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RESEARCH ARTICLE

Histological patterns of testicular biopsy in patients with azoospermia: single-institution experience

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Objective of this study was to identify the histopathological patterns and their frequency in testicular biopsy specimens from azoospermic patients and to categorize it according to Modified Johnsen scoring system. **Methods**: Testicular biopsies from male patients with clinical diagnosis of azoospermia were included in this study. All tissue samples were fixed in buffered 10% formalin, routinely processed and stained with Hematoxylin and Eosin. All cases were examined microscopically and categorized according to the histopathological patterns and Modified Johnsen scoring system. **Results**: Total 219 cases of testicular biopsies from 125 azoospermic male patients were evaluated, with 94 cases of bilateral testicular biopsy. The most prevalent age group was of 30-39 years (66.2%). The most common histological pattern was of Sertoli cell only syndrome (58.4%) while the least represented pattern was germ cell maturation arrest, seen in 4.6% cases. The most common Modified Johnsen score was 2 (66.7%). There was discordance in histologic pattern in both testes in 12.76% of patients who had bilateral testicular biopsy. **Conclusion**: Our study gives an insight on the most common histopathological patterns of azoospermic patients and emphasizes the need for a better national statistics and epidemiological studies of this entity. It also points out the significance of the bilateral testicular biopsy, as both, diagnostic and therapeutic procedure.

Keywords: infertility, histology, testis

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Introduction

Nowadays, infertility represents a major health issue that affects approximately 17,5% of adults worldwide as reported by WHO [1]. Azoospermia affects 10-15% of men experiencing infertility, and it is defined as a total lack of sperm cells in the semen. Depending on the presence of the obstruction in the extratesticular excurrent ducts, it is classified into obstructive (OA) and non-obstructive azoospermia (NOA), where 60% of cases are attributed to NOA [2,3].

In vitro fertilization can be the option for the treatment of infertility, if testicular sperm can be successfully retrieved. The latter can be easily done in OA because spermatogenesis is usually preserved. In men with NOA, an operative treatment called microdissection testicular sperm extraction (micro-TESE) is performed, because the spermatogenesis is limited to some seminiferous tubules, most likely dilated ones [4]. The rate of successful micro-TESE procedure is related to treated population characteristics, mainly age and histological pattern in testes [5,6]. Proper histopathological report in a diagnostic biopsy of the testis is invaluable for the prognosis of the micro-TESE procedure [4]. The most common morphological patterns identified in the testicular biopsies of NOA patients following negative sperm retrieval during a micro-TESE procedure are: hypospermatogenesis (HS), germ cell maturation arrest (GCMA), Sertoli-cell only syndrome (SCOS), seminiferous tubule hyalinization (STH) and mixed pattern (MP). Therefore, histopathological analysis is also critical for the proper selection of patients for the "second look" micro-TESE attempt, which increases the possibility for successful infertility treatment [7].

The aim of this study was to identify the histopathological patterns and their frequency in testicular biopsies among the azoospermic men, received at our institution for the past eight years.

Methods

Patients/Materials and study design

In this retrospective study we included testicular biopsies referred to the Department of Pathology, Faculty of Medicine, University of Sarajevo, in a period from January 2015 until May 2023, based on the clinical diagnosis of NOA on routine sperm analysis. Patient age and information about the side of testicular biopsy were extracted from the patient's medical records. Patients either had unilateral or bilateral testicular biopsy.

Pathological data

All testicular tissue samples were processed as routine, fixed in 10% buffered formalin, stained with Hematoxylin and Eosin (HE) and examined histologically by light microscopy. The biopsies were examined for histological uniformity within the same testis and between right and left testes. Histologic assessment implies histologic classification into one possible categories: HS, GCMA, SCOS, STH and MP [4,8].

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By hypospermatogenesis, it is considered reduced cellularity of germinal epithelium with present but reduced in number all stages of germ cells, including spermatogonia, spermatocytes, and spermatids. Germ cell maturation arrest marks the arrest of spermatogenesis at a specific cell stage, mostly at the primary or secondary spermatocytes (early and late maturation arrest). Sertoli cell only syndrome - tubules are normal or slightly decreased in diameter with a slightly thickened basement membrane, containing only Sertoli cells but no primary spermatocytes, spermatids or spermatozoa. The interstitium contains normal numbers of Leydig cells in the majority of cases. Seminiferous tubule hyalinization - small diameter tubules with thickened basement membrane and usually increased Leydig cells. If more than one pattern was observed in one biopsy (obtained from one side), the pattern was categorized as mixed.

The biopsy was labeled as a discordant pattern if the right and left testicular biopsies showed different patterns.

All biopsies were scored from 1-10 according to the Modified Johnsen scoring system for assessment of the level of spermatogenesis [9] as presented in Table I. For the mixed pattern and discordant pattern cases, the areas showing better histological patterns were selected for scoring in each case.

Statistical analysis

The data were analysed statistically using IBM Statistics SPSS version 25.0. Data were analysed according to type using the Chi-square test and Spearman's correlation test, where appropriate. Statistical significance was observed for p values of less than 0.05.

Results

A total of 219 cases of testicular biopsies of 125 males with azoospermia were evaluated, where 94 cases were bilateral testicular biopsies. The median age of patients was 36 years (range 23-49 years). The most prevalent age group was 30-39 years (66.2%). The smallest number of testicular biopsies was obtained from males in the age group younger than 30 (10%).

All the cases were categorized according to the mentioned histopathological categories as shown in Table II.

Table I. Modified Johnsen score for assessment of spermatogenesis.

Score	Level of spermatogenesis
1	No seminiferous epithelium, prominent sclerosis
2	Only Sertoli cells present
3	Spermatogonia present without other element of spermatogenesis
4	Spermatogenesis arrested at the level of primary spermatocyte
5	Many spermatocytes, no sperm or spermatids
6	Only few early spermatids without late spermatids or spermatozoa
7	No late spermatids and sperm; many early spermatids
8	Reduced number of sperm with less than 5 spermatozoa per tubules and a few late spermatids
9	Incomplete spermatogenesis with many late spermatids
10	Full spermatogenesis

The most common histological pattern was of Sertoli cell only syndrome (58.4%) followed by a mixed pattern found in 22.8% of cases. The least represented pattern was GCMA, seen in 4.6% of cases. The frequency of histologic pattern combinations among 50 cases of mixed pattern is shown in Table III.

The most common histological patterns are presented in Figure 1.

In 12 patients (12.76%) who had bilateral testicular biopsy, there was discordance in histologic pattern in both testes, most frequently showing a mixed pattern in one testis and seminiferous tubule hyalinization in the other. Although patients with a discordant pattern were older (38.17±6.7) compared to patients with same histologic pattern in both testes (35.71±5.0), there was no statistically significant difference (p=0.106).

All the cases were further categorized according to the Modified Johnsen scoring system. The most common Modified Johnsen score was 2 (66.7%) (seminiferous tubules containing only Sertoli cells). Results of the modified Johnsen scoring are shown in Table IV.

We investigated the distribution of histologic patterns among patients' age groups and found that the SCOS pattern was the most common in the age group of 30-39 years and least represented in the age group of patients younger than 30. In the age group of younger than 30 ,increases the percentage of patients with HS and STH (p=0.001). Patients with GCMA were older (median age 37.60±3.02), than patients with other histologic patterns, but without statistical significance (p=0.896). There was no significant correlation between patient's age and Johnsen's score (p>0.05).

Discussions

Male infertility, in comparison to female infertility, is generally not well reported for a number of reasons, ranging from the lack of the exact definition of a case, problems

Table II. Distribution of histological patterns in testicular biopsies.

Histologic pattern	n	%
HS*	15	6.8
GCMA†	10	4.6
SCOS‡	128	58.4
STH§	16	7.3
MP ‖	50	22.8
Total	219	100.0

*Hypospermatogenesis; †Germ cell maturation arrest; ‡Sertoli cell only syndrome; §seminiferous tubule hyalinization; ‖ Mixed pattern

Table III. Mixed histological patterns in the same testicular biopsy.

Mixed histological pattern	n	%
SCOS* and STH†	17	34
HS‡ and STH	9	18
SCOS and GCMA§	13	26
STH and GCMA	6	12
SCOS and HS	5	10
Total	50	100

* Sertoli cell only syndrome; † seminiferous tubule hyalinization; ‡ Hypospermatogenesis; §Germ cell maturation arrest

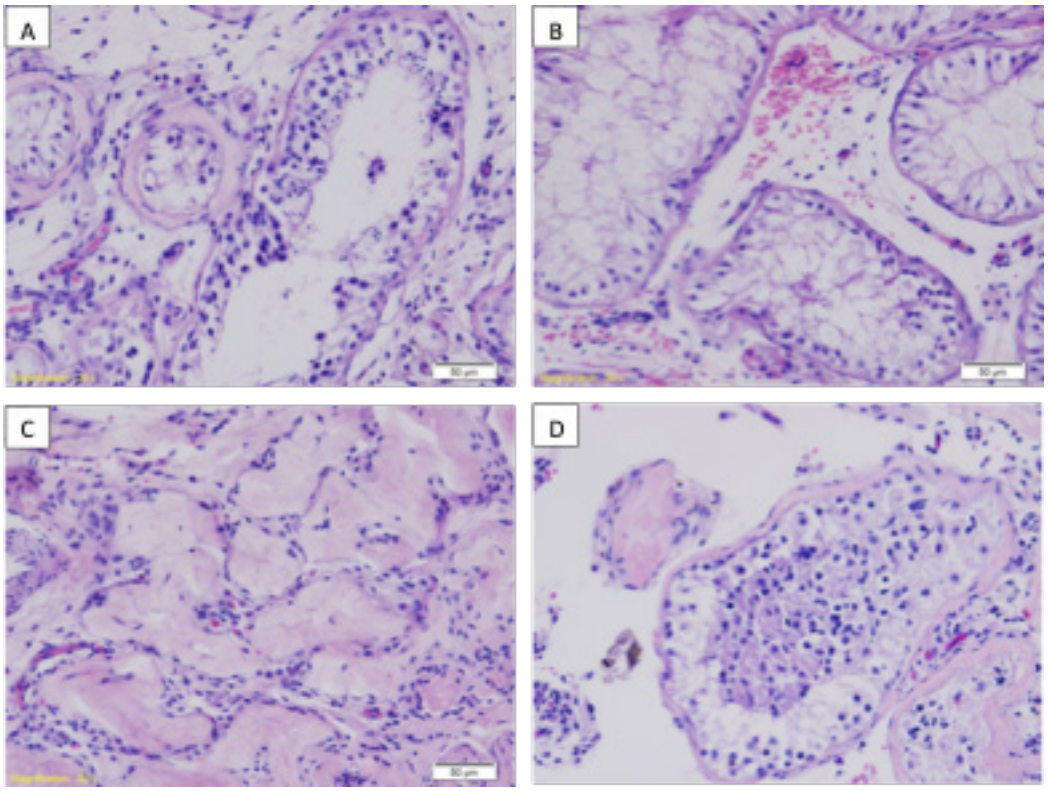

Fig. 1. Different histological patterns of testicular biopsy in azoospermic patients: A) Mixed pattern - Sertoli cell only syndrome and Hypospermatogenesis (HE, x200); B) Sertoli cell only syndrome (HE, x200); C) Seminiferous tubule hyalinization (HE, x200); D) Germ cell maturation arrest (HE, x200).

Table IV. The frequency of Modified Johnsen score in testicular biopsies.

MJS*	n	%
1	5	2.3
2	146	66.7
3	5	2.3
4	34	15.5
5	7	3.2
6	6	2.7
7	3	1.4
8	1	0.5
9	9	4.1
10	3	1.4
Total	219	100.0

* Modified Johnsen score

with national and regional statistics, to cultural issues. Thus, even the small regional statistics are valuable and can be considered as a step in the identification and adequate treatment of infertile men [10]. The aim of this study was to present the distribution of histopathological patterns found in testicular biopsies of a population of azoospermic men, referred to the Department of Pathology, Faculty of Medicine, University of Sarajevo for an eight-year period.

According to the review of the currently available literature, the reported age of infertile men undergoing testicular biopsy varies significantly. In our study, the mean age was 36 years, which is similar to the reports of the studies concluded in Central Europe and the Middle East [11,12]. In contrast, a younger median/mean age is regularly mentioned in the studies from Africa [10, 13, 14], thus emphasizing the potential geographical differences.

Pathohistological findings provide the confirmation of the nonobstructive/obstructive nature of azoospermia and the determination of the dominant pattern helps further diagnostics and therapy. Interestingly, in this study none of the biopsies revealed normal spermatogenesis while other authors present a frequency ranging from 5% to 35% for normal histology [11, 15]. This suggests the rigorous selection of patients to undergo an invasive procedure of testicular biopsy, based on the previously done diagnostics. Testicular biopsy can be considered only for azoospermic men, which is a case of our study, while other centers perform it even for individuals with oligospermia. On the other hand, this result could indicate poor and delayed overall referral of patients to the reproductive specialists.

In this study, the most common histological pattern was Sertoli cell only syndrome (58.4%) followed by a mixed pattern (22.8%) while the least represented pattern was GCMA (4.6%). This is in accordance with the study of Spahovic et al. [7] and Mushtaq [15]. SCOS is one of the most common, but also the most serious type of nonobstructive azoospermia and can have various etiology, including gonadotropin deficiency, cryptorchidism, orchitis, prostate cancer hormonal therapy, radiation, Klinefelter syndrome [4]. Contrary, other studies showed hypospermatogenesis as the dominant pattern [13, 16, 17]. In this study, a discordant pattern was found in 12.76 % of the biopsies. We can conclude that this result is in accordance with the data published previously, independently of the different methods of calculation [14,18]. These results em-

phasize the advantage of bilateral biopsies not only for the definite diagnosis but for the treatment options. As it is noted before, the absence of sperm in one testis does not necessarily rule out its presence/absence in another [14]. Symmetry of the testes, observed during a clinical exam, is not a relevant factor because a discordant pattern is found in approximately 21% of symmetrical testes in comparison to 26% of asymmetrical testes [18].

The most common Modified Johnsen score in our study was 2 (66.7%) (seminiferous tubules containing only Sertoli cells) which is lower than the median score reported in the study by Plas et al. [18] and in accordance with the results of other studies [15]. Possible explanations can be found in a rather small sample and relatively older patients.

Conclusion

In conclusion, our study gives an insight into the most common histopathological patterns found in testicular biopsies in cases of male infertility encountered in our department. It emphasizes the need for generally better national statistics and epidemiological studies of this entity. The results of our small study, in accordance with the currently available literature, point out the significance of the bilateral testicular biopsy, as both a diagnostic and therapeutic procedure.

Authors' contribution

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript;
NČ (Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing);
VM (Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – original draft);
SČ (Conceptualization, Methodology, Supervision, Validation, Visualization, Writing – original draft);
MB (Investigation, Methodology, Supervision);
MD (Investigation, Methodology, Supervision);
ELS (Supervision, Validation, Writing – review & editing);
SK-V (Conceptualization, Supervision, Writing – review & editing).

Conflict of interest

None to declare.

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