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Correlation between serum lipids profile with sperm parameters of infertile men with abnormal semen analysis

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Abstract

Background: One of the main laboratory tests for evaluation of infertility is semen analysis (SA). However, additional tests may be suggested for further diagnosis of male fertility potentials. The seminal fluid contains sperms, non-sperm cells, and various types of lipids and glucose.

Objective: The objective of this cross-sectional study was to correlate the sperm parameters with concentrations of cholesterol, triglyceride, LDL, and HDL in serum samples of infertile men with abnormal SA.

Materials and Methods: A total of 120 infertile men (aged 23-49 years) with abnormal SA were enrolled for this cross-sectional study. Sperm concentration and motility was evaluated using Makler chamber. While, normal morphology was done after Geimsa staining. Following 12 h of fasting, the blood samples were obtained for evaluation of cholesterol, triglyceride, LDL, and HDL levels. The lipid profiles were compared with the rates of normal and abnormal sperm parameters. Chi-square and fisher exact tests were used for data evaluation.

Results: 75.5% and 98% of the subjects with normal levels of triglyceride had abnormal sperm morphology and progressive motility, respectively. Also, abnormal levels of triglyceride and cholesterol were related with abnormal sperm morphology and motility. The levels of LDL and HDL were normal in 80% and 89.3% of the cases, respectively. The majority of the patients with normal LDL had abnormal sperm parameters.

Conclusion: The results showed that the concentrations of serum lipids were not generally related with the quality of semen parameters. Further studies on the role of lipid profiles of infertile men with sperm fertilizing potentials are necessary.

Key words*: Male infertility, Semen analysis, Cholestrol, Triglyceride, LDL, HDL.*

Introduction

Mammalian seminal plasma contains different types of lipids which are different in both structure and function. Some of these lipids are secreted by sex accessory glands, while others are from sperm membrane (1). Cholestrol is secreted into the seminal plasma by the prostate gland. It mainly protects the sperms' membrane integrity against environmental shock (2).

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In humans, sperm passage through the female reproductive tract is accompanied by a loss of cholesterol from the sperm membrane. This process is involved in the sperm capacitation, which takes several hours. Cholestrol also mediates the fusion of the sperm plasma membrane with the acrosomal membrane (3). Sebastian *et al* (1987) showed that human male infertility might be associated with altered lipid metabolism in seminal plasma (4).

Also, an alteration in the cholesterol: phospholipids ratio has been encountered to be the underlying problem in the sperm of infertile men with suboptimal capacitation and acrosomal reaction rates (5). Although, several reports have

evaluated the effect of hypercholesterolemia on the mechanism modulating sperm functional capacity (6, 7), there are no reports concerning the influence of cholesterol-enriched diets on sperm parameters, sperm maturation process, and fertilizing capacity (8).

In addition, Kulka and associates (1984) examined if ejaculates with normal or abnormal SA showed any correlation with triglycerides and phospholipids concentrations. Their results showed no connection between triglycerides concentration and sperm parameters of concentration, motility, and morphology. However, alterations in phospholipids concentrations were noticed with abnormal SA (9). In another study, high level of lipids was shown to be common in azoospermic males (10). Vigron *et al* (1989) also found that increased triglyceride have deleterious effects on spermatogenesis (11).

Interestingly, hypercholesterolemia as well as hypertriglyceridemia supplements in rabbits were involved with decreased sperm capacity in acrosome reaction (6). Recently, Erqun *et al* (2007) investigated the correlation of semen parameters with serum lipid concentrations among 18 infertile men (12). Their results showed that increase of very low density lipoprotein (VLDL) and triglyceride were significantly correlated with decreased sperm motion characteristics.

Therefore, the objective of this prospective cross-sectional study was to estimate the correlation between serum level of lipids (cholesterol, triglycerides, LDL, and HDL) with sperm parameters among 120 infertile men with abnormal SA. This may add some clues for diagnosis of male infertility, especially in cases with unknown etiology.

Materials and methods

Patients

The population of this cross-sectional study consisted of 120 Iranian men with abnormal SA. Men with normal SA were excluded from this study. The hormonal levels were shown to be normal in all infertile patients under study. Their infertility was shown to be related to their abnormal level of sperm parameters. None of the patients were under any types of medication at the time of study. The mean age was 32.6±6.2 years (23-49 years old), with infertility duration of 7.0±5.4 (2-15 years). Informed consent was provided to all men and the study was approved by our university's ethics committee.

Semen analysis (SA)

Every patient was asked to collect a fresh ejaculate into a sterile container. The ejaculate was allowed to liquefy for about 30 minutes. SA was done according to WHO criteria (13). Using the WHO criteria (normal sperm count, >20 10^6/ml; normal sperm progressive motility ≥50%; and normal sperm morphology ≥30%), the samples were divided into normal (excluded from the study) and those with single, double, or triple sperm defects. Following macroscopic evaluation, sperm count and motility were evaluated by adding 10 microliters of liquefied semen to Makler counting chamber under light microscopy (Nikon Co., Japan) at x200 magnification. For evaluation of sperm morphology, 20 microliters of semen was placed on a clean microscope slide to make a smear. Next, each smear was fixed in methanol (Merck co., Germany) for 5 minutes. Sperm morphology was assessed on smears with Geimsa staining (Merck Chemical Co., Germany). The percentage of sperms with normal morphology was determined by assessing 100 sperms under oil immersion (14).

Serum lipids assessment

Blood samples were obtained following 12 h of fasting. The levels of serum cholesterol, LDL, HDL, and triglyceride measurements were performed at the Yazd Central Laboratory. The measurements were done according to the manufacturing kits (Pars-Azmoon Co., Tehran, Iran). The levels of triglycerides were divided into four categories of normal (>150mg/dl), intermediate (150-199mg/dl), high (200-400mg/dl), and hypertriglyceridemia (>400mg/dl) (15).

Also, the levels of cholestrols were subdivided into three groups of normal (>200mg/dl), intermediate (200-239mg/dl), and hypercholestrimia (>240mg/dl). LDL concentrations were divided into three groups of normal with >130mg/dl, mild with 130-159mg/dl, and high level of <160mg/dl. Finally, HDL were categorized into low level (>35mg/dl) and normal level (<35mg/dl) (16).

Statistical analysis

Statistical analysis were performed using Fisher exact test and chi-square test to compare the frequencies between the cases with normal sperm parameters versus abnormal ones. P value less than 0.05 was considered significant.

Results

The results showed that 49 out of 120 of the infertile men had normal level of triglyceride, but the rest of them showed a high level of triglyceride (Table I). Almost 11% of them were shown with extremely high concentration of triglyceride. 75.5% and 98% of the subjects with normal levels of triglyceride had abnormal sperm morphology and progressive motility, respectively (p=0.00; Table I).

Also, triglyceride above normal level was related with abnormal sperm morphology and motility. In other words, only 15.4% and 0% of the subjects with hypertriglyceridemia were shown with normal morphology and normal progressive motility, respectively. There were no significant differences between sperm parameter of concentration with the level of triglyceride (Table I).

Our findings also showed that the level of serum cholesterol was normal in 83 of the subjects. It was shown to be high in 15.8% of the patients (Table II). 81.9% and 95.2% of the sperm samples from patients with normal concentration of cholesterol had abnormal sperm morphology and progressive motility, respectively (p=0.00). In addition, 47.4% and 94.7% of the subjects with high cholesterol had abnormal sperm morphology and progressive motility, respectively. As a result, the majority of patients with normal levels of triglyceride and cholesterol had abnormal sperm parameters of concentration, morphology, and motility.

The levels of LDL and HDL were normal in 80% and 89.3% of the cases, respectively (Tables III, IV). High level of LDL was noticed in almost 11% of infertile men. The majority of the patients with normal LDL and HDL had abnormal sperm parameters.

Abnormal rate of motility was significantly increased in subjects with normal as well as abnormal level of LDL (Table III). Infertile men with low HDL had sperm abnormal parameters (morphology=100%,motility=90.9%,concentration = 45.5%).

Table IV indicates that the rates of normal sperm concentrations were similar in cases with low and normal HDL concentration, when compared with abnormal ($<20x10^6$/ml) sperm concentration. In general, good level of LDL (below 129mg/dl), also good level of HDL (above 35mg/dl) were not related with high percentages of good sperm parameters (Tables III & IV).

Table I. The correlation between serum triglyceride concentrations and sperm parameters in 120 infertile men.

Sperm parameters	<150 mg/dl Normal (n=49)	150-199 mg/dl Mild (n=25)	200-399 mg/dl High (n=34)	• P J G O Very high (n=13)
Concentration($<20x10^6$/ml)	25 (51)	10 (41.7)	16 (47.1)	8 (61.5)
Concentration($\geq20x10^6$/ml)	24 (49) p= 0.87	14 (58.3) p= 0.38	18 (52.9) p= 0.71	5 (38.5) p= 0.39
Morphology (<30%)	37(75.5)	18(75)	27 (79.4)	11 (84.6)
Morphology (≥30%)	12 (24.5) p= 0.00	6 (25) p= 0.00	7 (20.6) p= 0.00	2 (15.4) p= 0.01
Progress. motility (<50%)	48 (98)	24 (100)	29 (85.3)	13 (100)
Progress. motility (≥50%)	1 (2) p= 0.00	0 (0) p= 0.00	5 (14.7) p= 0.00	0 (0) p =0.00

The values inside parentheses are %. n: number of cases. P: when normal values of sperm parameters are compared with abnormal values.

Table II. The correlation between serum cholesterol concentrations and sperm parameters in 120 infertile men.

Sperm parameters	<200 mg/dl Normal (n=83)	200-239 mg/dl Mild (n=18)	• P J G O High (n=19)
Concentration ($<20x10^6$/ml)	43 (51.8)	11 (61.1)	5 (26.3)
Concentration ($\geq20x10^6$/ml)	40 (48.2) p= 0.24	7 (38.9) p= 0.32	14 (73.7) p= 0.03
Morphology (<30%)	68 (81.9)	16(88.9)	9 (47.4)
Morphology (≥30%)	15 (18.1) p= 0.00	2 (11.1) p= 0.00	10 (52.6) p= 0.81
Progress. Motility (<50%)	79 (95.2)	17 (94.4)	18 (94.7)
Progress. Motility (≥50%)	4 (4.8) p= 0.00	1 (5.6) p= 0.00	1 (5.3) p= 0.00

The values inside parentheses are %. n: number of cases. P: when normal values of sperm parameters are compared with abnormal values.

Table III. The correlation between serum LDL cholesterol concentrations and sperm parameters in 120 infertile individuals.

Sperm parameters	<129 mg/dl Normal (n=96)	130-159 mg/dl Mild (n=11)	• P J G O High (n=13)
Concentration (<20x10^6/ml)	50 (52.1)	6 (54.5)	3 (23.1)
Concentration (≥20x10^6/ml)	46 (47.9) p= 0.53	5 (45.5) p= 0.75	10 (76.9) p= 0.04
Morphology (<30%)	79 (82.3)	8 (72.7)	6 (46.2)
Morphology (≥30%)	17 (17.7) p= 0.00	3 (27.3) p= 0.12	7 (53.8) p= 0.77
Progress. Motility (<50%)	91 (94.8)	11 (100)	12 (92.3)
Progress. Motility (≥50%)	5 (5.2) p= 0.00	0 (0) p= 0.00	1 (7.7) p= 0.01

The values inside parentheses are %. n: number of cases. p: comparing between normal values sperm parameters with abnormal parameters.

Table IV. Correlation between serum HDL cholesterol concentrations and sperm parameters in 120 infertile men.

Sperm parameters	<35 mg/dl Low (n=13)	• P J G O Normal (107)
Concentration (<20x10^6/ml)	5 (45.5)	53 (49.5)
Concentration (≥20x10^6/ml)	6 (54.5) ,p= 0.75	54 (50.5) p= 0.86
Morphology (<30%)	11(100)	80 (74.8)
Morphology (≥30%)	0 (0), p= 0.00	27 (25.2) p= 0.00
Progress. Motility (<50%)	10 (90.9)	102 (95.3)
Progress. Motility (≥50%)	1 (9.1) p= 0.00	5 (4.7) p= 0.00

The values inside parentheses are %. n: number of cases. p: comparing between sperm morphology <30% with ≥30%.

Discussion

It has been reported that phospholipids and fatty acid composition of spermatozoa are altered in men with infertility (15). It has been very difficult to pinpoint a strong correlation between the serum lipids concentrations and variations in seminal parameters. Padron *et al* (1989) examined the lipid composition and testicular function in mammals (10). Their results showed that high lipid levels exerted direct adverse effects at the testicular levels. This situation may alter the sperm maturation process in male reproductive tract and capacitation modification. Another finding from their study was that high lipid levels were common in patients with azoospermia. Ramirez-Torres and colleagues (2000) studied the incidence of hypercholesterolemia and hypertrigleceridemia among infertile men (17). Their findings showed that 65% of their cases had the aforementioned lipid defects. All our cases were with abnormal SA. In other words, at least one defect in sperm parameters of concentration, morphology, or progressive motility were observed when the ejaculates were analyzed. In parallel with our findings, Padron *et al* (10) evaluated the level of lipids among fertile and infertile individuals. Their results showed that lipid alteration was more common in azoospermic men. There was a correlation between abnormal semen quality with high levels of cholesterol and triglyceride. Our results showed that very high level of triglyceride (>400mg/dl) and cholesterol (>240mg/dl) were related with abnormal sperm parameters. But, it should be emphasized that normal levels of both triglyceride as well as cholesterol were also related with high sperm abnormality (Tables I & II).

Triglycerides, which are metabolic energy sources in spermatozoa, have substrates to produce glycerol in mature mammalian sperm (17). However, elevation in triglycerides concentration may have deleterious effects on spermatogenesis. It is possible to reduce the level of seminal triglyceride by centrifugation of seminal samples using culture media right after liquefaction (18). The majority of our patients had triglyceride levels above normal range. Our findings showed that half of our cases had abnormal level of triglyceride, and over 10% had hypertriglyceridemia. This rate is much higher when compared with normal population of our society (19). The normal level of triglyceride was not related with high quality of sperm parameters. However, it may interfere with sperm functional/ fertilization capacity. Diaz-Fontdevila and co-workers found that hypercholesterolemia and hypertriglyceridemia

was related with decreased capacity of sperm acrosome reaction in rabbits (6). Hypercholesterolemia also may have a detrimental effect on sperm capacity to penetrate and fertilize oocytes in rabbit (8). It has been shown that epididymal dysfunction in hypercholesterolemic animals may have detrimental effects on the cytostructural modifications and biochemical changes that occur during sperm epididymal maturation and may result both in decreased sperm motility and also in sperm morphometric abnormality (20). Our findings on cholesterol indicate that the majority of the cases had normal level of cholesterol. One-third of patients were shown with abnormal level of cholesterol, and over 15% had high level of cholesterol. This rate was similar to the cholesterol of normal young population in Iran (19).

Only very high level of cholesterol was correlated with abnormal sperm motility. In addition, levels of LDL and HDL were within normal range in the majority of our individuals. Both abnormal levels of LDL and HDL in the patients under study were much lower than normal population of our society (19). Abnormal levels of LDL and HDL were not involved with alterations in sperm parameters. Also, extreme levels of triglyceride and cholesterol are generally involved with alteration in sperm parameters, especially with morphology and motility characteristics.

Recently, it has been reported that increased VLDL and triglyceride as well as decreased testosterone values were significantly correlated with decreased sperm motion characteristics (12). Other semen parameters (e.g. sperm concentration, normal morphology) did not show any correlation with serum lipids concentration. In addition, Yamamoto *et al* (1999) found that only high level of cholesterol affected the sperm motility in rabbit. Their findings are similar with our observation in infertile human cases (8). In addition, Brinsko *et al* (2007) observed that sperm motility between fertile and infertile stallions were similar, but the ratio of cholesterol to phospholipids was 2.5 times greater in the seminal plasma of infertile stallions compared to fertile stallions (21).

They assumed that failure of sperm to capacitate and acrosome react is the primary dysfunction leading to infertility in these animals. Further analysis may determine the abnormalities that may be involved in the semen of unexplained infertile men, which interferes with ability of their spermatozoa to capacitate and undergo the acrosome reaction (22).

In conclusion, the results showed that the concentrations of serum lipids are not related with quality of semen parameters in infertile men. Further studies in lipid profiles of infertile men with unexplained etiology will help to further elucidate the underlying mechanism and probably lead to therapeutic options.

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