



Relationship between Lipid Profile and DNA Fragmentation Index with Abnormal Semen

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Abstract

Male infertility exists as a complex medical issue which depends on multiple physiological and biochemical factors particularly lipid metabolism and genetic integrity. The research evaluated how lipid profile characteristics relate to DNA fragmentation index (DFI) in Iraqi men who have either normal or abnormal semen test results. The study enrolled 90 male participants who included 46 men with normal semen characteristics between ages 20 to 45 and 44 men with abnormal semen parameters. Semen samples underwent analysis for standard parameters and lipid profile and DFI. The aberrant semen group displayed elevated triglyceride (TG) levels at 19.32 ± 5.60 mg/dl which exceeded normal group levels at 14.50 ± 4.62 mg/dl ($P < 0.01$). The analysis revealed no significant variation between cholesterol (CHO) levels ($P = 0.410$) but LDL levels of the aberrant semen group (6.38 ± 4.12 mg/dl) significantly diverged from the normal group levels (9.03 ± 3.43 mg/dl with $P < 0.01$). The DNA fragmentation index (DFI) levels of the aberrant semen group exceeded those of the normal semen group by a significant margin ($31.66 \pm 16.15\%$ versus $13.54 \pm 9.49\%$, $P < 0.01$). This indicates that men with poor semen quality typically present with sperm DNA damage. The research findings suggest that abnormal lipid metabolism together with increased DNA fragmentation plays a role in male infertility development particularly among men with abnormal semen analysis results.

Keywords: Semen quality, lipid profile, DNA fragmentation, and male infertility

Introduction

The absence of a clinical pregnancy after 12 months or more of consistent, unprotected sexual activity is known as infertility [1]. The inability of a man to conceive a child naturally in less than a year in a fertile woman is known as infertility [2]. Congenital or acquired

conditions, including varicocele, urogenital abnormalities, reproductive tract infections, genetic abnormalities, endocrine problems, and testicular Male reproductive potential can be decreased by systemic disorders, cancer, failure, and exposure to gonad harmful chemicals [3]. There are two types of infertility: primary and secondary. When

a man has never conceived a child, it is referred to as primary infertility. Secondary infertility happens when a man has previously gotten a woman pregnant, even if the woman is not the partner in the present relationship. There are two types of male infertility, commonly referred to as subfertility: complete and partial. Any of the following may be the cause: decreased sperm motility (asthenozoospermia), decreased sperm vitality (necrozoospermia), aberrant sperm morphology (teratozoospermia), or reduced spermatozoa (oligozoospermia). Most subfertility patients have an intrinsic testicular illness as their primary etiology. Males were considered infertile when their sperm parameters were below the WHO normal range [4]. Sperm DNA fragmentation (SDF) has also been connected to male infertility. Single and double-strand breaks in sperm DNA are referred to as SDF [5]. The relationship between male infertility and fat, however, needs further investigation. Obesity has reportedly been linked to modifications in the characteristics of semen, which result in decreased testicular volume, decreased semen quality, and compromised spermatogenesis [6]. The membrane structure along with fluidity and operational integrity of sperm cells in male reproductive physiology depends heavily on lipids. Seminal plasma oxidation levels together with systemic metabolic status depend on the lipid profile composition including total cholesterol, triglycerides, LDL and HDL. The integrity of sperm DNA suffers damage because of reactive oxygen species (ROS) that develop from abnormal lipid metabolism present in dyslipidemia [7]. Sperm cell genomic instability and chromatin degradation can be measured through DNA fragmentation

index (DFI) which serves as a standard metric to evaluate sperm DNA fragmentation. Elevated DFI results in diminished pregnancy success and impairs embryo formation together with reduced sperm fertilization abilities [8]. Research findings show that altered lipid profiles along with elevated triglycerides and low HDL cholesterol and high LDL cholesterol can worsen oxidative stress levels in male reproductive organs while causing DNA damage [9]. The high polyunsaturated fatty acid content in sperm membranes exposes them to high vulnerability when facing oxidative stress conditions. This peroxidation process damages spermatozoon membranes as it also triggers DNA strand breaks to accumulate [10]. The high polyunsaturated fatty acid content in sperm membranes exposes them to high vulnerability when facing oxidative stress conditions. This peroxidation process damages spermatozoon membranes as it also triggers DNA strand breaks to accumulate.

Materials and methods

Experimental design

The present study was conducted in a specialized infertility laboratory in Najaf Governorate. This work was carried out from November 2024 to May 2025. The study included (90) males suffering from primary and secondary infertility. The age of participants ranged from early adulthood to midlife. The duration of infertility was from (1-15) years. Written information consent was taken from the patients. Semen samples were taken from patients of the fertility center who had abnormal semen parameters (such as oligospermia and/or asthenozoospermia) and

those with normal semen parameters based on WHO criteria.

Diagnosis

1-Perform semen analysis according to WHO criteria to classify the sample as normal or abnormal.

2-Assess the blood lipid profile (total cholesterol, LDL, HDL, and triglycerides).

3-Calculate the DNA fragmentation index (DFI) using SCSA or TUNEL; a high DFI ($\geq 25\%$) alongside elevated LDL/triglycerides and low HDL indicates oxidative damage leading to increased DNA fragmentation.

Collection of samples

Following three to five days of sexual abstinence, patients and controls had their semen specimens directly collected in a sterile, dry, and clean disposable container by masturbating in a quiet room next to the seminal fluid analysis laboratory. The patient's name, age, and sexual abstinence at the time of sample collection were all written on the container. To allow for liquefaction, the obtained specimens were incubated for 30 minutes at 37°C . Following a brief period of meticulous mixing, the liquefied specimens were inspected under a microscope. Seminal fluid characteristics were evaluated and classified based on internationally recognized reference criteria to categorize infertile patients.

Statistical analysis

The Shapiro-Wilk test was used to confirm that the distribution was normal. To find out if there were any differences between the research variables, the Statistical Package of Social Sciences (SPSS) Version 27 software program (SPSS Inc., Chicago, Illinois, United States) was utilized. To ascertain the differences between the two groups, a two independent sample T test was used. The one-way analysis of variance (ANOVA) is the proper technique for comparing more than two group means.

Pearson's correlation coefficient, which was used to evaluate the relationship between the two variables, was used to examine the relationship between analyte levels within each research group. The MedCalc program (version 19.1.2, MedCalc Software Ltd, Belgium) (Statistical software package for the biomedical sciences) was used to create all correlation graphs a probability of less than 5% or 1% were considered statistically significant.

Result

The study results showed statistically significant differences ($P < 0.01$) between the control group and the patients group in semen parameters. The mean values of sperm concentration (64.13 ± 29.50 million/ml vs. 24.11 ± 11.39 million/ml), progressive motility ($62.37 \pm 9.79\%$ vs. $20.36 \pm 4.45\%$), normal sperm morphology ($65.74 \pm 5.88\%$ vs. $33.63 \pm 7.13\%$), and semen volume (4.30 ± 1.48 ml vs. 3.47 ± 1.58 ml) were significantly higher in the control group compared to the patients group. On the other hand, there was no statistically significant difference ($P = 0.185$) in round cell count between the two

groups (2.07 ± 1.03 million/ml in the normal group vs. 2.55 ± 1.89 million/ml in

the abnormal group).

Table (4-1): Comparison of the abnormal and normal semen groups' semen parameters.

Semen parameters	Control group n=46	Patients group n=44	P value	Shapiro-Wilk test for Normal distribution
Sperm concentration (Million/ml)	64.13 ± 29.50	24.11 ± 11.39	$<0.01^{**}$	W=0.9725, accept Normality (P=0.053)
Progressive motility (%)	62.37 ± 9.79	20.36 ± 4.45	$<0.01^{**}$	W=0.9771, accept Normality (P=0.1135)
Normal sperm morphology (%)	65.74 ± 5.88	33.63 ± 7.13	$<0.01^{**}$	W=0.9772, accept Normality (P=0.1152)
Semen volume (ml)	4.30 ± 1.48	3.47 ± 1.58	0.004^{**}	W=0.9794, accept Normality (P=0.1627)
Round cell (Million/ml)	2.07 ± 1.03	2.55 ± 1.89	0.185^{ns}	W=0.9752, accept Normality (P=0.0621)

Comparison of lipid profile levels and DNA fragmentation index of semen parameters of normal semen group and abnormal semen group.

The study results showed statistically significant differences ($P < 0.01$) between the healthy group and patients group in the

levels of certain lipid profile markers and the DNA fragmentation index. The levels of triglycerides (TG) were significantly higher in the abnormal semen group compared to the normal semen group (19.32 ± 5.60 vs. 14.50 ± 4.62 , $P < 0.01$). The normal semen group showed elevated LDL levels that exceeded the values of the

abnormal semen group (9.03 ± 3.43 vs. 6.38 ± 4.12 , $P < 0.01$). The abnormal semen group showed higher DNA fragmentation index values than the normal semen group (31.66 ± 16.15 vs. $13.54 \pm$

9.49 , $P < 0.0$). The two groups showed no significant variation in their total cholesterol (CHO) measurements according to statistical analysis ($P = 0.410$)

Lipid profile levels	Normal semen n=46	Abnormal semen, n=44	P value	Shapiro-Wilk test for Normal distribution
TG	14.50 ± 4.62	19.32 ± 5.60	$<0.01^{**}$	W=0.9712, accept Normality (P=0.0574)
CHO	22.84 ± 12.50	20.91 ± 9.24	0.410ns	W=0.9733, accept Normality (P=0.0604)
LDL	9.03 ± 3.43	6.38 ± 4.12	$<0.01^{**}$	W=0.9772, accept Normality (P=0.058)
DNA	13.54 ± 9.49	31.66 ± 16.15	$<0.01^{**}$	W=0.9636, reject Normality (P=0.074)

Discussion

The quality assessment between normal and sick semen populations revealed substantial dissimilarities. The aberrant group showed decreased sperm concentration and abnormal progressive motility and normal morphology according to statistical analysis with p-values below 0.01. These findings confirm World Health Organization recommendations about male infertility markers through tests of semen quality and volume [11]. The process of spermatogenesis gets affected when oxidative stress combines with testicular dysfunction alongside hormone imbalances

and environmental exposures. The abnormally low sperm concentration in the abnormal group could be explained by this finding [12]. Success in fertilization depends on progressive motility but this process decreased markedly in the aberrant group because of mitochondrial dysfunction and sperm tail structural damage [13]. The faulty semen group presented decreased sperm structures which demonstrates that proper sperm shape is necessary for successful reproduction. The presence of morphological defects in sperm cells can result from oxidative harm in conjunction with genetic abnormalities and sperm production disruption [14]. The

faulty semen group presented decreased sperm structures which demonstrates that proper sperm shape is necessary for successful reproduction. The presence of morphological defects in sperm cells can result from oxidative harm in conjunction with genetic abnormalities and sperm production disruption [15]. The presence of spermatozoa in the urine samples did not show a significant difference between normal and bad semen quality groups in this study thus indicating that inflammation or infection did not drive the distinction. Research indicates that leukocytospermia leads to elevated round cells that generate reactive oxygen species (ROS) which damage sperm function and reproductive potential [16]. The research analysis between normal semen and aberrant semen groups produced meaningful results for lipid profile levels and DNA fragmentation index (DFI). The aberrant semen group had higher Triglyceride (TG) levels compared to the normal group ($P < 0.01$) indicating a possible link between poor semen quality and hypertriglyceridemia. As stated by [17,18]. The presence of increased TG in seminal plasma seems to generate oxidative stress that transforms sperm membrane composition and diminishes their functional ability. Studies before this one demonstrated metabolic problems create harmful effects on male fertility. Patients from the abnormal semen group exhibited lower LDL values than those in the control group according to statistical analysis ($P < 0.01$). LDL plays a complicated role during sperm maturation within seminal plasma but science generally describes it as atherogenic in the bloodstream. Lower LDL levels may indicate issues with lipid homeostasis that compromise the integrity and functioning of sperm membranes [19]. It is possible that total cholesterol is not a

reliable indication of semen quality on its own because there was no discernible difference in the cholesterol level (CHO) between the two groups ($P = 0.410$). In this case, certain lipid subtypes or ratios—like LDL/HDL—may offer more information than the total cholesterol level. For instance, a study by Lu *et al.*, [20] found that elevated levels of LDL and HDL in seminal plasma were negatively associated with semen parameters, including semen volume, sperm concentration, and motility. The largest difference was seen in the DNA fragmentation index, where the abnormal semen group had significantly higher DFI values ($P < 0.01$). The known marker for poor sperm quality called DFI shows correlation to worsened embryo development and impaired fertilization ability and higher miscarriage risk [21]. Oxidative stress serves as the primary reason behind DNA breakage in sperm cells yet lipid dysregulation worsens this situation [22]. The increase in DFI observed among male patients with abnormal semen parameters strengthens the usefulness of DFI as an assessment tool during male fertility diagnosis and prediction.

Conclusion

This research demonstrates significant distinctions exist between people with normal semen characteristics compared to those with pathological semen characteristics regarding semen quality and lipid profile parameters and DNA fragmentation index. The relationship between elevated triglyceride levels and increased DNA fragmentation indicates abnormal semen properties because oxidative stress and lipid metabolism

dysregulation potentially contribute to male infertility. While total cholesterol showed no notable differences between the groups the lower LDL levels in the aberrant semen group might indicate altered lipid homeostasis with sperm function consequences. The significantly elevated DNA fragment index in the aberrant group shows how essential sperm DNA integrity remains for reproductive capabilities of males. Clinical diagnosis of male infertility must include lipid profile tests and DNA Fragmentation Index assessment due to the findings that guide future studies on how lipid metabolism affects sperm DNA integrity.

Ethical approval:

Official permission to conduct this study was granted by the Faculty of Science at the University of Kufa, as per the letter addressed to the Fertility Center. The procedures followed the ethical principles of the Declaration of Helsinki. Sample collection was conducted during the period 2024–2025, and written informed consent was obtained from all participants. The approval was granted on December 11, 2024.

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