Journal of Andrology, Vol. 25, No. 1, January/February 2004

High Levels of Sperm DNA Denaturation as the Sole Semen Abnormality in a Patient After Chemotherapy for Testis Cancer

Case Report

LESLIE DEANE, SHARON SHARIR, KEITH JARVI, AND ARMAND ZINI

From the Division of Urology, Department of Surgery, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

Systemic chemotherapy for the treatment of cancer is known to adversely affect male fertility potential. Sperm DNA damage is reported after cancer chemotherapy and is associated with semen 0abnormalities (Chatterjee et al, 2000; Morris, 2002). We report on a patient presenting for infertility evaluation with a history of chemotherapy for testis cancer, normal semen parameters, and high levels of sperm DNA denaturation (DD), a known marker of sperm DNA damage.

Case Report

A 31-year-old man presented for evaluation of secondary infertility of 2 years' duration. Three years before presentation he underwent a left radical orchiectomy and lymph node dissection followed by chemotherapy for testis cancer. The chemotherapy regimen included 2 cycles of VP-16 and cis-platinum followed by 3 cycles of VP-16 cis-platinum and bleomycin. Sperm was banked before treatment and a successful twin pregnancy with in vitro fertilization/intracellular sperm injection was achieved using banked sperm. The hormonal profile at the present evaluation was completely normal and included serum testosterone, follicle-stimulating hormone, luteininzing hormone, prolactin, thyroid-stimulating hormone, and estradiol. The patient submitted 2 semen analyses, 1 month apart. Standard semen parameters and sperm DD values are shown in Table 1 (with reference ranges for standard semen parameters based on World Health Organization criteria and for sperm DD based on the report of Evenson et al, 1999).

Sperm DD was evaluated by flow cytometry analysis of acridine orange-treated spermatozoa and expressed as the percentage of spermatozoa with denatured DNA (Evenson et al, 1999; Zini et al, 2001). All samples were run in quadruplicate (2 aliquots, each run twice through the flow cytometer) and the reported sperm DD values represent means of 4 readings. Interassay variability of sperm DD (<5%) has been verified by repeat assessments of control or reference semen samples. Over 300 aliquots of the same semen sample (''reference sample'') have been stored at −70°C for ongoing assessment of interassay variability. The reference sample is used to set the red and green photomultiplier tube voltage gains to give the same means for red and green fluorescence levels. A new reference sample is run every 6–10 samples to avoid drift (Zini et al, 2001).

Comment

Testis cancer and its treatments are associated with impaired fertility. Most patients will present with abnormal semen parameters initially, but this may improve to some extent over time. The effects of chemotherapy on sperm DNA integrity specifically are not completely known. Recent studies have demonstrated that chemotherapy may increase sperm germline mutations (Zheng et al, 2000).

The present case is unique in that the only identified abnormality accounting for this patient's infertility is the abnormally elevated sperm DD (~50%, which is 2 SD above the mean for the infertile group). Indeed, of the 164 consecutive semen analyses tested (from 141 men) over a 6-month period in our laboratory, high sperm DD levels were observed only once (the present case) in association with normal semen parameters. Of the 164 semen analyses, 33 (20%) had high levels of sperm DD (in excess of 30%) and the mean percentage of sperm DD (±SD) was 22% ± 15%. High levels of sperm DD (in excess of 30%) have been associated with very poor fertility in vivo (Evenson et al, 1999). There is good evidence to show that sperm DD is a valid marker of sperm DNA integrity (Zini et al, 2001).

To date, the reproductive consequence of sperm DNA damage is not completely understood. In particular, the possibility of iatrogenic transmission of abnormal genetic material to the offspring has not been discounted. The present couple has been counseled with respect to poten-

Correspondence to: Armand Zini, MD, Mount Sinai Hospital, 60 Murray St, 6th Floor, Toronto, Ontario, Canada M5G 1X5 (e-mail: azini@mtsinai.on.ca).

Received for publication July 14, 2003; accepted for publication September 25, 2003.

Semen parameters and percentage of sperm with DNA denaturation (sperm DD) in two consecutive semen samples

Sample #	Volume (mL)	Conc. (10^6/mL)	%Motility	%Normal Forms	Sperm DD
1	5.0	39	52	45	52.9
2	5.5	29	55	40	48.7
Reference range	(1.5–5.0)	>20	>50%	>30%	<30%

tial reproductive options (eg, assisted reproduction) and possible reproductive consequences of abnormal sperm DNA integrity. The couple will be followed with serial (biyearly) semen analyses and sperm DNA integrity.

References

Chatterjee R, Haines GA, Perera DMD, et al. Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukemia. *Hum Reprod.* 2000;15:762–766.

Evenson DP, Jost LK, Marshall D, et al. Utility of the sperm chromatin assay as a diagnostic and prognostic tool in the human fertility clinic. *Hum Reprod.* 1999;14:1039–1049.

Morris ID. Sperm DNA damage and cancer treatment. *Int J Androl.* 2002; 25:255–261.

Zheng N, Monckton DG, Wilson G, et al. Frequency of minisatellite repeat number changes at the MS205 locus in human sperm before and after cancer chemotherapy. *Environ Mol Mutagen.* 2000;36:134–145.

Zini A, Bielecki R, Phang D, et al. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. *Fertil Steril.* 2001;75:674–7.