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A Study of Seminal Hyaluronidase, Fructose, Lipid Peroxide and Zinc in Primary Male Infertility

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Summary

40 fertile control males and 90 primary infertile males were selected for the determination of seminal hyaluronidase, fructose, lipid peroxide and zinc levels. The levels of seminal hyaluronidase and zinc were significantly increased in all groups of primary infertile patients in comparison with control subjects ($P<0.001$). The maximum alterations in above biochemical parameters were found in azoospermic patients. Negative correlations were found in the levels of a) Hyaluronidase and Fructose b) Hyaluronidase & Lipid peroxide c) Lipid peroxide and Zinc d) Zinc and Fructose in control subjects and in primary infertile patients. Positive correlations were observed in the levels of a) Hyaluronidse and Zinc b) Lipid peroxide and Fructose in control subjects and primary infertile male patients.

Introduction

Approximately 15% of couples show primary infertility as they have been unable to achieve a pregnancy after one year of unprotected intercourse. Male factor is found to be responsible for 50% of infertility (Keye chang, et al., 1995). Hence an increasing interest has developed in the study of different biochemical parameters of male infertility.

Male infertility may be associated with one or more abnormalities such as obstruction of epididymis, local infection, immunological disroders etc. Semen analysis is the only step to investigage the male infertility which provides information about sperm count, morphology and motility. The biochemical analysis of semen can provide information about secretory function of the organs contributing to this fluid. Recent studies have been concerned with the biochemistry of semen as semen contains many secretions of prostate, seminal vesicle and other glands.

Hyaluronidase, fructose, free radicals and zinc play an essential role in fertilization (McRorie and Williaws 1974; Mann and Parson 1964; Halliwell 1994; Eliasson 1971). In view of noticing the role of above biochemical parameters in fertilization the present study was aimed to determine the levels of seminal hyaluronidase, fructose lipid peroxide and zinc in different groups of primary infertile patients and known fertile control subjects and to study correlation among these biochemical parameters in control subjects and primary infertile male patients if any.

Material and Methods

Present study included 40 fertile control male subjects and 90 primary infertile male patients. Male

persons who became father of children in the last year were grouped as fertile control subjects. Primary infertile male patients visiting to the infertility clinic at SCSM General Hospital, Solapur and who were unable to achieve a pregnancy at least one year after unprotected intercourse and for whom female partners have not shown any diagnosed cause of infertility were selected for the study in the age group of 21-45 years.

Semen samples were collected by masturbation in a sterile wide mouthed plastic container under all aseptic precautions, after at least four days abstinence and semen analysis were carried out within one hour after collection of samples. Depending on sperm count the study subjects were divided into four groups, viz.

Group I: Normozoospermic subjects (fertile controls). (sperm count > 20 million / ml.)
Group II: Oligozoospermic subjects. (Sperm count 10-20 million/ml.)
Group III: Severe oligozoospermic subjects. (Sperm count < 10 million/ml.)
Group IV: Azoospermic subjects. (Complete absence of sperm).

Semen samples were used for estimations of

1. Hyaluronidase activity by a spectrophotometric assay of Alfred Linker (Ulrich 1974).
2. Fructose level by the method of Roe (John et al., 1974).
3. Lipid peroxide level by the method of K. Satoh (Satoh, 1978).
4. Zinc level by Atomic Absorption spectrophotometer (Burtimovitz and Purdy 1977).

Statistical analysis was carried out by using students unpaired 't' test.

Results

Table No. (1) depicts the levels of hyaluronidase, fructose, lipid peroxide and zinc in the semen of fertile control subjects and different groups of primary infertile patients.

In the present study seminal hyaluronidase activity and zinc levels were significantly decreased ($P<0.001$) in all groups of primary infertile patients as compared to fertile control subjects. The seminal fructose and lipid peroxide levels were significantly increased ($P<0.001$) in all groups of primary infertile patients as compared with fertile control subjects.

Maximum depletion in the levels of seminal hyaluronidase and zinc and maximum elevation in the levels of seminal fructose and lipid peroxide were observed in azoospermic patients as compared with control subjects (Table No. 1).

Negative correlations ($P<0.001$) were found among I) Hyaluronidase and Fructose levels, ii) Hyaluronidase and Lipid peroxide levels, iii) Zinc and Fructose levels iv) Lipid peroxide and Zinc levels, in semen of fertile control subjects and primary infertile patients (Table No. II). While positive correlations ($P<0.001$) were noticed in I) Hyaluronidase and Zinc levels, ii) Lipid peroxide and Fructose levels in semen of fertile control subjects and in primary infertile patients (Table No. II).

Table No. I
Showing the comparison of levels of seminal Hyaluronidase, Fructose, Lipid peroxide and Zinc in fertile controls and different groups of primary infertile patients.

No.	Groups	n	Seminal Hyaluronidase Activity Units/lit.	Seminal fructose Mg/100ml	Seminal Lipid peroxide n mole MDA/ml	Seminal Zinc Mg/100ml
1.	Fertile Control	40	130.79 ± 31.37	320.80 ± 26.14	2.81 ± 0.62	21.81 ± 3.9
2.	Oligozoospermic	30	92.86 ± 6.7*	427.96 ± 18.62*	5.93 ± 0.57*	17.13 ± 1.2*
3.	Severe Oligozoospermic	30	56.21 ± 5.9*	456.76 ± 10.56*	6.68 ± 0.84*	15.37 ± 1.8*
4.	Azoospermic	30	19.27 ± 5.3*	552.03 ± 33.89*	8.22 ± 0.71*	13.04 ± 2.3*

Values are expressed as Mean ± S.D.
* → Indicates $P<0.001$
'n' → Indicates numbers of subjects

Table No. II
Depending correlations in different parameters in semen of fertile control subjects and primary infertile patients.

No.	Parameters	'r' value
1.	Hyaluronidase and Fructose	-0.8681*
2.	Hyaluronidase and Lipid peroxide	-0.9652*
3.	Hyaluronidase and Zinc	+0.9780*
4.	Lipid peroxide and Zinc	-0.9988*
5.	Lipid peroxide and Fructose	+0.9875*
6.	Zinc and Fructose	-0.8157*

* → Indicates P<0.001
- → Indicates negative correlation
+ → Indicates positive correlation

Discussion

From the observation it is evident that decrease in hyaluronidase activity in the present study is associated with decrease in sperm count in different groups of infertile subjects as compared with the control subjects (Table No. 1). These observations are in accordance with those of earlier workers (Savion M., et al., 1986; Mayade 1995).

Hyaluronidase is regarded as important in the fertilization process as the release of hyaluronidase by the spermatozoa is thought to be necessary for the passage for penetration of ovum by depolymerizing the matrix between cells of cumulus oophorus (Rogers and Morton 1973).

Hyaluronidase activity was found to be correlated significantly with sperm concentration, motility and percentage of sperm concentration with normal morphology. Hyaluronidase activity found in semen is sperm derived and hence it increases with increasing sperm number (Mayada 1995). Results from the present study are similar with this findings as activity corresponds to the fraction released from the sperm as evidenced by decreasing enzyme activity in semen with increase in severity of oligozoospermia.

Fructose is major carbohydrate in the semen and is the main energy source for spermatozoa. It is utilized for metabolic activity of the sperm and energy derived from the fructose can be utilized for the motility and vital role of sperm in the process of fertilization. Significant increase (P<0.001) in the concentration of fructose observed in the present study correlates well with findings of earlier workers (Mann and Parson 1950, Davis and McCune, 1950) and increase in the fructose level correlates with decreasing sperm count (Table No. 1). The low value of seminal fructose in normospermic subjects could result from utilization of fructose by spermatozoa (Davis and McCune, 1950).

Increased levels of seminal lipid peroxide in primary infertile patients in the present study (P<0.001) are relevant to result of earlier reports (Halliwell 1994; Selly et al 1991) are relevant to result of earlier reports (Halliwell 1994; Selly et al 1991). Mammalian spermatozoa are known to be highly sensitive to injuries caused by high oxygen concentration. Because polyunsaturated fatty acids in the phospholipid of human spermatozoa are highly susceptible to peroxidation, oxygen free radicals generated by spermatozoa may be involved in the production of spermicidal cytotoxic end products (Selly et al 1991).

Reduction in the levels of seminal zinc in primary infertile patients (P<0.001) from the present study are in accordance with the results of previous researchers (Eliasson and Lindhomer 1971; Madding et al, 1986). Zinc found in the semen is due to the secretions of prostate gland and reflects the prostatic stores. A certain local level of testicular zinc is required for the development of spermatozoa since zinc is essential for DNA synthesis and cell division (Madding et al, 1986). Zinc deficiency leads to a syndrome with dwarfism, hypogonadism, retardation of sexual maturation and increased oxidative stress.

In the present study increased production of lipid peroxide is associated with decrease in zinc as an antioxidant. An imbalance in this hormony may be responsible for the inactivation of hyaluronidase activity thereby leading to severity of oligozoospermia.

A further study including supplementation of antioxidants like zinc, Vit. E etc. in primary infertile males may enlighten on this aspect and also can assess effect of zinc supplementation by hyaluronidase activity in primary male fertility.

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