The Role of the Oxidative Stress Markers in Endotelial Dysfunction at Cardiovascular Disease Patients

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Although oxidative stress itself is directly associated with the occurrence of atherosclerosis, and together with endothelial dysfunction is a marker of atherosclerotic risk, none of these two markers were directly correlated with the presence of atherosclerosis. This study investigated the correlation between oxidative stress and endothelial dysfunction in people with cardiovascular disease. Furthermore, another objective of this paper is the direct study of the effect of a compound with antioxidant properties, selenium, on a model of endothelial dysfunction induced on human vascular fragments.

Keywords: oxidative stress, atherosclerosis, endothelial dysfunction, stress markers

Vascular endothelium is known as an organ indispensable for regulating vascular tonus and, in general, vascular homeostasis. Over the past 30 years, numerous research has been carried out that has highlighted the role of maintaining its integrity to halt the development of many pathologies. More specifically, an alteration of its integrity, called endothelial dysfunction, causes numerous adverse effects on vascular homeostasis, endothelial dysfunction through the major and initial stage of the development of atherosclerosis, a disease involved in cardiovascular / cerebrovascular events [1-3].

Recent research has suggested that the involvement of oxidative stress, namely the increase in the production of reactive oxygen species, whether or not accompanied by a decrease in antioxidant defense, is a key element in the production of endothelial dysfunction. This association is possible by interfering with these reactive oxygen species with nitric oxide, an event which causes them to decrease their bioavailability and implicitly causes endothelial dysfunction

Oxidative stress markers can be classified into: modified molecules as a result of interactions with ROS and molecules of the antioxidant system that change in response to increased oxidative/redox stress. The first category includes DNA, lipids, proteins, and modified carbohydrates.

Some of them have direct effects on the function of the molecule, while others only reflect the degree of oxidative stress in the body or the local environment [4-6].

The implication of ROS on the oxidation of various cellular components in CVD was recognized by the oxidative theory of atherogenesis. Due to increased content in double bonds, lipids are targets that are susceptible to oxidation and thus the best studied markers are those of lipid peroxidation: isoprostans (IsoPs) and malondialdehyde (MDA) [7-9].

Experimental part

a.Measurement of malondialdehyde (MDA)

MDA was determined spectrophotometrically from fresh serum obtained by centrifuging venous blood harvested from healthy individuals and CVD individuals. To 150 μL of serum was added 125 μL of 10% tricoloacetic acid, 125 μL of 5 mM ethylenediaminetetraacetic acid, 125 μL of 8% sodium dodecyl sulphate and 10 μL of 0.5 g / mL of butylhydroxytoluene. The resulting mixture was homogenized for 30 seconds, then incubated for 10 min, 500 μL of 0.6% thiobarbituric acid was added to the solution and then heated to 95 ° C for 30 min. The solution was then cooled and centrifuged (10000 g, 10 min) [10]. The sample absorbance was read on the spectrophotometer (532 nm) and compared to that of a standard (1,1,3,3-tetraethoxypropane 10 nmol / ml). Normal values: 2.35 - 2.67 μmol / mL.

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b.Measurement of serum glutathione

Serum glutathione was measured fluorometrically from fresh serum obtained by centrifugation of venous blood collected from healthy individuals and CVD individuals. The serum was then treated with 125 μL of 10% tricoloacetic acid and centrifuged (1900 g, 6 min). The supernatant was added to which phthalaldehyde (fluorescent agent) was added, *p*H 8.0, the absorbance being read on the excitation spectrofluorimeter: 350 nm; emission of 420 nm. Normal values of serum glutathione: 0.67-1.60 mEq / mL [11-13].

c.Determination of FMD

Assessment of endothelial function and endothelial dysfunction was performed by non-invasive measurement of flux-mediated vasodilation (FMD). By this method, vasomotor function can be quantified after nitric oxide dependent vasodilation. Furthermore, it allows the use of repeated measurements useful for assessing the effects of surgical or medicinal interventions that interfere with vascular function.

Measurement of FMD initially involves blood pressure measurement followed by longitudinal scanning of the brachial artery with arterial diathermy measurement (D1 - reference diameter.) Then the bladder of the strain gauge placed 50 mmHg over the predetermined blood pressure was swollen and held in position for 5 After 5 minutes the cuff is slowly decompressed to install the active throat. At one minute of active hyperemia, the longitudinal axis of the vessel, the vessel diameter D2, was measured [14-16].

FMD% was considered the procetual modification of the diameter obtained after the active hyperemia of the basal diameter:

$$FMD\% = \frac{D2(mm) - D1(mm)}{D1(mm)} x100$$

FMD was measured with an ultrasound (Aloka ProSound SSD 4000), under "a jeun" conditions, in a quiet and constant room. Patients were not allowed to smoke in the morning of the study, nor to consume drugs / vasoactive substances at least 12 hours prior to the experiments. The values taken in the study were the average of two consecutive determinations. Normal values:> 12% [17-19].

d.Determination of ox-LDL plasma concentration

The plasma concentration of ox-LDL cholesterol was measured by an immunoenzymatic method using solid-phase sandwich ELISA (Mercodia kit, Uppsala, Sweden)

e.Evaluation of vascular endothelium function through in vitro studies in organ bath

Experiments on vascular rings were performed in the presence of diclofenac (10 μmol/L) to avoid interference of arachidonic acid derivatives on endothelial function.

It has been shown that menadione produces endothelial dysfunction by increasing oxidative stress (by altering intracellular thiols), lowering intracellular glutathione and aATP, effects that produce cytotoxicity and loss of structural and functional cellular integrity and implicitly vascular endothelium dissorder with endothelial dysfunction

Results and discussions

In this study, we determined the value of FMD, as a marker of endothelial dysfunction, in the studied groups: patients with cardiovascular disease. healthy subjects as well as the determination of markers of oxidative stress: malondialdehyde and glutathione, but also ox-LDL-cholesterol (heavily atherogenic lipoprotein) to make a correlation between them in order to observe the existence of a direct link between endothelial dysfunction and oxidative stress patients. The results obtained are shown in Table 1.

Table 1
OXIDATIVE STRESS PARAMETERS, ox-LDL AND FMD FOR THE STUDIED GROUPS

	Control (n = 5)	**BCV (n = 5)**
FMD (%)	15.5 ± 3.12	10 ± 2.16
Malondialdehyde (μmol/mL)	2.40 ± 0.04	2.89 ± 0.09
Glutathione serum (mEq/mL)	0.93 ± 0.24	0.52 ± 0.37
Ox-LDL (U/L)	56.07 ± 10.17	70.19 ± 12.09

When comparing the FMD values from the BCV group we achieved a significant decrease of these vs. control (** p <0.01) (Fig. 1).

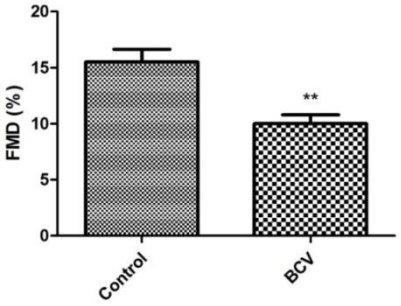

Fig. 1. Statistical comparison of FMD values obtained in BCV vs. Control

When comparing MDA values from the BCV group, we achieved a significant increase of these vs. control (*** p <0.001) (Fig. 2).

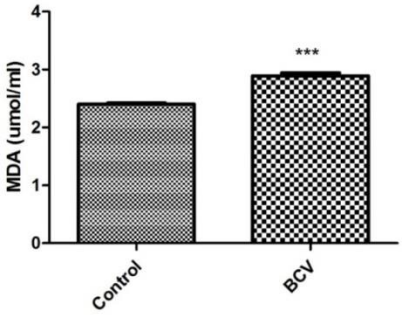

Fig. 2. The statistical comparison of MDA values obtained in BCV vs. Control

When comparing the GS and ox-LDL values from BCV we obtained a decrease, respectively a significant increase of these vs. control (* p <0.05) (Fig. 3 and 4).

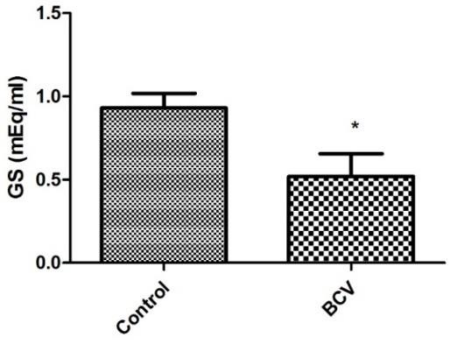

Fig. 3. The statistical comparison of the GS values obtained in the BCV vs. Control

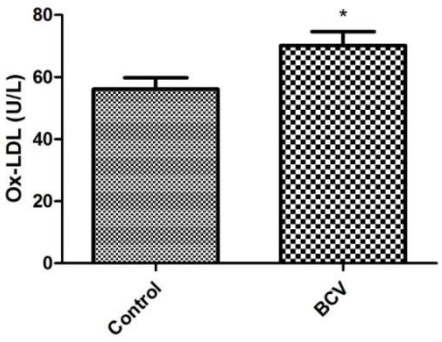

Fig. 4. The statistical comparison of the Ox-LDL values obtained in the BCV vs. Control

After obtaining the values for both batches, we sought to correlate the values of the oxidative stress parameters and the level of ox-LDL with FMD values obtained from clinically healthy patients or CVD patients. The results are shown in Figures 5-7.

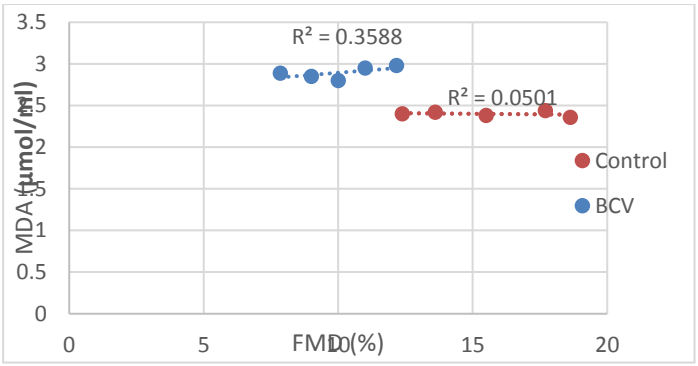

Fig. 5. Correlation of MDA with FMD values in control and CVD lots (R^2 = 0,3588)

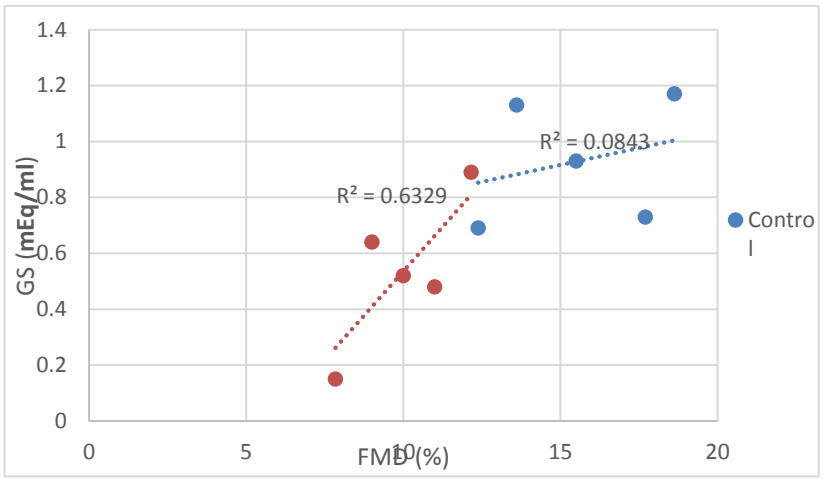

Fig. 6. Correlation of GS with FMD values in control and CVD lots (R^2 = 0,6329)

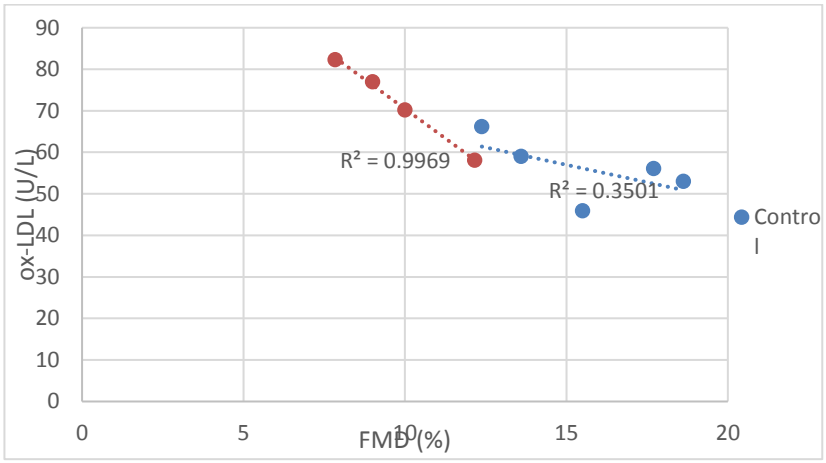

Fig. 7. Correlation of ox-LDL with FMD values in control and CVD lots (R^2 = 0,9969)

The results obtained have shown a significant decrease in FMD in patients with cardiovascular disease. This has also been described by other studies that consider FMD as the basis for assessing cardiovascular function and coronary risk (49). More importantly, DE evaluated through FMD can be considered a marker by which subjects with preclinical vascular disease can be identified.

When comparing ox-LDL values, a significantly higher value was seen in CVD patients, a result expected and described by many other studies that consider the increase in ox-LDL levels as an important step in the pathogenesis of atherosclerosis, a disease responsible for over 95% of cases of coronary artery disease (50).

When comparing the values of the oxidative stress parameters, we recorded an increase in serum malondialdehyde in CVD patients. Moreover, we also noticed a significant decrease in serum glutathione, an endogenous antioxidant in people with CVD. These results indicate the presence of significant oxidative stress in people with CVD.

To see exactly if the changes described above are responsible for the decrease in FMD or endothelial dysfunction, we sought to correlate the values obtained from the patients. Thus, in case of FMD correlation with serum malondialdehyde, we noticed the existence of a poor correlation for CVD patients. Moreover, in correlating the glutathione level we obtained a moderate correlation for the BCV group. In the case of the control group no correlations were obtained over the moderate ones. This indicates, in the present study, that the installation of oxidative stress produced by the decrease in antioxidant capacity is mostly responsible for endothelial dysfunction in people with CVD. Thus, we suggest that the decrease in antioxidant defense is the one that promotes the installation of

an endothelial dysfunction, the latter being installed, then leads to an increase in the oxidative stress by increasing the production of SRO.

Conclusions

The present study demonstrates, by direct decrease of endothelium-mediated vasodilation accompanied / correlated with changes in important parameters of oxidative stress, the direct involvement of oxidative stress, mainly produced as an alteration of endothelial antioxidant capacity, in the production of direct effects on vascular endothelium, in the case of people with cardiovascular disease. Moreover, ox-LDL is negatively and significantly correlated with flux-mediated vasodilation, suggesting direct involvement of plasma lipoprotein levels (oxidized due to oxidative stress) on promoting endothelial dysfunction.

Incubation of menadione ring-shaped rings results in endothelial dysfunction by action on both endothelium dependent vasodilation and endothelium-independent vasodilation. Furthermore, selenium administration appears to lead to an improvement in endothelial function, on endothelial dysfunction model due to menadione.

Thus, we can suggest that endothelial diffusion occurs as a result of the decrease in endogenous antioxidant capacity and the consequent increase in oxidative stress, which leads to an increase in LDL-cholesterol oxidation and implicitly an increase in the risk of developing cardiovascular events. Administration of selenium could improve endothelial function by protecting the body from eventual cardiovascular events associated with endothelial dysfunction.

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