Title: Defining the reference value of seminal reactive oxygen species in a population of infertile men and normal healthy volunteers
Objective: Reactive oxygen species (ROS) is recognized as an important cause of sperm dysfunction which leads to infertility in men. ROS levels in semen specimen can be tested by simple chemiluminescence assay; however, the diagnostics application of this test remains low amongst infertility specialists. This is mainly attributed to a lack of consensus on the reference value of this test. We sought to develop the normal and abnormal values of ROS in the semen samples from normal healthy volunteers and in a group of infertile men.

Design: Prospective study

Materials and Methods: The study included a randomly selected group of men (n = 33) with history of infertility. These men were evaluated by a male infertility specialist. Normal healthy control group consisted of 12 samples. Semen samples were analyzed for ROS in neat semen by chemiluminescence method using luminol as the probe. Each sample was tested for routine semen analysis and white blood cell count by peroxidase staining.

Results: Leukocytospermia was present in 0% donor specimens; while 18% (6/33) samples tested positive in patient group. ROS levels were significantly higher in infertile patients compared to controls (0.065 [0.02; 0.31] vs. (0.008 [0.0012; 0.06.] P <0.0014). Semen ROS levels in healthy non-infertile men had a reference value (median and 25%, 75% IQR) of 0.008 X 10^6 cpmp. The infertile patients were divided into 2 groups based on the reference ROS value from normal controls: ROS negative (<0.02 cpmp/10^6 sperm/mL) (n=21) and ROS positive (>0.02 cpmp/10^6 sperm/mL) (n=12). ROS levels showed no significant correlation with sperm parameters (motility, concentration and morphology) in either ROS negative or ROS positive group.

Conclusions: We have defined the normal and abnormal values of reactive oxygen species in human semen. These values may be used to identify men with seminal oxidative stress. Patients with abnormal ROS levels should be treated for any underlying pathology (varicocele or urogenital infection). These patients may benefit by an antioxidant supplement.

Support: None