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SEMINAL PLASMA LIPID PROFILES AND LIPID PEROXIDE LEVELS IN INFERTILE MEN#

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SUMMARY

The present study was undertaken to evaluate the seminal plasma lipids and lipid peroxide levels in fertile and normospermic infertile, azoospermic, oligospermic and asthenospermic men. Spermatozoa and seminal plasma contain considerable amount of lipids, in particular cholesterol and phospholipids, and thus susceptible to oxidative damage. The results show a significant reduction in total lipid and phospholipid levels in normospermic infertile men. Alteration in the lipid content of seminal plasma may adversely affect the membrane structure required for the occurrence of capacitation and acrosome reaction and also lead to an altered sperm function. Moreover, the results also showed significantly elevated seminal plasma lipid peroxide levels in all infertile men, remarkably in normospermic infertile men. It is concluded that in unexplained infertile couples, one of the reasons for failure of normal fertilisation process may be the perturbed seminal plasma lipid content brought about by free radical mediated lipid peroxidation.

Key Words : Infertility; lipids; lipid peroxides; seminal plasma.

INTRODUCTION

It is recognised that infertility is multifactorial and several male and female factors may contribute to its etiology. In approximately 10-15 percent of infertile couples no apparent cause can be found and such cases are categorized under unexplained infertility (1). In view of the rising prevalence of male infertility and growing interest in engineering new approaches to male contraception, knowledge of the cellular, biochemical and molecular events that regulate the fertilizing potential of human spermatozoa is highly desirable.

This paper is dedicated to late Dr. Lakshmi Singh
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Defective sperm function has been identified as the most frequent cause of human infertility (2). However, little is known about the etiology of this condition or the precise nature of defect(s) responsible for the loss of fertilizing potential. A significant new development in the field of male infertility research is related to oxidative stress which plays an important role in fertility disturbances (3). Human spermatozoa have been demonstrated to possess the capacity to generate reactive oxygen species (ROS), with the superoxide anion being produced as primary product that subsequently dismutates to hydrogen peroxide under the influence of intracellular superoxide dismutase (4).

Lipids and proteins are the major components of cellular membranes and are known to play important role in maintaining the structural and functional integrity of the cell and its organelles (5). Spermatozoa and seminal plasma contain considerable amount of lipids, particularly cholesterol and phospholipids (6). Therefore, spermatozoa are more susceptible to oxidative damage because of their high lipid content and their relative paucity of cytoplasmic enzymes responsible for scavenging ROS which initiate lipid peroxidation (7). In view of above considerations, the present study has been designed to investigate the lipid profiles and lipid peroxide levels in the seminal plasma of fertile and normospermic infertile, oligospermic, azoospermic and asthenospermic men.

MATERIALS AND METHODS

a. Selection of Patients:

The study included two groups of subjects:

Group – I : The male partners of the couples attending the infertility Clinic of the Department of Obstetrics and Gynaecology, Queen Mary's Hospital, King George's Medical College, Lucknow were included in this group.

Group – II : Age matched healthy males who had previously initiated at least one pregnancy and exhibited a normal semen profile (>20x10^6 spermatozoa/mL; >50% motility and >60% normal morphology) were considered in this group.

b. Exclusion Criteria :

1. Patients with illness like myocardial ischemia or infarction, hypertension, diabetes mellitus, renal insufficiency, hepatic disorder, pancreatic disorder, chronic obstructive airway disease and parasitic or inflammatory disorder, conditions which are known to alter the free radical system, were excluded from the present study.
2. Patients on anti-oxidants, vitamins like A,E, and/or C, or any other type of therapy were also excluded from the present study.

Collection of Semen Samples :

The semen samples were collected by masturbation after 3-4 days of abstinence into sterile plastic containers for analysis. After the collection of semen samples, the volume was recorded and an aliquot was taken to assess sperm motility after atleast 30 minutes were allowed for liquefaction to occur. A semen profile was constructed in accordance with the procedure described by the World Health Organization (8), which included sperm count, total sperm per ejaculate, percent normal morphology and percent motility.

The group-I was divided into four sub-groups based on semen quality (azoospermia, oligospermia, asthenospermia and normospermia). These groups were defined as: (1) azoospermia – no sperm seen in ejaculated specimen or centrifuged pellet of specimen; (2) oligospermia – sperm concentration less than 20 x 10^6 / mL; (3) asthenospermia– sperm concentration more than 20 x 10^6 / mL and motility less than 50% and (4) normospermia- sperm concentration >20 x 10^6 / mL and motility more than 50%, with more than 60% spermatozoa having normal morphology.

Preparation of seminal plasma :

The semen samples after liquefaction were centrifuged at 1200xg for 20 min for the separation of seminal plasma. The supernatant (seminal plasma) was recentrifuged at 10,000x g for 30 min to eliminate all possible contaminating cells.

Biochemical estimations :

The lipids of seminal plasma were extracted with chloroform:methanol (2:1) mixture as per the method described by Folch *et al* (9) and total lipids were estimated by the method described by Woodman and Price (10). Cholesterol was determined by the method of Zlatis *et al* (11). The method of Marinetti (12) was used for phospholipid estimation which is a modified method of Fiske and Subbarow (13). Protein content was quantified by standard technique of Lowry *et al* (14). Estimation of lipid peroxides was carried out according to the technique of Ohkawa *et al* (15).

Statistical Analysis

Data were analysed using Student's *t*-test and presented as mean ± S.D. A probability value of $P<0.05$ was considered to be significant.

RESULTS

Fertile donors were those whose partners became pregnant within two years of marriage. After atleast 3 days of abstinence, most of the donors selected had normal semen parameters according to World Health Organization criteria.

Normospermic men were those whose partners were found to be ovulatory with regular cycles with tubular patency, exhibiting no pelvic pathology or adhesion, no endometriosis, and no evidence of vaginal, cervical or uterine factors, which might disturb fertility. None of these men and their partners were using any contraceptive, yet they had no conception even after 2 years of marriage.

Oligospermic subjects were those with less than 20 x 10^6 spermatozoa/mL. While asthenospermic were those whose semen samples showed more than 20 x 10^6/mL spermatozoa and contained less than 50% of spermatozoa with adequate progressive motility. The general characteristics of the semen profiles in normal, oligospermic, asthenospermic and azoospermic men are presented in Table 1.

Significant decrease in total lipid profiles were observed in seminal plasma of all the groups studied, except in asthenospermia (Table 2). Cholesterol content was significantly low only in azoospermic men (Table 2). Phospholipid content in the seminal plasma was low in normospermic infertile, azoospermic and oligospermic subjects but moderately high in asthenospermic men (Table 2). However, no appreciable variations was found in the seminal plasma protein content in all the groups studied (Table 3).

Seminal lipid peroxide levels were significantly high in the normospermic infertile and asthenospermic men ($P<0.01$, Table 3). On the other hand, in azoospermic men lipid peroxide levels were significantly low.

DISCUSSION

Previously, there have been several reports of altered lipid levels in the seminal plasma and spermatozoa of infertile men (16-17). However, in the present investigation an attempt has

Table 1 : General characteristics of semen from normal fertile and infertile men.

Subjects	Semen volume (mL)	Sperm concentration 10^6/ mL	Motility (%)
Normal controls (27)	3.2 ± 0.5	128 ± 10	75 ± 5
Infertile Men			
- Normospermia (22)	2.8 ± 0.3	97 ± 4	64 ± 7
- Oligospermia (36)	2.5 ± 0.2	15 ± 4	26 ± 7
- Asthenospermia (9)	2.6 ± 0.3	65 ± 5	17 ± 2
- Azoospermia (14)	1.7 ± 0.4	—	—

Mean ± SD; Numbers in parentheses indicate total number of subjects.

Table 2 : Seminal total lipid, cholesterol and phospholipid levels from fertile and infertile men.

Subjects	Number of Subjects	Semen volume (mL)	Sperm concentration 10^6/ mL	Motility (%)
Normal controls (Fertile men)	27	161.01 ± 4.80	59.30 ± 2.28	29.21 ± 2.36
Infertile men				
- Normospermia	22	120.42 ± 4.37 *	51.47 ± 1.12 NS	16.74 ± 0.13 *
- Oligospermia	36	141.56 ± 3.78 *	46.31 ± 0.7 NS	22.92 ± 0.34 NS
- Asthenospermia	09	158.40 ± 5.01 NS	55.57 ± 2.0 NS	3.30 ± 0.53 NS
- Azoospermia	14	126.71 ± 2.24 *	37.68 ± 52 *	19.51 ± 1.30 *

Mean ± SD; *P<0.05; NS = Not Significant, in comparison with the normal group.

Table 3 : Seminal protein and lipid peroxide levels from fertile and infertile men.

Subjects	Number of Subjects	Protein (mg/L)	Lipid Peroxides (nM MDA/mL)
Normal controls	27	57.36 ± 4.08	1.83 ± 0.01
Infertile men			
- Normospermia	22	62.23 ± 1.8 NS	2.64 ± 0.12 **
- Oligospermia	36	51.72 ± 8.04 NS	1.95 ± 0.21 NS
- Asthenospermia	09	65.31 ± 6.84 NS	2.74 ± 0.32 **
- Azoospermia	14	48.37 ± 2.22 *	0.95 ± 0.40 ***

Mean ± SD. *P<0.05; **P<0.01; ***P<0.001. NS=Not Significant, in comparison with the normal group.

been made to compare the lipid content in fertile men with those of normospermic infertile, oilogospermic, asthenospermic and azoospermic men. Our results show significant reduction in total lipid levels in normospermic infertile men. These results are similar to those reported earlier in alcoholics (18) and in patients with chronic infections (19). It is well known that lipids in seminal plasma, apart from serving as energy source for spermatozoa during capacitation and fertilization process (20), also determine their structural integrity (21). As the spermatozoa pass from the caput to the cauda epididymidis, the incorporation of lipids in or on the cell membrane of spermatozoa is an important event in the maturation process (22). Furthermore,

lipid exchange occurs, more or less freely, between spermatozoa and seminal plasma (23). Therefore, it may be stated that decrease in the lipid content of seminal plasma in normospermic infertile men, as reported here, will not only have an adverse effect on the membrane structure required for the occurrence of capacitation and acrosome reaction but also lead to perturbance of sperm function.

Phospholipids, along with cholesterol, play important role in maintaining the cohesiveness of sperm membrane structure and their capability to withstand physical and physiological stress (24). There have been reports that cholesterol: phospholipid ratio influences the structural integrity and fluidity of membranes (21) and increase in this ratio is known to be associated with decrease in fertility (25). A decrease in the phospholipid concentration with more or less unaltered cholesterol, as reported here increases the cholesterol : phospholipid ratio in spermatozoa, which may also affect fertility. Such an increase in this ratio has also been reported earlier in spermatozoa from patients with unexplained infertility (20). Moreover, Sebastian *et al* (26) also reported diminished seminal plasma phospholipid levels in infertile men and they suggested a negative correlation between seminal neutral lipids and infertility.

Our results also reveal significantly elevated lipid peroxide levels in seminal plasma of normospermic and asthenospermic men. A significant increment in the lipid peroxide content of normospermic infertile men is remarkable. Lipid peroxidation is a free radical mediated phenomenon and it is a well known example of oxidative damage in cell membranes, lipoproteins and other lipid containing structures (27). Therefore, our results demonstrating high lipid peroxide levels in infertile men may be due to elevated oxidative stress. The lipids in spermatozoa are known to be susceptible to peroxidation and Aitken *et al* (28) have previously demonstrated the importance of lipid peroxidation in the pathophysiology of male infertility. Furthermore, as stated earlier, our results also indicate low seminal plasma lipid and phospholipid content in infertile men. This may be due to their increased breakdown by activated lipases and phospholipases. There are reports that these hydrolytic enzymes alongwith proteases, DNAses, etc., form secondary defence system against free radical mediated cellular injury (29). Moreover, anti-oxidants are known to be used up in conditions of elevated oxidative stress (30).

It has been widely reported that spermatozoa which are morphologically normal and have the optimum density, may be biochemically and functionally abnormal (31, 32). Therefore, on the basis of the results of the present study and as evidenced from the earlier studies, it may be seen that spermatozoa from infertile men, especially those showing a normal seminogram, are unable to actively participate in the normal fertilization process as a result of abnormal lipid composition. This may be due to elevated lipid peroxide content brought about by free radical mediated oxidative stress. However, further indepth studies are required to assess the status of other primary and secondary anti-oxidant defence systems in infertile male seminal plasma and spermatozoa *per se*.

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