Demonstration of the Site of Obstruction in Azoospermia by Biochemical Abstract Markers

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Abstract

This study was carried out on 25 patients with azoospermia and normal serum FSH levels signifying obstruction as its cause. To determine the site of obstruction semen transferrin levels, carnitine and fructose were estimated. Patients with an obstruction at the testis - epididymal junction had low levels of transferrin but normal levels of carnitine and fructose. Patients who had lower levels of transferrin and carnitine, but normal levels of fructose had obstruction at the epididymal level, probably in the tail of the epididymis. Obstruction of the ejaculatory ducts was signified by lower levels of all parameters in the semen. Our study showed that levels of transferrin, carnitine and fructose when used in conjunction with each other could localize the site of obstruction in azoospermia (JPMA 44:140, 1994).

Introduction

Azoosperrnia is a common and often curable cause of male infertility. To differentiate cases of azoospermia due to obstruction or due to seminferous tubular damage, serum levels of FSH should be done[1,2]. If the serum levels of FSH are within the reference range, the cause of azoospermia is obstruction somewhere in the male genital tract. If however, serum FSH is raised to twice or more than normal, the cause of azoospermia is testicular failure and further workup of the patient is useless. This study was done on normal levels of FSH in serum. By estimating the levels of transferrin, carnitine and fructose in the seminal plasma of such cases, it was postulated that the level of obstruction in such cases could be pinpointed.

Subjects and Methods

Subjects

The subjects included in the study were selected from all patients referred to AFIP for investigation of male infertility from civil and military hospitals of Rawalpindi and Islamabad. They belonged to the heterogenous healthy male population and their ages varied between 22-45 years. A total of 81 subjects were seen. Out of these 25 (3 1%) patients of azoospermia were selected for this study on the basis of initial semen analysis and serum levels of FSH. The criteria for selection included: (1) patients who had regular, unprotected intercourse for atleast 12 months without conceiving and (2) patients who had azoospermia on semen analysis. All patients were thoroughly examined clinically to see any skeletal or endocrinological abnormality. Secondary sex characters like facial, axillary, pubic hair, voice and gynecomastia were noted. Other signs of disease like varicocele, torsion or absence of testis, epididymitis were noted. All the patients followed a similar protocol for evaluation.

Controls

Eleven normal, healthy, pregnancy proven males were selected as controls who had no physical abnormality clinically and total sperm count more than 20 million/ml with sperm motility more than 50%, 15 minutes after liquefaction of semen and no pus cells in semen. Semen transferrin, carnitine and fructose levels were within normal limits. Blood hormone levels were also within the reference range.

Methods

Semen samples were taken in the laboratory from all cases after sexual abstinence of 3 days by method of masturbation without the use of lubricants. Sterile glass tubes were used to collect the samples. Three semen analysis reports were consulted before declaring a patient azoospermic. Alter the initial semen analysis report, the rest of the semen sample was centrifuged in a Heittich centrifuge at 2000g for 20 minutes. The pellet was discarded. The supernatant was used to determine fructose levels. Rest of the semen was stored at -70°C, after careful labelling. Fructose levels of semen were done immediately as fructose decomposes on standing. For evaluation of seminal transferrin and carnitine, the sample was removed from -70°, thawed to room temperature (22°C-25°C) and then evaluated for each parameter. The instructions of the manufacturers were followed explicitly in performing the tests. Commercially prepared kits were used for evaluation of biochemical tests. Semen transferrin was estimated by using kit supplied by Merck Seminal carnitine was done on a kit supplied by Boeh - ringer Mannheim. Estimation of semen levels of fructose was done on Dr. Lange Kit.

Blood Samples

Three blood samples at 20 minute intervals were taken from each patient, under aseptic conditions, from the anterior cubital vein using a canula. All three blood samples of each patient were pooled and spun at 6000g for 5 minutes in a Heittich room centrifuge. Sera was extracted and stored in three aliquots of 1 ml. each, labelled and stored at -70°C. For evaluation of FSH, LH and testosterone levels the sera were thawed to room temperature (22°C-25°C) and tested using standard radioimmunoassay techniques.

Statistical Analysis

The sample size did not follow the Gaussian distribution. Therefore non-parametric tests were used. The comparison of parameters between healthy subjects and azoospermic patients were done by using Mann-Whitney-U tests. Spearmans rank correlation tests was used to correlate different parameters with each other in the azoospermic group.

Results

Twenty five azoospermic patients with normal serum FSH level were selected for this study (Table I).

Table I. Total sperm count, motility of sperms and FSH levels in healthy subjects and patients of obstructive azoospermia.

Group	Total sperm count (Million/ml)		Motility (%)		FSH (mIU/ml)	
	Median	Range	Median	Range	Median	Range
Normal	65	(23-95)	70	(60-90)	4.5	(1.4-11.0)
Obstructive azoospermia	0	(0-0)	0	(0-0)	4.1	(1.2-8.3)

Levels of transferrin, carnitine and fructose were significantly lower in patients with obstruction azoospermia (FSH <1 0mlU/ml) than healthy subjects but there was no difference in FSH, LH and testosterone levels (Table II).

Table II. Comparison of biochemical parameters between healthy subjects and obstructive azoospermic patients (FSH < 10 mIU/ml).

Parameters	Healthy subjects (n = 11)		Obstructive azoospermia (n = 25)		P
	Median	Range	Median	Range	
FSH	4.5	(1.4-11.0)	4.12	(1.2-8.3)	NS
LH	5.8	(4.0-10.0)	7.1	(2.0-15.0)	NS
Testosterone	5.8	(4.0-8.9)	6.2	(3.8-10.5)	NS
Transferrin	74.0	(69.0-80.0)	50.0	(35.0-63.0)	<0.01
Carnitine	67.0	(40.0-72.0)	36.0	(15.0-70.0)	<0.01
Fructose	318.0	(260.0-390.0)	198	(152.0-282.0)	<0.01
Zinc	2.4	(1.2-6.8)	2.2	(0.2-14.0)	NS

To define the level of obstruction azoospermic patients were divided into three subgroups; A, B and C (Table III).

Table III. Level of obstruction in azoospermic patients (FSH < 10mIU/ml).

Parameter	A Testicular (n = 4)		B Epididymal (n = 5)		C Ejaculatory duct (n = 16)	
	Median	Range	Median	Range	Median	Range
Transferrin	52	(50-56)	60	(53-63)	48	(38-58)
Carnitine	51	(43-56)	33	(15-36)	34	(18-39)
Fructose	224	(205-282)	208	(206-272)	190	(180-198)

P Values:
Group A vs group B (i) Transferrin NS, (ii) Carnitine < 0.01, (iii) Fructosse NS
Group B vs group C (i) Transferrin < 0.01, (ii) Carnitine NS, (iii) Fructose < 0.01
Group C vs group A (i) Transferrin NS, (ii) Carnitine < 0.01, (iii) Fructose < 0.01

In group A, semen transferrin levels were below whereas those of carnitine and fructose were within the reference range. This signified obstruction before the epididymis. Group B consisted of patients with low levels of transferrin and carnitine but normal levels of fructose in their semen indicating post-epididymal obstruction. Group C had lower levels of all three parameters, i.e., transferrin, carnitine and fructose. This indicated a post-seminal vesicle obstruction.

Discussion

There was no difference in the serum concentrations of FSH, LH and testosterone between healthy subjects and patients of obstructive azoospermia. Clinical examination of these patients revealed firm testes of normal size. FSH levels are raised in azoospermia due to testicular failure and normal in obstructive azoospermia[1,3-5] as has also been observed in this study. As the cause of azoospermia is obstruction to the outflow of sperms from the testis and the spermatogenic elements (sertoli cells, seminiferous tubules, Leydig cells) are intact therefore the level of LH and testosterone are also normal[6]. Comparison of semen transferrin levels between healthy subjects and patients of azoospermia with an obstructive etiology showed that the levels of transferrin in patients of obstructive azoospermia were very low as compared to healthy subjects. There are two reasons for this. One is that in azoospermia due to any etiology (obstructive or non-obstructive) transferrin is always low[6-9]. The obstruction can be at the level of the testis -epididymal function, post-epididymal or post-seminal vesicle. In all three instances, transferrin levels in semen are low. However, even in complete bilateral obstruction of the outflow tract, the levels of transferrin do not fall to zero as the prostate gland contributes about 20% of transferrin to the seminal plasma. The rest of the transferrin (80%) comes from the testis[10]. Carnitine levels in semen of patients with obstructive azoospermia are lower than the levels in healthy subjects. Carnitine, which is an epididymal marker is greatly reduced in azoospermia. Seminal vesicles contribute some amount of carnitine to the seminal fluid. Therefore, the level will not be reduced to zero in obstructive azoospermia[11,12], Like seminal transferrin and carnitine, seminal fructose in patients of obstructive azoospermia was significantly lower as compared to healthy subjects. Patients of obstructive azoospermia were divided into three groups A, B and C, based on the levels of seminal transferrin, carnitine and fructose. Group A consisted of patients who had low levels of transferrin, but normal levels of carnitine and fructose in their semen. Group B had lower levels of transferrin and carnitine but normal fructose levels. Group C had lower levels of all three parameters. Comparison of Group A parameters with Group B showed that there was no difference in the levels of transferrin and fructose, but camitine was significantly different between the two groups. Dadoun et al[9]. designated patients of Group A as having obstruction at the testis epididymal junction, signified by the low transferrin, but normal carnitine and fructose levels. Comparison of Group B with Group C showed that transferrin and fructose were significantly different between the two groups but carnitine did not show any significant change. Group B consisted of patients who had post epididymal obstruction, most probably in the tail of the epididymis. This finding is similar to that of Dadoun et al[9]. Group C which consisted of patients with lower levels of all three parameters was compared with Group A, it showed a significant difference for carnitine and fructose but not for transferrin. Group C represented obstruction at the level of the ejaculatory ducts[9]. This study demonstrated that in obstructive azoospermia patients, biochemical markers, when used in relation with each other, can help to localize the site of obstruction. This can be of benefit to the patient as he does not need to go through invasive procedures like vasograms.

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