CREATINE KINASE AS AN INDICATOR OF SPERM QUALITY AND MATURITY IN MEN WITH OLIGOSPERMIA

JORGE HALLAK, RAKESH K. SHARMA, FABIO F. PASQUALOTTO, PAVITHRA RANGANATHAN, ANTHONY J. THOMAS, JR, AND ASHOK AGARWAL

ABSTRACT

Objectives. To determine the differences among the creatine kinase (CK) levels in the spermatozoa of subfertile men with mild, moderate, or severe oligospermia and to examine the differences in CK activity between infertile patients with various clinical diagnoses and a group of normal healthy donors (control). CK is a marker of sperm maturity that correlates with the sperm fertilizing capacity. Elevated levels are associated with an increased rate of functional abnormalities and increased cytoplasmic retention.

Methods. We compared the CK levels in 51 oligospermic men who could not initiate a pregnancy. Patients were categorized according to their degree of oligospermia as defined by the total sperm count: mild (greater than 10 to 40 × 10^6; n = 50), moderate (5 to 10 × 10^6; n = 11), and severe (less than 5 × 10^6; n = 10). These patients were further classified according to their diagnosis (ie, varicocele, n = 24; unexplained infertility, n = 17; vasectomy reversal, n = 9; and unknown diagnosis, n = 1). A separate group consisting of 25 healthy donors was included as a control group. A computer-assisted semen analyzer assessed the sperm characteristics, and the CK levels were measured using a CK test kit after the enzyme was extracted with Triton-X.

Results. The CK levels were significantly higher in the sperm of the severely oligospermic group (8.8 ± 6.5 IU/10^8 sperm) than in the moderate (0.50 ± 0.19 IU/10^8 sperm) and mild (0.49 ± 0.15 IU/10^8 sperm) groups (P < 0.0001). The mean CK level in the severely oligospermic group was 18-fold higher than that in the moderate (P = 0.03) and mild (P < 0.001) groups. The CK levels were significantly higher in all three infertile groups compared with the donor group (0.06 ± 0.01 IU/10^8 sperm). Patients with varicocele had the highest CK level (3.42 ± 2.56 IU/10^8 sperm) compared with patients in the vasectomy reversal group (1.73 ± 0.98 IU/10^8 sperm) and the idiopathic infertility group (0.26 ± 0.08 IU/10^8 sperm).

Conclusions. Elevated CK levels are associated with severe oligospermia, irrespective of the clinical diagnosis. CK may be a sensitive indicator of sperm quality and maturity in the follow-up of patients treated for male factor infertility.


Infertility is common in couples of childbearing age. In more than 60% of infertile men, varicocele or idiopathic infertility is the cause. Conventional semen analysis has limited value in predicting fertility. An important factor contributing to the development of sperm abnormalities appears to be the disruption of spermiogenesis, leading to the release of excess residual cytoplasm by the differentiating spermatozoa. A number of independent studies have indicated that defective sperm function is associated with elevated levels of certain key enzymes, such as creatine kinase (CK), lactate acid dehydrogenase, and glucose-6-phosphate dehydrogenase. In addition, docosahexaenoic acid has also been reported to be an excellent marker of sperm maturation and cytoplasmic retention. Even though these enzymes are not directly responsible for the loss of sperm function, they act as biochemical markers of sperm differentiation, thereby reflecting the presence of exfoliated precursor germ cells and retention of excess...
residual cytoplasm during the final stages of spermiogenesis.

CK levels in human sperm are an objective biochemical marker of sperm maturity and fertilizing potential.5–8,13–19 Immunocytochemical studies of CK levels in individual spermatozoa have demonstrated that increased CK concentrations reflect residual cytoplasm in sperm that was not extruded during late spermiogenesis.20–22 Interest in CK has been stimulated by studies suggesting that defective sperm function is associated with defects in spermiogenesis that leads to the release of immature spermatozoa from the germinai epithelium.7,8,10,13–16,18,19,23

The purpose of this study was to determine the differences between sperm CK levels in subfertile men with mild, moderate, or severe oligospermia. In addition, we examined the differences in sperm CK activity and the sperm characteristics between healthy donors and infertile patients with various clinical diagnoses.

**MATERIAL AND METHODS**

**SUBJECT SELECTION**

The Cleveland Clinic Foundation institutional review board approved this study. Fifty-one infertile men were included; all were referred to our andrology laboratory for semen specimen evaluation. Twenty-five healthy donors who were selected on the basis of World Health Organization guidelines for normal semen analysis (normospermia = total sperm count [TSC] greater than 40 million) served as controls.24 Of the 51 oligospermic men, 24 had a varicocele, 17 had idiopathic infertility, 9 had a vasectomy reversal, and 1 had an unknown clinical diagnosis. All 51 patients had a viable sperm count of less than \(10^9 \text{ sperm/mL}\). The CK levels (mean ± SE) in the three oligospermic groups and one nonoligospermic group are shown in Table I. The mean CK levels were log transformed and analyzed by analysis of variance testing. Comparisons were made across the four groups of men. \(P < 0.05\) was considered significant using analysis of variance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonoligospermia* ((n = 50))</th>
<th>Mild ((&gt;10–40 \times 10^6; n = 30))</th>
<th>Moderate ((5–10 \times 10^6; n = 11))</th>
<th>Severe ((&lt;5 \times 10^6; n = 10))</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients and donors</td>
<td>CK (IU/10^8 sperm)</td>
<td>0.12 ± 0.03</td>
<td>0.49 ± 0.15</td>
<td>0.50 ± 0.19</td>
<td>8.8 ± 6.5</td>
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<tr>
<td></td>
<td>log CK (IU/10^8 sperm)</td>
<td>−1.26 ± 0.10</td>
<td>−0.68 ± 0.10</td>
<td>−0.49 ± 0.12</td>
<td>0.24 ± 0.23</td>
</tr>
<tr>
<td>Patients</td>
<td>CK (IU/10^8 sperm)</td>
<td>0.27 ± 0.009</td>
<td>0.51 ± 0.16</td>
<td>0.50 ± 0.19</td>
<td>8.8 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>log CK (IU/10^8 sperm)</td>
<td>−0.74 ± 0.15</td>
<td>−0.65 ± 0.10</td>
<td>−0.49 ± 0.12</td>
<td>0.24 ± 0.23</td>
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</table>

**CK Estimation**

The CK levels were estimated using the CK kit (Sigma Chemical, St. Louis, Mo.). An aliquot of the ejaculate (250 µL) was transferred to a 15-mL polystyrene centrifuge tube. We removed the seminal plasma by washing the pellet with ice-cold imidazole buffer (0.15 M NaCl and 0.03 M imidazole at pH 7.0) at a ratio of 1:15 (vol/vol). The supernatant was decanted after centrifugation at 500g, and the pellet was resuspended in a 0.1% Triton X-100 detergent solution using a vortex for 20 seconds. The sample was centrifuged again at 500g, and the supernatant was analyzed for CK activity by measuring the change in the absorption of nicotinamide-adenine dinucleotide at 340 nm. The CK activity was expressed as international units/10^8 spermatozoa.

**STATISTICAL ANALYSIS**

Analysis of variance was used to compare the CK activity and semen parameters among the three groups of oligospermic patients and the donor group, and also among the four levels of oligospermia severity. The values were log transformed, and the analysis was performed by pairwise comparison that both included and excluded the donor group. CK activity was also compared among the groups, with pairwise \(P\) values adjusted for multiple range tests using the Tukey-Kramer method. The relationship between the sperm characteristics and CK levels was evaluated using the Spearman correlation coefficient among donors and patients. Statistical significance was assessed with two-tailed tests at the \(P < 0.05\) level using the Statistical Analysis System, version 6.12, statistical software package (SAS Institute, Cary, NC).

**RESULTS**

The CK levels (mean ± SE) in the three oligospermic groups and one nonoligospermic group are shown in Table I. The mean CK levels were highest in the sperm from patients with severe oligospermia compared with the levels from the mod-
erate and mild oligospermic groups. The CK levels in the severely oligospermic group were 18-fold higher than that in the moderate group \((P = 0.03)\) and mild oligospermia group \((P < 0.001)\). A similar association was seen when the CK values were log transformed. Using pairwise comparisons for total sperm concentration, all three oligospermic groups were significantly different from the non-oligospermic group \((P < 0.0001)\). When the donor group was included, the group with severe oligospermia (less than 5 million sperm) differed significantly from the moderate group \((P < 0.03)\) and the mild group \((P < 0.0002)\). Similarly, when the donors were excluded, the CK level in the severe oligospermia group was significantly higher than both the moderate oligospermia group \((P < 0.02)\) and the mild oligospermia group \((P < 0.0002)\).

Table II shows the CK levels (mean \(\pm SE\)) and sperm characteristics in the controls and oligospermic men, who were classified according to their clinical diagnosis. Patients with varicocele had the highest CK activity. The differences in CK levels between the controls and the three groups of oligospermic patients were significantly different \((P < 0.0001)\). Furthermore, the CK levels in the idiopathic infertility group were significantly different than the CK levels in the varicocele group \((P < 0.003)\) and vasectomy reversal group \((P < 0.04)\).

The sperm characteristics (concentration, motility, and morphology) were significantly lower in all patient categories compared with the control group (Table II). To examine whether there are sperm CK differences independent of the sperm concentrations, we divided the oligospermic group below a normal cutoff value using the donors. The 95th percentile CK value for controls was 0.189 IU. We considered the cutoff value of 0.189 IU or less as normal and a value greater than 0.189 IU as abnormal for determining the CK differences. A higher number of sperm from oligospermic men with CK values of 0.189 IU or less demonstrated a higher proportion of normal spermatozoa that were morphologically mature according to Kruger’s strict criteria.25

Table III shows the results of the correlation analysis for sperm characteristics and CK activity. The CK levels correlated negatively with the sperm concentration, TSC, percent motility, and normal sperm morphology. The CK levels correlated positively with the percentage of abnormal tail forms.

**TABLE II. Level of creatine kinase and other sperm characteristics in controls and oligospermic men, who are classified according to their clinical diagnosis**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls ((n = 25))</th>
<th>Varicocele ((n = 24))</th>
<th>Idiopathic ((n = 17))</th>
<th>Vasectomy Reversal ((n = 9))</th>
<th>(P) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (IU/(10^8) sperm)</td>
<td>0.06 (\pm) 0.01</td>
<td>3.42 (\pm) 2.56</td>
<td>0.26 (\pm) 0.08</td>
<td>1.73 (\pm) 0.98</td>
<td>0.0001</td>
</tr>
<tr>
<td>log CK (IU/(10^8) sperm)</td>
<td>-1.45 (\pm) 0.10</td>
<td>-0.28 (\pm) 0.13</td>
<td>-0.79 (\pm) 0.08</td>
<td>-0.32 (\pm) 0.26</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sperm concentration ((\times 10^6/mL))</td>
<td>78.77 (\pm) 9.70</td>
<td>6.10 (\pm) 0.91</td>
<td>9.68 (\pm) 1.29</td>
<td>6.25 (\pm) 2.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>65.99 (\pm) 3.22</td>
<td>32.95 (\pm) 2.51</td>
<td>33.84 (\pm) 2.22</td>
<td>21.18 (\pm) 4.40</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**TABLE III. Spearman correlation of sperm characteristics with creatine kinase activity**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Correlation with CK ((r))</th>
<th>(P) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration ((\times 10^6/mL))</td>
<td>-0.71</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total sperm count ((\times 10^9))</td>
<td>-0.67</td>
<td>0.0001</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>-0.53</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Morphology**

<table>
<thead>
<tr>
<th>WHO</th>
<th>Correlation with CK ((r))</th>
<th>(P) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal form (%)</td>
<td>-0.48</td>
<td>0.0001</td>
</tr>
<tr>
<td>Abnormal tail (%)</td>
<td>0.42</td>
<td>0.0002</td>
</tr>
<tr>
<td>Round cells (%)</td>
<td>-0.09</td>
<td>0.48</td>
</tr>
<tr>
<td>Kruger’s strict criteria (%)</td>
<td>-0.49</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Note:** Abbreviations as in Table I. Values presented as the mean \(\pm SE\). Comparisons were done between the different groups of infertile men with the controls; the clinical diagnosis could not be confirmed in 1 patient.

* \(P < 0.05\) was considered significant using the Tukey-Kramer method.

**COMMENT**

As spermatozoa enter the final stages of differentiation, they undergo a remarkable transformation whereby the cytoplasmic component of the cell is removed during the release of the mature spermatozoa from the Sertoli cell.1,2 In the case of human spermatozoa, any residual cytoplasm that remains associated with the spermatozoa after spermiation is retained in the mid-piece as an irregular cytoplasmic mass surrounding the mitochondria.2–4,16–20 If the amount of residual cytoplasm localized in the...
sperm mid-piece becomes excessive (more than one third the size of the sperm head), it is classified as a morphologic abnormality and described as a “cytoplasmic droplet.”22,24,25

CK is associated with other biochemical markers, namely reactive oxygen species (ROS), and is reflective of the oxidative stress in the pathologically abnormal spermatozoa.15,18 As a result of elevated levels of ROS and depressed levels of antioxidants, oxidative stress is associated with male infertility.20 Mature spermatozoa shed their extra cytoplasm as residual bodies in the adluminal area before being released into the seminiferous tubuli, and they characteristically have very low amounts of cytoplasm (low CK activity). Mature spermatozoa also show a higher concentration of the CK-M isoform that is expressed only during the last phase of spermiogenesis in elongated spermatids and in mature sperm.5,8,16,18,20,21 Studies suggest that the plasma membrane changes take place in spermiogenesis simultaneously with cytoplasmic extrusion and the expression of the new sperm-specific CK-M.5,11 That sperm cellular maturity may be a key underlying element of sperm functional integrity is also demonstrated by the expression pattern of β-1,4-galactosyltransferase, a protein that is present only on the surface of the sperm plasma membrane.27–30

CK values differed significantly among the various diagnostic groups. Compared with the controls, the highest CK values were seen in the varicocele group (50-fold), followed by the vasectomy reversal group (28-fold) and the idiopathic group (fourfold). In this study, we showed that the CK values correlated negatively with sperm concentration, TSC, and percent motility. Significantly elevated CK levels were seen in the group with severe oligospermia. Overall, the sperm concentration correlated negatively with the CK values. In the normospermic specimens with a higher percentage of mature spermatozoa, the CK values were significantly lower.

The observation that CK levels were highest in the varicocele group may have an important bearing on whether patients with varicocele will benefit from varicocele repair and how long couples should wait, especially if the wife is older than 35 years. For instance, a normospermic infertile patient with varicocele is a candidate for varicocele repair. Measuring the CK levels in this patient may enable us to better understand his sperm physiology. Therefore, we could potentially use the CK measurement in a patient with varicocele as a guide for choosing either surgical treatment or a type of assisted reproduction technique.

Sperm morphology correlated highly with CK activity. In our study, a large proportion of immature sperm was seen in the 25 healthy control subjects (TSC greater than 40 million). Similarly, we observed mature sperm in the mild and moderate oligospermic groups. Even though we did not examine the association of sperm concentration with sperm maturity, a lack of correlation between the concentration and sperm maturity has been previously shown.5–8,13 Our observation indicates that semen from both oligospermic and normospermic men contain similar proportions of mature and immature sperm. This may explain why many normospermic patients are infertile. In such cases, measuring their CK level may help identify idiopathic infertile men who have a normal sperm count (ie, lower levels of sperm maturity and fertilizing potential).

It is well known that oligospermia or asthenospermia, or both, is present in about 40% of infertile men and that effective treatments are lacking for these patients, mainly because the cause of the underlying condition is unknown.26 Also, a relationship between the incomplete extrusion of cytoplasm, oxidative stress due to the production of ROS, and lipid peroxidation has recently been reported.20,31 In oligospermic patients, the high level of ROS generation is also associated with a significant increase in diaphorase activity.30 This category of sperm may be more prone to oxidative stress induced as a result of pathologic conditions. Defective sperm function is causally associated with the induction and propagation of lipid peroxidation and oxidative stress in human spermatozoa. Fertile oligospermic men are reported to have lower CK levels than infertile men, even when the two groups have an identical sperm concentration.15,19 Future studies may demonstrate a correlation between CK levels, oxidative stress, and sperm DNA damage in oligospermic infertile patients. Docosahexaenoic acid content in human sperm has been shown to be inversely related to sperm maturation.14

What is the value of CK activity as an independent predictor of sperm quality in a logistic regression analysis model? CK activity, like Kruger’s morphology, was predictive of the fertilizing potential; however, sperm concentration did not provide any additional information.15 Mature and immature sperm are different in their degree of cytoplasmic retention.8 Fertility,32 morphologic and morphometric attributes,17 and zona pellucida-binding properties.18 Total CK activity in a given sample is important as a marker of sperm quality. However, the distribution of CK activity may be more important. We have observed in our unpublished work, along with other investigators,20 that semen samples with a low percentage of sperm with large cytoplasmic retention have high CK activity and a higher ability to produce ROS compared with sperm with a high percentage of
sperm with small cytoplasmic retention and low CK activity/sperm, even though the total CK activity may be similar. In such instances, determining the concentration of sperm with proximal cytoplasmic retention (immunohistochemical staining) may be more informative than the total CK activity. CK activity in the sperm is present in two isomeric forms, CK-M and CK-B isomers. The predictive value of CK-M ratios (CK-M/CK-M plus CK-B) in the assessment of fertility was tested in couples undergoing in vitro fertilization. \(^\text{32}\) Men with less than 10% CK-M ratios did not achieve pregnancy whether they had low or high sperm concentrations. Therefore, measuring the CK-M ratios, rather than sperm with proximal cytoplasmic retention in individual spermatozoa, may be more helpful as a marker of sperm quality and predicting infertility (rather than predicting fertility) in in vitro fertilization. Recently, a putative CK-M isoform, testis-expressed, 70-kDa, heat shock protein chaperone known as HspA2 (formerly CK-M) has been demonstrated. \(^\text{23}\) Immature sperm fail to express this HspA2 and show cytoplasmic retention and a lack of zona pellucida binding. Therefore, HspA2 may be an objective biochemical marker of sperm function in male infertility.

CONCLUSIONS

Elevated CK levels are associated with severe oligospermia, irrespective of the clinical diagnosis. Identifying men with diminished fertilizing potential and infertility, irrespective of the sperm concentration, may be an important factor in the workup of couples with male-factor or idiopathic infertility. CK may be a sensitive indicator of sperm quality and maturity in the follow-up of patients treated for male factor infertility.

REFERENCES

25. Kruger TF, Menkveld R, Stander FSH, et al: Sperm mor-


