Deterioration Of Sperm Morphology In Men Exposed To High Temperature

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Abstract. Occupational exposure to high temperatures adversely affects testicular function causing partial or complete spermatogenic arrest. This leads to oligoasthenoteratozoospermia (OAT) and azoospermia. This study reiterates that exposure to high temperature causes deterioration in sperm morphology and impairs motility. This inverse relationship of sperm function with elevated temperature has implication in clinical medicine both in understanding pathological states and for therapeutic measures.

Key words : Azoospermia, Oligozoospermia, Oligoasthenoteratozoospermia, Hyperthermia

Introduction :

About ten percent of males in the reproductive age group have severe defect in sperm production. Sperms are produced by a highly complex process of spermatogenesis. Any partial or complete disruption of this process results in spermatogenic arrest and finally leads to oligozoospermia and azoospermia respectively. In oligozoospermia the sperm concentration is less than 200 million/ml and in azoospermia there is complete absence of sperms in the semen.

For normal fertility, a man requires normal spermatogenesis, successful epididymal storage and normal sperm transport. Based on these functions the causes of infertility can be secretory or excretory. Secretory causes are due to spermatogenic arrest while in excretory cause, spermatogenesis is normal but there is obstruction to the outflow of spermatozoa. Besides this the fertility in males depends on normal linear progressive sperm motility and normal morphology. Sperm motility is the best predictor of fertility potential in man.

Several conditions can affect sperm motility and production of normal sperms. Some of the known conditions are exposure to high temperature, deletions of azoospermia factor loci (AZFd) and iatrogenic causes.

The normal human sperm measures 50-60 mm in length and has head, neck, middle piece and tail. The head is oval in shape and is 3-5 mm in length and 2-3 mm in width. The midpiece is slender, straight and regular in outline. It is aligned with the long axis of the head, and is approximately 7-8 mm in length. Its width is about one third of the head. The tail is slender, straight and regular in outline and is 40-45 mm in length.

In normal conditions testicular temperature is maintained 3°C lower than the core body temperature and is an important prerequisite for efficient spermatogenesis. Germ cells and Sertoli cells are highly sensitive to elevated temperature.

Various occupational factors and environmental agents have been shown to have deleterious effect on male reproductive function and it is difficult to elucidate the role of a single agent. Several confounding factors related to diet, lifestyle, stress and socioeconomic status also affect semen quality.

Though reports are available regarding the adverse effects of hyperthermia on semen quality but very few of these highlight the effect of high temperature on sperm morphology. The present paper reports the presence of increased number of morphologically abnormal sperms with impaired motility in males with occupational exposure to high temperature.

Materials and Methods :

Ninety two males within the age group of 22-35 years who were diagnosed as infertile at Urology clinic of All India Institute of Medical Sciences, New Delhi were included for this study. Couples are said to be infertile if the wife is unable to conceive after 1
year of regular unprotected intercourse. Twenty five fertile men whose wives had conceived within 1 yer of marriage were controls (control group). Information was recorded from patients and controls about their occupation, type of work performed, period of cohabitation, and recent illness, medication taken, exposure to irradiation, consumption of alcohol, smoking, eating habits, use of recreational drugs and family history in a predesigned performa.

Detailed general physical examination was performed for each patient. FSH and LH levels were assessed for each patient to determine the testicular damage and to confirm that the cause of infertility was secretary.

Semen samples were collected in a room near the laboratory and analysed for each patient. A minimum of 2 semen samples were collected at 1 month interval. For analyses, semen samples were allowed to liquefy at room temperature and seminal volume, pH, sperm count, motility and morphology were analysed according to guidelines in WHO manual (1992).

For assessing the sperm motility the microscope field was examined systematically and motility of each spermatozoa encountered was classified as A, B, C or D. Rapid linear progressive motility was motility grade A, while sperms with slow or sluggish linear or non linear motility were classified as having motility grade B. Non progressive motility was grade C and immotile sperms were classified as grade D.

For studying the sperm morphology a drop of semen was smeared on a clean glass slide. The smear was then air dried and fixed in a mixture of equal parts of ethanol and ether. The slides were then stained with 2% Giemsa for 30 minutes. Dried stained slides were scanned under oil immersion x100 objective to assess for morphological abnormalities of sperm head, midpiece and tail. A total of 100 sperms per sample were classified according to their morphology such as small, pin, large amorphous, tapered, double and triple head, dilated or bent midpiece and coiled or short thick tail.

The mean and standard deviation were calculated using standard statistical method and t test to study the significance from two samples assuming unequal variances.

**Results**:

Semen analysis was done in 92 infertile males and 25 controls. Mean age of the patients was 32 years. Of the 92 infertile males, 80 males had no sperms in their ejaculate and were classified as azospermic. Twelve males had a total sperm count less than 20 million sperms and were classified as oligozoospermic. In two of the twelve oligozoospermic males the total mean sperm count was 15 million with 75% of the sperms showing normal morphology. The mean seminal volume was 4±1.8 ml and the mean pH was 7.2 ± 0.08. These sperms had oval head with acrosomal cap covering more than one third of the head surface. The midpiece was cylindrical in shape and its width was less than one third the width of the head. The tails were long, slender and straight. About 20% sperms showed abnormal morphology.

Remaining 10 oligozoospermic males were found to have a very high percentage of morphologically abnormal sperms with impaired motility a picture characteristic of OAT and formed OAT group. These OAT cases were analyzed in detail and results were compared with control group (Table 1). Semen volume and pH in OAT group were slightly lower than the control group, which was not significant (p>0.05). The average total sperm count in OAT group was 12 million which was significantly (p<0.05) lower than the control group. The mean percentage of sperms with morphological abnormalities (figure 1 and 2) such as thick coiled tail, amorphous head, tapered head, double head and dilated midpiece were significantly higher in OAT group as compared to the control group (p < 0.05).

The mean percentage of sperms with motility grade C and D were significantly higher in OAT group than in the control group (p < 0.05) and motility grade A was significantly lower than the control group (p < 0.05). The mean percentage of sperms with motility grade B was lower than the control group which was not significant (p > 0.05).

The mean FSH and LH levels (21.2 ± 2 mlu/ml and 13.6±1 mlu/ml) were significantly higher.
(p<0.05) in the OAT group males as compared to the control group (3.54 ± 0.29) mlu/ml and 6.4 ± 0.1 mlu/ml).

**Correlation With Aetiological Factors**

In 11 azoospermic and 9 males with OAT, there was one common underlying aetiological factor. These males had been exposed to very high temperature at their work place for a period of 5-15 years. They were working in blast furnace (n=6), Cement, brick, glass and plywood preparation factory (n=6), melting tar (n=5), Dyers (n=3). Occupational exposure to consistently high environmental temperatures led to increased intratesticular temperatures in these men. There was no significant history of smoking, medication, viral illness or use of any recreational drugs in these males. In the rest of the 69 azoospermic males and 2 oligozoospermic males there were other aetiological factors which might have led to complete or partial spermatogenic arrest and resulted in azoospermia and oligozoospermia. The causes for spermatogenic arrest in these males were infections such as tuberculosis (n=5), mumps (n=5), diabetes mellitus (n=2), iatrogenic (n=3), genetic factors (n=18), trauma (n=1), dibromochloropropane (DBCP) exposure (n=1) and congenital causes such as undescended testes (n=5), hypogonadism (n=14) and idiopathic (n=17).

**Discussion :**

Temperature of the scrotum is 3°C lower than the core body temperature and this is an important prerequisite for optimal spermatogenesis. The germ cells and sertoli cells are highly sensitive to elevated temperature which causes partial or complete spermatogenic arrest (Martin et al, 1993). Previous studies have shown that some men have intrinsic defect in scrotal thermoregulation and about 80% of men with oligozoospermia have elevated scrotal temperatures (Zorgniotti and Sealfon, 1988). Even in normal fertile males fever, high summer temperatures and frequent hot baths, saunas are known to result in destruction of germinal epithelium and also induces transient oligozoospermia (Bedford, 1991; Miusset and Bujan, 1994). Bedford (1991) proposed that cauda epididymis is sensitive to high temperature. High scrotal temperature causes rapid disruption of absorptive and secretory function of cauda epithelium thereby changing protein composition of cauda fluid and causing reduction of its storage capacity. In present study 20

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OAT Group</th>
<th>Control Group</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3</td>
<td>3.2</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.21</td>
<td>7.24</td>
<td>NS</td>
</tr>
<tr>
<td>Total Sperm Count (million)</td>
<td>12</td>
<td>63</td>
<td>S</td>
</tr>
<tr>
<td>Abnormal morphology (%)</td>
<td>86.2</td>
<td>18.8</td>
<td>S</td>
</tr>
<tr>
<td>Thick coiled tail (%)</td>
<td>37.6</td>
<td>3.45</td>
<td>S</td>
</tr>
<tr>
<td>Amorphous head (%)</td>
<td>25.6</td>
<td>5.4</td>
<td>S</td>
</tr>
<tr>
<td>Tapered head (%)</td>
<td>23</td>
<td>6.15</td>
<td>S</td>
</tr>
<tr>
<td>Pinpoint head (%)</td>
<td>6.14</td>
<td>1.75</td>
<td>S</td>
</tr>
<tr>
<td>Dilated midpiece (%)</td>
<td>4.61</td>
<td>1.65</td>
<td>S</td>
</tr>
<tr>
<td>Short thick tail (%)</td>
<td>3</td>
<td>1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Motility grade D (%)</td>
<td>44</td>
<td>1.9</td>
<td>S</td>
</tr>
<tr>
<td>Motility grade C (%)</td>
<td>39</td>
<td>4.4</td>
<td>S</td>
</tr>
<tr>
<td>Motility grade B (%)</td>
<td>15</td>
<td>20.95</td>
<td>NS</td>
</tr>
<tr>
<td>Motility grade A (%)</td>
<td>2</td>
<td>72.7</td>
<td>S</td>
</tr>
</tbody>
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NS-Not Significant S-Significant
infertile males had exposure to high temperature at their workplace for a mean period of 8.5 years. It is possible that in these men exposure of testes to high environmental temperature led to an elevation of intratesticular temperature. This elevation of intratesticular temperature might have impaired spermatogenesis and led to production of morphologically abnormal sperms with impaired motility to complete absence of sperms in the semen. Dyers, welders, steel and furnace workers are exposed to high temperatures at their workplace and are reported to have impaired spermatogenesis (Tas et al, 1996).

Available literature suggests that short term exposure to high temperature may cause reversible changes in the seminiferous tubules whereas chronic exposure for 5-7 years had OAT while those exposed for 12-15 years had azoospermia.

Wang et al. (1997) reported that elevation of testicular temperature 1°C above the baseline depresses spermatogenesis by 14% and thus decreases sperm output. Exposure to high temperature also results in modification of sperm morphology. It is characterized by increase in the number of morphologically abnormal sperms. The mean value of sperms with abnormal morphology rises from 30 to 60% within 6-8 months of exposure to high temperature (Wang et al, 1997). They postulated that heating the testes induced a depression not only in the amount but also in the quality of sperm output.

Though a number of studies have reported about deterioration in semen quality on exposure to high temperature but to the best of our knowledge very few studies have reported about the increase in specific morphological abnormalities of sperms. In our study we found that 86.2% sperms had abnormal morphology. The most predominant abnormality was thick coiled tail (37.6%) and amorphous head (25.6%). Though percentage of sperms with morphological abnormalities rises sharply within 6-8 months of exposure (Wang et al, 1997; Mieusset et al, 1991; Levine et al, 1992), in the present study the cases had exposure for the mean period of 8.5 years. Wang et al. (1997) explained that elevated testiculoepididymal temperature decreases the synthesis of sperm membrane coating protein which in turn results in the production of morphologically abnormal sperms.

Only 2% sperms showed linear progressive motility and whereas majority (83%) of sperms had impaired motility (83%) of sperms had impaired motility (grade C and D). Increase in number of morphologically abnormal sperms results in impaired motility as normal intact sperm morphology is prerequisite for linear progressive motility. Gandini et al. (2000) postulated that sperm function is strictly correlated with sperm morphology and that sperm motility is the best predictor of fertility potential in man.

Functional and fully differentiated Sertoli cells are critical for development of quantitatively and qualitatively normal spermatogenesis. They also provide structural and functional support to the developing and differentiating germ cells as each Sertoli cell is in contact with 47 germ cells and 5 other Sertoli cells (Orth et al, 1988). High testicular temperature damages the Sertoli cells and their number decreases impairing spermatogenesis. This leads to a decrease in the number of germ cells with incomplete differentiation (Steger at al, 1999). Damaged Sertoli cells produce decreased amount of inhibin which decreases negative feedback on pituitary leading to increase in FSH level. This increase in FSH level is directly proportional to the Sertoli cell damage that indicates the severity of testicular damage. FSH levels are the most important endocrine parameter to evaluate testicular function (Bergmann et al, 1999). FSH and LH levels were elevated in azoospermia and in OAT cases. The difference in FSH and LH levels between OAT group and control group were statistically significant (p < 0.05).

One of the well known mechanism to explain spermatogenic impairment due to hyperthermia is activation of p53, a tumour suppressor gene which is expressed in testes (Rogel et al, 1985, Almon et al, 1993). Its level of expression is highest in pachytene spermatocytes (Schwartz et al, 1993). High scrotal temperatures cause condensation of nuclear chromatin which causes p53 activation which leads to cell cycle arrest. This prevents clonal proliferation of germ cells with damaged DNA. Morgentaler et al. (1999) postulated that p53 might
play a role in heat induced germ cell apoptosis. p53 is located on the nuclear membrane in the normal germ cells and is involved in quality control of germ cells. It translocates to the nucleoplasm with heat induced nuclear damage and induces germ cell apoptosis (Yin et al, 1997).

In the past decade, male reproductive health had declined with marked increase in the population of subfertile males (Carlsen et al, 1992). The sperm count has been falling at an alarming rate of 2% per annum for the last 20 years. This is believed to be due to increase in global temperature and environmental pollution. There has been an yearly decline of 2.6%, 0.3% and 0.7% in sperm concentration, sperms with normal motility and sperms with normal morphology (Tas et al, 1996).

Thus, the testes is remarkable as a biological system for its functional regulation by temperature. The testes function optimally at relatively cool temperature and high testicular temperatures impair spermatogenesis leading to OAT and azoospermia as has been seen in the present study. Observed temperature sensitivity of testes is implications in the growth of the testis, spermatogenesis leading to OAT and azoospermia temperatures impair spermatogenesis leading to OAT and azoospermia.

Acknowledgements:

One of the authors R Dada is receiving fellowship from Council of Scientific and Industrial Research (CSIR) and wishes to thank CSIR for its financial support.

References:


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Fig. 1. Semen microphotograph from an OAT case. A very high percentage of sperms with morphological abnormalities (x400).

Fig. 2. Semen microphotograph from OAT case showing
A Spermatozoa with coiled tail
B Spermatozoa with dilated bent midpiece
C Spermatozoa with double head
D Spermatozoa with triple head