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REVIEW

The Role of Red Blood Cell Deformability and Na,K-ATPase Function in Selected Risk Factors of Cardiovascular Diseases in Humans: Focus on Hypertension, Diabetes Mellitus and Hypercholesterolemia

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Summary

Deformability of red blood cells (RBC) is the ability of RBC to change their shape in order to pass through narrow capillaries in circulation. Deterioration in deformability of RBC contributes to alterations in microcirculatory blood flow and delivery of oxygen to tissues. Several factors are responsible for maintenance of RBC deformability. One of them is the Na,K-ATPase known as crucial enzyme in maintenance of intracellular ionic homeostasis affecting thus regulation of cellular volume and consequently RBC deformability. Decreased deformability of RBC has been found to be the marker of adverse outcomes in cardiovascular diseases (CVD) and the presence of cardiovascular risk factors influences rheological properties of the blood. This review summarizes knowledge concerning the RBC deformability in connection with selected risk factors of CVD, including hypertension, hyperlipidemia, and diabetes mellitus, based exclusively on papers from human studies. We attempted to provide an update on important issues regarding the role of Na,K-ATPase in RBC deformability. In patients suffering from hypertension as well as diabetes mellitus the Na,K-ATPase appears to be responsible for the changes leading to alterations in RBC deformability. The triggering factor for changes of RBC deformability during hypercholesterolemia seems to be the increased content of cholesterol in erythrocyte membranes.

Key words

Erythrocyte deformability • Na,K-ATPase • Cardiovascular risk factors

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Introduction

The biconcave shape of matured human red blood cells (RBC) is an essential property for their physiological function. This shape has two main advantages. Firstly, it allows larger surface area to volume ratio of RBC that is important for efficient diffusion of respiratory gases. Secondly, RBC is able to undergo deformation. The ability of the RBC to deform is one of the most important determinants of its survival in the circulation, since an average RBC measuring 7.2 micrometers in diameter has to pass through narrow capillaries including as small as 3-4 micrometers in diameter. For effective microcirculatory function and sufficient delivery of oxygen to the tissues RBC deformability is crucial (Schmid-Schonbein 1976). The clinical importance of RBC deformability is even more complex. It is also considered as a major determinant of RBC survival, maintaining the blood in fluid phase even at high hematocrit value and allowing the reduction of blood viscosity in larger vessels (Mokken *et al.* 1992). Rigidity and deformability of the RBC play very important role in tissue perfusion and in etiology of cardiovascular diseases (CVD) (Fornal *et al.* 2009, Penco *et al.* 2000). Decrease in RBC deformability caused by

genetic abnormalities, age of the cell, aging, hypertension, diabetes mellitus, dyslipidemia or any other factor increases blood viscosity and consequently impairs the proper tissue perfusion. This results in adverse outcomes in CVD (Fornal *et al.* 2010, Tomaiuolo 2014, Toth *et al.* 2014). RBC deformability is primarily determined *via* following three factors: deformability of the RBC membrane itself, intracellular viscosity and the surface area per volume ratio (Tomaiuolo 2014).

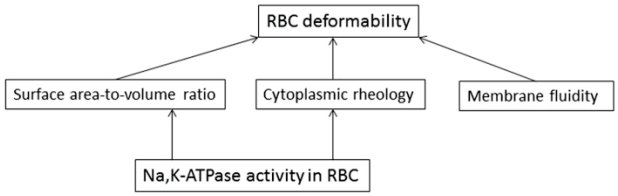

Fig. 1. Schematic presentation of determining factors regulating the RBC deformability in physiological conditions; role of the Na,K-ATPase activity.

Two of above mentioned determinants of RBC deformability are controlled by the activity of Na,K-ATPase in RBC (Fig. 1). Na,K-ATPase maintains the electrochemical gradient across the cell membrane, and in addition to its pump function acts as an essential component of the signal transduction mechanisms in the cells (Elimban *et al.* 2016). This enzyme is responsible for transport of Na^+ outside and K^+ ions inside the cell and resulting volume and water homeostasis. The importance of Na,K-ATPase in RBC is well visible in patients with stable coronary artery disease, since its activity negatively correlates with the severity of disease (Namazi *et al.* 2015). Considering these facts the aim of the present review is to provide insight into connection between Na,K-ATPase activity in RBC together with RBC deformability and various risk factors of CVD especially hypertension, diabetes mellitus and hyperlipidemia, based exclusively on papers from human studies.

Hypertension

Several studies of patients suffering from essential hypertension showed decreased deformability of RBC. It has been shown that deformability of RBC was impaired in patients with hypertension even under the treatment and that this impairment was inversely proportional to the mean arterial blood pressure (Odashiro *et al.* 2015). Decreased RBC deformability in hypertensives was accompanied with altered maintenance of intracellular homeostasis of calcium (David-Dufilho *et al.* 1992, Cicco *et al.* 2001) and sodium ions (Kazennov *et al.* 1990). As compared to normotensives, the patients with borderline hypertension displayed significantly lower activity of erythrocyte Na,K-ATPase, which indicated a hypothesis that in the blood of hypertensive subjects an enzyme inhibitor of erythrocyte Na,K-ATPase may circulate (Kazennov *et al.* 1987). Investigations of regulatory mechanisms of membrane functions in hypertension by means of electron spin resonance and spin labeling methods documented the relationship between endogenous Na,K-ATPase inhibitor (digitalis-like factor; DLF) and erythrocyte membrane fluidity in essential hypertension. The level of plasma DLF content was higher in hypertensive patients than in normotensive subjects and correlated with the decrease of erythrocyte membrane fluidity (Tsuda *et al.* 1992). Inhibition of the Na-K pump by digoxin *in vitro* correlates with reduced RBC deformability. However, this effect occurred at toxic doses of digoxin only. During *in vivo* testing the intake of digoxin (0.2 mg b.i.d.) in nine healthy volunteers, over a 5-day period did not alter the deformability of RBC and Na,K-ATPase activity (Wambach *et al.* 1985). The hypothesis that endogenous DLF might participate in essential hypertension led Devynck *et al.* (1987) to investigate the presence of plasma compounds interacting with digoxin antibodies. They observed positive correlations between systolic and diastolic blood pressure and apparent digoxin-like immunoreactivity of the plasma in man.

It was shown, that the higher intracellular concentration of sodium is in RBC, the higher is the blood pressure level. These intracellular sodium concentration changes that were accompanied with lowering the Na-pump activity, were more prominent in men than in women and in people whose both parents were hypertensive compared to those with only one parent being hypertensive (Zhao *et al.* 1984). In addition, comparison of normotensives with an average blood pressure 127/80 mm Hg and hypertensives with an average blood pressure 147/94 mm Hg (aged 23-27 years) resulted in decrease of RBC deformability in hypertensive subjects which was not accompanied by inhibition of the Na,K-ATPase (Hossmann *et al.* 1986). So the interrelationship between the DLF, Na,K-ATPase activity and deformability of RBC needs further clarification.

Beside the effect of endogenous DLF, another factor influencing the Na,K-ATPase activity and RBC

deformability has been hypothesized. The shape of RBC and consequent adaptability of human erythrocyte plasma membrane is determined by cytoskeleton underlying the membrane and connecting it to various localities in the intracellular space. The presence of various isoforms of tubulin was confirmed by proteomic studies in human RBC (Goodman *et al.* 2007). Later it was shown that tubulin in human RBC is distributed in three fractions: membrane bound, sedimentable and soluble compartments. In RBC from hypertensive patients membrane tubulin fraction was increased by approximately 150 %, what resulted in inhibition of Na,K-ATPase activity (Amaiden *et al.* 2011). Alongside with localization of tubulin in the cell, its chemical modification plays also important role in regulation of Na,K-ATPase and RBC deformability. Very important seems to be the detyrosination/tyrosination cycle of tubulin. In hypertensive patients the level of detyrosinated tubulin was higher in the sedimentable fraction. Consequent translocation of detyrosinated tubulin to the plasma membrane enhanced the inhibitory effect of tubulin on Na,K-ATPase activity resulting in reduced RBC deformability (Amaiden *et al.* 2015).

As a further factor influencing the functionality of RBC, the nitric oxide (NO) was identified. Based on the finding, that RBC contains functional NO synthase it was proposed that RBC synthesize and use NO to modulate its own physiology (Jubelin and Gierman 1996). Later it was shown that NO produced either in RBC or in endothelial cells increased the membrane fluidity and decreased the rigidity of cell membranes (Bor-Kucukatay *et al.* 2003). Furthermore, the greater effect of NO on the membrane fluidity of RBC in samples from hypertensive patients compared with normotensive subjects indicated that NO might actively participate in the regulation of rheological behavior of RBC and may have a crucial role in the improvement of microcirculation in hypertension (Tsuda *et al.* 2000). The lower deformability of RBC from hypertensive patients was accompanied with higher production of NO after acetylcholine stimulation as compared with RBC from healthy persons (Carvalho *et al.* 2006). Nevertheless, for complete information it should be added that excess of NO produced by leucocytes decreases the deformability of RBC (Korbut and Gryglewski 1993), so the NO concentration is of critical importance in this modulatory mechanism (Bor-Kucukatay *et al.* 2003).

Since oxidative stress plays a role in the pathophysiology of essential hypertension and Na,K-ATPase is modulated by the surrounding lipid microenvironment, lipid peroxidation may alter the interactions of this enzyme with the membrane components. Reduction of Na,K-ATPase activity and elevation of the thiobarbituric acid reactive substances may underlie the pathophysiological aspects linked to the prehypertensive status (Malfatti *et al.* 2012). Treated patients suffering from essential hypertension showed decreased oxidative stress and increased fluidity of RBC membrane, together with higher activity of erythrocyte Na,K-ATPase after supplementation with vitamins C+E (Rodrigo *et al.* 2014).

However, there were published also controversial data concerning the mechanical properties of RBC membranes in hypertensive patients. On the one side, a decrease in membrane fluidity was documented in hypertensive patients (Tsuda *et al.* 2000, 2011). The observation that lower membrane fluidity of erythrocytes was associated with higher plasma insulin level in hypertensive patients seems to be interesting (Tsuda *et al.* 2001). Membrane abnormality of RBC was attenuated with low salt intake, and, on the contrary, was more prominent with high-salt intake in essential hypertension (Masuyama *et al.* 1988). On the other side, no changes were observed in Na,K-ATPase activity and fluidity of RBC membranes (Pytel *et al.* 2012).

The importance of RBC deformability determination in patients suffering from essential hypertension was documented by correlation between this parameter and the levels of soluble vascular adhesion molecules-1 as well as soluble intracellular adhesion molecules-1 in circulation. Therefore, deformability of RBC may serve as a marker of endothelial dysfunction at least in hypertensives (Fornal *et al.* 2011).

Diabetes mellitus

Concerning the diabetes mellitus (DM), it is known for more than forty years that this disease is accompanied with impaired microcirculation resulting in relative tissue hypoxia (Ditzel and Standl 1975, Ditzel *et al.* 1978). In this impairment the worsened penetration of RBC into target tissues is involved (Le Devehat *et al.* 1994). DM induces numerous abnormalities of RBC including increased aggregation (Schmid-Schonbein and Volger 1976), increased membrane viscosity (Baba *et al.* 1979) and decreased deformability (McMillan 1975, Vague and Juhan 1983). Reduced viscoelastic properties of erythrocyte membranes have been attributed to the alterations in membrane lipid-protein interactions together with the increased glycosylation-derived internal

viscosity in juvenile DM (Watala *et al.* 1992). A scanning electron microscopy and atomic force microscopy have shown significant changes in membrane structure of RBC in type II DM (Buys *et al.* 2013). Another reason for decreased deformability of RBC in DM was ascribed to lower activity of the Na,K-ATPase leading to accumulation of intracellular sodium ions, with subsequent increase of free calcium ions in RBC due to competition in transmembrane exchange (Gardner and Bennett 1986, Juhan-Vague *et al.* 1986). The incubation of human RBC with glucose in higher concentration significantly lowered the Na,K-ATPase activity and increased the lipid peroxidation and protein glycosylation of RBC membranes compared with normal glucose-treated RBC (Jain and Lim 2000). The restoration of normoglycemia by insulin treatment in subjects with uncontrolled insulin dependent DM resulted in normalization of Na,K-ATPase activity, as well as the deformability of RBC after 24 h (Juhan-Vague *et al.* 1986). This result shows the importance to check the degree of glycemia control when studying rheological parameters in DM. The improvement of Na,K-ATPase activity in erythrocyte membrane was also observed after 24 weeks of metformin administration in type II diabetic patients (Chakraborty *et al.* 2011). The addition of pioglitazone, but not glimepiride, to metformin resulted in improvement of RBC deformability beyond improving blood glucose control in type II diabetic patients which was associated with an increase in plasma adiponectin levels (Forst *et al.* 2010). The similar situation was also observed in hypertensive men whose plasma adiponectin levels were lower than in normotensive men. The above mentioned hypoadiponectinemia was associated with decreased membrane fluidity of erythrocytes at least partially by the NO-dependent mechanism, since the plasma adiponectin levels were correlated with plasma NO metabolites (Tsuda 2006).

For longer time it was believed that the connecting peptide, so called C-peptide bridging the insulin A and B chains in the precursor proinsulin molecule is just a byproduct of insulin synthesis. Initial studies oriented to discovery of potential physiological role of proinsulin C-peptide brought negative results (Kitabchi 1970, Hoogwerf *et al.* 1986). However, later comparison of Na,K-ATPase activity in RBC from patients with type I DM, with patients suffering from type II DM and control subjects revealed that the mean enzyme activity was lower in the type I diabetic patients by 28 % as compared with control subjects. In type II diabetic patients, stepwise regression analysis showed that fasting C-peptide level was the only factor independently correlating with Na,K-ATPase activity (De La Tour *et al*. 1998). In addition, patients with type II DM with good residual C-peptide secretion were protected better from endothelial dysfunction that those with low C-peptide secretion (Manzella *et al*. 2003). These results indicated a potential role of C-peptide in regulating the Na,K-ATPase in RBC. Another investigation showed that *in vitro* preincubation of the blood samples from type I diabetic patients with human proinsulin C-peptide for 8 h restored the deformability of RBC, almost to values of samples from control subjects. In samples from control subjects, the preincubation with C-peptide did not modify the deformability of RBC. It was concluded that C-peptide is able to ameliorate the impaired deformability of RBC in patients suffering from type I DM and it was hypothesized that this effect is mediated by restoration of Na,K-ATPase activity, which is known to be attenuated in diabetic patients (Kunt *et al*. 1999). This hypothesis was confirmed by study of type I diabetic patients, in which C-peptide supplementation increased the erythrocyte Na,K-ATPase activity by about 100 %. There was found a linear relationship between plasma C-peptide levels and erythrocyte Na,K-ATPase activity. Using laser-diffractoscopic method a significant improvement of RBC deformability was observed after C-peptide administration in RBC of type I diabetic patients. Inhibition of the Na,K-ATPase by its specific inhibitor ouabain completely abolished the effect of C-peptide on RBC deformability (Forst and Kunt 2004). Further study was oriented to a question which part of the C-peptide consisting of 31 amino-acids is responsible for regulation of the Na,K-ATPase activity and consequent deformability of RBC. Incubation of blood samples from patients with type I DM and healthy controls with human C-peptide, C-terminal hexapeptide, C-terminal pentapeptide resulted in improvement of RBC deformability. This improvement was dependent on activation of Na,K-ATPase, as the presence of ouabain abolished the above effect. Incubation of RBC with the middle fragment comprising residues 11-19 of C-peptide did not show detectable effect, suggesting that the C-terminal residues of C-peptide are causally involved in this effect (Hach *et al.* 2008).

The lowered activity of Na,K-ATPase in RBC and consequent decrease of RBC deformability may also originate in polymorphism of the ATP1A1 gene which is encoding the α-1 isoform of the enzyme predominant in RBC. It was documented that polymorphism in the intron 1 of this gene is associated with lower enzyme activity in patients with C-peptide deficiency either with type I or type II DM, but not in normal individuals

(Vague *et al.* 1997, Jannot *et al.* 2002).

Beside the mechanism of polymorphism of the gene responsible for synthesis of α-1 isoform of the Na,K-ATPase and insufficiency of C-peptide further possible mechanism of the Na,K-ATPase regulation in DM was identified. Studies on RBC from diabetic patients resulted in lowering of Na,K-ATPase activity by 50 % alongside with increased membrane-associated tubulin content by more than 200 % as compared with normal subjects. It was proposed that increased glucose concentration in blood triggers tubulin-induced effects leading to microtubule formation and consequent Na,K-ATPase inhibition (Rivelli *et al.* 2012). Recently it was shown that high concentrations of glucose promote tubulin acetylation and translocation of this tubulin to the membrane, thus reducing deformability of RBC (Nigra *et al.* 2016).

Hypercholesterolemia

Another widely spread risk factor of CVD is hypercholesterolemia. Excess of plasma cholesterol level induces changes in basic properties of human erythrocyte plasma membrane, including its fluidity and the intensity of lipid peroxidation (Koter *et al.* 2002, 2003, Broncel *et al.* 2007). Increase of membrane cholesterol caused a progressive decrease in deformability of RBC, as measured by filtration (Cooper *et al.* 1975). Studies oriented to the influence of high cholesterol level in serum and in the LDL fraction showed significantly lower deformability of RBC in the group of hypercholesterolemic patients as compared with age-matched normocholesterolemic subjects (Kohno *et al.* 1997). A significant negative correlation between low-density lipoprotein (LDL) cholesterol level and Na,K-ATPase activity in RBC was also noticed in type II diabetic patients (Chakraborty *et al.* 2011). Hypercholesterolemic type II diabetic patients had significantly lower deformability of RBC compared with normocholesterolemic type II diabetic patients. These data suggest that elevated plasma cholesterol may impair RBC deformability by an additional effect to hyperglycemia in type II diabetic patients (Ercan *et al.* 2002). The filterability of RBC was significantly proportional to the HDL-cholesterol values, whereas it was inversely proportional to the triglyceride levels in apparently healthy subjects (Ejima *et al.* 2000).

One year lasting therapy of hyper-cholesterolemic patients with pravastatin, an inhibitor of hepatic hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase, reduced the serum cholesterol level by 23 % alongside with significant improvement of RBC deformability by approximately 20 %. Furthermore, significant relation between the improvement of RBC deformability and the reduction of serum cholesterol or LDL cholesterol were observed. The results suggested that RBC deformability was reduced in hypercholesterolemic patients, and that long-term cholesterol-lowering therapy could improve reduced RBC deformability, which may contribute to the improvement microcirculation and organ perfusion (Kohno *et al.* 1997). The administration of another HMG-CoA reductase inhibitor – atorvastatin (10 mg/day) improved RBC deformability after 1 month and 3 months of treatment except the lipid lowering effect (Szapary *et al.* 2004). Atorvastatin at 10 mg/day dose and simvastatin at 40 mg/day in a similar degree significantly increased the lowered activity of Na,K-ATPase in hyperlipidemic patients. However, simvastatin decreased stronger the membrane cholesterol content in RBC and erythrocyte membrane fluidity than atorvastatin (Broncel *et al.* 2007).

Unfortunately, filtration-based studies do not provide a direct measure of the viscoelastic constants, but their output is based on some parameters that are indirectly related to RBC deformability. The other approach – micropipette technique used to assess cell deformability tests the intrinsic viscoelastic properties of the membrane, but it is insensitive to the cell geometry and shape. Thus, different techniques may have different sensitivities to various factors involved in resulting deformability of RBC especially in condition of hypercholesterolemia (Chabanel *et al.* 1983, Tomaiuolo 2014). This may be possible explanation of controversial data concerning the deformability of RBC in hypercholesterolemic patients. The above mentioned lower deformability of RBC in hypercholesterolemia is in contrast to studies showing this parameter unchanged (Chabanel *et al.* 1983, Vaya *et al.* 1998).

Familiar type-2 hypercholesterolemia is autosomal dominantly inherited deficiency of the expression LDL receptor on the cell surfaces leading to excess of plasma LDL cholesterol and LDL deposition in the cells. The erythrocyte membranes of children affected by type-2 hypercholesterolemia revealed higher erythrocyte membrane cholesterol/phospholipid ratio than those of normolipemic individuals (Martinez *et al.* 1998). In adult patients suffering from type 2 hyper-cholesterolemia, beside the changes in the structure and fluidity of erythrocyte membranes, impaired function and structure of plasma membrane proteins, was observed. It

was documented by decreased concentration of thiol groups in membrane proteins followed by lower activity of Na,K-ATPase (Koter *et al.* 2004). Generally, a decrease in the accessible thiol groups correlates with increased aggregation of membrane proteins since a significant fraction of oxidized groups that can be involved in the formation of disulfide bridges (Soszynski and Bartosz 1997). Thus, free radical formation coexisting with hypercholesterolemia may lead to lipid peroxidation and result in modification of membrane proteins including Na,K-ATPase. The patients affected by type 2 hyperlipidemia that were not given medications known to affect lipid levels or interact with statins, and with body mass index (BMI) lower than 35, were given 10 mg of atorvastatin per day. The lowered erythrocyte membrane fluidity was significantly higher after 4 weeks and remained on the same level after 12 weeks of the atorvastatin therapy (Koter *et al.* 2002). Beside the statin therapy, the deformability of RBC was also improved in hyperlipidemic patients treated with fenofibrate (250 mg/day) for 8 weeks (Saklamaz *et al.* 2005).

Concerning the relationship between the erythrocyte membrane fluidity and the activity of Na,K-ATPase in RBC, interesting seems to be the observation that group of hypercholestrolemic patients with lowered enzyme activity and membrane fluidity of RBC after 2-month lasting supplementation of polyphenolic extract from *Aronia melanocarpa* showed significant decrease of erythrocyte cholesterol concentration (by 26 %) and a decrease of lipid peroxidation (by 40 %), and an increase of membrane fluidity. However, the membrane fluidity of RBC did not reach the values found in the control group despite the 2-month therapy and the activity of the Na,K-ATPase remained unchanged (Duchnowicz *et al.* 2012). So, it may be anticipated that in hypercholesterolemic patients the higher content of cholesterol and the state of oxidation of lipids directly in membranes plays primary role in regulating the deformability of RBC. It is in concordance with results of another study performed in patients diagnosed with coronary artery disease. It showed an increase in the level of lipid peroxidation (by 13 %) and total cholesterol (by 19 %), and a decrease in membrane fluidity (by 14 %) in the subsurface layers and in the deeper layers of erythrocyte membrane (by 7 %) isolated from patients with coronary artery disease in comparison to healthy controls. A significant decrease in catalase (by 10 %) and superoxide dismutase (by 17 %) activities were also observed. So, disorders in the antioxidant system were accompanied with changes in the membrane structure of RBC (Pytel *et al.* 2013). The NO levels achieved on RBC of hypercholesterolemic patients were higher against the NO levels for the control group. Moreover, besides the lower deformability of RBC obtained from hypercholesterolemic patients, the RBC produced higher NO levels after acetylcholine stimulation than RBC obtained from blood samples of healthy subjects (Carvalho *et al.* 2006).

LDL immunoadsorption apheresis is considered for appropriate LDL lowering when maximal drug therapy fails to achieve adequate LDL cholesterol reduction. Single LDL apheresis resulted in significant reduction of plasma viscosity, whole blood viscosity and erythrocyte aggregation index. However, long-term apheresis had no sustained effect on plasma and whole blood viscosity in hypercholesterolemic patients with coronary artery disease (Pusl *et al.* 2009). The patients with coronary artery disease and severe LDL hypercholesterolemia regularly treated with LDL apheresis were subjected to advanced direct adsorption of lipoproteins apheresis resulting in reduction of LDL cholesterol and improvement of various hemorheological parameters including RBC deformability (Otto *et al.* 2002).

Some other cardiovascular risk factors

Rheological blood behavior is influenced also by aging itself. The age-related decrease of RBC deformability appears to be due to the cumulative effect of various intra- and extra-cellular factors as age-related oxidative stress (Goi *et al.* 2005), altered sialic acid content (Huang *et al.* 2011) and Na,K-ATPase activity of the cell membrane (Maurya and Prakash 2013).

Obesity is known to be associated with elevated risk for CVD. Concerning the relationship of Na,K-ATPase activity and physical properties of RBC membranes various data were published. The increase of erythrocyte viscosity was present even in the absence of impaired glucose tolerance, diabetes, hypertension or hyperlipidemia (Rillaerts *et al.* 1989). The increased Na,K-ATPase activity was demonstrated in the RBC membranes of obese, normotensive, non-diabetic subjects together with decreased membrane fluidity, which become apparent without detectable changes in plasma lipids (Faloia *et al.* 1999). The long-term hypocaloric feeding lowered altered erythrocyte Na,K-ATPase in obese individuals (Pasquali *et al.* 1988). On the other hand results of another study support the hypothesis that abnormalities in the erythrocyte Na,K-ATPase system are not usual in the obese population but are probably present

only in a limited number of selected patients (Pasquali *et al.* 1985). However, measurement of erythrocyte deformability by ektacytometric techniques showed no significant difference between obese patients without other cardiovascular risk factors and controls (Sola *et al.* 2007).

The metabolic syndrome (MS) was identified as a multiplex risk factor for CVD which is one of the major causes of death. Overweight or obesity, abnormal lipid metabolism, hypertension, and insulin resistance are the major contributors in MS. The MS is believed to be a consequence of a sedentary lifestyle, excessive intake of calories and physical inactivity, although genetic factors are also involved. The membrane fluidity was lower in RBC from patients with MS than in the ones from control group. These alterations in RBC of patients with MS were ascribed to higher concentration of cholesterol in the membrane and an increased oxidative stress (Ziobro *et al.* 2013).

Changes in hemorheological parameters after ingestion of ethanol were studied in patients with ischemic cerebrovascular disease and elderly healthy men. The ingestion of 1 g/kg, but not 0.5 g/kg of ethanol resulted in impairment of RBC deformability in patients with ischemic cerebrovascular disease, while hemorheology was not significantly changed in healthy men (Nagai *et al.* 2001). The chronic alcohol consumption resulted in significant decrease of Na,K-ATPase activity in RBC and higher rigidity of erythrocyte membranes (Maturu *et al.* 2010).

Study of chronic cigarette smokers showed increased erythrocyte membrane lipid peroxidation and rigidity with decreased Na,K-ATPase activity and thiol groups in male volunteers smoking for 7-10 years at least 12±2 cigarettes per day (Padmavathi *et al.* 2010).

According to official statistical data there is a significant difference between pre-menopausal women and age-matched men in morbidity and mortality from CVD. Compared to female blood, male blood had higher viscosity and RBC aggregation and lower RBC deformability (Kameneva *et al.* 1999). The hormone (estrogen) replacement therapy significantly increased the membrane fluidity of RBC with a concomitant increase in plasma NO metabolite level in postmenopausal women (Tsuda *et al.* 2003).

Conclusion

Data presented in this review allow us to suggest that the interrelationship between the deformability of RBC and Na,K-ATPase function varies depending on the nature of cardiovascular risk factors. In patients suffering from hypertension as well as diabetes mellitus Na,K-ATPase appears to be mostly responsible for the changes leading to alterations in RBC deformability (Fig. 2). Changes of RBC deformability were commonly accompanied by changes in Na,K-ATPase activities.

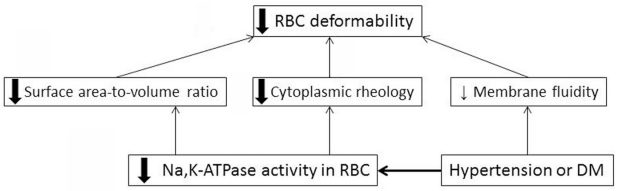

Fig. 2. Schematic presentation of determining factors regulating the RBC deformability in hypertension and diabetes mellitus; deterioration of Na,K-ATPase the most probably precedes decrease in RBC deformability.

The triggering factor for changes of RBC deformability during hypercholesterolemia seems to be the increased content of cholesterol in erythrocyte membranes resulting in decrease of membrane fluidity (Fig. 3). The decrease of membrane cholesterol resulted in improvement of hemorheological parameters including RBC deformability despite the unchanged Na,K-ATPase activity.

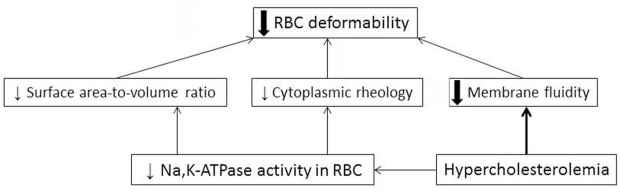

Fig. 3. Schematic presentation of determining factors regulating the RBC deformability in hypercholesterolemia; increase of membrane cholesterol caused a progressive decrease in membrane fluidity and consequently deterioration of RBC deformability.

In respect of treatment of CVD it could be challenging to determine possible effects of different interventions in CVD on Na,K-ATPase activity and consequent RBC deformability. Those approaches that result in improvement of microcirculation should be preferred. In respect of prevention of CVD any activity leading to improvement of Na,K-ATPase activity in RBC and consequent RBC deformability may be considered as simultaneous prevention of CVD.

Conflict of Interest

There is no conflict of interest.

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