ASSESSMENT OF DIFFERENTIAL CONTRIBUTION OF SPERMATOZOA AND LEUKOCYTES TO REACTIVE OXYGEN SPECIES PRODUCTION IN SEMEN USING NITROBLUE TETRAZOLIUM (NBT) REDUCTION TEST
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Objective: We examined the differential contribution of spermatozoa and neutrophils to ROS generating activity in semen using NBT test and determined the correlation between NBT results with levels of oxidative stress (OS) measured by chemiluminescence assay.

Design: A prospective study in a male infertility clinic

Materials/Methods: Semen samples were obtained from 21 infertility patients and 9 normal donors (group I). Seminal leukocytes were quantified by a myeloperoxidase test. Based on leukocyte concentrations in semen, patient samples were classified into two groups; leukocytospermic (group II, n = 8) and non-leukocytospermic (group III, n = 13). Levels of ROS were measured by chemiluminescence assay in neat (unprocessed) semen as well as in cell suspension following density gradient separation (47%/90%). Leukocytes were seen in fraction 1 (F1: seminal plasma/47% interface) and immature sperm separated in fraction 2 (F2: 47%/90% interface). Levels of ROS were expressed as X10^6 counted photons per minute/20 million sperm/mL. Total antioxidant capacity (TAC) was measured in seminal plasma by an enhanced chemiluminescence assay and results expressed as Trolox equivalent. A composite value of ROS-TAC score was calculated as an index for OS. NBT staining was performed by incubation of neat, F1 and F2 with an equal volume of 0.1% of NBT solution at 37°C for 30 minutes. A total of 100 immature sperm and 100 neutrophils were counted and scored. Neutrophils were scored as follows: cells filled with formazan (+++), intermediate density (++), scattered or few formazan granules (+), and no formazan detectable (-). Immature sperm were scored as follows: formazan occupied >50 % of cytoplasm (+) and >50% of cytoplasm (++).

Results: The percentage of NBT staining in leukocyte fraction (F1) in leukocytospermic group were significantly higher than donors (70% vs. 7%) and non-leukocytospermic group (14.5%; (P = 0.02 and 0.03, respectively). Levels of ROS in neat semen from leukocytospermic group was significantly higher than non-leukocytospermic group and donors (P = 0.01 and 0.04, respectively). The ROS-TAC score was significantly higher in donors (53.2 (47.5, 55.0)) than in the leukocytospermic patients (35 (30.5, 50.2)) (P = 0.04). A strong positive correlation was seen between ROS levels and positive NBT staining in leukocyte fraction (r = 0.7; P < 0.0001) and in fraction containing sperm with cytoplasmic retention (r = 0.72; P <0.0001). Also a significant correlation were found between NBT results and ROS-TAC score in leukocytes both in neat (r = -0.60, P = 0.0007) and F1 (r = -0.39, P = 0.04) and sperm with cytoplasmic retention in F2 (r = -0.38, P = 0.049).

Conclusions: Our results indicate that NBT reduction test can assess the differential contribution of seminal leukocytes and defective spermatozoa to ROS generation in semen. In addition, results of NBT staining were significantly correlated with levels of ROS and with ROS-TAC score. Therefore, the NBT test can be used as a routine procedure in clinical andrology laboratory without the need for expensive equipment.

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