

Controlling the levels of oxidative DNA damage improves patient's response to simvastatin therapy in primary male infertility

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Objectives Male primary infertility can occur for several reasons. In most of the cases, sperm sample examination itself may give information about the underlying cause, but it would not help in determining the management plan. Simvastatin treatment can help to reduce the degree of oxidative stress and improve sperm outcome. 8-hydroxydeoxyguanosine (8-OHdG) is a marker of oxidative DNA damage that can be used to assess the level of oxidative stress and the patient's response to a course of treatment.

Methods This is a randomized controlled trial study, which included 90 participants (55 males with primary infertility and a matching of 35 control subjects). The patients were recruited from Al-Najaf Teaching Hospital, Iraq. Patients were provided with simvastatin tablets (20 mg × once daily for 12 weeks). Semen samples were taken to assess sperm parameters such as morphology, motility and motility. In addition, urinary samples were also collected to measure levels of 8-OHdG, before and after the simvastatin treatment course.

Results This study showed significant reduction in the levels of urinary 8-OHdG in the patient group compared to the control group after the treatment course.

Conclusion This study provides evidence about the beneficial use of simvastatin to improve the sperms' outcome in males with primary infertility. In addition, the useful measurement of urinary 8-OHdG is to monitor the degree of oxidative stress and patient's response to medication.

Keywords Male infertility, oxidative DNA damage, 8-hydroxydeoxyguanosine, simvastatin therapy

Introduction

Primary infertility is the inability of the couples to conceive or to maintain their first pregnancy following a 1-year of regular unprotected sexual intercourse.¹ Primary infertility can occur either due to male problems or female problems or both. Primary male infertility may occur due to genetic causes and/or environmental causes.² 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 implicated in the poor sperm function and infertility.³ Therefore, it is important to identify the risk factors, conditions which affect normal sperm function.^{2,3}

Increasing levels of oxidative stress (OS) may negatively affect the process of spermatogenesis.⁴ This may occur due to the release of reactive oxygen species (ROS) which targets several steps in sperm formation and maturation leading to defective production of normal sperms.^{5,6} In normal condition, there is a balance between the ROS production and the antioxidant defense system,⁷ keeping minimal amount of ROS in the body. This low levels of ROS may be useful in some pathways like regulating normal sperm functions such as sperm capacitation, acrosomal reaction and sperm-oocyte fusion.⁶ However, the persistence of abnormally high levels of ROS in the body and in semen can overpower the antioxidant defense system leading to a condition of oxidative stress.⁷ Spermatozoa are particularly vulnerable to the damage induced by high levels of polyunsaturated fatty acids, besides, they have less amount of antioxidant enzymes.⁸ A recent study showed that the process of spermatozoa 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, causing loss of motility.⁹ Simvastatin possess a protective role against further spermatozoa lipid peroxidation and improves spermatogenesis.¹⁰ Studies showed that the beneficial effects of hydroxymethylglutaryl coenzyme A (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.¹⁰

Previously, a number of tests based on different methods such as flow cytometry and enzyme immuno-assay, have been proposed to assess the viability of different cell compartments of spermatozoa such as acrosome reaction, oxidative DNA damage and viability.^{4,11} Such assays have the advantage of being more statistically robust, accurate and quicker. 8-Hydroxydeoxyguanosine (8-OHdG) is a marker of oxidative DNA damage, which is measured in blood and urine to reflect the acute changes in levels of oxidative DNA damage.⁷ We have used these markers in our previous studies to assess the acute changes in the levels of oxidative stress in response to various oxidative stimuli.^{12–14}

Previous studies showed the beneficial effects of HMG-CoA reductase inhibitors in improving endothelium function and reducing progression of cardiovascular ischemic events. This is attributed to their lipid lowering effects as well as their anti-atherosclerotic effects on blood vessels.

In this study, we hypothesize that the primary male infertility may partially occur due to high levels of OS and oxidative DNA damage. Therefore, we propose the use of a course of simvastatin (20 mg/day), for about 12 weeks for this group and we measure semen parameters and urinary 8-OHdG before and

after the course of simvastatin treatment to see the beneficial effects of simvastatin on reducing the degree of oxidative DNA damage and improving the outcome of semen parameters.

Methods

Data collection

The study was approved by the human ethics committee, University of Kufa, Iraq. This is a randomized controlled trial study, performed on a total of 90 male participants divided into two study groups. Group 1 consisted of 55 participants who had primary infertility. An age matching control group (35 participants), were also included in the study (Group 2). The patients were randomly selected in the study following their referral to the Infertility Unit at Al-Najaf Teaching Hospital.

Selection of the study patients

Group 1 participants were included in this study based on their documented abnormal sperm parameters (motility, morphology and percentage of dead–live index). The study excluded patients with secondary infertility occur due to known diseases such as diabetes mellitus, hypopituitarism hypogonadism, testicular varicocele and venereal diseases. Other exclusion criteria included the use of medication which affects sexual function, hormonal therapy, impotence (difficult to collect semen through masturbation or coitus interrupts) and excessive alcohol consumption.

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

Design of study

Group 1 consisted of all the patients' participants. Patients were treated with 20 mg simvastatin tablets once daily (Ipca, Ipca Laboratories Ltd, Mumbai, India) for a 12-week period. All assays were performed before the start of medication and at the end of the treatment course. Group 2 consists of 35 normal control subjects with proven fertility (with a successful pregnancy within the last 12–24 months).

Semen collection and sperm preparation

After 3 days of sexual abstinence, semen samples were collected either through masturbation technique or through coitus interrupts.¹⁵ Ejaculate samples were collected in clean transparent plastic cups with wide opening and sealed after ejaculation, the specimens were placed in an incubator at 37°C for 30 min to allow liquefaction. The specimens were examined according to Zaneveld and Polakoski techniques¹⁵ and seminal leukocytes counts by positive myeloperoxidase staining (Endtz test).¹⁶

Spermatozoa were separated from seminal plasma by centrifugation. A total of 0.9 ml of cooled Triton X-100 (0.1 v/v%) was added to each 0.1 ml of pellets obtained from the sample. The samples were re-centrifuged for another 30 min and the supernatants were used for the tests.¹⁷

Oxidative DNA damage measures

The levels of oxidative DNA damage were measured using the urinary 8-OHdG ELISA kit (Cayman Chemical, MI, USA).¹⁸ The test utilizes an anti-mouse immunoglobulin G (IgG)-coated plate and a tracer consisting of an 8-OHdG-enzyme

conjugate. This format has the advantage of providing low variability and increased sensitivity compared with assays that utilize an antigen-coated plate.

Statistical analysis

The results were analyzed using SPSS (v14) and Microsoft Excel (Office 2007, Microsoft). All values were expressed as mean \pm SD. Statistical analysis was performed using a one-way ANOVA followed by a paired *t*-test to compare data provided in the pre- and post-simvastatin therapy. *P*-value was considered as "significant" at or below 0.05.

Results

Table 1 compares the sperm characteristics of the patients in Group 1 before and after simvastatin therapy:

Our study showed a significant improvement of sperm count and motility in Group 1 after simvastatin therapy (Table 1). By comparing the levels of urinary 8-OHdG in both study groups, there was a significant difference in the levels of these parameters in the patient group (Group 1) compared to the control group (Group 2), at $P \leq 0.001$ (Figure 1). Student's paired *t*-test showed a statistically significant reduction in the level of 8-OHdG in the patient study group (Group1) after initiation of simvastatin therapy (Fig. 1).

Discussion

Our previous studies showed that 8-OHdG levels can be increased in conditions associated with an imbalance between the degree of oxidative stress and the antioxidant defense system.^{7,12,13,19} In this study, the levels of 8-OHdG were significantly elevated in Group 1 compared to Group 2 ($P \leq 0.001$). However, there had been a significant reduction in the levels of this parameter in Group 1 in response to a 12-week of simvastatin treatment course (Fig. 1). This confirms two things: (1) The beneficial effects of simvastatin in reducing the lipid peroxidation, which reflects a marked reduction in the degree of oxidative stress and improving the outcome of sperm parameters (Table 1).^{5,20} (2) The accuracy of urinary 8-OHdG marker

Table 1. Characteristics of the patients involved in the study before and after simvastatin therapy

Seminal fluid findings of the patients study (Group 1)	Before simvastatin treatment	After simvastatin course
	Total = 55 patients	Total = 55 patients
Oligozoospermia (sperm count <20 million/ml)	46 (83.6%)	32 (58.2%)*
Hypospermia/Aspermia (sperm volume <1.5 ml)	12 (21.8%)	10 (18.2%)
Asthenozoospermia (motility <40%)	51 (92.7%)	39 (70.9%)*
Teratozoospermia (abnormal morphology >40%)	28 (50.9%)	25 (45.5%)
Oligoasthenozoospermia (motile density <8 million/ml)	18 (32.7%)	13 (23.6%)

*Significant difference between the study group before and after simvastatin therapy at $P \leq 0.05$.

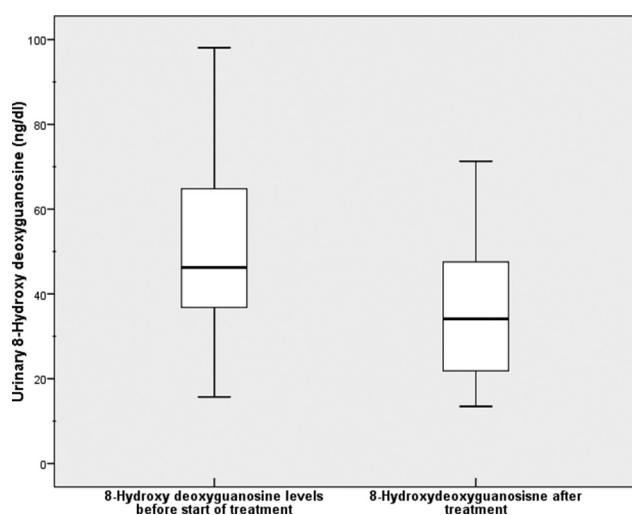


Fig. 1 NB: The levels (mean \pm SD) of urinary 8-OHdG in Group 2 was 20 ± 0.8 ng/dl.

is said to be used as an index of oxidative stress/oxidative DNA damage. It can also be used to monitor the patient's response to this medication.

The improvement noticed in sperms morphology in response to simvastatin therapy in Group 1 was occurred due to: (1) The beneficial effects of simvastatin in decreasing levels of lipid peroxidation (as noted through a reduction in MDA levels) and (2) the enhancement was seen in sperm morphology can help in suppressing further generation of reactive oxygen species, which helps to improve sperm formation and development.²¹

Conclusion

This study provided evidences about the beneficial use of simvastatin in improving sperm outcome in males with primary infertility. In addition, the useful measurement of urinary 8-OHdG is to monitor the degree of oxidative stress and patient's response to simvastatin medication. ■

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