A Novel Association Between Sperm Deformity Index and Oxidative Stress-Induced DNA Damage in Infertile Male Patients

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Objectives: Sperm deformity index (SDI) is a novel quantitative expression of sperm morphological quality that enhances both the predictive power and reproducibility of sperm morphology assessment. Morphologically abnormal spermatozoa are rich in cytoplasmic droplets, which contain NADPH oxidase capable of mediating oxidative stress (OS). The objective of our study was to investigate the impact of abnormal sperm morphology using SDI on NADPH-mediated reactive oxygen species (ROS) production and its correlation with sperm DNA damage.

Design: Prospective controlled study.

Materials and Methods: Semen samples were collected from 6 healthy donors with normal standard semen parameters according to WHO guidelines and 7 infertile male patients. Mature sperm were isolated using double density gradient (ISolate, 47% and 90%), re-suspended in BWW media and subdivided into 2 aliquots; the first was incubated with 5 mM NADPH up to 24 hours, while the second subset was incubated without NADPH to serve as control. Sperm morphology was assessed by strict Tygerberg’s criteria. The SDI was calculated by dividing the total number of deformities observed by the number of sperm evaluated. Sperm DNA damage was assessed using terminal deoxynucleotidyl transferase-mediated fluorescein-dUTP nick end labeling (TUNEL) assay by flow cytometry and ROS levels (x 10^6 counted photons per minute) by chemiluminescence assay using lucigenin as a probe.

Results: The median and interquartile values (25^th and 75^th percentiles) of SDI were higher in patients compared to donors both in whole ejaculate [1.89 (1.63, 2.17) vs. 1.62 (1.53, 1.8), P = 0.03] as well as in mature spermatozoa [2 (1.8, 2.1) vs. 1.53 (1.52, 1.58), P = 0.008]. Following NADPH incubation, a significantly higher DNA damage was seen after 24 hours in patients compared with donors [16.56 (11.29, 40) vs. 4.4 (3.92, 5.25), P = 0.007]. Similarly, in controls, DNA damage was higher in patients compared with donors [5.1 (3.87, 7.74) vs. 1.79 (2.87, 3.36), P = 0.03]. However, combining aliquots treated with NADPH (patients and donors) also showed higher incidence of DNA
damage than those not treated [10 (4.69, 24.85) vs. 3.85 (2.58, 5.1), \( P = 0.008 \)]. The increase in ROS levels was also more pronounced in aliquots exposed to NADPH [1.22 (0.3, 1.87) vs. 0.39 (0.1, 0.57), \( P = 0.03 \)]. SDI correlated with the percentage increase in sperm DNA damage following incubation for 24 hours (\( r = 0.5, P = 0.009 \)).

**Conclusions:** SDI is a useful tool in identifying infertile male patients with abnormal prevalence of OS-induced DNA damage. NAPDH plays a role in ROS-mediated sperm DNA damage, which appears to be more evident in infertile patients with semen samples containing high incidence of morphologically abnormal spermatozoa.

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