THIOBARBITURIC ACID REACTIVE SPECIES LEVELS AND OSMOTIC FRAGILITY IN HUMAN BLOOD IN NORM AND PATHOLOGY

Nikolay Kalaydzhiev[1], Elena Zlatareva[1], Avgustina Danailova[2*]
[1] Multiprofile Hospital for Active Treatment in Neurology and Psychiatry "St. Naum", Sofia, Bulgaria
[2] Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria
Corresponding author: avgustina_danailova@abv.bg

Abstract

As a primary source of biomarkers for different pathological states human blood is extensively investigated by many researchers. Therefore in this study we examine the TBARS (thiobarbituric acid reactive species) levels, an indicator for oxidative stress degree, in erythrocytes and in blood plasma, osmotic fragility as a marker for the erythrocyte membrane resistance to osmotic stress and the correlation between these parameters in norm and pathology.

Considering the results of the experiments performed, we establish lower TBARS levels in the blood plasma and erythrocytes and higher osmotic resistance in the samples obtained from the healthy controls, and significantly higher degree of oxidative stress and low osmotic resistance in patients with neurological disorders and co-morbidities. It is interesting, that the correlation coefficient, r (Pearson's r) between TBARS levels in the blood plasma and the erythrocytes osmotic fragility is negative for the control group and it is positive in the group of patients. We suppose that r is affected by both the primary disease and the co-morbidities. In conclusion, our data demonstrate that the TBARS levels and osmotic fragility are markers for predisposition to development of disorders and the Pearson's r could be applied as potential diagnostic marker for progressive diseases.

Key words: *TBARS levels, Osmotic fragility, Human blood*

Introduction

Human blood, as a biological fluid, consisting of blood plasma, erythrocytes, platelets and leukocytes, provides important information for health management, diagnosis and monitoring of different pathologies (Li et al, 2023, Cooley et al, 2023). Erythrocytes are the cells in the largest amount in the blood (Remigante, 2022; Möller Et al., 2023). These blood cells are high specialized and their lifespan is about 120 days. Their membrane structure consists phosphopipids in form of bilayer, stabilized by cholesterol, and peripheral and integral proteins, responsible about the shape, flexability, transport functions, blood groups, resistance of hemolysis of these cells (Gordon-Smith, 2013). Very important complex for the membrane integrity is the cytoskeleton, built by the proteins spectrin, actin, protein 4.1 and ankyrin (Gordon-Smith, 2009; Sudnitsyna et al, 2020).

There is a high concentration of hemoglobin in the erythrocytes, associated with their main function – the strictly regulated process of oxygen supply to the tissues and organs in the body (Remigante, 2022; Möller Et al., 2023). Because of the lack of nucleus and mitochondria in the mature erythrocytes, the biosynthesis of new proteins is impossible and the energy is provided by glycolysis (Mameri et al., 2021; Möller Et al., 2023). High content of polyunsaturated fatty acids in the phospholipid bilayer and the auto-oxidation of hemoglobin make erythrocytes sensitive to endogenous and exogenous sources of ROS (reactive oxygen species) and their morphological types, deformability, mechanical properties, resistance of hemolysis may be influenced (Morabito et al, 2020; Mameri et al., 2021; Besedina et al, 2022). Increased levels of oxidative stress in erythrocytes provoke accumulation of damaged proteins and oxidized lipids, which will affect cell structure and functions and will decrease the lifespan, affecting the membrane stability, causing preliminary ageing of the same cells, resulting in vascular damages, hypoxia, high risk of inflammations and tissue fibrosis (Remigante, 2022; Besedina et al, 2022).

ROS are the species to which oxygen converts to molecules with a high reactivity (Nosaka and Nosaka, 2017) and they can be free radicals and non-radical oxidants (Vona et al, 2021) such as superoxide anion radical ($O_2•^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•) (Nosaka and Nosaka, 2017), singlet oxygen ($^1O_2$) (Nosaka and Nosaka, 2017, Reczek and Chandel, 2017). ROS are exclusively toxic molecules and they are considered as inducing diseases (Reczek and Chandel, 2017).

Above mentioned primery products of the oxidation may affect many healthy cells, resulting in DNA oxidation, glycoxidation, protein oxidation, lipid peroxidation (Guan et al, 2021; Vona et al, 2021), cell membrane injuries, causing harmful effects on cell homeostasis, loss of membrane integrity, energy depletion, changes in signal transduction and triggering apoptosis (Mameri et al., 2021). The secondary product, such a malondialdehyde (MDA) and 4-hydroxynonenal can be find as a part of the product mixture of oxidation (De Leon and Chad, 2020).

TBARS levels in the blood plasma, which contains lipids, proteins, lipoproteins and large amount of water, can be used as a marker for the levels of plasma lipoproteins (more specifically low density lipoproteins, LDL) peroxidation (Grabež et al, 2020). The reasons about the lipid peroxidation in blood plasma can be iron ions-depended, copper ions-dependent, hydroxyl radical formation, etc (Brzezinska-Slebodzinska, 2001). It is known that the normal range of lipid peroxidation products in blood plasma, obtained from healthy volunteers is in the interval between 1.80–3.94 μM (De Leon and Chad, 2020).

Osmotic fragility is a susceptibility of erythrocytes to hemolysis, which can be provoked by incubation of these blood cells in a hypotonic solution and subsequent net flow of water into cells (Goodhead and MacMillan, 2017). The degree of osmotic resistance to lysis is determined by solution of sodium chloride in a concentration lower than this into the erythrocytes, what is the reason about the hemoglobin release in the media. The osmotic fragility test gives an information for effects of different physical and chemical factors, storage conditions, etc., on the osmotic properties of considered blood cells and it can be used for diagnosis of different pathological conditions (Walski et al, 2014; Radisic et al, 2020). Moreover it is known that the results of this test depend on the gender, age (Fang et al, 2022) and genetic factors (Issaian et al, 2021; Fang et al, 2022).

Here, we consider TBARS levels and osmotic fragility in human blood in norm and pathology, in particular Parkinson's disease (PD), with aim to establish new diagnostic criteria that can be used in addition to the conventional approaches.

Materials and methods.

Reagents. 2-Thiobarbituric acid, Trichloroacetic acid were purchased from Merck, Germany.

Blood sample preparation. Whole blood sampling from 8 healthy volunteers and 29 PD patients was carried out by venepuncture. Samples were collected in vacutainers with K_2EDTA. The components of the whole blood were separated by centrifugation for 15 minutes at 900 g and at 4 °C. The supernatant (blood plasma) was replaced in Eppendorf tubes. The pellet, containing erythrocytes, leukocytes and small amount of the blood plasma, was washed twice with 9 mM Phosphat-Buffered Saline (PBS) with 1 mM ethylene diamine tetraacetic acid (EDTA), PBS–EDTA buffer, pH 7.4, by centrifugation for 15 minitues at 900 g. After washing procedure, pure erythrocytes were diluted to haematocrit 35 %.

Spectrophotometry. For determination of TBARS levels and the degree of osmotic fragility UV-Vis spectrophotometer SPECORD PLUS Analytik Jena was used.

TBARS levels determination. The oxidative stress in the erythrocytes were established by TBARS levels detection in the samples, using the protocol, described in Morabido et al, 2020. In briefly, 10 % Trichloroacetic acid was added to 1.5 ml erythrocyte suspensions with Ht = 20 %. After 10 minutes centrifugation at 3000 g the supernatants was replaced in other tubes and 1 ml 1 % tiobarbituric acid was added to them. The samples were heated to 95 °C for 30 minutes and after cooling at room temperature, the absorptions at 453 and 532 nm were measured by the spectrophotometer. The concentration of TBARS were calculated as it is described in Morabido et al, 2020.

Osmotic fragility levels determination. In order to investigate the degree of osmotic fragility in erythrocytes from healthy volunteers and PD patients we applied the protocol, described in Morabito et al, 2020, with modifications. The washed in PBS–EDTA buffer solution erythrocyte samples were resuspended in 0.7 % NaCl solution to Ht=0.05 %. The absorption of released haemoglobin was measured by spectrophotometry at wavelength 405 nm.

Statistical analysis. All data are presented as average numbers ± SD (standard deviation). The Pearson's r (correlation coefficient) is determined by dint of Origin Pro 2018 software.

Ethical Considerations. The blood sampling is performed in the University multiprofile hospital for active treatment in neurology and psychiatry "St. Naum" (UMHATNP), Sofia, Bulgaria in accordance with the ethical standards of the Declaration of Helsinki and after signing an informed consent by the volunteers.

Results

The osmotic fragility degree of erythrocytes and TBARS levels in the blood plasma and erythrocytes, obtained from 8 healthy individuals (controls) and 29 patients with PD were investigated and the data are presented in Figure 1. Osmotic fragility levels in the samples, taken from the controls (Figure 1, Panel A) are higher than those, measured for the patients (Figure 1, Panel D), as our assumption is that this is due to changes in the membrane structure in the cells, isolated from the patients' whole blood, associated with the interaction between the typical for PD α-Synuclein and the erythrocyte membrane. Moreover, we have an information that the most of the PD patients suffer from co-morbidities (such as hypertension, kidney diseases and others), which may have influenced these results.

It is visible from the Figure 1, Panels B and E, that TBARS levels in the blood plasma for the PD patients are significantly higher than those, measured for the controls, typical for patients with PD and others disorders. This could be explained with the presence of oxidized proteins and lipoproteins in the patients' samples and it is some kind of a marker for predisposition for development not only for PD, but also to others different diseases. Considering Figure 1, Panels C and F, we can establish similar results, for the TBARS levels investigated in erythrocytes from PD patients and controls: As we can see, the levels of oxidative stress, determined for the group of patients have greater values compared to those of the healthy volunteers. Nevertheless, it makes the impression that the TBARS levels vary significantly in the individual patients, which is probably due to the co-morbidities, drug therapy and the nutritional habits.

Considering the data obtained for the TBARS levels in the erythrocyte samples of the controls (Figure 1, Panel C) and patients (Figure 1, Panel F), we establish close values of this marker for oxidative stress, within each of the studied groups, that show a higher degree of credibility of the obtained results. Therefore, we assume that the erythrocytes are more reliable source of information for the oxidative stress degree. Here we observe again higher TBARS levels in the group of PD patients than those of the control one, and we are convinced in with our thesis according to which the osmotic fragility degree and TBARS levels in the blood plasma and in erythrocytes are higher in the group of patients, compared to those in healthy volunteers.

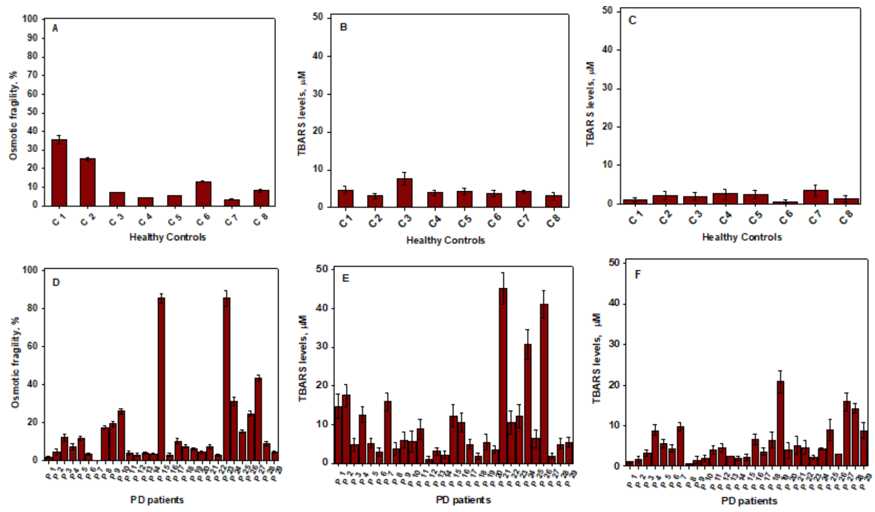

Figure 1. Osmotic fragility levels (Panel A), TBARS levels in the blood plasma (Panel B) and in erythrocytes (Panel C) determined for healthy controls. Osmotic fragility levels (Panel D), TBARS levels in the blood plasma (Panel E) and in erythrocytes (Panel F) established for the PD patients. Mean values ± SD.

We investigated the correlation between the osmotic fragility degree of the erythrocytes and TBARS levels in the blood plasma for each of the healthy volunteers and PD patients (Figure 2, Panels A and B, respectively). Applying Origin Pro 2018 software, we performed a statistical analysis and we established that the Pearson's r (a correlation coefficient) is a negative for the controls (-0.13916) and the same parameter determined for the PD patients is positive (0.11389).

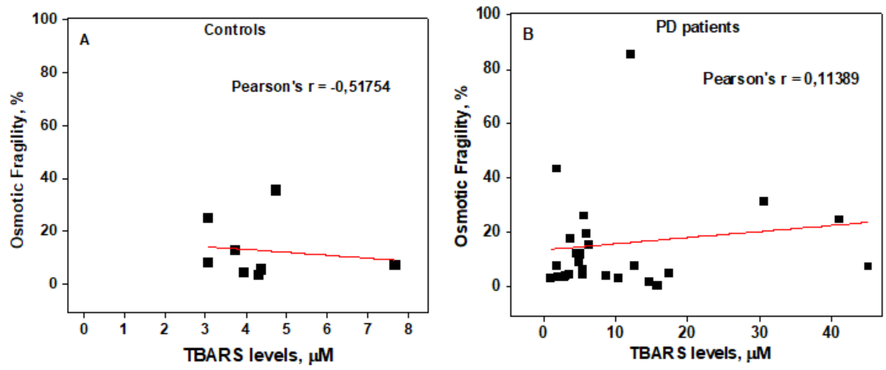

Figure 2. Correlation between TBARS levels in blood plasma and Osmotic fragility levels of the erythrocytes from healthy controls (Panel A) and PD patients (Panel B). Correlation coefficient Pearson's r is presented in each of the panels.

Our opinion is that the differ in the sign can be used as an additional indicator for the condition of the subjects and this correlation have a potential to be incorporated in the clinical practice, with purpose the physical health of investigated individuals to be monitored, and for early establishment of predisposition to neurological and others disorders.

Interesting, the differences in the sign of the Pearson's r can be found only when we analyse the relationship between TBARS levels in blood plasma and osmotic fragility levels of the red blood cells from healthy controls (Figure 2, Panels A and B), but the Pearson's r is negative about the erythrocytes for both control group and group of the PD patients (Figure 3, Panels A and B). In this sense, we reckon that the data obtained for the correlation between TBARS levels in blood plasma and osmotic fragility levels of the red blood cells from healthy controls and PD patients have good diagnostic and prognostic significance.

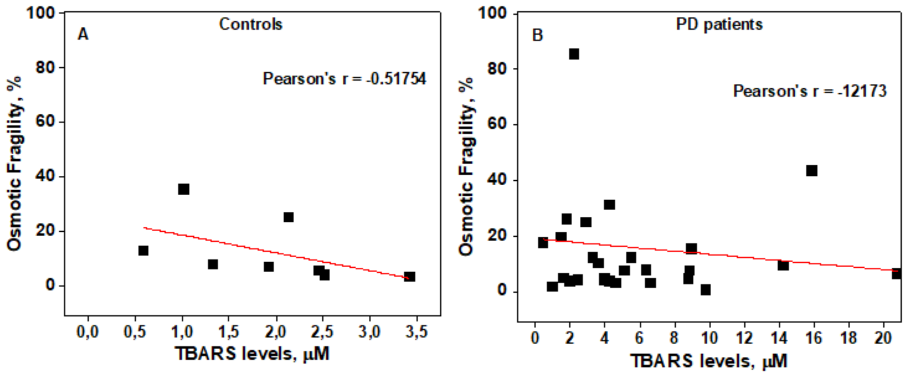

Figure 3. Correlation between TBARS levels and osmotic fragility levels of the erythrocytes from healthy controls (Panel A) and PD patients (Panel B). Correlation coefficient Pearson's r is presented in each of the panels.

Conclusions

The osmotic fragility degree of the erythrocytes and TBARS levels in the erythrocytes and in the blood plasma from healthy controls are lower than that, established for PD patients with co-morbidities.

The correlation coefficient (Pearson's r) between TBARS levels in the blood plasma and the osmotic fragility degree of the erythrocytes is negative for the control group and it is positive in the group of patients. Therefore, we reckon that r have diagnostic and prognostic significance for PD.

Our data demonstrate that the TBARS levels and osmotic fragility are markers for predisposition to development of disorders and the Pearson's r could be applied as potential diagnostic marker for chronic and/or progressive diseases.

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