EFFECT OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY ON SOME BIOPHYSICAL PROPERTIES OF HUMAN ERYTHROCYTES

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Abstract. The most widespread erythrocytes enzyme defect throughout the world is Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. The diagnosis of G-6-PD deficiency may be missed during acute hemolytic episode due to young RBC's which have good enzyme activity. However, this might be detected at a later stage when patient is asymptomatic. This calls for a detailed investigation of the patient with acute attack of hemolysis at a later stage. Blood samples were obtained from 45 G-6-PD deficient male patients with previously diagnosed disease {25 subjects were during hemolytic attack (Gp1) while the rest were outside acute hemolytic crisis (Gp2)} and 20 healthy male subjects. The effect of G-6-PD deficiency on erythrocytes of the previous groups was investigated by scanning electron microscope (SEM) complemented with dielectric spectroscopy. The quantification of the morphological shape changes revealed that Gp1 had a significant increase in spheroechinocytes as well as in the morphological index in comparison with those of all other groups. The overall electrical and morphological properties of the red cell membrane of these subjects modified to a great extent during hemolytic attack. This may have important diagnostic and research implications. Thus the combined application of dielectric spectroscopy and SEM can be used as an efficient manner for monitoring abnormalities in the blood and erythrocytes due to G-6-PD deficiency.

Key words: G-6-PD deficiency; erythrocyte; scanning electron microscope; dielectric spectroscopy; biophysical properties.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G-6-PD) is the most important enzyme of the pentose phosphate pathway, which generates reduced nicotinamide adenine dinucleotide phosphate (NADPH) and provides the most important defense mechanism for erythrocytes against oxidative stress [30]. G-6-PD deficiency is the most common genetic disorder with a worldwide distribution involving more than 200 million people. Deficiency of this enzyme leads to hemolytic crisis and favism

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in the case of oxidant attack to erythrocytes, especially in individuals from the Mediterranean region [15]. The diagnosis of G-6-PD deficiency may be missed during acute hemolytic episode due to young RBC's which have good enzyme activity. However, this might be detected at a later stage when the patient is asymptomatic.

Biochemical and molecular-biological analyses have indeed defined red-cell deficient G-6-PD, while biophysical characterization defines the corresponding membrane alterations. The combination of these approaches provides a clear understanding both of the molecular basis for red cell abnormalities and of the functional consequences of these abnormalities. Dielectric spectroscopy, that measures permittivity and conductivity as a function of frequency in a non-invasive way, provides insights into the structural and electrical properties of biological cells which have not only biophysical and medical scientific significance, but also might have useful clinical applications. Cha *et al*. [9] and Rigaud *et al*. [27] showed that electrical parameters can be a potential index for evaluating blood in clinical applications. Alterations in the physicochemical properties of erythrocyte membranes in a number of pathological states have been described by Butterfield *et al*. [8]. Bonincontro *et al*. [7] measured the microwave dielectric properties of normal and homozygous beta-thalassemic erythrocytes.

There are two major dispersion mechanisms of interest for cell suspensions in the frequency range below 100 MHz. The lower frequency dispersion is typically centered around l–10 kHz for RBC's [12]. The second one is the β-dispersion, which is known as structural relaxation and occurs at the radiofrequency range of 10 kHz to 10 MHz. Since the dielectric relaxation of this kind is sensitive to cell shape, dielectric spectroscopy is effective in detecting changes in shapes of erythrocyte [5]. The study of cell shape transformation of human erythrocyte is of great hematological interest because several clinical conditions are associated with erythrocyte shape changes [31]. The red blood cell membrane skeleton mostly determines the shape (discoid), deformability (rheological properties) and durability (half-life and resistance to shear stress) of the erythrocytes [1]. MODIFICATION of cytoskeletal composition and/or organization can alter erythrocytic properties and shapes.

The aim of the present study is to investigate the effect of G-6-PD deficiency on human erythrocytes by means of parallel application of two techniques: that examining cell morphology, scanning electron microscopy (SEM), and that analyzing membrane functionality; dielectric spectroscopy. The combined application of these techniques allowed an accurate description of the effects induced by G-6-PD deficiency on human erythrocytes in terms of morphological and functional parameters.

MATERIALS AND METHODS

SUBJECTS

This study was conducted at Kasr El-Eini Hospital after the approval of pediatric department (Faculty of Medicine, Cairo University) and the Local Ethics and Research Committee. Infants were consecutively enrolled if informed consent had been given by their parents.

Blood samples were obtained from 45 G-6-PD deficient male patients with previously diagnosed disease between the ages of 1 and 12 years {25 patients were during hemolytic attack (Gp1) while the rest was outside acute hemolytic crisis (Gp2)} and 20 healthy males (C) matched with the G-6-PD-deficient patients for age. The study was limited to males, as G-6-PD deficiency is an X-linked condition, and in males, who can be either normal or deficient hemizygotes, the G-6-PD status can be accurately determined. In females, on the other hand, a high number of G-6-PD-deficient heterozygotes may be encountered, a form of the condition which may be difficult to diagnose by biochemical methods [6]. Mothers of these patients (30–45 years) were apparently healthy and accepted as obligate heterozygous (Gp3). Written informed consents were obtained from all groups.

G-6-PD ACTIVITY DETERMINATION

Blood samples from all subjects were drawn into heparinized tubes. The samples were centrifuged at 1000×g for 10 min at 4 °C. Plasma and buffy coat were removed by aspiration, and erythrocytes were washed three times with ice-cold physiologic saline. Hemolysates were prepared by adding 1 volume of blood to 19 volumes of ice-cold deionized water. G-6-PD activities of hemolysates were assayed by the recommended modified Zinkham method [32]. Briefly, 50µl hemolysate was mixed with 850 µl of reaction mixture (2 mM $NADP^+$, 0.1 M MgCl2, 6 mM glucose-6-phosphate and Tris-HCl buffer (1 M, pH: 8)) and the absorbance at 340 nm was monitored for 10 minutes. G-6-PD activities were expressed as units / g of hemoglobin (one unit, U = 1 µmol of NADPH formed / min., 1 U = 1/60 micro katal).

RBC MORPHOLOGICAL ANALYSIS

The morphology of RBC's was studied using (SEM) according to Kaul *et al*. [24] as follows:

Blood samples were collected into 50 µg/ml heparin, stored at 4 °C and protected from light. Blood aliquot was fixed with 2.5% glutaraldehyde immediately after collection.

Erythrocytes were separated from plasma and buffy coats by centrifugation at 600×g for 10 minutes at 4°C and washed three times with: ice cold 5 m mol / l sodium phosphates, 145 mmol / l NaCl (pH 7.4) and post fixation in osmium tetroxide 1% respectively.

For observation, the erythrocyte samples were diluted in a solution of ethanol and propylene oxide (1:1, v/v), a drop was deposited directly onto the microscope support, air dried, coated with gold (by sputtering) and examined with SEM, Model Philips XL30 with accelerating voltage 30 kV, magnification of 10× up to 400.000× and resolution for w (3.5 nm).

To evaluate the erythrocyte shape changes quantitatively, stages of morphological alterations were estimated (as percentages) on electron micrographs as shown in the classification of Hsu *et al*. [22], as echinocytes 1, echinocytes 2, echinocytes 3 and spheroechinocytes. Type I echinocytes are described as irregularly shaped erythrocytes without defined spicules. Type II echinocytes have cellular projections that vary in length but maintain a disc-shaped appearance. Type III echinocytes are more spherical erythrocytes with high spiculation. Spheroechinocytes are spherical small erythrocytes (they have approximately two-thirds the diameter of normal RBC) with blunted spicules.

The morphological index was calculated according to Fujii *et al*. [18] as follows:

$$\textit{Morphological index} = \Sigma\,(\textit{morphological score}) \times [(\textit{number of distorted cells}) / (\textit{total cell number})],$$

where morphological scores of +1, +2, +3, and +4 correspond to each shape stage referred to above, respectively, the discocytes score was considered as 0. Approximately 500 cells were counted, distributed in five randomly selected fields.

DIELECTRIC MEASUREMENTS

Red blood cell preparation

After centrifugation of RBCs from fresh human blood, the buffy coat and plasma were removed. Then the RBCs were washed twice in phosphate buffer saline (PBS) with pH=7.4 (containing 5.8 mM phosphate buffer, 150 mM NaCl and 417 mg / l KCl) at 20 °C. Haematocrit (Hct) and mean cell volume were measured by means of an electric cell counter (Royco-cell 920A Coulter) before each dielectric measurement. Falling hemoglobin or Hct values, accompanied by elevated reticulocyte counts, classical hallmarks of hemolysis were inconsistently encountered in G-6-PD-deficient groups. While in some cases Hct value was lower in G-6-PD-deficient ones than in controls, in others hematological indices did not

reflect hemolysis. So, the Hct of the samples used for dielectric measurements was adjusted to standard Hct (40%) by adding or removing a calculated amount of autologous plasma obtained by centrifugation at 1400g for 6 min.

Measurements of dielectric properties of erythrocytes were carried out by means of a resonance technique. This method made use of a commercially available LCR bridge model HIOKI "3532 LCR HiTESTER HIOKI E.E. Corporation manufactured in Japan, together with a sample cell type PW 9510/60 manufactured by Philips. The sample cell has two squared platinum black electrodes, each having an area of one cm^2 with an inter-electrode distance of one cm. The sample cell was kept at 37±0.1°C in a temperature controlled incubator; then the dispersion characteristic in the frequency range 20 Hz to 10 MHz was investigated. Each run was taken five times and the data points are the average of these runs.

Correction of residual electrode polarization and determining dielectric parameters were performed in accordance with Ali [3].

The conductance of the sample G is given by

$$G_s = 1/R_s = \sigma A/d = 2\pi f \varepsilon_o \varepsilon'' A/d \tag{1}$$

where d is the distance between the two electrodes, A is the area of each electrode, f is the frequency, ε_o is the permittivity constant of free space and ε'' is the dielectric loss.

The capacitance of the sample is given by

$$C_s = \varepsilon' \varepsilon_o \frac{A}{d} \tag{2}$$

where ε' is the relative permittivity of the sample.

Cole-Cole equation [10] is given by

$$\varepsilon^* - \varepsilon_\infty = (\varepsilon_o - \varepsilon_\infty)/[1 + (i\omega\tau_o)^{1-\alpha}] \tag{3}$$

where α ($0 < \alpha < 1$) is the Cole-Cole spread parameter, τ is the relaxation time which is related to the critical frequency (f_c) by $\tau = 1/(2\pi f_c)$.

Knowing that $i^{(1-\alpha)} = e^{i(1-\alpha)\pi/2}$, $e^{i\theta} = \cos(\theta) + i \cdot \sin(\theta)$, and using the trigonometric relations for the subtraction of two angles, it could be shown that the real and imaginary parts of Cole-Cole equation can take the forms

$$\varepsilon'(f) = \varepsilon_\infty + \sum_n \frac{\Delta\varepsilon_n \left[1 + \left(f/f_{c_n}\right)^{1-\alpha_n} \sin\left(\alpha_n \pi/2\right)\right]}{1 + \left(f/f_{c_n}\right)^{2(1-\alpha_n)} + 2\left(f/f_{c_n}\right)^{1-\alpha_n} \sin\left(\alpha_n \pi/2\right)} \tag{4}$$

$$\varepsilon''(f)=\sum_n \frac{\Delta\varepsilon_n\left[\left(f/f_{c_n}\right)^{1-\alpha_n}\cos\left(\alpha_n\pi/2\right)\right]}{1+\left(f/f_{c_n}\right)^{2(1-\alpha_n)}+2\left(f/f_{c_n}\right)^{1-\alpha_n}\sin\left(\alpha_n\pi/2\right)}+\frac{\sigma_s}{2\pi f\varepsilon_o} \tag{5}$$

The dielectric data were fitted to the summation of multiple Cole-Cole dispersions in addition to a conductivity term [25] in which σ_s is the static ionic conductivity. Origin 6.1 software was used to handle the fitting process.

BLOOD IMPEDANCE

β dispersion can be expressed as a Cole–Cole locus [11] in which the imaginary part of the impedance is the ordinate and the real part is the abscissa. Fricke and Morse [17] suggested that the impedance of the blood in the β dispersion might be approximately simulated by a three-element circuit as shown in Fig. 1.

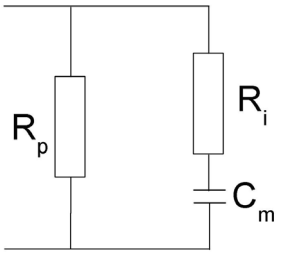

Fig. 1. Three element model of blood, R_p represents plasma resistance, R_i cell interior resistance and C_m cell membrane capacitance.

The values of the three elements of the model, R_p represents plasma resistance, R_i cell interior resistance and C_m cell membrane capacitance, could be determined by measuring the impedance amplitudes at three different frequencies [33]. The impedance of the circuit is calculated as

$$Z(\omega)=R_p\left\{\left(1+\omega^2C_m^2R_i^2\right)\Big/\left[1+\omega^2C_m^2\left(R_p+R_i\right)^2\right]\right\}^{1/2} \tag{6}$$

If three impedance amplitudes $Z(\omega_o)$, $Z(\omega_1)$, $Z(\omega_2)$ at different frequencies ω_o, ω_1, ω_2, are measured, the three elements can be obtained:

$$R_p=K_o^{1/2}Z(\omega_o) \tag{7}$$

$$R_i=R_p\Big/\left[\left\{K_0\left[K_1-K_0-F_1\left(1-K_0\right)\right]\Big/\left[K_1-K_0-F_1K_1\left(1-K_0\right)\right]\right\}^{1/2}-1\right] \tag{8}$$

$$C_m = (1/\omega_o) \left\{ (1-K_0) \Big/ \left[K_0 R_i^2 - (R_p + R_i)^2 \right] \right\}^{1/2} \quad (9)$$

where

$$F_1 = (\omega_1/\omega_o)^2,\ F_2 = (\omega_2/\omega_o)^2,\ K_1 = [Z(\omega_1)/Z(\omega_o)]^2,\ K_2 = [Z(\omega_2)/Z(\omega_o)]^2$$

and

$$K_0 = \frac{F_1K_2(1-K_1)(1-F_2) - F_2K_1(1-K_2)(1-F_1)}{F_1(1-K_1)(1-K_2F_2) - F_2(1-K_2)(1-K_1F_1)}$$

To evaluate the validity of the three-element model, the impedance–frequency (I–F) curves of blood samples were measured over the frequency range from 10 kHz to 1.2 MHz. The impedances at 100 kHz, 800 kHz and 1.2 MHz were used to calculate R_p, R_i and C_m from which theoretical I–F curves were obtained.

STATISTICS

All values have been expressed as mean ± standard deviation. The significance of the differences between each value presented by various groups was evaluated by the Student t-test and values with $p < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

The erythrocyte G-6-PD activity was lower than 1U / g Hb for hemizygous groups (Gp1 and Gp2), while it ranged between 1.8 – 4.70 U / g Hb for heterozygous group (Gp3). This is significantly low ($p < 0.001$) in comparison with the control group (5.25 – 8.73 U / g Hb).

The mothers of G-6-PD deficient male individuals should have at least one defective X chromosome; one of the X chromosomes in female cells is inactivated at the early stages of cell development and the active chromosome might be G-6-PD defective or normal. This is a random process and results in RBC with normal or absent G-6-PD enzyme activity. Therefore, obligatory heterozygous individuals expressed various levels of G-6-PD activity, but in most cases have lower enzyme activity compared to the control group (C), at a level determined by the ratio of the cells with deficient active X chromosome.

The G-6-PD deficient subjects are not different from normal subjects under normal conditions unless they are exposed to oxidant stress, e.g., digesting *Vicia faba* beans or using antimalarial, and analgesic drugs. Even in low levels, the existing enzyme capacity of deficient individuals is enough to survive their normal way of

living without causing a significant difference in structure and function of erythrocytes. In fact, normal persons use 0.1% of the maximum G-6-PD capacity in their erythrocytes [4]. This situation shows that, in normal conditions, the necessity for this enzyme is very limited.

Fig. 2 shows representative scanning electron micrographs of erythrocyte samples of the studied groups. Figs. 2(a) and 2(b) show normal discoid shape red cells of a control healthy subject and erythrocyte sample of heterozygote female with G-6-PD activity of 3.75 U / g Hb respectively. Fig. 2c shows red cells, with surface and margin changes, of homozygous case (Gp2). Higher magnification (×8000) of a crenated red cell is shown in Fig. 2d. Echinocytes II, III and spheroechinocytes (Fig. 2e) are present in the scanning electron micrograph of erythrocytes of a case with G-6-PD deficiency during attack (×2000). Statistical analysis was performed on the data collected from 500 erythrocytes distributed in five different fields for each sample.

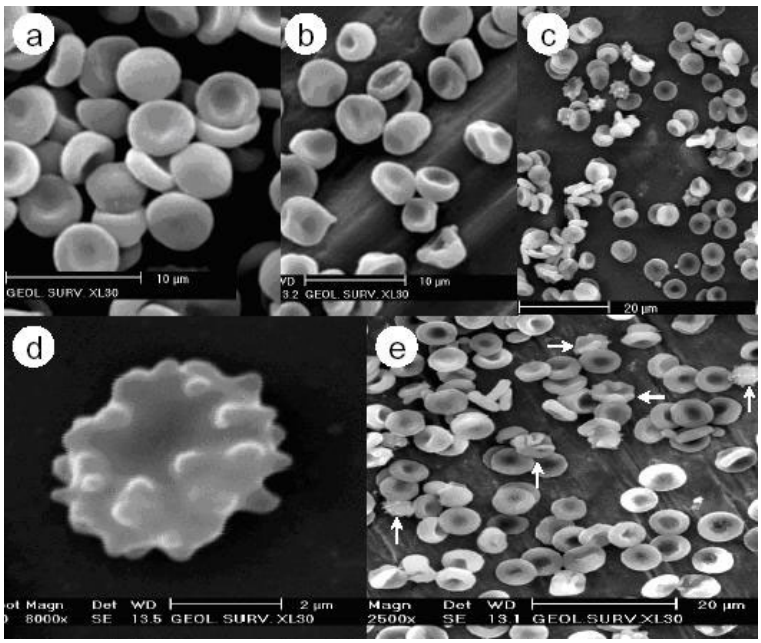

Fig. 2. Scanning electron micrographs of RBC's for representative cases of: Control healthy subject (a), heterozygote female with G-6-PD activity of 3.75 U / g Hb (b), homozygous G-6-PD deficient subject (c), higher magnification of a crenated red cell (d) and G-6-PD deficient subjects during attack (e). Arrows pointed to spheroechinocytes.

The present data revealed that Gp 1 had a highly significant number ($p < 0.01$) of spheroechinocytes and a high value of morphological index in comparison with those of all groups, as presented in Table 1. This may be interpreted as follows:

A basis for a quantitative theory of echinocyte transformation is provided by the bilayer-couple hypothesis (model), proposed by Sheetz and Singer [29]. According to this hypothesis, any factor which leads to expansion of the outer membrane leaflet relative to the inner one produces a tendency to form convex structures, as the echinocytic spicules. Conformational change of integral membrane proteins could lead to an expansion of one leaflet of the membrane double layer relative to the other one and in turn results in a shape change [20].

The primary metabolic consequence of G-6-PD deficiency is the diminished ability of the variant enzyme to generate sufficient NADPH to keep up with the requirement for reduced glutathione in a red cell population stressed by oxidizing agents. Depletion of cellular glutathione allows toxic oxygen products to damage red cell macromolecules, including hemoglobin, band 3, spectrin, membrane lipids, and other molecules [23]. Dulinska *et al*. [14] showed that G-6-PD deficiency causes impaired metabolism of RBC's and particularly ATP production, which might be the main reason for the change of elasticity and shape of erythrocytes. After cells exhaust their ATP they become spiculate spheres. Moreover, ATP depletion would lead to spectrin dephosphorylation, which was thought to rearrange the cytoskeleton and contract the inner monolayer [16]. G-6-PD-deficient erythrocytes are more susceptible to echinocytosis and do not recover the normal disk shape as readily as normal ones [2].

In order to clarify better the effect of G-6-PD deficiency on the red blood cell and to characterize the structural changes occurring at membrane level the electrical properties of erythrocytes were measured by means of frequency domain dielectric spectroscopy. This method has been employed previously on a large variety of cells and its effectiveness for investigating the electrical properties of cell membranes is well established.

Dielectric relaxation measurements were made for erythrocytes samples over a frequency range of 20 Hz to 10 MHz. The dielectric data for erythrocytes were fitted to several commonly used functions. First, an attempt was made to describe the data with single or double Debye relaxation processes. The resulting fit was rather poor as was that resulting from a single Cole-Cole relaxation process. A superposition of two Cole–Cole relaxation processes yielded an excellent fit (data not shown).

Cellular dielectric parameters, such as membrane capacitance and conductivity, have been shown to respond sensitively to changes in cell morphology and physiology. Sezdi *et al*. [28] showed that chemical alterations of RBC's during storage eventually affect the electrical properties of blood.

The changes in the Cole–Cole equation parameters: dielectric increment (Δ_1), static conductivity (σ), spread of relaxation time (α_1) and relaxation time (τ_1) of erythrocyte samples for the studied groups are shown in Fig. 3(a, b, c and d) respectively.

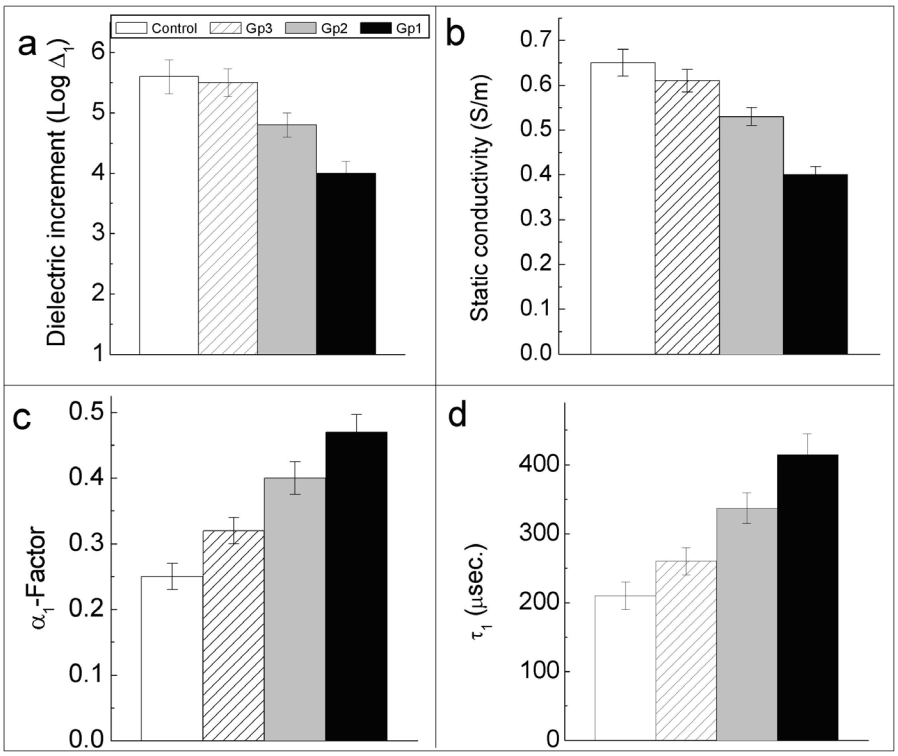

Fig. 3. Changes in the Cole–Cole equation parameters: dielectric increment (Δ1), static conductivity (σ), spread of relaxation time (α1) and relaxation time (τ1) (respectively) for erythrocyte samples of the studied groups.

Cell membranes are composed of lipids, and can be modeled electrically by a capacitor between intra- and extracellular electrolyte. When cells died, eventual rupture of the cell membrane occurred. Therefore, impedance between intra- and extracellular space for these cells decreased, since the membrane lost its insulating property [21]. This may give reason for the significant reduction of the dielectric increment (Δ_1), with the decrease in G-6-PD concentration (Fig. 3a).

In the present work, we use a frequency-domain dielectric spectroscopy approach to evaluate possible changes in the passive electrical parameters of the erythrocyte membrane, i.e., the membrane conductivity (a measure of the overall ionic transport across the cell membrane through ion channels and/or pores) and the membrane permittivity (which takes into account the static distribution of charges and/or polar groups across the cell membrane). As shown in Fig. 3b, a significant decrease of σ value was observed for Gp1 and Gp2 in comparison with the control group. Alteration in the passive membrane electrical properties indicates changes at molecular level in the structural organization of the membrane. As discussed before, due to G-6-PD deficiency the red blood cells undergo certain

physical and chemical changes such as shape transformation (Fig. 2), loss of surface charges and decrease in ion permeability of cell membranes. This affects the electrical impedance of blood since the latter depends on the state of blood cells and plasma. Furthermore, the change in lipid architecture of the inner layer could account for the decrease of the dielectric constant and conductivity [13].

The increase in α_1 (Fig. 3c) with the decrease in G-6-PD concentration, indicates a broadening of the dispersion and suggests that as different cells respond at different rate to changes in G-6-PD concentration the distribution in cell sizes broadens. The smallness of the change in α and its sensitivity as a fitting parameter make the change the most speculative, although similar changes are seen in other datasets. Variation of the relaxation time (τ_1) with G-6-PD concentration (Fig. 3d) is compatible with that of α_1 and may be explained upon the same reasons discussed previously.

This is in agreement with Fujii *et al*. [19] who showed that electrical properties of blood change with the deformation of red blood cells. Thus changes in ion concentrations and ATP affect the electrical properties of blood directly. Variation of the other Cole–Cole parameters (Δ_2, α_2 and τ_2) (data not presented) for the studied groups is compatible with that of Δ_1, α_1 and τ_1 and may be explained upon the same reasons discussed previously.

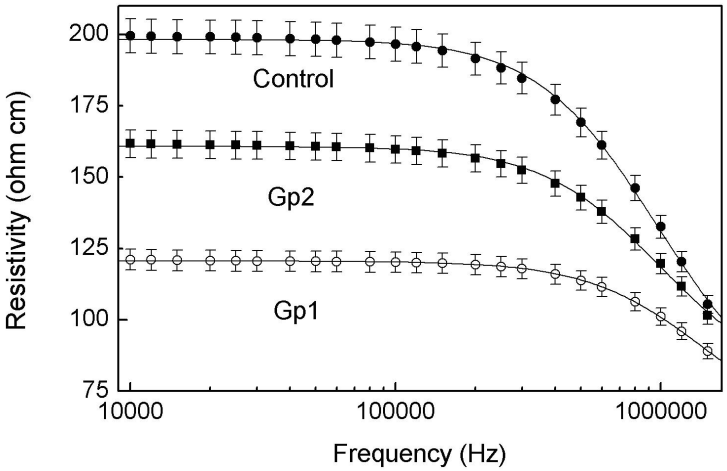

Fig. 4. Measured and calculated impedance–frequency curves for blood samples of healthy control subjects (C), G-6-PD deficient male patients during hemolytic attack (Gp1) and subjects outside acute hemolytic crisis (Gp2).

The present data revealed that the estimated dielectric parameters well reflected the changes occurring to erythrocytes due to G-6-PD deficiency. The degree of cell disruption as determined by the dielectric parameters well corresponded to those obtained by SEM. So, cell's passive electrical properties can

be used as a useful sensitive probe for studying the alteration of the membrane structure induced by G-6-PD deficiency in a non-invasive manner. This is in agreement with Ulgen and Sezdi [28] who showed that Cole-Cole parameters can be a potential index for evaluating blood in clinical applications.

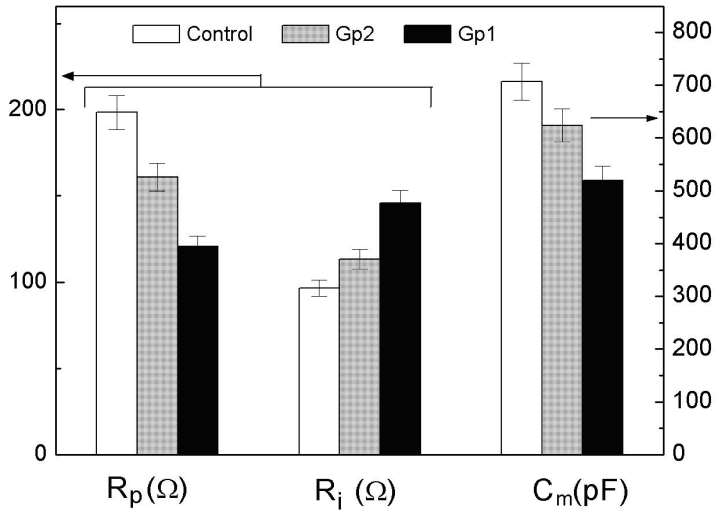

Fig. 5. Changes in red cell membrane capacitance (C_m), plasma resistance (R_p) and red cell interior resistance (R_i) for the studied groups.

Measured and calculated impedance–frequency curves for blood samples of healthy control subjects (C), Gp1 and Gp2, are shown in Fig. 4. As shown in Fig. 4, the measured and theoretical impedance–frequency curves agreed closely, and the impedance of blood could be approximately simulated by a three-element circuit. Hence the triple frequency technique seems useful for simultaneous measurement of red cell membrane capacitance, plasma resistance and red cell interior resistance. There is no significant change in the impedance–frequency curve of Gp3 in comparison with the control group (data not shown). Results (Fig.5) showed that the influence of G-6-PD on the blood is manifested as changes of red cell membrane capacitance, plasma resistance and red cell interior resistance. Changes in red cell membrane capacitance (C_m), plasma resistance (R_p) and red cell interior resistance (R_i) for the studied groups are shown in Fig. 5.

C_m is of special interest, since otherwise the properties of the membranes can only be studied with more complicated methods, such as electron microscopy and chemical analysis. As an easily measured parameter, C_m might be used for screening purposes (Zhao *et al*. [33] showed that measurement of membrane capacitance might be of clinical interest since higher values were found for blood samples from patients with high sedimentation rates).

As shown in Fig. 5, there is a significant decrease ($p < 0.01$) in C_m and R_p concomitantly with an increase in R_i with the decrease in G-6-PD concentration. It

should be noted that R_p and R_i are not the specific resistance of plasma and intercellular fluid themselves, but the contributions of plasma and cell interior to the total impedance of whole blood. Similarly, C_m is not the specific capacitance of the membranes, but the capacitance of the whole blood contributed by the cell membrane. This may explain the significant decrease in blood impedance for Gp1, as shown in Fig. 4.

CONCLUSION

The present data demonstrate that changes in membrane properties associated with overall erythrocyte shape transformation affect its electrical properties. Moreover, the data presented here clearly showed that dielectric differences between erythrocytes of G-6-PD-deficient subjects and their healthy counterparts are large. This demonstrates the capability to detect changes of erythrocyte membrane that can be correlated with structural alterations typical for certain blood diseases such as G-6-PD deficiency.

The overall electrical and morphological properties of the red cell membrane of these subjects modified to a great extent during hemolytic attack. This may have important diagnostic and research implications.

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