oxygen species, such as the superoxide anion, hydroxyl radical, and hydrogen peroxide. Lipid peroxidation can cause the loss of membrane integrity, which increases cell membrane permeability to lead to enzyme inactivation, structural damage to DNA, and cell death. Human spermatozoa are particularly susceptible to lipid peroxidation-induced damage because they contain high levels of polyunsaturated fatty acids and lack antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase.

The thiobarbituric acid reaction for malonaldehyde is the most convenient and widely used assay for lipid peroxidation. The outcome of this assay correlates statistically with results from other lipid peroxidation assay methods, including chemiluminescence, pentane or ethane formation, and colorimetric reactions based on the potassium iodide reduction of phospholipid hydroperoxides. Malonaldehyde is a stable product of lipid peroxidation and therefore can be used as an indirect measure of the cumulative lipid peroxidation. Also, the combination of ferrous sulfate and sodium ascorbate has been used to induce lipid peroxidation as well as to promote the breakdown of lipid peroxides into smaller alkenals such as malonaldehyde, the product most often measured in studies involving lipid peroxidation.

The semen quality in most patients with cancer before chemotherapy or radiation therapy is subnormal. In about 52% of patients with testicular cancer and 40% of those with lymphoma, the total sperm count is substantially reduced at diagnosis and before treatment. Physical and mental stress, genetic factors, and hormonal imbalances may all be responsible for the altered spermogenesis. Also, lipid peroxidation may have a role in carcinogenesis. The stimulation of lipid peroxidation in vitro by adding polyunsaturated fatty acids to the culture medium is cytotoxic to cancer cells. Elevated malonaldehyde levels have been reported in tumor tissue of patients with colorectal cancer; however, other studies have found no increase in malonaldehyde levels in the serum of patients with cancer. In our study, the malonaldehyde levels in cryopreserved semen specimens from patients with testicular or nontesticular cancer were similar to those observed in normal men. This finding suggests that the poor sperm quality in patients with cancer is not related to lipid peroxidation but might be caused by physical and mental stress, hormonal imbalance, or genetic factors. Similarly, the lack of difference in malonaldehyde levels in patients with testicular versus nontesticular cancer indicates that malignant cells in the testicles do not affect lipid peroxidation in the semen.

High levels of lipid peroxidation are reported in normospermic men with low in vitro fertilization rate, which increases after 1 month of treatment with an antioxidant. Our results suggest that antioxidant supplementation may not improve semen quality in patients with testicular or nontesticular cancer because the malonaldehyde levels in these patients were not elevated.

Both negative and positive correlations between malonaldehyde levels and sperm motility have been reported. However, we found no significant correlation between malonaldehyde levels and post-thaw sperm motility or patient age in patients with testicular or nontesticular cancer. Sperm motility and patient age did not affect lipid peroxidation in patients with cancer.

In conclusion, poor post-thaw semen quality in patients with cancer is not related to lipid peroxidation but may result from factors such as sperm membrane stress induced during the freeze-thaw process, physical and mental stress, hormonal imbalance, and genetic factors.

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REFERENCES


1985 to 1995 by patients with cancer who later died or were no longer interested in continuing to bank their sperm specimens. Cryopreserved semen samples from patients with testicular (n = 15) or nontesticular cancer (n = 16) [Hodgkin's disease, non-Hodgkin's lymphoma, or leukemia] and normal healthy volunteers (n = 20, control subjects) were used. Only patients with no history of chemotherapy or radiation therapy at the time of sperm banking were included, regardless of the cancer stage. The normal semen donors were selected on the basis of a normal semen analysis, including an ejaculate volume of 2.0 mL or greater, sperm concentration of 20 x 10⁹/mL or greater, motility of 50% or greater, and normal sperm morphology of 30% or greater.

**SEmen COLLECTION AND FREEZING**

Semen specimens from patients and normal donors were collected by masturbation after 2 to 3 days of sexual abstinence. The ejaculate was collected in a sterile container and allowed to liquefy at 37°C for 30 minutes. For pre-freeze and post-thaw semen analysis, 5 μL of the sample was loaded on a 20-μm counting chamber (Conception Technologies, La Jolla, Calif). Samples were analyzed on a computer-assisted semen analyzer (CASA, Cell-Trak, Model VP 110, Santa Rosa, Calif). To ensure the accuracy of CASA results, a manual assessment was done with each CASA analysis. Manual results were used for sperm count and percent motility if CASA results showed more than a 20% difference from manual readings. For cryopreservation, semen samples were diluted by dropwise addition of TEST-yolk buffer freezing medium (Irvine Scientific, Santa Ana, Calif) with uniform mixing for 5 minutes to give a final semen to freezing medium ratio of 1:1 (vol/vol). The samples were placed in 2.0-mL cryovials and put in a −20°C freezer for 8 minutes and thereafter in liquid nitrogen vapor at −79°C for 2 hours. The vials were then transferred to liquid nitrogen at −196°C. For post-thaw analysis, each vial was removed from the liquid nitrogen, thawed at room temperature for 5 minutes, and then incubated for 20 minutes at 37°C.

**LIPID PEROXIDATION MEasUREMENT**

Lipid peroxidation levels were measured using the thiobarbituric acid method. Ferrous sulfate and sodium ascorbate were used to promote the lipid peroxidation reaction. After thawing, the cryomedium was removed by centrifugation at 300g for 7 minutes, followed by washing in Ham's F-10 buffer (Life Technologies Inc., Grand Island, NY). The sperm concentration was adjusted to 20 x 10⁹/mL in Ham's F-10 buffer. To avoid chemical reaction, only glass test tubes were used for the assay.

The washed spermatozoa were incubated with 0.25 mL of ferrous sulfate (2.5 mM) (all chemicals were purchased from Sigma Chemical Company, St. Louis, Mo, unless otherwise stated) and 0.25 mL of sodium ascorbate (12.5 mM) for 1 hour at a 37°C water bath. Control tubes were incubated without the promoter. To precipitate proteins after incubation, 500 μL of 40% trichloroacetic acid was added. The sample was centrifuged at 1600g for 12 minutes, 1 mL of clear supernatant was collected, and 500 μL of 2% thiobarbituric acid in 0.2 N NaOH was added to the supernatant. The test tubes were boiled at 100°C for exactly 10 minutes. Malonaldehyde reacted with thiobarbituric acid to form a pink-colored product. The sample was cooled in crushed ice, and the optical density of the pink color product was measured at 534 nm with an Ultrospec III spectrophotometer (Pharmacia Biotech Inc., Piscataway, NJ). The malonaldehyde level was calculated by comparing the optical density of the sample with that of a malonaldehyde standard (malonaldehyde bis(dimethyl acetal)). The lipid peroxidation level was expressed as nM MDA/10⁶ sperm/hr.

**FIGURE 1. Scatterplots of malonaldehyde levels and post-thaw sperm motility in patients with testicular (A) or nontesticular cancer (B). Malonaldehyde levels and post-thaw sperm motility were not correlated in patients with testicular (Pearson's r = 0.33; P = 0.24) or nontesticular cancer (Pearson's r = −0.09; P = 0.74).

**STATISTICAL ANALYSIS**

A Student t test was used to compare the malonaldehyde level and post-thaw sperm motility between patients with testicular or nontesticular cancer and normal men, and a P value of less than 0.017 (with Bonferroni correction) was considered statistically significant. To assess the correlation between malonaldehyde levels and sperm motility or patient age, the Pearson correlation was used. All data were expressed as mean value ± SE. The SigmaStat statistical software package (Jandel Corporation, San Rafael, Calif, 1992) was used to analyze the data.

**RESULTS**

Malonaldehyde levels in patients with testicular (25.90 ± 1.00 nM/10⁹ sperm/hr; P = 0.58), or nontesticular cancer (24.48 ± 1.66 nM/10⁹ sperm/hr; P = 0.86) were not greater than those in normal healthy men (24.86 ± 1.43 nM/10⁹ sperm/hr) and did not significantly differ between patients with testicular or nontesticular cancer (P = 0.48). The median age was 27 years (range 18 to 40) in patients with testicular cancer, 23.5 years (range 18 to 38) in patients with nontesticular cancer, and 31.5 years (range 22 to 47) in normal men. No significant correlations were seen between malonaldehyde levels and age in patients with testicular (r = −0.07, P = 0.80) or nontesticular cancer (r = −0.11; P = 0.68).

Malonaldehyde levels and post-thaw sperm motility were not correlated in patients with testicular cancer or nontesticular cancer (Fig. 1).

Post-thaw sperm motility was 17.4% ± 2.0% in patients with testicular cancer, 16.7% ± 2.7% in patients with nontesticular cancer, and 26.1% ± 1.1% in normal men and was significantly lower in patients with testicular or nontesticular cancer than in normal men (P = 0.002 and P < 0.001, respectively). Sperm motility did not differ between the two groups with cancer (P = 0.83).

**COMMENT**

Lipid peroxidation damage to the sperm cell membrane results from the generation of reactive
EFFECT OF LIPID PEROXIDATION ON CRYOPRESERVED SEMEN QUALITY IN PATIENTS WITH TESTICULAR OR NONTESTICULAR CANCER

Y. WANG, R. K. SHARMA, AND A. AGARWAL

ABSTRACT

Objectives. Although pre-freeze and post-thaw semen quality in patients with cancer is poor, it is not clear whether lipid peroxidation affects semen quality. This study assessed (1) whether poor semen quality in patients with cancer is caused by lipid peroxidation, and (2) whether patient age or sperm motility is associated with lipid peroxidation.

Methods. Lipid peroxidation was measured by determining malonaldehyde levels using the thiobarbituric acid method. Malonaldehyde levels were measured in cryopreserved semen specimens from patients with testicular (n = 15) or non-testicular cancer (n = 16) and normal men (control subjects, n = 20). A computer-assisted semen analyzer was used to determine the sperm concentration and motility before and after cryopreservation.

Results. Malonaldehyde levels did not differ in frozen-thawed semen specimens among patients with testicular (25.90 ± 1.00 nM/10^6 sperm/hr) or non-testicular cancer (24.48 ± 1.66 nM/10^6 sperm/hr), and control subjects (24.86 ± 1.43 nM/10^6 sperm/hr). Malonaldehyde levels did not correlate with post-thaw sperm motility or patient age in patients with testicular or non-testicular cancer. Post-thaw sperm motility from patients with testicular or non-testicular cancer was significantly lower than that in normal subjects.

Conclusions. The poor post-thaw semen quality in patients with testicular or non-testicular cancer is not related to lipid peroxidation but may be caused by other factors such as sperm membrane stress induced during the freeze-thaw process. UROLOGY 50: 414–417, 1997. © 1997, Elsevier Science Inc. All rights reserved.

Testicular cancer, Hodgkin’s disease, and leukemia are among the most common malignant diseases affecting young men of reproductive age.1 Infertility is a major concern for men with malignant disease who are undergoing chemotherapy, radiation therapy, or surgery because most of these regimens cause sterility.2 Cryopreserving sperm is crucial to circumvent sterilization, but cryopreservation for patients with cancer is controversial because a substantial number of these patients have poor semen quality at the time of sperm banking, and sperm motility decreases by 25% to 50% after cryopreservation.3-4 The reason that sperm motion characteristics decline after cryopreservation is not clear, but the changes are great enough that it is well known that fertilization and pregnancy rates after cryopreservation are poor.4,5

Human spermatozoa are extremely susceptible to oxidative attack because they have a high polyunsaturated fatty acid content and poor antioxidant protection. As a result of oxidative stress, the polyunsaturated fatty acid membrane undergoes peroxidation, and the spermatozoa lose function.6 Because lipid peroxidation can cause irreversible loss of sperm motility, it can serve as a biochemical index of semen quality.7,8 The aims of this study were to assess (1) whether poor semen quality in patients with cancer is caused by lipid peroxidation, and (2) whether patient age or sperm motility in patients with cancer correlates with lipid peroxidation.

MATERIAL AND METHODS

SELECTION OF SUBJECTS

After obtaining approval from our institutional review board, we obtained semen samples that had been banked from