Functional state of the endothelium in men after mine-explosive injury during the war in Ukraine

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Abstract. Mine-explosive trauma (MET) is the most common trauma type and one of the main disability causes of the able-bodied contingent. Among the mechanisms of the cardiovascular and cerebrovascular disorders development after MET, endothelial dysfunction (ED) plays an important role.

The study aims - to assess the functional state of the microvessels and large arteries endothelium in men after a mine-explosive injury.

Materials and methods: the functional state of the skin microvessels endothelium was determined using a two-channel laser Doppler flowmeter BLF-21D "Transonic Systems Inc" (USA) during the test with the creation of reactive hyperemia. The function of the large arteries endothelium was determined using the D.S. Celermajer test, with post-occlusion reactive hyperemia according to the generally accepted method on the Nemio XG device, SSA-580A (Toshiba) with a high-frequency linear sensor with a central frequency of 7 MHz.

We examined men with mild MET (average age 37.7 ± 1.5 years). The control group was represented by 43 practically healthy men without MET (average age 41.8 ± 2.1 years).

Results: dysfunction of the microvessels endothelium was found in most persons with MET (69.1%), compared to men without MET (41.9%). Dysfunction of the large vessels endothelium was found in 40.4% of examined persons with MET. Persons with MET have a combination of large and small vessel endothelial dysfunction in 23.4% of cases, at the same time persons without MET have a higher proportion of isolated microvessel endothelial dysfunction (34.9%).

Conclusions: men with MET often have arterial endothelial dysfunction with microvascular endothelial dysfunction together, whereas men without MET have predominantly isolated microvascular endothelial dysfunction.

Keywords: mine-explosive injury; functional state of the endothelium; microcirculation.

Mine and explosive injury is a characteristic of military conflict trauma, which hurts long-term health [1]. It is known that the brain is critically dependent on a stable blood supply and is acutely responsive to metabolic needs. To balance the brain's blood supply, there is a vascular network of arteries, arterioles, capillaries, venules, and veins that is approximately 400 miles long. [2]. These vessels make up the neurovascular unit (NVU), and act in concert, regulating cerebral blood flow, vascular permeability and supply of microelements to the brain tissue [3]. The vessels that make up the NVU are blood vessels with an endothelial lining, smooth muscle cells (at the level of arteries, arterioles, and venules) and pericytes (at the capillary level, which help regulate vascular tone), neurons, and perivascular astrocytes. Endothelial cells form a monolayer consisting of intercellular tight and
connected junctions to form the blood-brain barrier (BBB). The capillary endothelium provides 85% of the BBB. This BBB forms a finely tuned barrier between the vasculature and the brain parenchyma [3].

The NVU-impaired function leads to inappropriate BBB changes in response to the altered metabolic needs of the damaged brain. BBB dysfunction disrupts the extracellular environment through protein and electrolyte leakage and can initiate microglial processes, activations, and damage [4]. There is evidence to suggest that these impairments last much longer than previously thought and may contribute to longer-lasting neuropathology after primary brain injury (BI). Given that vascular dysfunction is common and persistent after mild and severe BI and that neurodegeneration is a late consequence of some BI, it is likely that these two mechanisms may be related.

In addition, numerous studies have shown that patients after BI and concussion develop central dysregulation of sympathetic vasomotor tone, and decrease baroreflexes and heart rate variability [5, 6].

With MET, post-traumatic stress disorder (PTSD) develops, and many studies have shown its connection with cardiovascular diseases [7]. It has been suggested that this relationship can be explained by changes in the hypothalamic-pituitary-adrenal system, activation of the sympathetic nervous system, inflammation and behavioural changes [8].

Endothelial dysfunction is common and one of the main mechanisms of the development of cardiovascular and cerebrovascular occasions. Endothelial dysfunction (ED), including coronary ED and peripheral ED, is an important link in the initiation and progression of atherosclerosis [9, 10]. Atherogenesis is considered key in the development of atherosclerosis and clinically expressed cardiovascular diseases, including acute coronary syndrome [11, 12]. Although studies of endothelial function in the past few decades have focused mainly on coronary circulation, ED in peripheral arteries has also attracted considerable attention from researchers. It has been shown that peripheral artery ED is closely related to coronary artery atherosclerosis and is an independent prognostic predictor of coronary heart disease, heart failure and cerebrovascular diseases [10, 13].

To date, various non-invasive methods of assessing the function of the peripheral arteries endothelium are widely used in clinical research [10]. The results of blood flow ultrasound examination in the brachial artery with the determination of flow-dependent vasodilation correlate with coronary ED and cardiovascular diseases. Peak reactive hyperemia measured on the forearm provides information on the coronary atherosclerosis degree and severity and correlates with the coronary endothelium function [14]. Several studies prove the feasibility of assessing the endothelium functional state as a marker of cardiovascular occasion in patients at various stages of the continuum of cardiovascular diseases [15, 16].

Aim of the study

The study aimed to investigate the functional state of the microcirculatory link vessel endothelium and large arteries endothelium in men after MET.

Materials and methods

The study was conducted by the requirements of Ukrainian legislation and the principles of the Helsinki Declaration on Human Rights.

The persons included in the study signed consent to participate in the study before the examination (the examination program, information for the patient and the informed consent form were reviewed and approved at the meeting of the ethics committee of the Clinical Department of the D. F. Chebotarev Institute of Gerontology, National Academy of Medical Sciences of Ukraine 12.04.2020 (Protocol No. 4). The examined persons did not have cardiovascular diseases.

47 men with mild MET were examined. The average age of the examined was 37.7 ± 1.5 years.

43 practically healthy men represented the control group without MVT. The average age of the examined was 41.8 ± 2.1 years.

To rule out cardiac pathology, rhythm and conduction disorders, a standard electrocardiogram was recorded on a Yukard-200 device (Ukraine). None of the examinees had heart failure, all had sinus rhythm with a heart rate of < 80 beats per minute.
The functional state of the skin microvessels endothelium was determined using a two-channel laser Doppler flowmeter BLF-21D "Transonic Systems Inc" (USA) at rest, as well as during a functional test with the creation of reactive hyperemia [17].

The function of the large arteries endothelium was determined in a test with post-occlusion reactive hyperemia according to the generally accepted method [9] on an ultrasound device Nemio XG, SSA-580A (Toshiba) with a high-frequency linear sensor with a central frequency of 7 MHz. The study was conducted in the morning in a room with a comfortable temperature (≈22°), after 30 minutes of rest. During the test, the initial diameter of the brachial artery was measured 2-3 cm proximal to the elbow bend. Then the brachial artery was clamped with a tonometer cuff, the pressure of which exceeded the systolic pressure of the subject by 40-50 mm Hg. Art. Compression of the brachial artery lasted for 4 minutes, after which it was quickly decompressed and the diameter of the brachial artery was determined after 30 seconds. The increase in diameter was calculated according to the formula: \[\Delta D = \frac{\text{max diameter of the brachial artery after decompression} - \text{initial diameter}}{\text{diameter of the brachial artery before compression}} \times 100\%.

The nature of the distribution of the obtained variation series was checked using the Shapiro-Wilk W test, which confirmed the normal (Gaussian) nature of the data distribution.

Therefore, when performing statistical processing of the received data, the following methods are used: calculation of the arithmetic mean and its mean error (M ± m); carrying out an assessment of the probability of the obtained results difference in the compared groups using the Student's criterion. To compare the frequency of quality indicators occurrence, Pearson’s \(\chi^2\) test was used for connectivity tables with Yates correction.

**Results**

When determining the endothelium functional state, using the methodology of laser Doppler flowmetry and the D. S. Celermajer test, we found the presence of ED in 18 out of 43 men without MET (41.9%) and in 36 out of 47 examined (76.6%) with MET. This indicates a significant increase in the detection frequency of ED in persons with MET \( (\chi^2 = 0.00078, p = 0.0016). \)

Analysis of the ED structure showed that in people without MET, endothelial dysfunction is present due to the microcirculatory vascular channel ED. At the same time, in addition to the vessel endothelium dysfunction of the microcirculatory vascular link, people with MET also have either isolated large vessel dysfunction or a combination of large vessels ED and microcirculatory vessel channels ED (Tab.1).

<table>
<thead>
<tr>
<th>Endothelial function state</th>
<th>Persons without MET (n = 43)</th>
<th>Persons with MET (n = 47)</th>
<th>(\chi^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No violations, n%</td>
<td>25 58.14</td>
<td>11* 23.40</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Isolated microcirculatory vessel channels ED, n%</td>
<td>15 34.88</td>
<td>17 36.17</td>
<td>0.890</td>
<td>0.920</td>
</tr>
<tr>
<td>Isolated large vessels ED, n%</td>
<td>1 2.33</td>
<td>8* 17.02</td>
<td>0.020</td>
<td>0.048</td>
</tr>
<tr>
<td>Combination of large vessels ED and microcirculatory vessel channels ED, n%</td>
<td>2 4.65</td>
<td>11* 23.41</td>
<td>0.011</td>
<td>0.025</td>
</tr>
</tbody>
</table>

*Note:* statistically significant difference between groups, * - \(p<0.05\)
In the analysis of the data obtained during Laser Doppler flowmetry, in persons with MET, compared to the group of persons without MET, a slight decrease in the maximum volumetric skin blood flow velocity (SBFV) at the peak of reactive hyperemia (after compressing the vessels of the forearm for 3 min) and the maximum increase in SBFV was recorded (Tab. 2).

As can be seen from Tab. 2, the groups of examined persons practically do not differ in terms of the endothelial function state.

Table 2

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Groups</th>
<th>Persons without MET (n = 43)</th>
<th>Persons with MET (n = 47)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td>41.8 ± 2.1</td>
<td>37.7 ± 1.5</td>
<td>0.11</td>
</tr>
<tr>
<td>SBFV at rest, ml/min-100 g of tissue</td>
<td></td>
<td>1.15 ± 0.05</td>
<td>1.17 ± 0.04</td>
<td>0.77</td>
</tr>
<tr>
<td>SBFV maximum at the height of reactive hyperemia (3 min.), ml/min-100 g of tissue</td>
<td></td>
<td>6.72 ± 0.36</td>
<td>6.39 ± 0.28</td>
<td>0.47</td>
</tr>
<tr>
<td>SBFV increases at the height of reactive hyperemia, ml/min-100 g of tissue</td>
<td></td>
<td>5.45 ± 0.33</td>
<td>5.28 ± 0.27</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Note: statistically significant difference between groups, * - p< 0.05

Analysis of the functional microvessels endothelium state depending on the ED indicator (more or less than 100%) revealed that ED develops in persons with MET at a much younger age compared to persons without MET (p<0.05), which is reflected in Tab. 3.

Table 3

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Groups</th>
<th>Persons without MET (n = 43)</th>
<th>Persons with MET (n = 47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td>48.8 ± 3.2</td>
<td>37.3 ± 2.4</td>
</tr>
<tr>
<td>EF, %</td>
<td></td>
<td>67.2 ± 4.7</td>
<td>113.8 ± 3.0</td>
</tr>
<tr>
<td>SBFV at rest, ml/min-100 g of tissue</td>
<td></td>
<td>1.06 ± 0.07</td>
<td>1.21 ± 0.06</td>
</tr>
<tr>
<td>SBFV maximum at the height of reactive hyperemia (3 min.), ml/min-100 g of tissue</td>
<td></td>
<td>6.16 ± 0.71</td>
<td>7.09 ± 0.34</td>
</tr>
<tr>
<td>SBFV increases at the height of reactive hyperemia, ml/min-100 g of tissue</td>
<td></td>
<td>5.11 ± 0.66</td>
<td>5.70 ± 0.31</td>
</tr>
</tbody>
</table>

Notes:
- the probability of a difference compared to the persons without MET and impaired EF (EF<100%): * - p< 0.05;
- the probability of a difference compared to the persons without MET and preserved EF (EF>100%): # - p< 0.05.
Thus, we found a significantly higher frequency of DE development in persons with MET ($X^2 = 0.00078, p<0.05$). Their age is $8.8 \pm 1.3$ years younger than the age of persons without MET ($p<0.05$).

The assessment of the large arteries endothelium function (Tab. 4) revealed that the frequency of endothelial dysfunction (increase in diameter <10%) in persons with MET is four times higher (41.3%) than in persons without MET (11.1%) ($X^2 = 0.02, p=0.04$), and the age of those examined with MET and ED is $6.6\pm2.7$ years younger than the age of persons without MET.

### Table 4

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Persons without MET</th>
<th>Persons with MET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 18)</td>
<td>(n = 47)</td>
</tr>
<tr>
<td>EF&lt;10%</td>
<td>EF&gt;10%</td>
<td>EF&lt;10%</td>
</tr>
<tr>
<td>n = 3</td>
<td>n = 15</td>
<td>n = 19</td>
</tr>
<tr>
<td>Age, years</td>
<td>42.0 ± 5.5</td>
<td>41.8 ± 2.2</td>
</tr>
<tr>
<td>Before compression, mm</td>
<td>4.0 ± 0.3</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>After compression, mm</td>
<td>4.0 ± 0.3</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>EF of large vessels, %</td>
<td>0.0</td>
<td>32.1 ± 6.7*</td>
</tr>
</tbody>
</table>

Note: statistically significant difference between groups: * - $p<0.05$, ** - $p<0.01$

19 out of 47 examined men with MET (40.4%) had ED of large vessels. Most of them (11 out of 19, i.e. 57.9%) also had microcirculatory vessel channels DE. In persons without MET, the combination of microcirculatory vessel channels ED and large arteries ED was present only in 2 persons out of 43 examined (4.7%), while in men with MET, this combination was present in 23.4% of the examined ($X^2 = 0.01, p=0.025$).

The presence of ED in persons with MET may be one of the factors in the future development of cardiovascular and cardio-cerebral complications.

Conclusions.

1. Most men with MET have ED.
2. Large arteries ED was detected in a significant part of men with a previous MET (40.4%).
3. In men with MET, large-artery ED is often combined with microvascular ED, whereas men without MET have predominantly isolated microvascular ED.

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Conflicts of Interest: The authors declare no conflict of interest.
References


