Correlation Between Lipid Profile of Sperm Cells and Seminal Plasma With Lipid Profile of Serum in Infertile Men

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Background: Lipids play an important role in the functional activity of sperm cells. Objectives: The main goal of this study was to assess the correlation between the levels of cholesterol, phospholipids and triacylglycerols found in serum, with the lipid levels of semen in infertile men. Patients and Methods: Cholesterol, phospholipids and triacylglycerols in sperm cells, seminal plasma and serum were assayed in 60 infertile men. Results: There were no significant relationships between the concentration of sperm and seminal plasma cholesterol with serum cholesterol (r = 0.003, P = 0.9 and r = 0.055, P = 0.67, respectively), between the concentration of sperm and seminal plasma triglycerides with serum triglycerides (r = 0.16, P = 0.2 and r = -0.039, P = 0.77, respectively), or between the concentration of sperm and seminal plasma phospholipids with serum phospholipids (r = 0.18, P = 0.16 and r = 0.053, P = 0.69, respectively). Conclusions: These results suggest that serum cholesterol, phospholipids and triacylglycerols have no effect on the levels of cholesterol, phospholipids and triacylglycerols of spermatozoa and seminal plasma. Our findings suggest that sperm lipid content is regulated locally within the male reproductive tract.

Keywords: Cholesterol; Lipids; Phospholipid; Serum; Semen Analysis; Spermatozoa

1. Background

Lipids have an important role in the functional activity of sperm cells (1, 2). Sperm viability, maturity, capacitation and fertilization are affected by lipid components (3). Phospholipids and cholesterol are important components of human plasma membranes and they are required for membrane permeability, fluidity, and capacitation (4). Changes in the lipid composition of spermatozoa have been reported in infertile males (5, 6). In addition to alterations in the lipid composition of human spermatozoa, there have also been reports on changes in the serum lipid profile of infertile men. Ramirez-Torres et al. reported a 65% incidence of hyperlipidemia, including hypercholesterolemia and triglyceridemia in 106 male partners of infertile couples (7). Ergun et al. demonstrated a significant correlation between plasma lipid concentrations and sperm motility, and reported that hypertriglyceridemia may have deleterious effects on spermatogenesis (8). In animal studies, researchers examined the effects of a high-cholesterol diet on male fertility and concluded that a high-cholesterol diet resulted in a significant decline in fertility and sperm characteristics, decrease in sperm acrosome reaction kinetics, and detrimental effects on Leydig and Sertoli cell secretory capacity (9, 10). Although these studies suggest a link between dyslipidemia and infertility, no clear mechanism was reported. The question of whether the lipid profile of serum can have an effect on the lipid profile of semen, has not been answered. Although the destructive effects of serum lipid alterations, as well as semen lipids changes on reproductive ability are understood, the correlation between serum lipids and semen lipid concentrations is still unknown. To the best of our knowledge, little information is available about the correlation between serum lipids and semen lipids.

2. Objectives

The main goal of this study was to assess the correlation between the serum levels of cholesterol, phospholipids and triacylglycerol with the semen lipids of infertile men.
3. Patients and Methods

The population of this cross-sectional study consisted of 60 Iranian men with defined infertility, and without any liver or renal disease, thyroid disorders, diabetes mellitus, or history of using anti-hyperlipidemic drugs, or any medication affecting lipid metabolism such as statins or diuretics. Semen samples of infertile males were collected by masturbation following 3 days of abstinence. After liquefaction, semen volume, sperm concentration, total sperm count, morphology, and motility grades (a: rapid progressive; b: slow progressive; c: non-progressive; and d: immotile) were determined using World Health Organization standard procedures (11). Written informed consent was obtained from all those enrolled, according to the criteria of the Ethical Committee of Hamadan University of Medical Sciences. Blood samples were collected after overnight fasting from 60 subjects, and after serum isolation; samples were stored at -20°C until analyses.

3.1. Sperm Fractionation

Aliquots of 1 mL of the liquefied semen were layered on top of the upper layer of 40% and 80% Pure Sperm gradient (Nidacon International, Sweden), then centrifuged at 400 × g for 20 minutes (12). The resulting interfaces at 40% and 80% (fraction 1), 80% and pellet (fraction 2), and pellet (fraction 3), were isolated and transferred to separate tubes (13). An aliquot of fraction 3 was used to evaluate sperm motility, morphology, and concentration. Suspensions of sperm from the different pure sperm fractions were diluted in 2 mL phosphate buffer saline (PBS) and centrifuged at 800 g for 8 minutes; this washing was repeated. The pellet was then resuspended in 1 mL PBS and stored at -80°C (14). Sperm of fraction 3 were used for lipid extraction and determination of cholesterol, phospholipid, and triacylglycerol levels.

3.2. Extraction and Analysis of Lipids

Sperm cells of fraction 3 isolated by Pure Sperm gradient as well as seminal plasma were used for lipid extraction. Lipids of spermatozoa and seminal plasma were extracted with 6 volumes of chloroform-methanol (2/1, V/V), centrifuged at 800 × g for 3 minutes, and the resulting lower phase aspirated and dried under a stream of nitrogen (14). The cholesterol of the sperm cells and seminal plasma was assayed using Liebermann-Burchard reagent (15). The phospholipid level was determined using a modification of the method by Bartlett (16), and the triacylglycerol level of the sperm cells and seminal plasma was determined using acetyl acetone with the method of Gottfried and Rosenberg (17). Serum concentrations of cholesterol and triacylglycerols were measured by enzymatic methods (Pars Azmoon kits, Iran).

3.3. Statistical Analysis

Results were presented as mean ± SD. The correlation between the cholesterol, phospholipids and triglycerides of the serum, with the lipids of seminal plasma and sperm cells from fraction 3, was investigated using non-parametric Spearman’s coefficient (r). In addition, a non-parametric Spearman’s coefficient was used to determine the correlation between serum lipids with sperm morphology and motility.

4. Results

The main semen parameters of the groups are illustrated in Table 1. The concentrations of cholesterol, phospholipids, and triacylglycerols in seminal plasma, sperm fraction 3 and serum are shown in Table 2. There were no significant relationships found; between the concentration of sperm and seminal plasma cholesterol with serum cholesterol (r = 0.003, P = 0.9 and r = 0.055, P = 0.67 respectively), between the concentration of sperm and seminal plasma triglyceride with serum triglyceride (r = 0.16, P = 0.2 and r = -0.039, P = 0.77, respectively), or between the concentration of sperm and phospholipids, and triacylglycerols.

Table 1. Basic Parameters of Semen Sample in Total Subjects (n = 60) ± SD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Semen</th>
<th>Fraction 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility grade a, %</td>
<td>3.5 ± 3</td>
<td>7.7 ± 6.9</td>
</tr>
<tr>
<td>Motility grade b, %</td>
<td>17.85 ± 9.7</td>
<td>28 ± 11.8</td>
</tr>
<tr>
<td>Motility grade c, %</td>
<td>21.3 ± 11.9</td>
<td>24.5 ± 13.8</td>
</tr>
<tr>
<td>Motility grade d, %</td>
<td>57.2 ± 18.50</td>
<td>39.7 ± 13.7</td>
</tr>
<tr>
<td>Morphology, %</td>
<td>16.4 ± 7.2</td>
<td>25.6 ± 10.7</td>
</tr>
<tr>
<td>Pathologic head, %</td>
<td>60.4 ± 11.2</td>
<td>64.3 ± 11.8</td>
</tr>
<tr>
<td>Pathologic mid-piece, %</td>
<td>13.1 ± 6.1</td>
<td>6.7 ± 4.9</td>
</tr>
<tr>
<td>Pathologic tail, %</td>
<td>10 ± 7.5</td>
<td>3.4 ± 4.6</td>
</tr>
<tr>
<td>Concentration, 10⁶/mL</td>
<td>107.9 ± 49.6</td>
<td>28.8 ± 20.1</td>
</tr>
</tbody>
</table>

Table 2. Content (mean ± SD) of Cholesterol, Phospholipid and Triacylglycerol of Sperm Fraction 3, Seminal Plasma and Serum From Subjects (n=60).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cholesterol</th>
<th>Phospholipids</th>
<th>Triacylglycerols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm fraction 3, nmol/10⁶</td>
<td>0.63 ± 0.66</td>
<td>1.4 ± 0.95</td>
<td>0.19 ± 0.19</td>
</tr>
<tr>
<td>Seminal plasma, µmol/mL</td>
<td>1.45 ± 0.48</td>
<td>0.38 ± 0.23</td>
<td>5.40 ± 1.75</td>
</tr>
<tr>
<td>Serum, mmol/L</td>
<td>4.84 ± 1</td>
<td>1.2 ± 0.88</td>
<td>1.5 ± 0.71</td>
</tr>
</tbody>
</table>
seminal plasma phospholipids with serum phospholipids ($r = 0.18$, $P = 0.16$ and $r = 0.053$, $P = 0.69$, respectively). Generally, we did not observe any significant association between the levels of serum cholesterol, phospholipids and triglycerides with morphology and motility grades of sperm cells from the semen or fraction 3 ($P > 0.05$). However, the correlation between serum phospholipids with motility grade a from sperm fraction 3 ($r = 0.27$, $P = 0.03$) and serum triglycerides with motility grade b from sperm fraction 3 ($r = 0.26$, $P = 0.03$), were significant.

5. Discussion

The present study aimed to assess the correlation between serum lipid concentrations and variations in seminal lipid parameters in infertile men. We found no relationship between the concentration of cholesterol, phospholipids and triacylglycerols in serum, spermatozoa or seminal plasma of the infertile men under present examination, which is consistent with the findings of several other authors (18). Grizard et al. compared the effect of hypercholesterolemia and normocholesterolemia on the spermatozoa and seminal content of cholesterol and phospholipids. They suggested that hypercholesterolemia has no effect on cholesterol and phospholipid levels in spermatozoa and seminal plasma (18). Since cholesterol has a major role in the sperm membrane, which is essential for sperm cell function, it can be assumed that an increase of cholesterol level in the blood will also increase the cholesterol content of semen. This hypothesis was not confirmed in the presented study. There appears to be no correlation between the amount of cholesterol in the serum and in sperm or seminal plasma, suggesting that sperm cholesterol content is regulated locally within the male reproductive tract (4, 19, 20). For proper function of spermatozoa, cholesterol and phospholipids should be regulated accurately. In the male reproductive tract lipid homeostasis is done by testicular and post-testicular function (20, 21). Our results showed no correlation between serum lipids with sperm parameters, which is consistent with the findings of some other authors. Khalili et al. reported that the concentrations of serum lipids were not generally related to the quality of semen parameters (22). Nonetheless, our results are not consistent with the results of some other studies, in which animals were fed with a high-fat diet (9, 10). These results suggest that serum cholesterol, phospholipids and triacylglycerols have no effect on the levels of cholesterol, phospholipids and triacylglycerols in spermatozoa and seminal plasma, and in addition, they do not cause any alteration of semen parameters. Our findings suggest that sperm lipids levels are regulated locally within the male reproductive tract.

Acknowledgements

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Authors’ Contributions

Heidar Tavilani: design of the study and critical revision; Saeed Khosropour and Abozar Mohagheghei: statistical analysis and interpretation of data; Akram Vatannejad, Maryam Akbarzadeh, and Mojgan Atabakhsh: conducted the experiments.

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