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Erythrocyte aggregation and metabolic syndrome

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Abstract. Erythrocyte aggregation has been consistently associated with insulin resistance, central obesity and hypertension in the literature. Oxidative stress and chronic inflammation are almost always present in metabolic syndrome (MetS). Prooxidants and adipocytokines generated in MetS alter erythrocyte morphology, decrease erythrocyte deformability and increase whole blood viscosity (WBV). Increased WBV has been attributed to erythrocyte aggregation which in turn is greatly influenced by other rheological parameters, including its membrane surface charge and plasma fibrinogen concentration. The interplay of hemorheological factors, oxidative stress and inflammation has a detrimental effect in MetS due to the gross disturbance in microcirculation. The hemodynamic aspect of MetS needs further research and exploration.

Keywords: Aggregation, hemorheology, diabetes, obesity, dyslipidemia, hypertension

1. Background

Aggregation is the tendency of an individual erythrocyte to form a collection of doublets, triplets and so on. Two important parameters that determine the aggregation are the average size of rouleaux (aggregate size) and the rate at which erythrocytes adhere [8]. To form the aggregates of uniform size, erythrocytes come together through electrostatic force and are joined together by macromolecule bridging. This leads to the formation of rouleaux which later transforms into a sphere of uniform size. The driving force may be the reduction of the surface free energy. The aggregates contain not only erythrocytes but also the macromolecules binding them together [30]. Increases in haematocrit do not always increase the formation of aggregates due to the limited amount of macromolecules necessary to form the rouleaux [30]. Erythrocyte aggregation kinetics closely follow the von Smoluchowski's theory [8]. Impacting on both kinetics and aggregate size is the tendency for erythrocytes to repel each other due to the negative surface charge from sialic acid residues. Increased erythrocyte aggregation due to reduced sialic acid concentration in membrane has been reported in human pathology [84]. Erythrocytes are attracted to each other by van der Waals forces, and the balance of these two forces (repulsive and attractive) determines the most stable arrangement of erythrocytes in an electrolyte solution in the absence of other forces.

Metabolic syndrome (MetS) is the constellation of cardiovascular risk factors in an individual. It consists of multiple, interrelated risk factors of metabolic origin that appear to directly promote the development of CVD [29]. According to NCEP ATP III guidelines [29], the individual is said to be in the state of

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MetS if he/she fulfills three criteria from the following five criteria: waist circumference >102 cm in male and >88 cm in female; triglyceride (TG)≥150 mg/dl; high density lipoprotein cholesterol (HDL-C) <40 mg/dl in male and <50 mg/dl in female; blood pressure ≥130/85; and fasting glucose ≥110 mg/dl. The ultimate significance of MetS is that it helps identify individuals at high risk of both type II diabetes and cardiovascular disease (CVD). Oxidative stress [90] and inflammation [85] are almost always present in MetS and this further adds cardiovascular risk. Clinically, hypoxia has been associated with metabolic dysregulation of adipose tissue in obesity [98]. Furthermore, obstructive sleep apnea has been associated with MetS and its components [70]. Hypoxia associated with some metabolic diseases could be due to an altered hemorheology. Rate of oxygen release from the erythrocytes also depends upon erythrocyte morphology [50]. Using rat skeletal muscle venules as a model, Bishop et al. [9] demonstrated that erythrocyte aggregates *in vivo* decreases velocity of blood flow in venules. Reduction in flow velocity due to erythrocyte aggregates was markedly significant at low shear rate flow when compared to high shear rate flow [9]. Increased WBV is more marked at low shear rate flow [12, 15]. MetS has been constantly associated with various aspects of hemorheology [38, 39]. In this review, we discuss erythrocyte aggregation and its effect in different components of MetS including oxidative stress and inflammation.

2. Erythrocyte aggregation and diabetes mellitus

Erythrocyte aggregation plays an important role in the pathophysiology of blood circulation [97] and in the complications developed due to DM [57, 91, 110]. Increased erythrocyte aggregation has been correlated with the complications of DM [25]. Type II diabetic patients with clinically evident late complications have elevated erythrocyte aggregation regardless of the degree of metabolic control [25]. Husstedt et al. showed that increased degree of erythrocyte aggregation is related to the progression of neuropathy in DM subjects [44]. Nam et al. showed that erythrocyte aggregation was increased when they were incubated in glucose rich plasma. Increased erythrocyte aggregation was found with increased concentration of glucose which indicates hyperglycemia could be a factor that alters hemorheology [67]. Foresto et al. reported spherical shape of erythrocyte aggregates in DM instead of the common cylindrical shape of typical rouleaux [33]. Increased erythrocyte aggregation in DM was significantly correlated with glycosylated haemoglobin (HbA1c) in a study by Devehat et al. [53] which emphasizes the importance of glycemic control in DM. The complications of DM are probably due to altered hemorheological parameters like erythrocyte morphology, deformability, viscosity and aggregation. Thus, improvement in blood rheology through normalization of blood glucose and glycemic control could be beneficial to diabetic patients.

3. Erythrocyte aggregation and hypertension

Blood pressure depends on the shear rate of blood flow and total peripheral vascular resistance. These two factors in turn depend on several rheological factors like erythrocyte aggregation, deformability, hematocrit, vessel geometry and plasma viscosity [71, 89]. The mechanical and biochemical characteristics of erythrocytes are altered in hypertension [64]. There is a general membranous defect in the essential hypertension [66] with altered transportation of ions across the erythrocytic membrane [73–75]. In a study involving fifty two patients with essential arterial hypertension, it was shown that increased systolic, diastolic and mean arterial pressure were correlated with increased erythrocyte aggregation [106]. The hypertensive subjects showed increased WBV, decreased erythrocyte deformability and increased

erythrocyte aggregation when compared with normotensive subjects. The increased left ventricular mass in the hypertensive subjects was correlated with increased erythrocyte aggregation [106]. Erythrocyte aggregation index as well as disaggregation shear rate threshold (shear stress needed to break up the aggregated erythrocytes or shear resistance of erythrocyte aggregates) was found to be higher in hypertensive subjects when compared with normotensive subjects [80].

Treatment of renal anaemic patients with erythropoietin increases blood pressure [78]. Sandhagen suggested that decreases in erythrocyte fluidity could be an important reason for resultant increase in blood pressure [89]. Pirrelli has reported that altered hemorheology could be one of the numerous causes of hypertension [71]. He argued that chronic increased shear stress and hyperviscosity changes the physical conditions of vascular wall or in general changes the vascular geometry. Altered hemorheology and increased release of endothelial vasoactive factors induce an increase in peripheral resistance which can increase arterial hypertension [71]. Furthermore, the anti-hypertensive drugs, Ca++ channel blocker and ACE inhibitor have been shown to improve hemorheology in hypertensive subjects [49].

4. Erythrocyte aggregation and obesity

In 1999, Meiselman questioned whether altered hemorheology is the cause or effect of hypertension and argued that the resolution of the chicken versus egg problem has not been achieved [62]. In 2002, Bogar in an interesting way replied to the question and, in his own words said that hemorheology and hypertension are not "chicken or egg" but are two chickens from similar eggs. The egg he referred to was obesity. He claimed that being overweight and a sedentary life style are the major causes behind abnormal hemorheology and other metabolic interrelated diseases [10]. In a study by Vaya et al. 136 morbidly obese subjects and an equal number of normal weight healthy volunteers were recruited to study the effect of obesity on hemorheological parameters [100]. Morbidly obese was defined as body mass index (BMI) >40 kg/m^2. Fibrinogen, plasma viscosity, erythrocyte aggregation (M and M1) was significantly higher and erythrocyte deformability was significantly lower in obese subjects when compared with normal weight controls. No significant differences in hemorheological parameters were found between obese subjects when they were further divided into MetS and non-MetS group. Similarly, no differences in hemorheological parameters were found when obese subjects without any other components of MetS were compared with obese subjects with at least one other components of MetS [100]. This study suggests that obesity is the prime cause for the altered erythrocyte rheology. Similarly, Valensi et al. also showed that erythrocyte aggregation was higher in obese subjects when compared with normal weight individuals [99].

To study the effect of weight loss on hemorheological parameters, Fanari et al. recruited twenty obese subjects without any other cardiovascular complications and diabetes. Hemorheological parameters were measure before and after three months of treatment with low calorie diet. After dieting, erythrocyte aggregation was decreased when compared with the basal value [31]. The decrease in erythrocyte aggregation and improvement in hemorheology after weight loss has been reported in several other studies [41, 72]. Improvements in rheology after weight loss in obese subjects could be due to complex biochemical and endocrine changes after weight loss. In a study involving sixty-seven severely obese subjects (BMI $\geq$35), erythrocyte aggregation was shown to decrease after weight loss and it was claimed that increased erythrocyte aggregation in obesity is the result of increased insulin resistance rather that elevated fibrinogen or dyslipidemia [96].

Adipocytokines and inflammatory markers are high in obesity [42, 43]. CRP was shown to be high in morbidly obese cases and multivariate regression analysis showed that CRP predicted erythrocyte aggregability [100]. Berliner et al. [7] showed that erythrocyte aggregation could be used as a biomarker for

the detection of low grade systemic inflammation. Through the help of receiver operating characteristics curve, the authors showed that erythrocyte aggregation is superior to other commonly used markers such as fibrinogen and high-sensitive C-reactive protein (hsCRP) to demonstrate the ongoing inflammation [7]. In a group of 234 individuals with and without atherothrombotic risk factors, it was shown that fibrinogen contributed strongly towards the erythrocyte aggregation [2]. Thirty obese subjects without any underlying inflammatory or malignant conditions and thirty-five non-obese healthy volunteers were analysed for erythrocyte aggregation and markers of inflammation. Highly significant differences were noted for markers of inflammation and for the degree of erythrocyte aggregation between the two groups. There was also a significant correlation between BMI and the degree of erythrocyte aggregation and erythrocyte sedimentation rate, fibrinogen, hs-CRP, and leukocyte count. The authors concluded that increased erythrocyte aggregation in obesity could be due to the inflammation present in obesity [88].

5. Erythrocyte aggregation and dyslipidemia

The majority of the lipids present in the erythrocyte membrane originate from plasma lipoproteins as erythrocyte cannot synthesize lipids [36, 52]. Erythrocytes' membrane cholesterol content is high in hypercholesterolemic patients and LDL is mainly responsible for increased cholesterol delivery to erythrocyte [23, 92]. Increased level of cholesterol changes the erythrocyte membranous properties [47, 60]. Lipoprotein lipase (LPL) deficient mice were used to study the effect of severe hypertriglyceridemia on hemorheological parameters. Erythrocyte deformability and electrophoretic mobility was decreased whereas osmotic fragility was increased in LPL deficient mice when compared with control mice [109]. In the same way, erythrocyte of LPL deficient mice showed irregular protrusions on its surface as revealed from scanning electron microscopy [109].

After a direct absorption of lipoproteins (DALI) apheresis procedures for average of five consecutive sessions in six hypercholesterolemic patients, erythrocyte aggregation was reduced by 42% and WBV by 10% [11]. In average, after DALI apheresis, LDL-C was reduced by 66%, apolipoprotein-A (LpA) by 66%, very-low-density lipoprotein (VLDL)-cholesterol by 51%, TG by 28%, and fibrinogen by 18% [11]. Ten heterozygous familial hypercholesterolemic patients showed a significant reduction in plasma viscosity and erythrocyte aggregation along with LDL-C and fibrinogen after undergoing first four heparin-induced extracorporeal lipoprotein precipitation treatment [93]. Similarly, the beneficial effects of lipid lowering therapy by the drug statin in hemorheology have also been seen in other studies [1, 46, 56, 61]. A significant positive effect on blood hemorheology mediated by the efficient reduction of cholesterol indicates that dyslipidemia could be one of the prime causes of increased erythrocyte aggregation. Erythrocyte aggregation is also shown to be increased in subjects with primary hyperlipoproteinemia [65, 101]. Erythrocyte aggregation was higher in hyperglycemic patients when compared with normal controls and it was further higher in hypercholesterolemic hyperglycemic patients when compared to hyperglycemic patients alone [3].

Ultrasound duplex scans showed increased *in-vivo* erythrocyte aggregation in veins and arteries in hyperlipidemic subjects compared to normolipidemic subjects [21, 22]. In a multiple regression model, HDL2-C, LpA-I, and LpA-I/A-II emerged as significant factors influencing erythrocyte aggregation index and disaggregation shear rate threshold among hypercholesterolemic subjects [79]. HDL-C has been inversely correlated with erythrocyte aggregation in normal subjects as well as in coronary heart disease patients [87]. It has been demonstrated that erythrocytes permeability *in vivo* is impaired by high levels of TC and LDL-C [54]. HDL inhibits Ca2+ induced procoagulant activity on erythrocyte membranes [28]. HDL also competes with LDL for binding with erythrocyte membrane, competitively inhibiting

the LDL-C induced erythrocyte aggregation and thus decreases WBV [94]. HDL-C also plays a key role in protecting erythrocyte membranes against oxidative damage [32]. Thus, HDL-C may counteract the unfavourable effects of macromolecules such as fibrinogen to reduce the erythrocyte aggregation [79].

6. Erythrocyte aggregation and oxidative stress

The erythrocyte is exposed to free radicals from both within the cell and the extracellular environment. It is estimated that between 1 and 3% of oxygen consumed by aerobic cells becomes a reactive oxygen species [13]. Erythrocyte antioxidant defences are important in maintaining hemoglobin iron in the reduced state and for protecting the plasma membrane of the cell from lipid peroxidation. Erythrocyte possess multiple enzymatic and non-enzymatic antioxidant defense mechanisms to prevent oxidative damages, however during oxidative stress, these mechanisms can become exhausted [82, 83]. Fifty MetS cases and twenty-five healthy controls were recruited to study the erythrocyte redox balance and membrane properties in the study of Kowalczyk et al. [51]. Erythrocyte membrane fluidity and conformation changes of membrane proteins were studied by electron paramagnetic resonance technique. Subjects with MetS showed altered physical confirmation of erythrocytes' cytoskeleton proteins. There was an unfavorable alteration in erythrocyte lipid membrane fluidity and erythrocyte of MetS subjects showed increased osmotic fragility. Malondialdehyde (MDA) concentration in erythrocytes of MetS was significantly high whereas there were no significant changes in the activity of glutathione peroxidase, catalase and superoxide dismutase when compared with healthy controls. Membrane lipid peroxidation was considered as one possible cause of altered membrane properties in the study [51]. MDA can cause polymerization of membrane components and thus increase the aminophospholipid bilayer rigidity [17, 45]. Also, this peroxidant injury initiated in the lipid component of the membrane can be transmitted to neighboring substances such as membrane proteins [45]. These polymerization reactions consequently increase membrane rigidity.

Calcium was shown to decrease erythrocyte deformability [102]. Watanabe et al. has suggested that there is passive accumulation of calcium inside erythrocyte due to membrane disorganization by free radicals [102]. It is also suggested that increases in insulin concentration impairs the erythrocytic calcium-ATPase activity leading to increased intraerythrocytic calcium concentration [37]. Accumulation of calcium inside the cell further amplifies the damage of free radicals to the membranes [102]. Erythrocyte aggregation was increased and deformability was decreased by the effect of superoxide anion generated outside the erythrocyte membrane [5]. When superoxide anion was generated inside the erythrocyte membrane by the phenazine methosulfate, no significant increase in erythrocyte aggregation was noticed despite reduced deformability [5]. An antioxidant enzymes superoxide dismutase and catalase were shown to protect erythrocyte membrane alterations against activated granulocytes [4]. Incubation of healthy erythrocyte with oxidant diamide and ferrous sulphate/ascorbate decreased erythrocyte deformability and increased erythrocyte aggregation [19]. These alterations in hemorheology can be minimised by thiol containing antioxidants dithiothreitol and N-acetylcysteine [20]. Antioxidants also decrease erythrocyte aggregation induced by photodynamic treatment [6]. In the same way, Vitamin C has been reported to prevent cholesterol-induced microcirculatory changes in rabbits [34].

7. Discussion: The relationship between erythrocyte aggregation and metabolic syndrome

MetS has been associated with altered hemorheology [108]. Insulin resistance [96] and obesity [31] have been suggested as the main factors behind altered rheology in MetS. Decreased blood flow rate

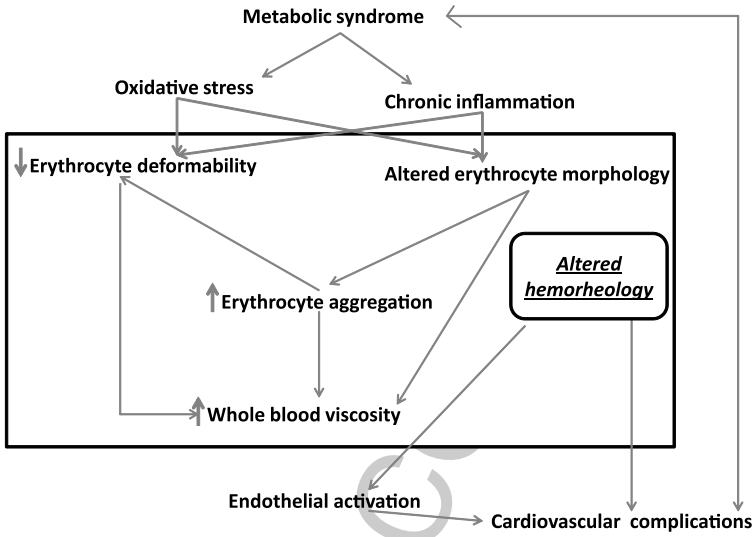

Fig. 1. Altered hemorheology is the bridge that links MetS with cardiovascular complications. Oxidative stress and chronic inflammation decreases erythrocyte deformability and alter erythrocyte morphology. Decrease deforming capacity and altered morphology increase erythrocyte tendency to aggregate. Decrease erythrocyte deformation, increase erythrocyte aggregation and altered erythrocyte morphology increases whole blood viscosity. Erythrocyte aggregates and morphologically altered erythrocyte could interact with the endothelium causing endothelial activation. Altered hemorheology and endothelial activation leads to cardiovascular complications.

and altered hemorheology has been shown previously in the microvasculature of the optic nerve among patients with primary open angle glaucoma [40, 104]. Lominadze et al. [59] clarified from their experiment in the rat model that the expression of erythrocyte protein is altered in hypertension and this alteration is responsible for increased erythrocyte/fibrinogen interaction rather than only due to increased fibrinogen concentration [59]. Lominadze and Dean [58] also suggested that a specific binding mechanism is involved in fibrinogen induced erythrocyte aggregation. The decrease in erythrocyte aggregation in diabetic subjects after intense management was not correlated with the changes in glycemic control, fibrinogen or lipid profile [18]. Hence, increased in erythrocyte aggregation in MetS could also be due to the alterations in erythrocyte intrinsic properties. In the study of Elishkevitz et al. [27] erythrocyte aggregation was associated with inflammatory markers but not with HbA1c. We believe that, inflammatory markers and prooxidant generated in MetS and its components [24, 35, 42, 55, 69, 76, 103, 105] are mainly responsible for altered rheology.

Oxidative stress decreases the erythrocyte deformability [45] and alter its morphology [95, 107]. Modifications of the membrane may lead less deformable erythrocyte to aggregate or may create a favourable environment to aggregate [81]. Rigid erythrocyte and erythrocyte aggregates damage blood vessels [71]. Morphologically altered erythrocyte and erythrocyte aggregates could interact with the endothelium further amplifying inflammation. Increased erythrocyte aggregation [16] and morphological transformation [63] ultimately leads to increased WBV and reduced microcirculatory flow which is unfavourable to cardiovascular system. Decreased blood flow rate and altered hemorheology has been shown previously in the microvasculature of the optic nerve among patients with primary open angle glaucoma. Blood pressure is raised following increased WBV [71, 89]. Increased erythrocyte aggregation has been associated with cardiovascular complications [68, 77, 86]. Peripheral vascular resistance is increased when WBV

increases which ultimately increases the pumping requirement of the heart [26]. This results in higher frictional force (shear stress) acting on the endothelium further increasing the risk of CVD [48] (Fig. 1). Intermittent hypoxia experienced by various tissues due to altered hemodynamics and hemorheology aggravate oxidative stress further complicating the scenario [14]. Thus, we propose altered hemorheology as the bridge that links MetS with CVD.

8. Conclusions

MetS is a state of oxidative stress and chronic inflammation. Prooxidant and inflammatory markers generated in MetS alter the hemorheology including erythrocyte aggregation. Cardiovascular complications of MetS are also associated with erythrocyte aggregation. Hence, Erythrocyte aggregation could be a useful biomarker in the assessment of MetS, its severity and progression. Since, MetS has a hemodynamic basis; its definition should be updated to incorporate hemorheological parameters.

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