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

Ghassan T. Alani * **MBChB, MSc, PhD**
Hedef D. El Yaseen** **BSc, PhD**

Fac Med Baghdad 2009; Vol. 51, No.3
Received Nov. 2008
Accepted Jan. 2009

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 coasty, 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. Theenzymes 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 (creatine 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

** Department of Physiology, College of Medicine, University of Baghdad.*
*** Department of Phisyological chemistry, College of Medicine, University of Baghdad.*

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 $1x10^6$ 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 0.2363±0.031 while in healthy 0.088±0.038 $U/10^8$ spermatozoa. A significant higher CK activity was observed (*P*=0.0001).

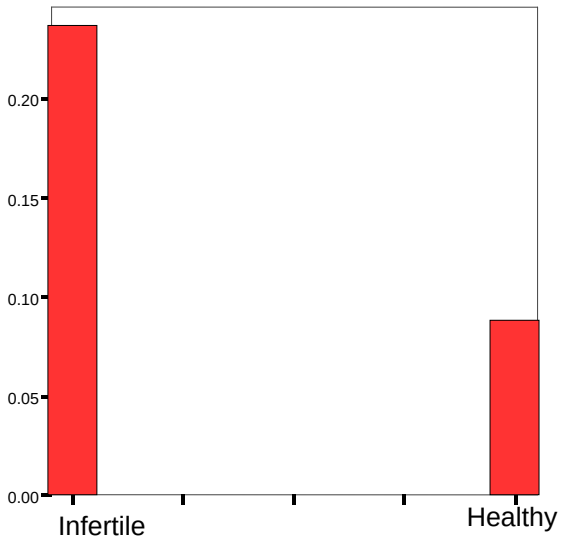

Figure 1: The levels of seminal creatine kinase ($U/10^8$ spermatozoa) in the studied samples (*P*=0.0001).

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.

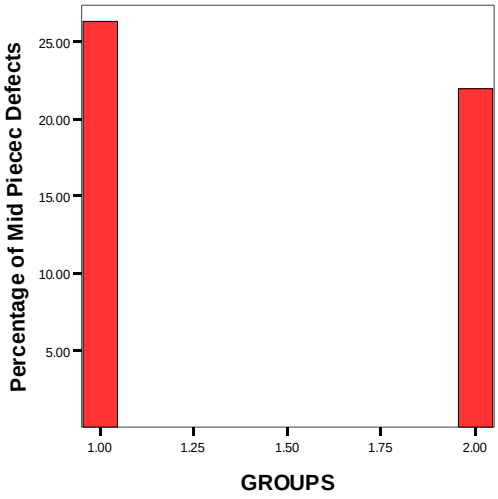

Figure 2: The percentage of sperms with mid piece defects in the studied samples (*P*=0.072).

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

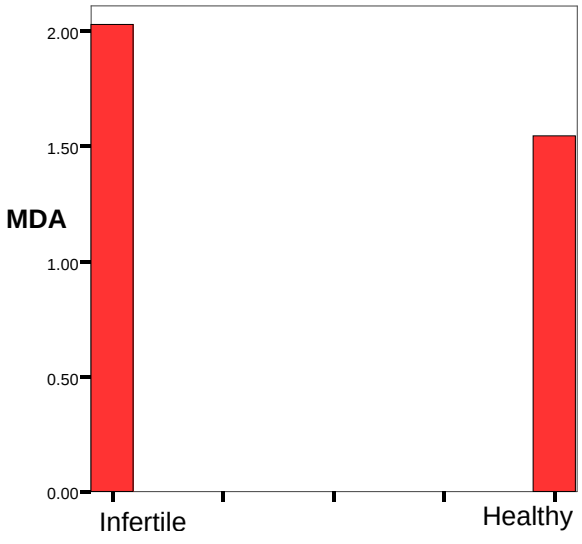

Figure 3: The levels of seminal malondialdehyde (MDA) (nmol/10^6) in the studied samples (*P*=0.006).

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).

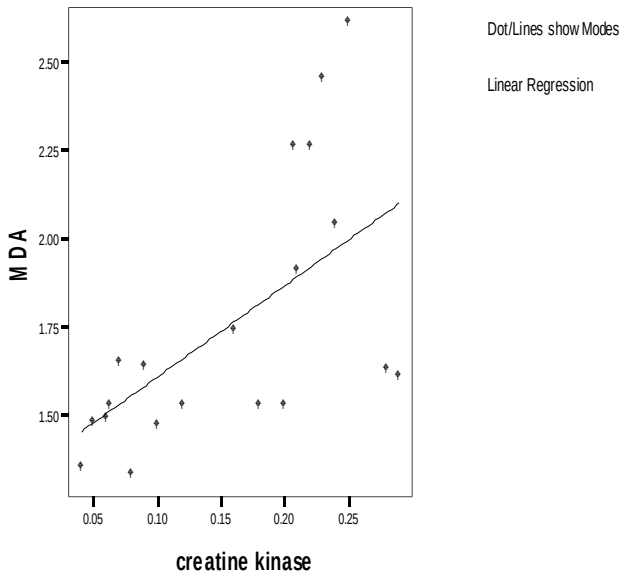

Figure 4: Correlation between seminal creatine kinase and seminal MDA (*P*=0.001; r=0.613).

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 oxido-reductase, calatyses 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:

1. World Health Organization (1999): WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction, 4th edition, Cambridge University Press, Cambridge.

2. Huszar, G., Stone, K., Dix, D., and Vigue, L., (2000): Putative creatine kinase M-isoform in human sperm is identified as the 70-kilodalton heat shock protein HspA2. Biol Reprod 63,925–932.

3. Guerin, J.F., Ali, H.B., Rollet, J., and Souchier, C., (1986): Glucosidase as a specific epididymal marker. Its validity for the etiologic diagnosis of azoospermia. J Androl, 7:165-172.

4. Huszar, G., and Vigue, L., (1990): Spermatogenesis-related change in the synthesis of the creatine kinase B-type and M-type isoforms in human spermatozoa. Mol Reprod Dev 25,258–262.

5. Huszar, G., Vigue, L., and Oehninger, S., (1994): Creatine kinase immunocytochemistry of human sperm-hemizona complexes: selective binding of sperm with mature creatine kinase-staining pattern. Fertil Steril 61,136–142.

6. Walliman, A.T., and Hemmer, W., (1994): Creatine kinase in non muscle tissues and cells. Mol cell Biochem, 133/134:193-220

7. Agarwal, A., Saleh, R.A., and Bedaiwy, M.A.,(2003): Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril, 79:829-843.

8. Sanocka, D., and Kurpisz, M., (2004): Reactive oxygen species and sperm cells. Reprod Biol Endocrinol, 2:12.

9. Agarwal, A., Gupta, S., and Sikka, S., (2006): The role of free radicals and antioxidants in reproduction. Curr Opin Obstet Gynecol, 18:325-332.

10. Saleh, A., and Agarwal, A., (2002): Oxidative stress and male infertility: from research bench to clinical practice. J Androl, 23: 737– 52.

11. Aitken, R.J., (1995): Free Radicals, Lipid Peroxidation, and Sperm Function, Reprod. Fertil. Dev. 7, 659–668.

12. Aitken, R.J., and Fisher, H. (1994): Reactive Oxygen Species Generation and Human Spermatozoa: The Balance of Benefit and Risk, Bioassays 16, 259–267.

13. Keskes-Ammar, L., Feki-Chakroun, N., Rebai, T., Sahnoun, Z., Ghozzi, H., Hammami, S., Zghal, K., Fki, H., Damak, J., and Bahloul, A., (2003): Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Arch Androl, 49:83-94.

14. Kao, S.H., Chao, H.T., Chen, H.W., Hwang, T.I., Liao, T.L., and Wei, Y.H., (2007): Increase of oxidative stress in human sperm with lower motility. Fertil Steril 2007.

15. Rao, B., Souflir, J.C., Martin, M., and David, G., (1989): Lipid peroxidation in human spermatozoa as related to midpiece abnormalities and motility. Gamete Res., 24:127-34.

16. Polansky, F.F., and Lamb, E.J., (1988): Do the results of semen analysis predict future fertility – a Survival Analysis Study. Fertility and Sterility 49 1059–106.

17. Lewis, S.E. (2007): Is sperm evaluation useful in predicting human fertility? Reproduction, 134 31-40.

18. Pasqualotto, F.F., Sharma, R. K., Kobayashi, H., Nelson, D. R., and Agarwal, A., (2001): Oxidative stress in normospermic men undergoing infertility evaluation. Journal of Andrology, Vol 22, Issue 2, 316-322.

19. Agarwal, A., Sharma, R.K, Nallella, K.P., Thomas A.J., Alvarez, J.G., and Sikka S.C., (2006): Reactive oxygen species as an independent marker of male factor infertility. Fertil Steril., Oct;86 (4):878-85.

20. Mazzilli, F., Rossi, T., Marchesini, M., Ronconi, C., and Dondero, F., (1994): Superoxide anion in human semen related to seminal parameters and clinical aspects. Fertil Steril., 62:862–8.

21. Plante, M., de Lamirande, E., and Gagnon, C., (1994): Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect normal sperm motility. Fertil Steril., 62:387–93.

22. Aitken, R.J., Irvine, D.S., and Wu, F.C., (1991): Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. Am J Obstet Gynecol., 164:542–51.

23. Esfandiari, N., Sharma, R.K., Saleh, R.A., Thomas, A.J., and Agarwal, A., (2003): Utility of the Nitroblue Tetrazolium Reduction Test for Assessment of Reactive Oxygen Species Production by Seminal Leukocytes and Spermatozoa. Journal of Andrology, Vol. 24, No. 6.

24. Verit, F.F., Verit, A., Kocyigit, A., Ciftci, H., Celik, H., and Koksal, M., (2006): No increase in sperm DNA damage and seminal oxidative stress in patients with idiopathic infertility. Arch Gynecol Obstet., 274(6):339-44.

25. Rajinder, S., Rakesh, K., and Ashok, A., (1998): relation between Creatine Kinase activity and semen characteristic in subfertile men. Int. J. Fertil., 43(4):192-197.

26. Dandekar, S.P., and Pakar, G.M., (1999): correlation between creatine kinase activity, lipid perodoxidation, and water test in male infertility. J Postgrad Med. 45:42-8.

27. Huszar, G., Corrales, M., and Vigue, L., (1998): Correlation between sperm creatine phosphokinase activity and sperm concentration in normospermic and oligospermic men. Gamete Re, 19.67-75.

28. Aitken, R.J., and Clarkson, J.S., (1988): Significance of reactive oxygen species and antioxidants in defining the efficacy of sperm penetration techniques. J Androl, 9:367-376.

29. Huszar, G., and Vigue, L., (1993): Incomplete development of human spermatozoa is associated with increased creatine phosphokinase concentration and abnormal head morphology. Mol Reprod Dev 34,292–298.

30. Huszar, H., (1994): The role of sperm creatine kinase in the assessment of male fertility. Reprod Med Rev. 3:179-197.

31. Clause, H.M., Cooper, T.G., and Birgit, K., (1998): Creatine Kinase activity in human spermatozoa and seminal plasma lacks predictive value for mal fertility in vitro fertilization. Fertility and sterility 1998; 69:4 727-734.