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Assessment of spermatozoal oxidative stress response to simvastatin in male infertility

Ahmed S. Al-Hilli,[a] Emad S. Ali,[b] Ala'a S. Ali,[c] Noorhan S. Mhao,[d] Najah R. Hadi,[e] Dina A. Jamil,[f] and Hayder A. Al-Aubaidy[f]

[a]College of Medicine, University of Kufa, Iraq.
[b]College of Pharmacy, University of Kufa, Iraq.
[c]Department of Dermatology & Venereology, Al-Sadder Teaching Hospital, Najaf, Iraq.
[d]Department of Gynecology & Obstetrics, College of Medicine, Kufa University, Najaf, Iraq.
[e]Department of Pharmacology, College of Medicine, University of Kufa, Iraq.
[f]School of Medicine, University of Tasmania, Hobart, Tasmania, Australia.
Correspondence to Najah R. Hadi (e-mail: drnajahhadi@yahoo.com).
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Objectives Inability to conceive may be caused by either female or male abnormalities. The aim of this study is to evaluate the effects of simvastatin medication on improving the degree of lipid peroxidation in patients with primary infertility and improving sperm formation and maturation.
Methods This is a randomized controlled study. It included 90 participants, 55 male patients who diagnosed with primary infertility compared with 35 normal control subjects. The patients were randomly selected from Al-Najaf Teaching Hospital, Iraq. The patients were divided into eight groups based on the cause of infertility. Patients were supplied by 20 mg simvastatin tablets once per day for a period of 12 weeks. Semen samples were assessed for lipid peroxidation before and after the course of the treatment.
Results There was a negative correlation between spermatozoal malondialdehyde level and percentage of active sperm motility. This was associated with a positive correlation between spermatozoal malondialdehyde level and abnormal sperm morphology.
Conclusion The current study suggests that simvastatin medication may be usefully administered to reduce the levels of lipid peroxidation in infertile males to improve sperm formation and maturation.
Keywords male infertility, lipid peroxidation, simvastatin therapy

Introduction

There are many factors that affect the progress of a successful pregnancy, some are related to the male and others are female's related issues. Infertility can be defined as failure to conceive following 1 year of regular unprotected sexual intercourse, it can negatively affect couples during the childbearing age.[1] The incidence of infertility is increasing in the United States as well as in the rest of the world, it is estimated that one of every six couples of childbearing age may be infertile.[2] Previous studies showed that defective sperm function is the most prevalent cause of male infertility, which is difficult to be treated as many environmental, physiological, biological, and genetic factors have been impacted in the poor sperm function and infertility.[3] Therefore, it is important to identify the risk factors and conditions which affect normal sperm function.[3,4]

Data accumulated over the past few decades indicate that male factor infertility plays a role in approximately 50% of infertile couples.[5] Human spermatozoa have unusually high levels of 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.[6] Thus, lipid peroxidation (LP) plays an important role in decline of the fertility rate among males.[6] 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-1 of lipid peroxide in the plasma membrane, which decomposes to malondialdehyde (MDA).[7]

In normal condition, there is a balance between reactive oxygen species (ROS) production and the antioxidant scavenging activities in male reproductive tract; therefore, only minimal amounts of ROS remain, and they are needed for regulating normal sperm functions such as sperm capacitation, acrosomal reaction, and spermoocyte fusion.[8] Abnormal high ROS in semen can overpower the antioxidant defense system leading to a condition of oxidative stress.[9] Spermatozoa are particularly vulnerable to the damage induced by high levels of polyunsaturated fatty acids, besides, they have less amount of antioxidant enzymes.[6] A recent study showed that the process of spermatozoal lipid peroxidation can damage the process of spermatogenesis through various ways; mainly by impairing cell membrane ion exchange that is essential for maintain normal sperm motility and this may cause loss of motility.[10]

Simvastatin is a 3-hydroxy 3-methyl-glutaryl coenzyme A (HMG-COA) reductase competitive inhibitor; it exerts a hypocholesterolemic action by stimulating an increase in low-density lipoprotein (LDL) receptors on hepatocyte membrane thereby increasing the clearance of LDL from the circulation.[11] Studies showed that the beneficial effects of HMG-COA reductase inhibitors on endothelium function and cardiovascular ischemic events may be attributed not only to their lipid-lowering effects but also to their anti-atherosclerotic effects on blood vessels.

In our study, we hypothesize that male infertility and sexual dysfunction may partially occur due to the process

of OS. Therefore, we propose to use markers of oxidative stress (like MDA as marker of lipid peroxidation) as an additional test in addition to the conventional tests used to determine sperm quality and dysfunction. This may provide additional tests to be routinely used when looking for male infertility. In addition, antioxidant supplementation of simvastatin can improve male sexual fertility.

Methods

Data collection

The study was approved by the human ethics committee, University of Kufa, Iraq. It is a randomized controlled study, carried out on a total of 90 male participants. About 55 participants were complaining of primary infertility. Patients were randomly selected in the study group following their referral to the infertility unit at Al-Najaf teaching hospital. The remaining 35 participants were selected as normal control subjects.

Selection of study patients

Participants patients were included in this study based on their documented abnormal sperm parameters (motility, morphology, and percentage of the dead-live index). The study excluded patients with secondary infertility occur due to other diseases like diabetes mellitus, hypopituitarism hypogonadism, testicular varicocele, and venereal diseases. Other exclusion criteria include the use of medication, which affects sexual function, hormonal therapy, impotence (difficult to collect semen via masturbation or coitus interrupts), and excessive alcohol consumption.

All participants provided written consent to their approval to participate in this study.

Design of study

The 55 patients included in this study were divided into eight groups based on the cause of their infertility. The sperm parameter was also considered based on the WHO criteria,[5] as shown in Table 1.

All patients were treated with 20 mg simvastatin tablets once per day (Ipca, Ipca Laboratories Ltd., Mumbai, India) for a 12 weeks period. All medical assays were carried out before start of medication and at the end of the treatment course.

The ninth group formed from the 35 normal control subjects with proven fertility (with a successful pregnancy within with the last 12 month).

Semen collection and sperm preparation

After 3 days of sexual abstinence, semen samples were collected either via masturbation technique or through coitus interrupt.[12] Ejaculated samples were collected in clean transparent plastic cups with a wide opening and sealed 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[12] and seminal leukocytes count by positive myeloperoxidase staining (Endtz test).[13]

Spermatozoa were separated from seminal plasma by centrifugation. Cooled 0.9 ml of triton x-100 (0.1%) was added to each 0.1 ml of pellets obtained from the sample. The samples were centrifuged again for another 30 min and the supernatant was used for the tests.[14]

Lipid peroxidation

The amount of MDA produced was measured using the thiobarbituric acid (TBA) reacting substance method.[15] MDA can react with TBA to form a pink colored product, which can be measured using spectrophotometer.

Statistical analysis

The concentration of MDA nm/mg = $a/L \times Eo \times D \times 10^6$

L = length bath

Eo = Extension coefficient 1.56×10^5. $m^{-1}.cm^{-1}$

D = dilution factor 6.7

The data were analyzed using SPSS (v14) and Microsoft Excel (Office 2007, Microsoft). All values were expressed as mean ± SD. Statistical analysis was performed using a one-way ANOVA followed by *t*-test to compare data pre and post simvastatin therapy. In addition, independent sample *t*-test was also used to compare the patents' groups with the control group. *P*-value was considered significant at or below 0.05

Results

Table 2 compares the levels of parameters of this study before and after simvastatin therapy.

By comparing the levels of MDA in all groups before and after simvastatin therapy, there was a significant reduction in the level of this marker in all the patients' groups compared to the control, Table 3.

Figure 1 shows the levels of the MDA in the study groups before and after simvastatin therapy, Fig. 1.

Table 1. **Grouping the patients based on the cause of their infertility**

Groups	Cause of primary infertility	Abbreviation	Number of participants
1	Asthenozoospermia	A	1
2	Asthenonecrozoospermia	AN	2
3	Asthenoteratozoospermia	AT	30
4	Asthenoteratonecrozoospermia	ATN	4
5	Oligoasthenoteratozoospermia	OAT	7
6	Oligoasthenotetronecrozoospermia	OATN	2
7	Oligoasthenozoospermia	OA	2
8	Teratozoospermia	T	7

Table 2. **The response to simvastatin medication, *P*-value significant at or below 0.05**

Groups	Active sperm motility%	Sluggish sperm motility%	Sperm morphology	Sperm viability	MDA levels
1-A	0.01	0.05	NS	0.05	0.01
2-AN	0.05	NS	NS	NS	NS
3-AT	0.05	0.05	NS	0.05	0.05
4-ATN	0.01	0.05	NS	NS	NS
5-OAT	0.01	NS	0.05	0.05	0.01
6-OATN	0.01	NS	0.05	NS	NS
7-OA	0.05	NS	NS	NS	0.01
8-T	NS	NS	NS	0.05	0.05

Table 3. **Comparing the values of the MDA levels (Umol/L) of the eight patients' groups to the control group before and after simvastatin therapy, *P* value significant at or below 0.05**

Groups	Before simvastatin therapy	After simvastatin therapy	*P* value difference (before and after simvastatin therapy)
1-A	0.71 ± 0.001**	0.58 ± 0.001*	0.01
2-AN	0.76 ± 0.27**	0.64 ± 0.43*	NS
3-AT	0.78 ± 0.12**	0.51 ± 0.16*	0.05
4-ATN	0.81 ± 0.16**	0.53 ± 0.16*	0.01
5-OAT	0.78 ± 0.15**	0.57 ± 0.15*	0.01
6-OATN	0.82 ± 0.19**	0.46 ± 0.14	0.01
7-OA	0.76 ± 0.12**	0.45 ± 0.1	0.01
8-T	0.89 ± 0.1**	0.57 ± 0.2*	0.01
9-Control	0.35 ± 0.16	0.35 ± 0.16	NS

*Significant difference between control and the designated patient's group ($P < 0.05$). **Significant difference between control and the designated patient's group ($P < 0.01$).

Discussion

For many years, infertility was (and still) the subject of a significant media attention and public discussion, especially in view of the wide advances process of assisted reproduction. Many proposed treatment modalities were proposed to enhance the production and improve sperm quality.[16–19]

In our study, we noticed a significant improvement in sperm most parameters in response to atorvastatin therapy in the eight pathological groups. Simvastatin reduced the degree of lipid peroxidation (as seen by an overall reduction in MDA levels), which is a key point in sperms formation and maturation.[3,4] To our knowledge, this is the first study of its type to investigate the benefits of simvastatin administration in improving male infertility. Simvastatin improved overall active sperm motility in all the pathological groups. Also, it helped to improve the sluggish sperm movement, sperm morphology, and viability in some of these groups. These findings supported by the previous studies, which showed that excessive lipid peroxidation can cause irreversible damage to sperm formation and maturation.[20]

Improving in sperms morphology in response to simvastatin therapy can be explained by (1) The beneficial effects of simvastatin in decreasing lipid peroxidation (as noted via a reduction in MDA levels), and (2) The decrease in the abnormal sperm morphology can help in suppressing further generation of ROS, which help to improve the sperm formation and development.[20]

Our study proved the beneficial effects of simvastatin therapy on improving sperm formation and maturation in association with a total reduction in lipid peroxidation representing an insight for a new treatment modality that can be used to boost the currently know treatment modalities for male infertility.

Conflicts of Interest and Source of Funding

Nothing to declare.

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