Original Article

Anatomy

Histomorphological Patterns of Testicular Biopsies in Azoospermic Infertile Males from a Tertiary Care Unit of Pakistan

1. Sadia Qazi 2. Atif Mazhar 3. Surriyya Sarwat

1, 2. Lecturers of Anatomy, College of Medicine, Alfaisal University, Al Maather Road, Riyad. Kingdom of Saudia Arabia 3. Asstt. Prof. of Anatomy, Sindh Medical University, Karachi

ABSTRACT

Objectives: The objective of this study was to find out the most prevalent histological phenotype in azoospermic testis in those infertile men who presented with lab diagnosis of azoospermia and to compare our data with international studies.

Study Design: Cross Sectional Hospital Based Study

Place and Duration of Study: This study was conducted at Anatomy Department, SMU, Karachi for duration of one year from January 2010 till January 2011

Materials and Methods: This study was carried out in 100 men with azoospermia, going for a trial of ICSI. All underwent Testicular Sperm Extraction (TESE) for the retrieval of sperm for ICSI and for histopathological diagnosis.

Results: The mean age of the studied group was 36.64 ± 5.217 years with 33.62 years as the lower limit and 35.66 years as the upper limit of 95% confidence interval. Body mass index was also calculated after height and weight measurement, which was observed as 28.22 ± 7.12 kg/m2 with 26.83 as the lower limit and 29.61 as the upper limit of 95% of confidence interval.

The microscopic assessment of testicular biopsy showed that normal spermatogenesis was found to be in 25% of cases, showing normal tubular diameter with the presence of all stages of spermatogenesis. 31% of testicular biopsies showed hypo-spermatogenesis, characterized by reduced population of germ cells seen in the tubules and alteration in the order of spermatogenesis. Maturation arrest was seen in 17% of cases, evident by a halt of maturation sequence, at the stage of primary spermatocyte. Abundant cells in division were visible but no spermatid or spermatozoa were seen. Sertoli cell only was apparent in 17% of cases in which the tubules were populated with by only sertoli cells with the complete absence of germ cells. Generalized fibrosis was seen in 13% of case s which showed the atrophic tubules had a thickened, convoluted basement membrane with a hyaline appearance surrounding a lumen obliterated by fibrous tissue.

Conclusion: Hypospermatogenesis was found to be the commonest pattern in testicular biopsies of studied population. This study supports the recommendation of bilateral testicular biopsies when investigating male infertility.

Key Words: Intracytoplasmic sperm injection, Body mass index, Hypospermatogenesis, Male infertility, Testicular biopsy

INTRODUCTION

World Health Organization (WHO) defines infertility as the absence of conception after at least 12 months of unprotected intercourse and about 10-15% of couples are considered infertile.[1] In about one in five infertile couples reproductive problem lies at the male side which is in contrast to the popular concept of sole involvement of the female factor.[2] It is often shocking for the men to know that the problem is related to them.

Now medical interventions can bypass many of the causes of male infertility. The past two decades have seen increasingly rapid advances in the field of assisted reproduction, so if conservative medical treatments fails ,there is still a hope of being successful by in vitro fertilization (IVF) which is one of the assisted reproductive technology (ART) techniques.[3]

Azoospermia which is the complete absence of sperm in the ejaculated semen is one the severe form of male infertility and is present in approximately 1% in all men and it is contributor of 15% of the total male infertility burden. It can be classified as obstructive where there is blockage of the male reproductive tract and non-obstructive where there is problem in spermato-genesis.[3,4]

Although a detailed history, physical examination, hormonal assays and semen analysis are the main tools for assessing male infertility, a testicular biopsy can provide valuable information to the urologist by further dividing men with azoospermia for the purpose of prognosis and treatment but the problem lies in the fact that this classification cannot be done without testicular biopsy.[5]As the sample is examined directly, it is also taken as gold standard for evaluating testicular function and permits the treating physician to take appropriate

steps or withholding the therapy in biopsy indicated hopeless cases after classifying the testicular lesions responsible for the infertility.[6]The testicular biopsy can help predict the chances of finding sperm on microdissection of the testicle which if retrieved can be used intracytoplasmic sperm injection ICSI, which revolutionized the treatment of azoospermic men.[6,7]

The objective of this study was to find out the most prevalent histological phenotype in azoospermic testis in those infertile men who presented with lab diagnosis of azoospermia and to compare our data with international studies.

MATERIALS AND METHODS

This was a cross sectional hospital based study, with a sample size of 100, conducted for duration of one year from January 2010 till January 2011 in which nonprobability, purposive sampling technique was used. The consent for conduction of research and collection of samples was taken from both hospital and patients. The study was started after approval from the institutional ethical committee. Those entire azoospermic male up to the age of 45 years were included in the study who wishes to be a father through IVF and were going for testicular biopsy for sperm search. The patients having age more than 45 years, bilateral cryptorchidism, testicular malignancy, congenital absence of vas deferens (CABVD) and varicocele were excluded from the study. All the cases underwent bilateral testicular biopsy under local anaesthesia. Biopsy samples were obtained with small curved scissors through a small incision on the scrotal skin by urologist. Half of the tissue was used for the IVF purpose and the rest was fixed in Bouin's solution for 24 hours. This preserved fine cellular detail, tubular architecture, Reinke crystalloids in Leydig cells and in particular the morphology of Sertoli cell nuclei so that it was easier to distinguish them from spermatogonia. Bouin's fixed specimens were then transferred to formalin after about an hour to prevent the tissue from becoming hard and difficult to section. The tissues were then immersed in 10% buffered formalin and processed as follows; firstly the tissue was immersed in ascending strengths of alcohol for dehydration. Xylene was used for clearing and then embedding was done with paraffin. The resulting blocks were sliced by rotary microtome into 3-5 micron section and picked up from water bath for mounting on slides. Staining with Haematoxylin and Eosin was done after drying and later examined under light microscope for diagnosis of the following diagnostic patterns. Testicular histology was scored on 1–10 scale according to the Johnson's modified scoring criteria. Each tubular spermatogenetic activity was evaluated according to the percentage of each histomorphological findings and then labelled as normal spermatogenesis, hypospermatogenesis, maturation arrest, sertoli cell only and generalized fibrosis.

Statistical analysis: The data was entered and analyzed on SPSS version 16. Mean ± standard deviation (SD) were computed for the quantitative variables like age, body mass index (BMI).Frequency distribution was calculated for all histological phenotypes.

RESULTS

The mean age of the studied group was 36.64 ± 5.217 years with 33.62 years as the lower limit and 35.66 years as the upper limit of 95% confidence interval. Body mass index was also calculated after height and weight measurement, which was observed as 28.22 ± 7.12 kg/m2 with 26.83 as the lower limit and 29.61 as the upper limit of 95% of confidence interval.

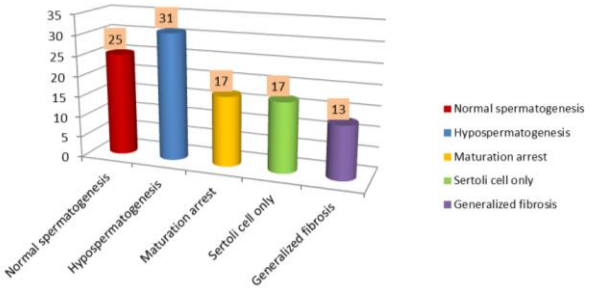

Figure No.1: Frequency Distribution of histological pattern

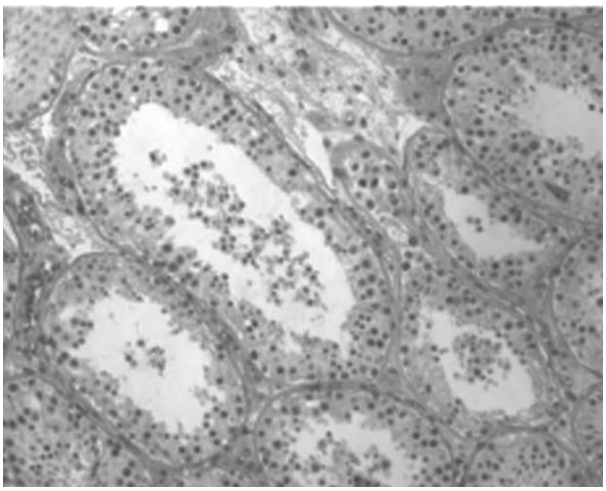

Figure No.2: Normal spermatogenesis at 10 magnification. Photomicrograph of section of testis displayed the seminiferous tubules which showed that all cell types of germ cell elements were represented along with a clear lumen. Haematoxylin eosin (H&E) stained.

92.2% of the males were suffering from primary infertility, while 7.8% were suffering from secondary infertility.

The microscopic assessment of testicular biopsy showed that normal spermatogenesis was found to be in 25% of cases, showing normal tubular diameter with the presence of all stages of spermatogenesis. 31% of testicular biopsies showed hypo-spermatogenesis,

characterized by reduced population of germ cells seen in the tubules and alteration in the order of spermatogenesis.

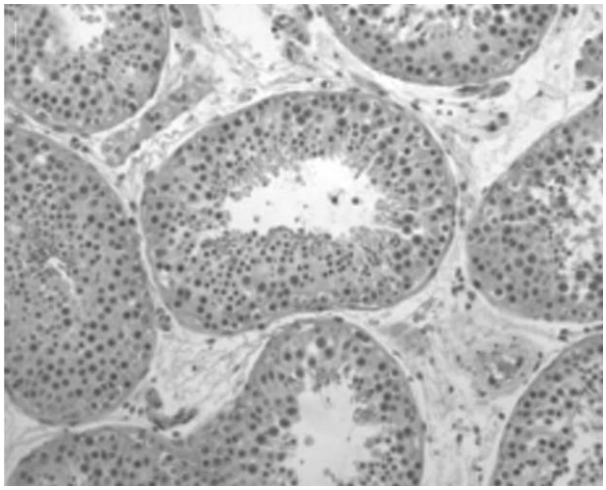

Figure No.3: Photomicrograph of human testis showing hypospermatogenesis at10 magnification, featured reduction in overall germ cells, tubular and luminal diameters. Haematoxylin eosin (H&E) stained.

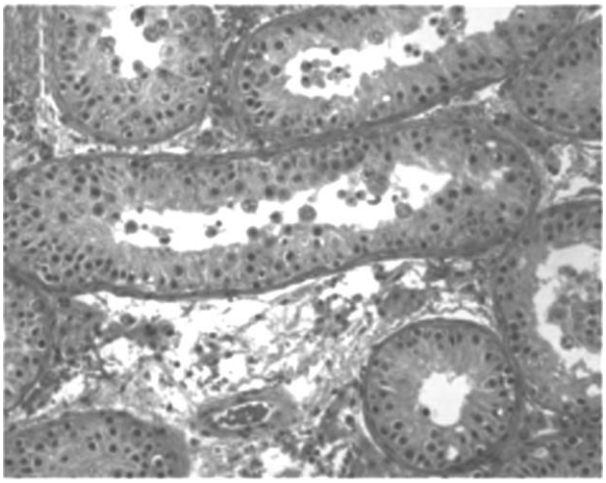

Figure No.4: Photomicrograph of human testis showing maturation arrest at 10 magnification. Section showing a generalized reduction in germ cell elements and tubular diameter. Note the absence of mature spermatid, while primary spermatocytes continue to be prominent. Cellular debris and degenerating spermatocytes can be seen. Haematoxylin and eosin (H&E) stained.

Maturation arrest was seen in 17% of cases, evident by a halt of maturation sequence, at the stage of primary spermatocyte. Abundant cells in division were visible but no spermatid or spermatozoa were seen.

Sertoli cell only was apparent in 17% of cases in which the tubules were populated with by only sertoli cells with the complete absence of germ cells.

Generalized fibrosis was seen in 13% of case s which showed the atrophic tubules had a thickened, convoluted basement membrane with a hyaline appearance surrounding a lumen obliterated by fibrous tissue.

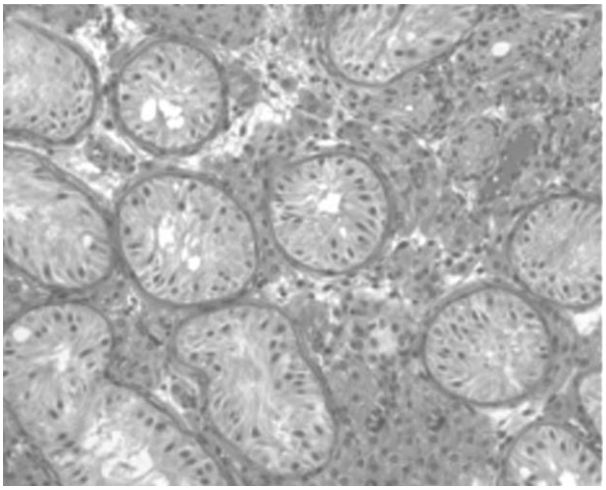

Figure No.5: Photomicrograph of human testis showed Sertoli only cell at 10 magnification, featured absence of germ cells large sertoli cells with reduction in tubular diameter. Clumps of leydig cells were also seen. Haematoxylin and eosin (H&E) stained.

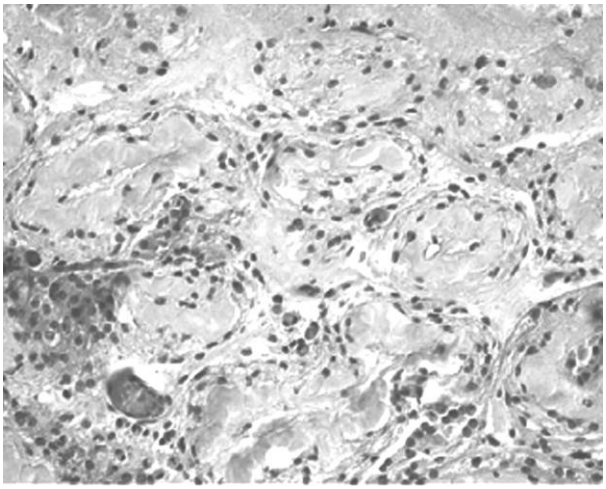

Figure No.6: Photomicrograph of section of human testis showed atrophy and generalized fibrosis, at 40 magnification/ hpf. The atrophic tubules had a thickened, convoluted basement membrane with a hyaline appearance surrounding a lumen obliterated by fibrous tissue. Haematoxylin and eosin (H&E) stained.

DISCUSSION

Infertility is a complex phenomenon and it is also a social taboo particularly in Asian countries. It is labelled as a shameful condition and many of the infertile couples hesitate to discuss it. Male factor infertility is usually not accepted in this part of the world where manhood is the esteem and both fertility and virility meant same to the society. Thus, it is a potentially emasculating condition, surrounded by secrecy and stigma.[8]

Our study showed significant frequency of spermatogenesis in (25%) of cases which suggests the obstructive aetiology, which was in agreement with the study done in Egypt,[9] On the other hand, others[9,10,11]

reported much higher incidence of normal spermatogenesis. Ragab et al.[9] from Egypt reported normal spermatogenesis in 24% of cases. Wong et al. [12]reported similar result (25%). Also, Colgan et al.[13] recorded normal histology in 20% of their cases. Brannen and Roth[14] reported a higher incidence of obstructive azoospermia (35%), and the same was reported by AlRayess et al.[10] documented (31%) normal spermatogenesis and Brannen et al[14] yielded 35%. Another study done in Nigeria by Thomas et al.[15], reported 38% cases of normal spermatogenesis .

Few other studies are in contrast with our findings and reported a much lower frequency of normal histological pattern. Meinhard et al.[16] reported 5% for obstructive azoospermia; Haddad et al.,[17] in a study from Jordan, reported 11.2% for obstructive azoospermia; and Nagpal et al.[18] reported similar results. A study done in Saudi Arabia by Abdullah L. et al.[19] reported 13 % frequency.

Hypospermatogenesis represented the most common finding 31% (29 cases, 29%) in the present study. Thomas[20] reported 20% and Wong et al[12] documented the incidence of 23%. A study in Iran[20] reported 36.6% cases of hypospermatogenesis in their studied population. Our finding is in agreement to the two studies done in Saudi Arabia[19,21] which showed the same results that is 29% and 25% respectively ,while it differs from two other studies from the same country[10,15] which revealed a much lower incidence of hypospermatogenesis that is 13% and 3.7%. Al Rayes[10] from Riyadh in his study of 230 testicular biopsies showed a 13% incidence of hypospermatogenesis, while Thomas and Jamal[15] from the western region of Saudi Arabia reported an incidence of 3.7% for hypospermatogenesis. Other international studies showed variable results. Haddad et al.[17] reported a high incidence for hypospermatogenesis (55.8%). Meinhard et al.[16] and Colgen et al.[13]also reported a high incidence of hypospermatogenesis of 46 and 49%, respectively. On the other hand, Thomas[20] and Wong et al.[12] reported 19% and 23%, respectively. Jamali et al. from Iran reported an incidence of 36.6%.

The incidence of GCMA in the present study was 17% which was in accordance with the Abdullah L. et al.[19] whose results showed 12%. The incidence was similar to that reported in several other studies.[10,20]This finding was in contrast with a study by Haddad et al.[17] reported a very low incidence in 1.7% of cases. Rashed et al.[9] which documented germ cell aplasia in 28% of cases, while Thomas[20] and Jamal[21] from Saudi Arabia reported low incidences of 5 and 7%, respectively.

Sixteen cases (16%) of Sertoli cell only syndrome was identified in 17% of cases in the present study. This finding correlates well with three other studies[18,19,20] in which similar figures were reported. Jamal[21] from the western region of Saudi Arabia reported an incidence of 16.5% while another study[15] reported an incidence of 27.2%. A similar high incidence (39%) was also reported by Al Rayes[10]. The difference in the incidence of Sertoli cell only syndrome between local studies as well as between our study and several international studies cannot be totally explained. The term Sertoli cell only syndrome should only be applied to a homogenous pattern with the absence of germ cells in any profile. Sertoli cell only syndrome is an irreversible change that can be associated with many underlying conditions. These include cryptorchid testis, orchitis, post radiation or chemotherapy, estrogen or androgen therapy and as a consequence of chronic hepatopathology.

Generalized fibrosis was evident in 13% of cases in the present study, which was in accordance with a study[19] who reported 16% of cases. Similar results were obtained by Thomas[15] and Jamal[21], Alsamawi[24], and Jamali and Hairiri[20], while much lower incidence was reported by Al Rayess[10], Nagpal[18] and Rashed et al.[9]

CONCLUSION

Our study distinguishes hypospermatogenesis as the most common histomorphological pattern among different spermatogenic defects studied in studied population.

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Address for Corresponding Author:
Dr. Surriyya Sarwat,
Assistant Professor of Anatomy.
Sindh Medical University.
Karachi.
R-857, Sector 15 A-2 Bufferzone Karachi.
Cell: 03332262245