Original Article

Evaluation of Seminal Fructose and Citric Acid Levels in Men with Fertility Problem

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Abstract

Context: Male infertility is a medical problem, attributed to 50% of infertility. Seminal plasma can be an anticipating factor as it comprises secretions of accessory sex gland, thus offering novel and precise ways to understand potential roles of these biochemical markers in male infertility. **Aim:** The objective of this study was to assess the correlation between biochemical markers and sperm parameters in envisaging male infertility. **Subjects and Design:** We enlisted 105 men with fertility issue as patients and 25 fertile men as controls to evaluate the sperm parameters and biochemical markers, namely fructose and citric acid in ascertaining male infertility. **Materials and Methods:** The semen samples from patients were collected properly and analyzed according to the World Health Organization-2010 manual. Later samples were centrifuged, seminal plasma was collected, and biochemical markers assessment was carried out by standard protocols. **Statistics:** Descriptive statistics, independent *t*-test, one-way ANOVA, and Pearson correlation were used for statistical analysis of different variables using SPSS 20.0. The mean sperm count and motility by all infertile conditions displayed a significant difference when compared with the controls ($P < 0.05$). **Results:** The mean fructose levels of oligozoospermia showed a nonsignificance difference when compared with controls ($P < 0.05$). Asthenozoospermia, asthenoteratozoospermia, and azoospermia had a significance difference ($P < 0.05$) for citric acid levels. Pearson correlation coefficient showed significant negative correlation of sperm count ($r = -0.564$) and sperm motility ($r = -0.574$) with fructose levels. Whereas seminal citric acid concentration had a positive correlation with sperm count ($r = 0.458$) and sperm motility ($r = 0.446$). **Conclusion:** Therefore, evaluation of certain biochemical markers of seminal fluid may benefit in understanding the functionality of accessory glands which subsidizes significantly to the seminal volume.

Keywords: *Accessory glands, citric acid, fructose, male infertility, seminal plasma*

Introduction

Procreation is one of the most essential aspects of mankind, and both the genders must be robust and normal to execute this process comfortably. The inability of couples to reproduce may result in stern psychological, social, and physical encumbrance; sometimes, this trauma ends in the loss of beloved ones. Today, 14%–30% of couples at their procreative age are enduring infertility,[1] and male factors are instrumental in nearly 50% of cases,[2,3] which is captivating global cumulative importance.[4,5] Male infertility is multifactorial, such as endocrine ailment, testicular catastrophe, testicular cancer, testicular instabilities, genital tract infection, varicocele, exposure to gonadotoxic substances,[6-8] smoking, advanced age, ejaculatory dysfunction, obstruction and

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abnormal functioning of accessory sex organs, prolonged exposure to heat, obesity, environmental pollutants,[9-11] poor Zinc and Vitamin C in food, excessive stress, and use of certain drugs.[12,13] The reproductive physiology of men affected by such factors produces lower sperm count, has deprived sperm motility, and shows anomalous sperm morphology, which are the key attributes of male infertility.[14] Semen is composed of concentrated suspension of spermatozoa, which is diluted by seminal fluid predominantly secreted by the seminal vesicles followed by prostate, with a slight contributions from the bulbourethral (Cowper's) glands and epididymis for the normal functioning of spermatozoa.[15-20] The seminal plasma is composed of an intricate assortment of organic and inorganic elements, that may not crucial for fertilization, yet it optimizes the suitable atmosphere for sperm motility, endurance and transport in the female reproductive expanse.[18,21] The first portion of the human ejaculate is principally composed of sperm and prostatic exudates i.e. citric acid, proteases, acid phosphatase and the later portion has fructose, prostaglandins, coagulating elements and bicarbonates for defending the acidic vaginal zone secreted by seminal vesicles.[16,17,22] Consequently, these biochemical secretions serve as a markers of their respective glands.[23] The seminal plasma has an excessive concentrations of fructose, which provides an anaerobic and aerobic source of energy for the sperm[24] and has been obliquely associated with progressive sperm motility and viscosity.[25,26] Evaluating fructose concentration of seminal plasma can display the status of seminal vesicles, endocrine anomalies and also potential ejaculatory duct obstruction, if any.[16,23,27] Citric acid is an essential organic acid, and its principle role is to maintain pH, convert protein, fat, and sugar into carbon dioxide.[24,28] It is a vital biochemical constituent of seminal plasma which not only rebounds the condition of the prostate, but also allied with coagulation and liquefaction of semen in humans.[29] Hence, it plays a vital part in sperm motility and hyaluronidase activity.[19,29] The importance of citric acid in altering sperm attributes during abstinence has been underrated, inspecting the levels of citric acid in seminal plasma thus may benefit to find the probable causes of male infertility. A proper counselling (brief medical history, physical inspection and imaging), simple semen analysis beside biochemical evaluation of seminal plasma is the prerequisite step to identify potential cause affecting viability, motility and morphology of spermatozoa[18,30] which laterally provides activity status of accessory sex glands.[31] Hence, assessing biochemical indicators for the identification of biological attributes of semen can help in establishing novel benchmarks that are precise and equitable in envisaging male infertility. Therefore, the current study was carried out to re-evaluate the efficiency of biochemical markers in assessing male infertility.

Materials and Methods

Study design

After gratifying the inclusion and exclusion standards for this study, a total of 105 men diagnosed with fertility issues as patients and 25 fertility-proven men with normozoospermia as controls were conscripted, who visited a renowned *in vitro* fertilization center, during the course of 2015–2018. The study was carried out in accordance with hospital ethics and guidelines and patients were aware of details of the study; written consent to partake for this study was obtained.

Semen collection and examination

After ejaculatory abstinence of 3–5 days, semen samples from patients and controls were collected in a sterile plastic container and examined after 30 min according to World Health Organization -2010 criteria.[18] Infertile groups were classified based on sperm concentration, motility, and morphology. Later, samples were centrifuged at 3000 rpm for 10 min and seminal plasma was stored at −20°C for fructose and citric acid estimation.

Estimation of fructose

Fructose is the source of energy for spermatozoa and acts as marker of seminal vesicle functionality. 20 µl of seminal plasma was mixed thoroughly with 220 µl distilled water, later the deprotinized with 50 µl of $ZnSO_4$ and 50 µl of NaOH. After 15 min of incubation, it was centrifuged at 2500 rpm and 200 µl of clear supernatant was mixed with Indole reagent followed by 32% hydrochloric acid. The mixture was incubated at 60°C for 20 min and after cooling readings were taken at 470 nm.[32]

Estimation of citric acid

Seminal citric acid is the marker of prostate gland functionality. 100 µl of seminal plasma and 100 µl of 50% trichloro acetic acid were mixed cooled in ice bath. After centrifugation at 2000 rpm for 15 min, 800 µl of anhydrous acetic anhydride was added to 100 µl of supernatant and incubated at 60°C for 10 min in a water bath. Later, dry reagent grade pyridine was added and incubated at 60°C for 40 min. Cooled on ice bath for 5 min and absorbance was measured at 400 nm.[33]

Statistical analysis

The data obtained were statistically interpreted and expressed in mean and standard error of the mean. Independent *t*-test was used to find whether the significant mean difference exists between patients

and controls along with Pearson correlation using statistical program IBM SPSS Statistics software Inc., version 20.0 (Armonk, NY, USA: IBM Corp.). Statistical interpretation was based on two-sided tests at a 0.05 significance level and correlation significant at the 0.01 level (two-tailed).

Results

In the current study, the mean age of patients and controls was 33.78 ± 0.42 and 32.56 ± 0.85 years, respectively, at the time of diagnosis. The men diagnosed with infertility problem were segregated into seven different infertile conditions [Table 1]. All infertile conditions exhibited significant mean difference for sperm count and motility with controls ($P < 0.05$). The fructose concentration was found higher in oligoasthenoteratozoospermia (139.83±0.19) and lower amongst asthenoteratozoospermia (47.85±2.74), when compared with rest other infertile conditions. Oligozoospermia (OL) had no significant difference with control for mean fructose concentration, although rest other conditions showed a fair significance ($P < 0.05$). AS + T displayed higher citric acid concentration when compared with controls, whereas rest other conditions exhibited marginal levels of citric acid. There was a significant difference among asthenozoospermia, AS + T, and azoospermia (AZ) with respect to control for citric acid concentration ($P < 0.05$). However, overall one-way ANOVA result showed a significant difference for sperm count, motility, and citric acid level when compared with controls ($P < 0.05$) except for fructose concentrations [Table 2]. Pearson correlation results exhibited significant negative correlation between sperm count ($r = -0.564$), sperm motility ($r = -0.574$), and fructose levels. Whereas seminal citric acid concentration had a positive correlation with sperm count ($r = 0.458$) and sperm motility ($r = 0.446$) [Table 3].

Discussion

The aim of this study was to ascertain the correlation between biochemical parameters in the seminal plasma and sperm parameters in controls and infertile patients. Our findings showed that fructose concentration decreases as the sperm concentration increases and vice versa.[34] This is because fructose is an energy reservoir,[35] and it is exploited by sperm for its metabolism and motility.[36] The elevated fructose concentration in our study with respect to AZ, OL + AS + T, OL, and severe oligoasthenoteratozoospermia could be either because of abridged sperm count, abnormal sperm morphology, and decreased sperm activity resulting in decreased utilization of fructose.[37,38] In our findings, low fructose concentration in AS + T [39,40] could be due to better motility of sperm or inflammation of seminal vesicle,[41] low levels of testosterone secretion,[42,43] or also due to anatomical anomalies.[30] Our findings revealed that fructose concentration is negatively correlated with sperm count and motility;[39] this correlation shows the utilization of fructose by sperm.[40] The increase in fructose content in teratozoospermia could be described

Table 1: Seminal parameters and biochemical marker concentration of individual infertile groups and controls

Patients (*n*)	Frequency (%)	Age (years)	Sperm count (mil/ml)	Sperm motility (%)	Fructose (≥13 µmole/ejaculate)	Citric acid (≥13 µmole/ejaculate)
AS	26.66	33.39±0.97	50.93±3.99*	32.54±1.24*	50.83±2.51*	42.18±1.62*
AS + T	17.14	34.06±0.91	31.61±3.27*	29.94±1.77*	47.85±2.74*	60.99±3.28*
AZ	15.23	32.63±0.84	0.00±0.00*	0.00±0.00*	127.98±0.67*	31.74±0.99*
OL + AS + T	3.8	35.50±1.76	10.75±0.25*	15.25±4.94*	139.83±0.19*	39.60±1.46
OL	3.8	34.50±2.50	9.75±1.11*	27.25±5.04*	115.18±1.30	36.58±1.35
SOL + AS + T	19.04	25.20±0.85	5.05±0.71*	9.45±1.67*	135.90±0.68*	36.05±0.64
T	14.28	32.87±1.23	32.27±4.28*	31.33±1.40*	131.77±1.19*	41.01±2.62
Control	-	32.56±0.85	62.40±4.45	43.04±0.70	104.29±2.79	36.70±1.65

*$P<0.05$ Defines the level of significance. All values are presented as mean±SE. *n*=Number, AS=Asthenozoospermia, AS + T=Asthenoteratozoospermia, AZ=Azoospermia, OL + AS + T=Oligoasthenoteratozoospermia, OL=Oligozoospermia, SOL + AS + T=Severe oligoasthenoteratozoospermia, T=Teratozoospermia, SE=Standard error

Table 2: Independent *t*-test for seminal parameters and biochemical marker concentration with respect to control versus infertile groups

Patients (*n*)	Sperm count (mil/ml)	Sperm motility (%)	Fructose (≥13 µmole/ejaculate)	Citric acid (≥13 µmole/ejaculate)
Infertile (105)	25.35±2.33	21.70±1.38	95.68±4.09	42.16±1.22
Fertile (25)	62.40±4.45	43.04±0.70	104.29±2.79	36.70±1.65
Sig level 0.05	<0.05*	<0.05*	0.31	0.03*

*$P<0.05$ defines the level of significance. All values are presented as mean±SE. SE=Standard error

Table 3: Pearson correlation coefficient among the study variables

	Count	Fructose	Citric acid
Motility	0.749**	−0.574**	0.446**
Count		−0.564**	0.458**
Fructose			−0.432**

**Correlation is significant at the $P<0.01$ level (2-tailed)

by its low utilization by spermatozoa with morphological defects.[40] Earlier studies reported that sperm with abnormal morphology may have poor or lack of motility and hence utilizes lower fructose.[30] The low levels of fructose in semen disturbs coagulation, sperm movement which could be due to genital tract inflammation.[37,44] The concentration of citric acid in seminal plasma acts as a dependable measure of prostate gland secretion; it plays a vital part in balancing osmotic equilibrium of semen which will affect the membrane activity and morphology of the spermatozoa.[40,45,46] Reduced concentration of citric acid have been found in severe or chronic prostatitis.[47] As it acts as gelling agent and helps in liquefaction of semen, indirectly benefit the sperm motility.[48] In our findings, symphonious to this, sperm count and motility showed positive correlation with citric acid.[48,49] In the current study, the negligible difference in the concentration of citric acid was found among all infertile patients except AS + T, which could be due to an improper activity of prostatic glands.[49]

Conclusion

Biochemical markers such as fructose and citric acid can be used for the recognition of biological attributes of semen which may assist in flourishing novel standards that are precise in anticipating and enhancing male fertility. These markers may not be a pertinent index of male reproductive dysfunction but in combination with alternative seminal characters could present effective manifestation of male reproductive function. Fructose is an indispensable liveliness resource for metabolism and motility of sperm; its absence is the mark of irregularity of seminal vesicle or ejaculatory duct impediment. The loss of citric acid in semen could be disablement of ejaculatory channels and could be a prior indication of prostate cancer. Therefore, evaluation of certain biochemical markers of seminal fluid may benefit in understanding the functionality of accessory glands which subsidizes significantly to the seminal volume.

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Conflicts of interest

There are no conflicts of interest.

References

1. Martinez G, Daniels K, Chandra A. Fertility of men and women aged 15-44 years in the United States: National survey of family growth, 2006-2010. Natl Health Stat Report 2012;51:1-28.
2. Jarow JP, Sharlip ID, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, *et al.* Best practice policies for male infertility. J Urol 2002;167:2138-44.
3. Thangaraj K. Genetics of male infertility: Indian scenario. Mol Cytogenet 2014;7:I19.
4. Povey AC, Stocks SJ. Epidemiology and trends in male subfertility. Hum Fertil (Camb) 2010;13:182-8.
5. Winters BR, Walsh TJ. The epidemiology of male infertility. Urol Clin North Am 2014;41:195-204.
6. Nieschlag E, Behre HM, Nieschlag S. Andrology: Male Reproductive Health and Dysfunction. Berlin: Springer; 2010.
7. Bayasgalan G, Naranbat D, Radnaabazar J, Lhagvasuren T, Rowe PJ. Male infertility: Risk factors in mongolian men. Asian J Androl 2004;6:305-11.
8. Cocuzza M, Alvarenga C, Pagani R. The epidemiology and etiology of azoospermia. Clinics (Sao Paulo) 2013;68 Suppl 1:15-26.
9. Kupis Ł, Dobroński PA, Radziszewski P. Varicocele as a source of male infertility – Current treatment techniques. Cent European J Urol 2015;68:365-70.
10. Jensen TK, Bonde JP, Joffe M. The influence of occupational exposure on male reproductive function. Occup Med (Lond) 2006;56:544-53.
11. Pizent A, Tariba B, Živković T. Reproductive toxicity of metals in men. Arh Hig Rada Toksikol 2012;63 Suppl 1:35-46.
12. Arcaniolo D, Favilla V, Tiscione D, Pisano F, Bozzini G, Creta M, *et al.* Is there a place for nutritional supplements in the treatment of idiopathic male infertility? Arch Ital Urol Androl 2014;86:164-70.
13. Kolesnikova LI, Kolesnikov SI, Kurashova NA, Bairova TA, Kolesnikova LI, Kolesnikov SI, *et al*. Causes and risk factors of male infertility. Bulletin of the Russian Academy of medical Sciences 2015;5:579-584.
14. Harris ID, Fronczak C, Roth L, Meacham RB. Fertility and the aging male. Rev Urol 2011;13:e184-90.
15. Weiske WH. Minimally invasive vasectomy with Fulguration technique: Experience in1000 patients in 12 years., Urologe. B.1994;34:448-52.
16. Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA. Campbell Walsh Urology: Expert Consult Premium Edition: Enhanced Online Features and Print. Vol. 4. Philadelphia: Elsevier Health Sciences; 2011.
17. Plant TM, Zeleznik AJ. Knobil and Neills Physiology of Reproduction. Amsterdam: Elsevier/Academic Press; 2015.
18. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. World Health Organization; 2010.
19. Amelar RD. Coagulation, liquefaction and viscosity of human semen. J Urol 1962;87:187-90.
20. Amelar RD, Hotchkiss RS. The split ejaculate: Its use in the management of male infertilitY. Fertil Steril 1965;16:46-60.
21. Insler V, Lunenfeld B, editors. Infertility: Male and Female. Edinburgh: Churchill Livingstone; 1986.
22. Orth JM. Proliferation of sertoli cells in fetal and postnatal rats: A quantitative autoradiographic study. Anat Rec 1982;203:485-92.
23. Aumüller G, Riva A. Morphology and functions of the human seminal vesicle. Andrologia 1992;24:183-96.
24. Mann T, Lutwak-Mann C. Male reproductive function and

the composition of semen: General considerations. In: Male Reproductive Function and Semen. London: Springer; 1981. p. 1-37.
25. Gonzales GF, Kortebani G, Mazzolli AB. Hyperviscosity and hypofunction of the seminal vesicles. Arch Androl 1993;30:63-8.
26. Fabiani R, Johansson L, Lundkvist O, Ronquist G. Prolongation and improvement of prostasome promotive effect on sperm forward motility. Eur J Obstet Gynecol Reprod Biol 1995;58:191-8.
27. Setchell BP, Waites GM. Changes in the permeability of the testicular capillaries and of the 'blood-testis barrier' after injection of cadmium chloride in the rat. J Endocrinol 1970;47:81-6.
28. Obidoa O, Ezeanyika LU, Okoli AH. Effect of scopoletin on male guinea pig reproductive organs. I. Levels of citric acid and fructose. Nutr Res 1999;19:443-8.
29. Marberger H, Marberger E, Mann T, Lutwak-Mann C. Citric acid in human prostatic secretion and metastasizing cancer of prostate gland. Br Med J 1962;1:835-6.
30. Lewis-Jones DI, Aird IA, Biljan MM, Kingsland CR. Effects of sperm activity on zinc and fructose concentrations in seminal plasma. Hum Reprod 1996;11:2465-7.
31. Plachot M, Belaisch-Allart J, Mayenga JM, Chouraqui A, Tesquier L, Serkine AM. Outcome of conventional IVF and ICSI on sibling oocytes in mild male factor infertility. Hum Reprod 2002;17:362-9.
32. Karvonen MJ, Malm M. Colorimetric determination of fructose with indol. Scand J Clin Lab Invest 1955;7:305-7.
33. Polakoski KL, Zaneveld LJ. Biochemical examination of the human ejaculate. In: Techniques of Human Andrology. Hafez ESE (Ed). Amsterdam: North-Holland;1977. p. 265-86.
34. Andrade-Rocha FT. Seminal fructose levels in male infertility: Relationship with sperm characteristics. Int Urol Nephrol 1999;31:107-11.
35. Mann T. Studies on the metabolism of semen; fructose as a normal constituent of seminal plasma; site of formation and function of fructose in semen. Biochem J 1946;40:481-91.
36. Schoenfeld C, Amelar RD, Dubin L, Numeroff M. Prolactin, fructose, and zinc levels found in human seminal plasma. Fertil Steril 1979;32:206-8.
37. Abdella AM, Omer AF, Al-Aabed BH. Biochemical markers in semen and their correlation with fertility hormones and semen quality among Sudanese infertile patients. Afr J Biochem Res 2010;4:255-60.
38. Rajalakshmi M, Sharma RS, David GF, Kapur MM. Seminal fructose in normal and infertile men. Contraception 1989;39 (3):299-306.
39. Gonzales GF, Garcia-Hjarles MA, Gutierrez R, Guerra-Garcia R. The secretory activity of the seminal vesicles and its relationship to sperm motility: Effects of infection in the male reproductive tract. Int J Androl 1989;12:286-94.
40. Said L, Galeraud-Denis I, Carreau S, Saâd A. Relationship between semen quality and seminal plasma components: Alpha-glucosidase, fructose and citrate in infertile men compared with a normospermic population of Tunisian men. Andrologia 2009;41:150-6.
41. Gonzales GF, Kortebani G, Mazzolli AB. Leukocytospermia and function of the seminal vesicles on seminal quality. Fertil Steril 1992;57:1058-65.
42. Moon KH, Osborn RH, Yannone ME, Bunge RG. Relationship of testosterone to human seminal fructose. Invest Urol 1970;7:478-86.
43. Gonzales GF, Garcia-Hjarles M, Napuri R. Corrected seminal fructose levels: Index of secretory activity of seminal vesicles. Arch Androl 1988;21:135-42.
44. Ludwig M, Kummel C, Schroeder-Printzen I, Ringert RH, Weidner W. Evaluation of seminal plasma parameters in patients with chronic prostatitis or leukocytospermia. J Urol 1999;4:1418-9.
45. Das S, Parveen S, Kundra CP, Pereira BM. Reproduction in male rats is vulnerable to treatment with the flavonoid-rich seed extracts of *Vitex negundo*. Phytother Res 2004;18:8-13.
46. Gavella M. A simple automated method for determination of citric acid levels in semen. Int J Androl 1983;6:585-91.
47. Kimmig J, Steeno O, Schirren C. Results of modern biochemical research in the field of andrology. Internist 1967;8:25-34.
48. Kavanagh JP. Isocitric and citric acid in human prostatic and seminal fluid: Implications for prostatic metabolism and secretion. Prostate 1994;24:139-42.
49. Costello LC, Franklin RB. Novel role of zinc in the regulation of prostate citrate metabolism and its implications in prostate cancer. Prostate 1998;35:285-96.