The concluding evidence suggests that ROS levels have a beneficial effect on sperm function and male fertility if they are within the normal physiological range and affect negatively in uncontrolled production. Therefore, accurate measurement and defined cutoff values of ROS levels in seminal ejaculates in a larger cohort of infertile men and controls with proven and negatives fertility are important. The measurement of seminal ROS may consequently, induce deleterious effects on spermatozoa function and, may consequently, alter normal physiological spermatozoa functions.

Introduction

Free oxygen radicals such as superoxide anion (O2¯), hydrogen peroxide (H2O2), and hydroxyl radical (OH•) are reactive oxygen species (ROS). The production of these radicals occurs during normal metabolism of the cell. In semen, ROS are produced by spermatozoa and play a vital role in many physiological processes of spermatogenesis, such as acrosome reaction, capacitation, mitochondrial sheath stability and fission with acrosome. Seminal plasma contains several antioxidant and has a strong antioxidant capacity. For thecontinuity of normal physiological spermatogenesis functions, a balance exists between seminal ROS and antioxidant capacity of ROS which extends the seminal plasma antioxidant capacity results in oxidative stress (OS), thereby inducing deleterious effects on spermatozoa function and, may consequently, lead to male infertility.

The harmful effects of higher levels of ROS have been documented in several sperm parameters including sperm concentration, motility, morphology, viability, and DNA fragmentation. Elevated ROS levels have also been reported in some clinical conditions (e.g., varicocele and reproductive tract infections) as well as in association with lifestyle (e.g., smoking). Similarly, low fertility and pregnancy rates were achieved in couples in which the male partner had elevated clinical conditions (e.g., varicocele and reproductive tract infections) as well as in association with lifestyle (e.g., smoking).

RESULTS

Material and Methods

Selection of Subjects

The study was approved by the Institutional Review Board. A total of 258 infertile men and 92 controls (with or without proven fertility) were enrolled in this study. All infertile men were attending the male infertility clinic. The clinics were selected based on their normal semen analysis according to the WHO guidelines, (2010).

Semen Collection and Processing

Semen samples from patients and controls were collected by masturbation after 2-3 days of abstinence period. The initial semen analysis was performed according to WHO (2010) guidelines. Measurement in whole ejaculates was carried out by using luminol-based chemiluminescence method. Samples were run in duplicate along with three tubes of blanks, negative and positive controls each. Chemiluminescence was measured for 15 minutes with a Berthold luminometer. Results were expressed as relative light units (RLU)/sec/10⁶ sperm. Test specificity, sensitivity and cutoff values were calculated by receiver operating characteristic curve (ROC).

Results

Significantly higher ROS levels were seen in infertile men compared to controls (p<0.001). The optimal cutoff value to distinguish between controls and infertility men was 102.2 (RLU/sec/10⁶ sperm). At this cutoff value the positive and negative predictive values of the test were 82.1% and 44.5% respectively.

Conclusions

The tubes were scored to ensure that the luminol was mixed with the rest of the reagents. All the tubes were placed in the luminometer in the following order: blank (tubes labeled 1-3), negative control (tubes labeled 4-6), test sample (tubes labeled 7-9) and positive control (tubes labeled 0-1). After setting at the tubes at their designated place the sample was analyzed for 15 minutes (Figure 3).

Calculating Results

The average RLU for per negative control, samples and positive control was calculated. Sample ROS was calculated by subtracting the negative control average from its average. Sample ROS = average RLU mean for sample – average RLU mean for negative control. The sample ROS was calculated by dividing it with it's average ROS (sample ROS: corrected sample ROS). Corrected sample ROS: Sample ROS concentration = XX (RLU/sec/10⁶ sperm).

Statistical Analysis

The Wilcoxon rank-sum test was used for group comparison with respect to quantitative variables and Chi square test with respect to categorical variables. The difference in the distribution of ROS levels between the two groups (control and patient) was assessed. A receiver operating characteristic (ROC) curve was used to assess the ability of ROS as a means of discriminating patients and controls. A cutoff value that maximized the sum of estimated sensitivity and specificity was shown. A p-value of <0.05 was considered significant.

Table 1. Percentage population of patients and donors above and below the new cutoff ROS Cutoff (RLU/sec/10⁶ sperm) Overall (n=350) Controls (n=92) Patients (n=258) p value y axis Sensitivity (%) Specificity (%)

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<td>&lt;0.001</td>
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Figure 1A-C: Representative curve showing ROS levels in infertile men and controls.

Figure 2: Preparing the tubes for ROS measurement.

Figure 3: Preparing the tubes for ROS measurement.

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