Creatine Kinase Level and Lipid Peroxidation Rate in Human Spermatozoa from Patients with Cancer

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Purpose: The present study assessed whether the poor semen quality in patients with cancer results from the inhibition of sperm maturation as indicated by creatine kinase or from increased oxidative stress as assessed by lipid peroxidation of the sperm membrane.

Methods: Cryopreserved semen specimens from patients with testicular ($n = 10$) and nontesticular ($n = 12$) cancer and normal healthy donors ($n = 14$) were analyzed for lipid peroxidation and creatine kinase levels.

Results: The levels of creatine kinase and malonaldehyde did not differ among testicular or nontesticular patients with cancer or normal healthy donors.

Conclusions: Poor semen quality in testicular and nontesticular patients with cancer is not related to creatine kinase or lipid peroxidation levels; it may be related to other factors.

KEY WORDS: sperm; creatine kinase; lipid peroxidation; testicular cancer; nontesticular cancer; cryopreservation.

INTRODUCTION

Testicular cancer, Hodgkin's disease, and leukemia are among the most common malignancies affecting men in their primary reproductive years (1). In addition to the effects of malignancy, a cause of infertility in these patients is the cancer treatment itself. It has been estimated that 70 to 100% of men undergoing treatment for Hodgkin's disease may be azoospermic by the time complete remission is achieved (2). Only a small percentage of these patients recovers spermatogenesis (3) and the prognosis for clinically significant recovery of spermatogenesis is poor (1).

In addition to sperm concentration and motion characteristics, various biochemical variables, such as creatine kinase and lipid peroxidation, help predict the fertilizing ability of spermatozoa and quality of spermatogenesis (4–8). Creatine kinase, a key enzyme in the energy synthesis and transport in the cell, does not directly influence fertilization; however, higher concentrations of the enzyme in spermatozoa are related to defects in spermatogenesis, such as extrusion of cytoplasmic droplets. Spermatozoa are extremely susceptible to lipid peroxidation, as they are rich in polyunsaturated fatty acids and lack antioxidant protection. Oxidative stress causes peroxidation of polyunsaturated fatty acids in the cell membrane and, hence, loss of sperm function (9). Therefore, creatine kinase and lipid peroxidation can serve as a biochemical index of semen quality (5).

The purpose of the present study was to determine whether the poor semen quality in patients with cancer results from inhibition of sperm maturation as indicated by creatine kinase levels or from increased oxidative stress as assessed by lipid peroxidation.

MATERIALS AND METHODS

Selection of Subjects

Cryopreserved semen samples from patients with testicular cancer ($n = 10$) or nontesticular cancer ($n = 12$; Hodgkin's disease, nonHodgkin's lymphoma, leukemia, or sarcoma) and normal healthy donors ($n = 14$) were used. The study was approved by our Institutional Review Board. Patients who had no history of chemotherapy or radiation therapy at the time of sperm banking were included, regardless of cancer stage. Healthy normal donors were selected on the basis of a normal semen analysis as determined by the WHO criteria (10).

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Assessment of Semen Characteristics and Semen Analysis

All specimens were collected by masturbation into a sterile cup at our facility after 48 to 72 hr of sexual abstinence. Specimens were allowed to liquefy at 37°C for 30 min before sperm concentration and other semen characteristics were evaluated.

Semen specimens were analyzed on a computer-assisted semen analyzer (CASA; Cell-Trak, Model VP 110, Version 4.22B; Motion Analysis, Santa Rosa, CA). For each measurement, 5 μ l of the sample was loaded on a 20- μ m counting chamber (MicroCell, Conception Technologies, Inc., La Jolla, CA) and analyzed for sperm count and motility by CASA and manually. An Olympus (New York) BH2-S microscope with a $\times 20$ positive phase-contrast objective was used for the manual evaluation (11).

Cryopreservation of Semen

Semen was slowly mixed with an equal volume of TEST-yolk buffer with glycerol (Irvine Scientific, Santa Ana, CA) in four equal aliquots at 5-min intervals so as to have a final semen-to-freezing medium ratio of 1:1. The mixed aliquots were frozen in cryogenic vials using a three-stage freezing procedure: exposure to -20°C for 10 min, exposure to -100°C nitrogen vapors for 2 hr, and finally, immersion in liquid nitrogen at -196°C until analysis (12). For thawing, the specimen was left at room temperature for 5 min and then at 37°C for 20 min.

Creatine Kinase Estimation

Creatine kinase was estimated using the procedure described by Huszar *et al.* (6), which involves determining the rate of reduction of nicotinamide adenine dinucleotide. An aliquot of specimen was transferred to a 15-ml conical centrifuge tube. The seminal plasma was removed by washing with ice-cold imidazole buffer (0.15 M NaCl and 0.03 M imidazole at pH 7.0) at a ratio of 1:15. The supernatant was decanted after centrifugation and the pellet resuspended in an equal volume of 0.1% Triton X-100 solution by vortexing for 20 sec. The suspension was then centrifuged for 10 min. To estimate creatine kinase activity, the supernatant was analyzed with a spectrophotometric creatine kinase kit (Sigma Chemical Co., St. Louis, MO). The results are expressed as units per 10⁸ sperm.

Lipid Peroxidation Measurement

Lipid peroxidation levels were measured using the thiobarbituric acid method (13,14). The seminal plasma was removed by washing in Ham's F-10 buffer (Life Technologies Inc., Grand Island, NY). The sperm concentration was adjusted to 20 $\times 10^6$ /ml in Ham's F-10 buffer.

The washed sperm were incubated with 0.25 ml ferrous sulfate (2.5 mM; Sigma Chemical Co.) and 0.25 ml sodium ascorbate (12.5 mM; Sigma Chemical Co.) for 1 hr in a 37°C water bath. To precipitate proteins after incubation, 500 μ l of 40% trichloroacetic acid (Sigma Chemical Co.) was added. The sample was centrifuged at 1600 g for 12 min, 1 ml of clear supernatant was collected, and 500 μ l of 2% thiobarbituric acid (Sigma Chemical Co.) in 0.2 N NaOH (Sigma Chemical Co.) was added to the supernatant. The test tubes were boiled at 100°C for exactly 10 min. The sample was cooled in crushed ice and the optical density measured at 534 nm with an Ultrospec III spectrophotometer (Pharmacia Biotech Inc., Piscataway, NJ). The malonaldehyde level was calculated from the optical density produced by malonaldehyde standards (Sigma Chemical Co.). The lipid peroxidation level is expressed as nanomoles of MDA per 10⁸ sperm per hour.

Statistical Analysis

Student's *t* test was used to compare the creatine kinase and malonaldehyde levels and postthaw sperm count and motility between normal men and patients with testicular or nontesticular cancer. A *P* value of <0.025 (with Bonferroni correction) was considered statistically significant. The Pearson correlation test was used to assess the correlation between creatine kinase and malonaldehyde values and sperm concentration or motility. A *P* value of <0.05 was considered statistically significant. The SAS statistical software package (SAS Institute Inc., Cary, NC) was used for analyzing the data.

RESULTS

Table I compares the groups on creatine kinase. Creatine kinase levels did not differ among patients with testicular and nontesticular cancer (*P* = 0.36). Similarly, creatine kinase levels in normal donors did not differ from those in patients with testicular and nontesticular cancer (*P* = 0.85, Table I). There was no

Table I. Comparison of Sperm Count, Motility, Creatine Kinase, and Lipid Peroxidation Levels Between Patients with Cancer and Normal Donors^a

Variable	Donors (<i>n</i> = 14)	Testicular cancer (<i>n</i> = 10)	<i>P</i> ^b	Nontesticular cancer (<i>n</i> = 12)	<i>P</i> ^b
Sperm count ($\times 10^6/\text{ml}$)	58.6 (40.4, 74.1)	15.3 (9.1, 45.2)	0.02	44.1 (32.0, 67.0)	0.95
Motility (%)	67.0 (60.7, 85.5)	24.16 (9.7, 28)	<0.001	15.5 (8.0, 22.5)	<0.001
Creatine kinase (U/ 10^8 sperm)	0.061 (0.037, 0.068)	0.097 (0.049, 0.253)	0.81	0.056 (0.038, 0.107)	0.85
Malonaldehyde (nM/ 10^8 sperm/hr)	22.61 (17.21, 25.35)	21.51 ^c (20.47, 32.67)	0.45	24.30 ^d (20.35, 41.63)	0.11

^a Data are expressed as median, with interquartile range in parentheses. Lipid peroxidation was measured as the rate of malonaldehyde production.

^b Compared to donors.

^c *n* = 7; malonaldehyde production could not be measured in three patients with testicular cancer.

^d *n* = 10; malonaldehyde production could not be measured in two patients with nontesticular cancer.

significant difference in malonaldehyde levels between patients with testicular and those with nontesticular cancer ($P = 0.79$). Malonaldehyde levels in patients with testicular and nontesticular cancer were similar to those in normal donors ($P = 0.48$ and $P = 0.11$, respectively).

Normal donors had a significantly higher sperm concentration compared to patients with testicular cancer ($P = 0.02$, Table I). Sperm motility was observed to be significantly higher in normal donors compared to patients with both testicular and nontesticular cancer ($P < 0.0001$, Table I). No significant correlations were observed between creatine kinase and malonaldehyde levels, sperm motility, or sperm concentration in normal donors and in patients with testicular and nontesticular cancer (Table II).

DISCUSSION

Patients with cancer have poor sperm quality before therapy. In about 52% of patients with testicular cancer

and 40% of patients with other cancer, the total sperm count is significantly reduced at diagnosis and at treatment (15). Sperm concentration and motility are poor predictors of the fertilizing ability of sperm in patients with cancer. However, biochemical indicators of spermatozoa maturity such as creatine kinase are independent of sperm concentration (16–18) and are considered important predictors of sperm maturity and fertilizing potential (7). Incomplete extrusion of cytoplasm, diminished sperm maturity, and abnormal sperm morphology are closely correlated with increased creatine kinase content and with increased lipid peroxidation (malonaldehyde production rate) in semen (7). The lability of the sperm membrane may make sperm more susceptible to lipid peroxidation in addition to unextruded cytoplasm. Changes in membrane phospholipids are related to lipid peroxidation rates (19). Sperm membrane damage can be caused by reactive oxygen species, such as the superoxide anion, hydroxyl radical, and hydrogen peroxide, which results in increased lipid peroxidation. Human spermatozoa are particularly susceptible to lipid peroxidation-

Table II. Correlation Analysis Results for Variables Examined in Normal Donors and Testicular and Nontesticular Cancer Patients

Variable	Motility (%)	Sperm count ($\times 10^6/\text{ml}$)	Malonaldehyde (nM/ 10^8 sperm/hr)
Creatine kinase (U/ 10^8 sperm)	$r = -0.17^b$	$r = -0.56$	$r = -0.53$
Testicular cancer (<i>n</i> = 10) ^a	$P = 0.63^c$	$P = 0.09$	$P = 0.28$
Nontesticular cancer (<i>n</i> = 12) ^d	$r = -0.14$	$r = -0.06$	$r = -0.27$
(U/ 10^8 sperm)	$P = 0.66$	$P = 0.85$	$P = 0.44$
Normal donors (<i>n</i> = 14)	$r = -0.05$	$r = -0.23$	$r = 0.29$
	$P = 0.87$	$P = 0.43$	$P = 0.31$

^a *n* = 7; malonaldehyde production could not be measured in three patients with testicular cancer.

^b Pearson correlation test.

^c *P* value of less than 0.05 considered significant.

^d *n* = 10; malonaldehyde production could not be measured in two patients with nontesticular cancer.

induced damage because the membrane has a high polyunsaturated fatty acid content, and sperm lack antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase (20).

In our study, levels of creatine kinase and malonaldehyde in cryopreserved semen specimens from patients with testicular and nontesticular cancer were similar to levels observed in normal men. These findings suggest that the poor sperm quality seen in patients with cancer is not related to sperm maturation in the late phase of spermatogenesis or oxidative stress but may be caused by other factors inhibiting spermatogenesis during the early phase.

In addition, the lack of difference in creatine kinase levels between patients with testicular and those with nontesticular cancer indicates that malignant cells in the testis do not affect sperm in the late phase of spermatogenesis. The lower concentration of sperm in patients with cancer compared to normal donors suggests that undifferentiated spermatogonial renewal and proliferation may be inhibited in these patients.

Testicular cancer, Hodgkin's disease, and non-Hodgkin's lymphoma adversely affect sperm count and motility in prefreeze and postthaw specimens (21). In our study, postthaw sperm motility was significantly lower in patients with both testicular and nontesticular cancer compared to normal controls. Although others have reported negative and positive correlations between lipid peroxidation and sperm motility in patients without cancer (3,22–24), we found no significant correlation between creatine kinase or lipid peroxidation and sperm motility in patients with cancer and normal men. Similarly, the malonaldehyde level was not elevated in patients with testicular or nontesticular cancer. Therefore, antioxidant supplementation may not be helpful in improving semen quality in these patients.

Sperm from patients with cancer can withstand stress induced during the freeze–thaw process similarly to spermatozoa from normal healthy donor (25). These sperm are probably mature sperm, as indicated by normal creatine kinase levels, therefore, it is essential to freeze any available sperm before beginning therapy in these patients.

One of the limitation of our study was that creatine kinase and lipid peroxidation levels were measured in only postthaw semen specimens. The reason for this was that patients with cancer generally have a very short time available for cryopreserving their specimens before the start of their therapy, and thus, their pre-freeze specimens cannot be used for research purposes. The postthaw specimens used by us were obtained

from patients who, after varying durations following cancer treatment, gave written authorization to discard their specimens from the sperm bank, as they did not feel the need for continued storage. Results from our own studies in normospermic men have shown that creatine kinase and lipid peroxidation levels are not different in fresh or frozen–thawed semen specimens (unpublished observation).

CONCLUSIONS

Creatine kinase and lipid peroxidation levels in semen do not differ significantly between patients with testicular or nontesticular cancer and normal healthy men. The lack of difference in creatine kinase levels in patients with cancer and normal healthy men suggests that the last phase of spermatogenesis is not affected in these patients. Similarly, semen quality in patients with cancer is not related to oxidative sperm membrane stress as indicated by the malonaldehyde level.

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