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VARIATION IN MEMBRANE ELECTROCHEMICAL POTENTIAL OF PLASMODIUM INFECTED ERYTHROCYTES

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ABSTRACT: Membrane electrochemical potential or zeta potential (ZP) is a feature of the molecular profile of the cell membrane and the two-dimensional arrangement of its charge-bearing molecules. *Plasmodium* species, the causative agents of malaria, are intracellular parasites that remodel host erythrocytes by expressing their own proteins on erythrocyte membranes. In the present study the variation in the ZP of erythrocytes of malaria patients was studied by comparing it with that of the normal healthy volunteers. 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. For healthy volunteers the average ZP value of the erythrocytes was found to be 22.57 ± 0.2984 mV and for malaria patients it was found to be 13.09 ± 0.8456 mV. The results revealed that there is a significant decrease in the ZP of the erythrocytes of malaria patients as compared with that of the healthy volunteers. Thus it can be concluded that plasmodium species remodels the host erythrocytes and hence reduces the membrane electrochemical potential of erythrocytes of the malaria patients.

INTRODUCTION: Mainly as a result of the survival advantage due to the protection against malaria conferred by the heterozygous state, inherited red cell disorders are the most common monogenic diseases affecting over a billion people around the world. Malaria is a serious infectious disease in humans which is caused by five species of the malaria parasites of the genus Plasmodium *i.e. P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*. Each year, up to 300 - 500 million people are infected with malaria parasites and a million (predominantly infants and young children) die as a consequence of the infection [1 - 2].

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Malaria infections are established by parasites released into the blood stream when parasite-infected female mosquitoes bite a vertebrate host; the consequent infection that follows occurs first in the liver followed by the erythrocytes. The clinical signs and symptoms of malaria infections in humans include chills, fever, body aches, headache, anemia, and spleen enlargement, while infections with *Plasmodium falciparum*, the most virulent, cerebral malaria-causing species, can be fatal [3]. The clinical symptoms of malaria manifest when parasites invade and multiply inside red cells.

A large number of interactions occur between various malarial and red cell proteins during all phases of the parasite life cycle **Fig. 1**, starting at the initial stages of invasion and continuing through 48 hr of intra - erythrocytic development and eventual rupture of the infected red cell at the end of the parasite life cycle [4]. Intracellular development of the parasite is accompanied by a number of striking structural, biochemical and

functional changes in red cells, a subset of which are strongly associated with parasite induced modifications to the red cell membrane. Infections with Plasmodium also induce cellular and molecular alterations to erythrocytes, such as cell adhesion [5 - 8], Band 3 clustering [9], erythrocyte-IgG association [10], increased hemichrome attachment to the host erythrocyte membrane [9], increased cell permeability [11], changes in erythrocyte rigidity[12-14] and with certain parasite species, the appearance of knob-like structures on the cell surface [15 - 19].

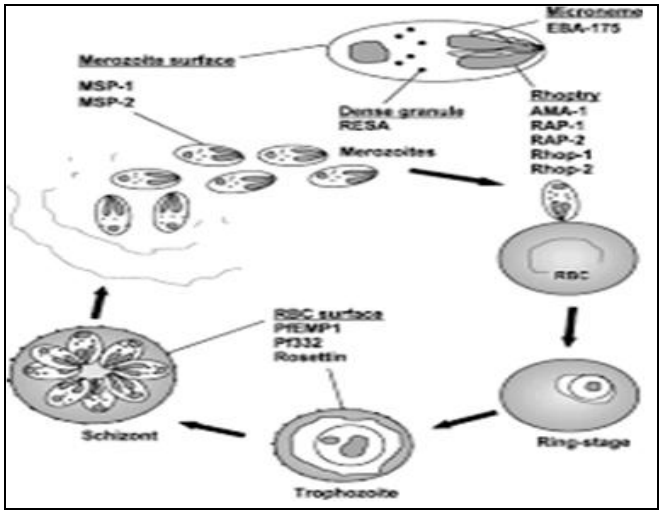

FIG. 1: RED CELL LIFE CYCLE OF MALARIA PARASITE

(The various parasite proteins (RESA, PfEMP1, Pf332, Rosettin) expressed at different stages of parasite development that interact with red cell membrane are indicated).

These features of human malaria parasites are reported to vary in different hosts but are not known for all parasite species and hosts at the erythrocyte level. *Plasmodium falciparum* causes the most deadly form of human malaria. During its stage of intra-erythrocytic development, the parasite dramatically remodels the membrane of its host red blood cell (RBC). In *P. falciparum* trophozoite-infected RBCs ('iRBCs') the most significant morphological membrane modification is the placement of electron-dense 'knobs' that protrude from the iRBC surface. Whereas, these knobs are complex structure which are formed by the aggregation of RBC and parasite-derived proteins. These modifications could significantly impact the electrochemical dynamics of the iRBC surface and the physical interaction of iRBCs with other cells [20 - 22]. Expression of parasite-derived proteins on the erythrocyte membrane disturbs the balance of endogenous host proteins and their structural integrity. Addition of new proteins induces protein-protein modifications and protein-lipid interactions at the erythrocyte membrane [23 - 26], as well as cytoskeleton remodeling, thereby resulting in changes in the net surface charge of the cell membrane.

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 cell surface charge is assessed by measuring their electrokinetic potential or zeta potential (ZP), which characterizes the electrical double-layer potential on the cell surface. Membrane electrochemical potential or zeta potential (ZP) is a feature of the molecular profile of the cell membrane and is determined by the net electrical charge of surface-exposed molecules. The RBC membrane is negatively charged and is surrounded by a fixed layer of cations in the medium. This fixed layer of cations is surrounded by a cloud-like diffused layer of a mixture of cations and anions. Within the diffused layer, Brownian motion of RBCs and the flow of medium create a 'shear' plane, which separates unfixed ions from those ions closely associated with the fixed layer. The potential at the shear plane is defined as the ZP [27 - 29]. Cell electrophoresis has been conventionally used to record the zeta potential of human cells [30].

Due to remodeling of the plasmodium infected host erythrocyte membrane, the changes in the net surface charge of the cell membrane can be studied by the variation in the zeta potential (ζ) of normal and plasmodium infected erythrocytes. This work was aimed at assessing the variation in the zeta potential of normal human erythrocytes and plasmodium infected erythrocytes using the cell electrophoresis technique using Zeta-Meter System 4.0.

MATERIALS AND METHODS:

Materials: Dextrose anhydrous (Merck), Distilled water, Lancet, Rectified spirit, Zeta meter system 4.0

Methods:

Blood Sample Collection: Blood samples of the malaria patients were collected from Malaria Department, Nagpur Municipal Corporation,

Nagpur and Dr. Varma Pathology Laboratory and Dr. Chinche Hospital, Katol. The volunteers were selected from Department of Pharmaceutical Sciences, R.T.M.N.U. Nagpur and blood samples were taken. The blood samples were collected by simple finger pricking procedure without causing any pain or difficulty to patient as well as to healthy human volunteers by using sterile Precision Glide™ Needle 26G × ½ (0.45 mm × 13 mm). In case of patient the blood sample was collected itself by the laboratory technician of the hospital and the pathology lab.

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.

Blood Suspension Preparation: Approximately 0.01 ml blood was transferred into 50 ml of freshly prepared isotonic dextrose solution. Mean values of the 10 readings was used to calculate the zeta potential according to the basic Helmholtz-Smoluchowski Eq. (1) as follows:

$$\text{Zeta Potential, } \zeta = \frac{4\pi \times Vt \times EM}{Dt} \quad .....(1)$$

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

Estimation of Zeta Potential of Normal and Plasmodium Infected Erythrocytes: The zeta potential of the normal and plasmodium infected erythrocytes was measured using Zeta meter System 4.0. Zeta potential is purely an electro kinetic property of the electrical double layer surrounding the system but 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.

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

Where; ζ_d = electrokinetic 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/sec, E = potential gradient in V/cm[10].

A special capillary cell called electrophoresis 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 electrophoresis 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 electrochemical potential or ZP 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 blood samples were obtained from normal healthy volunteers and malaria patients by simple finger pricking procedure and its suspension was prepared as mentioned above. The temperature of this suspension was measured and detection parameters for ZP measurements such as light intensity, focal plane and tracking duration were optimized for stable data collection. The electrophoresis cell was cleaned and calibrated using min-u-sil and the prepared blood suspension was placed under the zeta-meter stage and the mobility of individual erythrocyte was tracked by equipped Zeta meter-ZM4DAQ software using microscopically-acquired video images and data was recorded 10 times for each sample and average zeta-potential in mV was determined using a standard Helmholtz–Smoluchowski formula.

RESULTS AND DISCUSSION: The results of blood samples obtained from both patients and volunteers 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. **Table 1** shows the zeta potential (ZP) values of the erythrocytes of the healthy volunteers in milli Volts (mV) while **Table 2** shows the zeta potential (ZP) values erythrocytes of the malaria patients in mV. For healthy volunteers the average ZP value was found to be 22.57 ± 0.2984 mV and for malaria patients the ZP was found to be 13.09 ± 0.8456 mV. The results as shown in **Fig. 2** revealed that there is a significant decrease in the ZP of the erythrocytes of malaria patients as compared with the healthy volunteers.

TABLE 1: ZETA POTENTIAL OF ERYTHROCYTES OF NORMAL HEALTHY VOLUNTEERS

Sr. no.	Zeta Potential	Sr. no.	Zeta Potential	Sr. no.	Zeta Potential	Sr. no.	Zeta Potential
1	20.86 (1.73)	2	24.22 (1.14)	3	22.96 (0.4)	4	19.61 (0.34)
5	20.09 (1.77)	6	19.46 (0.93)	7	25.39 (1.95)	8	21.01 (0.97)
9	20.16 (0.36)	10	20.48 (1.02)	11	25.44 (1.02)	12	23.93 (1.24)
13	21.64 (0.97)	14	25.46 (0.82)	15	21.76 (0.51)	16	20.81 (0.73)
17	20.31 (0.34)	18	20.37 (0.6)	19	21.64 (0.97)	20	25.33 (0.76)
21	25.78 (0.68)	22	24.56 (1.32)	23	23.69 (0.73)	24	22.92 (0.71)
25	20.9 (0.6)	26	25.81 (1.05)	27	25.91 (0.38)	28	23.79 (0.53)
29	21.54 (2.13)	30	20.53 (0.67)	31	24.12 (1.36)	32	21.79 (0.55)
33	24.32 (0.89)	34	20.35 (0.66)	35	21.32 (1.04)	36	26.26 (0.96)
37	25.06 (0.65)	38	19.04 (0.67)	39	25.84 (1.49)	40	20.77 (2.52)
41	25.65 (1.62)	42	25.82 (1.65)	43	20.27 (0.85)	44	19.23 (0.9)
45	20.13 (1.34)	46	21.32 (1.04)	47	21.65 (0.98)	48	24.39 (2.81)
49	25.12 (0.61)	50	23.00 (0.66)	51	22.69 (1.13)	52	22.1 (0.54)
53	19.25 (1.92)	54	22.35 (1.77)	55	23.06 (1.67)		

Note: ZP values are mean of ten readings ( ) Figures denote ± standard deviation

TABLE 2: ZETA POTENTIAL OF ERYTHROCYTES OF MALARIA PATIENTS

Sr. no.	Zeta Potential	Sr. no.	Zeta Potential	Sr. no.	Zeta Potential	Sr. no.	Zeta Potential
1	0.78 (0.24)	2	11.81 (3.66)	3	14.00 (1.26)	4	10.71 (3.81)
5	13.39 (0.33)	6	17.07 (0.4)	7	15.48 (0.43)	8	14.36 (1.98)
9	15.01 (0.64)	10	17.12 (0.6)	11	11.04 (1.59)	12	11.81 (3.66)
13	9.77 (1.3)	14	0.15 (0.04)	15	17.42 (1.54)	16	16.22 (2.09)
17	16.56 (0.8)	18	15.7 (0.57)	19	16.47 (1.13)	20	16.7 (2.76)
21	6.51 (0.16)	22	16.82 (0.95)	23	6.38 (0.55)	24	16.31 (2.92)
25	16.92 (0.97)	26	17.26 (1.6)	27	10.30 (0.93)	28	15.01 (1.85)
29	11.00 (0.58)	30	14.69 (0.94)				

Note: ZP values are mean of ten readings ( ) Figures denote ± standard deviation

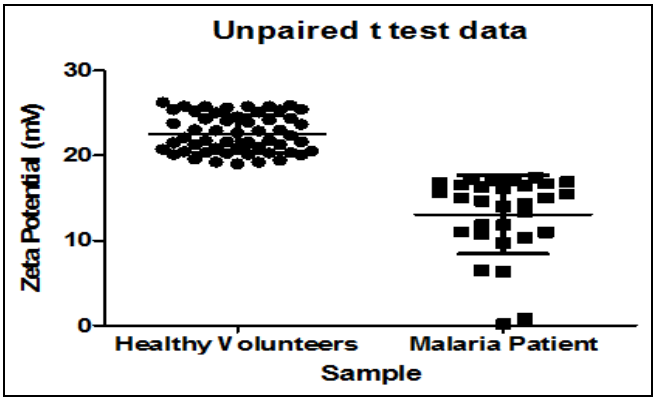

FIG. 2: COMPARISON OF ZETA POTENTIAL BETWEEN HEALTHY HUMAN VOLUNTEERS AND MALARIA PATIENTS

CONCLUSION: The Zeta potential values of the normal erythrocytes and plasmodium infected erythrocytes 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, the zeta potential measurements for the surfaces of non parasitized and parasitized (*Plasmodium*-infected) erythrocytes was examined. The electrochemical potential value obtained for Plasmodium-infected erythrocytes was found to be reduced in comparison with the non parasitized erythrocyte. Thus it is concluded that *Plasmodium* species are intracellular parasites that remodel host erythrocytes by expressing their own proteins on erythrocyte membranes and hence reduces the Zeta Potential of plasmodium infected 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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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest regarding this manuscript.

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