DIAGNOSTIC CRITERIA OF VIBRATION DISEASE ON THE BASIS OF THE ASSESSMENT OF BLOOD SERUM FATTY ACID COMPOSITION

UDC 616–001.34–074
Received 17.11.2012

© **I.A. Petrova,** Junior Research Worker, the Hygiene Department[1];
A.S. Gordetsov, D.Chem.Sc., Professor, Head of the Department of General Chemistry[2];
I.V. Fedotova, D.Med.Sc., Head of the Department of Hygiene[1]

[1]Nizhny Novgorod Scientific Research Institute of Hygiene and Occupational Pathology, Semashko St., 20, Nizhny Novgorod, 603950;
[2]Nizhny Novgorod State Medical Academy, Minin and Pozharsky Square, 10/1, Nizhny Novgorod, Russian Federation, 603005

The aim of the investigation was to justify new criteria of vibration disease diagnostics based on the study of the spectrum of higher fatty acids in blood serum of patients.

Materials and Methods. Blood serum of patients with degree 1 vibration disease (n=65) was under study. Higher fatty acids were determined in dry serum using capillary gas chromatography with preliminary acid methylation. Methyl ether content ratio was taken as the concentration of each acid.

Results. In vibration disease development there is the shift of blood serum fatty acid composition towards saturated acids (the same in men and women) that enables to distinguish this pathology among the others.

To assess quantitative composition of higher fatty acids, there were suggested the following coefficients reflecting the relationship of the total content saturated and polyunsaturated fatty acids of ω-6 (K_1) and ω-3 (K_2) family: K_1=0.9–1.5; K_2=16.0–28.5; in healthy people K_1=0.6–0.9; K_2=10.7–15.9 ($p \leq 0.01$).

The calculated value ranges of the suggested coefficients do not overlap, and if any one or more of the diagnostic criteria fall within the range of the stated limits, the presence of vibration disease can be expected.

Conclusion. The suggested coefficients can be used as criteria in vibration disease diagnosing, as well as in the assessment of lipid storage disease in case the efficiency of occupational disease therapy is determined.

Key words: fatty acid composition; blood serum; gas chromatographic analysis; vibration disease.

Vibration disease (VD) ranks one of the leading positions in occupational morbidity structure [1]. Despite incidence, this disease causes certain difficulties in diagnosis, since it is characterized by low clinical intensity at early stages [2]. Standard practice of VD diagnosis [2] is based on a patient's complex examination including history taking, clinical and laboratory data, as well as functional diagnostic techniques (cold test, skin thermometry, dolorimetry, determination of a vibration sensitivity threshold, dynamometry, rheovasography, stimulation electroneuromyography, electroencelography) aimed at detecting peripheral angiodystonic syndrome and vegetative-sensory polyneuropathy, which characterize VD symptom complex.

Another known VD diagnostic technique [3] consists in history taking, neurological status examination, biochemical blood assay, detection of angiospastic dysfunctions, disturbances of pain and vibration sensitivity. However, the main thing of this method is the results of electromyography in needle recording of biopotentials.

The mentioned VD diagnostic techniques are widely used in practice, though they do not provide clear quantitative assessment of essential diagnostic signs of the pathology that determines the necessity for expanding the range of laboratory methods in VD clinic picture.

The improved VD diagnostic technique [4] is based on quantitative analysis of a specific symptom complex of pathological changes in the body due to vibration effect. The same methods suggested by the authors are used as diagnostic procedures [3]. In addition, electromyographic indices of neuromuscular irritability is additionally recorded before and after the Trousseau–von Bonsdorff test, electrocardiography is performed to estimate deviation degree from optimal tightness on electrocardiogram in testing experimental subjects, as well as rheovasography of basilar circulation of brain, upper and lower limb vessels, X-ray examination or densitometry of supportive body frame with the study of quantitative deviation of simple and ionized calcium in blood serum. Taking into account the informative value of every index, we calculated the corresponding indices of disorders and an integral complex quantitative index of vibration pathology. This VD diagnostic technique is informative and safe, but of long duration, as it is related to the use of a large number of various diagnostic techniques and performing complicated mathematical calculations.

For contacts: Petrova Irina Alexandrovna, phone: 8(831)436-35-93, +7 920-016-97-08; e-mail: yes-ia@mail.ru

Currently, there is under way the work on search and validation of the most informative biomarkers, and their complexes to estimate vibration effect on human body [4–7].

Various cytochemical shifts are found in VD pathogenesis [8–11], e.g., in connection with metabolic disorder of lipids, which are the base of biological membranes and nervous tissue, which in their turn undergo a degeneration process under protracted exposure to local vibration [12]. Since metabolic processes and pathological changes of ester fragments of phospholipids are related to concentration dependences of corresponding fatty acids, it seems reasonable to study fatty acid composition of blood serum in VD patients as one of the indices of degenerative phenomena in nervous tissue.

The aim of the investigation was to justify new criteria of vibration disease diagnostics based on the study of the spectrum of higher fatty acids in blood serum of patients.

Materials and Methods. Blood serum of 65 people with degree I VD was under study. Mean age of patients was 56.0±10.0 years, total occupational life under local vibration being 21.6±3.6 years. To diagnose the mentioned pathology, the working men underwent the examination according to a standard practice [2]. All experimental subjects were found to have upper limb vegetative-sensory polyneuropathy syndrome, in five of them it was associated with peripheral angiodystonic syndrome; in addition, 30% of cases were revealed to have hypertensive disease. A control group consisted of 20 virtually healthy people comparable in age with a study group, with no exposure to vibration.

10 cm^3 of venous blood was taken on an empty stomach in the morning to study fatty acid composition of blood serum. The latter was kept in the dark for a day at room temperature till complete erythrocyte sedimentation. 2 cm^3 of an upper serum layer was put into a plate and left at 18–20°C to dry. Further preparation of dry blood serum, extraction of fatty acids by liquid extraction technique, and their transformation into methyl ethers were performed according to the technique [13]. Measurements were taken on gas chromatograph "Chromos GC-1000" (Russia) equipped by flame ionization detector and capillary column with polyethylene glycol phase modified by nitroterephthalic acid (ZB FFAP, 50 m×0.32 m×0.5 μm; Phenomenex, USA). Analysis conditions were the following: column temperature — 190°C, evaporator temperature — 200°C, detector temperature — 200°C. Injection volume was 1 μl, injection technique — by carrier gas (nitrogen) flow splitting 1:10. Fatty acids were identified by holding time

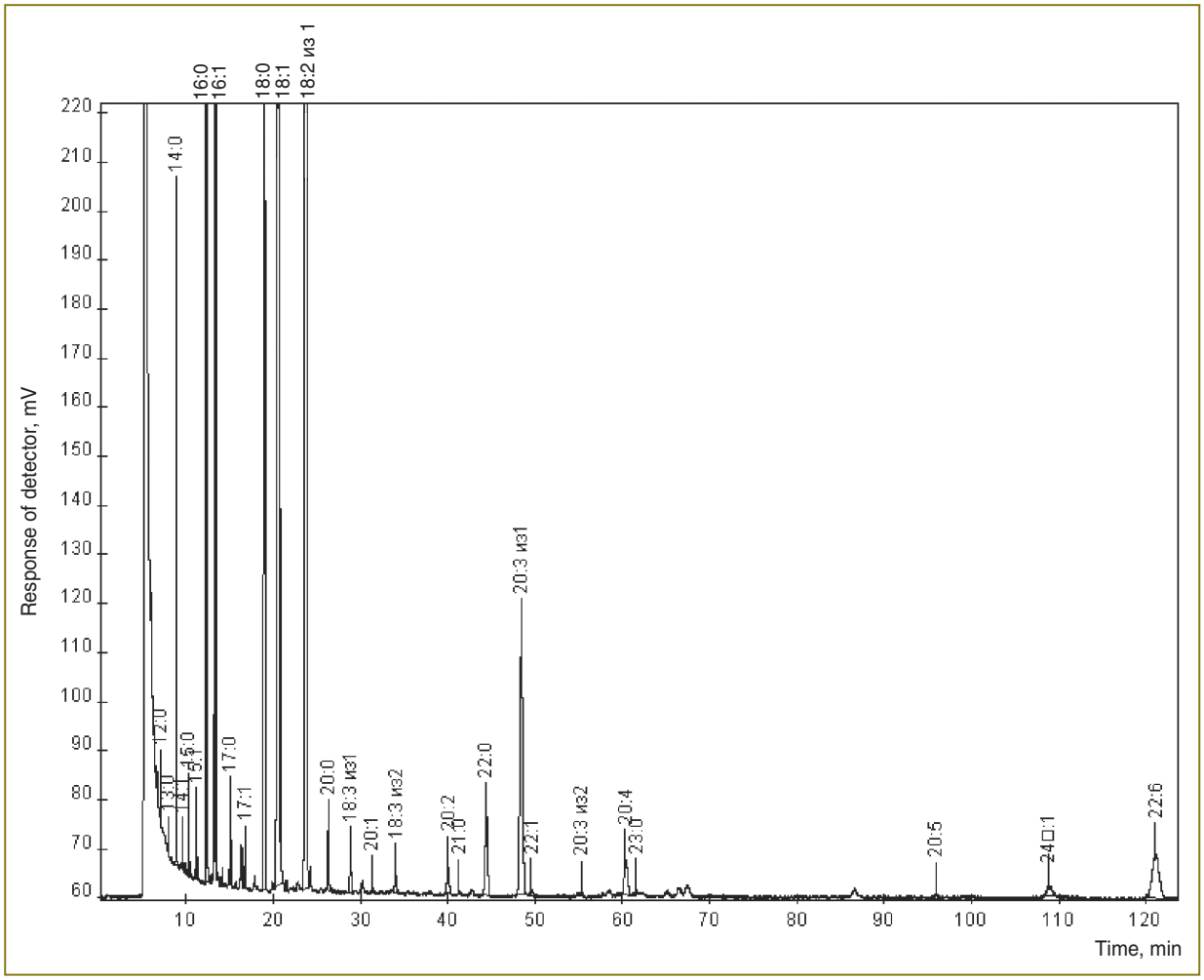

Fig. 1. Blood serum chromatograph of a patient with the diagnosis "degree I VD (vegetative-sensory polyneuropathy, peripheral angiodystonic syndrome)"; age — 50, length of work under local vibration — 22 years

using standards. Chromatographic data were processed on hardware and software complex "Chromateck-Analyzer" (Russia). Quantitative assessment of higher fatty acids (HFA) was performed using normalization principle of peak areas of their methylated derivatives. Relative level of the corresponding methyl ether was taken as concentration of each fatty acid. The data were statistically processed using "Biostatistics" program.

The values are expressed as M±m, where M is arithmetic mean, m is confidential interval (p=05). The reliability of mean value differences was determined using Student's test.

Results and Discussion. Qualitative HFA composition of the experimental subjects was represented by 27 components (Fig. 1) including:

11 saturated fatty acids (SFA): lauric $C_{12:0}$, tridecanoic $C_{13:0}$, tetradecanoic $C_{14:0}$, pentadecanoic $C_{15:0}$, hexadecanoic $C_{16:0}$, heptadecanoic $C_{17:0}$, stearic $C_{18:0}$, arachic $C_{20:0}$, heneicosanic $C_{21:0}$, behenic $C_{22:0}$, tricosoic $C_{23:0}$;

7 monounsaturated fatty acids (MUFA): myristoleic $C_{14:1}$, pentadecanoic $C_{15:1}$, palmitoleic $C_{16:1}$, heptadecanoic $C_{17:1}$, oleic $C_{18:1}$, icosenic $C_{20:1}$, erucidic $C_{24:1}$;

9 polyunsaturated fatty acids (PUFA): eicosandienoic $C_{20:2}$, linolic $C_{18:2}$ of ω-6 family, γ-octadecatrienoic $C_{18:3}$ ω-6, cis-8,11,14-eicosatrienoic $C_{20:3}$ ω-6, eicosatetraenoic $C_{20:4}$ ω-6, octadecatrienoic $C_{18:3}$ ω-3, cis-11,14,17-eicosatrienoic $C_{20:3}$ ω-3, cis-5,8,11,14,17-eicosapentaenoic $C_{20:5}$ ω-3, cis-4,7,10,13,16,19-docosahexaenoic $C_{22:26}$ ω-3 (Table 1).

Total SFA content in a control group was significantly lower than in VD patients. The observed growth of quantitative levels of this family acids in case of vibration pathology was statistically significant (p=0.002 in men, and p=0,000 in women) and was determined mainly by the increase in hexadecanoic acid content (p=0.005 in men, and p=0.000 in women).

The content of practically any MUFA acid in blood serum in VD group was significantly higher compared to that in a control group. However, quantitative level of total MUFA in VD patients statistically significantly differed from that in virtually healthy people (p=0.045 and p=0.046 in men and women respectively).

Total PUFA in cases with vibration pathology was significantly lower than that in a control group (p=0.003 in men, and p=0.000 in women). Quantitative composition of polyunsaturated acids of ω-3 family was observed to be unchanged in the process of VD formation, and this fact referred both to any particular acid, and to their total. The decrease of PUFA content was due to HFA of ω-6 family. VD subjects in relation to a control group were recorded to have significant fall in quantitative levels of linolic, cis-8,11,14-eicosatrienoic and eicosatetraenoic acids — on the average by 4.9, 1.5 and 0.6 mass% respectively that determined statistically significant difference and total PUFA content (p=0.005 in men, and p=0.000 in women) of ω-6 family.

It should be noted that there were found no differences in HFA profile formation in male and female VD patients (Fig. 2). It indicates that fatty acid composition of blood serum is unlikely to depend on gender, and being exposed to vibration undergoes similar changes.

Thus, quantitative fatty acid composition of blood serum of VD patients compared to controls is characterized by highly reliable changes of total content of PUFA (decrease),

Table 1

Analysis results of higher fatty acids of blood serum in subjects with vibration disease

Acid		Acid content, mass%		
		VD patients		Control
		male	female	
SFA	**Total**	**38.23±0.88**	**38.02± 0.62**	**32.77±1.17**
	lauric	0.49±0.14	0.38±0.15	0.08±0.03
	tridecanoic	0.10±0.03	0.06±0.02	0.02±0.01
	tetradecanoic	0.96±0.07	0.96±0.07	0.80±0.10
	pentadecanoic	0.27±0.03	0.22±0.03	0.22±0.04
	hexadecanoic	25.55±0.72	25.62±0.44	21.86±0.79
	heptadecanoic	0.27±0.04	0.25±0.03	0.30±0.03
	stearic	8.24±0.22	8.16±0.28	8.14±0.27
	arachic	0.33±0.07	0.25±0.05	0.29±0.06
	heneicosanic	0.29±0.04	0.26±0.03	0.20±0.02
	behenic	1.20±0.08	1.07±0.13	0.97±0.08
	ticosoic	0.46±0.06	0.79±0.33	0
MUFA	**Total**	**22.94±0.87**	**22.70±1.07**	**19.84±0.79**
	myristoleic	013±0.04	0.10±0.03	0.05±0.02
	pentadecanoic	0.38±0.09	0.30±0.09	0.60±0.08
	palmitoleic	1.87±0.24	1.89±0.19	1.61±0.37
	heptadecanoic	0.22±0.04	0.12±0.02	0.30±0.07
	oleic	19.97±0.69	19.29±0.99	18.03±1.25
	icosenic	0.12±0.03	0.07± 0.02	0.09±0.02
	erucidic	0.29±0.04	0.30± 0.04	0.32±0.04
PUFA	**Total**	**38.83±1.48**	**39.28±1.10**	**47.39±1.25**
	linolic ω-6	31.77±1.45	32.88±0.79	37.19±1.89
	octadecatrienoic ω-6	0.35±0.07	0.29±0.03	0.29±0.05
	octadecatrienoic ω-3	0.33±0.08	0.25±0.03	0.14±0.05
	eicosandienoic	0.07±0.02	0.03±0.01	0.04±0.01
	eicosatrienoic ω-6	4.57±0.28	3.79±0.46	5.64±0.39
	eicosatrienoic ω-3	0.06±0.01	0.06±0.02	0.04±0.02
	eicosatetraenoic ω-6	0.15±0.03	0.15±0.03	0.76±0.24
	eicosapentaenoic ω-3	0.03±0.03	0.05±0.01	0
	docosahexaenoic ω-3	1.51±0.11	1.77±0.29	2.04±0.29
ω-6	**Total**	**36.84±1.51**	**37.12±1.04**	**45.39±1.48**
ω-3	**Total**	**1.91±0.16**	**2.13±0.29**	**2.04±0.30**

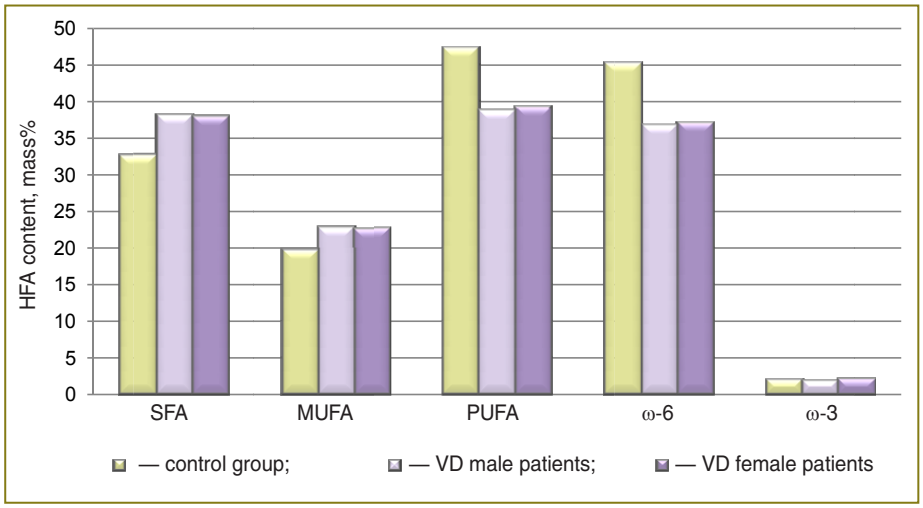

Fig. 2. Relative HFA content in blood serum

MUFA and SFA (increase). In addition, a recorded fall in quantitative level of HFA with polyunsaturated bonding is due to concentration change of acids of ω-6 family against the background of unchanged level of ω-3 family acids.

The observed growth of SFA and MUFA content resulted from the pathology development proceeds synchronically (See Fig. 2), i.e. there were revealed no changes of the total content of HFA with monounsaturated bonds in relation to SFA in the process of VD formation. In case of PUFA, there was an evident decrease in the content of these acids — of both ω-6 and ω-3 families — in relation to SFA. The content decrease of HFA with unsaturated bonds determines reduced antioxidant protection of the body recorded in VD. Moreover, the observed concentration decrease of ω-3 family PUFA occurs, in particular, due to a sharp fall in docosahexaenoic acid level supporting the theory that it is used for lipid mediator synthesis — neuroprotectin, which is activated under the threat of imbalance of normal nervous system functioning [14].

Deficiency of PUFA — of both ω-6 and ω-3 families — arising from vibration pathology is considered to be an unfavorable predictive factor with relation to functionality of the body on cellular level that determines the relevance of quantitative assessment of HFA nonsaturation degree reduction in blood serum of VD patients. In this regard, the authors suggest introducing coefficients showing the revealed changes by calculating the ratio of total SFA content to total PUFA of both families:

$$K_1 = \frac{\text{total SFA}}{\text{total PUFA } \omega\text{-6}}, \quad K_2 = \frac{\text{total SFA}}{\text{total PUFA } \omega\text{-3}}.$$

The ranges of values of the suggested coefficients (Table 2) in mentioned situations were found to be nonoverlapping. It proves the fact that a recorded shift in blood serum fatty acid composition in patients towards saturated acids in the course of VD formation is essential and statistically significantly differs from controls. Therefore, the suggested coefficients can be used as an additional diagnostic technique in VD diagnosis.

Table 2

Comparative ranges of K_1 and K_2 coefficient values

Coefficient	Control	VD patients	p
K_1	0.6–0.9	0.9–1.5	0.012
K_2	10.7–15.9	16.0–28.5	0.009

Conclusion. In vibration disease development there is metabolic imbalance of higher fatty acids towards the increase of their saturation, the same in men and women.

The study of quantitative composition of blood serum of VD patients and controls enabled to suggest the coefficients reflecting the relationship of the total content of saturated and polyunsaturated fatty acids of ω-6 (K_1) and ω-3 (K_2) family. The calculated value ranges of the suggested coefficients do not overlap, and if any one or more of the diagnostic criteria falls within the stated range, the presence of vibration disease can be expected.

The suggested coefficients can be used as criteria in vibration disease diagnosing, as well as in the assessment of lipid storage disease in case the efficiency of occupational disease therapy is determined.

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