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Research article

IMMUNOCHEMICAL MARKERS OF EFFECT UNDER EXPOSURE TO RISK FACTORS CAUSING VIBRATION DISEASE OF DIFFERENT ETIOGENESIS: COMPARATIVE ASSESSMENT

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In recent years, it has become especially vital to identify prognostic risks of health disorders in workers exposed to harmful occupational factors. This is necessary for substantiating an occupational origin of a disease and biomarkers of exposure and for optimizing the occupational risk assessment methodology.

The aim of this study was to compare and analyze immunochemical markers of effect (cytokines, heat shock proteins, and neuronal antibodies (AB)) in blood serum of patients with vibration disease (VD) induced by exposure to different types of vibration in order to substantiate the most informative diagnostic risk indicators concerning the disease development and clinical course.

Cytokines, heat shock proteins, and antibodies to regulatory proteins of nervous tissue were identified in blood by ELISA tests. We established unidirectional statistically significantly more apparent changes in patients who had VD caused by combined exposure to both whole body vibration and local vibration against those who had VD caused by exposure to local vibration only. These changes included hyperactivated pro-inflammatory reactions of the immune response (IL-1β, TNF-α, INFγ), growing concentrations of antibodies to proteins: S-100, MBP, NF-200, GFAP, and voltage-gated Ca-channel. The differences were that patients with VD under combined exposure to both types of vibration had greater production of pro-inflammatory IL-8 and HSP27 whereas people with VD caused by exposure to local vibration only had a decrease in HSP70 levels.

The study results confirmed more apparent neuro-immune inflammation in patients with VD caused by combined exposure to both whole body vibration and local vibration. This may indicate more significant risk factors of the disease and gives an opportunity to identify the most sensitive biomarkers eligible for diagnosing VD of different etiogenesis.

Keywords: *vibration disease, cytokines, heat shock proteins, neuronal antibodies, inflammation, local and whole body vibration.*

In recent years, it has become especially vital to identify prognostic risks of health disorders in workers exposed to harmful occupational factors. This is necessary for substantiating an occupational origin of a disease, biomarkers of exposure, and peculiarities of a biological response to exposures [1, 2]. Vibration disease (VD) is a well-known polysyndrome disease affecting both the peripheral and central nervous system[1] [3]. Cerebrospinal, thalamic, and cortical centers of vibrational sensitivity as well as the hypothalamus are involved in the pathological process [4, 5]. The leading VD syndromes include distal vegetative-sensory polyneuropathy and angiodystonia. Multiple studies have reported that dysfunctions in the nervous system are accompanied with changes in the immune system at any stage of VD occurrence and clinical course [6–9]. At present, workplaces in production typically involve combined exposure to both whole body and local vibration. Local vibration affects the body through the hands whereas whole body vibration affects the whole body in most cases and this has certain influence on the clinical course of the disease. At the same time, researchers predominantly give attention to pathogenesis, clinical course and diagnostics of VD caused by local vibration. Very few works provide some evidence

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[1] Mukhin N.A., Kosarev V.V., Babanov S.A., Fomin V.V. Professional'nye bolezni: uchebnik [Occupational diseases: textbook], the 2nd ed., edited and supplemented. Moscow, GEOTAR-Media, 2016, 512 p. (in Russian).

of additive effects produced by whole body and local vibration together [10]. However, there are no data in literature as regards comparative assessment of the neuro-immune response in patients with VD of different etiogenesis. Obviously, such data are necessary for developing the occupational risk assessment methodology and personified approaches to diagnostics and treatment of the disease.

In this study, our aim was to compare and analyze immunochemical markers of effect (cytokines, heat shock proteins, and neuronal antibodies (AB)) in blood serum of patients with vibration disease (VD) induced by exposure to different types of vibration in order to substantiate the most informative diagnostic risk indicators concerning the disease development and clinical course.

Materials and methods. We performed laboratory and immunological examinations of 137 men who had VD. The first group included 50 patients with VD caused by chronic exposure to local vibration (their age was 48.34 ± 0.88 years). Their occupations were drifts miners, working face miners, and welders-riveters. The second group was made of 53 patients with VD caused by combined exposure to whole-body and local vibration (their age was 52.21 ± 0.49 years). Their occupations were drilling unit operators, heavy truck drivers, and tracked vehicle drivers. The examined patients from these two groups had harmful working conditions at their workplaces; these conditions belonged to the hazard category 3.2 as per work intensity and the hazard category 3.3 as per work hardness. All the examined patients in the first and second group had an occupational disease diagnosed under occupational contacts with vibration. They did not have any comorbidity (obesity, diabetes mellitus, essential hypertension, etc.) or any chronic diseases in exacerbation. The third group included 34 healthy men who did not have any chronic diseases when they were examined (their age was 50.35 ± 1.69 years) and were not exposed to vibration at their workplaces. Biomaterials were sampled prior to the beginning of the COVID-19 pandemic. We estimated levels of pro-inflammatory cytokines (IL-1β, TNF-α, IL-2, IL-8, IL-10, IL-4, INFγ) in blood serum by ELISA tests using reagent kits manufactured by Vector-Best LLC (Novosibirsk). Heat shock proteins HSP27, HSP70 were quantified by ELISA tests using ELISA kits HSP70, HSP27 Assay Design (Enzo Life Sciences, USA). We identified antibodies (ABs) using standard test systems ELI-Neuro-Test produced by Immunkulus Moscow Scientific-Production Association and estimated levels of the most informative ABs of IgG class to the following proteins: S-100, NF-200 (neurofilament protein), GFAP (glial fibrillary acidic protein), MBP (myelin basic protein), and VGCC (voltage-gated Ca-channel).

The results were statistically analyzed using STATISTICA 6.0 applied software (StatSoft, USA). The distribution normality was checked using the Shapiro – Wilk test. The results are given as median (*Me*), lower (Q_{25}) and upper (Q_{75}) quartiles. The statistical significance was taken at $p < 0.05$.

The patients were examined in conformity with the ethical standards stipulated by the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects, as of 2000, and the Rules of Clinical Practice in the Russian Federation (the Order by the RF Public Healthcare Ministry issued on June 19, 2003).

Results and discussion. We compared levels of some pro- and anti-inflammatory cytokines in blood serum of the patients with VD depending on occupational exposures. This allowed us to reveal general regularities and differences between the compared groups. The patients from both the first and the second group had statistically significantly higher levels of pro-inflammatory IL-1β ($p < 0.00001$ and $p < 0.00001$ accordingly) and TNF-α ($p = 0.0006$ and $p = 0.001$ accordingly) than the healthy people in the third (control) group (Table).

In addition, we detected an increase in levels of another pro-inflammatory mediator IL-8 in the second group in comparison with the control one ($p = 0.003$) and the patients with VD caused by local vibration ($p = 0.0003$). The latter did not have any differences as per IL-8 levels from the control group. We analyzed

Table

Comparative assessment of some immunochemical indicators in patients with VD, *Me* (Q_{25}–Q_{75})

Indicator	Units of measurement	The first group (n = 50)	The second group (n = 53)	The third group (n = 34)
IL-1	pg/ml	12.57 (6.14–36.6) *p = 0.0000	10.52 (6.17–39.17) *p = 0.0000	3.4 (1.21–6.19)
IL-2	pg/ml	3.39 (2.37–5.39)	4.79 (2.44–7.71)	4.22 (2.67–6.33)
IL-4	pg/ml	0.01 (0.01–0.01)	0.01 (0.01–0.57)	0.01 (0.01–0.69)
IL-8	pg/ml	6.63 (1.47–8.52)	13.09 (6.65–29.77) *p = 0.003; •p^{1-2} = 0.0003	5.08 (1.41–13.40)
IL-10	pg/ml	0.58 (0.01–1.4)	0.01 (0.01–1.33)	0.01 (0.01–1.54)
TNFα	pg/ml	1.87 (1.50–2.86) *p = 0.0006	1.87 (0.84–3.3) *p = 0.001	0.73 (0.01–1.47)
INFγ	pg/ml	0.87 (0.01–2.24)	1.75 (0.72–21.7) *p = 0.0000; •p^{1-2} = 0.002	0.01 (0.01–1.16)
HSP27	pg/ml	2.93 (0.41–6.83)	7.53 (6.76–9.63) *p = 0.0003	1.7 (0.57–3.61)
HSP70	pg/ml	0.1 (0.04–0.36) *p = 0.019	0.39 (0.33–0.42)	0.37 (0.13–0.41)
S-100	arbitrary unit	0.585 (0.54 –0.686) *p = 0.00001	1.14 (0.942–1.19) *p = 0.000007; •p^{1-2} = 0.00004	0.285 (0.240–0.410)
GFAP	arbitrary unit	0.556 (0.483–0.618) *p = 0.00002	0.828 (0.525–0.903) *p = 0.000009; •p^{1-2} = 0.009	0.368 (0.310–0.430)
NF-200	arbitrary unit	0.565 (0.449–0.661) *p = 0.000001	0.813 (0.662–0.854) *p = 0.000001; •p^{1-2} = 0.0005	0.306 (0.250–0.320)
VGCC	arbitrary unit	0.582 (0.516–0.686) *p = 0.000005	0.833 (0.751–1.12) *p = 0.0000005; •p^{1-2} = 0.00001	0.215 (0.170–0.326)
MBP	arbitrary unit	0.453 (0.370–0.558) *p = 0.000004	0.679 (0.522–0.758) *p = 0.000005; •p^{1-2} = 0.0009	0.300 (0.270–0.360)

N o t e: * means difference from the third group; • means difference between the 1st and 2nd group.

levels of INFγ responsible for interaction between multiple cellular systems in three groups and revealed that the median of this indicator was statistically significantly ($p <$ 0.002) higher in the second group than in the first and third one ($p <$ 0.00001). At the same time, the indicator tended to grow in the patients from the first group.

Heat shock proteins (HSPs) or stress proteins are important indicators able to provide the most adequate description of a non-specific cell reaction to external stimuli. Several studies reported that circulating extracellular proteins could have some immune-regulatory properties and immune cells, in their turn, could be a source of HSP extracellular pools [9, 11, 12]. Given that, it seemed advisable to include comparative analysis of changes in certain markers into our study (HSP27 and HSP70) since they describe the functional state of cells (Table). Our investigation of extracellular HSP27 indicated that its levels were statistically significantly higher in the patients from the second group as opposed to the third group (p = 0.00003); local vibration only induced its ascending trend. This fact might be

evidence of direct damage to cells that facilitated release of the aforementioned proteins and their exit to the extracellular space [13]. HSP70 levels in blood serum identified in the patients from the second group did not have any difference from the control group; still, the patients from the first group with VD caused by local vibration had statistically significantly lower levels of this protein against the control ($p < 0.019$). This decrease in HSP70 levels in blood serum identified in the patients in the first group obviously indicates the protein accumulates inside cells [14]. Some authors believe that heat shock proteins can obtain some autoantigen properties and this may induce vessel injury [15] and endothelium membrane injury, the most susceptible targets under VD. This fact was evidenced by some experimental research [16]. Therefore, to get a comprehensive and complex insight into peculiarities of immune biochemical processes in patients with VD of different etiogenesis, we investigated neurospecific antibodies (ABs), which were the most informative under occupational diseases of the nervous system. The data provided in the Table indicate that the patients with VD, both from the first and second group, had statistically significantly ($p < 0.05$) higher levels of AB to the following proteins: S-100; MBP; NF-200; GFAP; voltage-gated Ca-channel (VGCC). The analysis also revealed that the median values of all the identified auto-ABs were statistically significantly higher in the patients with VD caused by combined exposure to whole body and local vibration than in the patients with VD induced by local vibration only. The most apparent differences were established for ABs to S-100 protein as their levels were almost twice as high in the patients with VD caused by combined exposure to whole-body and local vibration ($p < 0.00004$) than in the patients with VD caused by exposure to local vibration. Excessive ABs concentrations persisting for a long time are known to be able to promote immune-metabolic dysfunctions in involved nervous tissues with their intensity varying from mild to complete destruction of the said tissues[2] [17]. We should remember that S-100 multifunctional proteins could produce both protective and destructive effects on nervous tissues depending on their concentration. S-100 proteins regulate interaction between glia and neurons in general thereby providing functional homeostasis of brain cells [18]. Taking into account literature data and our study results, we can conclude that changes in the nervous system are more apparent in patients with VD caused by combined exposure to whole body and local vibration. This may indicate that risk factors of the disease occurring under such exposure are more significant.

The identified peculiarities of immunochemical indicators in patients with VD of different etiogenesis were consistent with and confirmed by the results of neurophysiological examinations performed on the same people. Registration of somatosensory evoked potentials revealed greater changes in neurons of the central afferent pathways in the somatosensory zone of the cortex and cervical section of the spinal cord in the patients with VD caused by combined exposure to whole body and local vibration [19]. Neuroenergy mapping identified some distinguishing features in the same patients, namely, an increase in constant potential levels in patients with VD caused by combined exposure was determined in the central region and in the right temporal region under exposure to local vibration [20]. This allows identifying the most informative biomarkers for risk factors causing VD of different etiogenesis.

Conclusion. Therefore, we established unidirectional statistically significantly more apparent changes in patients who had VD caused by combined exposure to both whole body vibration and local vibration against those who had VD caused by exposure to local

[2] Poletaev A.B. Molekulyarnaya dispanserizatsiya (novye podkhody k rannemu proyavleniyu patologicheskikh izmenenii v organizme cheloveka: metodicheskie rekomendatsii dlya vrachei [Molecular clinical examination (new approaches to early signs of pathological changes in the human body: methodical guidelines for doctors]. Moscow, Immunkulus, 2014, 80 p. (in Russian).

vibration only. These changes included hyper-activated pro-inflammatory reactions of the immune response (IL-1β, TNF-α, INFγ), growing concentrations of antibodies to proteins of nervous tissue: S-100, MBP, NF-200, GFAP, and voltage-gated Ca-channel; they were quite different from effects caused by exposure to local vibration. The patients with VD under combined exposure to both types of vibration had greater production of pro-inflammatory IL-8 and HSP27 whereas people with VD caused by exposure to local vibration had a decrease in HSP70 levels. The study results confirmed greater risks that neuro-immune inflammation would develop in patients under combined exposure to whole body and local vibration and made it possible to identify the most sensitive biomarkers (IL-1β, TNF-α, INFγ, ABs to S-100) eligible for VD diagnostics and prediction of its clinical course.

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