OXIDATIVE STRESS AND INCREASED LEVELS OF APOPTOSIS (CYTOCHROME C, CASPASE 3 AND 9) IN PATIENTS WITH MALE-FACTOR INFERTILITY

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Objective:
Increased production of reactive oxygen species (ROS) results in abnormal seminal quality, DNA damage and the inability of the spermatozoa to fertilize. Mitochondria produces ROS during oxidative phosphorylation, and mitochondrial cytochrome C, a trigger of apoptosis activates caspase 9 and 3 resulting in mitochondrial and subsequently nuclear DNA damage. The objective of the study was to determine the pathway of apoptosis leading to mitochondrial DNA damage. We evaluated, 1) the apoptosis factors in the semen of patients with male-factor infertility, and 2) the association between semen quality and apoptosis in the presence of oxidative stress.

Design:
Prospective study at a male infertility clinic.

Materials/Methods:
Semen specimens were obtained from 35 infertile patients and 8 normal healthy donors. Following semen analysis, samples with total sperm counts of 5 to 10 X 10^6 sperm were processed for extraction of total protein. 30ug total protein was separated by SDS-polyacrylamide gel electrophoresis. Cytochrome C, caspase 9 and caspase 3 level were quantified by western blot using corresponding antibodies and detected on X-ray film. The amount of the expressed protein was quantified using the IQmac v1.2. For each protein, the positive control was taken as 1.00. ROS level was measured in washed sperm suspensions by chemiluminescence assay.

Results:
Infertile patients had significantly poor semen parameters compared to donors (concentration, p<0.001, motility p<0.03, curvilinear velocity p<0.03, linearity p< 0.001, WHO sperm morphology p<0.003, and Kruger’s strict criteria p<0.01) compared to donors. Significantly higher levels of ROS (X 10^6 counted photons per minute (cpm) were seen in patients [median and 25% and 75% interquartile range, 4.15 (0.26, 40.16)] compared to donors [0.06 (0.02, 0.29, p<0.01]. Increased levels of cytochrome C were seen in patients compared to donors [2.78 (2.21, 43.65) vs. 1.5 (1.25, 2.2) p<0.01]), caspase 9 [2.52 (0.9, 4.28) vs. 0.56 (0.32, 1.02), p<0.006], and caspase 3 [6 (4.85, 7.63) vs. 1.69 (1.66, 2.67), p<0.01]. Semen parameters (motility, concentration, and morphology) were negatively correlated with caspase 9 and 3 (p<0.05). ROS was positively related with cytochrome C (r = 0.43, p<0.03), caspase 9 (r = 0.56, p<0.001), and caspase 3 (0.65, p<0.01).

Conclusions:
Our study demonstrates the positive relationship between increased sperm mitochondrial DNA damage as evident by higher oxidative stress and apoptosis in patients with male-factor infertility. Antioxidants aimed at reducing ROS production (during spermiogenesis, sperm storage/ transit in the genital tract, or infection) may play a role in decreasing apoptosis and in turn improving the sperm quality.

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