A Study of Sperm Morphology in Cases of Varicocele

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Abstract

Background: Varicocele is the most common treatable cause of male infertility affecting the young couple's .Varicocele is associated with increased incidence of abnormal sperm abnormalities including sperm count, sperm concentration, motility & abnormal morphology of sperm. Abnormally shaped sperm are not capable of fertilising the egg, because they do not function properly leading to male infertility. In testing for teratozoospermia, (abnormal sperm) sperm are collected, stained and analyzed under a microscope to detect abnormalities. These abnormalities may include heads that are large, small, tapered, or pyriform or tails that are abnormally shaped. *Patients & Method*: The study was conducted at R.D. Gardi Medical College, Ujjain between 8th January 2015 to 30th July 2015. Total of 50 patients in age group of 18-45 with clinically proved cases of varicocele from surgical outpatient department were included in the study. The semen sample was evaluated by routine examination including total count, motility & emphasis done on sperm morphology by staining the smear by Paponicolaou method. *Results*: Over all of the total 50 cases the most prounced abnormality was in head 33 (16.5%) followed by abnormality of tail 27 (13.5%) & mid piece constitute (12%).

Keywords: Male Infertility; Semen Parameters; Varicocele; Teratozoospermia.

Introduction

Varicocele is defined as abnormally dilated testicular (pampiniform plexus) in the scrotum, which is normally secondary to internal spermatic vein reflux. Varicoceles detected on a physical examination in the erect position are called clinical varicoceles. Almost all varicoceles are detected after puberty and there is a prevalence of about 11-15% among adult men [1,2] 35% of men with primary infertility and up

to 80% of men with secondary infertility. Varicoceles are considered to be the commonest correctable cause of male infertility [5].

Their incidence among infertile men is 20-40% about three times greater than in the general population [6]. Studies have shown that varicocele causes progressive duration-dependent injury to the seminiferous epithelium.

Clinical Classification of Varicocele

Classification	Defination		
Grade III (Large)	Easily palpable		
Grade II (medium)	Palpable at rest without (Valsalva maneuver), invisible.		
Grade 1 (small)	Palpable with Valsalva maneuver only		
Subclinical not palaple	Vein larger than 3 mm on ultrasound; Doppler reflux on Valsalva maneuver		

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Diagnosis of Varicocele by Physical Examination

Varicoceles are typically diagnosed by physical exam. They are most easily detected when the patient has been standing in a warm room for several minutes. Often, varicoceles will expand when the patient bears

down or strains, as the blood reverses flow back toward the testicle. Most experts agree that only varicoceles detected by physical exam are clinically significant, which means they should be considered for treatment.

Diagnosis of Varicocele by Clinical & Imaging Methods

In patient whose clinical diagnosis was not confirmed underwent sonography of the scrotum in the department of radiology by a senior consultant using high-resolution gray scale and color Doppler sonography for confirmation.

Varicocele was detected clinically in 45 patients, where as sonography alone detected 05 cases of varicocele

Materials and Methods

The study was carried out during 8 th January 2015 to 30 th July 2015, in R.D. Gardi Medical College, Ujjain, in department of pathology. 50 patients suffering from varicocele were subjected for seminal analysis &

emphasis was only focused on sperm morphology. A written consent was taken from each patient who participitated in the study.

Inclusion Criteria

The patient's in the age group of 18 -45 suffering from varicocele were included in the present study.

The patients under study were given verbal instructions about the method of ejaculation by masturbation. A sterile petridish was provided to each patient to ejaculate the sample in isolation. A previous counseling was done to avoid the stress of producing the sample.

Exclusion Criteria

Patients suffering from varicocele along with other testicular diseases were not included in the present study.

Azoospermic patient were not included in the study.

The patient's who were unable to ejaculate the semen sample in laboratory only were not included in the study.

Age in years		Number
18-25		20
26-35		20
36-45		10

Semen Smear Processing

The following factors were taken into consideration while preparing the seminal smear:

All the smears were prepared by *feathering method* immediately after the semen sample was liquefied. Each smear was prepared in such a way that there was minimum of overlap of the spermatozoa. The angle of the dragging slide & the speed of smearing the smear were taken into account.

All the smears were air dried & fixed in 95 % ethanol for 15 minutes & stained by *Papanicolaou stain*.

Examination of the Slide

Examination was done using bright field optics at x 1000 magnification with oil immersion lens.

Each field was examined from right to left.

100 sperms were counted in each smear.

The number of normal & abnormal sperms was noted with laboratory counter.

Observation & Results

All the 50 semen samples show moderate to marked degree of morphological abnormalities of spermatozoa. The following abnormalities were noted in order of frequency in different segment of spermatozoa > abnormalities of head, abnormalities of principal piece (tail) & in last abnormalities of middle piece. The morphological abnormalities of spermatozoa were highest in age group of 36-41 year age group. In our study each defect for head, neck & tail was counted as a sperm with 3 defects. For example if the sperm has only one defect in head it was counted as sperm with one defect but if the same sperm is having defect of tail & neck also it was counted as sperm with 3 defect. The abnormalities were tabled as (+), (+++) & (++++) abnormalities.

Teratozoospermia is a condition of increased number of abnormally shaped sperms in the semen that affects fertility in males. Patient with varciocele had dual impact of the disease which results in impaired male infertility.

The following are morphological abnormalities in each, head, Neck & tail.

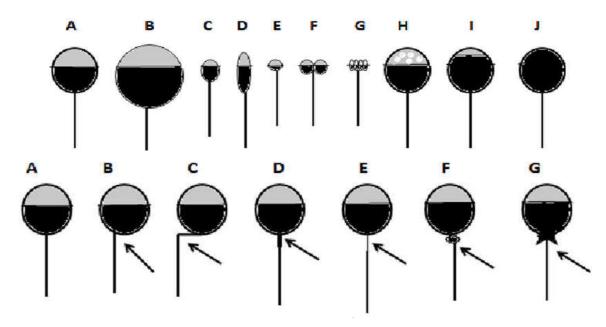
Normal head(A)	Tail	
Giant head(B)	Normal neck (A)	Normal (A) tail
Micro head ©	Asymmetrical neck (B)	Double tail (B)
Pear shaped head(D)	Bent Neck ©	Small tail ©
Amorphous head(E)	Thick neck (D)	Hair pin (D)
Double head (F)	Thin neck (E)	Coiled tail(E)
Tapering head(G)	Irregular neck (F)	Absent tail(F)
Vacuolated head(H)	Cytoplasmic appendages (G)	Terminal droplet (G)
Small acrosome head(I) Absent head(J)		Broken tail (H)

Images showing Abnormalities of Head

Normal (A), Giant (B) Microhead (C), Pear shaped (D), Amorphous (E), Double(F), Tapering (G), Vaculoated (H), Small acrosome head(I), Absent head(J), Absent nucleus (J).

Images of Neck & Mid Piece Abnormalities

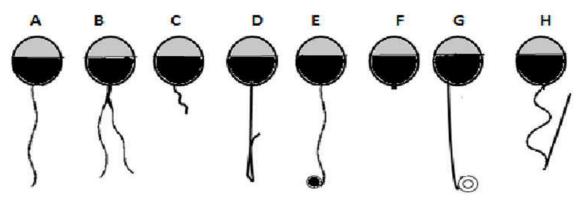
Normal neck (A), asymmetrical neck (B), bent neck (C), thick neck (D), thin neck (E), irregular neck (F), cytoplasmic appandeges (G). Defects are shown by black arrow.



Images of Tail Abnormalities

Normal(A), double tail (B), small tail (C), hairpin

(D), Coiled (E), absent tail (F), terminal droplet (G), & broken tail (H)



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Age	% of normal	% of abnormal	Head defect	Neck defect	Tail defect	Overall
18-25	69	31	14	10	07	31
26-35	66	34	14	09	10	34
36-45	72	28	10	06	12	28

Most common abnormalities in order of frequency in different segment is as under in the present study

Head abnormality	%	Mid piece Abnormality	%	Prinicipal piece abnormality	%
Amorphous(10)		Bent(13)		Small (10)	
Tapering(7)		Thick (10)		Absent(8)	
Vacuolated		Thin(02)		Double(6)	
Small(3)		Irregular(02)		Coiled(2)	
Micro(3)		<u> </u>		Hair pin(2)	
Noacrosome(1)				Broken(1)	
Double(1)				` '	
Multiple(1)					
Giant(1)					
Round(1)					
Pear(1)					
Total (33)		Total (27)		Total (29)	

Single sperm defect in 20 patients (40) % Double sperm defect (20) patients (40) % Triple sperm defect in (10) patients (20)%

Discussion

Concept of Normal Sperm Morphology

Each spermatozoon is made up of a head, neck, middle piece (mid piece), principal piece and end piece. Since the end piece is difficult to see with a light microscope, the spermatozoa for all practical purposes is considered to made up of head (and neck) and tail (mid piece and principal piece). For a spermatozoon to be considered normal, both its head and tail must be normal. All borderline forms were being considered abnormal. By the strict application of criteria of sperm morphology, relationships between the percentage of normal forms and various fertility end points (timeto-pregnancy (TTP), pregnancy rates in vivo and in vitro) have been established. WHO, in 2010 state that a person with 5% normal morphological sperm can produce the child.

Head Abnormalities

Are common, if it is present attachment in the oviduct & to the egg will be impaired. The form of the sperm head is more important than its dimensions. An eyepiece micrometer was used to distinguish between normally and abnormally sized sperm heads. Normally less than 6% of sperms are of amorphous type & percentage of tapering form should not exceed

more than 4%. In our study the amorphous forms account 10 % & tapering form account 7 %.

Tail: It impart motility to sperms, Coiled tails may indicate epididymal dysfunction

The abnormal tail morphology will interfere with the sperm ability to swim in the oviduct & through the Zona pellucida.

The abnormalities of semen parameters in infertile men with varicocele were first objectively described by Macleod in 1965 57. In that study, Macleod observed that the vast majority of semen samples, obtained from 200 infertile men with varicocele, were found to have an increased number of abnormal forms, decreased motility and lower mean sperm counts. This 'stress pattern', which is also characterized by an increased number of tapered forms and immature cells, was also reported in other studies. However, other investigators have shown that the characteristic stress pattern is not a sensitive marker for varicocele, and believe that it is not diagnostic of this pathology.

Clinical Implications of Morphological Abnormal Spermatozoa

- · Lower fertilizing potential
- Increased DNA fragmentation
- Increased incidence of structural chromosomal aberrations
- Immature chromatin and
- Aneuploidy
- Defective spermatogenesis and some epididymal pathologies

Conclusion

Varicocele is one of the most debatable issues in cases of male infertility due to its changes in sperm parameters. The exact pathophysiology of teratozooaspermia in cases of varicocele is not clearly understood. The present study shows the significance of altered sperm morphology which can be one of the components of infertility.

The presence of increased number of abnormallyshaped spermatozoa with multiple abnormalities can negatively affect fertility.

If varciocele patient shows teratozooaspermia of high degree the chances are conception are markedly reduced. Men with the dual disease varciocele & teratozospermia have to undergo assisted reproduction treatment in order to fertilize an egg.

Acknowledgement

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