Use of Simvastation as antioxidant drug significantly decreases lipid peroxidation by utilization of malondialdehyde (MDA) level assay as an indicator of spermatozoal oxidative stress in male infertile patient

Ahmed S. Al-Hilli, M ed. M Sc, ph.D *
Ala’a S. Ali M. B. CH., D. V. D ***

Abstract:
A randomized blind controlled study is carried out on a total of 55 patient with primary male factor infertility in Maternity and Childhood Teaching Hospital in Najaf Province between April 2005-June 2006. The distribution of patients in group I were classified into 8 subgroups according to pathological cause of infertility and group II consisted of 35 normal proven fertility volunteer men served as a control group. All patients were given simvastation tables at dose 20 mg t twice daily for a period of 3 months. Semen samples and spermatozoal malondialdehyde level (MDA) level were assessed in all infertile subgroup patients before administration and after termination of treatment while group II were assisted as a control group. The present results showed that there was a negative relation between malondialdehyde level (MDA) and active sperm motility % and positive relation between MDA and abnormal sperm morphology. This study suggests that simvastation might be administered as an antioxidant drug to reduce lipid peroxidation and the later as as an index of malondialdehyde (MDA) level assay in oxidatively stressed infertile patients.

Keywords: spermatozoal oxidative stress, lipid peroxidation, simvastation.

This study is attributed to College of Medicine Kufa University, Najaf/ Iraq. All requests should be addressed to:
*Asst. Prof. of Reproductive Physiology Dr. Ahmed S. Al-Hilli, College of Medicine, P.O Box (498) Najaf / Iraq,
Email: Ahmedalhilli@yahoo.com
Introduction:

Successful pregnancy results from an interaction between myriad physiological process in both men and women any disturbance in this interactive system, whether in a man or women can result in an inability to have a biological child (1). Infertility can be defined as the failure of a couple to achieve pregnancy after a year of regular unprotected intercourse, it is a significant problem for couples of childbearing age (2). In United States, one of every six couples of childbearing age may be infertile (3). Despite the enomorous progress in research and reasoning, most of the blame for infertility, until recently, was placed on the female only during the last 15-20 years advances in understanding of gonadal/ sperm function and dysfunction led to dramatic increase in our knowledge of male infertility (4). Defective sperm function is the most prevalent cause of male infertility and difficult to treat, many environmental, physiological, biological and genetic factors have been implicated in the poor sperm function and infertility, thus, it is very important to identify the factors, conditions which affect normal sperm function (5).

Data accumulated over the past few decades indicate that male factor infertility plays a role in approximately 50% of infertile couples (6). Human spermatozoa have unusually high levels of either linked lipids and a high contact of unsaturated fatty acids groups such as docasa hexaenoyl (22:6 chain) and the major lipid composition of human spermatozoa (7). Lipid peroxidation (LP) is broadly defined as oxidative deterioration of polyunsaturated fatty acids (PUFA) which contain more two carbon-carbon double bonds (29), it is a physiologically process occurring in all cells that are rich in lipids, especially PUFA, it is also plays a significant role in etiology of defective sperm function, the onset of LP susceptible forms leads to progressive accumulation of hydroperoxides one of lipid peroxide in the plasma membrane, which decomposes to malondialdehyde which is an index of lipid peroxidation damage (9,10).

Oxidative stress OS is a condition associated with an increased rate of cellular damage induced by oxygen (11) and oxygen derived oxidants commonly known as reactive oxygen species (ROS) have been implicationed in over a hundred of diseases states which ranged from arthritis and connective tissue disorders to carcinogenesis, aging, toxin exposure, physical injury infection and acquired immune deficiency syndrome, the role of oxidative stress in infertility and methods for counteracting its impact on reproductive tissue with antioxidant is still in its infancy (12). A study mentioned that oxidative stress arise as a consequence of excessive production of ROS and impaired antioxidative defective mechanisms (13), and also it is proposed that OS precipitates the range of pathologies that currently are thought to afflict the reproductive function (14). In the context of human reproduction, a balance exists between ROS production and antioxidant scavenging activities in male reproductive tract, as regulating balance, only minimal amounts of ROS remain, and they are needed for regulating normal sperm functions such as sperm capacitation, acrosomal reaction and sperm – oocyte fusion (15,16), while, the production of excessive amounts of ROS in semen can overwhelm the antioxidant defense mechanisms of spermatozoa and seminal plasma can cause oxidative stress. Spermatozoa are particularly susceptible to damage induced by quantities of polyunsaturated fatty acids and their cytoplasm contain low concentration of scavenging enzymes (17). Recently, a study pointed out that lipid peroxidation of human spermatozoa impairs cell membrane ion exchange that is
essential for maintaining normal sperm motility and may cause loss of motility\(^{(18)}\). Infertility and sexual dysfunction indicate that seminal oxidative stress tests has diagnostic and prognostic capabilities beyond these of conventional tests of sperm quality or dysfunction and OS can accurately discriminate between fertile and infertile men and identify patients with a clinical diagnosis of male factor infertility who are likely to initiate patient with a clinical diagnosis of male infertility from antioxidant supplementations incorporation of such tests into routine andrology laboratory practice may be of particular importance to the future management of male infertility\(^{(19)}\). 

Simvastatin is a 3-hydroxy 3-methyl-glutaryl coenzyme A (HMG-CoA) reductase competitive inhibitor that is derived synthetically from fermentation of \textit{Aspergillus terreus}, it exerts a hypocholesterolemic action by stimulating an increase in LDL receptors an hypocholesterolemic action by stimulating an increase in LDL receptors on hepatocyte membrane thereby increasing the clearance of LDL from the circulation\(^{(20)}\). Recent evidence suggests that the beneficial effect of HMG-CoA reductase inhibitors on endothelium function and cardiovascular ischemic events may be attributed not only to their lipid lowering effects but also atherosclerotic vessel walls and this indicates that simvastatin treatment preserved in markers of oxidative stress, these beneficial endothelial effects of simvastatin are likely to occur independently of plasma lipid concentration and to be mediated by its antioxidative action\(^{(20,21)}\). On the basis of recent evidences that simvastatin reduces intracellular cholesterol biosynthesis and preserve endothelial function. In the present study, similar adoption had been proposed and applied being simvastatin might serve with antioxidant prosperities in the field of male infertility, in preserving sperm function with following aims: to identify the clinical significance of simvastatin as antioxidant drug on spermatozoal oxidative stress in the field of male infertility. To establish an assay for accurate and reliable assessment of spermatozoal oxidative stress.

**Patients and methods:**

**Data collection:**

A randomized blind controlled study is carried out on a total of 243 patients with primary male factor infertility (barren marriage for more than one year). infertile patient were selected after their referral to the Infertility Unit at Maternity and Childhood Teaching Hospital in Najaf Province at the period between April 2004 and June 2005 , and Cancer Research Unit in Kufa Medical College. Out of the total number of patients, only 55 patients are submitted for this study and 35 normal proven fertility volunteer men served as control group for comparison purpose.

**Selection of study patients:**

The referred infertile patients with proven abnormal sperm parameters (motility, morphology and dead-live%) are exclusively registered in this study on the basis of their spermogram disruptive spermatogenesis and with normal sexual function while patients with infertility that will interfere with infertility-related origin excluded from the study viz: hypopituitarism hypogonadism, diabetes mellitus testicular varicocele, venereal disease, leukocytopermia. Other allied exclusions that interfere with fertility were also obtained by history namely: drug and hormonal therapy, Heavy smoking and heavy drinking, below and beyond age group 20-50 year and any patient with erection dysfunction , impotence and who had difficulties in semen collection by masturbation or
coitus interrupts. All patients participating in the study were accept with verbal consent, and patients included in treated group were given simvastation a(simlo) tables, Ipca, Ipca laboratories ltd, Mumbai, India at dose 20mg twice daily for a period of 3months. All assays were carried out before giving any treatment and reviewed after termination of the period of 3months.

**Design of study:**
The distributions of 90 men in the study are fallen into 2 main groups, the first group composed of:
55 patients were classified into 8 groups according to the pathological cause of infertility of sperm parameter, this had been considered in regard to scores of WHO criteria (6).
- Asthenozoospermia (A), n=1
- Asthenonecrozoospermia (AN), n=2
- Asthenoteratozoospermia (AT), n=20
- Asthenoteratonecrozoospermia (ATN), n=4
- Oligoasthenoteratozoospermia (OAT), n=7
- Oligoasthenoteratonecrozoospermia (OATN), n=2
- Oligoasthenozoospermia (OA), n=2
- Teratozoospermia (T), n=7

The second group (control suggest) consist of 35 healthy normal with proven fertility volunteers (donors) initiated a successful pregnancy within with the last 12 month.

**Semen Collection:**
Samples of ejaculates were collected from married patients by masturbation technique or coitus interrupts after 3-5 days of sexual abstinence (22). Ejaculate samples were collected in clean transparent plastic cups with wide opening and precise sealing after ejaculation, the specimen was placed in an incubator at 37°C for 30 min to allow liquefaction. The specimen was examined according to Zaneveld and Polakoski techniques (22) and seminal leukocytes counts by positive myeloperoxidase staining (Endtz test) (23).

**Sperm preparation:**
All masturbated semen samples liquefied after 30 minutes at room temperature, spermatozoa were separated from seminal plasma by centrifugation at 500x rpm for 30 minutes. The supernatant was precisely measured by a graduated centrifuge test tube and discarded. Homogenized buffer consisted of (11.9 gms of menthol, 4.8 gms of sucrose, 0.09 gms of EDTA in 250 ml of distilled water adjusted the pH to 7.4 with tris-base). How organized buffer was kept in refrigerator at 4°C. The samples were hand homogenized were subsequently centrifuged for 10 minutes at 3000 rpm. Cooled 0.9 ml of triton x-100 (0.1 %) was added to each 0.1 ml of pallets obtained from the sample. The samples were centrifuged again at same rpm for half an hour in a centrifuge, the supernatant was used (24).
Lipid peroxidation:
Determination of Malondialdehyde (MAD).
The amount of MAD produced was used as an index of lipid peroxidation, the procedure carried out according to thiobarbituric acid (TBA) assay by Mihra and Vchiyama (25).

The principle:
Malondialdehyde react with thiobarbituric acid (TBA) to form a pink colored product.

Procedure:
500 ml of homogenate was added to 3ml (of 1%) phosphoric acid, 0.1 ml of 0.6% TBA and 0.5 ml of 2.0% butylated hydroxytoluene (BHT) in 95% methanol. The samples were heated in boiling water both for 45 minutes, cooled and 4.0 of butanol was added. The butanol phase was separated by centrifugation at 3000 rpm. All values were expressed as n moles MAD/mg of proteins using spectrophotometer Cocil- 1011. England in measurements.

Calculations:
The concentration of MDA nm/mg=a/LxEoxDx10^6
L = length bath
Eo = Extension coefficient 1.56 × 10^5 . m^-1 . cm^-1
D = dilution factor 6.7
Data were analyzed using inbuilt functions within the statistical package SPSS UK version 10 Surrey UK. Least significant difference (LSD) had been applied for difference between means at level of significance 0.05 and all hypothesis testing two tailed p<0.005 was considered statistically significant.

Results:
Table (1-8) presented (mean ±se) comparison of post-simvastation (40 mg daily for 3 months) treated infertile patients of sperm function parameters (sperm motility %, sperm morphology % and viability %) in each sperm pathological subgroups (n=55) with control mean values of normal fertile volunteers (n=35) respectively.

Table (1) showed that high significant increase (p<0.05) of active sperm motility % in A, AT, ATN, OAT and OATN subgroups (p<0.05) where as AN,OA subgroups show significant increase (p<0.05).

Table (2) demonstrated that there was a high statistically significant increase (p<0.05) of sluggish motility % in A,AT and ATN subgroups respectively.

Table (3) depicted that there is only A and AT subgroups cited the high significant difference decrease (p<0.05) fall in OAT,OATN and OA subgroups but AN and T subgroups did not show significant results (p>0.05).

Table (4) presents the high statistically significant differences (p<0.05) of malondialdehyde (nmol/mg of protein) in AT and T subgroups and statistically significant results in A,OAT and OA subgroups while, neither AN nor ATN subgroups showed significant difference (p>0.05) . When p-value a of MAD compared to p-values b of abnormal sperm morphology % surprisingly , subgroups with high statistically significant differences and statistically significant differences (P<0.05) showed a relatively close p-values in the same subgroups, namely: A, AT, OAT and T subgroups. The close similarity of those p-values ascertain that there is a causative contributing factor behind the results of the variables. Moreover , the results of OAT and OATN...
Table (5) depicted that there was a high statistically significant decrease \( (p<0.05) \) of malondialdehyde in only T subgroup when compared to control mean, the values are \( (0.58 \pm 6.78 \times 10^{-2} \) vs \( 0.36 \pm 3.70 \times 10^{-2} \) consecutively. While, AN subgroup showed a significant results \( (p<0.05) \) values \( 0.44 \pm 5.00 \times 10^{-2} \) vs \( 0.36 \pm 3.70 \times 10^{-2} \) all other subgroups showed statistically insignificant results \( (p>0.05) \). Table (6) presented clearly the highest means of abnormal sperm morphology \% that show significant decrease \( (p<0.05) \) in AT, OATN and T subgroups whereas , A , ATN and OA subgroups show significant values in most pathological post-treated subgroups and the relation with MAD.

Table (7) that shows inverse relation between abnormal sperm morphology and MAD level.

Table (8) revealed the high statistically significant increase \( (p<0.05) \) of variable sperm\% in post – treated patients in all sperm pathological subgroups except T subgroups showed significant increase \( (p<0.05) \) while, OAT did not show significant difference \( (p>0.05) \). Obviously, the cause of significance in most post – treated pathological subgroups are due to the raise in mean values when compared to the control value.

**Discussion:**

In recent years, infertility has become the subject of significant media attention and public discussion, particularly in light of new advances in the technology of assisted reproduction \(^{(26)}\). A variety of medications have been developed in an attempt to improve the sperm quality in turn modify the male infertility potential \(^{(27, 28)}\).

In light of the present finding, there was an obvious improvement in most sperm function parameters in post-treated sperm pathological subgroups in which simvastation virtually participating in inhibition of mean value of malondialdehyde (MDA) level that reflected a decrease of lipid peroxidation which is the key of spermatozoal oxidative damage \(^{(18)}\). No update literatures on simvastation administration were available in field of infertility to compare our find with other studies.Regarding the results of simvastation on sperm motility \% are presented in tables (1,2,3) our results were supported with other studies \(^{(39,30,32,33)}\) that mentioned lipid peroxidation can cause irreversible loss of sperm motility and MAD serves as biochemical index pf lipid peroxidation. The present results of simvastation on abnormal sperm morphology \% depicted a shiny picture when pointed out to a possible positive reaction between p-value (a) of abnormal sperm morphology and p-values (b) of MAD level table(7). In our belief, there are many interpretations might be collectively contribute to sperm morphology improvement (1): as simvastation decreases lipid peroxidation that cause high significant decrease \( (p < 0.05) \) in mean of MAD value that actively initiate oxidative stress \((7),(2)\): the decrease in abnormal sperm morphology \% itself may play an important role in suppression of ROS production created by abnormal sperm \(^{(34)}\). However, it is known that there is a potential negative impact of oxidative stress (OS)on both spermatogenesis and spermiogenesis causing an increase in teratogenic (sperm droplet) , therefore, the direct effect of OS polyunsaturated fatty acid (PUFA) of mature sperm results in an increment in the lipid peroxidation that interfere with sperm function parameters and consequently impair male fertility \(^{(35,36)}\). In this study, there was high statistically significant increase \( (p<0.05) \) of viable sperm \% in post- treated AT subgroup and significant difference in OAT subgroups table (6).
Obviously, there was a possible inverse relation between p-value (a) of viable sperm % and p-value of MAD, this ideally may express the lowering evidence of lipid peroxidation (MAD) of sperm plasma membrane culminating in decrease of spermatozoal oxidative stress that reflected improvement of sperm viable %, this result agree with (37). On the basis of mode action of simvastation (HMG-COA reductase inhibitor) that reduces intracellular cholesterol biosynthesis and serum cholesterol (38), principally, the present study had been proposed the possible antioxidant property and adopted the idea, that minimizing the lipid peroxidation of plasma membrane of sperm by its, the cholesterol-lowering action, that corroborate with our present findings. Lastly, the present study suggested that simvastation could be of clinical significance in management of male infertility in preserving function of sperm and subsequently might improve positively sperm function parameters with co-existence of such relationship to simvastation.

References: