Study the effect of Seminal Fructose and Citric Acid Levels in Men with Infertility

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Abstract:

Background: Male infertility, related to 50 percent of infertility, is a medical problem. Seminal plasma can be an anticipating factor as it contains accessory sex gland secretions; thereby offering new and specific ways to understand possible roles in male infertility of these biochemical markers.The purposes of this study was to evaluate the association between biochemical markers and sperm parameters in the perception of primary and secondary infertility in men with sterility.

Materials and Methods:

We recruited 163 men with fertility subject as patients (57 primaries, 56 secondary) and 50 fertile men as controls to assess the sperm parameters and biochemical markers, namely fructose and citric acid in determining male infertility (primary and secondary).Patient semen samples were correctly obtained and rendering was analyzed in the manual of the World Health Organization-2010. Samples were centrifuged later, seminal plasma was obtained, and standard procedures were used to test biochemical markers.

Results:

seminal Fructose concentration in patient with in infertility (with primary and secondary) were significantly higher than control group.On the other hand, there was no significant ($P>0.05$) when comparing between primary with secondary infertility, and there was a significant decrease ($P < 0.05$) in seminal plasma Citric acid conc. in infertile men (with primary and secondary) subjects with compared to control. On the other hand, there was no significant ($P>0.05$) when comparing betweenprimary with secondary infertility. Pearson correlation coefficient showed significant negative correlation of sperm count ($r = -0.404$), sperm concentration ($r = -0.354$), and sperm motility ($r = -0.420$) with fructose levels. Whereas seminal citric acid concentration had a positive correlation with sperm count ($r = 0.314$) sperm concentration ($r = 0.339$), and sperm motility ($r = 0.446$).

Conclusion:

Therefore, evaluation of certain seminal fluid biochemical markers maybe helpful in understanding the functionality of accessory glands that significantly subsidize the seminal volume and Find the difference between primary and secondary sterility using biochemistry parameters in semen.

Keywords: Male infertility (primary and secondary) accessory glands, citric acid, fructose, seminal plasma

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Introduction

Infertility is a disorder with psychological, cultural, medical consequences resulting in trauma, stress, particularly in a social set-up like ours, with a strong emphasis on childbearing. The International Committee for Monitoring Assisted Reproductive Technology, World Health Organization (WHO),Infertility is a reproductive system disorder described as failure to achieve clinical pregnancy after 12 months or more of regular sexual intercourse[1]. Infertility may be split into primary infertility and secondary infertility.primary male infertility is when the man has never impregnated a woman, while secondary male infertility is when a man has impregnated a woman irrespective of the outcome of the pregnancy [5]. men with secondary infertility, in general, have better chance of future fertility [5]. The male infertility can be complete or partial termed as subfertility.Infertility can be permanent (irreversible) or subfertility which means the probability of spontaneous conception may be decreased [5]. A man is accountable in about20% of infertility among couples, and subsidize to infertility with woman in another 30-40% [6].

Infertility is a public health problem during the reproductive age, affecting about 10-15% of couples attempting to achieve pregnancy in worldwide[2]. Male infertility is a problem of the reproductive system, and the word infertility itself means no fertile,and that would be equal to sterility[3]. sterility means that a man is totally unable to have a child[4]. conception after at least 12 months of unprotected sexual intercourse[3, 5].Infertility can either be primary or secondary;

One of the main features of infertile men, whether primary or secondary infertility, that is produced by their reproductive system is the decrease in sperm count, it has poor sperm motility, and shows the irregular formation of sperm [6].Semen consists of condensed sperm suspension, which is dissolved by seminal fluid mainly secreted by seminal vesicles followed by prostate, with a small contribution of bulbourethral (Cowper's) glands and epididdymis to normal sperm activity[7-10].The seminal plasma consists of a complex mixture of organic and inorganic components, which may not be essential for

fertilization, but it optimizes the correct environment for sperm motility, stamina and transportation in the female reproductive expanse[9, 11].The first fraction of the human ejaculate consists primarily of sperm and prostatic exudates, i.e. citric acid, proteases, acid phosphatase, and the later part contains fructose, coagulating components, and bicarbonates to secure the seminal vesicles ' acidic vaginal zone[7, 8, 12].Such biochemical secretions also act as the markers of their respective glands[13]. The seminal plasma has excessive amounts of fructose, which provides an anaerobic and aerobic energy source for the sperm[14] and has been obliquely correlated with progressive sperm motility and viscosity[15, 16].

Evaluating the fructose concentration of seminal plasma may show the status of seminal vesicles, endocrine abnormalities as well as possible obstruction of the ejaculatory duct, if any[7, 13, 17]. The presence of citric acid in human seminal fluid was first demonstrated in1929 by Schersten[18]In order to explain the role of citric acid in human semen, several theories have been advanced. The production of citric acid is essential for the ejaculate's coagulation-lysis mechanism [19] Several authors suggest that the action of citric acid is correlated with the activity of hyaluronidase [20].While others argue that citric acid may play an important role in stabilizing the extracellular environment, which is compulsory for normal sperm viability and function [19]. A model for androgenic regulation at the level of the membrane-bound Na / K ATP-dependent pump was proposed [20].This includes a sodium and citrate co-transport mechanism, which is claimed to be equally abundant in prostate fluid [19].Therefore, the assessment of biochemical markers for the identification of semen's biological attributes will help to establish new criteria that are reliable and fair for the prediction of male infertilityand also to determine and know if there were differences between primary and secondary sterility, Consequently, the current study was conducted to re-evaluate the efficacy of biochemical markers in male infertility assessment.

Materials and Methods

Study design

After gratifying the inclusion and exclusion standards for this study, a total of 163 men diagnosed with fertility issues as patients and 25 fertility-proven men with normozoospermia as controls were conscripted, who visited a "infertility center" at Basra hospital for Obstetrics and childrenin Basrah Governorate-Iraq during the period from January 2019 till end of August 2019. 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

Written informed consent was obtained from all participants after recruitment. semen was collected from seventy-eight men aged (26-60) years old, about163 suffered from infertility men (57 primary, 56 secondary) and 50 healthy fertile men as control, by masturbation into a sterile plastic specimen cup at the Center. Subjects were instructed to abstain from ejaculation for at least 72 hours prior to performing a routine the semen sample. The sample was liquefied for at least 20 minutes, but no longer than 1 hour prior to performing a routine semen analysis, which included measurements of volume, sperm count, sperm

motility, and sperm concentration.The Samples were collected from "infertility center" at Basra hospital for Obstetrics and children in Basrah Governorate-Iraq.

Ten Ml of well mixed semen was put on a clean microscope slide and covered with cover slip. Assessment of sperm motility should begin immediately to avoid artifacts caused by either a temperature decrease or dehydration of the preparation. Spermatozoa with pin heads or free tails should not be counted. Percentage of sperm motility (S.M) was assessed as following: The total number of spermatozoa in each motility group was divided on the total number of spermatozoa assessed in each field, Sperm analysis was carried out according to the World Health Organization [13] guidelines.In proven fertile controls, the sperm count(S.C) ranged from 20 –120 million sperm /ml. Sperm concentration(S.CONC.) was determined by counting the two sides of a haemocytometer, Samples were subsequently centrifuged for 10 min at 3000 rpm and seminal plasma was processed at -20 ° C for measurement of fructose and citric acid.

Estimation of fructose

Fructose is the sperm source of energy and serves as a marker of the functionality of the seminal vesicle. 50µLof seminal plasma was mixed thoroughly with 500 µL distilled water, after that, take from the diluted 50 µL and mix with 3 ml of the matrix solution using a commercially available Colorimetric kit Fructose(Catalog No: E-BC-K134,Elabscience Biotechnology,USA) according to manufactures recommendations, mixed fully and incubate in boiling water bath for 8min. Cool the tubes with running water. Read at 285nm[19].

Estimation of citric acid

Seminal citric acid is the marker of the functionality of the prostate gland. 100 µL of seminal plasma and 900 µL of Reagent (1) were mixed cooled in ice bath. mixed fully,using a commercially available Colorimetric kit Citric Acid (Catalog No: E-BC-K351,Elabscience Biotechnology, USA) according to manufactures recommendations, then centrifuge at 11000 g for 10 min at 4°C.700 µL of anhydrous acetic anhydride was added to 100 µL of supernatant and added 100 µL,100 µL of Reagent(4), Reagent(5),respectively Mixed fully and stand at room temperature for 30 minutes, Read at 545 nm[20].

Statistical analysis

The collected data were interpreted statistically and expressed in mean and normal mean error. Independent t-testing was used to assess whether the mean difference between patients and controls occurs along with Pearson correlation using IBM SPSS Statistics Software Inc. Version 20.0 (IBM Corp., Armonk, NY, USA). Statistical analysis was based on two-sided research at a significance level of 0.05 and meaningful correlation at a level of 0.01 (two-tailed).

Results

One hundred and sixty-three subjects were included in this study. From total infertility men subjects, 57 subjects were classified as primary infertility(never having conceived), meanwhile the other 56 were classified as secondary(infertility after having conceived at least once before),As much as 50 healthy men (fertile, had history of at least one having a child) were considered as control group.

There was significant difference in mean Fructose conc.(F.CONC.)in seminal plasma among study groups (P <0.05); the level was highest in infertility patients(with primary and secondary) subjects (3.0 ± 1.3; 2.9± 2.0 mg /mL) with compared to control(1.8 ± .62 mg /mL), respectively,On the other hand, there

Groups	N	Mean± SD*	SE	Range	95% C.I.	
					Lower	Upper
Control	50	1.8 ± .62	0.1	0.45 – 2.4	1.5	2.0

was no significant (P>0.05) (3.0 ± 1.3 (mg /mL) Vs. 2.9± 2.0 (mg/mL) when comparing primary with secondary, Table (1), Figure (1).

Table (2)showed thatwas a significant decrease (P < 0.05) in seminal plasma Citric acid CONC. (C.A CONC.) level in infertile men (with primary and secondary) subjects (3.10 ± 1.3; 3.17 ± 1.6mmol/L) with compared to the healthy control (4.5 ± 1.2mmol/L), respectively. in constant, there was no significant (P>0.05) (3.10 ± 1.3 (U/mL) Vs. 3.17 ± 1.6 (U/mL) when comparing primary with secondary, Table (2), Figure (2).

The data obtained in Table (3), showedthe mean age of patients and controls at the time of diagnosis in the current study was (32.8 ± 8.8 and 27.3± 3.8 years) respectively. On the other hand, that was asignificant (P < 0.05)(29.8 ± 7.2 yearsVs.36.6 ± 9.3years)when comparing primary with secondary, and All infertile patient exhibited significant mean difference for sperm count and motility with controls (P < 0.05).

hat as it may, by and large one-way ANOVA result appeared a critical distinction for sperm tally, motility, Fructose and citric Acid level when compared with controls (P < 0.05). in table (4).

Pearson relationship comes about shown critical negative relationship between sperm count (r = −0.404), sperm concentration (r = 0.354), sperm motility (r = −0.420), and fructose levels in infertile men (primary and secondary).

Whereas seminal citric acid concentration had a positive correlation with sperm count (r = 0.314), sperm concentration (r = 0.339), and sperm motility (r = 0.446) in infertile men (primary and secondary). Table (5).

Table (1) Seminal plasma of fructose conc. in infertile men (1º, 2º) patients (primary &secondary) and healthy control

1º	57	3.0 ± 1.3	0.3	0.3 – 4.9	2.4	3.6
2º	56	2.9± 2.0	0.4	0.3 – 9.0	1.9	3.9

					95% C.I.

**P < 0.001 compared to control, and the value *p >0.05(SN) when comparing primary with secondary.

Data are presented as mean standard deviation (SD); SE: standard errors; n: number of the subjects; range: difference between the highest and lowest values in the set; 95% CI: confidence interval/limits (lower and upper). p>0.05: p-value not significant, p<0.05: p-value significant; p<0.01: p-value highly significant, indicating the level of significance in comparison with the correspondingcontrol value.

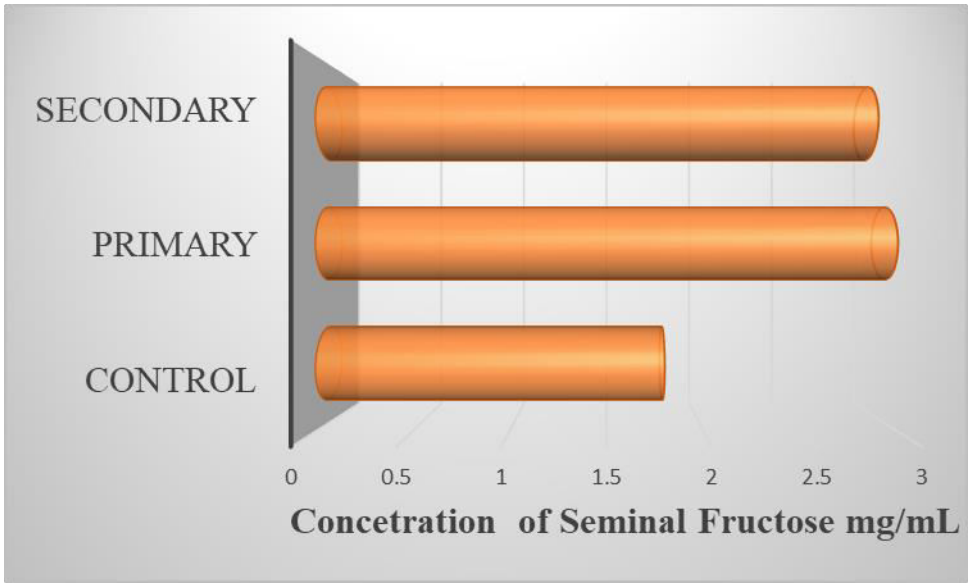

Figure (1) Seminal plasma fructose in control and in infertile men groups

Table (2) Seminal plasma of citric acid in infertile men (1º, 2º) patients (primary &secondary) and healthy control.

Groups	N	Mean ± SD*	SE	Range	Lower	Upper
Control	50	4.5 ± 1.2	0.2	1.5 – 7.0	3.9	5.0

Groups	N	Age Means ± SD	S.C Mil/ml	S.M (%)	S. CONC. Cell/ml	C.A CONC. > 10.5 (µmol/L)	F. CONC. >2.5 (mg/mL)

**p < 0.001compared to controls and the *p > 0.05 (NS) when comparing primary and secondary

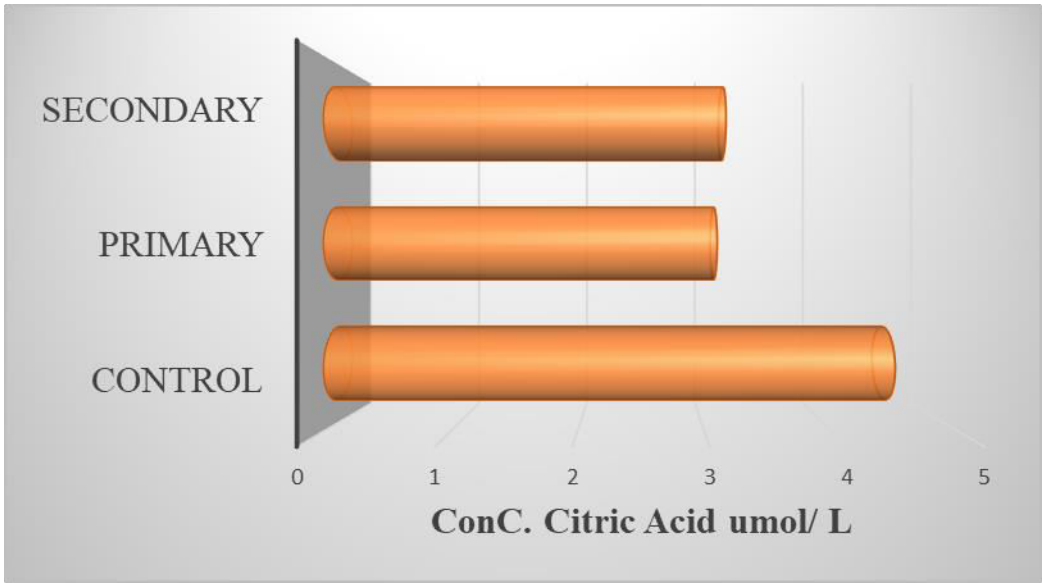

Figure (2) Seminal plasma citric acid in control and in infertile men groups

Table. (3) Seminal parameters and biochemical marker concentration of infertile patient (primary and secondary) and control

Control	50	27.3 ± 3.8	100.8 ± 26.1	73.3± 8.8	26.3± 9.3	4.5 ± 1.2	1.83 ± 0.62
Infertile	163	32.8 ± 8.8	48.28 ± 37.1	34.8± 22.3	13.9± 10.1	3.1± 1.4	2.98 ± 1.66
P-value		P >0.05	P<0.01	P<0.01	P<0.01	P<0.05	P<0.05

Where: n: number of the subjects, values represent Means ± SD (Standard deviation), SE: Standard Errors, P- value: N.S (P > 0.05), S (P < 0.05) was to be considered statistically significant, in comparison with the corresponding control value.

Table. (4) Independent t-test for seminal parameters and biochemical marker concentration with respect to control versus infertile groups

Wheren: number of the subjects, Values represent Means± SD (standard deviation), SE: Standard Errors, P- Value: N.S(N. S (P > 0.05), S (P < 0.05) was to be considered statistically significant, in comparison with the corresponding control value.

Table (4) Pearson correlation coefficient among the study variables

Groups	N	Age Means±SD	S.C Mil/ml	S.M (%)	S. CONC. Cell/ml	C.A CONC. > 10.5 (µmol/L)	F. CONC. >2.5 (mg/mL)
1º	57	29.8± 7.2	40.4±33.9	34.8±22.3	13.1×10^6 ± 13.9	3.10 ± 1.3	3.0 ± 1.3
2º	56	36.6± 9.3	56 ± 39.2	39.2 23.0	22.3×10^6 ± 19.9	3.17 ± 1.6	2.9± 2.0
P-value		P< 0.05	P > 0.05	P>0.05	P > 0.05	P>0.05	P>0.05

	Motility	Fructose	Citric Acid
Count	**0.222****	**−0.404****	**0.314****
Motility	**1**	**−0.420****	**0.358****
Fructose	**−0.420****	**1**	**−0.240****
CONC.	**0.749****	**−0.574****	**0.540****

****Correlation is significant at the P< 0.001 level (2- tailed)**

Discussion

Infertility may be a common issue confronted by the clinician and might emerge from male variables, female components or both. In spite of the fact that barrenness is customarily separated into primary and secondary sorts, the etiology isn't continuously different[21].Semen investigation is a necessarily portion of assessment of a barren couple. In guys with irregular semen parameters, counting diminished sperm number or sperm motility, and sperm concentration.

The point of this ponder was to discover the relationship between biochemical parameters within the seminal plasma and sperm parameters in controls and fruitless patients, and if there a difference between primary and secondary infertility depending on this study. Our discoveries appeared that fructose concentration diminishes as the sperm concentration increases and bad habit versa, andthere was no significant (P>0.05) (3.0 ± 1.3 (mg /mL) Vs. 2.9± 2.0 (mg/mL) when comparing primary with secondary[22, 23]Usually since fructose is a vitality reservoir,[24]and it is abused by sperm for its digestion system and motility[25].

The elevated fructose concentration in our consider with regard to infertile conditions (primary and secondary)Could be due either to shortened sperm count, abnormal sperm morphology, and reduced sperm activity resulting in reduced fructose use[26-28].

In our findings the seminal fructose concentration is higher in oliogozoospermia and in azoospermia andlowerasthenospermia[26]. Fructose concentration in norrmozoopermia is significant lower than oligozooseprmia[13]. that an increase in sperm concentration is often accompanied by a decrease in fructose concentration in seminal plasma, because sperm using fructose as the primary source of energy[25, 27] [10,11Could be due to increased sperm motility or seminal vesicle inflammation,[41].little levels of testosterone secretion,[28, 29]or also due to anatomical anomalies[30].

On the other hand, the results show the negative correlations between seminal fructose and sperm count, sperm concentration, and progressive motilitywhen seminal fructose decreases, sperm count, sperm concentration, and progressive motility increase, seminal fructose may decrease lower than normal.this result is compatible with[31]. Fructose in semen is the source of energy of every sperm activities. The higher of sperm count, sperm concentration, and motility asked for more energy, so fructose is lower [32, 33]. Normal seminal fructose concentration confirms the role of testosterone and the function of vesicles and vas deferens are normal [34].Earlier studies have shown that sperm with an irregular morphology may have weak motility or lack it and therefore use lower fructose[30]. The low semen fructose levels disturb coagulation, which may be due to inflammation of the genital tract[26, 35].

In this work, there was a significant decrease ($P < 0.001$) in seminal plasma Citric acid level in infertile men (with primary and secondary) subjects (3.10 ± 1.3; 3.17 ± 1.6mmol/L) with compared to control (4.5 ± 1.2mmol/L), respectively. On the other hand, there was no significant ($P>0.05$) (3.10 ± 1.3 (U/mL) Vs. 3.17 ± 1.6 (U/mL) when comparing primary with secondary,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.[36-38] .Reduced concentration of citric acid has been observed in serious or chronic prostatitis[39].Since it acts as a gelling agent and helps in semen liquefaction, sperm motility has indirectly benefited[40].In our studies, sperm count and motility have shown positive correlation with citric acid.[40, 41].

Conclusion

The mean age of all infertile men was 32.8 years old. ranged between 18-60 years. Men in the primary infertile group were significantly younger than men in secondary infertile group (29 versus 34 years; $p<0.001$).

Through this study, I did not find evidence to give a difference to the primary and secondary sterility using the biochemical markers fructose and citric acid and where the results there were no significant ($P>0.05$) between the level of fructose concentration and citric acid in both cases.

Biochemical indicators such as fructose and citric acid can be used to identify semen's biological properties that can help develop new standards thatare effective in predicting and improving male fertility. Such markers may not be a valid indicator of male reproductive dysfunction but could provide an important expression of male reproductive function in conjunction with alternative seminal characters.Such markers may not be a valid indicator of male reproductive dysfunction but could provide an important expression of male reproductive function in conjunction with alternative seminal characters.

Fructose is a vital resource for sperm metabolism and motility; its lack is an indication of seminal vesicle irregularity or an impediment to the ejaculatory duct. Citric acid deficiency of semen may be a malfunction in ejaculatory channels and may be a previous sign of prostate cancer.Hence, assessment of

certain biochemical markers of seminal liquid may advantage in understanding the usefulness of accessory organs which subsidizes essentially to the seminal volume.

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