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

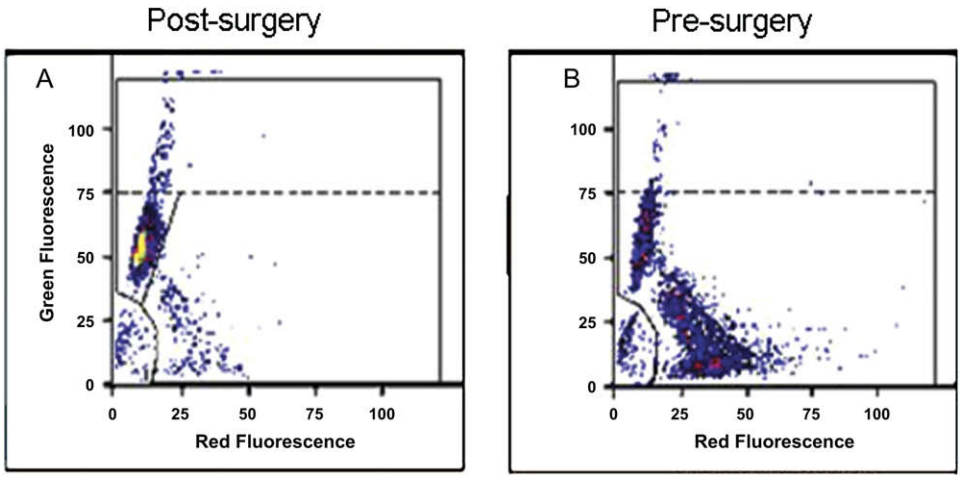
Ninety percent of patients showed a decrease in percent DNA fragmentation index 3 to 6 months after varicocelectomy ($P<.01$) (Fig. 2). The average percent change was 24% DNA fragmentation index including a 2% and −28% DNA fragmentation index change. Excluding these two outliers the average percent change 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

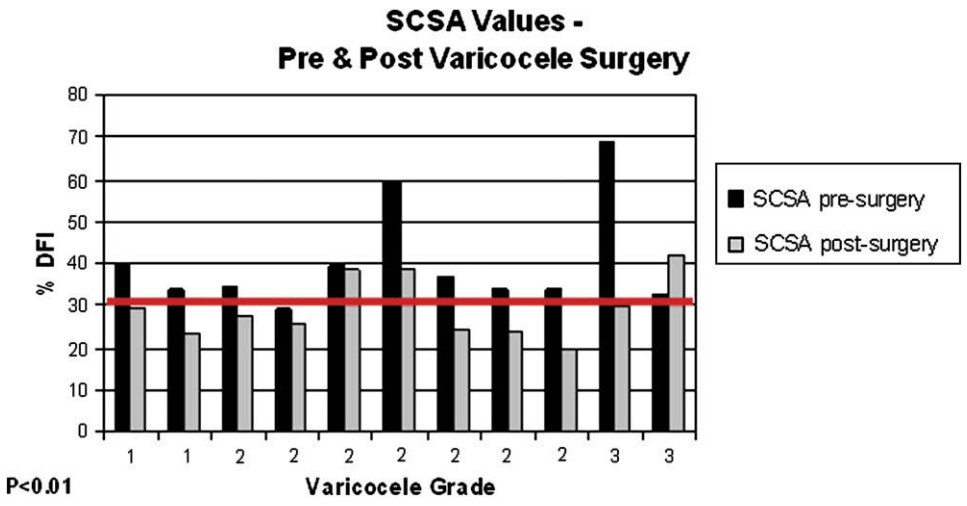
SCSA cytograms after surgery (**A**) and before surgery (**B**). Each dot represents one of 5,000 sperm cells. Note in **B** the 60% of sperm characterized by high red fluorescence because of fragmented DNA.



Werthman. Sperm DNA integrity after varicocelectomy. Fertil Steril 2008.

FIGURE 2

SCSA values before and after surgery. Ninety percent of patients showed a decrease in percent DNA fragmentation index (DFI) 3 to 6 months after varicocelectomy ($P<.01$).



Werthman. Sperm DNA integrity after varicocelectomy. Fertil Steril 2008.

size from grade 3 to grade 2. This persistent varicosity could be a reason that the DNA fragmentation level did not improve by 4 months after surgery. The DNA fragmentation index was not rechecked at a later date because the patient's wife conceived 3 months after the last DNA fragmentation index measurement, and it is possible that it improved but required a longer time period. Another patient had a preoperative SCSA value of 28.9% and a postoperative SCSA value of 25.5%, which kept this patient in the "good" statistical group of sperm DNA integrity.

Pregnancy rate (PR) has been shown to improve significantly after varicocele repair. Ashkenazi et al. (13) investigated PR in two groups of patients with infertility after varicocele repair. Group 1 consisted of 12 couples with mechanical female infertility and 10 with normal female fertility. Male partners from both groups had subfertile semen in the presence of a varicocele. After varicocele repair, group 1 and group 2 patients achieved a 20% and 40% PR, respectively. Yamamoto et al. (14) found a 31% increase in PR after varicocelectomy, whereas no pregnancy occurred before surgery.

Varicocelectomy also appears to be more cost-effective than immediate ART treatment. Schlegel (15) found the cost per delivery with intracytoplasmic sperm injection (ICSI) to be $89,091 (95% confidence interval [CI], $78,720 to $99,462), whereas the cost per delivery after varicocelectomy was $26,268 (95% CI, $19,138 to $44,646). Schlegel concluded that the treatment of a varicocele-associated male factor infertility with surgical varicocelectomy is more cost-effective than primary treatment with assisted reproduction.

The exact pathways of sperm DNA damage caused by varicocele are unclear but may be due to apoptosis or oxidative stress. Lin et al. (16) found significantly greater apoptosis in patients with varicocele than in controls ($P<.005$). Chen et al. (1) measured the apoptotic index in patients with varicocele and fertile controls. The apoptotic index was calculated by dividing the number of terminal deoxynucleotidyl transferase–mediated deoxyuridine-5′-triphosphate nick end labeling stained spermatozoa by the total number of Hoechst 33258–stained sperm cells for 300 sperm. A significantly higher apoptotic index was identified in the varicocele group than in the fertile controls ($P<.0001$). In our opinion, the percent increase in terminal deoxynucleotidyl transferase–mediated deoxyuridine-5′-triphosphate nick end labeling staining does not reflect only apoptotic cells but shows DNA fragmentation by other mechanisms.

In contrast, Henkel et al. (17) found that when sperm DNA fragmentation was elevated, there was no significant relationship to the early apoptotic markers, annexin V binding and Fas expression, indicating that sperm DNA damage may be due to reactive oxygen species (ROS) rather than apoptosis.

Spermatozoal membranes are susceptible to damage by oxidative stress through ROS because they are rich in polyunsaturated fatty acids (18). The biomarker 8-hydroxy-2′-deoxyguanosine is considered an excellent method of assessing the extent of ROS-induced DNA damage. Chen et al. (1) examined oxidative damage in sperm DNA of patients with varicocele. The results showed that 8-hydroxy-2′-deoxyguanosine levels in sperm DNA were significantly higher in the varicocele group compared with the subclinical varicocele and normal young males group. Hendin et al. (19) found

significantly increased levels of oxidative stress irrespective of fertility status in infertile and fertile patients with varicocele in comparison with a control group. Results from Saleh et al. (2) have shown that sperm DNA fragmentation was significantly increased in patients with infertility with varicoceles. Results from the same study indicated that infertile patients with varicocele had significantly higher levels of oxidative stress than the infertile patients without varicocele and controls.

Recently, Zini et al. (3) assessed sperm DNA damage before and 6 months after varicocelectomy. Decreases in sperm DNA fragmentation were found after varicocele repair ($P<.05$). Agarwal and Said (18) summarized the effects of ROS on DNA through chromatin cross-linking, chromosome deletion, DNA strand breaks, and base oxidation. Reactive oxygen species activity appears to be important for mediating apoptosis by inducing cytochrome c and caspases 9 and 3, which can result in increased single- and double-strand breaks (20). It would appear that elevated sperm DNA fragmentation, caused by ROS, and apoptotic events may all play a part in the pathogenesis of male infertility.

A large number of studies have reported on the relationship between the extent of sperm DNA fragmentation and pregnancy outcome. A recently published meta-analysis indicated couples were 7, 7.3, approximately 2.0, and 1.6 times more likely to achieve a pregnancy if their DNA fragmentation index was <30% for in vivo, IUI, routine IVF, and ICSI, respectively (21).

Although the statistical threshold for ICSI has yet to be determined, patients with an elevated DNA fragmentation index using ICSI fertilization have shown a trend toward miscarriage. In a recent study, fertile donor semen samples were compared with semen samples from patients with repeated pregnancy loss. The results showed that men of couples with repeated pregnancy loss had a fourfold greater amount of sperm DNA fragmentation than in donor sperm (10). In another study, slightly lower clinical PRs and higher miscarriage rates of the elevated sperm DNA fragmentation group resulted in an ongoing PR that was almost half of the group showing lower levels of sperm DNA fragmentation (6). In another study where ICSI was used as the ART procedure, patients were two times more likely to miscarry if their sperm DNA fragmentation was elevated (8). Zini et al. (3) found a trend toward an increased spontaneous pregnancy loss rate when the DNA fragmentation index was >30% ($P=.50$). An earlier study predicted 39% of the miscarriages due to elevated sperm DNA fragmentation (9).

Varicocelectomy appeared to be beneficial in this small study by lowering preoperative elevated levels of sperm DNA fragmentation in 90% of the study patients. In contrast to the findings of Zini et al. (3), where preoperative sperm DNA fragmentation levels were below the statistical threshold of 30%, this study specifically examined patients who had preoperative levels >30% DNA fragmentation index and exhibited a subsequent significant decrease of percent DNA fragmentation index values after surgery. Although pregnancy outcome data are the deciding factor for varicocele repair, a noninvasive approach such as the evaluation of sperm DNA fragmentation before varicocele repair can be another tool to help the patient and physician choose the best method for infertility treatment. Men with high levels of sperm DNA fragmentation and varicoceles may want to consider varicocele repair before proceeding with other fertility treatments to lower the degree of DNA damage and thereby potentially increase the chances of success.

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