

Biochemical Parameters in Evaluation of Oligospermia

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In Pakistan, male infertility constitutes about 35% of all cases of infertility. The dysfunction can be at the level of the central nervous system or somewhere in the male genital tract. A study was conducted to screen cases of male infertility using various biochemical parameters. A total of 81 cases were screened. Oligospermia was seen in 13 (16%) patients. They were divided into severe and mild to moderate oligospermia. In severe oligospermia, there was an increase in serum FSH level with decrease in seminal transferrin and carnitine suggesting damage to sertoli cells. In mild to moderate oligospermia, serum FSH were normal with levels a slight decrease in the levels of transferrin and carnitine in semen indicating that sertoli cells were not significantly defective and the dysfunction could be somewhere else (JPMA 44:137, 1994).

Introduction

Oligospermia is an important cause of male infertility. It is essential to carry out a systematic and complete workup to define the exact cause and to see if any help can be given. Seminal plasma examination is the most important indicator of the disorder¹. Based on this many biological tests including serum hormonal levels are done. Various substances are secreted by different parts of the male genital tract. These include transferrin secreted by the sertoli cells, carnitine which may be indicative of epididymal function, fructose which is a product of seminal vesicles and zinc secreted by the prostate which is responsible for sperm motility. Our study was aimed to find out the type of oligospermia and to evaluate these biochemical parameters in determining the level of dysfunction²⁻⁸.

Patients and Methods

Subjects

The oligospermic patients were selected out of patients referred to Armed Forces Institute of Pathology, Rawalpindi from civil and military hospitals of Rawalpindi and Islamabad, for investigation of male infertility. They belonged to the heterogeneous healthy male population and their ages varied between 22-45 years. A total of 81 subjects were seen. Out of these 13 (16%) patients of oligospermia were selected for this study on the basis of their initial semen analysis. The criteria for inclusion were (1) patients who had regular, unprotected intercourse for at least 12 months without conception of their partner and (2) patients who had sperm counts less than 20 million/ml, but a sperm motility more than 50% of normal.

Controls

Eleven normal healthy males whose partners had been pregnant were selected as controls. Their ages varied between 23-45 years. Protocol for collection of semen and sera samples was the same as for oligospermic patients. The criteria for selection as controls also included that there was no physical abnormality clinically, a semen count of more than 20 million/ml with motility more than 50% of normal 15 minutes after liquefaction of semen and absence of pus cells in semen. The levels of transferrin, carnitine, fructose and zinc in semen were normal. Blood hormone levels were also within reference limits.

Methods

All patients were examined clinically to exclude skeletal, endocrine disorders. Secondary sex characteristics like facial, axillary and pubic hair, voice and gynecomastia. Other signs of disease like varicocele, torsion, absence of testis and epididymitis were noted. All patients were subjected to the same protocol for evaluation purposes. Semen samples were taken from all patients in the laboratory after a minimum sexual abstinence period of three days by method of masturbation without use of lubricants. Sterile glass tubes were used to collect samples. Three semen samples were evaluated before a patient was declared oligospermic. After the initial semen analysis report, the rest of the semen sample was centrifuged in a Heitich centrifuge at 2000g for 20 minutes. The pellet was discarded. The supernatant was used to determine fructose levels. Rest of the semen was aliquoted and stored at -70°C after careful labelling. Fructose levels of semen were done quickly as fructose decomposes on standing. For evaluation of seminal transferrin, carnitine and zinc, the sample was removed from -70°C, thawed to room temperature (22°C-25°C) and then processed for each analyte. Commercially prepared kits were used for biochemical tests. Transferrin levels in semen were measured by Merck Kit No.11538; carnitine levels by L-Carnitine kit of Boehringer Mannheim, GmbH, Germany; fructose levels were estimated by Dr. Lange kit; zinc by kit provided by Wako Chemicals, Japan code No.998-14901. The serum levels of FSH, LH and testosterone were estimated by RIA kits of Diagnostic Products Corporation, Los Angeles.

Hormone analysis

Three blood samples at 20 minute intervals from interior cubital vein were taken from each patient, under aseptic conditions, using a canula. All three blood samples of each patient were pooled and spun at 6000g for 5 minutes in a Heitich centrifuge at room temperature. Sera was extracted and stored in three aliquots of one ml each, labelled and stored at -70°C. For hormone levels, the sera were thawed to room temperature (22°C-25°C) and the measurements were done according to the standard routine radioimmunoassay procedures as per instructions of the manufacturers.

Statistical analysis

The sample size did not follow the Gaussian distribution. Therefore non-parametric tests were used. The comparison of analyte between healthy subject and oligospermic patients were done using Mann-Whitney-U test. Spearman's rank correlation test was used to correlate different parameters with each other in the oligospermic group.

Results

Out of SI subjects, 13 cases (16%) were found to be oligospermic on semen analysis. These oligospermic cases were again divided into two groups: (A) severe oligospermia: 7 (9%) patients and (B) mild to moderate oligospermia: 6 (7%) patients.

Table I. Total sperm count, motility of sperms in healthy subjects and oligospermic patients.

Group	Total sperm count (million/ml)		Motility (%)	
	Median	Range	Median	Range
Normal	65	(23-95)	70	(60-90)
Mild to moderate oligospermia	10	(7-18)	80	(50-90)
Severe oligospermia	3	(2-4)	60	(50-80)

Table I shows that the sperm count in group A and B ranged from 2-18 million/ml which was much lower than healthy controls while no difference was observed in the motility in three groups. The serum concentrations of FSH in the severely oligospermic group (group A) were significantly higher than healthy age matched subjects with no difference in LH and testosterone levels. Serum transferrin and carnitine levels in severely oligospermic group of patients were significantly lower compared to healthy age matched controls. The levels of fructose and zinc in semen of both the groups were not significantly different (Table II).

Table II. Comparison of biochemical parameters between healthy subjects and severely oligospermic patients (< 5 million/ml). Results were compared by using Mann-Whitney U test.

Parameters	Healthy subjects (n = 11)		Severely oligospermic patients (n = 7)		P
	Median	Range	Median	Range	
FSH	4.5	1.4-11.0	12	9.0-16.0	<0.01
LH	5.8	4.0-10.0	8.2	1.0-16	NS
Testosterone	5.8	4.0-8.9	4.7	3.0-9.6	NS
Transferrin	74.0	69.0-80.0	54.0	50.0-60.0	<0.01
Carnitine	67.0	40.0-72.0	47.0	27.0-80.0	<0.01
Fructose	318.0	260.0-390.0	288.0	255.0-400.0	NS
Zinc	2.4	1.2-6.8	2.3	0.6-3.6	NS

Table III. Correlation of parameters in severely oligospermic patients. Results were calculated by using Spearman's rank correlation test.

1st parameter	2nd parameter	R	P
Transferrin	Total sperm count	0.89	<0.01
Transferrin	Motility	0.42	NS
Carnitine	Total sperm count	0.68	<0.05
Carnitine	Motility	0.25	NS
Fructose	Testosterone	0.25	NS
Zinc	Motility	-0.51	NS

Table III shows a significant correlation of semen transferrin with total sperm count, but not with motility of sperms. The same was true for semen carnitine. There was no correlation between semen levels of fructose and serum concentration of testosterone in this group. Seminal zinc did not show any correlation to motility of sperms in this group. Patients of mild to moderate oligospermia (group B) did not show any difference of serum levels of FSII, LII and testosterone when compared with healthy subjects. The values of semen transferrin and carnitine were significantly lower in this group when compared with healthy subjects. Semen fructose and zinc were not significantly different between the two groups (Table IV).

Table IV. Comparison of biochemical parameters between healthy subjects and mild to moderately oligospermic patients (5-20 million/ml). Results were compared by using Mann-Whitney U test.

Parameters	Healthy subjects		Mild to moderately oligospermic patients		P
	(n = 11)		(n = 6)		
	Median	Range	Median	Range	
FSH	4.5	1.4-11.0	8	2.1-17.0	NS
LH	5.8	4.0-10.0	5.9	2.1-20.7	NS
Testosterone	5.8	4.0-8.9	5.6	2.1-9.2	NS
Transferrin	74.0	69.0-80.0	61.0	53.0-68.0	<0.01
Carnitine	67.0	40.0-72.0	61.0	33.0-65.0	<0.01
Fructose	318.0	260.0-390.0	290.0	264.0-365.0	NS
Zinc	2.4	1.2-6.8	2.0	1.3-5.8	NS

Semen transferrin did not show any correlation with sperm count or motility. Seminal carnitine correlated significantly with the total count but not with motility of spermatozoa. All other parameters did not show any correlation with each other (Table V).

Table V. Correlation of parameters in mild to moderately oligospermic patient. Results were calculated by using Spearman's rank correlation test.

1st parameter	2nd parameter	R	P
Transferrin	Total sperm count	0.54	NS
Transferrin	Motility	0.36	NS
Carnitine	Total sperm count	0.72	<0.05
Carnitine	Motility	-0.17	NS
Fructose	Testosterone	0.54	NS
Zinc	Motility	-0.42	NS

Discussion

Different analytes were assessed in semen of oligospermic patients to locate exact site of dysfunction and to comment on their prognosis. Subjects showing total sperm count of less than 20 million/ml were placed in the oligospermic group⁹. These were further divided into two groups as recommended by earlier workers^{7,10-12}. Group A included subjects who had severe oligospermia with a total sperm count of less than 5 million/ml and group B which consisted of patients with mild to moderate oligospermia with a total sperm count more than 5 million/ml but less than 20 million/ml. When the hormonal profile of this group A was compared with those of healthy subjects, significant differences were observed in levels of FSH whereas LH and testosterone were normal. It was also seen that median concentration of FSH was higher in this group compared to healthy subjects. Our findings are in agreement with similar studies reported by others^{10,11,13,14}. FSH acts on the sertoli cells and initiates the process of differentiation of spermatids. Sertoli cells also produce a protein called Inhibin which has a negative effect on FSH secretion. Damage to the germinal epithelium resulting in loss of control of FSH secretion. LH acts on Leydig cells to produce testosterone which acts on differentiating spermatozoa and secondary sex characteristics. Damage to the germinal epithelium will not affect secretion of testosterone. Hence, levels of LH and testosterone should be normal. Our findings of normal levels of LH are against the findings of Haim et al.¹⁵, where the LH levels were significantly raised in severely oligospermic patients. However, our criteria of severe oligospermia was a total sperm count of less than 5 million/ml; whereas they¹⁵ had a criteria of less than one million/ml, which probably accounts for total loss of germinal epithelium, thus resulting in testicular failure which leads to increased levels of FSH and LH. Transferrin levels in severe oligospermic patients were found to be significantly lower than in the controls, Our findings are similar to those of previous workers^{2,16-19}. Transferrin levels were also significantly correlated with total sperm count in severely oligospermic patients. These were in accordance with the findings of other workers^{2,16-19}. Transferrin levels decrease significantly with a decrease in total sperm count and this decrease is progressive as oligospermia increases. The reason for this is not known, but it shows that seminal transferrin levels can be used as an indicator of sertoli cell function. As reported by others, motility of sperms and transferrin did not however, show any significant correlation^{2,3,20}. Since motility is acquired in epididymis, the sperms are

therefore not influenced by transferrin. Carnitine levels in severely oligospermic group were significantly lower than controls which has also been observed in previously reported studies^{5,12,21}. Low carnitine levels correlated significantly with total sperm count^{12,22} but Carter et al.²³ did not find any such relationship. Carnitine levels are also found to be low when motility of sperm is low. If the motility of sperms is normal, carnitine levels are also normal. This was also noted by Salinas- Sanchez et al. and Bornman et al.^{4,5}. Fructose levels in semen of severely oligospermic patients appeared normal^{24,25} but Raja lakshmi et al.²⁶ suggested that a decrease in the number of sperms results in an increase in fructose levels in semen as sperms utilize fructose for energy. Zinc levels are normal in severely oligospermic patients^{27,28}. The reason for this is that since zinc is responsible for motility, therefore with oligospermia, it may not be affected. In mild to moderate oligospermia (group B) differences in serum concentrations of FSH, LH and testosterone when compared with normal controls, remain insignificant. Since FSH in mild to moderate oligospermic patients remained normal, it was assumed that the cause of oligospermia in such cases was not germinal failure. This is in agreement with previous findings^{7,13}. Semen transferrin levels in this group were lower than controls, but were a little higher than those of severely oligospermic patients^{2,15,16}. When transferrin was compared with the total sperm count in mild to moderately oligospermic patients, no correlation was found, which was in contrast to the findings in severely oligospermic patients where transferrin correlated significantly with the total sperm count pointing to the fact that transferrin levels could be used as an index of sertoli cell function. Similarly a fall in transferrin levels with decrease in sperm count and rise of FSH level would signify germinal failure. Seminal carnitine in mild to moderately oligospermic subjects was significantly lower when compared with healthy controls, but was a little higher than those of severe oligospermia. It related significantly to the total sperm count but not to motility. This was similar to the findings in severe oligospermia, hence proving once again that since motility was normal, carnitine did not relate to it. Seminal fructose levels again did not show any difference in both groups of oligospermia. Zinc levels remained insignificant in mild to moderate oligospermia compared with healthy controls. This study indicates that semen transferrin level is an indicator of sertoli cell function. Its association with serum levels of FSH are indicative of extent of destruction of sertoli cells. The levels of the two parameters are helpful in assessing the prognosis in a case of severe oligospermia. A rise in serum FSH indicates germinal failure. It is recommended that serum levels of FSH and seminal levels of transferrin should be repeatedly done in oligospermia to monitor the progress.

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