Study Of Semen Acid And Alkaline Phosphatase In Relation To Sperm Count And Motility

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Abstract:

In the present study phosphatase levels (Acid and Alkaline) were compared with sperm count and motility in 100 subjects with varying conditions. All the subjects presented were between 21-45 years of age. Samples of semen were collected from 100 cases comprising of the different grades of sperm concentration and motility. Human seminal fluid contains significant amount of acid phosphatase and alkaline phosphatase. The activities of the acid phosphatase and alkaline phosphatase vary considerably in different normal individuals. The activity of acid phosphatase showed progressive rise with decrease in the sperm concentration. No significant correlation observed between seminal acid phosphatase and sperm motility and seminal alkaline phosphatase with sperm count as well as motility.

Key Words: : Acid Phosphatase, Alkaline Phosphatase, Sperm count, Motility.

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INTRODUCTION:

The terms acid and alkaline phosphatase were first proposed by Davies[1] to distinguish phosphatases with widely different pH optima. The difference between the two groups of enzymes is also reflected in their substrate specificity. Alkaline phosphatase will hydrolyse Ssubstituted monoesters of phosphorothioic acid (R-SP03H2) and not O-substituted monoesters of phosphorothioic acid (R-OPOjH^ and the reverse applies to acid phosphatase[2] Another major distinction lies in the activation by Mg2+ and inhibition by metal chelating agents of alkaline

phosphatase and not acid phosphatase[3].

It has been known for a long time that human seminal plasma is a rich source of acid (prostatic) phosphatase[4,5] were the first to report an alkaline phosphatase activity (at pH 9-0) in human seminal fluid, although with a very much lower activity than the acid phosphatase. We were interested in this alkaline phosphatase as a possible example of a seminal metaloenzyme and so attempted to identify optimal conditions for its measurement. However, we were unable to demonstrate a pH optimum in the alkaline region, hence we have considered the possibility that the low phosphatase activity under alkaline conditions is due to acid phosphatase.

Materials And Methods : In the present study, 100 cases of male infertility under investigations were studied during the period April'99 to October 2000. All cases were from OPD patients of Guru Gobind Singh Hospital, Jamnagar. Semen samples were collected by masturbation after 3 days of abstinence in the laboratory side room in clean, dry, biological inert container protected from cold.

Sperm count, motility and morphology were studied using standard technique. Semen alkaline phophatase and acid phosphatase was measured using principle given by Kind and King[6].

Observation And Results : In all 100 cases sperm count, sperm motility, seminal acid and alkaline phosphatase levels were carried out. Semen was collected in the side laboratory as an OPD procedure. The subjects were aged between 21 years to 45 years.

Though the study is relation of seminal phophatase level (acid and alkaline phosphotase) sperm count and motility, all the cases were studied in detail and history and clinical findings were recorded.

There were 08(08%) cases between age group 20-25 yrs, 40 (40%) cases between 26-30 yrs age group, 36(36%) cases between 31-35 yrs, age group 14(14%) cases between 36-40 yrs. age group and 02(02%) case were of above 40 yrs. age. Most of subjects under investigation were in the age group 26-35 yrs.

Most of the cases which presented were

having sperm concentration below 50 million /ml(73%). Out of these 73 cases (73%), 16 cases showed azoospermia and 19 cases showed sperm count between 41-50 million/ml and 12,11,8,7 cases presented with sperm concentration 11-20, 31-40, 1-10. 21-30 million /ml respectively. Out of 16 azoospermic subjects 12 were due to testicular origin and remaining 4 had under gone vasectomy.

TABLE : 1 Sperm Count In 100 subjects

Sperm Count (million / ml)	No. of cases	Percentage
Azoospermia	16	16
1 to 10	08	08
11 to 20	12	12
21 to 30	07	07
31 to 40	11	11
41 to 50	19	19
51 to 60	12	12
61 to 70	07	07
71 to 80	05	05
81 to 90	03	03
Total	100	100%

42 cases had motility of 40% , 27 cases presented with motility of 41 to 60% while remaining 15 cases had motility above 60%

TABLE : 2 Sperm Motility In 100 Cases

Sperm Motility (Percentage %)	No. of cases	Percentage (%)
Azoospermia	16	16
Occasionally motile	05	05
Few to 20	12	12
21-40	25	25
41-60	27	27
61-80	15	15
TOTAL	100	100%

The activity of acid phosphatase decreased as sperm count increased. The value of seminal acid phosphatase level was significantly elevated in azoospermic subjects. Subjects having normal count (50-90 million/ml) had normal acid phosphatase level.

TABLE : 3 Relation of Seminal Acid Phosphatase Level With Sperm Count:

Sperm count (Million/ml)	No. of cases	Acid Phosphatase (KAU/ml) Level (Mean)+SD
Azoospermia	16	8836.25 +169.169
1 to 10	08	8131.25 +

		168.677
11 to 20	12	942.66 + 285.221
21 to 30	07	6988.14 + 140.140
31 to 40	11	6338.09 + 125.895
41 to 50	19	5461.06 + 156.325
51 to 60	12	4877.00 + 107.356
61 to 70	07	3944.00 + 130.677
71 to 80	05	3631.00 + 116.833
81 to 90	03	2446.3 + 73.059
Total	100	

Table shows inverse relationship between sperm count and acid phosphatase level.

TABLE : 4 Relation of Seminal Alkaline Phosphatase Level With Sperm Count:

Sperm count (Million/ml)	No. of cases	Alkaline Phosphatase (KAU/ml) Level (Mean)+SD
Azoospermia	16	0.527 +0.013
1 to 10	08	0.480 + 0.009
11 to 20	12	0.410 + 0.014
21 to 30	07	0.520 + 0/009
31 to 40	11	0.630 + 0.010
41 to 50	19	0.690 + 0.001
51 to 60	12	0.730 + 0.015
61 to 70	07	0.720 + 0.019
71 to 80	05	0.680 + 0.011
81 to 90	03	0.670 + 0.018
Total	100	

Above table shows that there is no relation between sperm count and seminal alkaline phosphatase level.

TABLE : 5 Relation of Seminal Acid Phosphatase Level With Sperm Motility.

Sperm Motility Percentage(%)	Acid Phosphatase Level(KAU/ml) Mean+SD
Occasionally Motile	3400.000 +102.47
Few -20	4471.670 +124.99
21-40	5702.240 +120.75
41-60	4815.000+ 107.58
61-80	4218.533 +094.85

Above table shows that there is no significant correlation between seminal acid phophatase and sperm motility.

TABLE : 6 Relation of Seminal Alkaline Phosphatase Level With Sperm Motility.

Sperm Motility Percentage(%)	Alkaline Phosphatase Level(KAU/ml) Mean+SD
Occasionally Motile	0.410 +0.009
Few -20	0.480 +0.013
21-40	0.360 +0.018
41-60	0.310+ 0.019
61-80	0.490 +0.008

Above table shows no significant correlation between seminal alkaline phophatase with sperm motility.

TABLE :7 Relation of Seminal Acid Phosphatase And Alkaline Phosphatase Level With Sperm Count:

Sperm count (Million/ml)	Acid Phosphatase (KAU/ml) Mean +SD	Alkaline Phosphatase (KAU/ml) Mean+SD
Azoospermia	8836.250 +169.169	0.526 +0.013
1 to 10	8131.250 + 168.677	0.480 + 0.009
11 to 20	942.660 + 285.221	0.410 + 0.014
21 to 30	6988.142 + 140.140	0.520 + 0/009
31 to 40	6338.090 + 125.895	0.630 + 0.010
41 to 50	5461.058+ 156.325	0.690 + 0.001
51 to 60	4877.000 + 107.356	0.730 + 0.015
61 to 70	3944.000+ 130.677	0.720 + 0.019
71 to 80	3631.000 + 116.833	0.680 + 0.011
81 to 90	2446.330 + 73.059	0.670 + 0.018

Table shows inverse relation between seminal acid phosphatase and sperm count. Maximum values of acid phosphatase levels obtained in azoospermic group. Alkaline phosphatase values had no significant correlation with sperm count.

Discussion: In the present study, semen analysis of 100 cases was carried out, which included sperm count, motility, seminal acid and alkaline phosphatase level. Relation of seminal phosphatase level with sperm count and motility was studied in the department of pathology, Shri M.P. Shah Medical College, Jamnagar, during the period of April '99 to October 2000

In the present study, semen analysis was performed in 100 subjects between 21-45 years age.

Present study showed the presence of significant amount of acid phosphatase and alkaline phosphatase in human seminal plasma. Seminal acid phosphatase level was significantly higher (8836.250 169.169 KAU/ml) in azoospermic (16 cases) group.
In the present study, varying levels of seminal acid phosphatase were observed in 27 normal subjects (51-90 million /ml) ranging from 2446.33 + 73.059 to 4877.00 + 107.356 KAU/ml of semen and seminal alkaline phsophatase level was ranging from 0.670 + 0.018 to 0.730 + 0.015 KAU/ml respectively.

As sperm count increases, the seminal acid phosphatase level decreases. Inverse relationship between the activity of human seminal acid phosphatase and the concentration of spermatozoa in the present study was found.

In the present study, seminal acid phosphatase level had no relation with the percentage of motility of spermatozoa. Occasional motile sperm (5 cases) having 3400.000 + 102.470 acid phosphatase level and few-20, 21-40, 41-60 and 61-80% motile sperm had acid phosphatase level 4471.666 +124.99, 5606+120.75, 4815.000 +107.58, 4218.533 + 94.85 respectively.
Samnel et al[7] observed highest level of seminal acid phosphatase in vasectomised azoospermic patients. They showed reverse relationship of seminal acid phosphatase activity against sperm concentration.

V.S. Jathar et al[8] had found that the activity of seminal Acid Phosphatase showed a fall as the sperm count increased. As compared to normal (fertile group), significantly higher mean values of seminal acid phosphatase activity were observed in oligospermic (subfertile group) and azoospermic group.

In the present study, also inverse relation of seminal acid phosphatase level with sperm count was observed which was comparable with above studies.

Upadhaya M. Et al [9] studied 176 males attending infertility clinic and 88 controls awaiting elective vasectomy. They also found that the activity of seminal acid phosphatase was higher in the former group.

Vaishwanar and Abhayankar[10] did not find any significant correlation of seminal acid phosphatase with concentration of sperm. No significant difference in the activities of seminal acid phosphatase for azoospermic and non-azoospermic men was observed by them.

Compared to this, present study showed inverse relation between seminal acid phosphatase and sperm count which differs from study of Vaishwanar and Abhayankar[10]

S. And A. Carpin et al[11] showed the significant decrease in prostatic acid phosphatase ejaculate levels among 95 varicocele patients than in the control group. They also observed the significant low sperm count, sperm motility and ejaculate volume in the varicocele group than in the control group.

R.P. Das et al[12] had studied the relation of acid phosphatase in human semen to sperm count and motility. They observed that seminal acid phosphatase showed positive correlation with sperm count and motility. Singh g. et al[13] had studied 56 patients. Out of these, 19 were normospermic, 18 were mildly oligospermic and 19 were severely oligospermic (sperm count 1-20 million/ml). They found that individuals with very severe oligospermia showed a positive correlation of seminal acid phosphatase.

R.P. Das et al[12] found positive correlation between sperm motility and seminal acid phosphatase level as shown in table which was different from present study. Present study did not find any relation of seminal acid phosphatase to sperm motility

Carpino. A et al[14] (1994) investigated relation of seminal acid phosphatase level with sperm motility in 3 idiopathic asthenozoospermic and 20 normal subjects. They found lower level of prostatic acid phosphatase in asthenozoospermic patients and they had evaluated direct correlation with sperm motility in all subjects.

Above studies differ from present study. Vaishwanar and Abhayankar[10] found no significant correlation between the acid phosphatase and the semen characteristics like sperm motility and live / dead cells. Upahyaya M et al[9] studied 176 males attending an infertility clinic and 88 controls

awaiting elective vasectomy. They observed that the semen acid phosphatase level had no definite relationship to ejaculate volume, sperm motility, viability and morphology.

Le-Calve M. et at[15] studied no significant relation between seminal acid phosphatase level with motility in normozoospermic (N=41) and asthenozoospermic group(N=29)

Present study also showed no significant relation between seminal acid phosphatase level and sperm motility which was quite comparable with the above studies.

Samnel et al[7] did not observe significant correlation between alkaline phosphatase level and sperm count in their study on fertile, subfertile and vasectomised azoospermic individuals.

V.S. Jather et al[8] studied 60 normal adults and compared with 60 obligospermic and 24 azoospermic Indian subjects. They also did not show any relationship between seminal alkaline phosphatase level and sperm count as show in table.

In the present study, no significant correlation between seminal alkaline phosphatase and sperm count made out which was similar to the above studies. Lewin-L M et al[16] studied total alkaline phosphatase activity in 30 samples of human semen which were measured colorimetrically, using p-nitrophenylphosphate as substrate. They found no significant relation between the enzyme activity and the sperm count, and semen volume.

Present study is comparable with above studies. R.P. Das et al[12] (1975) found positive correlation of seminal alkaline phosphatase level with sperm count which was not comparable with present study.

Relation of Seminal Alkaline Phosphatase With Sperm count And Motility. R.P. Das et al[12] had studied the relation between activity of alkaline phosphatase and the sperm count and motility of spermatozoa in freshly ejaculated human semen under varied conditions. They showed positive correlation of alkaline phosphatase level with the sperm count and motility. This study differs from present study.

While Lewin-LM et al[16] found no significant relation between the alkaline

phosphatase and sperm count, motility and semen volume. Present study is comparable with this study.

Relation of Seminal Acid Phosphatase Level With Sperm Count And Motility. Upadhyaya M et al[9] studied 176 males attending an infertility clinic and 88 controls awaiting elective vasectomy. The semen acid phosphatase activity was found to be significantly higher in the former group. However, no definite relationship of semen acid phosphatase level to ejaculate volume, sperm motility, viability and morphology could be made out.The above study was consistent with the present study.

Conclusion: In the present study, relation of phosphatase levels (Acid and Alkaline) were compared with sperm count and motility in 100 subjects with varying conditions. All the subjects presented were between 21-45 years of age. Samples of semen were collected from 100 cases comprising of the different grades of sperm concentration and motility. Human seminal fluid contains significant amount of acid phosphatase and alkaline phosphatase. The activities of the acid phosphatase and alkaline phosphatase vary considerably in different normal individuals. The activity of acid phosphatase showed progressive rise with decrease in the sperm concentration. No significant correlation observed between seminal acid phosphatase and sperm motility and seminal alkaline phosphatase with sperm count as well as motility.

References

1. Davies, D.R. (1934) The phosphatase activity of spleen extract. Biochem. J. 28, 529.
2. Hollander, V.P. (1971) Acid phosphatases. In the Enzymes, 3rd edn, Vol. IV, pp. 449-498. Ed. P. D. Boyer. Academic Press, New York.
3. Fernley, H.N. (1971) Mammalian alkaline phosphatases. In The Enzymes, 3rd edn., Vol. IV, pp. 417-447. Ed. P. D. Boyer. Academic Press, New York.
4. Kutscher, W. & Wolbergs, H. (1935) Prostatic phosphatase. Z. Physiol. Chem. 236, 237-240.
5. Gutman, A.B. & Gutman, E.B. (1941) Quantitative relations of a prostatic component (acid phosphatase) of human seminal fluid. Endocrinology, 28, 115-

118.

6. Kind P.R.N. & King E.J. 1954. Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrine. Journal of Clinical Pathology. 7(4): 322-326.
7. Sammel Nurm, Ines Musacchio, J Fanne A, Epstein. : Variations in seminal plasma constituent from fertile, Subfertile and vasectomised azoospermic men. Fertil. Steril. 23(5) : 357, 1972.
8. V.S. Jathar and Rashmi Hirwe, Shanta Desai and R. S. Satoskar. : Seminal fructose citric acid and phosphatase levels and their relation to the sperm count in man. Ind. J. Physiol. Pharmac. 186-190,1977.
9. Upadhyaya M. Hibbard B. M, Walker S. M. : Seminal acid phosphatase in relation to fertility. Acta. Obst. Gyneco. Scandinavica. 65(1) : 49 -52, 1986.
10. Vaishwanar P.S. and Abayankar H. N. : Acid phosphatase and pH in human semen. Ind. J. Exp. Biol. 9 : 261, 1971.
11. S. and A.Carpin, M. Button, M. Maggiolin, C. Giachett. F. Seidit. : Fructose, Prostatic acid phosphatase and Zinc levels in the seminal plasma of varicocele. Int. J. Fertil. 35(4) : 249 : 252, 1990.
12. R.P. Das, Somnath Roy and A.K. poddar : Relation of phosphatases in human semen to sperm count and motility. Ind.J.Med.Res.63(9):1323 -1326, 1975.
13. Singh – G. Adaikan P. g, Ng – Y.K. : Is seminal prostatic acid phosphatase a reliable marker for male infertility ? singapore Med. J 37(6) : 598- 599, 1996.
14. Carpino – A, Sisci – D, Aquila – S, Salernon M, Sicilano – L, Sessa – M. : Adnexal gland secretion markers in unexplained asthenozoospermia. Arch. Androl. 32(1) : 37-43, 1994.
15. Le- Calve M, Seagalen.J, Quernee-D, La Yanlt – M. T, Lescoat – D. : Diamino Oxidase activity and biochemical markers in human seminal plasma. Hum. Reprod. 10(5) : 1141 -1144, 1995.
16. U. Lewin, LM, Golan R., Soffer Y. Kaufman S., Yulzary Y. Zaidman J., Alkaline phosphatase in human semen : An investigation using enzyme inhibition and gel electrophoresis. Eur. J. of clin. Chem. and clin. Biochem 31(12):811-814, 1993.