Histopathologic Patterns of Testicular Biopsies in Men with Infertility and Azoospermia in a Nigerian Tertiary Hospital

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ABSTRACT

Infertility is a common public health problem worldwide. It is defined as inability to conceive after one year of unprotected sexual intercourse. Investigations for male infertility include seminal fluid analysis (SFA) and azoospermia is one of the abnormal findings. Testicular biopsy is frequently done on such patients to unravel the testicular cause of infertility.The aim of this work was to study the pattern of testicular histopathological findings in patients with azoospermia among men with infertility in our facility. We retrospectively evaluated testicular histology reports of twelve (12) patients between 2018 and 2020 who underwent bilateral testicular biopsy for azoospermia. Biodata of these patients alongside with the type of infertility, duration of marriage was entered into a proforma designed for this study. Patterns of histopathology were further scored using the modified Johnson Scoring system and recorded. Data were entered into the statistical package for social science (SPSS) version 20.0 software and analyzed. Mean age of patients was 39.75±6.122 years ranging from 34-53years. Primary infertility predominated. Mean duration of marriage was 4.92 ± 2.392 years ranging from 2 to 10 years. Men in their 4th decade of life formed the majority (58.3%). The most commonly encountered histopathological pattern was seminiferous tubule hyalinization in 41.7% of the cases with a modified Johnson Score of 1 dominating the picture (58.3%). Our patients were relatively young with majority having poor prognostic testicular histologic pattern. This was also seen in both local and international studies.

Keywords: Histopathologic patterns, Testicular biopsy, men with infertility, Azoospermia

INTRODUCTION

Infertility is defined as inability to conceive after one year of unprotected sexual intercourse.[1] It is further divided into primary when conception has never occurred and secondary when conception has occurred previously regardless of the outcome. Infertility is a global health problem being reported in 15% of couples and quoted to be higher (15-45%) in Sub-Saharan Africa with same high values reported in Nigeria (20-30%).[2,3,4,5] Researchers have reported that about 20% of infertility cases are male factor related while 30-40% are contributory from both partners.[6,7]Azoospermia is defined as complete absence of sperm in the ejaculate and it is present in about 1% of all men and as high as 10-15% men with infertility.[8] Aetiology of azoospermia may be due to pre-testicular, testicular and post-testicular factors.[7] Pre-testicular causes are due to extra-gonadal endocrine origin which is reported to be relatively rare while testicular causes are mainly intrinsic testicular disorders of spermatogenesis. Post-testicular factors are due to ejaculatory dysfunction, absence of the vas deferens or obstruction. Pre and post testicular causes are considered correctable, while testicular disorders except those due to varicocele are irreversible.[7,9]The aim of this study was to evaluate the histopathological pattern of azoospermic testes and to differentiate between obstructive and non-obstructive types. With these, we can develop protocols in future for management of such patients.

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MATERIALS AND METHODS

This was a retrospective study of twelve (12) patients with azoospermia who underwent bilateral testicular biopsy from January 2018 to December 2020. As a norm, patients were counseled on this procedure and informed consent duly obtained. Open

bilateral biopsy of the testes was done and specimens transported in Bouin's solution to the histopathology laboratory. All results were retrieved from their folders and entered into a proforma together with patient's biodata, type of infertility and duration of marriage. Data were transferred to spread sheet and analysed using SPSS version 20.0 software. Frequency table was constructed for categorical variables and mean and standard deviation for continuous variables were also done. Histopathological classification of testicular biopsy results was done and age of patients was correlated with other variables. Pvalue < 0.05 was set as significant. The testicular biopsy results were categorized into different histological patterns[10] and scored using the modified Johnson Scoring system from 1 to 10[11] as follows:

Score 10: There is full spermatogenesis

Score 9: Incomplete spermatogenesis with many late spermatids

Score 8: There are less than 5 spermatozoa per tubules and a few late spermatids

Score 7: There are many early spermatids but no spermatozoa or late spermatids.

Score 6: There are few early spermatids but no spermatozoa or late spermatids

Score 5: there are many spermatocytes but no spermatozoas or spermatids

Score 4: There are few spermatocytes but no spermatozoas or spermatid

Score 3: There are only sperm atogonias

Score 2: Only presence of Sertoli cells and no germinal epithelial cells

Score 1: There is no seminiferous epithelium

RESULTS

Twelve (12) patients with infertility who had bilateral testicular biopsy for azoospermia were retrospectively studied. Mean age of all patients was 39.75 $\pm$ 6.112 years ranging from 34 to 53 years. Men with primary infertility were younger than those with secondary infertility (Table 1). Mean duration of marriage was 4.92 $\pm$ 2.392 years ranging from 2 to 10 years. For the men with primary and secondary infertility, their mean duration of marriage was 4.78 $\pm$ 1.858 and 5.33 $\pm$ 4.163 years respectively. Most men were in their 4th decade of life [n=7(58.3%)]. (Table 2) Men with primary infertility formed the majority [n=9(75%]. (Table 2)The histopathological classification oftesticular biopsy results showed seminiferous tubule hyalinization as the most common pattern [n=5 (41.7%)].(Table 3) There were 2 cases of mixed histopathological pattern which was equally distributed between seminiferous tubule hyalinization-sertoli cell only syndrome and germ cell maturation arrest-sertoli cell only syndrome. (Table 4) On grading of the histologic pattern with modified Johnson; score of 1 formed the majority [n=7(58.30%)]. (Table 5) Age of patients correlated significantly with the type of infertility (r=.616, P <0.5). (Table 6).

Table 1: Means of continuous variables

Variables	Means and Std	Range
Age (Years)	39.75+6 .122	34-53 years
Age with Primary infertility	37.67 + 3.905	34-44 years
Age with secondary infertility	46.00 + 8.185	37-53 years
Duration of Marriage (years)	4.92 + 2.392	2-10 years
Duration for primary infertility	4.78 + 1.858	2-8 years
Duration for secondary infertility	5.33 + 4.163	2-10 years

Table 2: Frequency of Categorical variables age range and type of infertility

Age range			
Age (Years)	**Frequency(n)**	**Percent (%)**	**Cumulative %**
30-39	7	58.3	58.3
40-49	4	33.3	91.7
50-59	1	8.3	100.0
	12	100.0	
Type of Infertility			
Primary	9	75.0	75.0
Secondary	3	25.0	100.0
Total	12	100.00	

Table 3:Histopathological Classification of testicular Biopsies (n=12)

Histopathological classification	**No. of cases**	**Percentages (%)**
Normal Spermatogenesis	0	0.0
Hypospermatogenesis	1	8.3
Maturation arrest	3	25.0
Sertoli cell syndrome	1	8.3
Seminiferous tubule hyalinization	5	41.7
Mixed pattern	2	16.7
Discordant pattern	0	0.0
	12	100.0

Table 4: Mixed histopathological pattern in the same Testicular biopsy (n=2)

Mixed Histological patterns	**No. of cases**
*Seminiferous tubule hyalinization and sertoli cell only syndrome	1
*Maturation arrest and sertoli cell only syndrome	1

Table 5: Modified Johnson Score category (n=12)

Johnson scoring	**Number of cases (n)**	**Percentages (%)**
8	1	8.30
7	1	8.30
5	1	8.30
4	1	8.30
2	1	8.30
1	7	58.30
Total	12	100.0

Table 6: Correlations

Age Vs histopathological patterns	r =.103,	P>.05
Age Vs type of infertility	r=.616,	P<.05*
Type of infertility Vs histopathological pattern	r= - .154,	P >.05

DISCUSSION

Testicular biopsy is a vital tool in the diagnosis of gonadal causes of azoospermia, directs therapeutic goals and reproductive prognosis and provides histologic information that distinguishes obstructive from non-obstructive azoospermia. This distinction is necessary as obstructive causes usually respond to microsurgical procedures, while the non-obstructive type may be irreversible except in impaired spermatogenesis caused by varicocele.[7,9] Obstructive azoospermia is not a rare condition among azoospermic men - it occurs in a range of 20 - 42% in many studies.[7,8,12-14]

Mean duration of infertility in this study was 4.92 years. This compares with a previous report (5.0 years) in the South Eastern part of Nigeria and 4.9 years from a Mongolian infertility study.[15,16] The mean age of the patients was 39.75 $\pm$ 6.122 years. They were older than patients with azoospermia in other studies.[17,18] This may be due to cultural practices of early marriage in that population. Men with primary infertility were younger than those with secondary infertility ($P > .05$) and the duration of marriage before presentation for evaluation was also shorter. Patients with primary infertility are most likely to present early because of lack of conception, while men with secondary infertility having fathered children before may likely present late for evaluation. Primary infertility group formed the majority (75%). Generally, in infertility clinics in Sub-Saharan Africa, several studies have shown that men with secondary infertility dominate.[19,20] Another study in Nigeria reported more men with primary infertility in their cohort.[18]

In this study, none of the patients had normal spermatogenesis indicating a predominantly primary testicular failure. This compares with previous studies in Nigeria where a high incidence of primary testicular failure was reported with very low proportion of normal spermatogenesis.[12,13,18] Azoospermia with normal spermatogenesis is usually due to ductal obstruction probably from previous sexually transmitted infection.[21] We believe that educational awareness on this infection and improved medications for treatment have reduced the incidence in Nigeria.

Hypo-spermatogenesis was seen in 8.3% which corresponds to modified Johnson Score7. This was lower than in other reports that documented between13-19%.[12,17,22] In hypo-spermatogenesis, viable and intact spermatozoa may be harvested for fertilization and when they are absent, the prognosis remains poor. This condition may be due to environmental and industrial toxins that disrupt testicular function or some preexisting genetic testicular dysfunctions.[23] In the post-Gulf war, Haddad *et al.* reported a high incidence of this condition (55.8%). This confirms atmospheric or environmental toxins as key factor.[24]

Germ cell maturation arrest was recorded in 25% of men corresponding to Johnson Score of 6-3. This is far higher than other reported studies and lower than a study in Brazil that focused on spermatozoa retrieval for intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermic men.[17,22,24,25] This condition is usually due to genetic factors, radiation exposure, chemotherapeutic drugs, excessive alcohol intake and chronic marijuana use.[26] The germ cells are usually arrested at either the primary or secondary spermatocyte stage and no spermatids are formed.[10]

Sertoli cell only syndrome was reported in 8.3% of cases representing Johnson Score of 2. It was lower than in other studies, similar to another report and higher than same study elsewhere.[17,22,24,27,28] It is a histologic pattern where no germ cells are seen in any profile and said to be an irreversible condition.[29] Documented underlying causes include cryptorchid testis, orchitis, post-irradiation and chemotherapy administration, antiandrogens and oestrogen therapy and of course chronic liver disease.[29] Genetic causes have also been advocated as in deletion of human azoospermia factor (AZF) gene located in the long arm of chromosome Y.[30]

Seminiferous tubule hyalinization was seen in 41.7% corresponding to Johnson

score of 1. In this study, this histologic pattern dominated the picture and consistently higher than figures reported both locally and internationally.[17,22,24,26,27] This wide discrepancy is difficult to explain but may be due to the population studied and biopsy selection criterion between different urologists and even differences in the interpretation of histology results. One study in Nigeria suggested a past history of inflammation in the testis as one of the possible causes.[12]

Mixed pattern was reported in 16.7% of cases (n=2) and consisted of both sertoli cell only syndrome-seminiferous tubule hyalinization and sertoli cell only syndrome - maturation arrest respectively. In one report, it was reported in 9.43% and 9.0% respectively, giving a lower incidence than in our centre.[17,31]

We did not encounter any discordant variant in our samples. This might not be completely true in our population. It might be due to the small sample size in our study and selection bias. McLachlan *et al.* commented that this is a common finding in many testicular histopathologies in men with azoospermia and in another report by Abdullah *et al*, 5% of the population studied had discordant picture.[31,32] Testicular biopsy discriminates between obstructive and non-obstructive causes of azoospermia and in the former can have a predictive role in the possibility of harvesting viable spermatozoa for intracytoplasmic sperm injection(ICSI). The obstructive type can further guide cost effective tubal surgeries for a remedy. Even in the irreversible poor-prognostic patterns like sertoli cell only syndrome (SCOS) and tubule hyalinization, the urologist can have the courage to guide factual counseling to the patient and rest from a futile attempt for conception.

In our study, seminiferous tubule hyalinization and germ cell maturation arrest were prominent histological findings and those with modified Johnson score of 1 were more than 50%, giving a bleak prognostic outcome. In war-torn areas and industrialized countries, the picture is more of a worsening histologic pattern possibly due to air pollution, social cultural practices and with some genetic undertones. A multicentre study needs to address this life depressing situation. In this study, we found that age of patients correlated positively with histopathological patterns ($r = .616$, $P < .05$). This agrees with the fact that the older a man, the more likely that his fertility potential drops.

LIMITATION

This was a retrospective study with its inherent flaws; there may be inter-observer variability among the results of the different histopathologists who reviewed the slides; the sample size was small and may skew the results. A prospective large multi-centre study is considered in future.

CONCLUSION

Bilateral testicular biopsy and a careful look at the histology will help in the further management of men with azoospermia. These histologic patterns in our study, similar to many others globally,indicate the need for an effective educational campaign against harmful socio-cultural habits, advocacy with government on ways to eradicate industrial pollution and also genetic counseling, when necessary.

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