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Can we improve the fertility outcomes in obese males with idiopathic subfertile normozoospermia?

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

[a]Department of Medicine, College of Medicine, Kufa University, Najaf, Iraq.
[b]Department of Gynecology & Obstetrics, College of Medicine, Kufa University, Najaf, Iraq.
[c]Department of Dermatology, Sadder Medical City, Najaf, Iraq.
[d]Department of Pharmacology, College of Medicine, Kufa University, Najaf, Iraq.
[e]School of Medicine, University of Tasmania, Hobart, TAS, Australia.
Correspondence to Prof. Najah R. Hadi (email: drnajahhadi@yahoo.com).
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Objective Despite the presence of numerous tests of sperm quality and function, no single laboratory test can determine with accuracy and precision whether a man is fertile or not. It is important to evaluate the oxidative stress (OS) in male and female reproductive tracts especially because of the results have diagnostic and prognostic value in the management of infertility. A randomized double-blind controlled study was carried out on a total of 55 patients with primary male factor infertility in Maternity & Childhood Teaching Hospital.
Methods The distributions of patients in group 1 were classified into eight subgroups according to pathological cause of infertility and 35 normal proven fertility volunteer men severed as a control group. Simvistatin (SIMLO) tablets (Ipca Laboratories Ltd. Mumbai, India) were given to all the patients, at dose of 20 mg twice per day for a period of 3 months. Seminal creatine kinase (CK) was assessed pre- and post-treatment.
Results The results showed that high significant decrease ($P < 0.05$) of CK activity in asthenozoospermia, asthenoteratozoospermia, oligo asthenoteratozoospermia and teratozoospermia. While the most influence subgroup was in teratozoospermia that showed about 2.5-fold lower than pretreated patients.
Conclusion This study suggests that simvistatin may be used as antioxidant treatment and CK assessment could be used as indirect index of OS in infertile patient.

Introduction

There is an increased evidence of the presence of an abnormal semen parameters among overweight/obese males, which may cause subfertility.[1] Obesity can contribute to a higher incidence of male factor of unexplained infertility.[2] An individual can be defined as an overweight if their BMI is 25–30 kg/m², and obese if their BMI exceeds 30 kg/m².[3] A combination of an increasing sedentary lifestyle and unfavorable diet in the world has resulted in an increased number of overweight and obese children and adults.[3] According to WHO, approximately 1.9 billion adults were classified as being overweight and 600 million were obese in 2014.[4] This came in association with the reported decline in semen's quality and men's reproductive potential over the past 75 years.[5] In contrast to extensive knowledge of the effects of obesity on female fertility, not much data is available concerning the obesity effects on male's infertility, even after discovering that there is a three-fold increase in the incidence of obesity in patients with male factor infertility, demonstrating the needs for a greater clinician's awareness in this area.[6] The etiology is unknown in about one-third of male factor infertility. In 15–20% of these cases, doctors are unable to find a specific cause of infertility, so it is labeled as a cause of "unexplained infertility."[2,7]

Carnitine plays an important role in regulating Sertoli cell function, protecting sperm against oxidative stress (OS), reducing apoptosis of spermatogenic cells and inhibiting sperm aggregation.[8] L-carnitine and acyl-L-carnitine are highly concentrated in epididymis and are important for metabolism and maturation of sperms. In a double-blind cross-over trial infertile patient, receiving either L-carnitine or placebo a significant improvement in sperm quality (sperm concentration and forward motility) was observed in the L-carnitine group.[9] In addition, the largest improvements were noted in men with the poorest semen quality.[9] In another study by the same investigators, a combination therapy with L-carnitine and acetyl-L-carnitine was given to 60 infertile men and seminal outcomes were observed.[10] Whether it's true or not this form of supplementation can result in significant improvements in pregnancy rate remains unknown.[10] L-carnitine is required during oxidation of lipid for transport of fatty acid from the cytosol into mitochondria for generation of energy. L-carnitine exerts a substantial anti-oxidation, anti-cytokines and anti-apoptotic action providing multi-mechanisms protective effects for the cells.[11]

L-carnitine has antioxidant activity that combines both free-radical scavenging and metal-chelating properties.[12] L-carnitine protects all membrane and DNA against damage induced by free oxygen radicals and has a pivotal role in the mitochondrial oxidation of long-chain fatty acids, which increases energy production to the cells.[12] Mitochondrial dysfunction may lead to incomplete detoxification of the free radicals, which may leads to oxidative damage to the micromolecules, such as lipids, proteins and DNA. L-carnitine has free-radical scavenging activity and ability to scavenge superoxide anion and inhibits lipid peroxidation (LP), thereby, conferring protection against damage induced by hydrogen peroxide.[13–15]

Sperm membrane plays an important role in fertilization capacity. Sperm membrane harbors highest concentration of polyunsaturated fatty acids (PUFA) than other human cells, sperms with highest concentration of PUFA are thought to

have the most normal morphology.[9] Reactive oxygen species (ROS) can cause instability of the membrane permeability through effects on PUFA as these fatty acids are extremely sensitive to OS. Indeed, the most protective anti-peroxidative mechanisms are thought to maintain cell membrane stability.[10]

The use of anti-estrogens is helpful in altering the intrinsic hypothalamo-gondal hormone axis. The most popular of these drug is clomiphene citrate (CC), a selective oestrogen receptor modulator (SERM), acts by reducing hypothalamic and pituitary sensitivity to estrogen, anti-estrogens increase pituitary outcome of luteinizing hormone (LH) and follicular stimulating hormone (FSH), thus, stimulating both testosterone production and spermatogenesis.[16] Despite of at least 20 clinical trials with CC for male infertility in the last 30 years, there is still debate about the value of anti-estrogens for male infertility. There is emerging literature to suggest that CC may play an important role in a subgroup of infertile patients to overcome acquired hypogonadotropic hypogonadism, stimulating the pituitary level due to prolactinemia, sickle cell disease and diabetes mellitus.[17] Other selective estrogens receptor modulator drugs, including tamoxifene citrate, which act in a similar fashion to CC.[18,19]

This study aims to identify the pharmacological effects of a combination of CC and L-carnitine on reducing the levels of spermatozoal LP and improving the outcomes in obese male with idiopathic subfertile normozoospermia.

Patients and Methods

This is a randomized prospective study carried out at the Maternity and Childhood Hospital, Al-Najaf, Iraq. The study included 55 obese (BMI over 30 Kg/m^2) males diagnosed with idiopathic subfertile normozoospermia for over 1 year. Participants were divided into two study groups: Group 1 consisted of 35 participants and they were given CC (Asia Pharmaceutical Industries, 50 mg once a day) and L-carnitine (Ultimate Nutrition INC Farmington, CT 06034USA at dose 1000 mg twice a day) for 3 months. Group 2 consisted of 20 participants who did not receive any medication except placebo. In addition, the study included 10 healthy fertile males (Group 3) who initiated a successful pregnancy within the last 2 years, were served as control subjects.

The study excluded patients with proven normal sperm parameters and infertility factor that might interfere with fertility-related origin. Like hypogonadotropic hypogonadism, varicocele, cryptorchidism, venereal disease, leucocytospermia, drug and hormonal therapy, abnormal sexual function (erectile dysfunction and impotence) and any patient who had difficulties in semen collection by masturbation or coitus interrupts.

All participants of this study were received participants information sheet and they were given enough time to read the sheet and discuss their questions with the chief investigators. Written consents were collected from the participants before being actively enroll in the study. Full history and complete physical examination were conducted at the beginning of the study.

Semen Collection and Preparation

Semen samples were collected from all participants either by masturbation or coitus interrupts after 3–5 days of sexual abstinence. Collected samples were left for 30 min before start of the laboratory analysis to permit liquefaction. Each specimen was divided into aliquot. The first part was used to examine in details all macroscopic and microscopic examinations according to WHO criteria.[20] Quantitation of seminal granular leucocytes were assessed according to Endtz test.[21] The second aliquot was used for a preparation of sperm homogenization buffer for measurement of malondialdehyde (MDA) as an index of a spermatozoal lipid peroxidation. All investigations were carried out before and after period of 3 months treatment for whole participants.

Spermatozoa were separated from seminal plasma by centrifugation at 500x rpm for 30 min. The supernatant was precisely measured by a graduated centrifuge test tube and discarded. The supernatant was used for enzymatic measurements.[22] Homogenizing buffer added to the pellet fraction. Homogenizing buffer consisted of (11.9 gms of manitol, 4.8 gms of sucrose, 0.09 gms of EDTA in 250 ml of distilled water adjust the pH to 7.4 with tris-base). Homogenized buffer was kept in refrigerator at 4°C. The samples were hand homogenized and were subsequently centrifuged for 10 min at 3000 rpm. Cooled 0.9 ml of Triton X, 100 (0.1%) was added to each 0.1 ml of pellet obtained from the sample, the samples were centrifuged again at 8000 rpm for half an hour in a centrifuge.

Determination of Malondialdehyde (MDA)

The MDA was determined by the use of thiobarbituric acid (TBA) assay of Mihra and Uchiyama.[23] MDA react with TBA to form a pink colored product. Sperm homogenate (500 ml) was added to 3 ml of 1% phosphoric acid, 1.0 ml of 0.6% TBA and 0.15 ml of 2.0% butylated hydroxytoluene (BHT) in 95% methanol. The samples were heated in a boiling water bath for 45 min, cooled and 4.0 ml of butanol was added. The butanol phase was separated by centrifugation at 3000 rpm. All values were expressed as nmoles/mg of protein using spectrophotometer Cecil, 1011 England in measurements.

Calculations

MDA concentration (nmol/mg) = $(A/L \times Eo) \times D \times 10^6$
A = absorption
L = light bath
Eo = extension coefficient (molar absorptivity)
1.56×10^5 $m^{-1}.cm^{-1}$
D = dilution factor 6.7.

Radioimmunoassay (RIA)

A 5 ml venous blood was drawn from all the participants (Groups 1, 2 and 3) around 9 am, and blood samples were kept on a rack for 30 min for clotting. Blood samples were centrifuged at 3000 rpm for 15 min to allow separation of serum. The sera were aspirated and kept at −20°C frozen until analyzed. LH, FSH, and testosterone hormone (T) were assessed using the (immunotech a Beckman Coulter Company, cat.# 2125, immune-radiometric assay kit, reference 1M 2125-1M 3301 and mini gamma counter, LKB-Wallac).

Statistical Analysis

Data were analyzed using inbuilt functions within the statistical package of SPSS version.[18] Mean and standard error or means were measured for the continuous variables. Analysis of variance (ANOVA) and least significant difference (LSD) between

means at the level of significance is (0.05). All hypothesis testing two-tailed considering a significant *P* value at or below 0.05.

Results

Table 1 showed the sperms characteristics of Group 1 ($n = 35$) before the treatment and after the 3 months of the treatment. There were significant increase in the levels of sperm counts ($P = 0.02$), sperm active motility ($P = 0.001$), sperm viability ($P = 0.01$), and sperm morphology ($P = 0.01$), in this group after the end of the 3-months treatment. In addition, there were significant improvement in the levels of MDA after the treatment ($P = 0.001$) (Table 1).

Table 2 compared the results of Group 1 ($n = 35$) with Group 2 ($n = 20$) after 3 months of the treatment. It showed a significant rise in the levels of sperm counts ($P = 0.045$), sperm concentration per volume ($P = 0.01$), sperm active motility ($P = 0.01$) in Group 1 compared to Group 2. In addition, the levels of MDA were significantly reduced in Group 1 compared to Group 2 (Table 2).

We have also compared the control group (Group 3) with both Group 2 and 1 at the end of the 3 months and we have found that there is significant improvement for the most of the sperm parameters in Group 1 compared to Groups 2 in relation to Group 3 parameters (Table 3).

Discussion

There is a complex mechanism linking obesity, male's idiopathic subfertility and infertility. In spite of the clear shortage of exploration of the underlying mechanisms with lack of effective therapeutic interventions, the present study calls for a greater clinician awareness of the effects of obesity on male's infertility; to have a better understanding of underlying mechanisms and avenues for mitigation of treatment.

From this study (and previous studies), we notice the significant effects of obesity on male's subfertility, and how management of obesity can improve the fertility outcomes in this population.[24] Many health experts believe that overweight and obesity need to be controlled to reverse both unhealthy consequences associated with obesity and the negative impacts on male's fertility.[25,26] The increase in the incidence of obesity has a substantial social health impact. Contrasting reports have been published whether overweight and obesity affect male fertility.[1] Cabler et al. hypothesized that there is a corresponding improvement in male's fertility and fecundity after treating obesity and obesity should be considered as one of the etiological factors for male's infertility.[24]

Obesity increases the risk of hypogonadtropic hypogonadism. In animal models, it was found that obesity can cause leptin insensitivity in the hypothalamus, which leads to a decrease in "KISS1" gene expression, which in turn, alters the release of gonadotropin-releasing hormone (GnRH).[1] Aromatase inhibitors are one of the available treatment options for obese males facing infertility problems, especially if they have elevated estrogen and lowered testosterone levels.[27] These facts support our findings of the presence of abnormal sperm parameters in obese males and how these parameters can be improved by giving the appropriate treatment which are directed to reducing body weight. In addition, the finding from other studies which further support our study, is that the treatment with aromatase inhibitors can lead to normalization of patients testosterone, LH, FSH hormone levels and normalization of spermatogenesis.[28,29]

The reduction in the levels of MDA in Group 1 after treatment (Table 1), can be partially explains the improvement in male's fertility due to the use of "L-carnitine." This is a proved medication, which can helps to reduce the degree of LP and improving the overall antioxidants levels of sperms.[9,10] And also supports Abdulrazik's study that mentioned L-carnitine is required during oxidation of lipids for transport of fatty acids from the cytosol to mitochondria for generation of energy.[11] L-carnitine exerts a substantial anti-oxidant, anti-cytokines and anti-apoptotic activity providing multi-mechanisms proactive effects for cells.[11] A previous study done to monitor the aging process had mentioned that L-carnitine possess anti-oxidant activity that combines both free-radicals scavenging and metal-chelating properties.[12]

Findings of Table 2 consolidate the data in Table 1 in regards to the significant reduction of the MDA levels in Group 1 compared to Group 2 at the end of the treatment course. This confirms the effects of L-carnitine in reducing the production of ROS and improving the levels of LP (MDA levels). This resulted in improving sperm vitality % in obese subfertile patients after failure to respond of treatment patient to integrated therapy of empirical medicine.[7,26]

Table 3 demonstrated improving in the overall sperm parameters and the MDA levels in Group 1 compared after the end of the treatment course. This is reflected by the insignificant results of these parameters in Group 1 compared to Group 3. Something which is not seen when comparing Group 2 to Group 3.[30] It is worth mentioning here that the etiological cause in nearly one-third of male's infertility is still unknown, and the presence of men with idiopathethic infertility who have been successfully treated by empirical therapeutics modalities is not high.[31]

Conclusion

This study suggests that a drug combination of CC and L-carnitine can be effective treatment to reduce the spermatozoal LP and improves sperm vitality in obese male with idiopathic subfertile normozoospermia.

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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