Creatine Kinase Activity and Malondialdehyde in the Seminal Plasma of Normospermic Infertile Males

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Summary:

**Background:** Normospermia might be a major problem to the doctor and the infertile couple because the male seminal sample has an accepted seminal parameters during the routine seminal examination and the female partner will be claimed for the infertility and she will suffered from costly, painful, time consuming, non indicated investigations and treatments. Our purpose was to measure sperm creatine phosphokinase (CK) activity, which reflects cytoplasmic retention in immature spermatozoa and malondialdehyde in the seminal plasma which is a marker of oxidative stress in normospermic infertile males' seminal samples.

**Patient and methods:** Nine infertile men with aberrantly normal standard seminal analysis parameters where included in this study and fifteen fertile men samples where used as control. The seminal kinase and seminal malondialdehyde were calculated in addition to the standard seminal analysis.

**Results:** Significant higher levels of creatine kinase and malondialdehyde in the normospermic infertile samples (p=0.0001; p=0.006 respectively) and also significant positive correlation between the seminal creatine kinase and seminal malondialdehyde (p=0.001; r=0.613). These markers did not correlate with the percentage of mid piece abnormalities in the studied samples.

**Conclusion:** The seminal plasma creatine kinase and seminal malondialdehyde might be accepted methods to differentiate infertile samples from healthy despite the presence of accepted ranges of standard seminal analysis.

**Keywords:** Normospermia, Oxidative stress, Creatine Kinase.

Introduction:

Traditionally, the diagnosis of male infertility has relied on microscopic assessment and biochemical assays to determine human semen quality. The conventional parameters given most importance have been the concentration, motility, and morphology of sperm in the ejaculate. Some laboratories have added additional tests, including estimations of vitality, anti-sperm antibodies, contaminant cells, and total motile counts before and after sperm preparation for assisted conception. Normospermia means that the results of the standard seminal analysis are accepted according to the WHO guide regarding motility, concentration, morphology, viscosity etc (1). Spermatogenesis, a complex process of male germ cell development, encompasses spermatogonial proliferation, meiosis and spermiogenesis. The spermiogenetic events that eliminate the surplus cytoplasm, such as development of the acrosome, tail growth, along with cytoplasmic extrusion, result in mature sperm (2). Developmental defects may occur in both the cytoplasmic or nuclear compartments, which can result in the production of immature sperm. Studies on the enzymatic status of spermatozoa are specialized and are limited to few enzymes. The enzymatic profile of spermatozoa should constitute a good indication of functional metabolic activity. The enzymes present in seminal fluid are shown to be derived from secretions of seminiferous tubules, spermatozoa, epididymis, seminal vesicles and prostate gland. Thus, the estimation of different enzymes in semen permits one to obtain markers of seminal quality (3). Creatine kinase (creatin phosphokinase, CK) in human sperm is a marker of cytoplasmic retention and, thus, diminished sperm maturity (4). Immature sperm with cytoplasmic retention were not able to bind to the zona pellucida (5). Creatine kinase catalyses the reversible phosphorylation of ADP to ATP or creatine to creatine phosphate, thus maintaining an immediately accessible energy reservoir in the cell (6). Cells requiring high energy such as spermatozoa are characterized by high creatine kinase activity. In the etiology of male infertility, there is growing evidence that damage to spermatozoa by reactive oxygen species (ROS) play a key role (7). Spermatozoa contain large quantities of polyunsaturated fatty acids (PUFA). Therefore, they are susceptible to ROS-induced damage. It has been suggested that ROS induce membrane lipid peroxidation in sperm (8). The seminal plasma is...
well endowed with an array of antioxidants that act as free radical scavengers to protect spermatozoa against oxidative stress. Seminal plasma contains a number of enzymatic antioxidants such as superoxide dismutase (SOD) and catalase. In addition, it contains a variety of non-enzymatic antioxidants (9). Oxidative stress from excess ROS, such as hydrogen peroxide, superoxide anions, and hydroxyl radicals present either from increased production or reduced antioxidant protection are thought to be a major cause of sperm dysfunction (10). One of the primary mechanisms by which this occurs is via the stimulation of a lipid peroxidation cascade in the plasma membrane (11). Sperm are particularly susceptible to ROS-mediated injury due to their high level of unsaturated fatty acids and inability to repair damage. Numerous studies have reported associations between oxidative stress, structural and function damage (12). Available data on the impact of oxidative stress on sperm are based on the measurement of seminal plasma and sperm levels of malondialdehyde (MDA) by the thiobarbituric acid-reacting substance (TBARS) assay (13). The strong prognostic value of levels of seminal oxidative stress on assisted reproductive techniques (ART) outcomes has been reported by many studies (14). The aim of the present study was to assess seminal plasma levels of Malondialdehyde and Creatine kinase in normozoospermic infertile males.

Patients and methods:
Nine patients (age range of 31.56±6.126 years) were included in this study referred from the department of male infertility and seminal fluid analysis lab in Kamal Al-Samera'y hospital for infertility and assisted reproductive techniques in the period from 1/5/2007 to 20/8/2007. All the patients were with a history of at least 1 year duration with regular unprotected intercourse. After a good history including duration of the infertility, developmental history of the patient, past medical history, past surgical history such as pelvic or retroperitoneal surgery, history of previous pregnancy or abortion of the female partner and full gynecological history and examination for the female partner, including imaging and hormonal assay by specialists to exclude the obvious causes of female factors of infertility, freshly ejaculated semen samples were obtained in a near privet room by masturbation into a wide mouthed sterile specimen jar after at least 3 days of sexual abstinence. The standard seminal analysis was done after an accepted liquefaction time according to the WHO manual and ranges (1). For controls the seminal samples 15 healthy fertile men were used (i.e., father of a child within the last 12 months and with no history of infertility or any abnormalities that might affect the fertility) with mean age (29.84±6.434). The morphological characters were studied by slides stained with Giemsa staining. Morphological assessment was performed at (100x) oil immersion bright field objective and a differential morphological count on at least 100 spermatozoa was performed on each slide. Results were expressed as the percentage of normal spermatozoa, head defects, neck and midpiece defects, and tail defects. Samples with more than 1x10⁸ leukocytes were not included in this study. The seminal MDA levels were analyzed using thiobarbituric acid (TBA) method according to methods described by Rao and coworkers 1989 (15). The seminal creatine kinase was assisted in the seminal samples using creatine kinase testing kit.

Results:
Figure (1) shows the levels of seminal creatine kinase in the studied samples. The level in infertile samples was 0.236±0.031 while in healthy 0.088±0.038 U/10⁸ spermatozoa. A significant higher CK activity was observed ($P=0.0001$).

![Creatine kinase](image1)

Figure 1: The levels of seminal creatine kinase (U/10⁸ spermatozoa) in the studied samples ($P=0.0001$).

In figure (2) the percentage of sperms with mid piece defects in the studied samples. The percentage in infertile samples was 26.28±6.50 while in healthy 21.94±7.21. A non significant correlation ($P=0.072$). Up to 50% was the normal accepted range according to the WHO.
Figure 2: The percentage of sperms with mid-piece defects in the studied samples ($P=0.072$).

While figure (3) show the levels of seminal malondialdehyde in the studied samples. The level in infertile samples was $2.023 \pm 0.39$ while in healthy $1.54 \pm 0.118$ nmol/10$^6$ spermatozoa. A significant higher CK activity was observed ($P=0.006$).

Figure 3: The levels of seminal malondialdehyde (MDA) (nmol/10$^6$) in the studied samples ($P=0.006$).

Also in figure (4) we could observe correlation between seminal creatine kinase and seminal MDA. A significant positive correlation ($P=0.001$; $r=0.613$).

Discussion:

Male infertile patient’s semen analysis still provides the fundamental information on which clinicians base their initial diagnosis, so it is imperative that it is performed as accurately as possible. In the two decades since the WHO manuals have been our core reference points. The poor power of semen analysis in predicting future fertility was first highlighted in the mid-1980s (16). In the present days, it has become apparent that a basic semen analysis is insufficient for the determination of the fertility status of individual men (17). The most relevant findings of this study were (i) a significant elevation of seminal plasma MDA levels in normozoospermic infertile samples compared with fertile men (ii) a significant elevation of seminal plasma creatine kinase levels in normozoospermic infertile samples compared with fertile men. The results of seminal MDA levels were in common with the findings of Pasqualotto and coworkers 2001 (18) and Agarwal and coworkers 2006 (19) who suggested that oxidative stress is associated with male factor infertility and the presence of oxidative stress was irrespective of whether these patients have normal or abnormal semen parameters. Oxidative damage is common for spermatozoa during epididymal maturation and storage. Human spermatozoa are highly susceptible to oxidative injury but are naturally protected from such injury by the antioxidant properties of seminal plasma. ROS plays a central for sperm physiology such as sperm maturation and capacitation. Abnormal ROS production is associated with defective sperm.
function (20). A fine balance between ROS production and recycling is essential for spermatogenesis. Excessive generation of seminal ROS, mainly by neutrophils but also by immobile sperm, morphologically abnormal sperm, or morphologically normal but functionally abnormal sperm, could be a cause for male infertility (21). The incidence of spontaneous pregnancy was negatively correlates with ROS production (22). Agarwal and coworkers 2006 (19) showed a harmony with the results of this study and they even suggested that high ROS is an independent marker of male factors of infertility. They also suggest the inclusion of ROS measurement as part of idiopathic infertility of oxidative stress in infertile normospermic men might explain previously unexplained cases of infertility otherwise attributed to female factors. In contrast, Verit and coworkers 2006 (24) did not find any relationship between oxidative stress and infertility in normozoospermic infertile men. They suggested that the pathophysiology of idiopathic infertility cannot be explained by seminal oxidative stress. The present study showed that mean of creatine kinase was significantly higher in infertile samples. These results are in agreement with results of previous studies studying infertility (25) (26). Huszar and coworkers 1998 (27) studied the CK level in the seminal plasma as a marker of sperm immaturity and correlate it with the plasma MDA levels. These observations were in harmony with results of the present study. They found also that 12% of normospermic samples showed similar levels of both markers as oligozoospermia samples. The CK activity measurements in the direction of ATP synthesis is based on a three step reaction. In the first step, CK catalyses synthesis of ATP from creatine phosphate and ADP. In the second step the ATP is utilized for glucose-6-phosphate synthesis in the presence of hexokinase. In the third step the glucose-6-phosphate is oxidised to 6-phosphogluconate with reduction of NADP TO NADPH which is measured with an optical density change at 340 nm. Here, the glucose 6-phosphate dehydrogenase (G6PDH) which is an oxidoreductase, catalyses the oxidation of glucose-6-phosphate to 6-phosphogluconate. This step is an important step of the hexosemonophosphate shunt, as it is through this shunt that dihydro nicotinamide adenine dinucleotide phosphate (NADPH) is generated by spermatozoa. This NADPH is the major source of electrons responsible for production of free radicals (O2) by human spermatozoa (28). It is an important factor for the disruption of spermiogenesis leading to retention of excess of residual cytoplasm by differentiating spermatozoa. Evidence for the hypothesis has come from a number of independent studies indicating that sperm function is frequently associated with elevated activities of certain key enzymes including creatine kinase (29). These enzymes are not thought to be evaluation. Treatment with antioxidants may be beneficial in such patients. The main sources of oxidative stress in the seminal plasma are leukocytes and abnormal morphological sperm cells (23). And since leukocytospermia were not included in this study, and a non significant difference was found between the percentages of sperms with abnormal mid piece between normospermic infertile and fertile samples, this might raise the suggestion that the structurally normal but functionally abnormal sperms might be a source of oxidative stress in the seminal plasma. This is in harmony with a study by Pasqualotto and coworkers 2001 (18) who concluded that the presence directly responsible for loss of sperm-function but rather to act as biochemical markers of normality of sperm differentiation. Such errors of spermiogenesis can result in creation of oxidative stress. This could be proved by a positive association between the CK content of human spermatozoa and induction of peroxidative damage (30). In the present study, both groups had accepted ranges of percentage of sperms with abnormal mid piece according to WHO (1). Surprisingly, there was no significant different in both group studied despite the high MDA levels and CK activities which associated with cytoplasmic retention in the sperms (P>0.05). The creatine kinase activity was measured in different patients samples in a comparative study done by Clause and coworkers 1998 (31), the seminal CK in seminal plasma was measured in normal, oligozoospermia and azoospermia samples and was found to be highest in azoospermia samples. This mean that the source of CK still present despite the non presence of sperms in the seminal plasma and at a very high activity. They concluded that Creatine Kinase activity in human spermatozoa and seminal plasma lacks predictive value for mal- fertility. The seminal plasma MDA level and the CK activity may be reliable methods to differentiate the infertile samples from healthy despite the accepted ranges of standard seminal analysis but further investigation is indicated with larger number of patients and more complex tests like sperm apoptosis and DNA damage of the sperms to get a further understanding of the subject of idiopathic male infertility.

References:
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