Study the Spermatozoal Lipid Peroxidation and Creatine Kinase Activity on Sperm Parameters after Use of Simvastatin in Infertile Male Patients

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Abstract

The present study was carried out on a total of 55 patient with primary male factor infertility in Maternity and Childhood Teaching Hospital and Cancer Research Center, Kufa Medical College in Najaf Province between April 2005 - June 2006. The distribution of 55 patients in group I was classified into 8 subgroups according to the pathological cause of infertility and 35 normal proven fertility volunteer men served as a control group. All patient group was given simvastatin (simlo) tablets lpca, lpca laboratories Ltd Mubai: India at dose 20 mg twice per day for a period of 3 months. Spermatozoal malondialdehyde and creatine kinase (CK) were assessed before and after termination of treatment period. The present results showed a statistically significant decrease ($p<0.05$) in malondialdehyde MDA level and creatine kinase CK activity. Data also pointed out that there was a positive relation between spermatozoal MDA level and CK activity in most Simvastatin treated sperm pathological subgroups. This study might suggest and adopt an evidence of the action of simvastatin in lowering lipid peroxidation as MDA level and CK activity as an indicator of sperm parameters improvement due to antioxidant properties in patients with spermatozoal oxidative stress.

Key Words: Lipid peroxidation, creatine kinase, sperm, antioxidant.

دراسة تأثير مضادات الاكسدة بإستخدام عقارsimvastatin على فعالية اكسدة الشحوم و مستوىcreatine kinase في صفات السائل المنوي لمرضى العقم الذكري

الخلاصة

اجريت هذه الدراسة على (٥٥) مريضاً من الذكور ممن يعانون من العقم الاولي الابتدائي في وحدة علاج العقم في مستشفى النجف للولادة والاطفال وللفترة من نيسان ٢٠٠٥ الى حزيران ٢٠٠٦ وقد صنف هؤلاء المرضى الى ثمانية مجاميع ثانوية طبقاً للسبب المرضي للعقم وقد تم مقارنتهم مع (٣٥) شخصاً طبيعياً كمجموعة ضابطة . تم اعطاء هذه المجاميع عقار (Simvastatin) وبجرعة (٢٠ ملغم) مرتين في اليوم ولفترة ٣ أشهر . بينت هذه الدراسة وجود انخفاض معنوي في مستويات (Creatine Kinase & Malondialdehyde) في السائل المنوي للمرضى المعالجين بالعلاج المذكور مبينة دوره كمادة مانعة للأكسدة في تحسين صفات السائل المنوي في مرضى العقم الذي يعانون من ارتفاع في مستويات مضادات الاكسدة.

Introduction

Successful pregnancy results from an interaction between myriad physiological processes in both men and women and any disruption in this interaction system, whether in a man or woman, can result in an inability to have a biological child [1]. Despite the enormous progress in research and reasoning, most of the blame of 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 [2,3,4]. Defective sperm function is the most prevalent cause of male infertility and difficult condition to treat [5]. Data accumulated over the past few decades indicate that male factor infertility plays a role in approximately 50% of infertile couples [6,7]. 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 [8,9]. Standard semen analysis using light microscope is widely used in most laboratories for the initial evaluation of the male partner of an infertile couple, however, diagnosing defective sperm function by standard semen analysis is difficult because spermatozoa are highly specialized cells that express a diverse array of biological properties to achieve fertilization [10]. Recent study displayed that high levels of seminal reactive oxygen species (ROS) have been reported in up to 20-40% of infertile men [11]. Increased understanding of the role of oxdative stress (OS) in the pathophysiology of human sperm function has therefore become imperative in the study of human fertility. Moreover, a study indicated that high levels of ROS are detected in semen of (25-40%) of infertile men [12,13]. Spermatozoa are particularly susceptible to the damage induced by excessive ROS because their plasma membranes contain large quantities of polyunsaturated fatty acids (PUFA) [14] and their cytoplasma contain low concentration of scavenging enzymes [15]. A positive relationship was found between the rate of lipid peroxidation (LP) as measured by malondialdehyde (MDA) formation in sperm fractions separated by Percoll and creatine kinase (CK) activity and concluded that tests like lipid peroxidation and creatine kinase can be used to confirm loss of sperm function [16,17].

Lipid Peroxidation (LP) is broadly defined as oxidative deterioration of polyunsaturated fatty acid (PUFA) which are fatty acids that contain more than two carbon-carbon double bonds [18,19]. It is a physiological process occurring in all cells that are rich in lipids, especially PUFA. Lipid proxidation plays a significant role in the etiology of defective sperm function and the onset of LP in susceptible forms leads to the progressive accumulation of hydroperoxides in the sperm plasma membrane, which decomposes to malondialdehyde (MDA) which is an index of lipid peroxidation damage [20].

Creatine Kinase (CK) (E.C. 2.7.3.2) catalyzes the reversible phosphorelation of the ADP to ATP or creatine to creatine phosphate, thus maintaining an immediately accessible energy reservoir in the cell [21]. A number of independent studies have indicated that defective sperm function is associated with elevated levels of certain key enzymes such as creatine kinase [22-27]. Other studies mentioned that CK activity is inversely correlated with spermatozoal fertilizing potential [16,23] Simvastatin is an 3 hydroxy 3 methly-glytaryl coenzyme

A (HMG-CoA) reductase competitive inhibitor that is derived synthetically from fermentation of *aspergilus terreus*, it exerts a hypocholesterolemic action by stimulating an increase in LDL receptors on hepatocyte membrane thereby increasing the clearance of LDL from circulation [28]. Recent evidence suggests 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 cholesterol lowering independent (direct) effects on atherosclerotic vessel wall and this indicates that simvastatin treatment preserved the endothelial function associated with decrease in markers of oxidative stress, these beneficial endothelial effects of simvastatin are likely to occur independently of plasma lipid concentrations and to be mediated by its antioxidant properties of simvastatin treatment in the filed of male infertility in preserving sperm function parameters.

The present study was designed with following aims:

(1) To identify the clinical significance of simvastatin on lipid peroxidation (LP) and creatine kinase (CK) as antioxidant drug and subsequently on spermatozoal oxidative stress in field of male infertility.

(2) To identify the relation between spermtazoal LP and sperm function parameters.

(3) To identify the relation between spermatozoal CK on sperm function parameters.

Patients and Methods

Data collection: The present study was 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 2005 and June 2006, 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 spermiogram disruptive spermatogenesis and with normal sexual function while patients with infertility that will interfere with fertility-related origin were excluded from the study viz: hypopitutarism 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 (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 distriputiobs of 90 men in the study were fallen into 2 main groups, the first group composed of 55 patients was 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].

1- Asthenozoospermia (A),n=7
2- Asthenonecrozoospermia (AN),n=5
3- Asthenoteratozoospermia(AT),n=6
4- Asthenoteratonecrozoospermia(ATN),n=5
5- Oligoasthenoteratozoospermia (OAT),n= 8
6- Oligoasthenotetronecrozoospermia (OATN),n=9
7- Oligoasthenozoospermia (OA),n= 7
8- Teratozoospermia (T),n=8

The second group (control) consisted 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 after3-5 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^{o}C for 30 min to allow liquefaction. The specimen was examined according to Zaneveld and Polakoski techniques [29] and seminal leukocytes counts by positive myeloperoxidase staining (Endtz test) [30].

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). Homogenized buffer was kept in refrigerator at 4^{o}C. The samples were hand homogenized and 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 [31]

Lipid peroxidation: determination of spermatozoal 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 [32].

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 unite.of butanol was added. The butanol phase was separated by centrifugation at 3000xg 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.

Results

The results in all tables presented as (mean±SE) a comparison of post simvastatin (40mg daily for 3

months) treated infertile patients on sperm function parameters in each sperm pathological subgroup (n=55) with control group mean values of normal fertile volunteers (n=35).

Table (1) demonstrated that there was a high significant increase ($p<0.05$) of sluggish motility % in A, AT and ANT subgroup, respectively. However, there were also two subgroups with significant difference ($p<0.05$), the subgroups were OAT and OATN consecutively. The remaining AN, OA and T subgroups were not statistically significant ($p>0.05$).

Table (2) depicted that there was only A and AT subgroups cited the high significant decrease ($p<0.05$) of sperm immotile % respectively. While significant results ($p<0.05$) fall in OAT, OATN and OA subgroups. Moreover, AN, T subgroups did not show significant results ($p>0.05$).

Table (3) revealed the high statistically significant increase ($p<0.05$) of viable sperm % in post-treated patients in all sperm pathological subgroups except T subgroup that showed significant increase ($p<0.05$), while, OAT did not show significant difference ($p>0.05$).

Table (4) displayed the highest means of decrease of abnormal sperm morphology % that showed high statistically significant decrease ($p<0.05$) in AT, OATN and T subgroup whereas A, ATN and OA subgroups showed significant values ($p<0.05$) while, AN, OAT subgroups showed insignificant statistical results ($p>0.05$).

Table (5) showed that there was a high statistically significant decrease ($p<0.05$) of malondialdehyde in only T subgroup when compared to control mean consecutively. While, AN subgroup showed significant result ($p<0.05$). All other subgroups did not show statistically significant results ($p>0.05$)

Table (6) demonstrated that high statistically significant decrease ($p<0.05$) of creatine kinase fall in A, AT, and T subgroups. While, the subgroups that did not show statistical significant difference ($p>0.05$) in AN, ATN, OATN and OA. The post-treated value was 2.5 fold lower than pre-treated value in T subgroup.

Table (7) presented that there was a high statistically significant result ($p<0.05$) in all subgroups and without any exception. Creatine kinase level was approximately 6 fold, 7 fold and 11 fold higher in post-treated mean value than control mean value in T, A and OA subgroups respectively .

Discussion

In the present study, sperm motility % showed high significant increase ($P<0.05$) of sluggish motility % in A, AT and ANT subgroups and there was only high significant decrease ($P<0.05$) of sperm immotile % in A and AT subgroups table (1,2). In our belief, this improvement of sperm motility might be due to decrease in sperm lipid peroxidation namely MDA level and to the decrease in CK activity as well, these results are supported by [16-20]. Obviously, in the present study, the result of viable sperm showed that there are highly significant increase ($p<0.05$) in all simvastatin post-treated subgroup except T subgroup that showed significant difference ($p<0.05$). While, OAT subgroup showed insignificant effect ($p>0.05$), table (3), Our explanation of this result may be explain the effect of simvastatin act on decrease of lipid peroxidation as assessed by MDA level index, the decrease was about more than 1.5 fold lower than control mean value tables (6,7). In addition, there was statistical decrease ($p<0.05$) in abnormal sperm morphology table (4) this result might be related and ascertained by the result

of creatine kinase (CK) that showed a high significant decrease of CK activity in A, AT, OAT and T table (6), the most possible influenced subgroup in this decrease of CK is T subgroup that showed about (2.5) fold lower than the pre-treated patients. In light of our findings this study may attribute the decrease in CK activity to the decrease in lipid peroxidation that cites to the decrease in MDA level which may play the main role in decrease of ROS production with inhibition of other ROS-generating sources these results are corroborated with [24-26]. Interestingly, a study by Dandekar and Parkar (2002) correlated between CK activity and lipid peroxidation in male infertile patients and concluded that a positive correlation was observed between CK and lipid peroxidation [31] however, our data were consistent to Dandekar and Parkar results in accord to pre-treatment results, in addition to that simvastatin treatment act in decrease lipid peroxidation namely malondialdehyde level causing reduction the percentage of abnormal sperm form mid piece cytoplasmic surplus, this reason might be contribute to overwhelm the inverse effect of ROS on spermatozoal oxidative stress status that infertile spermatozoa have underwent.

No available data to compare our results with them but, generally speaking, our interpretation regarding these statistically significant results might come from the antioxidant effect of simvastatin treatment. It is clear that the improvement of sperm parameters in the involved pathological subgroups, in the present study specifically abnormal sperm form is interpreted probably were due to decrease in lipid peroxidation that may reflect its action on this remission by a dual reasons first, minimizes ROS production by decrease the lipid peroxidation [34] second, reinforces the decrease in sperm abnormal morphology % that may contribute again in lowering ROS stimulation as well this result was consistent with [35-41]. From this point of view our results are supported by other studies that mentioned increase ROS production result in an increase of morphologically abnormal cytoplasmic surplus [42]. However, the present study suggests that remission of sperm parameter variables is related with co-existence of this relationship. In decline of initiation and production of ROS in sperm of infertile patients in different pathological subgroups that may reflect on decrease in sper matozoal oxidative stress on the other hand, spermatozoa themselves are particularly susceptible to ROS damage by lipid peroxidation because their plasma membrane contain large quantities of polyunsaturated fatty acids and low concentration of scavenging enzymes and in this regard, our data corroborate with [43,44]. Lastly, in conclusion, the present study adapted the suggestion that simvastatin might have antioxidant properties resulting in decrease LP by use MDA level assay while the later may cause lowering of spermatozoal ROS in dual ways this might contribute in improvement of sperm parameter variables in different pathological subgroups.

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Table 1 Mean of **sperm sluggish motility %** of infertile patients after **Simvastatin** 40mg daily for 3 months treatment and control group of normal fertile volunteers (n=35) versus each pathological subgroup. Data are presented as mean ±(SEM).

Pathological subgroups (n=55)	Post Simvastatin treatment		P value
	mean	**±SEM**	
Asthenozoospermia (n=7)	28.36	±2.86	**0.00****
Asthenonecrozoospermia (n=5)	31.50	±1.50	**0.24**
Asthenoteratozoospermia (n=6)	31.40	±2.89	**0.00****
Asthenoteratonecrozoospermia(n=5)	21.50	±3.52	**0.00****
Oligoasthenoteratozoospermia (n=8)	33.00	±5.04	**0.03***
Oligoasthenoteratonecrozoospermia (n=9)	32.50	±12.50	**0.01***
Oligoasthenozoospermia (n=7)	25.00	±15.00	**0.79**
Teratozoospermia (n=8)	22.57	±3.53	**0.65**
Control group (n=35)	**16.08**	**±0.99**	

** Highly significant value P < 0.05 when value 0.00 (2-tailed)
* Significant value less than P < 0.05
Insignificant value P > 0.05

Table 2 Mean of **sperm immotile % of** infertile patients after **Simvastatin** 40mg daily for 3 months treatment and control group of normal fertile volunteers (n=35) versus each pathological subgroup. Data are presented as mean ±(SEM).

Pathological subgroups (n=55)	Post Simvastatin treatment		P value
	mean	**±SEM**	
Asthenozoospermia (n=7)	23.09	±2.83	**0.00****
Asthenonecrozoospermia (n=5)	35.00	±5.00	**0.23**
Asthenoteratozoospermia (n=6)	26.20	±3.01	**0.00****
Asthenoteratonecrozoospermia(n=5)	31.75	±6.43	**0.70**
Oligoasthenoteratozoospermia (n=8)	39.14	±5.77	**0.02***
Oligoasthenoteratonecrozoospermia (n=9)	17.50	±2.50	**0.05***
Oligoasthenozoospermia (n=7)	41.00	±21.00	**0.02***
Teratozoospermia (n=8)	18.85	±2.94	**0.73**
Control group (n=35)	**20.14**	**±1.01**	

** Highly significant value P < 0.05 when value 0.00 (2-tailed)
* Significant value less than P < 0.05
Insignificant value P > 0.05

Table 3 Mean of **viable sperm %** of infertile patients after **Simvastatin** 40mg daily for 3 months treatment with control group (n=35) of normal fertile volunteers versus each pathological subgroup. Data are presented as mean ±(SEM).

Pathological subgroups n=55	Post Simvastatin treatment		P value
	mean	**±SEM**	
Asthenozoospermia (n=7)	59.54	±2.21	**0.00****
Asthenonecrozoospermia (n=5)	50.00	±10.00	**0.00****
Asthenoteratozoospermia (n=6)	59.00	±1.70	**0.00****
Asthenoteratonecrozoospermia(n=5)	50.00	±8.15	**0.00****
Oligoasthenoteratozoospermia (n=8)	53.42	±4.68	**0.16**
Oligoasthenoteratonecrozoospermia (n=9)	65.00	±0.00	**0.00****
Oligoasthenozoospermia (n=7)	55.0	±10.00	**0.00****
Teratozoospermia (n=8)	61.42	±1.82	**0.01***
Control group (n=35)	**68.91**	**±1.08**	

** Highly significant value P < 0.05 when value 0.00 (2-tailed)
* Significant value less than P < 0.05
Insignificant value P > 0.05

Table 4 Mean of **sperm abnormal morphology %** of infertile patients after **Simvastatin** 40mg daily for 3 months treatment with control group of normal fertile volunteers (n=35) versus each pathological subgroup. Data are presented as mean ±(SEM).

Pathological subgroups (n=55)	Post Simvastatin treatment		P value
	mean	**±SEM**	
Asthenozoospermia (n=7)	39.00	±2.56	**0.01***
Asthenonecrozoospermia (n=5)	39.00	±11.00	**0.10**
Asthenoteratozoospermia (n=6)	48.45	±1.67	**0.00****
Asthenoteratonecrozoospermia(n=5)	43.50	±4.69	**0.01***
Oligoasthenoteratozoospermia (n=8)	46.57	±3.06	**0.01***
Oligoasthenoteratonecrozoospermia (n=9)	65.00	±5.00	**0.00****
Oligoasthenozoospermia (n=7)	50.00	±10.00	**0.01***
Teratozoospermia (n=8)	52.71	±2.68	**0.00****
Control group (n=35)	**32.22**	**±1.28**	

** Highly significant value P < 0.05 when value 0.00 (2-tailed)
* Significant value less than P < 0.05
Insignificant value P > 0.05

Table 5 Mean of **Malondialdehyde** (nmol/mg) of infertile patients after **Simvastatin** 40mg daily for 3 months treatment with control group of normal fertile volunteers (n=35) versus each pathological subgroup. Data are presented as mean ±(SEM).

Pathological subgroups (n=55)	Post Simvastatin treatment		P value
	mean	**±SE**	
Asthenozoospermia (n=7)	0.54	$\pm 2.96 \times 10^{-2}$	**0.34**
Asthenonecrozoospermia (n=5)	0.44	$\pm 5.00 \times 10^{-3}$	**0.06**
Asthenoteratozoospermia (n=6)	0.71	$\pm 3.17 \times 10^{-2}$	**0.15**
Asthenoteratonecrozoospermia(n=5)	0.70	$\pm 6.55 \times 10^{-2}$	**0.45**
Oligoasthenoteratozoospermia (n=8)	0.84	$\pm 6.08 \times 10^{-2}$	**0.31**
Oligoasthenoteratonecrozoospermia (n=9)	0.67	$\pm 3.50 \times 10^{-2}$	**0.40**
Oligoasthenozoospermia (n=7)	0.66	$\pm 7.50 \times 10^{-2}$	**0.38**
Teratozoospermia (n=8)	0.58	$\pm 6.78 \times 10^{-2}$	**0.00****
Control group (n=35)	**0.36**	**$\pm 3.70 \times 10^{-2}$**	

** Highly significant value P < 0.05 when value 0.00 (2-tailed)
* Significant value less than P < 0.05
Insignificant value P > 0.05

Table 6 Effect of **simvastatin** (40mg daily for 3 months) on CK ($U/10^8$) in infertile patients (n=55) in each pathological subgroup (n=55). Data are presented as mean ±(SEM).

Pathological subgroups (n=55)	CK $U/10^8$ sperm		
	Pre	Post	**P value**
Asthenozoospermia (n=7)	8.46 ±0.23	4.36 0.46	**0.00****
Asthenonecrozoospermia (n=5)	8.41 ±0.59	4.43 ±1.79	**0.34**
Asthenoteratozoospermia (n=6)	9.05 ±0.21	5.25 ±0.30	**0.00****
Asthenoteratonecrozoospermia(n=5)	8.24 ±0.26	5.66 ±0.80	**0.65**
Oligoasthenoteratozoospermia (n=8)	8.95 ±0.27	5.85 ±0.42	**0.00****
Oligoasthenoteratonecrozoospermia (n=9)	9.03 ±0.30	4.81 ±0.89	**0.17**
Oligoasthenozoospermia (n=7)	8.21 ±9.50 $x10^{-2}$	6.89 ±0.91	**0.41**
Teratozoospermia (n=8)	**8.41 ±0.48**	**3.66 ±0.72**	**0.00****

** Highly significant value P < 0.05 when value 0.00 (2-tailed)
* Significant value less than P < 0.05
Insignificant value P > 0.05

Table 7 Mean of **Creatine Kinase** U/10^8 of infertile patients after **Simvastatin** 40mg daily for 3 months treatment with control group of normal fertile volunteers (n=35) versus each pathological subgroup. Data are presented as mean ±(SEM).

Pathological subgroups (n=55)	Post Simvastatin treatment		P value
	mean	**±SE**	
Asthenozoospermia (n=7)	4.36	±0.46	**0.00****
Asthenonecrozoospermia (n=5)	4.43	±1.79	**0.00****
Asthenoteratozoospermia (n=6)	5.25	±0.30	**0.00****
Asthenoteratonecrozoospermia(n=5)	5.66	±0.80	**0.00****
Oligoasthenoteratozoospermia (n=8)	5.85	±0.42	**0.00****
Oligoasthenoteratonecrozoospermia (n=9)	4.81	±0.89	**0.00****
Oligoasthenozoospermia (n=7)	6.89	±0.91	**0.00****
Teratozoospermia (n=8)	3.66	±0.72	**0.00****
Control group (n=35)	**0.61**	**±5.71 x10^{-2}**	

** Highly significant value P < 0.05 when value 0.00 (2-tailed)
* Significant value less than P < 0.05
Insignificant value P > 0.05