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EVALUATION OF SEMEN QUALITY IN A LOCAL LIBYAN POPULATION

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Abstract : Semen analyses was carried out in a population of 1250 randomly selected Libyan males. Two semen samples collected from each volunteer were subjected to the routine analyses following the World Health Organization recommendations. The Libyan population had a higher percentage of men with sperm density in the range of 40-60 millions/ml. The percentage of men with sperm counts above 100 millions/ml is comparatively lower than that is reported by MacLeod and Gold (9). The seminal plasma transferrin levels showed a positive correlation with sperm density and alpha-glucosidase activity with sperm motility.

Key words : semen quality transferrin alpha-glucosidase activity Libyan population

INTRODUCTION

Seminal plasma is rich in proteins. Some of these proteins are reported to be derived from blood plasma and some like lactoferrin, acid phosphatase and transferrin from the reproductive grandular secretion (1). Of these seminal plasma proteins transferrin is reported to be secreted by Sertoli cells and reflect its function (2, 3). In the present study the semen composition of local Libyan population from Benghazi were presented. We had measured seminal plasma transferrin and alpha glucosidase activity in some of these patients. The enzyme alpha glucosidase (EC. 3.2.1.20) which is reported to be present in several mammalian species (4) was analysed in the seminal plasma of these patients with a view to find out whether it shows any relationship to sperm density and motility like transferrin.

METHODS

Semen samples collected from 1250 local Libyan subjects over the period of 1985 to 1990 in Benghazi were subjected to routine analyses following the recommendations of World Health Organization procedure (5). Volume of the ejaculate was measured in a graduated cylinder and sperm counts were performed in a haemocytometer. Total sperm counts were calculated as the product of the sperm count and the volume of the ejaculate. Fructose and citric acid were estimated by routine methods. A 10 microlitre of the undiluted semen was placed on microscope slide and covered with an inverted microscope, and the percentage of total (progressive and non-progressive) motility was calculated. Sperm motility in the ejaculate was measured at room temperature 30 to 60 min after collection.

Samples were stored at –20°C, thawed to room temperature, mixed and centrifuged at 2,000 x g for 10 min and the supernatant is used for the assay. Transferrin was assayed in 10-fold diluted samples by a specific radioimmunoassay (RIA) (6). The working range of standards were between 0.2 to 25 mg/L with an inter-assay coefficient of variation lying below 8%. Alpha glucocidase activity was measured after diluting the seminal plasma with 4 volumes of 50 mM ammonium acetate at pH 5.0 and titrated for 30 min at 4°C. Ethanol precooled at –20°C was added slowly until 30% (v/v) were obtained. The mixture was stirred for an additional 30 min centrifuged at 10,000 g for 15 min and the supernatant was treated with ethanol to reach a concentration of 70%. The precipitate

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contained the enzyme activity which was measured by a specific assay based on the spectrophotometric determination of p-nitrophenol released from the hydrolysis of p-nitrophenol-alpha-glucoside (PNPG) in citrate phosphate buffer pH 3.8 containing 50 mM sodium phosphate, 100 mM KCl, 1 mM EDTA and 5 mM $MgCl_2$. One milliunit of enzyme activity is defined as the amount of enzyme that catalyzes the hydrolysis of PNPG in 1 min at 37°C (7). The protein was determined by Lowry et al method (8). The seminal plasma transferrin and alpha-glucosidase activity were measured in 15 subjects in each group classified according to their sperm density. Regression curves were calculated by the least squares method, and the correlation coefficient, was calculated. Student 't'-test (two tailed) was used to determine whether two groups of data were significantly different. The 0.05 level of probability ($P<0.05$) was used as the minimal criterion of significance.

TABLE I : Seminal parameters analysed in a local population.

	Parameters analysed	*Values*
1.	Number (N)	1250
2.	Age (Year)	30±15
3.	Semen Volume (ml)	3.0±1.5
4.	Sperm density (millions/ejaculate)	185.0±180.0
5.	Seminal plasma fructose (mg/dl)	280.0±50.0
6.	Seminal plasma citric acid (mg/dl)	465.0±85.0
7.	Seminal plasma transferrin (μg/ml)	65.0±10.0
8.	Alpha glucosidase (mμ/mg protein)	5.0±1.5

RESULTS

The volume of the ejaculate varied from 0.5 to 7.5 ml with a mean of 3.00 ml and standard deviation of 1.5 ml. Sperm counts ranged from 0.5 millions to 685 milions per ml with a mean of 85 millions per ml and standard deviation of 65 millions. The range of total sperm counts was 1 to 2500 millions per ejaculate with a mean of 185 millions per ejaculate and a standard deviation of 100 millions per ejaculate. The large standard deviations are due to wide ranges found in different parameters studied which was also observed by other workers. The median values, 5 and 95 percentiles are presented in Tables I and II. The results of sperm counts per ml and total sperm counts were compared with the data reported earlier for other

TABLE II : Frequency distribution of sperm counts in local population.

	Sprem count millions/ml			*Number*	*Total sperm count millions/ejaculate*			*Number*
	5	*Median*	95		5	*Median*	95	
1.	<10	.0			<50.0			
	1.5	5.5	9.0	75(6%)	19.5	34.5	48.5	105(8.4%)
2.	10.1-20.0				50.1-100			
	11.5	15.75	19.5	100(8%)	50.5	75.0	98.5	155(12.4%)
3.	20.1-40.0				100.1-200.0			
	20.5	29.5	39.5	200(16%)	101.0	150.0	195.0	150(12%)
4.	40.1-60.0				200.1-300.0			
	40.5	50.5	59.5	325(26%)	205.0	245.5	290.0	405(32.4%)
5.	60.1-80.0				300.1-400.0			
	61.0	69.5	79.0	215(17.2%)	301.5	345.0	390.0	305(24.4%)
6.	80.1-100				400.1-500.0			
	80.5	89.0	99.5	130(10.4%)	401.0	450.0	495.0	75(6%)
7.	>100				501-700			
	100.5	180.0	225.0	205(16.4%)	501.0	595.0	695.0	55(4.4%)

TABLE III : Comparison of frequency distribution of sperm counts with earlier studies.

Sperm counts million/ml	MacLeod and Gold 1951		Nelson and Bunge 1974	Rehan et al 1974	Smith et al 1978	Sultan Sheriff 1983	Present 1992
	fertile %	infertile %	%	%	%	%	%
10	2	9	4.7	2	9.6	7.3	6
10.1-20	3	5	15.5	5	9.5	6.5	8
20.1-40	12	13	30.8	16	20.7	24.0	16
40.1-60	12	11	21.0	18	15.5	18.7	26
60.1-80	14	13	14.3	21	12.2	12.3	17.2
80.1-100	13	9	6.7	13	9.4	10.0	10.4
>100	44	38	7.0	24	23.1	21.2	16.4
Number of subjects	1000	1000	386	1300	2543	1500	1250

populations (9-12) (Tables III, IV). Higher percentage of men in the present population had a sperm density of 40 to 60 millions per ml compared to other populations. Significant differences in the mean sperm counts per ml between different populations and the present one were observed (x^2 test-P<0.05). Statistically no significant differences were found for the values for mean semen volume and mean total sperm counts (millions per ejaculate) of different studies considered (x^2 test and P>0.05) in each case. The seminal concentration of fructose and citric acid did not show any correlation with sperm density.

Seminal plasma transferrin levels showed a positive correlation with sperm density (r = 0.65; P<0.05). The seminal plasma alpha-glucosidase activity did not reveal any such relationship with sperm density. Its activity was high in semen samples containing >50% motile spermatozoa and was low in semen samples with poor motility. (r=0.55; P<0.05) Tables V and VI; Figs. 1 and 2).

TABLE IV : Comparison of frequency distribution of total sperm counts reported by MacLeod and Gold (1951), Smith et al (1978), Sheriff (1983) and the present report.

Total sperm count millions/ejaculate	MacLeod and Gold 1951		Smith et al 1978	Sheriff 1983	Present study
	fertile %	infertile %	%	%	%
50	7	15	36.0	27.0	8.4
50.1–100	8	10	17.7	20.7	12.4
100.1–200	20	18	21.9	20.0	12.0
200.1–300	19	17	11.7	17.6	32.4
300.1–400	15	13	6.1	4.8	24.4
400.1–500	9	9	2.5	4.3	6.0
>500	22	18	4.1	5.6	4.4
Number of subjects	1000	1000	2.43	1500	1250

TABLE V : Seminal plasma transferrin and alpha-glucosidase activity at different sperm densities.

Sperm density	Transferrin	Alpha-glucosidase
1. <10.0	50.0±10.0	4.50±1.5
2. 10.1–20.0	65.0±9.5 *	4.75±1.0
3. 20.1–40.0	72.5±11.0*	5.0±1.0
4. 40.1–60.0	82.0±9.5**	5.0±1.25
5. 60.1–80.0	95.0±10.5@	4.75±1.0
6. 80.1–100	102.0±12.5@	5.10±1.5
7. >100.0	105.0±10.0@	5.40±1.5
8. Azoospermia	45.0±7.5	4.85±1.0

Values are Mean ± SD of 15 cases per group
*P<0.05; **P<0.01; @P<0.001 when compared to azoospermic samples.

TABLE VI : Levels of alpha glucosidase activity.

Subjects	Alpha glucosidase activity mμ/mg protein
Controls (> 50% motility)	5.10 ± 1.0 (15)
Patients (0–50% motility)	4.05 ± 0.8 (15)
Patients (0–20% motility)	3.10 ± 0.6* (8)

Values represent the Mean ± SEM
*Statistically different from controls, P<0.05

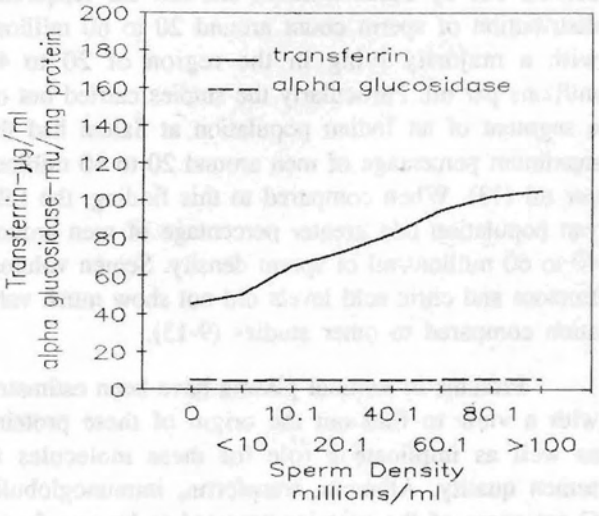

Fig 1 : Seminal plasma transferrin, alpha glucosidase activity and sperm density.

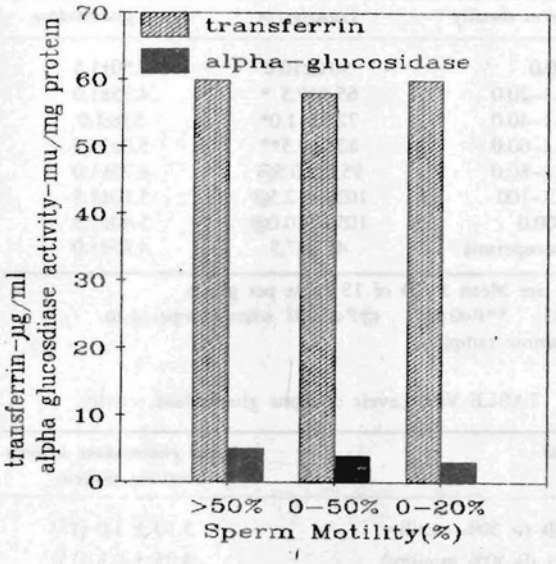

Fig 2 : Seminal plasma transferrin alpha glucosidase activity and sperm motility.

DISCUSSION

The studies carried out by Nelson and Gold (9) revealed that greater percentage of population had a sperm density > 100 millions per ml with a decline shown by other studies (9-13). The latter studies carried out by other workers showed the frequency distribution of sperm count around 20 to 60 millions with a majority lying in the region of 20 to 40 millions per ml. Particularly the studies carried out on a segment of an Indian population at Salem had the maximum percentage of men around 20 to 40 millions per ml (13). When compared to this finding, the Libyan population had greater percentage of men around 40 to 60 millions/ml of sperm density. Semen volume, fructose and citric acid levels did not show much variation compared to other studies (9-13).

Proteins in seminal plasma have been estimated with a view to find out the origin of these proteins as well as implicate a role for these molecules in semen quality. Albumin, transferrin, immunoglobulin G are some of the proteins reported to be transferred from the blood plasma into the semen, and proteins like lactoferrin as well as proteins involved in coagulation-liquefaction are derived from seminal vesicles and prostate (1). Of these proteins seminal plasma transferrin level is reported to show a positive correlation with sperm density and made people to suggest that it is secreted by Sertoli cells. Its level in the semen could be taken as a marker of Sertoli cell function. (1-3). A recent study has shown that plasma transferrin levels did show a positive correlation and such a positive correlation with sperm density is not specific for transferrin but also to other proteins like albumin and B2 microglobulin. On the basis of these findings they suggested that all of these proteins get transferred from the circulation in the lower genital tract and its rate of transfer is controlled by a substance produced by the sperm (3). In the present study we found that seminal plasma transferrin did show a positive correlation with sperm density with the levels of this protein being lowest in azoospermia. Along with this protein we estimated alpha-glucosidase activity to find out whether it shows such a positive correlation with sperm count. This enzyme is believed to be important for sperm metabolism in providing glucose from hydrolysis of maltose and may play role in sperm maturation by removing oligosaccharides from glycoprotein coat of the spermatozoa during its epiddymal transit (4). In the present study, the enzyme did not show a relationship with sperm density. But it did show a positive correlation with sperm motility. Epididymis is the site in which the spermatozoa attains progressive mobility. A positive correlation observed between the enzyme activty and sperm motility appears, therefore rational.

Further studies are being carried out to elucidate the relationship between the enzyme activity and sperm motility. Thus, studies carried out on different populations help in evaluating the semen quality in different races and provides basis for a comparative study to assess male reproductive function. The effects of changing patterns of living as well as the influence of xenobiotic factors on semen quality need to be studied, time and again, to set normal standards of semen parameters and to gain greater insight into male reproductive function.

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