Significance of sperm characteristics in the evaluation of adolescents, adults and older men with varicocele

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ABSTRACT

Background: No reports have been published about age-related sperm malformations in varicocele patients. Aim: To investigate the distribution of abnormal sperm characteristics in adolescents, adults and older men with varicocele. Setting and Design: Records of semen analysis of 143 men aged 14 to 53 years who had evident left-sided varicocele detected by physical examination and confirmed by doppler sonography were selected. Materials and Methods: Sperm concentration, vitality, motility, morphology, hypoosmotic swelling test (HOST) and morphology were measured in adolescent males aged 14 to 20 years (n=31), men 21 to 30 years (n=48), 31 to 40 years (n=40) and older men over 40 (n=24) and compared with a control group of fertile men with no varicocele (n=27) and with a group of infertile men with varicocele (n=26). Statistical Analysis: One-way analysis of variance and the Kruskal-Wallis test were used to compare varicocele groups. Comparisons with the control group and infertile group were performed using the unpaired t-test and the Mann-Whitney test. The discriminating ability of significant sperm characteristics in evaluating the sperm quality of varicocele men was also analyzed using receiver operating characteristics curve to select the cut-off level providing the best combination of sensitivity and specificity. Results: Varicocele men displayed similar impairment of vitality, motility and HOST. Sperm morphology analysis revealed a prevalence of small head, slightly and severely amorphous head and particularly combined anomalies in the study groups. Sperm concentration fell within the normal range of the World Health Organization manual. Differences were not significant between the study groups and when compared with infertile group (P>0.005). However, a comparative study of the varicocele groups and the infertile group with the control group revealed significant differences in sperm vitality, motility, HOST, morphologically normal sperm, pin-headed, tapered and combined anomalies. Morphologically normal sperm and combined anomalies showed higher accuracy in identifying poor sperm quality in varicocele men (83.7% and 77.9%, at cut-off levels of 9% and 38%, respectively). Conclusions: Varicocele harms equally the sperm characteristics of adolescents, adults and older men. Apparently, it affects sperm quality more adversely than it does sperm production.

KEY WORDS: Varicocele, male factor infertility, semen, sperm morphology

Varicocele has been clearly identified as an important cause of male infertility. However, the influence of varicocele on men’s reproductive capacity has been subject to debate due to its markedly diverse effects on the testicles. Varicocele’s elementary pathologies include variations in size, intratesticular temperature, hydrostatic pressures in the internal spermatic vein, different degrees of venous stasis and alterations in the hypotalamic-pituitary-gonadal axis, which often cause deleterious effects on spermatogenesis. Varicocele can adversely affect sperm concentration, motility, morphology and membrane, acrosome and chromatin integrity. Likewise, it increases the levels of reactive oxygen species in the semen and causes sperm apoptosis, which is also implicated with impaired fertility. The controversy persists concerning why not all men suffering from varicocele have seminal abnormalities and generally father children, whereas others fail to achieve a pregnancy, even after varicocele repair. Ultimately, the effects of varicocele on fertility vary between individuals and the underlying reasons for these variations are not yet completely understood.

Varicocele is seldom found in boys below the age of 10 years. However, it has been detected in young men after puberty with an ever-increasing incidence that reaches 15-20% at the age of 20 years. These percentages are similar to those found in adults. Although it is a well-known fact that many patients with varicocele have poor semen quality and that semen...
analysis provides reliable information in this respect, no reports have been published about age-related sperm abnormalities and their significance for male fertility. The purposes of the current study were: 1) to evaluate sperm characteristics in adolescents, adults and older men with varicocele; 2) to identify age-related differences in sperm quality; and 3) to identify sperm features predictive for infertility in varicocele patients.

Materials and Methods

Study population

Records of semen analysis performed in the Semen Research Unit between January 2002 and December 2004 were reviewed and the results of 145 men (adolescents and adults) who had evident left-sided clinical varicocele detected by physical examination and confirmed by Doppler sonography were identified. Varicoceles were classified as Grade 1 if they could be palpated only after the Valsava maneuver; Grade 2 if they were palpable without the Valsava maneuver; and Grade 3 if they were visible. The distribution of varicocele grades I to III was as follows: adolescents: I = 8, II = 15, III = 8; adults: I = 53, II = 28, III = 31. A retrospective analysis of these laboratory records was then carried out by the author. Twenty-seven records of fertile men without varicocele (mean age 34.4 years old and interquartile ranges 31.0-36.5) who had fathered offspring during the previous one to two years following evaluation of their fertility status or after the treatment of female partners were also included as control group. Likewise, the study evaluated records of men (mean age 32.1 years old and interquartile range 29.8-35.5) having left-sided varicocele and history of infertility of at least one year without achieving a pregnancy with unprotected intercourse (n=26). Patient’s records, including those of the control group and infertile group were reviewed thoroughly to exclude the presence of leukocytospermia, bacteriospermia, sperm autoimmunity, endocrine abnormalities, testicular injury (traumatism, orchitis and torsion), maldescended testes and those with previous history of varicocelectomy. Azospermic samples were also excluded from the study.

Laboratory procedures

Semen was collected by masturbation at the laboratory, after three to five days of sexual abstinence and examined as soon as liquefied. Semen with abnormal liquefaction was evaluated 60 min after ejaculation. Sperm concentration (million sperm per milliliter), vitality, percentage of rapidly progressive motile spermatozoa (a) and the total percentage of progressively motile spermatozoa (a+b) were analyzed according to the WHO semen manual.[14] Sperm counts were performed using a Neubauer counting chamber, whereas the sperm morphology analysis was assessed by light microscope in semen smears stained by a modified Leishman blood staining method, as described previously.[15] Spermatozoa were recorded according to the WHO manual[14] as normal, small head, large head, slightly amorphous, severely amorphous, round-headed, double-headed, pin-headed, tapered, angular, abnormal midpiece (bent, abnormal insertion), cytoplasmic droplet, double, coiled and short-tailed and combined anomalies (two or more anomalies of head, mid-piece and tail). The hypoosmotic swelling test (HOST) was also performed according to the technique of Jeyendran et al.[16]

Statistical analysis

Basic descriptive statistics (means ± standard deviation) were calculated for the study groups. Statistical analysis of the means between adolescents, adults and older men with varicocele was performed using one-way analysis of variance and the Kruskal-Wallis test, for normality and non-normality distribution, respectively. A P value less than 0.05 was considered significant. Differences between patients with varicocele and men of proven fertility were evaluated using the unpaired t-test and the Mann-Whitney U-test for normality and non-normality distribution. The discriminating ability of the individual sperm characteristics in evaluating the sperm quality of varicocele patients from control group was analyzed using receiver operating characteristics (ROC) curves (MedCalc, version 8.1.0.0, Mariakerk, Belgium) to select the cut-off level providing the best combination of sensitivity and specificity. The sensitivity of the test was defined as the percentage of individuals with poor sperm quality (disease), whereas its specificity was defined as the percentage of individuals with good sperm quality (free from disease).

Results

The study groups were composed of adolescent males 14 to 20 years old (n=51), men 21 to 30 years old (n=48), 31 to 40 years old (n=40) and older men over 40 (n=24) with varicocele and the control group of men without varicocele and with proven fertility (n=27). A study group composed of infertile men having varicocele (n=26) was also compared with the study groups and control group. The results of the semen parameters analyzed in the study are presented in Table 1. Data are shown as mean ± SD. With the exception of sperm count, impaired sperm quality could be observed in all the study groups of varicocele patients, with a decrease in the vitality, rapidly progressive motility, total progressive motility, HOST and morphologically normal sperm. The percentages of these sperm characteristics were far below the current reference values.[15] However, there were no significant differences between the study groups (P>0.05).

On the other hand, when sperm characteristics of varicocele patients were compared with the control group, differences were significant in the percentage of rapidly progressive motility (P=0.0098 for adolescents, P=0.0104 for men between 21-30 years, P=0.0014 for men between 31-40 years and P=0.0083 for older men), total progressive motility (P=0.0009, P=0.0324, P=0.0114 and P=0.0130, respectively) and morphologically normal spermatozoa (P=0.0252, P=0.0002, P=0.0274 and P=0.0082, respectively). Differences were also significant in the percentages of vitality and HOST for adolescents (P=0.0281 and P=0.0089, respectively), men between 31-40 years (P=0.0095 and P=0.0135, respectively) and older men (P=0.0102 and P=0.0012, respectively), in the percentage of pin-headed sperm (P=0.0116 for men between 21-30 years and P=0.0104 for older men), tapered sperm (P=0.0127 for adolescents and P=0.0029 for men between 21-30 years) and combined anomalies (P=0.0004 for adolescents, P=0.0004 for
men between 21-30 years and $P=0.0077$ for men between 31-40 years). Sperm concentration and morphologically abnormal forms, namely small, large, slightly and severely amorphous, round-headed, double-headed, mid-piece defects and tail defects did not present significant differences ($P>0.05$). Results ($P$-value) are shown in Table 2.

The infertile group of men with varicocele was also compared with the adolescents, adults and older men with varicocele. The differences were not significant in all the semen parameters analyzed ($P>0.05$). However, when the infertile group was compared with the control group, differences were significant in sperm vitality ($P=0.0072$), rapid progressive motility

<table>
<thead>
<tr>
<th>Parameter</th>
<th>≤20 years vs. control group</th>
<th>21-30 years vs. control group</th>
<th>31-40 years vs. control group</th>
<th>&gt;40 years vs. control group</th>
<th>Infertile men vs. control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Vitality</td>
<td>0.0281</td>
<td>NS</td>
<td>0.0908</td>
<td>0.0102</td>
<td>0.0072</td>
</tr>
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<td>Rapid motility (a)</td>
<td>0.0098</td>
<td>0.0104</td>
<td>0.0014</td>
<td>0.0083</td>
<td>&lt;0.0001</td>
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<td>Total motility (a+b)</td>
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<td>0.0324</td>
<td>0.0114</td>
<td>0.0130</td>
<td>0.0033</td>
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<td>NS</td>
<td>0.0133</td>
<td>0.0012</td>
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<td>0.0252</td>
<td>0.0002</td>
<td>0.0274</td>
<td>0.0082</td>
<td>0.0014</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Slightly amorphous</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<td>Round-headed</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Double-headed</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pin-headed</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cytoplasmic droplet</td>
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<td>NS</td>
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<td>Tapering</td>
<td>0.0127</td>
<td>0.0029</td>
<td>NS</td>
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<td>Abnormal mid-piece</td>
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<td>Double tail</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Combined</td>
<td>0.0004</td>
<td>0.0004</td>
<td>0.0077</td>
<td>NS</td>
<td>0.0034</td>
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</table>

*Hyposmotnic swelling test. NS – nonsignificant
(P<0.0001), total progressive motility (P=0.0033), HOST (P=0.0269), morphologically normal sperm (P=0.0014) and combined anomalies (P=0.0034). Sperm concentration and morphologically abnormal forms, namely small, slightly and severely amorphous, round-headed, double-headed, pinheaded, tapered, mid-piece defects and tail defects did not present significant differences (P>0.05). Results (P-value) are shown in Table 2.

The study also evaluated the individual sperm characteristics that presented significant differences compared with the control group to investigate their discriminating power in identifying infertile men [Table 3]. Morphologically normal sperm and combined anomalies yielded higher accuracy in the identification of poor sperm quality in varicocele men (83.7% and 77.9%, at cut-off levels of 9% and 38%, respectively).

**Discussion**

The results of this study show that a similar decrease of the sperm vitality, motility, membrane integrity and number of normal forms of spermatozoons in adolescents, adults and older men might be regarded as evidence for an age-independent impairment of the sperm quality in men with varicocele. Since the sperm concentration was found to fall within the current reference value[14] in the study groups, it seems that varicocele affects sperm quality more adversely than it does sperm production.

Teratozoospermia was also found in the study groups, with a prevalence of small head, slightly and severely amorphous heads, pin-headed and combined anomalies, following the decrease of normal sperm forms. The above findings might be a manifestation of underlying changes in the sperm structure due to the adverse effects of varicocele on testicular function. The sperm malformations have varied on a case-by-case basis as noted in the study groups, with a pronounced prevalence of combined anomalies. Substance data about morphological defects in varicocele men are available, including the presence of combined anomalies.[17-19] However, the prevalence of these sperm malformations is now emphasized for the first time.

The seminal profile of varicocele was first described by MacLeod[20] in infertile men. He reported an abnormal seminal pattern with oligozoospermia, asthenozoospermia and a teratozoospermia characterized by a marked increase in immature germinal cells, especially early spermatids, amorphous and tapered forms. MacLeod introduced the concept of ‘stress pattern’, based on the prevalence of tapered forms higher than 15%, which was associated with varicocele. This assumption was supported by further reports[21-23] and so it was thought that the ‘stress pattern’ was pathognomonic of varicocele. However, others have not been able to detect significant differences in sperm morphology or even in sperm count.[24-25] In spite of many descriptions of the prevalence of tapered sperms in infertile men, no data consistently support the hypothesis that the ‘stress pattern’ is characteristic of or unique to varicocele, thus rendering it useless for diagnostic purposes. In the current study the frequency of tapered sperms was not increased.

In addition, some studies have demonstrated a loss of testicular mass as well as abnormalities in the hypothalamic-pituitary-gonadal axis in adolescents and these might prove critical for future fertility.[26] However, the time-dependent detrimental effect upon male fertility is still dubious, since not all of these patients experience infertility in adult life if left untreated.[27] In adolescents who were followed prospectively, progressive decline in sperm quality was observed in untreated patients.[28-29] In adult life, it is believed that varicocele also causes progressive damage.[30,31] since the prevalence of varicocele in secondary infertility is higher than in primary infertility.[32,33] Accordingly, men who were able to father children can develop infertility over time on account of the deleterious effects of varicocele on testicular function. Progressive decline of sperm quality has been reported by a number of investigators,[30-32] whereas others, however, failed to detect it.[33-35] Nevertheless, it is a well-known fact that many factors beyond varicocele can also affect the reproductive capacity of men with secondary infertility.[36] In fact, few prospective studies have hitherto assessed the pathological process of age-related varicocele and no consistent evidence has been produced to determine whether varicocele does exert progressive deleterious effects on testicular function over time. The current study does not claim to provide any convincing proof in this respect either, since seminal abnormalities were similarly distributed across the study groups and were not age-related. This finding is similar to the one reported by Hishikawa and Fujisawa.[37] On the other hand, it does show clearly that differences in sperm quality are evident in proven fertile men.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC</th>
<th>SE</th>
<th>CI 95%</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cut-off</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitality</td>
<td>0.525</td>
<td>0.140</td>
<td>0.282-0.760</td>
<td>100.0%</td>
<td>20.0%</td>
<td>74%</td>
<td>0.8585</td>
</tr>
<tr>
<td>Rapid motility (a)</td>
<td>0.674</td>
<td>0.143</td>
<td>0.417-0.870</td>
<td>100.0%</td>
<td>50.0%</td>
<td>15%</td>
<td>0.2234</td>
</tr>
<tr>
<td>Total motility (a+b)</td>
<td>0.708</td>
<td>0.124</td>
<td>0.451-0.893</td>
<td>54.5%</td>
<td>85.7%</td>
<td>29%</td>
<td>0.0945</td>
</tr>
<tr>
<td>Hypoosmotic swelling test</td>
<td>0.722</td>
<td>0.137</td>
<td>0.465-0.902</td>
<td>66.7%</td>
<td>75.0%</td>
<td>54%</td>
<td>0.1039</td>
</tr>
<tr>
<td>Normal head</td>
<td>0.837</td>
<td>0.096</td>
<td>0.590-0.964</td>
<td>100.0%</td>
<td>70.0%</td>
<td>9%</td>
<td>0.0005</td>
</tr>
<tr>
<td>Pin-headed</td>
<td>0.583</td>
<td>0.143</td>
<td>0.332-0.806</td>
<td>50.0%</td>
<td>66.7%</td>
<td>2%</td>
<td>0.5607</td>
</tr>
<tr>
<td>Tapered</td>
<td>0.723</td>
<td>0.159</td>
<td>0.466-0.903</td>
<td>75.0%</td>
<td>71.4%</td>
<td>3%</td>
<td>0.1614</td>
</tr>
<tr>
<td>Combined</td>
<td>0.779</td>
<td>0.120</td>
<td>0.525-0.935</td>
<td>85.7%</td>
<td>81.8%</td>
<td>38%</td>
<td>0.0202</td>
</tr>
</tbody>
</table>

Table 3: Receiver operating characteristic curve analysis of vitality, rapidly progressive motility, total progressive motility, sperm membrane integrity (HOST), morphologically normal sperm, pin-headed, tapered and combined anomalies for cut-off values.
without varicocele. Also, sperm characteristics in fertile men with varicocele were distributed similarly to adolescents, adults and older men and differences were not significant. On the other hand, poorer sperm quality could be observed in regard to the control group with significant differences in vitality, motility, morphologically normal sperm and combined anomalies. Thus, it seems that men with varicocele may have poor semen quality and might consequently also be infertile. In this way, semen analysis may serve as a critical tool in identifying infertile men with varicocele.

Also, it should be emphasized that the group of younger men presented similar results to those of adults and older men and the differences were not significant. Nevertheless, this study has used records of semen analysis of individuals between 14 and 20 years old, which could be in different phases of seminal development. Since normal ranges have not been standardized for younger men, normal semen quality will undoubtedly vary according to the age of the patient. Thus, it is recommended that the records of semen analysis from younger men must be evaluated with due clinical caution taking into account this possibility, before the choice of varicocele repair is made.

The areas under the ROC curve suggest the accuracy of some sperm characteristics in the discrimination of good and poor sperm quality of varicocele patients. Among all the sperm characteristics of the study population, percentages of morphologically normal sperm and combined anomalies display the highest predictive power (83.7% and 77.9%, respectively). So, it is expected that men with <9% of morphologically normal sperm and >38% of combined anomalies present poorer sperm quality and it seems that they also have impaired sperm vitality, motility and sperm membrane integrity. These are probably candidates for varicocele repair.

**Conclusion**

Varicocele affects equally the sperm quality of adolescents, adults and older men, as shown by concurrent decrease in the vitality, motility, sperm membrane integrity and number of normal forms of spermatozoons. Varicocele seems to affect sperm quality more significantly than it does sperm production, while multiple sperm abnormalities particularly combined anomalies, can be found in the semen analysis, subject to a case-by-case variance. Accordingly, semen analysis could offer a reliable source of information in identifying individuals who would most benefit from treatment, based on the distribution of sperm malformations. It is expected that men with <9% of morphologically normal sperm and >38% of combined anomalies present impaired sperm quality, thus also helping to support the choice of varicocele repair.

**References**

3. The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. World Health Organization.
28. Cozzolino DJ, Lipshultz LI. Varicocele as a progressive lesion:

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