Significant decrease in sperm deoxyribonucleic acid fragmentation after varicocelectomy

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Objective: To measure sperm DNA integrity values before and after varicocelectomy in patients with elevated preoperative levels of sperm DNA fragmentation.
Design: Retrospective.
Setting: Private urology clinic.
Patient(s): Eleven patients with grade 1, 2, or 3 varicocele.
Intervention(s): Varicocelectomy.
Main Outcome Measure(s): Sperm DNA fragmentation values were assessed before and after varicocelectomy.
Results(s): Ninety percent of the patients showed a significant decrease in sperm DNA fragmentation levels.
Conclusions(s): Although this study was small, 10 of the 11 patients with varicocele showed a significant decrease in sperm DNA fragmentation after varicocele repair. Elevated sperm DNA fragmentation has been shown to have a significant negative effect on pregnancy outcome with use of in vivo, IUI, routine IVF, and to a lesser extent intracytoplasmic sperm injection fertilization; therefore pregnancy outcome may improve after varicocelectomy. (Fertil Steril® 2008;90:1800–4. ©2008 by American Society for Reproductive Medicine.)

Key Words: Varicocele, SCSA, male infertility, sperm DNA fragmentation, DFI

Varicoceles are found in approximately 15% and 19% to 41% of the general and infertile populations, respectively, and have long been recognized as a common cause of infertility (1). The exact pathways of damage by varicocele are difficult to explain and may be due to apoptotic events, oxidative stress, or heat. Saleh et al. (2) found that sperm DNA fragmentation was significantly increased in patients with infertility with varicocele in comparison with patients with normal results on genital examination. Recently, Zini et al. (3) showed decreases in sperm DNA fragmentation after varicocele repair.

Sperm DNA damage is multifactorial and may be due to many environmental conditions such as chemotherapy, radiation, some prescription medications, air pollution, smoking, pesticides, chemicals, heat, assisted reproductive technology (ART) preparation protocols, and various pathologic conditions including cryptorchidism, cancer, fever, age, infection, leukocytospermia, and varicocele among others (4). Elevated levels of sperm DNA fragmentation have been significantly associated with a negative pregnancy outcome (5–10). If varicocele repair can decrease elevated sperm DNA fragmentation, pregnancy outcomes should generally improve. The purpose of this study was to evaluate sperm DNA integrity before and after varicocelectomy in patients with elevated preoperative levels of sperm DNA fragmentation.

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MATERIALS AND METHODS

Study Group

This study is a retrospective analysis of 11 consecutive men with clinical varicocele and high levels of sperm DNA fragmentation as measured by the Sperm Chromatin Structure Assay (SCSA). The patients were referred to a male fertility clinic for evaluation and treatment because they and their partners had experienced more than a year of infertility and had at least one abnormal parameter on their semen analysis. Each patient had a scrotal ultrasound to confirm the presence of either unilateral or bilateral varicocele and had a semen sample analyzed for level of DNA fragmentation with use of the SCSA. All patients had a DNA fragmentation index over 27% to 30% (fair to poor sperm DNA integrity) and had no other potential obvious reasons for high levels of sperm DNA fragmentation and infertility except for the presence of a varicocele(s). Eight patients had unilateral left varicoceles, one a recurrence after surgery performed elsewhere, and three patients had bilateral varicoceles. All patients underwent mini-incision microsurgical inguinal varicocele repair in the outpatient setting performed by a single surgeon (P.W.) as described by Goldstein et al. (11). All patients had reevaluation of their semen parameters and level of sperm DNA fragmentation 4 to 6 months after surgery.

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Sperm Chromatin Structure Assay

Unless otherwise mentioned, all reagents used in this study were obtained from Sigma Diagnostics, St. Louis, Missouri. The SCSA protocol has been previously described by Evenson et al. (12). Approximately 0.25 mL of raw semen was transferred to a 2 mL cryovial (Fisher Scientific, Hanover Park, IL) without cryoprotectant, flash frozen in a liquid nitrogen dry shipper, and then transported to SCSA Diagnostics, Inc., for SCSA testing. For each semen sample, 5,000 individual sperm cells were evaluated by flow cytometry. Flow cytometry data were used to create the DNA fragmentation index histogram profile of the entire sperm population, and computer gates were used to quantify the percentage of sperm with high levels of DNA fragmentation (percent DNA fragmentation index) based on the increased ratio of red (fragmented DNA) to green (native DNA) fluorescence in individual sperm. See Figure 1 for presurgery and postsurgery cytograms. All semen samples were measured twice by SCSA to ensure accuracy of the results. The two measures did not differ by 2% to 3%.

The SCSA statistical groups are as follows: $\leq$15% DNA fragmentation index = excellent sperm DNA integrity; >15% to <30% DNA fragmentation index = good sperm DNA integrity; $\geq$30% DNA fragmentation index = fair to poor sperm DNA integrity. It is important to note that a DNA fragmentation index value above 30% does not preclude a normal, term pregnancy. A >30% DNA fragmentation index, if consistent over time, does mean that the male partner is statistically placed into a group of men that demonstrate a longer time period to establish a natural pregnancy, more routine IVF cycles, increased risk of spontaneous miscarriages, or no pregnancy.

Statistical Analysis

Statistical analysis was performed with use of the paired *t*-test with SAS software (version 8; SAS Institute Inc., Cary, NC).

RESULTS

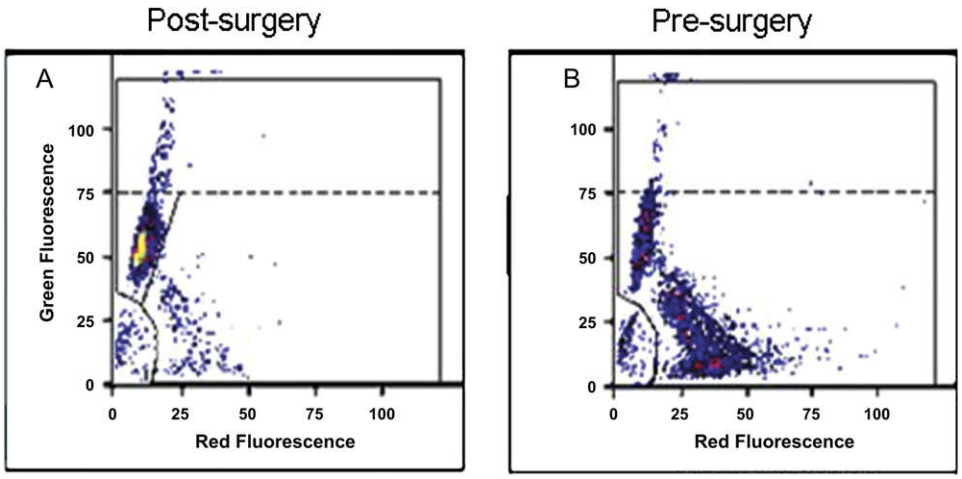
Ninety percent of patients showed a decrease in percent DNA fragmentation index 3 to 6 months after varicocelectomy ($P<.01$) (Fig. 2). The average percent change was 24% DNA fragmentation index including a 2% and −28% DNA fragmentation index change. Excluding these two outliers the average percent change was 33% DNA fragmentation index. Seven of the 11 patients showed decreases in sperm DNA fragmentation that brought their values below the SCSA statistical threshold of 30% for increased statistical potential of a pregnancy.

DISCUSSION

Although 90% of the patients showed an improvement in SCSA values, this study was too small to make any clear recommendations regarding the beneficial effects of varicocelectomy on elevated sperm DNA fragmentation. It is interesting to note that repair of a grade 3 varicocele resulted in the greatest percent change in sperm DNA fragmentation (58%); however, this patient had the highest DNA fragmentation index in the study. Although it appears optimistic that varicocele repair may decrease sperm DNA fragmentation, not every patient benefited from the minimally invasive procedure of varicocelectomy; one of the patients showed a percent increase of 28% DNA fragmentation index after varicocele repair. However, this patient did not have complete resolution of the varicocele, but the varicocele decreased in

FIGURE 1

SCSA cytograms after surgery (**A**) and before surgery (**B**). Each dot represents one of 5,000 sperm cells. Note in **B** the 60% of sperm characterized by high red fluorescence because of fragmented DNA.



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