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Research Article

Dynamics of endothelial function indexes in patients with post-Covid syndrome using a combination drug of ethylmethylhydroxyperidine succinate/vitamin B6

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Abstract

Introduction: After the COVID-19 pandemic, public healthcare has faced a new problem, the persistence of symptoms, the most significant of which is undue fatigue. The definition of coronavirus infection as an endothelial disease suggests a possible relationship between asthenic syndrome and endothelial dysfunction. **Aim**: to evaluate endothelial function in patients after COVID-19 before and after treatment with combination drug of ethylmethylhydroxyperidine succinate (EMHPS)/vitamin B6.

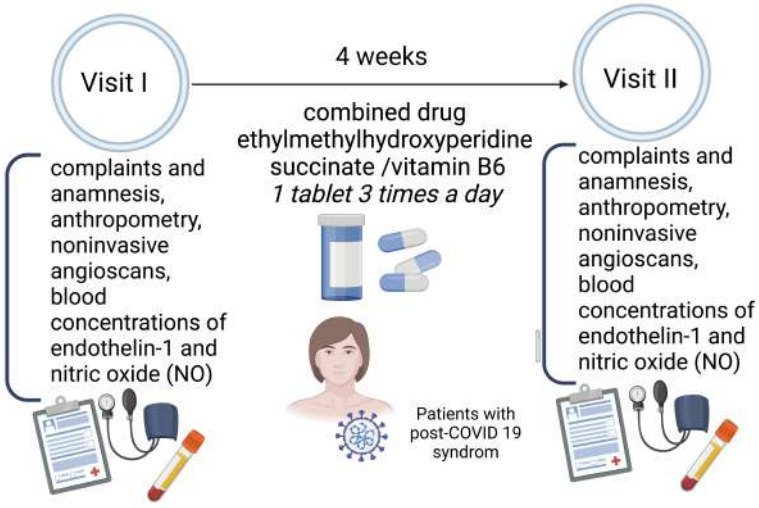
Materials and Methods: A prospective study included 33 participants, 82% women and 18% men aged 22 to 68 years after COVID-19. There were two site visits made in the course of the study and a 4-week treatment course between the visits.

Results: After the treatment, the index of endothelium-dependent vasodilation increased in women and decreased in men. The differences were statistically insignificant. The number of subjects with a normal level of endothelium-dependent vasodilation increased from 7 to 11 after the treatment. The index of vascular wall stiffness corresponded to the mean age of the examined participants and did not statistically change, although women tended to decrease stiffness and men to increase it. The initial level of nitric oxide was lower in women and statistically significantly increased after treatment, while it remained unchanged in men.

Discussion: The study confirms a prolonged course of the post-covid. We assume that the virus destroys endothelial cells. Endothelial dysfunction is known to be associated with an increased cardiovascular risk.

Conclusion: The indicators of endothelial function changed in patients after treatment with the drug. It is necessary to perform endothelial function studies in more patients after COVID-19.

Graphical abstract:



Keywords

antioxidant/vitamin combination, asthenia, COVID-19, endothelial dysfunction, endothelin, fatigue, nitric oxide (NO), noninvasive angioscanning

Introduction

For patients who have had a coronavirus infection, the resolution of the underlying symptoms can mark the beginning of a long road to recovery due to the possible occurrence of post-Covid syndrome. Currently, there are insufficient outpatient cases to identify its main manifestations and the frequency of its development. However, such patients have undoubtedly added to the total number of people with symptoms of a worsening course of somatic diseases. This dictates the need to search for drugs to correct post-Covid syndrome. As data on the development of the coronavirus infection accumulated, it became clear that some patients were experiencing persistent symptoms, particularly fatigue (Rudroff et al. 2020).

It was at the beginning of the COVID-19 pandemic that the concept of a new coronavirus infection as an endothelial disease was formulated, explaining the pathophysiological mechanisms of its manifestations. The necessity of using endothelial biomarkers and functional noninvasive tests for risk stratification of COVID-19 patients was outlined (Evans et al. 2020). Recent recommendations suggest subsequent evaluation of endothelial function and arterial stiffness markers in patients suffering from COVID-19 for early detection of long-term adverse effects (Lambadiari et al. 2021).

SARS family coronaviruses penetrate the cell through angiotensin-converting enzyme 2 (ACE 2) expressed on vascular endothelial cells of different diameters, from large main trunks to the microcirculatory bed (Hamming et al. 2004). This explains SARS-CoV-2 tropism to almost all body tissues and the diversity of clinical manifestations of COVID-19. The course of the disease is complicated by the development of widespread endotheliitis in the heart, lungs, kidneys, liver and gastrointestinal tract (Varga et al. 2020). Researchers from Zurich have shown that endothelial dysfunction in various organs occurs due to a direct viral lesion from SARS-CoV-2, as confirmed by the presence of viral cells, and can be a generalized systemic inflammatory response. In addition, the induction of apoptosis and pyroptosis can be largely responsible for endothelial cell damage in patients with COVID-19 (Varga et al. 2020). The data accumulated during the pandemic show that patients with pre-existing endothelial dysfunction (patients with arterial hypertension (AH), coronary heart disease (CHD), and type 2 diabetes mellitus) are at high risk of severe disease and death from COVID-19, which also confirms the association of new coronavirus infection with endothelial damage.

Endothelial dysfunction is characterized by changes in endothelial functions: disturbed balance between vasodilatory and vasoconstrictor factors; a proinflammatory state, including increased expression of adhesion molecules; forming of a procoagulant and antifibrinolytic phenotype; increased vascular permeability (Xu and Zou 2009). The endothelium produces several vasoconstrictors and vasodilators, such as endothelin-1, angiotensin-2, nitric oxide and prostacyclin, which regulate vasomotor tone as well as inflammatory cell activity and thrombosis. Endothelial damage is associated with neutrophil activation and expression. In addition to inflammation, coagulation and fibrinolysis are host responses to infection and damage. Endothelial cells coordinate this response, moving from their normal antithrombotic, anti-inflammatory, and profibrinolytic phenotype to an activated state of endothelial dysfunction. The endothelium actively regulates hemostasis by producing various proteins, including prothrombotic substances (von Willebrand factor), clotting limiting molecules

(thrombomodulin) and fibrinolytic factors (plasminogen activators) (Vassiliou et al. 2020).

Indirect endothelial damage through the release of inflammatory mediators can contribute to endothelial dysfunction through impaired nitric oxide metabolism resulting in impaired endothelium-dependent vasodilation and increased arterial stiffness. Numerous changes in endothelial dysfunction parameters, subclinical inflammation, as well as marked elevation of c-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and IL-6 levels are detected in patients who have recovered from the new coronavirus infection. Corresponding inflammatory changes in patients with post-Covid syndrome are similar to those observed in patients with atherosclerosis. The values of endothelium-dependent vasodilation in patients suffering from COVID-19 are also similar to those in patients with atherosclerosis (Jud et al. 2021).

COVID-19 endotheliitis may explain systemic microcirculatory dysfunction in various vascular beds and its clinical consequences in patients with COVID-19. In severe COVID-19 cases, extensive microthrombosis provoked by endothelial dysfunction is accompanied by a significant increase in D-dimer level and a decrease in total platelet count (Zhou et al. 2020). This provides the basis for the search for therapeutic measures aimed at endothelium stabilization in COVID-19 and post-COVID syndrome.

Antioxidants, such as ethylmethylhydroxyperidine succinate (EMHPS), are crucial for the normalization of endothelial function. The effectiveness of EMHPS is most pronounced when there is enough pyridoxine and magnesium in the body. With their insufficiency, the neuroprotective and antioxidant effect of EMHPS is reduced. In this regard, their combined use seems to be the most promising therapy for the post-viral syndrome. In addition, pyridoxine and magnesium neutralize homocysteine and reduce its level in the blood. Elevated homocysteine levels contribute to endothelial damage and an increased risk of atherosclerosis and adverse vascular events (Gromova et al. 2016). The research aims to evaluate endothelial function in patients who have recovered from COVID-19 before and after treatment with the combination drug of ethylmethylhydroxyperidine succinate/vitamin B6.

Materials and Methods

Group description

The interventional prospective study was conducted in the Clinical and Diagnostic Center of Immanuel Kant Federal State Educational Institution of Higher Professional Education in April-May 2022. All the participants signed their written informed consent. The study, approved by the independent ethics committee of the Clinical Trial Center of Immanuel Kant Baltic Federal University, followed the recommendations outlined in the Declaration of Helsinki (Minutes № 30 of 05/Apr/2022). Patient group characteristics: the inclusion criteria were age over 18 years, COVID-19 suffered within the last 1-6 months, and complaints of undue and excessive fatigue. There were 33 participants in the study, including 27 women (82%) and 6 men (18%) aged 22 to 68 years, with a mean age of 47 (37; 57) years. The duration of coronavirus infection ranged from 52 to 173 days, with an average of 95 (70; 121) days. All patients included in the study had recovered from COVID-19 as outpatients.

Study design

The study design included two face-to-face visits, before and after taking the ethylmethylhydroxyperidine succinate/vitamin B6 combination drug. All the subjects underwent collection of complaints and medical history, anthropometry, noninvasive angioscans and assessment of endothelin-1 and nitric oxide (NO) concentration in blood.

Anthropometric indices included height and body weight measurement with BMI calculation and waist circumference measurement. Non-invasive angioscans were performed by contour analysis of peripheral pulse waves recorded with photoplethysmography using an AngioScan-01 device (AngioScan Electronics LLC). We initially assessed the stiffness index (SI) reflecting pulse wave velocity (PWV) of major arteries, the ratio of the subject's height to the time between systolic and diastolic components of pulse wave (Δt) measured in m/s. Then we performed a reactive hyperemia test based on the activation of nitric oxide synthesis by endothelial cells in response to shear stress with an increased blood flow rate in the brachial artery. For this purpose, we placed a manometer cuff on the upper arm of a subject with 200 mm Hg pressure for 5 minutes after which air was released from the cuff and the pulse wave signal level was recorded again. At the end of the test, an increase in pulse wave amplitude (endothelium-dependent vasodilation) was automatically calculated. A two-fold or greater increase in pulse wave amplitude indicates preserved endothelial function.

Identification of serological markers of endothelial dysfunction

Endothelin-1 (ET-1) is a powerful vasoconstrictor peptide produced by endothelial cells and degraded mainly in the pulmonary vasculature (Jin et al. 2020). Serum endothelin-1 (ET-1) levels were determined by enzyme immunoassay using the ELISA Kit for Endothelin 1 test system (Cloud-Clone Corp., Houston, TX, USA), with a minimum sensitivity of 2.71 pg/mL. The method relies on a competitive inhibition reaction between biotin-labeled ET-1 and unlabeled ET-1 contained in a patient's serum/plasma with endothelin-1-specific antibodies. We measured optical density using a Bio-Rad 680 microplate photometer (USA) at a wavelength of 450 nm.

According to the literature, nitric oxide (NO) metabolites in blood serum are determined mainly by nitrates since nitrites are easily oxidized and practically absent in blood (Tsikas 2007). The concentration of stable NO degradation products ($NO2^-$/ $NO3^-$) was determined in serum by a spectrophotometric method using a commercial microplate nitric oxide assay kit (Nitric Oxide Assay Kit A013-2, Cloud-Clone Corp., IS 101, USA). The kit is a one-step method for the quantitative measurement of NO in blood serum (plasma), tissues, and other biological fluids in vitro. Optical density (λ=550 nm) was measured on a CLARIOstar Plus multimodal plate reader, BMG LABTECH, Germany).

This kit determined total NO: nitrites and nitrates (When interacting with biological substrates, nitric oxide (II) is oxidized to nitrates and nitrites, the total content of which in blood serum was indirectly determined by its in vitro content).

Statistical analysis

Statistical processing of the data was performed using Microsoft Excel spreadsheet editor. Continuous variables were presented as median and interquartile intervals (Me, 25%; 75%), reliability of differences was assessed using Mann-Whitney U-test. The critical level of significance for the null statistical hypothesis (p) was 0.05. At $p<0.05$, the differences were considered statistically significant. Correlation analysis was performed according to Spearman. The strength of the correlation was assessed: at $r<0.25$ it was weak, at $0.25<r<0.75$ it was moderate.

Results

Mean age, anthropometric indexes, and the number of examined persons diagnosed with AH and CHD in male and female subgroups were not statistically different (Table 1).

Table 1. Clinical characteristics of patients

Indicators	Women	Men
Number, n (%)	27 (82)	6 (18)
Age, years	47 (40; 58)	46 (35; 54)
BMI, kg/m2	25.0 (20.1; 28.3)	26.1 (23.0; 25.3)
Waist circumference, cm	83.7 (71.5; 94.5)	90.5 (81.3; 99.5)
AH, n (%)	6 (22)	1 (17)
CHD, n (%)	1(4)	0 (0)

Note: There were no statistically significant differences found; BMI – body mass index; AH – arterial hypertension; CHD – coronary heart disease.

The assessment of endothelial function revealed that the mean value of a pulse wave amplitude increase in response to shear stress (endothelium-dependent vasodilation) in the examined patients before receiving the combination drug of ethylmethylhydroxyperidine succinate/vitamin B6 was on average lower than the norm in women, and corresponded to the norm in men (Table 2). At the same time, there was an increase in EDVD (endothelium-dependent vasodilation) observed in women and its decrease in men. The lack of statistical significance can probably be attributed to the small number of subjects. In general, the number of subjects with normal EDVD levels (more than 2.0) increased from 7 to 11 after taking the combination drug of ethylmethylhydroxyperidine succinate/vitamin B6.

According to the results of noninvasive angioscanning, the index of vascular wall stiffness (SI) corresponded to the mean age of the examined; after the combination drug ethylmethylhydroxyperidine succinate/vitamin B6 administration it did not change statistically, though, in women, there was a trend to a decrease in stiffness and in men – to its increase (Table 2).

The correlation analysis revealed statistically significant associations of noninvasive angioscan indices with the age of patients in the studied group: a moderate negative association of EDVD and age ($r=-0.33$; $p<0.05$) and a moderate positive association of SI stiffness index with age ($r=0.5$; $p<0.05$).

Table 2. Results of noninvasive angioscans before and after administration of the combination drug of ethylmethylhydroxyperidine succinate/vitamin B6

Indicator	Women		Men	
	Before treatment	After treatment	Before treatment	After treatment
EDVD	1.6 (1.2; 1.9)	1.8 (1.3; 2.0)	2.1 (1.4; 2.9)	1.7 (1.4; 1.9)
SI, m/s	7.7 (7.3; 7.9)	7.5 (7.0; 8.0)	7.7 (6.9; 8.3)	8.0 (7.7; 8.2)

Note: There were no statistically significant differences found; EDVD – endothelium-dependent vasodilation; SI – index of vascular wall stiffness.

The study of serological markers of endothelial dysfunction also revealed gender differences. The initial level of endothelin-1 was higher in women than in men, while after treatment, it slightly decreased in women and increased in men. Nitric oxide level before treatment in women was lower than in men and statistically significantly increased after the administration of the combination drug of ethylmethylhydroxyperidine succinate/vitamin B6, whereas in men it did not change (Table 3). To assess the relationship between the vasodilator and vasoconstrictor links of endothelial function regulation in the examined patients, we calculated a conditional coefficient – the ratio of NO/ET1 concentration. The decrease in this index can reflect the degree of endothelial dysfunction in terms of vasodilation/vasoconstriction balance. This index increased in women after treatment and decreased in men (Table 3).

Table 3. Dynamics of serological markers of endothelial dysfunction before and after administration of the combination drug of ethylmethylhydroxyperidine succinate/vitamin B6

Indicator	Women		Men	
	Before treatment	After treatment	Before treatment	After treatment
ET-1, pg/mL	193 (155; 215)	181 (152; 215)	167 (154; 171)	208 (150; 256)
NO, μmol/L	6.3 (5.3; 5.9)	7.5 (6.2; 8.2)*	6.8 (6.1; 7.7)	6.8 (5.7; 7.8)
NO/ ET-1, u.s.	0.034 (0.027; 0.035)	0.044 (0.026; 0.056)	0.041 (0.038; 0.043)	0.029 (0.024; 0.034)

Note: * – differences are statistically significant ($p<0.05$); NO – nitric

Discussion

The study confirms the prolonged course of the post-Covid. It included patients with complaints of undue and excessive fatigue that persisted an average of 3.1 months after coronavirus infection indicating prolonged asthenization after the disease.

More recently, there has been a hypothesis formulated that prolonged fatigue and asthenia after coronavirus infection are initiated by endothelial cell ageing and impaired barrier function caused by angiotensin II affected by SARS-CoV-2 (Sfera et al. 2021). The outcome of endothelial ageing is the disruption of the blood-brain barrier, which contributes to the entry of the virus into the CNS. It is also assumed that the virus destroys not only endothelial cells but also intestinal epithelial cells, damaging the intestinal barrier and facilitating the penetration of microbes and/or their lipopolysaccharides into the host tissues, namely skeletal muscles and brain. The disruption of the intestinal barrier and the subsequent translocation of the virus cause aberrant immune responses in the patient, ranging from cytokine storm to excessive tolerance, which probably causes the development of post-Covid asthenia symptoms (Sfera et al. 2021).

The severity of endothelial dysfunction is known to be associated with an increased cardiovascular risk. Monitoring endothelial function contributes to quantitative risk assessment and allows for early interventions to reduce the incidence of adverse events in patients. However, few studies have focused on changes in endothelial biomarkers in patients recovering from the new coronavirus infection. A prospective longitudinal multicenter cohort study showed that levels of endothelial dysfunction markers, such as vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and macrophage inflammatory protein-1β (MIP-1β), increase in the post-Covid period, potentially contributing to atherosclerosis and cardiovascular complications (Tong et al. 2022). In another 3-month study, persistent abnormal levels of endothelial biomarkers, proinflammatory cytokines, and chemokines (VCAM-1, ICAM-1, TNF-α, MIP-1α, and MIP-1β) persisted in patients who had recovered from COVID-19 and were particularly high in those with severe COVID-19 (Lambadiari et al. 2021). It found that during four months after SARS-CoV-2 infection, the levels of thrombomodulin, von Willebrand factor and impaired pulse wave velocity and endothelium-dependent vasodilation elevated in patients' blood, which was associated with impaired endothelial glycocalyx (Lambadiari et al. 2021). These patients had such residual symptoms as shortness of breath, cough, chest pain, and fatigue. The latter was associated with increased oxidative stress, arterial stiffness and LV myocardial longitudinal deformation: patients experiencing fatigue for months after COVID-19 infection had more pronounced changes in global LV longitudinal deformation, pulse wave velocity and malondialdehyde compared to patients without this symptom. This shows the importance of strict outpatient monitoring of "well-recovered patients" who may have subclinical cardiovascular complications (Lambadiari et al. 2021).

Conclusion

There is a need for larger prospective clinical studies with defined cardiovascular endpoints to determine the main effects of SARS-CoV-2 on vascular and endothelial function and the pathophysiological mechanisms underlying them (Lambadiari et al. 2021). A comprehensive evaluation of markers of endothelial dysfunction in coronavirus-infected patients should be performed dynamically and at certain intervals.

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Conflict of Interest

The authors declare that the study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interests.

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