Changes in Molecular Size and Shape of Hemoglobin of Systemic Lupus Erythematosus

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Abstract

Different hemoglobin derivatives concentration and electrical conductivity were measured for patients suffered from, in addition the dielectric relaxation in the frequency range 100KHz up to 10MHz of Hb molecule of systemic lupus patients compared to normal control. Abnormal dynamic motion of Hb was found, a lack of changes in the size and/or shape of Hb. Also, the dielectric results indicating that the molecular shape tends to deviate from the spherical form as the activity (severity) of the disease increases.

Key Words: Systemic lupus – Dielectric properties – Abnormal motion – Hemoglobin.

Introduction

SYSTEMIC Lupus Erythematosus (SLE) is a chronic, usually life-long, potentially fatal autoimmune disease [1]. Lupus is one of many disorders of the immune system known as auto immune disease. In auto immune diseases the immune system turns against parts of the body it is designed to protect. This leads to inflammation and damage to various body tissues. Lupus can affect many parts of the body, including the joints, skin, kidneys, heart, lungs, blood vessels and brain although people with the disease may have many different symptoms, some of the most common ones include extreme fatigue, painful or swollen joints (arthritis), unexplained fever, skin rashes and problems [2].

Each person with Lupus has slightly different symptoms that can range from mild to severe and may come and go over time. SLE is a complex multi-system disease, this complexity makes it difficult to monitor, in particular, there are problems in differentiating potential reversible organ dysfunction, due to disease activity, from irreversible organ damage [3]. Accurate diagnosis of systemic lupus erythematosus is important because treatment can reduce morbidity and mortality [4,5].

SLE is a chronic inflammatory disease believed to be a type III, hypersensitivity response with potential type II involvement [6]. Lupus is a treatable symptomatically, mainly with corticosteroids and immunosuppressant through there is currently no cure [7].

SLE has no single diagnostic marker; instead it is identified through a combination of clinical and laboratory criteria [8].

Remission of SLE is not uncommon but often is punctuated flares [9], in a six year prospective cohort study [10], disease flares occurred at a rate of 0.2 per year per patient.

Infections and disease of the cardiovascular, renal pulmonary and central nervous systems are the most frequent causes of death in patients with SLE [11].

Since 1950s, the five year survival rate for patients with SLE has increased from 50% to a range of 91-97%. Mortality rates for SLE are particularly high in children [12].

The degree of severity depends on different factors [13,14,15].

a- The severity of the inflammatory response.
b- Frequency of episodes.
c- The degree of organ or system involvement.
d- Vital organs or systems, such as lungs. Kidneys, nervous system, joints, skin and others are affected in 50% to 75% of SLE patients. Infections followed by kidney failure are the chief causes of death in SLE patients [16,17,18].
Material and Methods

Heparinized blood specimens were obtained from 60 cases of which 15 cases were normal control (12 females-3 males), while other cases 45 were patients suffering from SLE (40 females-5 males) treated in Air Force Hospital. Patients were classified into three groups according to the American Rheumatology Association Criteria for the classification of SLE:

- Fifteen patients with inactive SLE (G1), where 14 females and one male.
- Fifteen patients with active SLE (G2), where 12 females and 3 males.
- Fifteen patients with infection and SLE (G3), where 14 females and one male.

All patients were subjected to detailed clinical examination in the Air Force Hospital.

Multicomponent spectrophotometric method for the simultaneous determination of four hemoglobin derivatives.

The millimolar extinction coefficients were put into four linear equations with the four unknown concentrations of hemoglobin pigments (CHbO2, CHbCO, CMetHb and CSHb).

\[
A_{500} = 5.05 C_{HbO2} + 5.35 C_{HbCO} + 9.04 C_{MetHb} + 7.2 C_{SHb} \quad (1)
\]

\[
A_{569} = 11.27 C_{HbO2} + 14.27 C_{HbCO} + 4.1 C_{MetHb} + 8.1 C_{SHb} \quad (2)
\]

\[
A_{577} = 15.37 C_{HbO2} + 10.0 C_{HbCO} + 4.1 C_{MetHb} + 8.1 C_{SHb} \quad (3)
\]

\[
A_{620} = 0.24 C_{HbO2} + 0.33 C_{HbCO} + 3.35 C_{MetHb} + 20.8 C_{SHb} \quad (4)
\]

Where the absorption bands at wavelengths 500, 569, 577 and 620nm represent the absorption maxima of Met-Hb, HbCO, HbO2 and SHb, respectively.

The above linear system of equations can be represented in the matrix form as:

\[
\begin{bmatrix}
5.05 & 5.35 & 9.04 & 7.2 \\
11.27 & 14.27 & 4.10 & 8.1 \\
15.37 & 10.0 & 4.10 & 8.1 \\
0.24 & 0.33 & 3.35 & 20.8
\end{bmatrix}
\begin{bmatrix}
C_{HbO2} \\
C_{HbCO} \\
C_{MetHb} \\
C_{SHb}
\end{bmatrix} =
\begin{bmatrix}
A_{500} \\
A_{569} \\
A_{577} \\
A_{620}
\end{bmatrix}
\]

This linear system of equations was solved by mathematical manipulation, using the Gaussian elimination method. For matrix calculation [19], to yield the following equations:

\[
C_{SHb} = \frac{9.060234 A_{500} - A_{577} - 2.6960235 A_{569} - 35.295898 C_{SHb}}{66.750821} \quad (7)
\]

\[
A_{569} = \frac{2.2316831 A_{500} + 16.074415 C_{MetHb}}{7.968118 C_{SHb}} \quad (8)
\]

\[
A_{620} - 5.35 C_{HbCO} + 9.04 C_{MetHb} - 7.2 C_{SHb} = \frac{5.05}{C_{SHb}} \quad (9)
\]

Where A500, A569, A577 and A620 are the absorbances of hemoglobin solution at the wavelengths 500, 569, 577 and 620nm, respectively.

The results calculated through the use of the last four equations were found to be identical to those obtained by using a computer program for matrix calculation.

Determination of conductivity:

Conductivity of hemoglobin solution was determined using a conductivity meter type digimeter L21/L21C aqua lytic autotemp. Comp. Mignon-Germany, with a rod electrode in protective PVC tube temperature consistent up to 100°C. Measurements were performed at constant frequency (1500Hz sine from in the range of 0 to 200 micro Siemens/cm).

The conductivity measurements were carried out for hemoglobin solution of concentration 1.3x10^-4 M at room temperature washed with running distilled water.

Dielectric measurements:

The dielectric dispersion for 5% aqueous solution of Hb was measured at 25°C in frequency range 0.1 and 10MHz for SLE patients compared to normal control using a Loss Factor meter type 1033, RFT, Funkwerk Erfurt. Gerny. The Hb samples were measured by the cell type pw 9510/60, manufactured by Philips. The sample cell has two squared platinum black electrodes each having an area of 0.8x0.8cm with an intermediate distance of 1cm. The cell with the sample is kept at 25°C ±0.1 in a temperature controlled incubator Kottermann type 2771 Germany. The value of 'ε’ (Relative permittivity of the sample in the cell) was calculated at each frequency from the constant K (the cell constant that depend on the cell dimensions) and C_o (The residual capacitance) and the measured values of C, also the loss tangent (tan δ) was obtained from the measured values of the resistance R and C in farad as:

\[
\tan \delta = \frac{1}{2\pi f RC} = \varepsilon'' \frac{1}{\varepsilon}
\]
The dielectric loss $\varepsilon''$ was calculated from the relation $\varepsilon'' = \varepsilon'\tan\delta$, the conductivity ($\sigma$) was then calculated from the relation:

$$\sigma = 2\pi f \varepsilon'\varepsilon_0 = C/K$$

For spherical macromolecules the dielectric relaxation time depends on the viscosity of the liquid $\eta$ and its absolute temperature $T$. Viscosity measurements of each Hb solution was carried out with an ostwald viscometer at concentration of 5% and 25ºC bidistilled water was used first at fixed volume to pass through certain height of the Ostwald’s capillary, then the efflux of water $t_2$ is determined three times and an and an average value was taken also the averaged efflux times $t_1$ for both the control and the Hb of SLE patients were determined, then the viscosity coefficient $\eta_1$ of each sample was calculated as:

$$\eta_1 = \frac{(f_1t_1)}{(f_2t_2)}$$

Where $\eta_2$ is the viscosity coefficient of water, $f_2$ and $f_2$ are the densities of water and solute molecules respectively.

Data were analyzed statistically by using the common t-test.

**Results**

Hb of different derivatives of SLE patients as compared to control are shown in Table (1). The obtained data revealed that Met-Hb, S-Hb were increased significantly in the blood of SLE patients, the highly rise was appeared in G3 in the content of Met and S-Hb as compared to (G1&G2) as well as control group.

A high significant decrease in oxy Hb was appeared in G3 as compared to G1 and G2 as well as the control group. The value of oxy Hb gives the actual functional Hb concentration, thus on the base of the concentration of oxy Hb, the degree of the severity of the disease appeared.

Table (1): Hemoglobin of different ligand derivatives of SLE patients compared to control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>SHb</th>
<th>Met Hb</th>
<th>HbCO</th>
<th>HbO2</th>
<th>Total Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm/dl</td>
<td>%</td>
<td>gm/dl</td>
<td>%</td>
<td>gm/dl</td>
</tr>
<tr>
<td>Control</td>
<td>0.0341±</td>
<td>0.263</td>
<td>0.313±</td>
<td>0.243</td>
<td>0.3061±</td>
</tr>
<tr>
<td></td>
<td>0.00023</td>
<td></td>
<td></td>
<td></td>
<td>0.00026</td>
</tr>
<tr>
<td>G1</td>
<td>0.346±</td>
<td>3.23</td>
<td>0.437±</td>
<td>3.12</td>
<td>0.401±</td>
</tr>
<tr>
<td></td>
<td>0.00051</td>
<td></td>
<td>0.00063</td>
<td></td>
<td>0.00068</td>
</tr>
<tr>
<td>G2</td>
<td>0.389±</td>
<td>4.26</td>
<td>0.473±</td>
<td>4.53</td>
<td>0.423±</td>
</tr>
<tr>
<td></td>
<td>0.00039</td>
<td></td>
<td>0.000028</td>
<td></td>
<td>0.00072</td>
</tr>
<tr>
<td>G3</td>
<td>0.499±</td>
<td>4.39</td>
<td>0.595±</td>
<td>4.92</td>
<td>0.441±</td>
</tr>
<tr>
<td></td>
<td>0.00063****</td>
<td></td>
<td>0.000023**</td>
<td></td>
<td>0.00073</td>
</tr>
</tbody>
</table>

Table (2) illustrates that the electric conductivity of Hb of SLE patients compared to reference group. A significant increase was detected in patients with SLE as compared to control, the pronounced increase was detected in G3 than other groups.

Figs. (1-4) illustrate The results of the relative permittivity $\varepsilon'$, the dielectric loss $\varepsilon''$ & conductivity S were measured in the frequency range 0.1 to 10MHz for normal control and SLE patients (G1, G2, and G3) respectively. These figures indicate that Hb has a critical frequency $f_c$ ranging from 0.7MHz at 25ºC & 5% aqueous solution of hemoglobin.

Table (3) illustrates the values of the static $\varepsilon_s$ and infinite $\varepsilon_\infty$, dielectric constant, dielectric increment per gm per 1000ml, cole-cole, the relaxation time $\zeta\beta$, viscosity coefficient $\eta$ in poise and molecular radius in nm for SLE patients compared to reference group.

The molecular radius of Hb ($r$) was calculated from the data of relaxation time $\zeta\beta$ through:

$$r = \frac{4\pi\eta \zeta\beta}{kT}$$

The results indicated that the radius of Hb molecule increased as well as the relaxation time with the degree of the severity of the diseases from G1 to G3 compared to normal control. The dielectric increment ($\Delta\beta$) per gm per liter was calculated from

$$\Delta\beta = \frac{\varepsilon_s - \varepsilon_\infty}{C}$$
Changes in Molecular Size & Shape of Hemoglobin of SLE

Table (2): Electrical conductivity of Hb of SLE as compared to control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Conductivity Ms/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.312±0.532</td>
</tr>
<tr>
<td>G1</td>
<td>49.596±0.564 *</td>
</tr>
<tr>
<td>G2</td>
<td>66.21±0.706 **</td>
</tr>
<tr>
<td>G3</td>
<td>79.16±0.836 ***</td>
</tr>
</tbody>
</table>

* p<0.05.
** p<0.01.
*** p<0.001

Where C is the concentration of Hb solution in gm/liter. The results of the dielectric increment indicated a higher value in G3 compared to other groups as well as normal control.

Figs. (5-8) show cole-cole plot ($\varepsilon''$ Vs $\varepsilon'$) for normal control and SLE patients (G1, G2 and G3) respectively. From these figures, the values of the cole-cole parameter ($\alpha$) for all samples are deduced and given in Table (3), these results revealed that there is a wide distribution of the relaxation times of Hb molecules of patients with SLE concomitant with the severity of the disease as compared to reference group. The curve fitting analysis has shown that, the cole-cole model gave a better fit for the dielectric data.
Table (3): Values of the static $\varepsilon_s$ and infinite $\varepsilon_\infty$, dielectric constant, dielectric increment per gm per 1000ml, cole-cole parameter $\alpha$, relaxation time $\tau$ in sec., viscosity coefficient $\eta$ in poise and molecular radius in nm for SLE patients compared to reference group.

<table>
<thead>
<tr>
<th></th>
<th>$\varepsilon_s$</th>
<th>$\varepsilon_\infty$</th>
<th>$\Delta\beta$</th>
<th>$\alpha^*$</th>
<th>$\beta^*$</th>
<th>$\eta$</th>
<th>$\tau$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.01±0.54</td>
<td>104.01±0.62</td>
<td>0.53±2x 10^{-3}</td>
<td>0.01132±4x 10^{-4}</td>
<td>0.259±2.8x 10^{-4}</td>
<td>0.0215±2.9x 10^{-4}</td>
<td>3.316±0.015</td>
</tr>
<tr>
<td>G1</td>
<td>75.86±0.58</td>
<td>105.26±0.66</td>
<td>0.65±2x 10^{-3}</td>
<td>0.01006±4.2x 10^{-3}</td>
<td>0.298±4.6x 10^{-3}</td>
<td>0.212x 10^{-4}</td>
<td>3.422±4.3x 10^{-3}</td>
</tr>
<tr>
<td>G2</td>
<td>74.22±0.78</td>
<td>114.93±0.68</td>
<td>0.87±2x 10^{-3}</td>
<td>0.10004±4.8x 10^{-3}</td>
<td>0.326±5x 10^{-3}</td>
<td>0.0201±3.8x 10^{-5}</td>
<td>3.536±4.6x 10^{-3}</td>
</tr>
<tr>
<td>G3</td>
<td>72.16±0.82</td>
<td>118.23±0.73</td>
<td>0.986±1.6x 10^{-3}</td>
<td>0.10015±5.3x 10^{-3}</td>
<td>0.358±5x 10^{-3}</td>
<td>0.0196±4x 10^{-3}</td>
<td>3.612±3.9x 10^{-3}</td>
</tr>
</tbody>
</table>

*Fitted parameters from the computer program.

Discussion

Almost 85% of SLE patients experience problems associated with abnormalities in the blood, about half of SLE patients are anemic [20].

Iron deficiencies resulting from excessive menstruation, iron deficiencies caused by some of the treatments and a specific anemia called hemolytic anemia, which destroy red blood cells, can occur with very high levels of the anticardiolipin antibody [21].

In the present study, the high Met-Hb level found in Hb of SLE patients and its positive dependents on the degree of the severity of the disease may be due to zinc deficiency [22,23]. Zinc is necessary for the normal function of the immune system [24,25]. The immune system is strongly influenced by zinc, because it is one of the most highly proliferative organs [26,27].

Sulphohemoglobin is characterized by its inability to carry oxygen and cannot be converted back to Hb, some drugs can be able to induce the formation of S-Hb in patients with systemic lupus [28,29,30].

Hb concentration gives the actual functional hemoglobin (oxy Hb). It gives the actual degree of anemia of subjects, even when the total Hb concentration is within the normal range.

The electrical conductivity of Hb which illustrates the hydrophobic groups that appeared on the molecular surface, the new groups by which unfolding become exposed to the surface of this globular protein are responsible for the increase of the electric conductivity of Hb of SLE patients, the pronounced rise was appeared in G 3 as compared to normal control, which in turn led to changes in hydrophobic/hydrophilic ratio (tertiary structure).

Dielectric relaxation technique, gives more useful informations about some biophysical properties of the molecule such as the relaxation time.
The change in the tertiary structure of \( \alpha \) patients and the fattening of the curve increases \( \zeta \) 2- SCHUR P.H.: Systemic lupus erythematosus in L. Golden, with different values of the parameter Hb molecule results in a change in its molecular shape from nearly spherical to non spherical form as the disease goes to severity, is similar, the shift towards lower or higher frequencies \( f \) as indicated from the \( \beta \) dispersion in Figs. (1-4) is attributed to changes in molecular radius. Since smaller molecules have shorter relaxation times and hence larger critical frequencies [31-34].

There is a marked increase in the dielectric increment (86.03%) for \( G_3 \), (65.28%) for \( G_2 \) and (22.64%) for \( G_1 \) as compared to reference control, it may be presumed that the activity of the disease may result in the variation of the dielectric increment.

Theoretical treatment of the dielectric relaxation data to calculate the Cole-Cole parameter \( \alpha \) for the Hb of different clinical categories of Systemic lupus disease as compared to normal control, illustrates another form of the conformational changes in the hemoglobin of SLE patients, the values of \( \alpha \) show a very wide distribution of relaxation time (0.01132 for control group to 0.10015 for \( G_3 \)). The shape of Hb molecule in the reference group is not completely spherical, so Cole-Cole plot \( (e^\nu \text{ Vs } e^\tau) \) is nearly semi circle. The shape of Hb molecule tend to deviate from the spherical form as the degree of the activity of the disease increase (Figs. 5-8) i.e an increase in the unfolding, the different degree of unfolding of Hb as a globular protein coincides with the change in hydrophobic / hydrophilic ratio. The change in the tertiary structure of Hb molecule results in a change in its molecular shape from nearly spherical to non spherical form with different values of the parameter \( \alpha \).

References
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