Original Article

Influence of Oxidative Stress on Functional Integrity of Human Spermatozoal Membrane.

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Abstract

Objective : To study the relationship between lipid peroxidation of spermatozoa and changes in functional integrity of human spermatozoa membrane in male subjects. **Materials and Methods :** Sixty eight male partners of infertile couples were included in the study. Status of oxidative stress was assessed by determining malondialdehyde (MDA) in seminal plasma. Functional integrity of sperm membrane was tested subjecting the sperm to hypo-osmotic test (HOS). The seminal plasma MDA levels were compared with seminogram parameters as well as with the results of HOS test using Pearson's coefficient of correlation (r) and significance of coefficient of correlation calculated from the table. **Result :** A significant but weak negative correlation was observed between seminal plasma MDA level and sperm concentration ($r=-0.33, p<0.05$), sperm motility ($r=-0.37, p<0.05$), sperm morphology ($r=-0.37, p<0.05$), and percentage of HOS positive spermatozoa ($r=-0.33, p<0.05$). Percentages of HOS positive spermatozoa also exhibited a significant but weak negative relationship with sperm concentration ($r=-0.47, p<0.01$), sperm motility ($r=-0.48, p<0.01$), sperm morphology ($r=-0.49, p<0.01$). **Conclusion :** Lipid peroxidation of spermatozoa is likely to affect the functional integrity of the spermatozoa membrane.

Key words ; - oxidative stress, spermatozoa, membrane integrity

Introduction

Semen analysis is the fundamental and time honored test in the work up of infertility investigation. Despite the constant advances in the technique of semen analysis and efforts to standardize the seminogram parameters, no single seminogram parameter is an absolute predictor of man's fertilizing capacity.

Therefore, many sperm function tests are included in routine semen analysis such as Acrosome reaction, Hypoosmotic swelling test (HOS) and Zona free hamster oocyte penetration tests. These functional tests have been found to predict the fertilizing capacity of human spermatozoa in vitro and in vivo with some accuracy¹. But except Hypo osmotic swelling test, which determines the physiological integrity of sperm plasma membrane, all other tests are time consuming, expensive and require special facilities and skills². The HOS test has been proved to be a reliable test in predicting male fertility potential in those subfertile males who have greater possibility of conceiving with timid intercourse following ovulation induction³. The human sperm membrane is under constant threat of oxidative damage due to large quantities of poly unsaturated fatty acids

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Table 1

Seminogram characteristics, seminal MDA concentration and results of hypo osmotic swelling test in normal and abnormal ejaculates

Group	Sperm count (10 ⁶ /ml) ^a	Percent sperm motility ^a	Percent sperm forms ^a	Percent swollen spermatozoa after hypoosmotic treatment ^a	Seminal MDA conv. (nmol/ml) ^a
Normal					
Ejaculates (n=34)	89.26 ±28.09	55.41 ±8.31	37.85 ±5.71	62.89 ±13.03	1.46 ±0.50
Abnormal					
Ejaculates (n=34)	12.20 ±7.50	27.26 ±9.87	19.76 ±4.00	46.66 ^b ±17.93	1.94 ^b ±0.72

a :- Values are means ± SD

b :- Value significantly different (p<0.01) from normal

(PUFA) in their plasma membrane and relative lack of scavenging antioxidant enzyme in their cytoplasm. Oxidative stress induced damage to sperm membrane may be responsible for defective sperm function thereby contributing significantly to male infertility⁴.

The present study is aimed at studying the effect of lipid peroxidation on functional integrity of sperm plasma membrane by evaluating the level of seminal MDA (Malondialdehyde- a lipid peroxidation product) and assessing the response of spermatozoa to hypo osmotic stress.

Material and Methods

Sixty eight male partners of infertile couples with infertility duration of more than 1 year referred were included in the study after thorough physical examination and local genital examination in the surgery department. Subjects with varicocele, hydrocele, cryptorchidism and those suffering from chronic systemic disease such as TB, DM, history of exposure to spermatotoxic or gonadotoxic substances were excluded from the study. Semen samples were obtained by masturbation after 3 days of abstinence in a clean wide mouthed plastic container. Semen analysis was performed on SQA II B auto analyzer (Ranbaxy

Diagnostics, New Delhi) after complete liquefaction. Ejaculates were classified into 2 broad categories based on WHO standard criteria for semen analysis⁵.

Group I- Normal ejaculates (34)-sperm concentration>20x10⁶, motility>50%, sperm morphology>30%

Group II – Abnormal ejaculates (34)- sperm concentration <20x10⁶, motility < 50% sperm morphology <30%

Hypoosmotic swelling test (HOS) : Spermatozoa were subjected to hypoosmotic stimulus by incubating 0.1 ml of liquefied semen with hypo-osmotic solution at 37° C for 30 minutes (reagents for this test were supplied by National Institute of Health and Family Welfare, Munirka, New Delhi). Spermatozoa were examined with phase contrast microscope. Swellings of the sperm were identified by coiling of spermatozoa tails. At least 100 spermatozoa were counted and percentage of swollen sperms was calculated. Samples showing more than 60% of swollen spermatozoa were classified as HOS positive and those with less than 60% as HOS negative⁶. Estimation of seminal plasma malondialdehyde (MDA) concentration: Seminal MDA concentration was measured by thiobarbituric acid reaction method⁷.

Results of HOS and seminal MDA concentration were composed with normal and abnormal ejaculates to determine the significance of difference between the mean using Student's "t" test.

Correlation between response of spermatozoa to HOS stress and difference in seminogram parameters (Sperm concentration, motility and morphology) as well as with seminal MDA concentration was evaluated by finding coefficient of correlation (r) in pooled data.

Results

The mean sperm count, percentage of motile sperm, percentage normal morphology and seminal MDA concentration as well as percentage of swollen spermatozoa in normal and abnormal ejaculates was as shown in Table 1. In about 50% (34 out of 68) of ejaculates, HOS test was negative (less than 60% swollen spermatozoa). The percentage of HOS positive test was 76.5% (36 out of 34) of normal ejaculates and 23.5% (8 out of 34) in abnormal ejaculates. (Figure 1 and 2)

A significant but weak positive correlation was observed between HOS response and sperm concentration ($r=0.47$, $p<0.01$), percentage sperm motility ($r=0.48$, $p<0.01$) and percentage of morphologically normal spermatozoa ($r=0.49$, $p<0.01$) in a pooled data (Figure 3,4,and 5).

A significant but weak positive correlation was observed between seminal MDA concentration and sperm count ($r=-0.33$, $p<0.05$), sperm motility ($r=-0.37$, $p<0.05$), sperm morphology ($r=-0.27$, $p<0.05$) and HOS positive test ($r=-0.33$, $p<0.05$) when pooled data was used (Figure 6,7,and 8). Seminal MDA concentration in normal and abnormal ejaculates was significantly different ($p<0.05$).

The percentage of swollen spermatozoa in abnormal ejaculates was significantly less than in normal ejaculates ($p<0.001$).

Discussion

Spermatozoal plasma membrane is the structure where processes such as motility, acrosomal exocytosis and sperm oocyte fusion occur. These processes are important steps in successful fertilization.

Many environmental, Physiological, and genetic factors have been implicated in the poor sperm functions and infertility. Free radical induced oxidative damage to spermatozoa is one such condition which is recently gaining a considerable attention for its role in inducing poor sperm function and infertility.

Spermatozoal membrane is also the major site of lipid peroxidation. Reactive oxygen species is produced by a variety of semen components including immotile or

morphologically abnormal spermatozoa, leucocytes and morphologically normal but functionally abnormal spermatozoa.

Hypo-osmotic stress test is the simplest preliminary test which could be used as a preliminary marker test for sperm tail damage by reactive oxygen species⁸.

Lipid peroxidation of spermatozoa membrane is responsible for causing perturbation of membrane structure and function (transport processes, maintenance of ion and metabolite gradient, receptor mediated signal transduction)⁹

In the present study, significant higher values of MDA together with poor response of spermatozoa to hypo osmotic stress in abnormal ejaculates as compared to spermatozoa in normal ejaculates indicated that lipid peroxidation has caused adverse changes in the spermatozoal functional integrity. Such oxidative damage to spermatozoal membrane also resulted in impairment of sperm motility, and sperm morphology as evidenced by negative correlation between seminal MDA levels and sperm motility. Sperm morphology and functionally intact membrane is responsible for normal sperm motility and morphology as seen by a positive correlation between HOS positive test and sperm motility and sperm morphology.

Our findings are in accordance with those reported by Dandekar, Nadkarni and Kulkarni⁸.

From the present study it became clear that lipid peroxidation may alter sperm membrane functional integrity adversely and contribute to abnormal sperm function, thereby interfering with normal process of fertilization.

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