Dielectric spectroscopy of red blood cells in a randomized sample of anemic and non-anemic Egyptian first trimester pregnant and non-pregnant women in the rural district of Alexandria city: a primitive screening approach

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Abstract

In Egypt, iron-deficiency anemia (I.D.A) is the most prevalent public health problem related to socioeconomic and lifestyle. Despite ferritin assay is the gold standard for (I.D.A), it is expensive and can be conflicted with other diseases. Hereby, new screening tools were emerged to monitor I.D.A. A sophisticated physical method depending on the electrical relaxation of R.B.Cs in anemic first trimester pregnant Egyptian women of a rural district in Alexandria was conducted. I.D.A was confirmed in all participant anemic women through calculated ferritin saturation percent. The dielectric permittivity and conductivity curves between control non-anemic non-pregnant and non-anemic pregnant women showed slight significant discrimination. These findings may be a primitive approach for I.D.A screening and further physical parameters will be needed to strengthen the idea.

Keywords: Iron deficiency anemia, ferritin saturation, pregnancy, dielectric spectroscopy.

1. Introduction

In the world of irritable lifestyle, a disrupting health guideline is accompanied. Anemia, a case of decreased hemoglobin (Hb) level with insufficient oxygen delivery to organs, is a public health problem that affects populations. unexpectedly at the era of advanced technology and highly developed medical care, pregnant women may experience a predominant type of microcytic low ferritin anemia known as iron deficiency anemia (I.D.A) with increased risk of low birth-weight infants in both rich and poor countries \(^{(1)}\).

Globally, 29% of all adult reproductive females are diagnosed anemic (Hb is lower than 11 g/dl), among 38% pregnant and 29% for non-pregnant women \(^{(2)}\). I.D.A was estimated as 4.3% -20% in developed countries and 30%-48% in developing ones. In Egypt, it is the most prevalent public health problem
especia, especially in pregnant women related to socioeconomics and lifestyle (3,4). Hb is an iron-containing metallocprotein found inside red blood cells (R.B.Cs). Hb assay is the primary indicator of anemia diagnosis (5,6). Another protein called ferritin stores iron in nearly all living cells, especially liver and immune cells. Transferrin is a ferritin-carrier protein that transports iron to the site of hemoglobin production whenever the need for. For (I.D.A) diagnosis, ferritin assay is the gold standard. In developing countries, ferritin determination is expensive and cannot be routinely done (7).

Another attempt to determine (I.D.A) in pregnant women depend upon economically affordable R.B.CS indices. Unfortunately, theses indices are not reliable because they can be affected by other factors like; leukemia, chronic inflammatory disease, infections. On the other hand, few studies have been carried upon electrical and magnetic properties of (R.B.Cs.) (8).

Therefore, a surrogate need to elucidate (I.D.A) mainly in women has emerged. Studying the electrical properties of human blood cells is a promising non-invasive to overview the physiological changes and membrane integrity of the cells (9,10). Besides, it can be important in a biomedical applications such as diagnosis and therapy. Despite R.B.Cs. can be considered a primitive cell with no organelles, their electric behavior is very complicated. The electrical and magnetic properties of (R.B.Cs) are mainly related to the presence of unpaired electrons of Fe ion depending on its ionization state. In 1897, Stewart et al (11), declared that (R.B.Cs) are almost perfect nonconductors at direct current. The resistance of whole blood was proportional to (R.B.Cs) concentration. Hence, by the grace of Moore et al works electro-physical characteristics of (R.B.Cs) were outlined.

The external membrane is non-conducting that is evoking their resistivity, while the internal is one-half plasma conductivity but non-conducting for direct current. Based on the above, R.B.Cs behave as a dielectric at sufficiently high frequency. Dielectric spectroscopy (impedance) is an affordable noninvasive tool to predicate the physiological changes inside the cells. This technique depends mainly on physical characteristics of red blood cells hemoglobin under the effect of electric fields of different range of frequencies depending on permittivity ( capacitance of the cells) and conductivity (ability to pass the current (9,10).

Hereby, the current work concerns about the dielectric spectroscopy behavior in randomized Egyptian anemic pregnant women in comparing with anemic nonpregnant and normal control age matching Egyptian women district of Alexandria city, under different values of frequencies.

2. Materials and methods

Subjects

A sample size of total (n=100) women was incorporated in the current study. They were categorized into three groups. First anemic pregnant of (n=35) 1st-trimester pregnant woman of the age range of (25-35) and hemoglobin level less than 10 g/dl referred to general Abo Kir hospital, gynecology & obstetrics department, Alexandria, Egypt. Another (n=35) pregnant women of the same age and socioeconomic level and hemoglobin level more than 10 g/dl. The last 30 non-pregnant non-anemic women were chosen as a control group. All participant women are to freely volunteer to participate and informed consent were written prior to their inclusion, according to ethical guidelines. Sophisticated physical and gynecological examinations were carried out to all participants by a specialized physicians to collect the anthropometric and gynecological data as shown in Table 1.

For all study groups, ferritin was measured to distinguished iron deficiency anemia and other inflammatory origin, serum iron, ferritin, and total iron binding-capacity (TIBC) were measured spectrophotometrically according to prescribed kit
procedures\textsuperscript{(12)}. Ferritin saturation percentage (FSP) was calculated from equation 1

\[ FS\% = \frac{\text{Iron}}{\text{TIBC}} \times 100 \]  
\textbf{Equation 1}

Anemia was diagnosed based on hemoglobin level lower than 11g/dl, and FSP below 12\%.

**Red blood cells preparation**

The blood samples (about 2 ml) were drawn from the participants on heparin vacutainer tubes immediately under definite medical supervision and sterile tools. The tubes were centrifuged at a moderate speed to fractionate whole blood and buffy coat and plasma were discarded and only the R.B.Cs layer was used for the measurements. R.B.Cs’s fraction was washed triplicates with an isotonic solution to avoid cell aggregation and then washed cells were divided into two-part one for hematological and serological study, the other for dielectric measurements.

**Dielectric measurement**

resistance and capacitance of R.B.C’s suspension were monitored at 5 and 50 kHz frequencies via two square form silver electrodes connected to LCR meter (Hioki, 3532, LCR Hitester Japan, physics department, faculty of Science, Damanhour University, Egypt) at room temperature (24 ± 0.1°C). Measurement cell was designed as two cubic silver electrodes of area (A) 1x1 mm\(^2\) and 5 mm apart (d). The permittivity (\(\varepsilon\)) and conductivity (\(\sigma\)) of the R.B.Cs were calculated from Equations (45 and 45) as a function of capacitance as insulator (C) and conductance as conductor (G) at microwave frequencies range (1 kHz -5 MHz).the conductivity and permittivity frequency – dependence non-linearity were fitted by least squares regression.

\[ C = \varepsilon \frac{A}{d} \]  
\textbf{Equation 2}

\[ G = \sigma \frac{A}{d} \]  
\textbf{Equation 3}

**Statistical analysis**

All variables were tested for normality using the Kolmogorov–Smirnov test. Consequently, Parametric and non-parametric tests of variables with normal distribution were carried out. The Statistical Package for the Social Sciences (SPSS) software version 18 was used for analysis. The all study group were compared one –way ANOVAs.

3. Results

For all study cases, a frequency dependence response curves were noticed in Figure 1. Concerning permittivity, the trend begins with step-like increment followed by sudden decrease till the plateau stage \((9-14)\). A contrary behavior occurred with conductivity curves. At the current microwave frequency range, only \(\beta\)-dispersion of dipole relaxation may be mainly concerned during the permittivity response, \(\alpha\)-dispersion (ionic relaxation) could not occur as it is related to lower frequency range \((11)\).

Comparing group conductivity, the control begins with a significant little lower degree than non-anemic and anemic pregnant groups respectfully. In accordance with previous work, there is a strong relation between electrolytes concentration in cytoplasm and conductivity alternating current frequencies. As a result, R.B.C. count and as the resistance increase with increasing R.B.Cs count \((7)\). These findings are matching with ours as from figure the contribution of hemoglobin dispersion may be effective as mentioned by Pauly and Schwan 1966 \((11)\), thus we can notice the relative higher level of at lower frequency permittivity in control and non-anemic pregnant groups than in an anemic pregnant group.

**Conclusion**

From the previous data obtained, there is a slight significant difference in dielectric (conductivity and permittivity) response to the anemic and non-anemic pregnant at the first trimester in comparing with non-anemic non-pregnant women under the current experimental conditions. We can conclude that dielectric spectroscopy may provide noninvasive monitoring of I.D.A but, we strongly recommend
more work to be done at different stages of pregnancy and accompanied more physical parameters to help in an accurate and appropriate monitor of I.D.A women during pregnancy.

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Table 1: Anthropometric characters of all incorporated women in the study Including control non anemic (CNA) and pregnant non anemic (PNA) , anemic (PA), *p<0.1.&**p< 0.05, p<0.4

| Age** | R.B.CS* | Hemoglobin* | Hematocrit* | Iron* | TIBC | FSP* |
|-------|---------|-------------|-------------|-------|------|------|
|       | (*10^6/L) | (g/dl) | (%) | (µg/ml) | (µg/ml) | (%) |
| Control | CNA | 27.9±3.6 | 4.43±0.6 | 12.38±0.8 | 37.14±2.3 | 0.6±0.2 | 7.16±0.4 | 15.01±4.4 |
| Pregnant | PNA | 34.54±1.1 | 4.54 | 12.73±3.3 | 38.18±0.3 | 0.56±0.0 | 3.28±0.0 | 17.66±6.9 |
|         | PA | 31.08±3.8 | 2.13±0.4 | 8.67±0.5 | 26±1.5 | 0.32±0.0 | 3.09±0.0 | 10.39±1.0 |

Figure 1: Graphical representation of dielectric responses (A) permittivity and (B) conductivity of R.B.Cs. of all study groups control non anemic, nonanemic pregnant (NAP), and anemic pregnant (AP) women.
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