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Efficacy of tamoxifen and L-carnitine on sperm ultrastructure and seminal oxidative stress in patients with idiopathic oligoasthenoteratozoospermia

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Summary

Idiopathic oligoathenoteratozoospermia (iOAT) is a common finding in the evaluation of male infertility. Oxidative stress (OS) may underlie its pathology. Tamoxifen and L-carnitine are used to treat idiopathic male infertility. The aim of this work was to detect the efficacy of tamoxifen and L-carnitine on sperm parameters, sperm ultrastructure and seminal OS in iOAT patients. Sixty patients were recruited for this study and divided into three groups; the 1st was treated with tamoxifen, 2nd with L-carnitine and 3rd with both drugs. Semen analysis, malondialdehye (MDA) level and transmission electron microscopy were performed before and after three months treatment. The first group showed significant improvement in MDA levels, sperm concentration, sperm morphology, ultrastructural head, acrosomal and mitochondrial anomalies ($P < 0.01$). Other parameters were not significantly improved. In the 2nd group, significant improvements in MDA, sperm motility, sperm morphology, ultrastructural mitochondrial and tail anomalies were detected ($P < 0.01$). No significant improvement in the other parameters. Third group showed improvement in MDA, all semen parameters and all ultrastructural anomalies ($P < 0.01$). In conclusion, tamoxifen and L-carnitine are effective in improving seminal OS, semen parameters and sperm ultrastructure. Combination of both drugs is superior to monotherapy.

Introduction

Oligoasthenoteratozoospermia (OAT) is a condition of abnormally low sperm concentration, motility and normal sperm morphology in the ejaculate. OAT affects approximately 30% of all infertile men. About 30% of all OAT patients are diagnosed as idiopathic (Bonanomi *et al.*, 2002; Singh *et al.*, 2012). Idiopathic oligoasthenoteratozoospermia (iOAT) is defined as a decrease in the sperm concentration, sperm motility and normal sperm morphology of unknown aetiology after thorough clinical examination, instrumental, or laboratory investigations (Bonanomi *et al.*, 2002). There are many causes of OAT which are usually overlapping. It is still difficult to detect the primary cause (if any) of iOAT, and more than one cause is needed to affect sperm patterns. Various factors are included in the aetiology of OAT such as age (Shamsi *et al.*, 2008), genetic factors (Ferlin *et al.*, 2006; Imken *et al.*, 2007), genital infections (Momoh *et al.*, 2011; Jungwirth *et al.*, 2012), environmental pollutants (Hauser *et al.*, 2007; Mendiola *et al.*, 2008), smoking (Olooto, 2012), hormonal abnormalities (Sharma *et al.*, 2001), varicocele (Jungwirth *et al.*, 2012; El Taieb *et al.*, 2013), and drugs (Brezina *et al.*, 2012).

The exact pathogenesis of iOAT is still unclear. The accepted pathogenesis is that the above mentioned aetiologies lead to increased reactive oxygen species (ROS) concentration and to increased oxidative stress (OS) which negatively affect spermatogenesis. In turn, impaired spermatogenesis leads to increase ROS concentration and to increase OS. ROS can affect sperms by many ways including lipid peroxidation, mitochondrial damage, apoptosis and DNA damage (El-Taieb *et al.*, 2009). Spermatozoa, like all other aerobic cells are particularly susceptible to OS induced damage because of the large polyunsaturated fat content in their membranes. Men

with high ROS may have a lower fertility potential compared to those with low ROS. High levels of ROS in semen have been correlated with reduced sperm motility and damage to sperm nuclear DNA (Makker *et al.*, 2009; Iacono *et al.*, 2013).

Reactive oxygen species change lipid/protein ratio of membranes by affecting polyunsaturated fatty acids and lipid peroxidation which causes functional irregularities of several cellular organelles (Ferramosca *et al.*, 2013). OS causes reduction in mitochondrial functionality which may lead to decrease in sperm motility (Ferramosca *et al.*, 2013). ROS are known to attack DNA inducing strand breaks and other oxidative-based damage in spermatozoa (El-Tohamy, 2012; Khalil *et al.*, 2012). ROS also can cause various types of gene mutations such as point mutations and polymorphism, resulting in decreased semen quality (Sharma *et al.*, 2004). Spermatozoa have large quantities of polyunsaturated fatty acids in their plasma membrane and low levels of scavenging enzymes in their cytoplasm. Therefore, it is subject to peroxidation from elevated seminal ROS levels (Hadwan *et al.*, 2013). One of the by-products of lipid peroxidation is malondialdehyde (MDA). This by-product has been used in various biochemical assays to monitor the degree of peroxidative damage sustained by spermatozoa (Aitken & Fisher, 1994).These pathological effects of OS on spermatozoa have been found by researchers to be correlated with sperm parameters. The most important negative correlation is between OS and sperm concentration (Saraniya *et al.*, 2008), motility (Agarwal *et al.*, 2008) and morphology (Aydemir *et al.*, 2008).

L-carnitine is highly concentrated in the epididymis and plays a crucial role in sperm metabolism and maturation. Many studies support the conclusion that L-carnitine can significantly improve both sperm concentration and total sperm counts among men with astheno or oligoasthenozoospermia (Sheikh *et al.*, 2007; Peivandi *et al.*, 2010). Many human clinical trials have found that L-carnitine therapy can optimise sperm motion parameters in men with astheno or oligoasthenozoospermia (Vicari *et al.*, 2002; Lenzi *et al.*, 2003; Sigman *et al.*, 2006). The mechanism of action of L-carnitine is not fully understood. It may accelerate lipid metabolism and has a pivotal role in mitochondrial β-oxidation of long-chain fatty acids for cellular energy production (Kerner & Hoppel, 1998). It has an effect in Sertoli cell–spermatogenic line interaction (Lenzi *et al.*, 2003). In addition, carnitine administration increases prostaglandin E2 concentration (Vicari & Calogero, 2001), which affects sperm count (Cavallini *et al.*, 2004). Moreover, carnitine protects cell membrane and DNA against damage induced by ROS, so it acts as an 'anti-ageing' substance, protecting against damage induced by ROS (Aram *et al.*, 2012).

Anti-oestrogens are the most commonly used therapy for idiopathic infertility. Oestrogen antagonists as empiric medical therapy for idiopathic male infertility may increase spontaneous pregnancy rate, improve sperm concentration and percentage of sperm motility (Chua *et al.*, 2013).Tamoxifen is an anti-oestrogen drug, that is, widely used to treat breast cancer. Tamoxifen citrate was introduced as an empirical treatment for iOAT, and it was found that tamoxifen improved total sperm count, motility and functional sperm fraction after 3 and 6 months (Adamopoulos, 2000). The exact mechanism by which tamoxifen increases sperm parameters is not fully understood. It has a stimulatory action on gonadotrophin secretion, its direct effect on Leydig cell function, and because of its production of 5a-dihydrotestosterone in tubules and epididymis (Cavallini *et al.*, 2004; Vandekerckhove *et al.*, 2007). Tamoxifen is extensively metabolised to give 4-hydroxytamoxifen (OHTAM) and *N*-desmethyltamoxifen (Fromson *et al.*, 1973). Besides their chemopreventing function, tamoxifen and OHTAM, which are extremely lipophilic (Custódio *et al.*, 1993), induce multiple cellular effects, including antioxidant actions as they are strong intramembranous scavengers of peroxyl radicals (Custódio *et al.*, 1994).

In this study, we measured MDA as an indicator of ROS levels in iOAT patients before and after treatment by L-carnitine and tamoxifen. We compared efficacy of L-carnitine and tamoxifen on semen parameters in iOAT patients. We compared the effects of L-carnitine and tamoxifen on sperm ultrastructure in iOAT patients.

Patients

Sixty patients with iOAT were recruited for this study. They were selected between May 2011 to August 2013 at Andrology clinic, Department of Dermatology, Venereology and Andrology, Qina University Hospital, South Valley University. Patients were divided into three groups: first group (20 patients) were treated by tamoxifen 20 mg day^{-1} for 3 months, second group (20) patients were treated with L-carnitine 3 g day^{-1}, and the third group were treated with both drugs.

Full history taking, complete general examinations, genital local examination and complete investigations were carried out to exclude any detectable cause of OAT. Patients with iOAT syndrome were selected according to the following inclusion criteria: sperm concentration less than 15 million per millilitre, forward progressive sperm motility <32%, normal sperm morphology <4%, leucocytes concentration <1 million per millilitre according to WHO definition of leucocytospermia (World Health Organization, 2010), negative semen culture, no detectable varicocele either by clinical examination or by scrotal

coloured duplex, normal hormonal assay, no smoking or smoking history in the last six months, no history of chronic, systemic or infectious disease known to impair spermatogenesis and normal genital examination. A written consent was signed by each patient included in this study. The study was approved by our local ethical committee.

Semen analysis

Semen analysis was performed at our Andrology clinic according to WHO guidelines (World Health Organization, 2010). Sperm parameters were determined by a blind physician pre- and post-treatment. Sperm concentration per millilitre, percentage of progressive and total sperm motility and percentage of normal sperm morphology were determined.

ROS measurement

Malondialdehye levels were measured as per thiobarbituric acid (TBA) method (Hsieh *et al.*, 2006). 0.1 ml of seminal plasma was added to 0.9 ml of distilled water in a glass tube; to it 0.5 ml of TBA reagent was added and then heated for 1 h in a boiling water bath. After cooling, the tube was centrifuged for 10 min at 4000×*g* and the supernatant absorbance was read on a spectrophotometer at 534 nm (Das *et al.*, 2009).

Electron microscopy examination

An aliquot of each semen sample was prepared for transmission electron microscope (TEM). Samples were fixed in (4% paraformaldehyde resolved in 0.1 M cacodylate buffer +1% glutaraldehyde; Merck, Cairo, Egypt), Then, agarose pellets were made: 1% agarose (high gelling agarose resolved in phosphate buffer solution; Sigma, Cairo, Egypt) was warmed to 80°C. The semen samples were washed in phosphate buffer solution four times, and then, samples were warmed up to 60°C. One percent agarose was put on the samples and then mixed, centrifuged and cooled in crush ice for 3 h. Subsequently, pellets were collected in 0.1 M cacodylate buffer. All samples were embedded for 1 day. The embedding procedure included exchange of the 0.1 M cacodylate buffer (15 min), then 3 h in 1% osmium tetroxide (2% OsO4 resolved in 3% potassium hexacyanoferrat), then, twice washing for 15 min. Then samples were dehydrated in ascending grades of ethanol (every step: twice for 15 min: 70, 80, 96 and 100% alcohol). Then, samples were put in medium 1,2 propylen oxide twice for 15 min. Then in mixture 1 : 1,2 propylenoxide – epoxy resin for 3 h, then in absolute epoxy resin overnight on the shaker. The following day all samples were embedded in fresh epoxy resin in special embedding forms and put into the oven (60°C) for 24 h (Glauret, 1986; Haidl *et al.*, 1991).

All samples were sectioned semi thin (about 1, 5 μm) and then ultra thin (about 80 nm) with the Ultracut E (Reichert–Jung, Munich, Germany). The ultrathin sections were put on copper grids (150 mesh; Balzers, Milton keynes, UK) and stained with uranyl acetate (Agar, Essex, UK) and lead citrate (Merck, Cairo, Egypt). After all, samples were washed and examined on a Jeol Jem 1010 (Jeol, Tokyo, Japan). In each sample, 200 sections in the head and 200 sections in the tail were examined to detect the percentage of normal head, mid-piece and tail, as well as the percentage of different head and tail anomalies in both study groups.

Statistical analysis

Mean standard (mean ± SD) of sperm parameters quality in iOAT patients was analysed by descriptive statistic. The relationship of the MDA levels with sperm count, motility, morphology and semen volume was also compared. Samples *t*-test and linear regression model were applied to compare seminal MDA and sperm quality in all the samples.

Results

This study was conducted on 60 iOAT patients classified into three groups. The mean ages for all groups were (35.26 ± 10.07), (33.5 ± 7.69) and (32.55 ± 65) respectively. There was no statistically significant difference regarding the mean ages between three groups ($P = 0.58$) (Table 1).

I-MDA measurements

Malondialdehye concentrations in seminal plasma were (2.54 ± 0.6) before tamoxifen therapy and decreased to (1.55 ± 0.5) after therapy with a high significant difference (Table 2). In L-carnitine-treated group MDA was (3.44 ± 0.4) before treatment decreased to (2.45 ± 0.3) after therapy with a highly significant results (Table 3). In

Table 1 Descriptive statistics of the patients' age

Age	Statistic
Mean	37.1
95% confidence interval fr mean	32.7–41.4
5% trimmed mean	36.7
Median	34.0
Standard deviation	10.5

Table 2 Malondialdehye (MDA) concentration and sperm parameters before and after tamoxifen therapy

Sperm parameters	Before (mean ± SD)	After (mean ± SD)	*P* value[a]
MDA concentration (nmol ml^{-1})	2.54 ± 0.6	1.55 ± 0.5	<0.001
Semen volume (ml)	2.73 ± 1.5	2.93 ± 1.2	<0.01
Sperm concentration ($\times 10^6$ ml^{-1})	7.27 ± 3.2	9.54 ± 2.6	<0.01
Progressive sperm motility (%)	16.52 ± 5.8	20.32 ± 2.7	0.072
Normal sperm morphology (%)[a]	2.38 ± 3.7	7.02 ± 4.3	<0.001
Head anomalies	44.8 ± 8.1	41.7 ± 6.5	<0.001
Tail anomalies	8.84 ± 3.8	8.80 ± 3.8	0.19

[a]Wilcoxon signed ranks test was used.

Table 3 Malondialdehye (MDA) concentration and sperm parameters before and after L-carnitine therapy

	L-carnitine (mean ± SD)		
Sperm parameters	Before	After	*P* value[a]
MDA concentration (nmol ml^{-1})	3.44 ± 0.4	2.45 ± 0.3	<0.001
Semen volume (ml)	3.75 ± 1.5	3.95 ± 1.2	<0.01
Sperm concentration ($\times 10^6$ ml^{-1})	6.27 ± 3.2	7.54 ± 2.6	0.61
Progressive sperm motility (%)	14.50 ± 5.7	22.30 ± 2.6	<0.001
Normal sperm morphology (%)[a]	3.40 ± 3.7	8.04 ± 4.3	<0.001
Head anomalies	43.8 ± 8.1	42.7 ± 6.5	0.32
Tail anomalies	7.83 ± 2.7	5.29 ± 2.7	<0.05

[a]Wilcoxon signed ranks test was used.

the third group, MDA was (2.49 ± 0.3) before therapy and became (1.50 ± 0.2) after therapy with also significant result (Table 4).

II-Semen parameters

In tamoxifen therapy, the average values of semen volume, sperm concentration, progressive sperm motility, normal morphology, head anomalies and tail anomalies before and after treatment are shown in Table 2. There was a statistically significant association between tamoxifen and improvement in semen volume, sperm concentration, normal sperm morphology and head anomalies. ($P < 0.01$, <0.01, <0.001, <0.001), but not with the progressive sperm motility and tail anomalies ($P = 0.072$ and 0.19 respectively).

Table 4 Malondialdehye (MDA) concentration and sperm parameters before and after combined tamoxifen and L-carnitine therapy

Sperm parameters	Before (mean ± SD)	After (mean ± SD)	*P* value[a]
MDA concentration (nmol ml^{-1})	2.49 ± 0.3	1.50 ± 0.2	<0.001
Semen volume (ml)	2.70 ± 1.5	2.90 ± 1.2	<0.01
Sperm concentration ($\times 10^6$ ml^{-1})	8.29 ± 3.2	10.56 ± 2.6	<0.01
Progressive sperm motility (%)	17.55 ± 6.8	25.35 ± 3.7	<0.001
Normal sperm morphology (%)[a]	9.38 ± 2.6	14.02 ± 3.2	<0.001
Head anomalies	40.5 ± 6.1	37.4 ± 4.5	<0.001
Tail anomalies	9.80 ± 3.8	7.26 ± 3.8	<0.001

[a]Wilcoxon signed ranks test was used.

In the L-carnitine group, the average values of semen volume, sperm concentration, progressive sperm motility, normal morphology, head anomalies and tail anomalies before and after treatment are shown in Table 3. There was a statistically significant association between L-carnitine and improvement in semen volume, progressive sperm motility, normal sperm morphology and tail anomalies. ($P < 0.01$, <0.001, <0.001 and <0.05) respectively, but not with the sperm concentration and head anomalies ($P = 0.61$ and 0.32).

In the third group, the average values of semen volume, sperm concentration, progressive sperm motility, normal morphology, head anomalies and tail anomalies before and after treatment are shown in Table 4. All the above mentioned parameters were improved after combined therapy with high statistically significant results ($P < 0.01$, <0.01, <0.001 < 0.001, <0.001 and >0.05) respectively.

III-TEM findings

Transmission electron microscopy findings of tamoxifen treated group showed percentages of normal head, binucleated head, nuclear vacuolations, nuclear inclusions, abnormal nuclear density, acrosomal inclusions, mitochondrial anomalies, normal tail, absent axoneme, missing central singlet and missing outer doublets before and after treatment are shown in Table 5. There was a statistically significant association between tamoxifen treatment and improvement of percentages of normal head, binucleated head, nuclear vacuolations, nuclear inclusions, abnormal nuclear density, acrosomal inclusions and mitochondrial anomalies ($P < 0.001$), but not with normal tail, absent axoneme, missing central singlet and missing outer doublets ($P = 0.09$, 0.08, 0.07 and 0.09 respectively).

Table 5 Transmission electron microscopy findings before and after tamoxifen

	Before (mean ± SD)	After (mean ± SD)	*P* value
Normal head (%)	52.56 ± 4.66	73.39 ± 6.14	<0.001
Binucleated head (%)	7.65 ± 1.59	5.06 ± 1.11	<0.001
Nuclear vaculations (%)	20.26 ± 4.37	12.22 ± 2.16	<0.001
Nuclear inclusions (%)	18.24 ± 3.98	10.44 ± 2.09	<0.001
Abnormal nuclear density (%)	7.13 ± 1.58	4.51 ± 0.87	<0.001
Acrosomal inclusions (%)	8.74 ± 1.93	5.06 ± 1.73	<0.001
Mitochondrial anomalies (%)	32.03 ± 5.25	18.78 ± 2.65	<0.001
Normal tail (%)	46.59 ± 3.62	50.83 ± 4.94	0.09
Absent axoneme	6.35 ± 1.54	5.44 ± 1.04	0.08
Missing central singlet (%)	10.59 ± 2.43	9.39 ± 1.46	0.07
Missing outer doublets (%)	23.26 ± 4.27	21.67 ± 2.19	0.09

Data are presented and standard deviation (SD) $P < 0.001$ = highly significant.

In the L-carnitine treated group TEM findings showed that percentages of normal head, binucleated head, nuclear vacuolations, nuclear inclusions, abnormal nuclear density, acrosomal inclusions, mitochondrial anomalies, normal tail, absent axoneme, missing central singlet, missing outer doublets before and after treatment are shown in Table 6. There was a statistically significant association between L-carnitine therapy and improvement of percentages of abnormal nuclear density, acrosomal inclusions, mitochondrial anomalies, normal tail, absent axoneme, missing central singlet and missing outer doublets. ($P < 0.001$), but not with normal head, binucleated head, nuclear vacuolations and nuclear inclusions ($P = 0.16$, 0.07, 0.06 and 0.06 respectively).

In patients treated with both drugs, TEM findings showed that percentage of normal head, binucleated head, nuclear vacuolations, nuclear inclusions, abnormal nuclear density, acrosomal inclusions, mitochondrial anomalies, normal tail, absent axoneme, missing central singlet and missing outer doublets before and after treatment are shown in Table 7. All ultrastructure abnormalities were improved after treatment with both drugs with statistically significant results ($P < 0.001$) (Figs 1–8).

Table 6 Transmission electron microscopy findings before and after L-carnitine

	Before (mean ± SD)	After (mean ± SD)	*P* value
Normal head (%)	51.56 ± 4.66	53.39 ± 6.14	0.16
Binucleated head (%)	6.65 ± 1.59	5.06 ± 1.11	0.07
Nuclear vaculations (%)	19.26 ± 4.37	16.22 ± 2.16	0.06
Nuclear inclusions (%)	20.24 ± 3.98	19.44 ± 2.09	0.06
Abnormal nuclear density (%)	8.12 ± 1.59	5.50 ± 0.88	<0.001
Acrosomal inclusions (%)	9.75 ± 1.94	6.07 ± 1.74	<0.001
Mitochondrial anomalies (%)	33.04 ± 6.27	19.79 ± 3.67	<0.001
Normal tail (%)	44.57 ± 3.60	62.81 ± 4.92	<0.001
Absent axoneme	7.36 ± 2.54	4.45 ± 2.04	<0.001
Missing central singlet (%)	7.58 ± 2.42	3.38 ± 1.45	<0.001
Missing outer doublets (%)	20.23 ± 4.27	12.64 ± 2.19	<0.001

Data are presented and standard deviation (SD) $P < 0.001$ = highly significant.

Table 7 Transmission electron microscopy findings before and after tamoxifen and L-carnitine

	Before (mean ± SD)	After (mean ± SD)	*P* value
Normal head (%)	49.55 ± 4.66	70.38 ± 6.14	<0.001
Binucleated head (%)	5.60 ± 1.57	3.01 ± 1.09	<0.001
Nuclear vaculations (%)	16.25 ± 3.37	8.21 ± 1.16	<0.001
Nuclear inclusions (%)	21.25 ± 3.99	13.45 ± 2.10	<0.001
Abnormal nuclear density (%)	5.11 ± 1.55	2.49 ± 0.84	<0.001
Acrosomal inclusions (%)	7.70 ± 1.93	4.02 ± 1.73	<0.001
Mitochondrial anomalies (%)	30.02 ± 5.25	16.77 ± 2.65	<0.001
Normal tail (%)	40.53 ± 2.62	58.77 ± 3.94	<0.001
Absent axoneme	5.34 ± 1.53	2.43 ± 1.03	<0.001
Missing central singlet (%)	9.50 ± 2.43	5.40 ± 1.46	<0.001
Missing outer doublets (%)	18.21 ± 3.20	10.62 ± 1.12	<0.001

Data are presented and standard deviation (SD) $P < 0.001$ = highly significant.

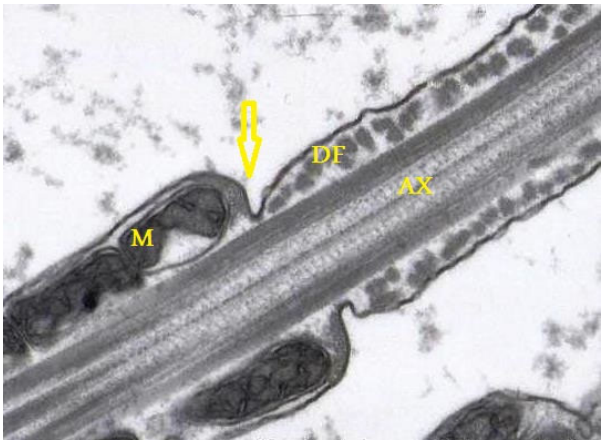

Fig. 1 Longitudinal section of a normal spermatozoon at the junction between the midpiece and tailpiece showing normally distributed mitochondria (M) (arrow), outer dense fibers (DF) and axoneme (AX) (Mag. 12000).

Fig. 2 Transverse Section in midpiece of a normal spermatozoon under TEM showing normal mitochondria (M), dense fibers (DF) and axoneme (AX) (Mag. 50000).

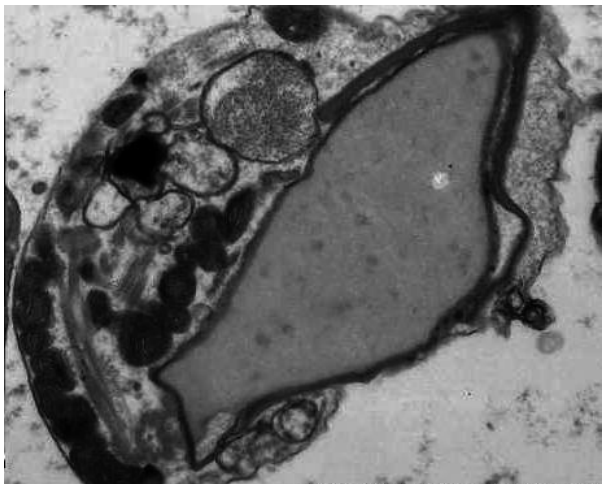

Fig. 3 Longitudinal section in an abnormal coiled spermatozoon of idiopathic oligoathenoteratozoospermia patients showing abnormal nuclear density, acrosomal redundancy and mitochondrial anomalies (Mag. 15000).

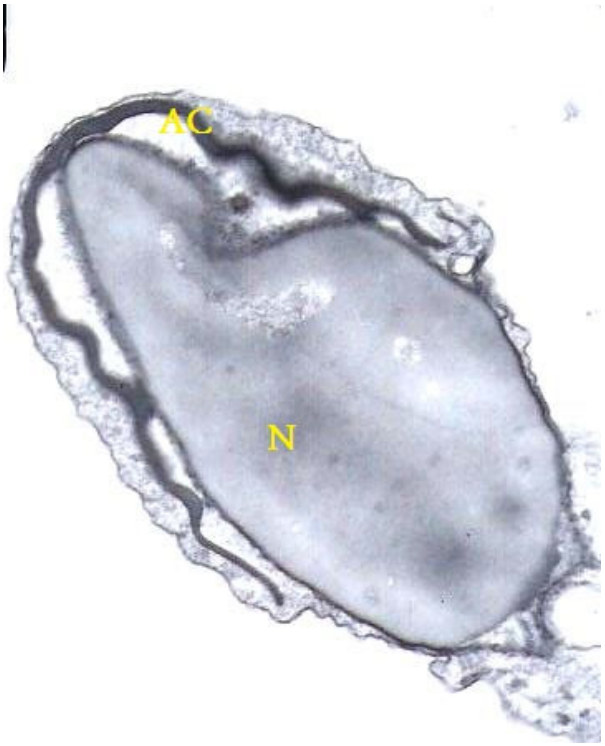

Fig. 4 Longitudinal section in the head of an abnormal spermatozoon of idiopathic oligoathenoteratozoospermia patients showing multiple nuclear vacuoles (V) within the context of the nucleus (N). The acrosome (AC) shows areas of redundancy (Mag. 15000).

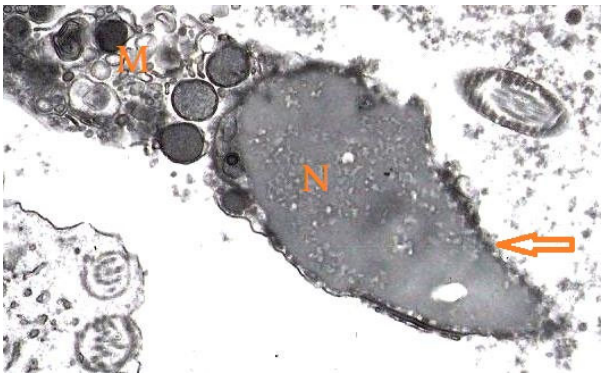

Fig. 5 Longitudinal section of abnormal spermatozoon of idiopathic oligoathenoteratozoospermia patients showing mitochondrial disorientations, abnormal nuclear density and abnormal Acrosome (arrow) (Mag. 15000).

Discussion

Idiopathic oligoathenoteratozoospermia is one of the most common findings in the era of male infertility workshops. It has no exact pathogenesis until now. The most accepted theory is that an undetectable cause leads to accummulation of ROS with resulting OS with its negative feedbacks on spermatogenesis (El-Taieb *et al.*, 2009; Iacono *et al.*, 2013). ROS play a fundamental role in the aetiology of male factor infertility; indeed, high ROS concentrations have been detected in 25–40% of infertile male semen sample (Agarwal *et al.*, 2003; Venkatesh *et al.*, 2009).

In this context, we measured MDA as an OS indicator in patients with iOAT before and after treatment with tamoxifen, L-carnitine and with both drugs. We detected

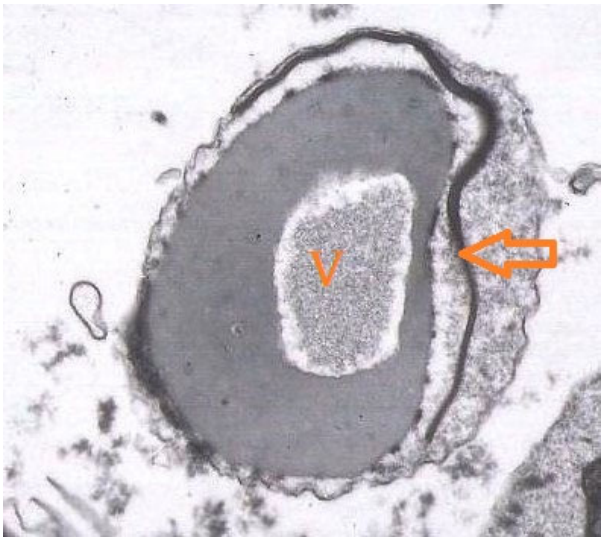

Fig. 6 Transverse section in the head of an abnormal spermatozoon of idiopathic oligoathenoteratozoospermia patients showing nuclear vacuole (V) within the context of the nucleus (N) and acrosomal redundancy (arrow) (Mag. 15000).

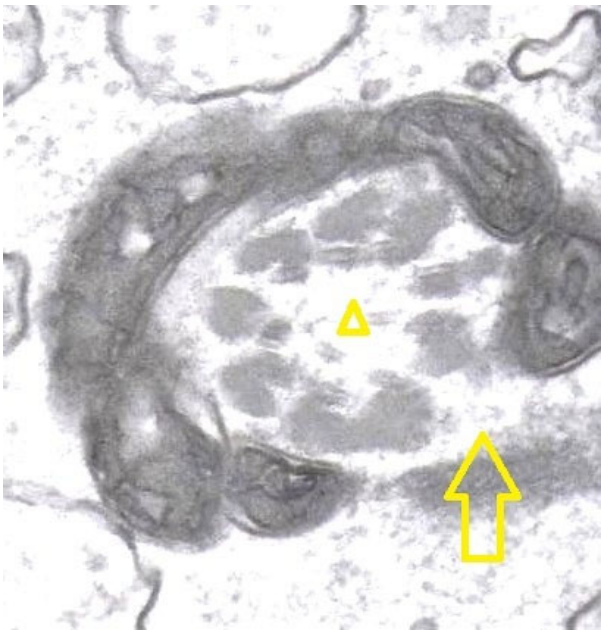

Fig. 7 Transverse section in the midpiece of an abnormal spermatozoon of iOAT patient showing abnormal mitochondria (M), absence of central singlets (CS) (Mag. 40000).

that MDA seminal plasma concentration was decreased after therapy with statistically significant results in all study groups. These results agree with the widely published data detected high levels of ROS in patients with abnormal semen parameters regardless the method of ROS and OS measurement. Desai *et al.* (2009) used the

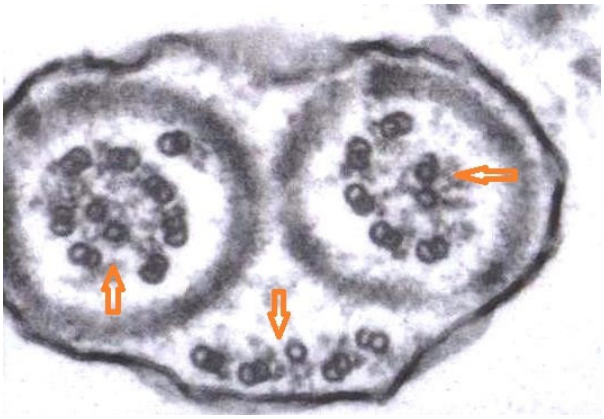

Fig. 8 Transverse Section of three tail pieces of an abnormal spermatozoa of idiopathic oligoathenoteratozoospermia patient showing absence of outer doublet microtubules (OD) and presence of an extra axonemal like structure enclosed by the same flagellar membrane (arrows) (Mag. 50000).

chemiluminescence assay to measure levels of ROS. They found higher levels of ROS in infertile patients with abnormal sperm parameters as compared with the normal controls. Higher levels of ROS have been detected in patients with idiopathic male infertility and abnormal semen parameters than those in normal controls (Pasqualotto *et al.*, 2008; El-Taieb *et al.*, 2009).

In the present study, all sperm parameters except tail anomalies were improved after tamoxifen treatment. This agrees with a number of uncontrolled trials suggesting improvement in all semen parameters and pregnancy rates with the use of anti-estrogens for idiopathic infertility (Allag & Alexander, 1979; Bartsch & Scheiber, 1981; Adamopoulos, 2000; Vandekerckhove *et al.*, 2000). Kotoulas *et al.* (1994) concluded that tamoxifen exerts a beneficial effect on sperm density and number of live spermatozoa, but it has no substantial effect in sperm motility and morphology. Adamopoulos *et al.* (1997) found that men with idiopathic infertility treated with tamoxifen (10 mg twice daily) for 3–6 months had a significant increase in sperm concentration, functional fraction and testicular volume compared with placebo. In a placebo controlled study, combined treatment with tamoxifen citrate and testosterone undecanoate was found to improve sperm variables and lead to a higher incidence of pregnancy in subfertile patients with idiopathic oligozoospermia (Adamopoulos *et al.*, 2003).

In patients treated with L-carnitine only, there was improvement in sperm motility, morphology and semen volume, and no significant improvement regarding sperm concentration. These results agree with previous reports of positive effects on sperm motility and morphology (Costa *et al.*, 1994; Afsaneh *et al.*, 2004; Sethumadhavan

& Chinnakannullg, 2006). These effects can be explained by the findings that L-carnitine plays a role in the energy metabolism, which positively affects sperm motility, maturation and the spermatogenic process (Oehninger & Kruger, 2007; Xin-Zhou & Zhai, 2007). In contrast to our findings, there are previous reports of a good increase in sperm count after L-carnitine therapy in a group of patients with idiopathic asthenozoospermia and treatment with L-carnitine prevents mitochondrial dysfunction (Moncada *et al.*, 1992; Vitali *et al.*, 1995; Scallet *et al.*, 2001). Sigman *et al.* (2006) concluded that carnitine supplementation demonstrated no clinically or statistically significant effect on sperm motility or total motile sperm counts in men with idiopathic asthenozoospermia.

Patients treated with both drugs showed significant improvement in all sperm parameters with superior efficacy than each drug alone. The combination therapy increased sperm concentration, percentage of progressive sperm motility and percentage of normal sperm morphology. Up to our knowledge, there is no previous work evaluated the efficacy of combination of tamoxifen and L-carnitine on sperm parameters and seminal OS in iOAT or in male infertility. This superior efficacy could be explained by combination of the mechanism of action of the two drugs.

Regarding sperm ultrastructure, tamoxifen treatment significantly improved head, acrosomal and mitochondrial ultrastructural anomalies. Tail anomalies showed no significant improvement. This could explain the improvement in sperm concentration and failure of tamoxifen to improve the sperm motility. Up to our knowledge, no previous work demonstrated the effect of tamoxifen on sperm ultrastructure.

Treatment with L-carnitine significantly improved nuclear, acrosomal, mitochondrial and tail anomalies but failed to improve head anomalies. This concedes with its positive effect on sperm motility. Our findings on L-carnitine agree with those of Abd El-Baset *et al.* (2010) who studied the efficacy of L-carnitine on sperm morphology in subfertile males by light and electron microscopy. They found no statistically significant differences in the fertile control group. In the oligo group, they observed highly significant decrease in the mean per cent of head defects, cytoplasmic droplet and mitochondrial sheath defects after treatment. In both astheno and terato groups, highly significant decrease in the mean per cent of head defects, mid-piece defects, cytoplasmic droplet and mitochondrial sheath defects were observed after treatment.

Conclusion

Our study concluded that both tamoxifen and L-carnitine reduce OS levels in patients with iOAT. Treatment with tamoxifen alone improved mainly sperm concentration and some morphological parameters but did not improve the sperm motility. L-carnitine improved sperm motility and some morphological parameters but not sperm concentration. Combined treatment improved all parameters.

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