Chapter

Alteration in Zeta Potential of Erythrocytes in Preeclampsia Patients

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Abstract

Erythrocyte is one of the earliest and extensively analyzed blood cells in blood physiological and clinical studies. The erythrocyte membrane is negatively charged and sialic acid residues are responsible for most of the negative charge at the cell surface. This negative charge on the red blood cells (RBC) surface is believed to prevent RBC aggregation. This charge varies in different disease condition which can be determined by zeta potential (ZP) values. The present study deals with alteration in zeta potential of erythrocytes in preeclampsia patients. The mean erythrocytic ZP of control pregnant women taken during third trimester was found to be 21.64 ± 0.3122 mV whereas; when erythrocytic ZP of preeclampsia patients was measured it was found to be 15.13 ± 0.1393 mV which was significantly less than that of control pregnant volunteers. Alteration in zeta potential value was accompanied by endothelial damage which is able to mechanically deform and hemolyze erythrocytes as they pass through the capillaries. It was also observed from determination of lipid peroxidation of erythrocytes, that there is formation of higher concentration of malondialdehyde within the erythrocytes of preeclampsia patients. The data suggest that, in preeclampsia there is excessive accumulation of oxidative stress which causes injury to vascular endothelial cells by generation of lipid peroxides and detachment of sialic acid residues. As a result there is alteration in the net negative surface charge on RBCs extracellular membrane which leads to alteration in zeta potential value. Thus it can be concluded that zeta potential value of erythrocytes can act as a screening test to anticipate pregnancies at high risk for this complication.

Keywords: zeta potential, preeclampsia, erythrocytes, sialic acid, lipid peroxidation, endothelial damage

1. Introduction

Preeclampsia, first described more than 100 years ago, is a major complication of pregnancy, and is associated with increased maternal and fetal mortality and morbidity [1]. It is a hypertensive disorder of human as well as primate pregnancy characterized by a generalized inflammatory state and endothelial dysfunction, resulting in disseminated microangiopathic disease with vasospasm and hypercoagulation [2]. It occurs in 5–7% of all pregnancies and is a leading

cause of maternal and fetal morbidity and mortality [3] together with bleeding and infection. Preeclampsia places the mother at risk of convulsions (eclampsia), kidney failure, liver failure and death [4]. Clinically, preeclampsia is defined as hypertension (blood pressure ≥ 140/90 mmHg) and proteinuria (urinary protein ≥ 300 mg/24 h). It is also associated with pathological edema, coagulation abnormalities and decreased uteroplacental blood flow [5–7]. Despite numerous basic, clinical and epidemiologic studies that have been conducted over the past half-century [8], there is no reliable test to identify women at risk for developing this disorder, thus it is important to develop a predictive screening test early in pregnancy so that we can anticipate pregnancies at high risk for this complication [9]. Recently there is a growing interest in characterizing RBC membrane defects in several diseases, as changes in membrane structure contribute to the pathophysiology of the disease process [10].

The cell-surface charge is the key biophysical parameter that depends on the composition of the cytoplasmic membrane and the physiological condition of cells. The general picture of the membrane structure of erythrocyte is that of 'Bilayer lipid membrane' based on the Gorter-Grendel bimolecular leaflet model with a thickness of about 100 Å. The N- and C-terminal segments of 'Glycophorin', the major glycoprotein that spans the RBC membrane may contribute to the surface charge. From an electrophoretic point of view, the human erythrocyte behaves as a 'macropolyanion'. The dominant ionogenic species is the carboxyl group of N-acetyl neuraminic acid or sialic acid. Other ionic components which are involved in charge contribution are the acidic and basic groups of amino acids of proteins [11]. When such a charged particle suspended in a liquid is placed in an electric field, electrophoresis migrates it towards the oppositely charged electrode [12]. This migration is calculated as velocity or mobility of erythrocyte which is used to calculate zeta potential.

Zeta potential is an electrochemical property of cell surfaces that is determined by the net electrical charge of molecules exposed at the surface of cell membranes [13]. So long as the zeta potential of the system remains constant, the fluidity of the system also remains constant. But if the ZP of the system is lowered then the stability of the system undergoes progressive changes [10]. A number of reports indicate that blood levels of lipid peroxidation products are elevated in women with preeclampsia relative to normal pregnancy [14]. It is also associated with oxidative stress and is responsible for the production of reactive oxygen species (ROS) which are the contributory factors for vascular endothelial cell dysfunctioning [15]. Injury to endothelial cell leads to activation of the clotting cascade and promotes platelet aggregation and clot formation [16] which is able to mechanically deform and hemolyze erythrocytes as they pass through the capillaries [17]. A substantial amount of evidence demonstrates that red cell aggregation increases in preeclampsia compared with normotensive pregnant women [18, 19]. Given the effect of endothelial cell injury and red cell aggregation, the purpose of this chapter is to study the effects of the alteration in erythrocytic zeta potential in preeclampsia patients as assessed by the cell electrophoresis technique using zeta-meter system 4.0.

The following materials are required to study the zeta potential, dextrose (Merck), distilled water, lancet, rectified spirit, zeta meter system 4.0. Blood was collected from voluntary donors with preeclampsia (n = 88) and control pregnant women (n = 60) under treatment in Dalvi memorial hospital and Daga memorial hospital, Nagpur. None of the subjects (both control and patients) were addicted to any drug/smoking. Each volunteer provided written consent for the study of their blood sample.

2. Preparation of isotonic dextrose solution

A 5% w/v dextrose solution was prepared by dissolving 5 g of anhydrous dextrose (Merck) in 100 ml of distilled water.

3. Preparation of blood suspension for zeta potential measurement

Approximately 0.01 ml blood is transferred into 50 ml of freshly prepared isotonic dextrose solution. Mean values of the 10 readings are used to calculate the zeta potential according to the basic Helmholtz-Smoluchowski equation as follows:

$$\text{Zeta potential}, \zeta = \frac{4\pi \times V_t \times EM}{D_t}. \quad (1)$$

where V_t = Viscosity of suspending liquid in poises at temperature 't', EM = Electrophoretic mobility at actual temperature, D_t = Dielectric constant, ZP = Voltage in electrostatic units.

4. Estimation of zeta potential of prepared blood sample by zeta meter system 4.0

The zeta potential of the RBCs was is measured using zeta meter system 4.0. Zeta potential is purely an electro kinetic property of the electrical double layer surrounding the system but not the surface of the system itself. The value of zeta potential gives an indication about the stability of the system under study. This quantity is measured by determining the mobility/velocity of the particle under an applied electric field. The value of zeta potential can be obtained from the equation given by Helmholtz-Smoluchowski.

$$\zeta_d = (4\pi\eta/\epsilon)\, V \quad (2)$$

where ζ_d = electro kinetic potential/zeta potential, η = viscosity of dispersion medium, ϵ = dielectric constant of the dispersion medium, V = v/E (mobility of the particle), v = velocity of the particle in cm/s, E = potential gradient in V/cm [20].

A special capillary cell called electrophoretic cell is used for the measurement of zeta potential. The capillary is embedded inside a chamber having electrodes at either of the two ends. Sample is placed from any one end of the electrophoretic cell and electrodes are connected to the cell and electric field at specific voltage is applied (200 V). Charged particles move towards oppositely charged electrode and their velocity is measured and expressed in terms of electro kinetic potential/zeta potential which indicates the mobility of particle under applied electric field. Recently this method is widely used for determining the membrane potential of biological membranes.

In this experiment, fresh capillary blood samples were obtained from volunteer by puncturing the skin with a lancet and blood suspension was prepared as described in above procedure. Prior to zeta potential measurement temperature of the RBC suspensions were measured and detection parameters for ZP measurements such as light intensity, focal plane and tracking duration were optimized for stable data collection. The RBC suspensions were then added to the previously cleaned and calibrated (using min-u-sil) zeta-meter cell placed under the zeta-meter stage and the mobility of individual RBCs was tracked by equipped

Zeta meter-ZM4DAQ software using microscopically-acquired video images, and data were recorded 10 times for each sample and average zeta-potential in mv was determined using a standard Helmholtz-Smoluchowski formula.

5. Result

The results of blood samples obtained from both patients and control are expressed as mean values with standard deviation. Comparison between different groups and interpretation of results are based on 'two-sample t test' with software PRISM 5. Differences between the groups were considered significant at $p < 0.05$ which indicates that the control and other patient groups differ significantly from one another in all situations. The ZP of erythrocytes was measured by the cell electrophoresis technique using zeta meter system 4.0 at the minimum voltage required for the movement of the erythrocytes to travel a fixed distance. In a study conducted in India, **Table 1** shows the zeta potential (ZP) values of the erythrocytes of control pregnant women in mV while **Table 2** shows the zeta potential (ZP) values erythrocytes of preeclampsia patients in mV [21] (reference of your work). The mean erythrocytic ZP of control pregnant women taken during third trimester was found to be 21.64 ± 0.3122 mV whereas; when erythrocytic ZP of preeclampsia patients was measured it was found to be 15.13 ± 0.1393 mV. The results as shown in **Figure 1** revealed that there is a significant decrease in the ZP of the RBC of preeclampsia patients as compared with the control pregnant women.

Sr. No.	Zeta potential	Sr. No.	Zeta potential	Sr. No.	Zeta potential
1	−23.02 (1.95)	22	−26.28 (1.1)	43	−21.36 (1.41)
2	−21.87 (1.4)	23	−24.07 (1.94)	44	−18.13 (1.6)
3	−17.43 (1.33)	24	−19.34 (0.88)	45	−18.46 (1.98)
4	−24.52 (2.6)	25	−16.82 (0.64)	46	−18.11 (1.69)
5	−19.71 (0.77)	26	−19.52 (1.92)	47	−18.04 (0.84)
6	−22.46 (0.92)	27	−19.02 (2.04)	48	−16.38 (0.83)
7	−16.59 (0.64)	28	−19.83 (1.87)	49	−16.41 (2.5)
8	−20.10 (1.39)	29	−21.26 (0.67)	50	−19.88 (1.7)
9	−24.49 (2.36)	30	−19.62 (1.46)	51	−21.15 (1.03)
10	−21.07 (2.28)	31	−20.73 (1.12)	52	−20.52 (0.84)
11	−17.27 (3.59)	32	−20.11 (0.57)	53	−21.02 (2.76)
12	−24.49 (0.93)	33	−25.59 (1.57)	54	−22.68 (1.79)
13	−21.54 (1.71)	34	−20.57 (0.88)	55	−22.50 (1.2)
14	−26.78 (0.99)	35	−25.73 (1.9)	56	−25.21 (2.15)
15	−19.39 (2.47)	36	−21.68 (1.63)	57	−25.11 (2.77)
16	−22.44 (1.12)	37	−21.13 (1.6)	58	−24.19 (2.28)
17	−20.48 (1.62)	38	−19.68 (1.81)	59	−18.43 (0.77)
18	−20.50 (0.83)	39	−19.96 (1.61)	60	−19.34 (2.75)
19	−23.15 (1.54)	40	−20.75 (1.63)		
20	−21.06 (1.44)	41	-20.36 (2.43)		
21	-21.42 (1.43)	42	-25.65 (1.23)		

Table 1.
Zeta potential of normal pregnant women [21].

Sr. No.	Zeta potential	Sr. No.	Zeta potential	Sr. No.	Zeta potential	Sr. No.	Zeta potential
1	−16.00 (1.42)	23	−16.00 (0.94)	45	−15.62 (0.93)	67	−16.75 (1.62)
2	−13.94 (0.4)	24	−16.16 (0.87)	46	−12.91 (1.58)	68	−14.13 (0.27)
3	−14.45 (0.29)	25	−14.67 (0.8)	47	−15.99 (1.15)	69	−16.5 (1.21)
4	−16.7 (2.1)	26	−15.53 (1.08)	48	−14.94 (0.68)	70	−16.41 (2.5)
5	−14.68 (1.21)	27	−16.74 (2.27)	49	−14.02 (0.35)	71	−15.27 (1.18)
6	−14.00 (0.71)	28	−16.84 (1.12)	50	−16.58 (0.77)	72	−14.28 (0.57)
7	−16.81 (1.03)	29	−16.53 (1.74)	51	−16.14 (0.57)	73	−13.39 (0.34)
8	−13.44 (0.68)	30	−16.49 (1.12)	52	−15.92 (1.35)	74	−16.07 (0.43)
9	−14.75 (0.4)	31	−14.38 (1.12)	53	−16.68 (1.11)	75	−14.28 (0.4)
10	−15.59 (1.11)	32	−13.71 (0.48)	54	−14.07 (0.73)	76	−13.82 (1.86)
11	−15.51 (1.09)	33	−14.86 (0.85)	55	−14.07 (0.95)	77	−16.71 (1.44)
12	−16.14 (1.52)	34	−16.24 (1.15)	56	−12.13 (0.67)	78	−14.38 (1.12)
13	−12.08 (1.08)	35	−15.24 (1.67)	57	−13.55 (0.62)	79	−14.81 (0.55)
14	−16.59 (0.64)	36	−16.59 (0.89)	58	−16.59 (0.22)	80	−15.32 (0.34)
15	−16.45 (0.73)	37	−14.1 (0.57)	59	−16.89 (0.58)	81	−16.38 (0.83)
16	−14.61 (1.79)	38	−13.86 (1.28)	60	−14.96 (1.26)	82	−13.72 (0.83)
17	−12.12 (0.39)	39	−12.42 (0.83)	61	−16.77 (0.42)	83	−13.72 (2.36)
18	−15.87 (2.1)	40	−13.86 (0.82)	62	−15.28 (0.36)	84	−16.28 (0.94)
19	−15.18 (1.1)	41	−16.92 (1.74)	63	−16.52 (1.32)	85	−12.05 (1.05)
20	−13.72 (0.83)	42	−15.61 (1.08)	64	−15.44 (0.6)	86	−14.65 (0.6)
21	−13.52 (0.48)	43	−13.55 (0.62)	65	−14.81 (1.52)	87	−16.72 (0.46)
22	−16.83 (1.08)	44	−14.67 (0.8)	66	−16.82 (0.64)	88	−13.82 (1.86)

Table 2.
Zeta potential of preeclampsia patients [21].

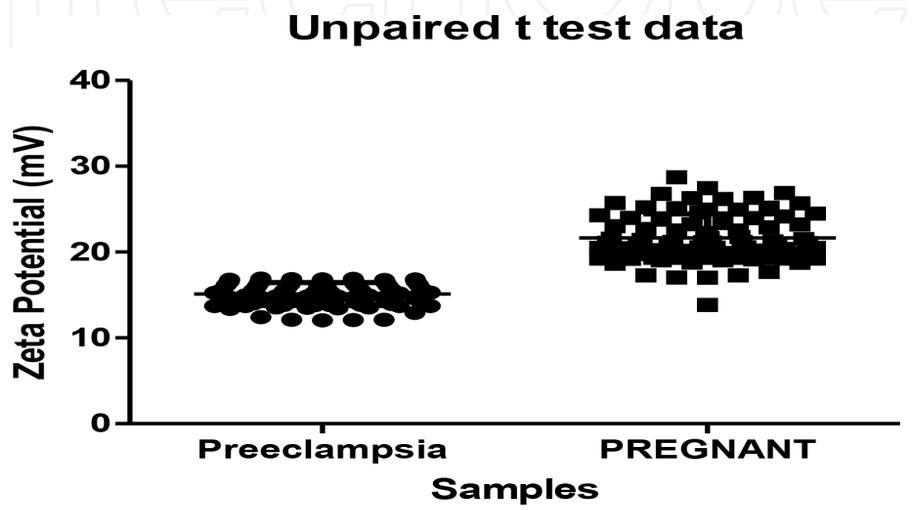

Figure 1.
Comparison of erythrocytic zeta potential of preeclampsia patients and control pregnant women [21].

6. Discussion

Zeta potential (ZP) is an electrochemical property of cell surfaces that is determined by the net electrical charge of molecules exposed at the surface of cell membranes. Membrane proteins contribute to the total net electrical charge of cell surfaces and can alter ZP through changes in their intermolecular interactions [22]. Erythrocyte is one of the earliest and extensively analyzed blood cells in blood physiological and clinical studies [23]. The erythrocyte membrane is negatively charged and the major contributor to the negative ZP of RBCs is sialic acid, which is abundantly present on the RBC surface [24, 25]. This negative charge on the RBC surface is believed to prevent RBC aggregation [25]. The physiologic changes during normal pregnancy affect red cell aggregation [26]. The aggregation properties of the cells depend, in turn, on the shape and concentration of red blood cells (RBCs) as well as the presence of sticky proteins [27]. In a cross-sectional study, Ozanne et al. demonstrated that red cells aggregation increases during the course of normal pregnancy [28]. Whereas, in preeclampsia red blood cell aggregation is further increased when compared with normotensive pregnant women; this increase could be due to either the changes in fibrinogen system or functional abnormalities of erythrocyte membranes [22]. Andrea et al. observed that erythrocyte aggregation was increased in all the hypertensive pregnant patients compared with the normotensive pregnant controls, regardless of both the onset (chronic or pregnancy-induced) of hypertension and the status of plasma macromolecules. Thus, concluded that increased erythrocyte aggregation may be due to either conformational changes of the membrane occurring during hypertension or a redistribution of the ionic charges on the two surfaces of the membrane [23]. The membrane zeta potential and the morphology are the important structural and functional parameters of erythrocytes. They affect the deformability and the circulation of erythrocytes in a blood vessel. On the other hand, they influence the affinity, aggregation, metabolism and immunity of the cell [25]. Accordingly, in this study, we performed systematic measurements of the membrane zeta potential during preeclampsia and comparison was done between blood samples from pregnant women as control vs. preeclampsia patients.

7. Conclusion

The zeta potential values of the erythrocytes of pregnant women and preeclampsia patients were determined by cell electrophoresis technique using zeta meter system 4.0. Zeta potential (ZP) is a characteristic signature for the diagnosis of hemolytic diseases, studies of membrane permeability, and alterations leading to destruction of erythrocytes. To investigate the properties of the erythrocyte membrane, we examined the zeta potential measurements for the surfaces of erythrocyte of preeclampsia patients during each trimester. The electrochemical potential value obtained for preeclampsia patient erythrocytes was found to be reduced in comparison with the normal pregnant women erythrocyte. Thus it is concluded that increased erythrocyte aggregation may be due to either conformational changes of the membrane occurring during hypertension or a redistribution of the ionic charges on the two surfaces of the membrane and hence reduces the zeta potential of preeclampsia patients' erythrocytes. Measurement of zeta potential is an easy and relatively quick way to detect molecular changes that have occurred on the membrane surface of erythrocytes.

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