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Full Length Research Paper

Biochemical markers in semen and their correlation with fertility hormones and semen quality among Sudanese infertile patients

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Several biochemicals in semen are secreted by the accessory glands in the reproductive tract. These biochemicals can be used as diagnostic predictors for the disorders in the male reproductive system. To assess the level of biochemical markers in semen, their relation to fertility hormones and spermogram among Sudanese infertile patients were studied. The biochemical markers studied were fructose, citric acid, zinc and neutral -glucosidase. Their levels in semen were estimated using analytical photometry, spectrophotometry and atomic absorption spectrometry. Estimation covered 500 infertile males (150 azoospermic, 150 oligospermic, 100 asthenozoospermic and 100 with abnormal sperm morphology), as well as 100 normospermic control males. Fertility hormones were assayed in patients and controls by ELISA. Seminal neutral -glucosidase and citric acid levels were found significantly reduced in azoospermic and oligospermic patients, while zinc levels was reduced in all infertile patients ($p < 0.05$). Semen fructose level was found within the normal range. Significant negative correlation was noticed between neutral -glucosidase and both follicle stimulating and luteinising hormones (in azoospermic patients), and prolactin hormone in oligospermic patients ($r < 0.05$). 2.7% of azoospermic patients had Sertoli cell syndrome only. 13% of the infertile patients had varicocele, and this was associated with a significant increase in FSH and LH and a decrease in seminal neutral -glucosidase, citric acid and zinc ($p < 0.05$). 9.6% of the patients studied had dysfunctional sexual problems and was associated with a significant increase in prolactin. On the other hand, 7.2% of these patients were smokers and this was associated with a significant reduction in semen volume and levels of neutral - glucosidase and zinc ($p < 0.05$). There was conflicting association between biochemical markers in semen with both reproductive hormones level and semen quality in the infertile patients, but neutral -glucosidase level was the only biochemical markers in semen that correlated well with both gonadotropins hormones (negatively/inversely) and the semen quality.

Key words: Biochemical markers, reproductive hormones, male infertility, seminal plasma, -glucosidase.

INTRODUCTION

Male fertility depends on the proper function of a complex system of organs and hormones The testis produce the spermatozoa, which begin partially embedded in nurturing amoebae-like cells known as Sertoli cells, which are located in the lower parts of the seminiferous tubules. As they mature and move along, they are stored in the upper part of the tubules (Vaclav et al., 1993). When the sperm has completed the development of its head and tail, they are released from the cell into the epididymis. The epididymis is a convoluted canal in which final steps of sperm maturation and development take place. The spermatozoa are stored in the epididymis for a long time, and pass through it during ejaculation. The epididymis sustains maturation process that results in the acquisition of progressive motility and fertilizing ability by spermatozoa. The epididymis synthesizes certain compounds that are secreted in the semen. These include protein,

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carnitine, lipid, glycerylphosphorylcholine, neutral -glucosidase, carbohydrates, steroids and other small molecules (Vaclav et al., 1993).

Semen or ejaculate is the fluid discharged from the penis during the time of orgasm. Like blood, semen consists of two compartments, cellular compartment (spermatozoa), and noncellular compartment (seminal plasma). Thus, it contains the sperm, which sometimes results in pregnancy following vaginal sex with a female. Semen is a whitish, milky fluid, slightly viscous, containing water and small amounts of salt, protein, fructose, citric acid and other substances (Setchell and Waites, 1970). Spermatozoa make up only about 5 to 10% of the volume of semen. The bulk of the seminal plasma, the fluid portion of semen, is contributed by the male accessory organs of reproduction. 40 to 80% of semen is produced by the seminal vesicles. The secretions include fructose for sperm nutrition, prostaglandins, coagulating substances and bicarbonate to buffer the acidic vaginal vault. Most of the remainder is generated by the prostate (10 to 30%). Prostatic secretions in humans contain enzymes and proteases to liquefy the seminal coagulum, high levels of citric acid, acid phosphatase, phospholipids and spermine. Small amount of seminal fluid comes from the bulbourethral (Cowper) glands (Orth, 1982). The seminal plasma contains a very complex range of organic and inorganic constituents. Fructose is the main energy source of sperm cells. A reduction of fructose level below normal value is often due to an obstruction of the ejaculatory ducts or absence of the seminal vesicles, especially when associated with a low ejaculate volume and a thin, watery consistency. Some of the other components of semen serve to increase the mobility of sperm cells; sperm function best in a slightly alkaline environment (Setchell and Waites, 1970).

Although, the seminal plasma may not contain factor that are absolutely essential for fertilization, the secretion may nevertheless optimize condition for sperm motility, survival and transport in both the semen and the female reproductive tract (Vaclav et al., 1993). The increased or decreased level of these factors visualizes the abnormalities that occurred along the male reproductive tract. Fructose secretion is under androgenic regulation, but many factors such as frequency of ejaculation, blood glucose levels and nutritional status can also affect the seminal plasma fructose concentration (Vaclav et al., 1993). Zinc, is accumulated and released in the prostatic fluid; the anatomical localization of Zn^{2+} in the human prostate, is in the epithelium of the glands. Dihydrotestosterone may initiate the synthesis of zinc binding protein required for the accumulation of zinc within the prostatic cells and lumen. As the epithelial cells become saturated with zinc, this cation may switch off testosterone reduction by binding to thiol group at or near the cofactor binding site of the 5-reductase. Following ejaculation, 5-reductase activity may be restored, allowing the renewed accumulation of zinc within the prostate (Carol, 1998).

In the early stages, the sperm cannot swim in a forward direction and can only vibrate its tail weakly. By the time the sperm reaches the end of the epididymis, however, it is matured and looks like a microscopic squirming tadpole. The ability of a sperm to move forward rapidly and straight is probably the most significant determinant of male fertility (MacSween and Keith 1995).

In the penis, the sperm first pass into one of two rigid and wire- like muscular channels, called the vas deferentia. Muscle contractions in the vas deferens from sexual activity propel the sperm along past the seminal vesicles. Each vas deferens then joins to form the ejaculatory duct. This duct, which now contains the semen containing the sperms, passes down through the urethra, but during orgasm, the prostate closes off the bladder so urine cannot enter the urethra. The semen is forced through the urethra during ejaculation, the final stage of orgasm when the sperm is literally shot out of the penis (Vaclav et al., 1993). It is important to emphasize the relationship between the germ cells and the Sertoli cells, with the reproductive hormones that are essential for the successful development of normal spermatogenesis, FSH (follicle-stimulating hormone) and testosterone, act through the Sertoli cells since the receptors for these hormones are located on these cells and are not located on germ cells (Cyril et al., 1994; Plant and Marshall, 2001; Walker and Cheng, 2005).

According to our knowledge, this study was the first work in Sudan in this field. It aimed to correlate the concentrations of the biochemical markers (semen fructose, citric acid, zinc, and neutral -glucosidase) to fertile hormones and spermogram in infertile Sudanese males, and highlight their importance in male infertility.

MATERIALS AND METHODS

Study subjects

500 infertile males were enrolled for this study in Khartoum State (Sudan). It was carried out between July 2005 and October 2007. The subjects were males attending the fertility clinics that complained of inability to achieve pregnancy for at least one year after marriage (with no apparent chronic or acute disease), and whom their wives had shown no diagnosed causes of infertility (hormone test, laparoscopy).

The infertile patients studied were divided into four groups according to World Health Organization (WHO) standards for semen quality: azoospermic patients (n = 150), oligospermic patients (n = 150), asthenozoospermic patients (n = 100) and patients with abnormal sperm morphology (n = 100). The control group included 100 fertile males who had fathered a child during the last two years, and with normal spermogram. Consent for the study was obtained from all enrolled subjects.

Demographical data were collected via a structural interview that was conducted during the first visit. A basic medical, surgical, reproductive and family history was recorded. A complete physical and genital examination was carried out. All subjects submitted a semen specimen and a blood sample.

Hormones analysis

Venous blood was collected in a plain tube for the estimation of the fertility hormones: follicle stimulating hormone (FSH), luteinising hormone (LH), prolactin and total testerone. The separated serum was assayed by the enzyme-immunolinked assay (ELISA) (Tietz, 1988; Wisdom 1976; Shome and Parlow, 1974; Baker, 1974; Chopra et al., 1971). Kits were provided from DIMA GmbH Robert-Bosch-Breite 23, 37079 Goettingen Germany.

Semen analysis

Semen samples were obtained by masturbation and collected in sterile polystyrene containers after 3-5 days of abstinence. Samples were analyzed according to the WHO criteria (WHO 1999). Seminal plasma was separated from the spermatozoa by centrifugation and divided into two containers: one metal-free polypropylene container for the estimation of zinc by atomic absorption spectrometry (AAS) with the Zeeman background correction method, and using an analytical quality control for accuracy (Michael et al., 1996) and the second container was for the estimation of: Neutral - glucosidase (NAG) according to Cooper et al. (1990) and Guerin et al. (1986). Kits were produced by FertiPro N. V., Industriepark Noord 32, 8730 Beemem and Belgium. Citric acid was estimated according to Tietz et al. (1999) and Young et al. (1975) and fructose according to WHO Manual (1999). Analytical photometers and Jenway 6305 spectrophotometer were used for the estimation.

Quality control of assays

Samples representing the normal and pathological level for all the measured analysts in serum/or semen were used for quality control. Results ± 2SD of the target values were considered acceptable. The batches with all controls been within permissible were accepted.

Statistical analysis

Data were expressed in mean, standard error, and standard deviation. They were analyzed by student t- test, one-way analysis of variance (ANOVA) and a coefficient of correlation (r) with a significance fixed at $p = 0.05$.

Ethical consideration

Consent for this study was taken from the infertile patients and the healthy volunteers.

RESULTS

500 Sudanese infertile males were studied. Duration of infertility was 2-15 years in azoospermic patients, 4 to 18 years in oligospermic patients, 3- 14 years in asthenozoo-spermic patients, and 2 to 11 years in patients with abnormal sperm morphology. The control group constituted 100 fertile males with normal spermo-gram.

Clinical examination and laboratory investigations revealed that 2.7% of azoospermic patients were suffering from Sertoli-cell syndrome, 13% of the infertile patients had varicocele and 9.6% of the infertile patients had sexual dysfunction.

Seminal NAG (neutral-acetyl- -D-glucosaminidase) and citric acid levels were significantly reduced in azoospermic and oligospermic patients, whereas zinc was reduced in all infertile subjects. Seminal fructose was slightly increased in oligospermic patients and subjects with abnormal sperm morphology. However, it was decreased in azoospermic and asthenozoospermic patients as shown in Tables 1 and 2.

Tables 3a, 3b, 4a and 4b show the correlation of seminal biochemical markers with fertility hormones in the infertile patients studied. In azoospermic patients, a slight correlation was evident between NAG and testosterone and between fructose and citric acid with prolactin. Furthermore, a significant inverse negative correlation was recorded between NAG and FSH and LH in this group of patients. In oligospermic patients correlation was clear between citric acid with LH, prolactin and NAG. In addition, a significant negative correlation was also noticed between seminal zinc, LH and prolactin in such patients. On the other hand, fructose correlated well with prolactin in asthenozoospermic patients.

DISCUSSION

Jeyendran et al. (1995) measured the neutral - glucosidase (NAG) activity in seminal plasma of infertile patients. They found an inverse correlation of NAD activity with the spermatogram abnormalities. Semen NAG level in this study was significantly reduced among infertile patients which is in agreement with the findings of other workers (Jeyendran et al., 1995). Since NAG is secreted by the epididymis that is under the control of the testosterone hormone, the high significant reduction of NAG level recorded in azoospermic patients may be due to obstruction of the first part of the ejaculatory duct next to the epididymis (Vaclav, 1993).

Seminal fructose level was found to be increased in oligospermic and abnormal sperm morphology patients. This may have resulted from the reduced sperm count and activity leading to low consumption of the synthesized fructose. On the other hand, seminal fructose level was decreased in azoospermic and asthenozoospermic patients. This is explained by partial or complete obstruction of the seminal ducts or ageing of the accessory glands that secrete fructose (Buckett et al., 2002).

Seminal citric acid level was slightly reduced in asthenozoospermic patients and significantly reduced in azoospermic and oligospermic patients. Since seminal citric acid is secreted mainly from the prostate gland, any partial or complete obstruction of the ejaculatory ducts may reduce its level in semen. Abnormal testosterone secretion and accessory glands infection may also lead to reduction of seminal citric acid level.

Generally, zinc and citric acid concentrations as well as NAG activity were found to decrease significantly with

Table 1. Descriptive analysis of seminal biochemical markers in infertile patients and control group.

Seminal biochemical marker	Azoospermic patients (n = 150)	Oligospermic patients (n = 150)	Asthenozoospermic patients (n = 100)	Abnormal sperm morphology patients (n = 100)	Control group (n = 100)
NAG (mIU/ml)	11.2 ± 0.3	18.2 ± 0.5	23.1 ± 0.48	22.8 ± 0.5	24.6 ± 0.9
Fructose (mmol/l)	12.9 ± 0.73	13.2 ± 0.69	12.1 ± 0.81	13.4 ± 0.87	13.0 ± 0.39
Citric acid (mmol/l)	1.9 ± 0.09	2.2 ± 0.15	2.6 ± 0.19	2.8 ± 0.20	3.3 ± 0.16
Zn (mg/dl)	11.3 ± 0.27	11.5 ± 0.25	12.8 ± 0.4	12.6 ± 0.45	19.3 ± 0.6

n = the number of subjects. Data are presented as M ± SE.

Table 2. Dunnetts T3 multiple analysis of seminal biochemical markers in infertile patients and control group.

Seminal biochemical marker	Statistic	Azoospermic patient *versus* control	Oligospermic patient *versus* control	Asthenozoospermic patient *versus* control	Abnormal sperm morphology patient *versus* control
NAG	Mean difference	13.4	6.5	1.5	1.9
	Significance	0.000‡	0.000‡	0.78	0.51
Fructose	Mean difference	0.07	- 0.2	0.83	- 0.46
	Significance	1.0	1.0	0.98	1.0
Citric acid	Mean difference	1.4	1.1	0.7	0.5
	Significance	0.00‡	0.00‡	0.07	0.5
Zinc	Mean difference	8.0	7.6	6.7	6.6
	Significance	0.00‡	0.00‡	0.00‡	0.00‡

‡Significant at the level p < 0.001.

Table 3a. Statistical correlation of seminal biochemical markers with fertility hormones in azoospermic and oligospermic patients (Azoospermic patients).

Seminal biochemical marker	Statistic	Testosterone	FSH	Prolactin	LH	Zinc
NAG	Person correlation	0.14	- 0.25	0.09	- 0.20	0.08
	Significance (2-tails)	0.1	0.02‡	0.3	0.01‡	0.32
Fructose	Person correlation	-0.01	0.02	0.15	- 0.02	-0.11
	Significance (2-tails)	0.9	0.8	0.08	0.8	0.19
Citric acid	Person correlation	- 0.03	- 0.1	- 0.11	0.03	0.01
	Significance (2-tails)	0.7	0.2	0.19	0.76	0.95
Zinc	Person correlation	0.07	0.05	- 0.1	0.04	-
	Significance (2- tails)	0.4	0.5	0.2	0.7	-

FSH = Follicle stimulating hormone; LH = luteinizing hormone; ‡correlation was significant at p < 0.05.

increase in seminal abnormalities. This is quite obvious in infertile azoospermic patients. NAG level is considered the most important marker because it affects the maturation and the acquisition of spermatozoa motility (Vaclav, 1993).

Seminal NAG, fructose and citric acid in the test groups recorded relation with seminal zinc. There was weak positive relation between NAG, Zn and fructose in infertile

Table 3b. Statistical correlation of seminal biochemical markers with fertility hormones in azoospermic and oligospermic patients (Azoospermic patients) (Oligospermic patients).

Seminal biochemical marker	Statistics	Testosterone	FSH	Prolactin	LH	Zinc
NAG	Person correlation	0.11	0.05	0.16	0.05	-0.15
	Significant (2-tails)	0.2	0.6	0.05*	0.5	0.07
Fructose	Person correlation	0.03	0.03	0.08	0.07	0.07
	Significant (2-tails)	0.7	0.7	0.3	0.4	0.4
Citric acid	Person correlation	0.07	- 0.11	0.07	- 0.25	-0.12
	Significant (2-tails)	0.3	0.17	0.4	0.02‡	0.13
Zinc	Person correlation	- 0.03	- 0.08	0.2	0.20	- -
	Significant (2-tails)	0.7	0.3	0.02‡	0.03‡	

*Correlation was significant at $p < 0.001$; FSH = follicle stimulating hormone; LH = luteinizing hormone; ‡correlation was significant at $p < 0.05$.

Table 4a. Statistical correlation of seminal biochemical markers with fertility hormones in asthenozoospermic patients and abnormal sperm morphology patients (Asthenozoospermic patients).

Seminal biochemical marker	Statistics	Testosterone	FSH	Prolactin	LH	Zinc
NAG	Person correlation	- 0.04	0.06	0.08	- 0.09	-0.07
	Significant (2-tails)	0.7	0.5	0.4	0.4	0.5
Fructose	Person correlation	0.1	0.03	0.17	0.09	-0.08
	Significant (2-tails)	0.3	1.0	0.08	0.4	0.4
Citric acid	Person correlation	0.01	0.01	- 0.05	0.1	-0.16
	Significant (2-tails)	1.0	1.0	0.6	0.3	0.12
Zinc	Person correlation	0.14	0.15	0.07	- 0.13	-
	Significant (2- tails)	0.5	0.6	0.5	0.2	-

Table 4b. Statistical correlation of seminal biochemical markers with fertility hormones in asthenozoospermic patients and abnormal sperm morphology patients (Asthenozoospermic patients; abnormal sperm morphology patients).

Seminal biochemical markers	Statistics	Testosterone	FSH	Prolactin	LH	Zinc
NAG	Person correlation	0.01	0.01	- 0.1	- 0.08	0.12
	Significance (2-tails)	0.9	1.0	0.3	0.4	0.3
Fructose	Person correlation	0.16	0.13	0.16	0.1	0.1
	Significance (2-tails)	0.12	0.2	0.12	0.2	0.3
Citric acid	Person correlation	- 0.07	0.16	0.08	0.06	-0.14
	Significance (2-tails)	0.5	0.12	0.4	0.5	0.17
Zinc	Person correlation	0.14	0.02 0.8	0.07	0.1	-
	Significance (2-tails)	0.2		0.5	0.3	-

patients with azoospermia since these parameters are affected by testosterone level, and accessory glands secreting activity. This appeared clearly in azoospermic patients. The secretory activity of the accessory glands is affected by the level of seminal zinc. This phenomenon was demonstrated by the strong relation between the zinc level and seminal volume and liquefaction in azoospermic and oligospermic patients.

Testosterone is essential for all steps of spermatogenesis hence any reduction in testosterone concentration may affect sperm quality. Hunt et al. (1992) deduced the important role of zinc in testosterone production and its need in the spermatogenesis process. They also observed the positive relation between testosterone and zinc in healthy male volunteers fed on zinc-restricted diet. As a consequence of zinc deficiency, serum testosterone concentration and seminal volume per ejaculate were reduced in this study.

The fertility hormones levels in infertile patients reflected a relationship with seminal zinc level. The positive correlation of prolactin hormone with seminal zinc indicates the stimulating effect of prolactin on the prostate gland that secretes zinc. The inverse correlation of LH with seminal zinc in oligospermic patients suggests the feed-back stimulation of zinc deficiency on LH secretion by the pituitary gland.

Conclusion

Seminal NAG is the best epididymal marker in semen that showed significant correlation with both gonado-tropins hormones (negatively/inversely) and the semen quality, which can aid in the differential diagnosis of obstructive and nonobstructive azoospermia in men with infertility and replace the semen aspiration technique.

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