

The Predictive Value of Glucose-fructose Ratio in Seminal Plasma

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Abstract: Seminal fluid glucose and fructose were assayed, using standard procedures in 50 subjects made up of 24 normospermic, 18 oligospermic and 8 azospermic patients. The results revealed that the concentration (mean \pm S.D mg dl⁻¹) of glucose and fructose in normospermic subjects were 39.50 \pm 10.51 and 143.33 \pm 19.92 respectively while the glucose-fructose ratio was 0.28 \pm 0.70. The glucose level in azospermic subjects (27.0 \pm 5.23) were significantly lower than those in oligospermic subjects (31.56 \pm 6.41) which were significantly lower than those for normospermic subjects (39.50 \pm 10.51) (P<0.05). In contrast, the fructose levels in azospermic subjects (206.75 \pm 9.60) were significantly higher than those in oligospermic subjects (189.17 \pm 17.99) which were also higher than those of normospermic subjects (143.33 \pm 19.92) (P<0.05). The glucose-fructose ratio tends to follow the same pattern as glucose. The glucose values do not appear to distinguish between degrees of sperm motility, whereas fructose and glucose-fructose ratio did. Fructose was significantly lower in sperm motility of between 50-100% than in those of motility of below 40% (P<0.05).

Key words: Spermatozoa • fructose • glucose • sperm motility

INTRODUCTION

Approximately 15% of couples attempting their first pregnancy meet with failure. Most authorities define these patients as primarily infertile if they have been unable to achieve a pregnancy after one year of unprotected intercourse [1]. Data available over the past 20 years reveals that in approximately 30% of cases pathology is found in the man alone and in another 20% both the man and the woman are abnormal. Therefore, the male factor is at least partly responsible in about 50% of infertile couples [2].

In the bid to enhance the investigation of infertility in males, many workers have reported on some biochemical composition of human semen, including glucose and fructose [3]. However, reports on glucose-fructose ratio levels in human seminal plasma in Nigeria are relatively scarce. This paper attempt to establish the predictive values of glucose-fructose ratio in seminal plasma of Nigerian males [4].

Less than 10% of ejaculate is Spermatozoa. The remainder being seminal plasma which is a composite mixture of secretions from various accessory genital glands. The p^H of semen is approximately 7.4 and contains 0.1-1.0% citric acid, 0.04-0.4% fructose and 0.01% ascorbic

acid together with glyceryl phosphorylcholine, inositol, sorbitol and various enzymes. Seminal plasma acts as an activator, lubricant and a diluent for spermatozoa [5].

Sugars in seminal plasma include glucose and fructose. Ejaculated sperms meet most of their energy requirement by utilization of fructose from the seminal vesicle. Spermatozoa contain mitochondria which can metabolize fructose completely to carbon dioxide and water by the combination of fructolysis and TCA cycle activity. The Mitochondria of the sperm are unique. They are the only Mitochondria known to contain lactate dehydrogenase. In all other cells, this enzyme is confined to the cytosol. This enables sperm mitochondria to oxidize lactate obtained by fructolysis and makes the shuttle for the transport of these reducing equivalents into the mitosol unnecessary [6].

MATERIALS AND METHODS

A total of 50 samples were collected from the Human Reproductive/Infertility Laboratory of the Federal Medical Center Asaba. The samples were collected according to the WHO 1993 procedure [7]. They were collected by masturbation after 3-5 days of sexual abstinence into sterile universal containers and were

analyzed within 2 hours where possible or stored at -20°C until analyzed. Semen was transferred into a centrifuge tube and centrifuged at 40000g for 30minutes. After centrifugation the top layer of the supernatant was carefully taken off and transferred into another tube. The supernatant was stored at 20°C until analysis of the glucose and fructose contents

Semen analysis: Using a graduated tube, the semen samples were diluted 1 in 20 with sodium bicarbonate. The Neubauer counting chamber was filled with the diluted semen and left for 3-6 minutes in a moist condition for the spermatozoa to settle. Using the $\times 10$ objective of the microscope with the condenser iris closed sufficiently to give a good contrast, the numbers of spermatozoa in an area of 25 sq mm were counted.

The number of spermatozoa in 1 ml fluid was calculated from the formula

$$\text{No. of cells} = \frac{N \times DF \times 10^5}{A \times D}$$

Where

N = Number of cells counted

DF = Dilution factor

A = Area

D = Depth

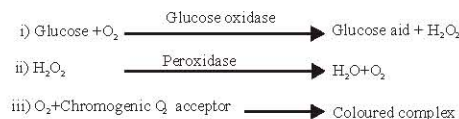
Determination of glucose: The amount of glucose in seminal plasma was determined by the glucose oxidase method.

Procedure: To 3.5 ml of diluent (1.0 g phenol, 6.5 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 8.0 g NaCl in H_2O and made up to 1 litre) and 0.5ml of 0.3N NaOH in tubes met for sample, standard and blank. 0.1 ml sample (seminal fluid), standard and water were added respectively. The content were mixed and centrifuged at 3000 rpm for 5 minutes.

To 1.5 ml of colour (10 g Na_2HPO_4 , 1.0 g sodium azide, 300 mg 4-amino phenazone, 10 ml BDH glucose oxidase and 17 ml 0.1% peroxidase dissolved in distilled water and made up to 1 litre) in tubes meant for sample, standard and blank, 0.5 ml of supernatant were added respectively. The contents were mixed and incubated for 10 minutes at 37°C. The absorbance of the samples and standard were measured against reagent blank at 490 nm.

Principle: Glucose in the presence of atmospheric oxygen is oxidized to glucuronic acid and hydrogen peroxide under the catalytic action of glucose oxidase. The hydrogen peroxide produced is picked up by a

chromogenic oxygen acceptor to form a colour which is proportional in intensity to the concentration of glucose present in the sample.



Determination of fructose: The amount of fructose in seminal plasma was determined by the Selliwanoff's method.

Procedure: To 3.5 ml of diluent (1.0 g phenol, 6.5 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 8.0 g NaCl in H_2O and made up to 1 litre) and 0.5 ml of 0.3N NaOH in tubes meant for sample, standard and blank. 0.5 ml of sample (seminal fluid) and standard fructose (100 mg of fructose dissolved in 0.3% benzoic acid and made up to 100ml) were added respectively. The content were mixed and centrifuged at 3000 rpm for 5 minutes.

To 5.0 ml of selliwanoff's reagent (50 mg Resorcinol dissolved in 33 ml conc Hcl and diluted to 100 ml with water) in glass tubes meant for samples, standard and blank, 0.5 ml of supernatant were added respectively. The content were mixed and placed in a boiling water bath (100°C) for 5 minutes, removed and cooled. The absorbance of the sample and standard were read against reagent blank at 450 nm.

Principle: Hot hydrochloric acid converted fructose to hydroxymethyl furfural which links with resorcinol to produce a red coloured compound. The intensity of colour produced is directly proportional to the amount of fructose present in the sample.

Calculations:

$$C_T = AT/A_S \times C_S$$

A_T = Absorbance of test

A_S = Absorbance of standard

C_S = Concentration of standard

C_T = Concentration of test

RESULTS AND DISCUSSION

Table 1 shows the semen concentration ($\bar{X} \pm \text{S.D}$) of glucose, fructose and glucose-fructose ratio in azospermic, oligospermic and normospermic males. The mean fructose tends to decrease as the cell count

Table 1: Relationship between sperm count and the concentration (X±SD) of glucose, fructose and glucose-fructose ratio

Sperm count	n	Glucose (mg dl ⁻¹)	Fructose (mg dl ⁻¹)	Glucose- fructose ratio
(a) Azospermic	8	27.00±5.23	206.75±9.60	0.13±0.03
(b) Oligospermic	18	31.56±0.41	187.17±17.99	0.17±0.03
(c) Normospermic	24	39.50±10.51	143.33±19.92	0.28±0.07
a vs b		P<0.05	P<0.05	P<0.05
a vs c		P<0.05	P<0.05	P<0.05
b vs c		P<0.05	P<0.05	P<0.05

Table 2: Relationship between sperm motility and the concentration (X±SD) of glucose, fructose and glucose-fructose ratio

Sperm motility (%)	n	Glucose (mg dl ⁻¹)	Fructose (mg dl ⁻¹)	Glucose- fructose ratio
0-40	13	32.62±8.85	184.54±22.05	0.18±0.06
50-100	29	37.86±8.51	153.31±27.65	0.26±0.08
		P>0.05	P>0.05	P>0.05

Table 3: Results obtained from correlation coefficient calculation using the pairs of parameters shown where 'a' and 'B' are the constants in the equation Y= a + bx and n is the number of pairs of data tested

	n	r	a	b
Seminal glucose and oligospermic cell count	18	-0.52	-5.84	0.39
Seminal glucose and normospermic cell count	24	-0.34	135.24	-1.75
Seminal fructose and oligospermic cell count	18	+0.47	0.47	0.17
Seminal fructose and normospermic cell count	24	-0.35	56.24	0.05
Seminal glucose-fructose ratio and Normospermic cell count	24	0.7	-0.98	96.1
Seminal glucose-fructose ratio and Oligospermic cell count	18	-0.2	91.94	-98.41
Seminal glucose-fructose ratio and Sperm motility (0-40%)	13	0.3	23.38	23.91
Seminal glucose-fructose ratio and Sperm motility (50-100%)	29	0.08	56.48	28.7

increases. When the mean of glucose concentration of azospermic and oligospermic subjects was compared with those of normospermic subjects there was a statistical increase ($P<0.05$). There was a significant decrease ($P<0.05$) in the means of fructose concentration in azospermic and oligospermic subjects when compared with normospermic controls. There was a significant increase ($P<0.05$) in the means of the glucose-fructose ratio of azospermic and oligospermic subjects when compared with those of normospermic subjects.

There was a generalized significant decrease ($P<0.05$) when the means of fructose concentration and glucose-fructose ratio in semen with sperm motility of less than 40% were compared with those with sperm motility greater than 50%. Whereas there was no significant difference ($P>0.05$) between the mean of

glucose concentration in semen with sperm motility of less than 40% as compared with those with sperm motility greater than 50%.

There was a moderate negative correlation between glucose level and normospermic cell count ($r = -0.34$) and between glucose level and oligospermic cell count ($r = -0.5$) and between fructose level and normospermic cell count ($r = -0.35$) but a weak positive correlation between fructose level and oligospermic cell count ($r = +0.47$).

There was a progressive decrease in the mean fructose concentration as the cell count increases and as sperm motility increases. Ejaculated sperm meet their energy requirement by utilization of fructose from the seminal vesicle. The more motile the spermatozoa, the more the energy utilized especially if the cells are actively motile.

Higher means glucose levels found in normospermic males than oligospermic and azospermic males. This is because ejaculated semen, the spermatozoa prefers fructose as energy source thereby sparing glucose. Bacterial infections of semen are a common feature with oligospermic and azospermic subjects. These micro-organisms utilize the glucose content of seminal fluid leading to low glucose levels. very low seminal glucose-fructose ratio was also observed in oligospermia and azospermia compared to normospermic control, with azospermia having glucose-fructose ratio of less than half of normospermic (0.28 ± 0.07).

CONCLUSION

Seminal glucose and fructose level show some significant differences between normospermia, oligospermia and azospermia. The value of fructose has been used as an index of sperm viability with little regard to the glucose level. However, the glucose-fructose ratio appears to clearly distinguish the three levels of sperm cell count and motility and is thus recommended as an adjunct to other established biochemical parameters for the investigation of male infertility.

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