Relationship Between Creatine Kinase Levels and Clinical Diagnosis of Infertility

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Purpose: The creatine kinase level indicates sperm maturity and correlates with the spermatozoal fertilizing potential. The relationship between creatine kinase levels in subfertile men and their clinical diagnosis was examined.

Methods: Patients with unexplained infertility ($n = 34$), varicocele ($n = 20$), postvasectomy reversal ($n = 7$), or cancer ($n = 22$) were included in this prospective clinical study. The control group consisted of healthy normal donors ($n = 15$).

Results: The median and interquartile range values of creatine kinase for each group were as follows: normal donors, $0.061 \text{ U}/10^8 \text{ sperm}$ (0.056 to $0.076 \text{ U}/10^8 \text{ sperm}$); idiopathic male factor, $0.119 \text{ U}/10^8 \text{ sperm}$ (0.061 to $0.190 \text{ U}/10^8 \text{ sperm}$); varicocele, $0.392 \text{ U}/10^8 \text{ sperm}$ (0.209 to $1.494 \text{ U}/10^8 \text{ sperm}$); postvasectomy reversal, $0.589 \text{ U}/10^8 \text{ sperm}$ (0.425 to $4.043 \text{ U}/10^8 \text{ sperm}$); and cancer, $0.068 \text{ U}/10^8 \text{ sperm}$ (0.047 to $0.168 \text{ U}/10^8 \text{ sperm}$). Sperm creatine kinase levels were significantly higher in patients with varicocele compared to normal donors ($P = 0.0001$), cancer patients ($P = 0.0002$), and men with idiopathic infertility ($P = 0.0009$). Sperm concentration and creatine kinase level were inversely correlated in patients ($r = -0.7$, $P < 0.001$) but not in normal donors.

Conclusions: Semen quality is poorer in subfertile patients with clinical varicocele and postvasectomy reversal than in cancer patients and patients with idiopathic male infertility. That the creatine kinase levels in cancer patients were similar to those of normal donors suggests that the final phase of spermatogenesis may not be altered in men with cancer; thus semen from these patients should be banked to ensure fertility after cancer treatment.

KEY WORDS: cancer patients; postvasectomy reversal; idiopathic infertility; sperm creatine kinase; varicocele.

INTRODUCTION

Infertility is common in couples of childbearing age. Approximately half these cases are related to a male infertility factor (1). In more than 60% of infertile men varicocele or idiopathic infertility is the cause (2). Other important causes of infertility are testicular failure and postvasectomy reversal. Testicular and nontesticular cancer (Hodgkin's disease and leukemia) also affect young men of reproductive age and treatments for these cancers may cause infertility. Conventional semen analysis, which assesses semen characteristics such as ejaculate volume, sperm concentration, percentage motility, and normal sperm morphology, has limited value in predicting fertility (3,4). Diagnosis of defective sperm function is difficult, and identification of sperm dysfunction may therefore require alternative methods of assessing sperm quality, such as assaying lipid peroxidation, acrosin, hyaluronidase, or creatine kinase (5–7).

Creatine kinase is involved in synthesis and utilization of energy in the sperm (8). This enzyme does not play any direct role in fertilization itself. However, several studies indicate that it may be important in male fertility. Fertile oligospermic men are reported to have lower sperm creatine kinase activity than infertile men, even when the two groups have identical sperm concentrations (9). Creatine kinase levels predict sperm quality better than semen analysis (10). Spermatozoal creatine kinase levels indicate sperm maturity, with higher levels being found in immature spermatozoa that retain their cytoplasmic droplets (11). Finally, creatine kinase activity is inversely correlated with spermatozoa fertilizing potential (11,12).

The purpose of this study was to examine (a) differences in sperm creatine kinase levels between normal healthy donors and subfertile patients with various diagnoses and (b) the relationship, if any, between sperm creatine kinase levels in subfertile men and their clinical diagnosis.

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METHODS

Selection of Subjects

This study was approved by our Institutional Review Board. We selected 61 subfertile patients with a clinical diagnosis of idiopathic infertility ($n = 34$), varicocele ($n = 20$), postvasectomy reversal ($n = 7$), or cancer ($n = 22$: testicular cancer, $n = 10$; and nontesticular cancer, $n = 12$) who were referred to our laboratory for semen evaluation. Healthy normal donors ($n = 15$) were selected on the basis of a normal semen analysis (13).

Assessment of Semen Characteristics and Semen Analysis

Semen specimens were collected after 48 to 72 hr of sexual abstinence by masturbation into a sterile cup. Specimens were allowed to liquefy at 37°C for 30 min before evaluating sperm concentration and other semen characteristics. Semen specimens were analyzed on a computer-assisted semen analyzer (CASA; Motion Analysis; Cell-Trak, Model VP 110, Version 4.22B; Santa Rosa, CA). For each measurement, a 5- μ l aliquot was loaded on a 20- μ m counting chamber (MicroCell, Conception Technologies, Inc., La Jolla, CA) and analyzed for sperm count and motility. Sperm concentration and motility were verified manually under an Olympus BH2-S microscope (Olympus; Tokyo) with a X20 positive phase-contrast objective. Sperm morphology was assessed by WHO criteria (13).

Creatine Kinase Measurement

Creatine kinase levels were measured using the procedure described by Huszar and Vigue (1). An aliquot of semen was transferred to a 15-ml conical centrifuge tube. Seminal plasma was removed by washing with ice-cold imidazole buffer [0.15 M NaCl and 0.03 M imidazole, pH 7.0, at a ratio of 1:15 (v/v)]. The supernatant was decanted after centrifugation and the pellet resuspended in 0.1% Triton X-100 by vortexing for 20 sec. The suspension was centrifuged for 10 min. The supernatant was removed and its creatine kinase activity estimated using a spectrophotometric kit (Sigma Chemical Co., St. Louis, Mo). Results are expressed as units per 10^8 sperm.

Statistical Analysis

The Spearman correlation rank-sum test was used to determine correlations between creatine kinase and various sperm characteristics. The Kruskal-Wallis test was used to compare creatine kinase levels in semen from subfertile patients and normal men. A P value of < 0.05 was considered significant. The Wilcoxon rank-sum test with the Bonferroni correction was used for any pairwise comparisons. All statistical analyses were performed using the SAS statistical software package (SAS Institute Inc., Cary, NC).

RESULTS

Age, abstinence, and ejaculate volume did not differ among the five groups. The median and interquartile values for the creatine kinase level in the patient groups were as follows: idiopathic infertility, 0.119 U/ 10^8 sperm (0.061 to 0.173 U/ 10^8 sperm); clinical varicocele, 0.392 (0.209 to 1.494 U/ 10^8 sperm); post vasectomy reversal, 0.589 (0.425 to 4.043 U/ 10^8 sperm); patients with cancer, 0.068 (0.047 to 0.168 U/ 10^8 sperm); and normal donors, 0.061 (0.056 to 0.076 U/ 10^8 sperm). The creatine kinase level was significantly higher in patients with varicocele compared to normal donors ($P = 0.0001$), cancer patients ($P = 0.0002$), and men with idiopathic infertility ($P = 0.0009$). No significant differences in creatine kinase level were observed between normal donors and patients with cancer or idiopathic infertility (Table I, Fig. 1). The levels of creatine kinase in patients with varicocele

Table I. Comparison of Creatine Kinase Activity in Various Clinical Groups^a

Group	Creatine kinase (U/ 10^8 sperm)		
	Q1	Median	Q3
Donor ($n = 15$)	0.056	0.061	0.076
No known male factor ($n = 34$)	0.061	0.119	0.190
Varicocele ($n = 20$)	0.209	0.392	1.494
Cancer ($n = 22$)	0.047	0.068	0.168
Postvasectomy reversal ($n = 7$)	0.425	0.589	4.043

^a Results were analyzed by Kruskal-Wallis test: $P = 0.001$ was significant. Significant group differences occurred: donor vs no known male factor, donor vs varicocele, donor vs postvasectomy reversal, no known male factor vs varicocele, no known male factor vs postvasectomy reversal, varicocele vs cancer, and postvasectomy reversal vs cancer.

Fig. 1. Creatine kinase activity in patients with various clinical diagnoses. Boxes cover the middle 50% of data values, between the lower and the upper quartiles. The central horizontal line is the median and vertical lines indicate 95% of the data.

and those with postvasectomy reversal were higher than in patients with other diagnoses and normal donors.

Sperm concentration significantly correlated with creatine kinase level in patients ($r = -0.7$, $P < 0.001$). Similarly, a negative correlation between sperm concentration and creatine kinase level was seen in other groups of patients. However, as expected, semen in specimens from normal donors showed no such correlation (Table II).

CONCLUSIONS

The sperm creatine kinase levels in our normal donors were similar to those reported in normospermic men (1). Subfertile patients have elevated levels of creatine kinase compared to normal donors (unpublished reports from our laboratory). Subfertile patients in this study who had clinically identifiable male infer-

tility factors, such as varicocele or postvasectomy reversal, had significantly higher sperm creatine kinase levels compared to patients without an identified male factor. In patients with varicocele or postvasectomy reversal, spermatozoa may be biochemically immature and may have less chance of fertilizing the ovum. Creatine kinase is a biochemical marker for cellular maturity (14–16). Infertile couples with an identifiable male factor have been reported to have a lower mean sperm penetration assay score than infertile couples with no known male factor (17).

The creatine kinase levels in men with testicular and nontesticular cancers were similar to those in normal donors. This finding suggests that sperm produced by cancer patients may be biochemically mature, and it supports earlier reports of no relationship between diagnosis of cancer and infertility in patients with Hodgkin's disease or leukemia (18, 19). Poor semen quality in patients with cancer may be explained by nonspecific conditions, such as anorexia, fever, and stress. Recent studies have determined that conception can be achieved even with poor-quality spermatozoa obtained from cancer patients when assisted reproductive procedures such as in vitro fertilization are used (20–23). These reports indirectly support our observations that sperm from these patients may be mature, with normal levels of creatine kinase and capable of fertilization.

Inhibition of sperm maturation (spermiogenesis) may be accompanied by arrest of spermatogenesis. The negative correlation between sperm concentration and creatine kinase observed in all patient groups may be explained by inhibition or arrest of spermatogenesis accompanying inhibition of spermiogenesis. However, in some oligospermic men, a low sperm count may not be accompanied by maturation arrest as is evident from normal creatine kinase levels in our cancer patients. This difference may explain why some oligospermic men are fertile. We hypothesize that some maturation factors responsible for the completion of spermiogenesis are deficient in patients with a high creatine kinase levels. These maturation factors may be secreted by epithelial cells of the duct and need to be identified. Coculture of sperm with these factors may help complete spermiogenesis and improve fertilizing ability.

In conclusion, a clinical diagnosis of varicocele or postvasectomy reversal in subfertile patients indicates a poor semen quality compared to that in subfertile patients in whom no clinical diagnosis is evident. Patients who have varicocele or postvasectomy reversal and elevated levels of creatine kinase may need

Table II. Correlation Analysis Results for Creatine Kinase with Semen Characteristics in Various Clinical Groups

Group	Spearman coefficient (P) ^a				
	Concentration (10 ⁶ /ml)	Motility (%)	Velocity (μm/sec)	Linearity (%)	Normal forms (%) ^b
Donors ($n = 15$)	-0.13 (0.65)	0.15 (0.61)	-0.15 (0.59)	-0.11 (0.59)	0.19 (0.52)
No known male factor ($n = 34$)	-0.40 (0.02)	-0.13 (0.47)	-0.13 (0.65)	0.39 (0.02)	0.41 (0.02)
Varicocele ($n = 20$)	-0.51 (0.02)	-0.06 (0.81)	0.35 (0.14)	0.04 (0.85)	-0.31 (0.20)
Postvasectomy reversal ($n = 7$)	-0.82 (0.02)	-0.39 (0.37)	-0.20 (0.70)	-0.71 (0.11)	-0.75 (0.05)
Cancer ($n = 22$)	-0.55 (0.01)	-0.09 (0.69)	—	—	—

^a $P < 0.05$ considered significant.^b Determined using World Health Organization criteria.

surgical correction before invasive assisted reproductive procedures such as *in vitro* fertilization or intracytoplasmic sperm injection are recommended. The similar levels of creatine kinase in cancer patients and normal donors suggest that the final phase of spermatogenesis may not be altered in cancer patients. Therefore, semen from these patients should be banked before initiating chemotherapy or radiation therapy to ensure future fertility.

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