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Comparative Content of Neuron-Specific Enolase in Human Blood Serum and Seminal Plasma

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We studied the content of neuron-specific enolase (NSE) in 69 paired samples of blood serum and seminal plasma from men with azoospermia (n=11) and oligoastenozoospermia (n=10) and from men with fertile ejaculate (n=48). NSE concentration was determined by ELISA (Vector-Best kit). The median concentration and the interquartile range of the NSE content in seminal plasma were 65.7 (47.9; 83.4) ng/ml and 24.33 times (p<0.000001) exceeded those for blood serum 2.7 (1.45; 4.0) ng/ml. There were no differences in the content of NSE between the groups for both seminal plasma and blood serum. The content of NSE in seminal plasma did not correlate with the content of NSE in blood serum, and also did not depend on the content of spermatozoa. A weak negative correlation (r=-0.341; p=0.0057) was found between the age of the examinees and the level of NSE in seminal plasma, but not in blood serum.

Key Words: *neuron-specific enolase; ejaculate; seminal plasma; semen; blood serum*

Neuron-specific enolase (NSE) is a form of glycolytic enzyme enolase. The study of NSE in the cerebrospinal fluid is used to assess the state of the tissue of CNS [1,3,8], and in the blood serum – for the diagnosis and monitoring of neuroendocrine tumors [5,6]. There are published data on the detection of NSE during cytochemical examination in the organs of the male reproductive system. Thus, the expression of NSE was detected in Leydig cells [11], spermatogonia [4], spermatozoa [7], and cells of male reproductive organ neoplasms [10,13]. However, we found no reports on the study of this enzyme in human seminal plasma.

The aim of the study to determine NSE concentration in blood serum and seminal plasma samples of men of different ages and different fertility as a potential biomarker of norm or pathology.

MATERIALS AND METHODS

A single-stage observational case–control study was conducted in compliance with the ethical principles of the Helsinki Declaration of the World Medical Association.

The study included 69 paired samples of biological material (blood serum and seminal plasma) of men who were examined to clarify the causes of infertile marriage. The main group consisted of subjects with the absence of sperm in the ejaculate (azoospermia; n=11). The comparison group included patients with subfertile ejaculate samples (oligoastenozoospermia; n=10). The control group consisted of men (n=48) who met the criteria for ejaculate fertility in accordance with WHO recommendations [2]. The characteristics of the examined and their biomaterial are presented in Table 1.

Blood serum and seminal plasma were separated by centrifugation at 3000g for 20 min. Depersonalized remnants of biomaterial samples preserved and stored at -40°C were used for the study.

The concentration of NSE was determined by ELISA using NSE-ELISA-BEST kit (T-8476; Vector-Best).

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TABLE 1. Characteristics of the Examined Individuals (*M*±*SD*, Me (25%;75%), min-max)

Parameter	Group 1 (azoospermia; *n*=11)	Group 2 (oligoastenozoospermia; *n*=10)	Group 3 (normal; *N*=48)	Kruskal—Wallis *H* test
Age, years	36.3±9.1	33.4±7.0	34.0 ± 6.8	*H*=0.7277789
	34.0 (29.0; 42.0)	31.5 (30.0; 36.0)	34.0 (29.0; 38.5)	*p*=0.6950
	26-56	27-51	21-52	
Volume of ejaculate, ml	4.1±1.6	4.2±1.7	4.1±1.2	*H*=0.0504747
	3.9 (3.2; 4.8)	4.1 (2.5; 5.0)	4.1 (3.3; 4.9)	*p*=0.9751
	2.0-8.0	2.2-7.2	2.0-7.2	
Sperm concentration, 10^6/ml	0±0	8.83±3.83	88.91±54.67	*H*=44.81736
	0 (0; 0)	8.85 (7.0; 11.3)	81.55 (53.6; 110.1)	*p*<0.0001
	0	2.0-14.1	15.6-252.0	
Sperm content, 10^6/ejaculate	0±0	36.08±26.11	334.38±174.84	*H*=44.81736
	0 (0; 0)	28.6 (17.6; 47.5)	303.1 (201.4; 439.9)	*p*<0.0001
	0	2.0-87.4	49.9-716.2	

The sensitivity of the test system used was 0.5 ng/ml, linearity — up to 130 ng/ml.

The optical density of the samples was measured on a StatFax 3200 vertical photometer (Awareness). The accuracy was controlled by measuring optical density of a control sample with a target NSE content of 11.97 ng/ml (permissible limit 9.5-14.5 ng/ml). In this study, the measurement results were 11.4 and 11.0 ng/ml, which indicated acceptable accuracy of the assay.

Statistical processing of the obtained results was carried out using the Statistica 7.0 software (StatSoft, Inc.). The parameters of descriptive statistics were calculated for each data array: arithmetic mean (*M*), standard deviation (*SD*), median (Me) and interquartile range (25%; 75%), as well as minimum (min) and maximum (max) values. The data arrays were evaluated for the presence and severity of outliers, the nature of data distribution was evaluated using the Shapiro—Wilk test. The obtained results made it possible to reject the null hypothesis about the normal nature of their distribution, which justified the use of nonparametric methods of comparative statistical analysis. Independent samples were compared using the Kruskal—Wallis *H* test, and the Wilcoxon's test was used to compare dependent samples. Correlations were evaluated using Spearman's correlation coefficient (*r*). For the maximum acceptable probability of error of the first kind (*p*), a value of the level of significance equal to or less than 0.05 was taken.

RESULTS

The median concentrations of NSE in different biomaterials of the examined subjects differed by 24.33 times ($p<0.000001$): 65.7 (47.9; 83.4) ng/ml for seminal plasma; min-max range 19.6-165.1 ng/ml and 2.7 (1.45; 4.0) ng/ml for blood serum; min-max range 0.1-4.2 ng/ml.

The concentration of NSE in the biological materials significantly differed between the groups, but no significant differences within the groups were found (Table 2).

There were no significant correlations between the content of NSE in blood serum and in seminal plasma (r=-0.125; p=0.32) (Fig. 1, *a*) and between NSE concentration in seminal plasma and common indicators of fertility of ejaculate. For instance, the coefficient of correlation between the level of NSE and sperm content was r=0.018 (p=0.8876) and between the NSE content and the number of actively motile spermatozoa r=0.0998 (p=0.4324). At the same time, a significant weak correlation was revealed between the age of the examined subjects and the content of NSE in the seminal plasma: r=-0.341473 (p=0.005754) (Fig. 1, *b*). This regularity was described by linear regression equation (95% probability):

NSE concentration (ng/ml)=114.3-1.228×age (years);

This regularity can be explained by age-related involution of body tissues and a decrease in reproductive potential. This conclusion is supported by published data that spermatozoa with reduced expression of NSE during cytochemical detection are characterized by reduced fertility (for example, after cryopreservation). For instance, it was shown that spermatozoa with initially normal NSE activity recover better after cryopreservation than cells with reduced activity of this enzyme [9]. These observations are supported by the results of a study of farm animals, indicating the important role of the normal content of NSE in the seminal plasma in the prognosis of fertility [12].

Thus, the content of NSE in seminal plasma is 24.33 times ($p<0.000001$) higher than its content in

TABLE 2. NSE Content in Seminal Plasma and Blood Serum in the Examined Groups (*M*±*SD*, Me (25%;75%), min-max)

Parameter	Group 1 (azoospermia; *n*=11)	Group 2 (oligoastenozoospermia; *n*=10)	Group 3 (normal; *N*=48)	Kruskal—Wallis *H* test
NSE concentration in seminal plasma, ng/ml	76.22±42.73	77.69±34.80	70.52±30,18	*H*=0.1193781
	75.5 (39.5; 118.0)	69.5 (47.3; 98.8)	64.45 (49.2; 82.05)	*p*=0.9421
	19.6-129.2	42.3-136.9	25.2-165.1	
NSE concentration in blood plasma, ng/ml	2.13±1.53	1.93±1.45	2.26±1.12	*H*=2.014406
	2.35 (0.5; 3.3)	2.05 (0.4; 2.9)	2.2 (1.25; 3.00)	*p*=0.3652
	0.2-4.1	0.1-4.2	0.1-4.2	
Wilcoxon's *W* test	*p*<0.000001	*p*<0.000001	*p*<0.000001	—

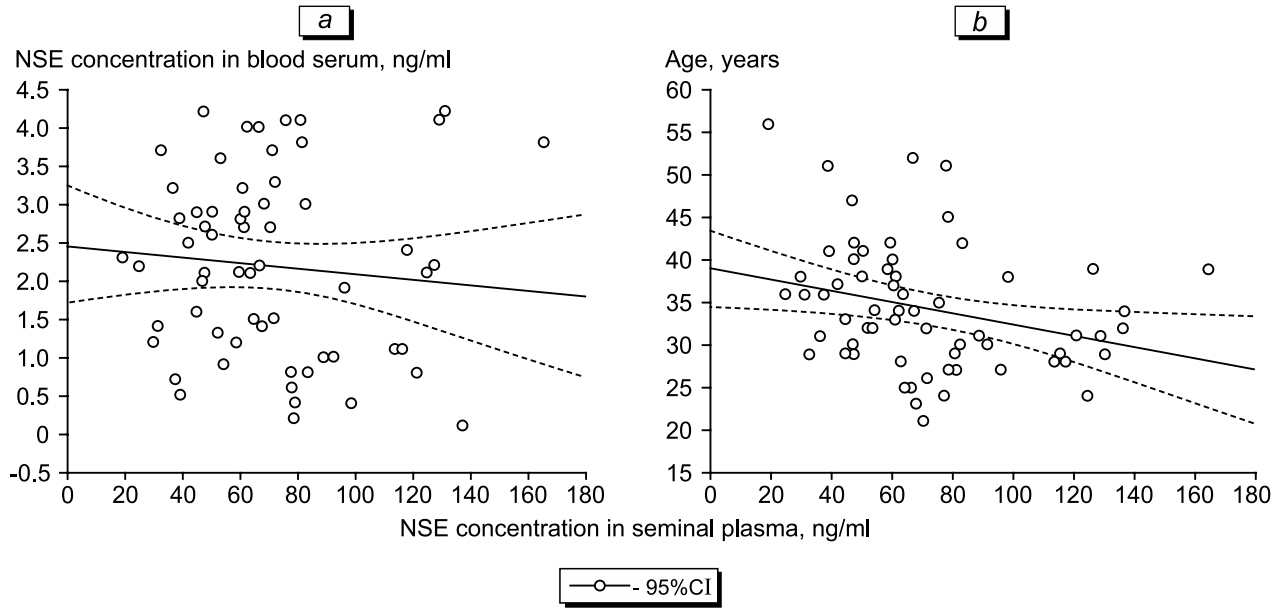

Fig. 1. Dependence of NSE concentration in seminal plasma on its concentration in blood serum (*a*) and on the age of the examined subject (*b*).

the blood serum. There was no significant correlation between the content of NSE in seminal plasma and blood serum. We also revealed no significant differences in the content of NSE in seminal plasma and serum between the groups of subjects with azoospermia, oligoastenozoospermia, and subjects with normal sperm count. The content of NSE in seminal plasma, but not in the blood serum decreases with age.

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