Characteristics of Cryopreserved Semen from Men with Lymphoma

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Purpose: *This study compared the pretreatment semen quality in patients with Hodgkin's disease and non-Hodgkin's lymphoma with a group of healthy donors. We also examined the differences in prefreeze and postthaw semen quality among the different stages of Hodgkin's disease.*

Methods: *The study included 89 patients with Hodgkin's disease, 18 with non-Hodgkin's lymphoma, and 50 healthy sperm donors.*

Results: *In patients with Hodgkin's disease, the prefreeze and postthaw semen characteristics were significantly lower than those of the healthy donors. Similar results also were seen in patients with non-Hodgkin's lymphoma. No significant differences in the prefreeze semen quality were seen in patients with different stages of cancer.*

Conclusion: *Patients with Hodgkin's disease and non-Hodgkin's lymphoma in our study had poor semen quality when compared with healthy donors both before and after cryopreservation. As cancer therapy significantly impairs reproductive potential, sperm banking should be offered to these men before the start of their therapy.*

KEY WORDS: Semen cryopreservation; male infertility; lymphoma; assisted reproduction.

INTRODUCTION

Lymphomas, the seventh most common cancer type, are a group of malignant diseases of lymphoreticular origin. Hodgkin's disease (HD) originates in the lymph nodes, 90% within the gonads. Non-Hodgkin's lymphoma (NHL) usually arises from parenchymal tissues of organs (1).

Multimodality treatment approaches and sophisticated diagnostic tools have markedly increased survival for lymphoma patients over the last few decades, and cure has become a realistic goal (1,2). For example, survival from Hodgkin's disease has risen from 52% in the 1960s to more than 80% in the 1980s (3). The reported incidence of NHL has risen 50%, but cure rates are 40% to 50% (4). However, the price that male patients may pay for the cure is long-term or permanent azoospermia from the high gonadal toxicity of the treatment. This may be particularly devastating to the many patients who are within their reproductive years at the time of diagnosis (5,6). Most men with lymphoma have poor semen quality even before they start their treatment (7,8). However, recent advances in assisted reproductive techniques (ART) and cryopreservation methods have made sperm banking a realistic option for patients with lymphoma (9).

Sperm banking before cancer treatment may help these men preserve their fertility, but fertility cannot be assured without answering two questions. First, how does the quality of semen in the lymphoma patient who has not yet undergone cancer therapy compare to semen quality in healthy men? Second, is a lymphoma patient's semen more susceptible to damage by cryopreservation than semen from healthy men?

The aims of this study were (a) to examine pretreatment semen quality in patients with HD and NHL, (b) to compare prefreeze and postthaw semen quality in these patients with those of healthy donors,

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and (c) to examine differences in prefreeze and postthaw semen quality in men with different stages of HD.

MATERIALS AND METHODS

Patient Selection

This study was approved by the institutional review board of the Cleveland Clinic Foundation and written consent was obtained from all subjects.

We reviewed the records of 89 patients with HD and 18 patients with NHL who were referred to the sperm bank at our institution for sperm cryopreservation between 1982, when the sperm bank was established, and 1997. The results were compared to those of 50 normal healthy donors. Patient information was obtained from their medical records and when necessary by phone calls to the patients or to the referring physician. Patients with HD were subsequently staged using the Cotswold classification (10).

Donor Selection

Inclusion criteria were an ejaculate volume of at least 2 ml and a sperm concentration of at least 20×10^6/mL of which at least 50% were motile and 30% had normal sperm morphology according to the World Health Organization (WHO) criteria (11).

Assessment of Semen Variables

Semen specimens were collected by masturbation after 48–72 hr of sexual abstinence and liquefied at 37°C for 30 min. Five microliters of the specimen were loaded on a 20 μl Microcell counting chamber (Conception Technologies, San Diego, CA) and analyzed by a computer-assisted semen analyzer (Cell Trak Semen Analyzer, CTS Version 4.0, Motion Analysis Corporation, Palo Alto, CA). Manual verification of the semen analyzer results was performed by microscopic examination.

Semen Cryopreservation

Sperm were cryopreserved with a glycerol-based cryoprotectant, TEST, Yolk buffer (Irvine Scientific, Santa Ana, CA). An aliquot of the freezing medium equal to 25% of the original speciment volume was added to the specimen and gently mixed for 5 min using an aliquot mixer (Hema-Tek, Miles Scientific, Elkhart, IN). This procedure was repeated until the volume of the cryoprotectant added equaled the volume of the ejaculate. Cryovials were frozen at −20°C for 8 min and then in liquid nitrogen vapor at −100°C for 2 hr. The vials were then transferred to liquid nitrogen at −196°C for long-term storage. On the day after the semen was frozen, a vial was removed and thawed by incubation at 37°C for 20 min. A 5 μl aliquot was analyzed as described above.

Statistical Analysis

All statistical analyses were performed with the SAS statistical software package (SAS Institute Inc., Cary, NC). The pairwise Wilcoxon rank-sum test was performed to compare the prefreeze and postthaw semen analysis results of the healthy donors and the patients. In addition, age and ejaculate volumes were compared using the pairwise Wilcoxon rank-sum test. The comparison between the four stages of HD was done by using Kruskal–Wallis test. A P value of less than .05 was considered significant, and all summary statistics are presented as median and ranges or interquartile ranges.

RESULTS

Patients with HD were significantly younger than the healthy donors (median 27, range 23–30 years vs. median 29, interquartile range 23–35 years; $P = 0.01$). The ejaculate volume of patient group showed no difference from that of the healthy donors ($P = 0.39$). Similarly, there was no difference in age or ejaculate volume between the patients in four stages of HD. Patients with NHL showed no difference in age or ejaculate volume from normal donors [age 26 years (20–34 years) vs. 29 years (23–35 years); $P = 0.28$; ejaculate volume 2.7 ml (2.1–4.4) vs. 2.4 mL (1.8–3.5); $P = 0.35$]. Patients with NHL were not classified by stage due to the small number of cases in each category.

Prefreeze Semen Quality in Patients with HD

Median total motile sperm count (TMS), percent motility, curvilinear velocity (VCL), and linearity (LIN) were significantly lower in patients with HD than in healthy donors (Table I). Sperm motion characteristics showed no statistical difference between the four stages of HD.

Table I. Sperm Characteristics Before and After Cryopreservation in Healthy Donors and Patients with Lymphoma[a]

Sperm characteristics	Healthy donors (n = 50) median (IQR)	Hodgkin's disease (n = 89) median (IQR)	Non-Hodgkin's lymphoma (n = 18) median (IQR)	P^b	P^c	P^d
Motile sperm count						
Prefreeze	129 (61.5–240)	27.7 (11–94.9)	81.1 (19.5–134)	.0003	.03	.87
Postthaw	59.1 (23–90.8)	20.2 (4.4–48.7)	20 (3.7–55)	.03	.27	.72
% change	−53.7 (−64.4–42.5)	−55 (−68.4–−33.8)	−65.9 (−71–−45.6)	.38	.96	.58
Motility (%)						
Prefreeze	63.5 (50–74)	39 (24–54)	46.5 (32–56)	.0001	.002	.42
Postthaw	33 (26–40)	18 (10–29)	20.5 (12–30)	.0001	.0009	1.0
% change	−47.2 (−57.6–−30.6)	−58.7 (−69.5–−33.9)	−58.7 (−69.5–−33.9)	.54	.94	.74
VCL (μm/sec)						
Prefreeze	49.9 (37.1–56.4)	42.4 (34.6–49)	39.2 (34–49.2)	.006	.34	.45
Postthaw	40.6 (32–46)	32.1 (28.3–38.8)	33 (27–40.1)	.0001	.13	.46
% change	−22.2 (−34.1–−6.7)	−21.4 (−34–−6.5)	−20.6 (−40.9–−7.3)	.8	.64	.74
LIN (%)						
Prefreeze	6.2 (4.4–39)	4.9 (4.3–5.7)	5.4 (4.6–6.7)	.0001	.06	.1
Postthaw	6.2 (5–37)	5 (4.2–5.5)	5.5 (4.8–7.8)	.0001	.36	.01
% change	2 (−12.7–12.5)	1.9 (−11.6–11.2)	−1.1 (−4.7–4.4)	.91	.49	.44
ALH (μm)						
Prefreeze	2.7 (2.4–3.2)	2.6 (2–3)	2.9 (1.8–3.6)	.45	1.0	.64
Postthaw	2.2 (1.8–2.5)	2.2 (1.8–2.5)	2.8 (1.8–3.2)	.72	.01	.08
% change	−23.5 (−34.4–−11.6)	−12.8 (−25.1–−1.9)	−10.5 (−25.3–6.8)	.27	.07	.27

[a] IQR, Interquartile range (25% and 75%); VCL, curvilinear velocity; LIN, linearity; ALH, amplitude of lateral head displacement.
[b] Difference between donors and patients with HD.
[c] Differences between donors and patients with NHL.
[d] Difference between patients with HD and patients with NHL.

Postthaw Semen Quality in Patients with HD

Median TMS, percent motility, and LIN were significantly lower in patients with HD than in healthy donors (Table I). Sperm motion characteristics showed no statistical difference between the four stages of HD. Also, the percentage change in sperm motion characteristics between prefreeze and postthaw semen quality did not differ significantly between the patients and the healthy donors. Percentage change in linearity was the only significant difference between the four stages of HD.

Prefreeze Semen Quality in Patients with NHL

Median TMS, percent motility, and LIN were significantly lower in the patients than in healthy donors.

Postthaw Semen Quality in Patients with NHL

Median percent motility and amplitude of lateral head displacement (ALH) were significantly lower in the patients than in healthy donors. The percentage change between prefreeze and postthaw semen quality did not differ significantly between the patients and the healthy donors in any of the sperm motion characteristics. In addition, prefreeze and postthaw semen characteristics in patients with NHL did not differ significantly from those of the patients with HD, except for postthaw LIN (P = 0.01).

DISCUSSION

Lymphomas are among the most common neoplasms affecting young men of reproductive age. The effect of therapy on fertility of men with lymphoma has become more apparent as advances in treatment improve survival rates (12). Regimens of chemotherapy, radiotherapy, surgery, or combinations of these treatments frequently result in permanent infertility. The duration and severity of spermatogenic suppression are dependent on the agents involved, the duration of the treatment, and the total dose of the agents (13,14). Azoospermia has been reported in more than 80% of men with HD after MOPP (nitrogen mustard, vincristine sulphate, procarbazine, and prednisone) therapy (6). Pryzant *et al.* (2) found that pelvic irradiation is a

major risk factor for permanent sterility in lymphoma patients; only 20% of men treated with doses ranging from 1.78 Gy to 5.3 Gy regained normospermia. Therefore, pretreatment sperm banking may be the only way to preserve fertility, and pretreatment semen characteristics become important.

Our study shows that those patients with HD and those with NHL have poorer prefreeze semen quality than healthy donors before they start cancer therapy. In certain types of malignancy, such as testicular cancer, the cancer may be directly related to the poor semen quality (15). However, in patients with lymphoma, poor semen quality may be caused by incidental factors, such as fever or general stress (16). The possibility that lymphomas have a direct effect on spermatogenesis was raised after it was recognized that patients who were azoospermic at diagnosis had previously fathered children (16), and spermatogenesis was thought to recover after treatment (17). We found no relationship between the deleterious effects of the disease on spermatogenesis and the stage of the disease, which is in agreement with other studies (17–19).

We also found that postthaw semen quality is poorer than that of healthy donors. We found no difference in prefreeze and postthaw semen characteristics between patients with HD and those with NHL. In addition, the percentage change from the prefreeze to postthaw semen quality did not differ significantly between the patients and the healthy donors. This suggests that cryopreservation damages the same proportion of spermatozoa in patients with lymphoma and in controls (20).

Therefore, cryopreserving spermatozoa before starting treatment seems to be an effective method to circumvent the sterilizing effect of cancer therapy in patients with lymphoma (21). This is crucial, especially with the tremendous progress in assisted reproductive techniques, which enables men with the most severe forms of infertility to establish pregnancy (22,23).

Although there are no clinical data about whether these cryopreserved sperm could transmit lymphomas and genetic abnormalities to the children, such a theory deserves attention (1). Lymphoma may be transmitted by grafts of both fresh and frozen mouse ovarian tissues. However, these grafts were intact ovarian tissue with all its blood-borne elements, so no extrapolation can be made about whether germ cells alone can pass abnormalities to the offspring (24).

CONCLUSION

In conclusion, overall semen quality in lymphoma patients involved in this study is poor both before and after cryopreservation when compared with those of the healthy donors. There is no correlation between semen quality and the type of lymphoma or disease stage (in HD). The effects of the cryopreservation process on patients with lymphoma and on healthy donors are the same. We recommend that before cancer therapy begins, sperm banking be offered to all lymphoma patients who may wish to conceive. These patients then have a chance to have children through assisted reproductive techniques.

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