Original Article

A Study of Teratozoospermic Index in Pakistani Men

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Objective: **To determine the teratozoospermic index (TZI) of proven fertile males and compare this with that of infertile males.**

Study Design: **Cross-sectional comparative**

Place and Duration: **It was carried out at Islamic International Medical College Rawalpindi and Islamabad Clinic Serving Infertile Couples Islamabad, from July 2005 to July 2006.**

Materials and Methods: **50 healthy fertile males were selected, while another 50 infertile males were recruited as controls. Their sperm morphology was determined according to Tygerberg's strict criteria. And the Teratozoospermic index was calculated. The sampling technique used was convenience non-probability. Inclusion criterion for proven fertile males was pregnancy achieved within one year of marriage with successful coituses. In case of infertile males it was failure to achieve pregnancy without the use of assisted reproductive techniques, with no infertility factors in the female partner. The semen samples were obtained at the laboratory after 3 to 4 days of sexual abstinence with clear written and oral instructions given to the subjects before the collection of the sample.**

Results: **The infertile group was found to be statistically older than the proven fertile group i.e. (36.60 versus 31.32 years). TZI was significantly less in the proven fertile group (*P* < 0.021). TZI ranged from 1.25 to 2.09 in the proven male group and from 0 to 2.28 in the infertile group**

Conclusion: **TZI increases the clinical value of semen analysis and should also be used to differentiate between fertile and infertile males in addition to other semen parameters.**

Key Words: ***Sperm morphology, Fertile males,*** **Teratozoospermic Index**

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Introduction

The estimation of sperm concentration, motility and morphology is the mainstay of the assessment of male reproductive health.[1] Decreased sperm concentration has been associated with decreased fertility.[2] Sperm motility has also been associated with the fertiliy.[3]

The examination of sperm morphology by more standardized and stringent criteria, however, has enhanced objectivity and decreased intra-laboratory variability. The World Health Organization (WHO) has also recommended that strict criteria should be applied when assessing the morphological normality of the spermatozoon. This has led to the establishment of lower threshold levels for normality.[1]

Morphologically abnormal spermatozoa often have multiple defects i.e. head defects, mid-piece defects and tail defects.

The following categories of defects are usually found[1,4]

Head defects: Large, small, tapered, pyriform, round and amorphous heads. Vacuolated heads (>20% of head area occupied by unstained vacuolar areas) or those with small acrosomal cap (<40% of head area) and double heads or combination of above are head defects.

Neck and mid-piece defects: Normally neck/ mid-piece and tail should form an angle of 90° to the horizontal axis of head. Bent, asymmetrical insertion of mid-piece into the head, thick or irregular shaped mid-piece, abnormally thin mid-piece (i.e. no mitochondrial sheath), or any combination of these are considered as mid-piece defects.

Cytoplasmic droplets which are usually located in the mid-piece should not be greater than one-half of a normal sperm head.

Tail defects: Short, multiple, hairpin, broken, bent tails, irregular width, coiled tails or any combination of these are the defects found in sperms.

In earlier practice, when multiple defects were present only one defect was recorded. It is now

customary to record total number of sperm defects divided by the number of defective spermatozoa, called multiple anomalies index (MAI) or teratozoospermic index (TZI). The MAI or TZI values should read between 1.00 (i.e. each abnormal sperm has only on defect) to 3.00 (i.e. each abnormal sperm has head, mid-piece and tail defects). For a spermatozoon to be classified as normal the size and shape must be within normal limits.[5-6] The aim of the present study was to determine the TZI of proven fertile males and compare this with that of infertile males in our population.

Materials and Methods

This was a cross-sectional comparative study comparing a fertile population with an infertile group. It took place at the Islamic international medical college, Rawalpindi and Islamabad clinic serving infertile couples, Islamabad, from July 2005 to July 2006. It was a convenience non probability sample. A total of 100 subjects were divided in two groups each containing 50 subjects. Husbands of fifty pregnant women attending the antenatal clinic at Railway hospital, Rawalpindi were enrolled in the study, whose semen samples were collected for analysis. Another fifty infertile men were recruited into the study as a control group, as they consulted at the Islamabad clinic serving infertile couples, Islamabad. Proforma was completed and an informed consent was obtained. Inclusion criteria for proven fertile males were pregnancy achieved within one year of marriage with successful coituses. For Infertile males it was failure to achieve pregnancy without the use of assisted reproductive techniques, with no infertility factors in the female partner. The exclusion criteria was secondary infertility, high grade fever, tuberculosis, mumps, orchitis or any chronic debilitating illness, vericoceole, sexually transmitted diseases, any drug affecting male fertility e.g. beta-blockers, anti-neoplastic agents etc.

The semen samples were obtained after 3 to 4 days of sexual abstinence at the laboratory and the subjects were given clear written and oral instructions. Sperm morphology was assessed by strict criteria by preparing a stained slide of sperms from the ejaculate[6] after liquefaction. A clean dry glass slide was labelled with patient's number and a 5 - 10µl drop of ejaculate was placed on the slide and a thin smear was made using edge of another glass slide or a cover slip the smear was dried in air and fixed by spraying ethyl alcohol. The slide was dipped in the Giemsa stain for 3 – 5 minutes and washed under running tap water and then dried in air. Sperm morphology was assessed under oil immersion at x100 magnification of microscope using ocular micrometer [ocular micrometer was calibrated with stage micrometer to measure the exact size] The ocular micrometer has many large squares which have four small squares and each of the four small squares contains 25 smallest squares, each measuring one micrometer. The sperm head, mid-piece or tail was brought over the micrometer to measure the exact size. 100 sperms were counted at random measuring carefully their head, mid-piece and tail size. At least two observations were taken. Teratozoospermic index (TZI) was calculated by the formula[1]:

Total No. of defects
No. of sperms with defects

The following is an example of the calculation of TZI:

Number of sperms counted	200
Number of normal sperms	10
Percentage of Normal sperms	5%
Number of sperms with defects (200-10)	190 (95%)
Number of sperms with head defects	180 (90%)
Number of sperms with mid-piece defects	34 (17%)
Number of sperms with tail defects	24(12%)
Total number of defects (180+34+24)	238

Teratozoospermic index
(Total number of defects/ Number of sperms with defects) = 238/190 1.24

Results were entered into SPSS version 10.0. Descriptive statistics were used to calculate means and standard deviations for numerical data. These were compared using t-tests at a confidence level of 95%. Frequencies were calculated for categorical data and these were compared using chi-square tests.

Results

Table I shows Mean ± SD of weight and age of the proven fertile and infertile groups. The difference is significant in both of these ($P < 0.000$). These results suggest the possible role of weight and age in the fertility potential of males. When the ages of the subjects in both the groups were compared, the infertile group was found to be statistically older than the proven fertile group i.e. (36.60 versus 31.32 years).

Table I: Demographic Data of Proven Fertile and Infertile Group (n=50 each)

Group	Weight (Kilograms)	Age (Years)
Proven Fertile	74.26 ± 6.49	31.32 ± 6.10
Infertile	81.58 ± 4.03	36.60 ± 6.28
P-Value	< 0.000*	< 0.000*

* ***P*** = Significant

Table II gives distribution of the subjects in upper, middle and lower classes of proven fertile and infertile groups. The difference between the two groups is significant ($P < 0.000$) with infertile group predominantly comprising of upper and middle class and the proven

fertile comprising mainly the lower class. Table III presents Mean ± SD teratoozospermic index in proven fertile and infertile group. TZI was significantly less in the proven fertile group (***P*** < 0.021). TZI ranges from 1.25 to 2.09 in the proven male group and from 0 to 2.28 in the infertile group.

Table II Socio-economic Status of Proven Fertile and Infertile Group (n=50 each)

Group	Upper Class	Middle Class	Lower Class
Proven Fertile (n=50)	8	18	24
Infertile (n=50)	23	26	01
P-Value		< 0.000*	

P = Significant

Table III Teratoozospermic/Multiple Anomalies Index of Proven Fertile and Infertile Group (n=50 each)

Group	Teratoozospermic/ Multiple Anomalies Index
Proven Fertile (n=50) (Mean ± SD)	1.62 ± 0.19
Infertile (n=50) (Mean ± SD)	1.83 ± 0.57
P-Value	< 0.021*

* ***P*** = Significant

Discussion

To be of clinical value, the methods used for semen analysis should be standardized and threshold values for fertility and infertility should be calculated for various parameters used in standard semen analysis. Since there are so many different methods for semen evaluation, especially sperm morphology that it would be difficult to standardize the methods used for semen analysis. The two classifications most widely accepted are the WHO (1987 & 1992) and the Tygerberg strict criteria.[1,7]

Inconsistency between different methods of sperm morphology assessment has been identified by Ombelet *et al*[8] and others[9,10] who suggested that the semen analysis methodologies should be standardized.

Previously only one defect was recorded during sperm morphology assessment, with priority given to head defects over mid-piece and to mid-piece defects over tail defects.[1] The WHO recommends to record multiple anomalies index (MAI) or teratozoospermic index (TZI).[11] These indices of multiple sperm defects are predictors of sperm function both *in vivo* and *in vitro*[1] and have been associated with the probability of occurrence of conception among couples with fertility problems[12].

In the study of Slama R *et al*[12] the morphology parameters proved to be strongly associated with time to pregnancy, in particular MAI or TZI proved to be significantly related to the probability of a clinically recognized pregnancy. Its variations were associated with variations in time to pregnancy on the whole ranges of MAI, without any clear threshold pattern.

In our study the mean TZI value was found to be 1.62 in the proven fertile group, which is consistent with the WHO value of 1.60[1] (i.e. lower pregnancy rates when above this value in untreated infertile couples).

Conclusion

TZI increases the clinical value of semen analysis and should also be used to differentiate between fertile and infertile males in addition to other semen parameters.

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