Indian J Physiol Pharmacol 2000; 44 (4) : 456–460

RELATIONSHIP OF SEMINAL PLASMA TRANSFERRIN WITH SEMINAL PARAMETERS IN MALE INFERTILITY

RAJAY N. BHARSHANKAR* AND JYOTSANA R. BHARSHANKAR

Department of Physiology,
Govt. Medical College,
Nagpur – 440 003

(Received on March 13, 2000)

Abstract : This study was undertaken to investigate whether there is relationship between seminal plasma transferrin and seminal parameters which included sperm count, motility and morphology. The study included 100 male subjects in the age group of 23–41 yrs including 7 proven fertility, 6 post-vasectomised and 87 subjects were of idiopathic infertility. Estimation of seminal plasma transferrin concentration was done by using Mancini's single radial immunodiffusion technique. Study of the seminal parameters (Sperm count, Motility and Morphology) was done by using guidelines of WHO Manual.

Mean seminal plasma transferrin concentration in proven fertility subject was 5.35 mg/dl (± 2.07) and in normozoospermic subject was 4.63 mg/dl (± 2.50) which was significantly higher ($P<0.001$) than those of oligozoospermic, azoospermic and post-vasectomised subjects. Coefficient of correlation between seminal plasma transferrin concentration and sperm count was statistically significant ($r = 0.3087$, $P<0.001$).

The seminal plasma transferrin concentration was correlated with the percentage of motile sperms and was statistically significant. However no correlation could be demonstrated with various grades of motility. Statistically significant correlation was not found between transferrin and sperm morphology. The present study demonstrates that seminal plasma transferrin concentration is correlated with sperm count and percent motile sperms. Thus sertoli cell secretion-transferin has a positive influence over spermatogenesis and can be used as a marker of testicular function.

Key words : seminal plasma sperm seminal plasma transferrin

INTRODUCTION

In assessment of infertility, affecting about 15–18% of population (1) seminal examination alone is inadequate. Therefore biochemical constituent such as seminal plasma transferrin, produced by sertoli cells has been studied and correlated with seminal parameters like sperm count, motility and morphology.

Setoli cells are the most important intratesticular cells, which are not till date physiologically assayable with use of specific cell markers. Human sertoli cell cultures are capable of secreting transferrin, which

*Corresponding Author

is 1–4% of total protein secreted (2). Spermatogenesis takes place in the folds of sertoli cell cytoplasmic membrane, which creates intratubular microenvironment. Transferrin supplies iron to the developing and differentiating germ cells. Transferrin is an essential factor for sertoli cells and the cycle of production of this protein seems to be linked to the cycles of seminiferous epithelium (3). Thus germ cells appears to interact with sertoli cells to modulate the pattern of transferrin secretion. Hence the seminal plasma transferrin can be taken as marker of testicular function.

Many authors worldwide have studied seminal plasma transferrin levels in relation with seminal parameters. We wanted to demonstrate the same in the population of central India.

METHODS

The study included 100 male subjects in the age group of 23–41 yrs. Out of which 7 subjects were of proven fertility, 43 normozoospermic, 24 oligozoospermic, 20 azoospermic and 6 post-vasectomised subjects. The study subjects attended the semenology lab in the department of Physiology, Govt. Medical College, Nagpur for semen analysis. The written consent was obtained. Study period was of 22 months from Jan., 97 to Oct., 98.

After 4–5 days of abstinence, subjects were instructed to collect the semen sample in the sterile, dry, clean glass container by masturbation in the lab itself without using any lubricant or condoms. Semen samples were allowed to liquefy. Semen analysis was done according to WHO manual within 1 hour of collection (4).

The liquefied semen samples were centrifuged at 1000 g for 15 minutes to get the supernatant for estimation of seminal plasma transferrin. Mancini's Single Radial Immunodiffusion technique was followed for the estimation (5). Anti-transferrin antibodies were obtained from a serum of rabbit from immunology section of Microbiology Dept. Govt. Medical College, Nagpur. The mixture of purified agar (150 mg) and barbitone buffer (10 ml) was boiled vigorously till gel particles fully dissolves. Then appropriate amount of pre-warmed mono-specific anti-transferrin antibody (3%) was mixed with fluid gel at 56°C. This antibody incorporated gel was poured on glass slides and allowed to set at 4°C for 30 minutes. The 5 µl of undiluted seminal plasma was poured into the wells which were punched out from antibody incorporated gel on glass slides. These slides were kept at 4°C for 48 hours. After completion of diffusion time, diameter of ring precipitation was measured with tri-partigen scale. Transferrin concentration related to measured diameter was directly read from the table of reference value prepared in laboratory.

Data was analysed by using analysis of variance (ANOVA) test, student 't' test and coefficient of correlation.

RESULTS

The subjects with proven fertility had highest mean sperm count (130 ± 20) expressed in millions/ml as compared to normozoospermic (107.67 ± 78.34) and oligozoosperic subjects (19.1 ± 12.9). Similarly seminal plasma transferrin concentration is higher than normozoospermic and oligozoospermic group. The coefficient of correlation between seminal plasma transferrin concentration and sperm count is significant (r = 0.3087, P<0.001). Mean differences in seminal plasma transferrin concentration between various groups is statistically significant (ANOVA; F–7.486, P<0.001) Table I.

The seminal plasma transferrin concentration in subjects having grade 3 and grade 4 sperm motility was higher than subjects having decreased motility but not to the level of significance (P>0.05). The difference between the mean seminal plasma transferrin concentration in group having 60% or more motile sperms and in group having less than 60% motile sperms is statistically significant (P<0.05) Table II.

There was no correlation between seminal plasma transferrin and sperm morphology in any of the groups Table III.

TABLE I: Depicting seminal plasma transferrin concentration in different groups.

Groups (n)	*Transferrin (mg/dl) (Mean±SD)*	*Anova Test*
Proven fertility (7)	5.35±2.07	F value–7.486
		df–2, 71
Normozoospermic (43)	4.63±2.50	
		(P<0.001)
Oligozoospermic (24)	2.63±1.76	
		Highly significant

TABLE II: Relationship of seminal plasma transferrin concentration with sperm motility.

Motility		*n*	*Transferrin (mg/dl)*	*'t' test result*
Qualitative:	Grade 2–4	52	4.147±4.07	t=0.4112
	Grade 0–2	21	3.753±2.568	df=71 (P>0.05)*
Quantitative:	>60%	50	4.377±2.365	t=1.99
	<60%	20	3.128±2.41	df=68 (P<0.05)**

*Not significant
**Significant

TABLE III: Correlation of seminal plasma transferrin concentration with sperm morphology.

Groups (n)	*% of sperms with normal morphology*	*Transferrin (mg/dl)*	*Coefficient of correlation (r)*
Proven fertility (7)	66.66±5.16	5.35±2.07	–0.204 (NS)
Normozoospermic (43)	63.60±6.84	4.63±2.50	–0.191 (NS)
Oligozoospermic (24)	49.58±16.01	2.62±1.75	–0.256 (NS)

NS-Not significant

DISCUSSION

Transferrin is an iron binding protein in serum and later found in seminal plasma (6). Sertoli cells as shown by their monolayer culture mainly produce this protein. Various authors have suggested that its measurements could provide indications for functional activity of sertoli cells. Different authors have used different methods for transferrin estimation in fertility proven subjects (7, 8, 9, 10) and the value is similar to that of present study (5.35 mg/dl).

The positive correlation between sperm count and seminal plasma transferrin concentration observed in present study confirms the previous reports (11). Therefore, seminal plasma transferrin levels appears to reflect the sertoli cell function and the relationship between this function and germ cells.

Spermatogenesis and spermiogenesis takes place within the folds of sertoli cell membrane. This close proximity of maturing sperms forms the ideal topographic basis for metabolic exchange. Sertoli cells serves as an intermediatery in the transport of iron from serum transferrin to developing germ cells (2). Transferrin has important role in supplying iron to testicular cells, which has growth promoting effect (12). Transferrin binds to the spermatocytes with high affinity (13) and plays an important role in development and differentiation of spermatogenetic cells.

The seminal plasma transferrin levels in normozoospermic infertile subjects and in those with proven fertility is not significantly different. So seminal plasma transferrin concentration is not associated with sperm fertilizing capacity. Similar were the findings of Suldo et al (14). However some authors suggested low transferrin was associated with poor fertilizing ability of human oocyte *in vitro*. Synthesis of transferrin may be important in the penetration of the vesting layer of human oocyte by the sperm (14). Human seminiferous tubules and sertoli cells are devoted to the storage and or production of transferrin whereas spermatocytes and spermatids use transferrin (15).

We found positive correlation with transferrin and percentage of motile sperms. This finding is similar to the findings of Zalata et al (16). Transferrin concentration mainly depends upon progressive sperm motility, ATP content, and sperm transferrin receptors. Transferrin seems to act to provide iron for proliferation and differentiation of germ cells whereas maturation of sperm motility takes place in epididymis and vas deference (17).

There is no significant correlation between the seminal plasma transferrin concentration and sperm morphology in any of the group. Other workers also found no correlation between sperm morphology and transferrin (18, 19).

Thus seminal plasma transferrin concentration is the direct index of the functional harmony between sertoli cells and germ cells in the microenvironment of seminiferous tubules. Further studies are required to draw more definitive conclusion regarding its relevance to the male fertility potential.

ACKNOWLEDGEMENTS

We gratefully acknowledge Dr. Geeta A. Kurhade and Dr. Sanjay P. Zodpey for their important guidance in writing this manuscript and for statistical analysis.

REFERENCES

1. Gopalkrishnan Kamala. Research in Infertility. *ICMR Bulletin* 1996; 26 (10): 97–104.
2. Skinner MK, Griswold MD. Sertoli cell synthesize and secrete transferrin like protein. *J Biol Chem* 1980; 255 (20): 9523–9525.
3. Mather JP, Gunsalus GL, Musto NA et al. The hormonal and cellular control of Seroli cell secretion. *J Steroid Biochem* 1993; 19: 41–51.
4. World Health Organization. Laboratory manual for the examination of human semen and semen-cervical mucus interaction, Edited by MA Belsey, R. Eliasson, AJ Gallegos, Ks Moghissi, CA Paulsen, MRN Prasad. Singapore Press Concern, 1980.
5. Mancini G, Carbonara, Hermans JF. Immunochemical quantification of antigen by single radial-immunodifusion. *Int J Immunochem* 1988; 20 (1): 15–20.
6. Quinlivan WLG. Analysis of proteins in human seminal plasma. *Arch Biochem* 1968; 127: 680–687. As quoted by ref no. 7.
7. Lizana J, Blad E. Immunonephelometry of specific proteins in human seminal plasma. *Clin Chem* 1983; 29 (4): 618–623.
8. Holmes SD, Lpshultz LI, Smith RG. Transferrin and gonadal dysfunction in man. *Fertil Steril* 1982; 38: 600–604.
9. Foresta C, Manoni F, Businaro V, Donadel C, Indino M, Scandellari C. Possible significance of transferrin levels in seminal plasma of fertile and infertile men. *J Androl* 1986; 7 (2): 77–82.
10. Tauber PF, Zaneveld LJD, Propping D and Schumacher GFB. Components of human spit ejaculates I. spermatozoa, fructose, immunoglobulins, albumin, lactoferrin, transferrin and other plasma proteins. *J Reprod Fertil* 1975; 43 (2): 249–267.
11. Mallea L, Mas J, Padron RS, Diaz JW. Transferrin in seminal plasma of fertile and infertile men. *Andrologia* 1988; 20 (1): 15–20.
12. Perez-Infante V and Mather JP. The role of transferrin in the growth of testicular cells lines in serum free medium. *Exp Cell Res* 1982; 142: 325–332.
13. Holmes SD, Bucci LR, Lpshultz LI and Smith RG. Transferrin binds specifically to pachytene spermatocytes. *Endocrinology* 1983; 113: 1916–1918.
14. Sueldo Carlos, Marrs RP, Berger T, Kletzky OA and Brien. Correlation of semen transferrin concentration and sperm fertilizing capacity. *Am J Obstet Gynecol* 1984; 150: 528–531.
15. Vannelli BG. Immunostaining transferrin receptors in human seminiferous tubules. *Fertil Steril* 1986; 536–541.
16. Zalata A, Hafez T, Schoonjans F and Comhaire F. The possible meaning of transferrin and its soluble receptors in seminal plasma as a marker of the seminiferous epithelium. *Human Reprod* 1996; 2 (4): 761–764.
17. Guyton AC and Hall JE. *Textbook of Medical Physiology* 1996; 9th ed W. B. Saunders Company. pg 1006.
18. Fuse H, Ohta S, Sakamoto M and Katavama T. Seminal plasma transferrin concentration : Relationship with seminal parameters and plasma hormone levels. *Unrol Int* 1992; 49: 158–162.
19. Chan SY, Loh TT, Wang C, Tang LC. Seminal plasma transferrin and seminiferous tubular dysfunction. *Fertil Steril* 1986; 45 (5): 687–691.