyalinization

complaint of azoospermia aged 18–60 years, where they attended for testicular biopsy evaluation in Al-Najaf center for histopathology during a period of 3 years (2020–2022). Clinical data regarding age and clinical presentation were obtained from histopathological reports from the archives for each patient.

As mentioned in the archived histopathological reports, all biopsies were preserved in 10% formalin, processed, sectioned, and stained with hematoxylin and eosin (H&E), and examined under a light microscope for scoring and categorization according to the modified Johnson's system which is ranging from 1 to 10 as follows[2];

10 Complete spermatogenesis and perfect tubules
9 Many spermatozoa present but disorganized spermatogenesis
8 Only a few spermatozoa present
7 No spermatozoa but many spermatids present
6 Only a few spermatids present
5 No spermatozoa or spermatids are present but many spermatocytes present
4 Only a few spermatocytes present
3 Only spermatogonia present
2 No germ cells present
1 No germ cells or sertoli cells are present.

Statistical analysis of the results of histopathological examination was done using the Pearson's Chi square test and Fischer's exact test at ≤ 0.05 level as a significant cut-off.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 701 (including the number and the date in November 15, 2021) to get this approval.

Results

Total cases of 180 testicular biopsies from azoospermic men were evaluated and categorized according to the above-mentioned histopathological patterns as shown in Table 1 and Figure 1. In this study, germ cell maturation arrest was the most frequent histological pattern. It formed 46.67% of submitted cases. Germ cell aplasia/sertoli cell-only syndrome has formed 30% and is considered the second most frequent histological pattern, whereas seminiferous tubule hyalinization/interstitial fibrosis has formed the least frequent pattern (10%). A considerable proportion of cases were found to have normal spermatogenesis (13.33%) [Table 1].

Table 1: Histopathological classification of testicular biopsies in azoospermic males (2020–2022)

Histopathological classification	Number of cases	%
Normal spermatogenesis	24	13.33
Germ cell maturation arrest	84	46.67
Germ cell aplasia/sertoli cell-only syndrome	54	30
Seminiferous tubule hyalinization/interstitial fibrosis	18	10
Total	180	100

Significant *P* value ≤ 0.05

Table 2: Germ cell maturation arrest types

Types of maturation arrest	Number of cases	%
Early maturation arrest	72	85.7
Late maturation arrest	12	14.3
Total	84	100

Significant *P* value ≤0.05

Table 3: Categorization of testicular biopsies according to the modified Johnson's scoring system

Johnson's scoring	Number of cases	%
10	2	1.11
9	6	3.33
8	16	8.89
7	5	2.78
6	7	3.89
5	36	20
4	26	14.44
3	10	5.56
2	54	30
1	18	10
Total	180	100%

Significant *P* value ≤ 0.05

There was a significant difference in the prevalence of these histopathological patterns of testicular biopsies (*P* value < 0.05).

Maturation arrest in the current study is further subdivided into early and late arrest. Early arrest is reported more frequently (85.7%) than late arrest (14.3%) with a significant difference between these two groups (*P* value < 0.05) as shown in Table 2.

The score of spermatogenesis in all submitted cases was also categorized according to the modified Johnson's scoring system as shown in Table 3 and Figure 2. It has been found that score 1 formed 10% which represents seminiferous tubule hyalinization/interstitial fibrosis. Whereas score 2 formed (30%) which was given to germ cell aplasia/sertoli cell-only syndrome. Scores 3–7 formed the largest proportion collectively (46.67%), which represents germ cell maturation arrest (early and late).

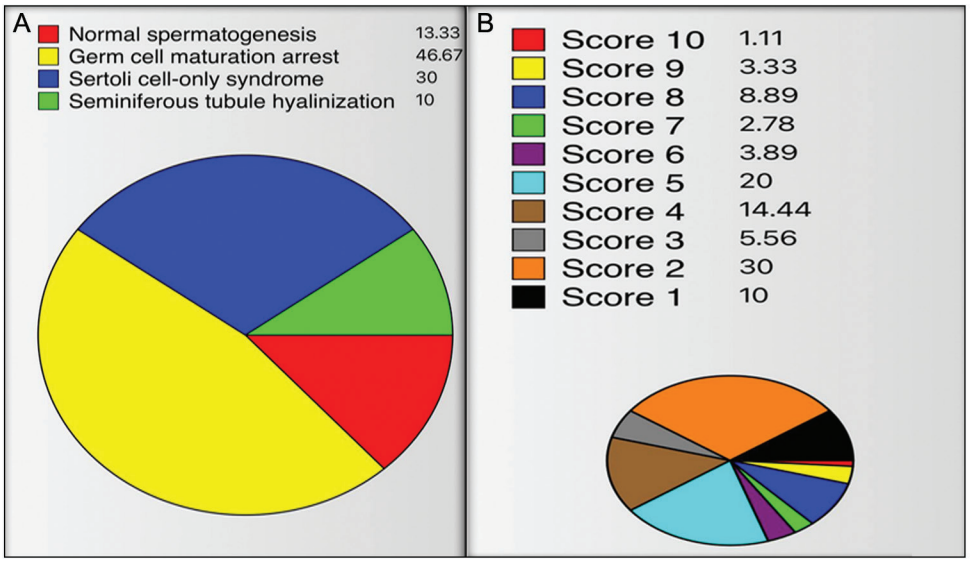

Figure 2: (A) Histopathological classification of testicular biopsies. (B) The modified Johnson's scoring system

Score 8 (8.89%), score 9 (3.33%), and score 10 (1.11%) have been given to normal spermatogenesis. There was a significant difference in comparison among these scores (P value < 0.05).

Discussion

Male infertility is a poorly studied problem due to social reasons.[2] Male infertility due to azoospermia has various causes such as genetic disorders, lifestyle habits, medical illnesses, and medications.[15] They have been divided into pretesticular, testicular, and post-testicular causes.[15] In about 28% of male infertility the cause is unknown (idiopathic).[16]

Pretesticular causes are non-obstructive causes that may be induced by hypothalamic or pituitary lesions (congenital or acquired), and both are characterized by the inability to produce GnRH and thus affects the production of sperm. These cases can get benefits and improvement as they respond well to hormonal replacement therapy.[17] Other pretesticular causes include some medication (Nitrofurantoin and Colchicine)[18] or radiation.[16]

Testicular causes or testicular failure are non-obstructive conditions that may be related to testicular agenesis, cryptorchidism, inflammation, radiation, or surgery.[2,16] One of the most important and preventable inflammatory lesions of the testis is mump.[19] Other causes are medications such as cyclophosphamide (which may cause testicular atrophy), diabetes, and tumor.[16,18]

The known post-testicular causes are absolute obstructive lesions. The obstruction may be due to the absence of one part or more of the testis such as epididymis or vas deferens that carry sperms away from the testis.[20] Congenital bilateral absence of vas deferens is a genetic disorder associated with carrying or having cystic fibrosis.[21] Another important cause of obstruction is varicocele which affects 15% of adults and only 20% of those with varicocele will have some degree of infertility.[22,23] Varicocele is associated with a decrease in sperm number and motility and may cause testicular atrophy if longstanding which indicated the need for surgery.[23]

In this study, 13.33% of cases showed normal spermatogenesis and scored as: score 10 (adequate and organized), 9 (adequate but disorganized), and 8 (normal spermatogenesis with a reduction in the number of spermatozoa). So hormonal profile and family history and other clinical data have been discarded; however, they remain essential complementary data for a full assessment of the etiological factors. So, a full evaluation of azoospermic patients is not so easy to say restricted to testicular pathology and remains enquiring answers for clinical and cytogenetic questions.

This necessitates introducing advice for these patients to complete their investigation with a genetic profile related to azoospermia and to recommend enquiring full family and medical history of each patient before doing a testicular biopsy.

In this study, 13.33% of cases showed normal spermatogenesis and scored as score 10 (adequate and organized), score 9 (adequate but disorganized), and score 8 (normal spermatogenesis with a reduction in the number of spermatozoa). This proportion is less frequent in comparison with other studies: Huma *et al.* (16.98%),[2] Layla *et al.* (29.29%),[16] Ramadan *et al.* (30%),[24] and Anita *et al.* (6.5%).[14] This means that obstructive lesions such as varicocele in our patients were less frequent; however, it formed a considerable problem and the patient needs to be fully investigated so the invasive testicular biopsy technique should be saved to the end. This group

of patients may get benefit from effective treatment such as the microsurgical reconstruction of the obstructed ducts.[25] Also, these patients may get benefit from isolating some viable intact spermatozoa for fertilization purposes (artificial insemination or IVF).[26,27]

The most prevalent histopathological pattern was germ cell maturation arrest, which comprised 46.67% and scored as 3–7 according to the modified Johanson's scoring system. In comparison with other studies: Huma *et al*. (15.09%),[2] Layla *et al*. (12%),[16] Ramadan *et al*. (38%),[24] and Anita *et al*. (25%).[14] This higher proportion of cases with maturation arrest is significantly different. This finding gives us the suggestion of the urgent need for introducing a screening tool for early detection of any testicular lesion and pick up those cases prone for the development of azoospermia. The size of such a problem is undetermined as many of these cases may have some genetic or chromosomal abnormality and nowadays a limited genetic test in our region has been introduced. Most of these patients were presented with early arrest (85.7%) in comparison with late arrest (14.3%), which means that there is a delay in clinical presentation and most patients are in real need of early consultation.

In this study, germ cell aplasia/sertoli cell-only syndrome has formed a large proportion of cases (30%), with spermatogenesis of score 2, which is almost close to other studies: Huma *et al*. (30.18%),[2] Ramadan *et al*. (32%),[24] and higher than other: Layla *et al*. (16%),[16] Anita *et al*. (18.75%).[14] This histological pattern is not specific, and may be related to or associated with many etiologies such as chromosomal abnormalities, orchitis, cryptorchidism, radiotherapy or may be due to embryological failure of germ cell migration to gonadal ridges.[28,29]

Seminiferous tubules hyalinization with interstitial fibrosis (score 1) was the least frequent histological pattern, yet it formed about 10% of cases. This percentage considered low when compared to other studies, Huma *et al.* (7.54%),[2] Layla *et al*. (16%),[16] and Anita *et al*. (34.375%)[14] with significant difference (P value < 0.05). This means that primary testicular lesion in our region is less frequent, and most of them were presented in late stage. Another contributory evidence for this low prevalence is most patients usually did not undergo testicular biopsy since the testis is small in size and can be diagnosed by clinical, hormonal, or cytogenetic data. This pattern may be seen in Klinefilter's syndrome, cryptorchidism, inflammation, endocrine, or in obstructive causes of azoospermia.[13]

Also, many cases of azoospermic testicular biopsies have shown some proportion of intermixing different histological patterns and spermatogenesis scores in same patient. However we have depended on the predominant histological pattern and spermatogenesis score for classification and finalization of results. In contrast, these results mean that there were some intermixed etiological factors playing a role in the pathogenesis of azoospermia, in that some of them were related primarily to hormonal factors, others to inflammatory lesions and in part to some genetic etiology. So histological pattern remains not specific and not pathognomonic for reflecting the etiological factors of azoospermia.

Conclusion

We can conclude that germ cell maturation arrest is the most frequent pattern of azoospermic patients in our study, followed by germ cell aplasia/sertoli cell-only syndrome. Seminiferous tubule hyalinization/interstitial fibrosis was the least frequent change, whereas normal and adequate spermatogenesis formed a considerable proportion that needs to be further investigated. Histological pattern remains not specific and not pathognomonic in reflecting the etiologic factors of azoospermia. So, full evaluation of azoospermic patients for clinical and cytogenetic aspect which are complementary to histopathological examination for the final diagnosis.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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