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Assessment of Some Biochemical Parameters and Dielectric Relaxations in *β*-Thalassemic Children

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Abstract

Thalassemias are considered as a group of inherited disturbances or blood disorders characterized by the formation of abnormal hemoglobin. *β*-thalassemia is the most familiar type of thalassemia worldwide and in Egypt. *β* -thalassemia is classified into three types; minor, intermediate and major. Thalassemia minor resembles anemia with mild iron-deficiency, whilst Thalassemia major is the most severe; patients with the major form need regular blood transfusion stay alive. Patients with the intermediate form show mild to moderate anemia and do not need regular blood transfusion therapy. Therefore, it was thought to be of value to compare the three types on the bases of various biochemical parameters and dielectric properties of blood in the frequency range up to 5 MHz. Sixty children with *β*-thalassemia were involved in the research. They were divided into three equal groups, according to their thalassemia type, in addition to the same group of control. All children were subjected to thorough clinical examination, and the following blood analyses were performed: hemoglobin electrophoresis, complete blood picture "CBC", serum ferritin and creatinine, several vital enzymes, total/direct bilirubin, albumin, and other dielectric parameters. Significant differences were found between thalassemia blood and the control, especially in the case of thalassemia major blood. Dielectric relaxations were also detected in thalassemia blood, indicating possible changes in the RBC membranes of thalassemia blood due to possible variations of the surface charges on the RBC membranes and the reduction of glycophorin sialic acid content. Therefore, the present work reveals significant variations between the biochemical and dielectric properties of the three types of thalassemia blood and control blood, and these differences may be of value in the treatment of thalassemia in children.

Keywords: *β*-thalassemia, antioxidant, CBC, liver enzyme, dielectric, relaxations
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1. Introduction

According to Rund and Rachmilewitz [1], thalassemias are a group of inherited disturbances or blood disorders characterized by the formation of abnormal hemoglobin. Disorders of globin constraints (α) or (β) lead to rupture and erythrocyte damage [2]. Deficiency or reduced synthesis in α-globin constraints gives rise to α-thalassemia. β-thalassemia is the most familiar type of thalassemia. It is characterized by decreased synthesis and production of normal adult hemoglobin (HbA), the prevailing type of hemoglobin found in the blood from shortly after birth until death. β-thalassemia is the most common type with 3.5 to ≥ 9% and a gene frequency of 0.03% [3]. The annual total occurrence of symptoms of thalassemia is 1:100 individual population and 1:10,000 individual population in the European countries [4]. In the worldwide population, about 5% has a globin alternative, with only about 1.7% having the α- or β-thalassemia signs, whereas about 4.4 of every ten thousand of live children are affected. Previous research indicates that in Egypt, it was estimated that 1,000:1.5 million live babies born per year would catch thalassemia disease. Galanello and Origa [3] suggested the following classifications of β-thalassemia: thalassemia minor, thalassemia intermedia, and thalassemia major. A thalassemia minor individual is said to be heterozygous because they have as only one of the β-thalassemia gene copies, with one normal gene of the β-chain. Thalassemia minor resembles anemia with mild iron-deficiency, so people who fall into this category do not need treatment. A patient with thalassemia major, on the other hand, is said to be homozygous for β-thalassemia as they have two genes for β-thalassemia and no normal β-chain gene. Accordingly, the individual with thalassemia major is an ill person. A new-born baby with this major type appears completely normal because their hemoglobin at birth is fetal hemoglobin (HbF) that contains two α-chains, similar to HbA, and two γ-chains unlike HbA. Within the first month following birth, anemia however becomes fatal. The baby becomes unable to grow healthily because normal hemoglobin gets replaced by defective hemoglobin and hemolytic anemia results. It is because of this condition that primary thalassemia patients need regular blood transfusions to stay alive and maintain a quality of life. Thalassemia intermedia, on the other hand, shows mild to moderate anemia without the need for regular blood transfusion therapy [4-6]. To bear in mind the items described above will be of value when comparing the biophysical and biochemical parameters and oxidative stress that may be present in some children suffering from the three types of β-thalassemia. Moreover it will be useful when studying the dielectric characteristics of the blood of children in each category as compared to those characteristics of the blood of the children who are controls.

2. Materials and Methods

2.1 The grouping of children with β-thalassemia

Sixty children with β-thalassemia were involved in the study. They were divided into three equal groups; the first group had thalassemia minor, the second group had thalassemia intermedia, and the third group had thalassemia major, in addition to the same group of control. All participants were of the same socio-economic standard. They lived in similar living conditions and had similar dietary habits. All children were first subjected to a thorough clinical examination. Venous blood samples (5 ml) were collected from each subject's antecubital vein under aseptic conditions into two different tubes; a) an EDTA containing tube for dielectric measurement and CBC (automated measurement), and b) an empty tube that was immediately centrifuged at 5000 rpm to obtain serum. The serum of each sample was then frozen at -80°C until further analysis. Blood samples were also obtained from healthy volunteers who served as controls and the control subjects' blood was treated identically.

The blood from each subject was taken by qualified people who performed the following analyses: Haemoglobin Electrophoresis using full automated capillary electrophoresis [7], complete blood picture "CBC" [8], serum ferritin [9], serum creatinine [10], alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase [11], Glutathione-S-Transferase (GST) [12], Total Antioxidant Capacity (TAC) [13], Catalase Assay (CAT) [14], Superoxide Dismutase (SOD), Glutathione Reductase (GR) [15], Glutathione peroxidase (GPx), Gamma-Glutamyl transferase (GGT) [12], Lipid Peroxide (Malondialdehyde; MDA) [16], total/direct bilirubin, and serum albumin [10]. Furthermore, the degree of oxidative stress was assessed by determining total antioxidant activity (Biodiagnostic, Egypt). The principle of this method depends on the reaction of antioxidants in the sample with a defined amount of exogenously provided H_2O_2. The antioxidants in the serum blood sample eliminate a certain amount of the added hydrogen peroxide. The residual H_2O_2 is determined calorimetrically by an enzymatic reaction involving the conversion of the exogenously added 3,5, dichloro-2-hydroxyl benzenesulphonate to a pink-colored product. The absorbance of each sample was read at 505 nm against a blank in which the serum blood sample was replaced with distilled water. In each sample, the total antioxidant concentration was calculated according to the following equation:

$$\text{Total antioxidant concentration (mM/l)} = (\text{Abs of blank-Abs of sample}) \times 3.33 \quad (1)$$

The level of malondialdehyde (MDA) as a measure of lipid peroxidation was also determined by a ready-for-use colorimetric kit (Biodiagnostic, Egypt).

2.2 Dielectric relaxation measurements for the blood

A home-made electric cell takes the shape of a parallel-plate capacitor made of silver containing the sample material (plate distance 1 cm and plate area 0.0001 m^2). The electrodes were connected to an LCR meter (Model Hioki, 3532-50 LCR HiTESTER),which measures the capacitance and resistance with accuracy ± 0.05%, over a frequency range from 42Hz-5MHz.

Capacitance C, and conductance G, were used to calculate the relative permittivity, real (ε') and imaginary (ε") and real conductivity (σ') of RBCs, using the following equations [17].

$$C = \varepsilon' \varepsilon_0 A/d \quad (2)$$

$$G = \sigma A/d \quad (3)$$

Where, C (Farad) and R (Ohm), are the capacitance and resistance of the capacitor between the two measuring electrodes, A (m^2) is the surface area of the electrode, d (m) -the separation distance between the two electrodes, ε'-the relative permittivity (Farad/meter), ε0-the permittivity of vacuum (8.85×10^{-12} F/m), and σ is the electrical conductivity (Siemens/m).The imaginary parts of complex permittivity ε″ and conductivity σ″ were calculated according to the relation [18, 19].

$$\varepsilon'' = (\sigma - \sigma_L)/2\pi f \varepsilon_0 \quad (4)$$

Where σL is the low-frequency limiting conductivity taken at 42 Hz, and εh is the high frequency limiting permittivity taken at 5 MHz.

2.3 Statistical analysis

All values are presented as the mean ± standard deviation (±SD) and were analyzed by the Statistical Package for Social Science (SPSS) version 17. A paired t-test was used to compare two mean parameter values for the same element and the level of significance was set at a P-value of 0.05 or less. All individuals were subjected to full clinical examination and did not show clinical symptoms

or signs of any other problems except thalassemia**.** The study protocol was revised and ethically approved by the Zagazig University Hospital, Zagazig University, Egypt.

3. Results and Discussion

3.1 Variation of hemoglobin (Hb), red blood cells (RBCs) and white blood cells (WBCs)

The results of this study revealed that with respect to the types of hemoglobin that existed in the blood of the control group and thalassemia groups, it is clear that fetal hemoglobin was absent in both the control group and the thalassemia minor group, and was significantly higher in both the thalassemia intermedia and thalassemia major groups. Both Hemoglobin (Hb) and Hemoglobin A (HbA) decreased from the thalassemia minor to thalassemia major groups, while HbA2 increased significantly from T-Minor to TM. All the hemoglobin variations are illustrated in Table 1.

Table 1. Variation of the different hemoglobin in thalassemia groups and control

Type of Hb	Statistic	Groups: n = 20			
		Control	T-Minor	TI	TM
Hb F **%**	Range	00	0.2-1.7	7.9-40	46-95
	Mean±SD	00±00	1.08±0.57*	23.78±9.98*	66.51±15.89*
Hb A **%**	Range	97.6-99.1	92.9-97.3	55-89	0-50
	Mean±SD	98.4±0.47	95.17±1.88*	72.99±10.13*	28.77±15.65*
Hb A2 **%**	Range	0.9-2.4	2.5-6.4	1-7.8	2.1-16
	Mean±SD	1.6±0.47	3.84±1.44*	3.23±1.70*	5.25±3.20*
Hb**%**	Range	11.4-14.3	8-11.9	2.7-9.5	3.5-9
	Mean±SD	12.3±0.93	9.95±1.30*	7.18±1.90*	7.46±1.55*

* Significance at the level ($P < 0.05$)

Fetal hemoglobin, or Hb F, is usually absent in the blood, but it appeared in the blood of thalassemia patients in an increasing order from T-Minor to T-M, with minimum concentration in T-Minor. Hb F is the major oxygen transport protein in the newborn child and it has a much greater exceptional ability to bind oxygen than adult does hemoglobin. Therefore, the higher concentrations of it in T-I and T-M blood types are able to compensate for the cessation of hemoglobin in these patients. Adult hemoglobin or HbA, is the most common form of hemoglobin and comprises about 97% of adult hemoglobin. It exists in erythrocytes to transfer oxygen from the lungs to tissues. In the present data, this hemoglobin was found at 98.4% in the control 'normal' blood and declined gradually from the T-Minor to the T-M bloods. This indicates the need for these patients to have blood transfusion. Hemoglobin A2 is different from other forms of hemoglobins in that it is composed of two α-chains and two δ-chains, and may comprise 1-3% of adult hemoglobin. The average level found in this work for the normal control blood was 1.6%. Elevated concentrations in thalassemia patients are a useful marker of the occurrence of thalassemia, as indicated by Ou *et al*. [20]. Normal hemoglobin, Hb, as analyzed in this work declined across the groups, reaching its lowest level in the T-M group. This indicates the presence of anemia and the need for these patients to have blood transfusion. It must be mentioned that there are several types of anemia produced by different abnormalities in hemoglobin. An example is microcytic hypochromic anemia, which is a standard abnormal hematological parameter in clinical practice and is usually caused by a deficiency in iron and thalassemia traits.

RBCs were not radically changed the in the T-Minor group blood. However, a significant decrease in RBCs occurred in both the TI and TM type bloods when compared to the controls, and this was also seen in the TM type in comparison to the TI blood type. HCT% was observed to be decreased significantly with respect to the control group moving from the T-Minor towards the TM group, with the largest reduction occurring in the TM group whereas MCV was observed to be decreased significantly in TI with respect to the control group. With respect to MCH, it had decreased significantly in the T-Minor group compared to the control but then started to increase in the TI and TM groups, approaching the control level in both cases. The values of MCHC did not change significantly, fluctuating around the control value. On the other hand, the platelets, WBCs and monocytes observed in the T-Minor group had decreased significantly when compared to the controls. Furthermore, significant increases were seen in both the TI and TM groups, results which may be attributable to blood transfusions. The granulocytes and lymphocytes fluctuated around the control value, with non-significant changes. The results of RBCs, WBCs counts and indices variations are illustrated in Table 2.

Table 2. Variation of RBCs indices in thalassemia groups and control

Element type	Statistic	Groups: n = 20			
		Control	**T-Minor**	**TI**	**TM**
RBCs (x 10^6)	Range	3.05-5.31	3.6-6.11	1-4.29	1.43-4.22
	Mean±SD	4.69±0.62	4.91±0.84	2.96±0.90*	3.04±0.68*
HCT%	Range	32.2-38.6	23-33.1	7-26.9	9.8-27.7
	Mean±SD	34.37±2.15	28.51±3.41*	19.84±5.67*	19.76±4.37*
MCV (fl)	Range	61.3-73.6	57.9-77.3	52.9-72.3	67.6-72.7
	Mean±SD	65.96±5.51	67.61±6.06	59.58±8.14*	70.20±1.78
MCH (Pg)	Range	23.8-26.9	17.7-26.3	18.7-28.5	18.6-30
	Mean±SD	25.09±1.05	20.85±3.41*	24.68±3.13	24.78±3.09
MCHC (g/dl)	Range	34.1-36.7	33.5-37.2	32.2-41.8	33.8-44.1
	Mean±SD	35.79±1.15	34.88±1.29	36.42±2.53	37.54±3.01
Plts (x 10^3)	Range	238-387	141-312	100-1096	44.5-1118
	Mean±SD	278.9±44.56	245.9±54.31*	479.0±29.57*	433.32±26.75*
WBCs (x 10^3)	Range	4.2-15	5.3-11	3.9-68.3	3.3-88.2
	Mean±SD	8.61±3.73	7.52±1.69*	24.23±3.08*	29.81±9.83*
Lymphocyte	Range	22-63.1	36.1-50.2	33.6-77.6	34.7-79
	Mean±SD	46.84±12.22	41.59±4.42	50.66±16.52	55.79±15.78
Monocyte	Range	1.9-5.6	1.8-2.7	4-7.7	3.9-13.2
	Mean±SD	3.32±1.19	2.3±0.30*	5.73±1.30*	6.64±2.70*
Granulo-cytes	Range	34.4-76.1	47.7-61.9	14.8-69.1	3.7-13.2
	Mean±SD	49.84±13.19	56.11±4.35	43.57±17.33	37.56±17.04

* Significance at the level ($P < 0.05$)

The RBCs counts (TI and TM) and MCV (TI) in thalassemia patients were decreased significantly which affected the hematocrit value. Moreover, the mean corpuscular hemoglobin level was reduced due to the decrease in Hb concentration, but the mean corpuscular hemoglobin concentration of TM increased. The results of low levels of hemoglobin, HCT, and the change in

hematological parameters are related to the type of thalassemia. The present results are in good agreement with those reported by Mankad *et al.* [21]. According to this report, the clue for thalassemia is the low mean corpuscular volume (MCV) at < 78 fl or the low mean corpuscular hemoglobin (MCH) at <27 pg, which are seen in our results. Platelets show increased levels in T-I and T-M. This increase in platelets can respond to an abnormality on the vessel wall rather than to hemorrhage, resulting in inappropriate platelet adhesion, and the formation of a clot within an intact vessel that may result in arterial obstruction.

For white blood cell counts and differentiation, the increase in WBCs, lymphocytes and monocytes that is accompanied by a reduction in granulocytes is all an indication of internal inflammation, which is produced from anemia and seen in thalassemia patients. However, Mankad *et al.* [21] attributed the increase in WBCs to the probability of the existence of a large number of immature (nucleated) red blood cells, which the cell counter may mistakenly identify as white blood cells.

3.2 Liver enzymes

ALT did not change with respect to the control level except in the case of TM. However, there was a significant difference between T-Minor and TM only. AST increased significantly in the cases of TI and TM, with no variation in T-Minor. Furthermore, there was a significant difference between T-Minor and both TI and TM, with no difference between TI and TM. Albumin did not show any change in concentration in any of the thalassemia groups with respect to the control level. Bilirubin Total increased significantly in the cases of T-Minor and TM, with no variation in TI. There was also a significant difference between T-Minor and TM, with no difference between TI and TM. Bilirubin Direct increased significantly in all thalassemia groups except in the T-Minor group and between each two groups, i.e. between T-Minor and TM, and between TI and TM. Ferritin concentration increased significantly in all thalassemia groups with respect to the control and between each two groups, i.e. between T-Minor and both TI and TM, and between TI and TM. The concentrations in TI and TM were very high, with the concentration in TM the highest. Variations of the afore-said parameters in thalassemia blood and the control groups are illustrated in Table 3.

Table 3. Variation of liver and kidney metabolites in thalassemia groups and control

Parameter	Groups: n = 20			
	Control	**T-Minor**	**TI**	**TM**
Creatinine (mg/dL)	0.74± 0.07	0.78± 0.10	0.79±0.19	0.79± 0.19
GGT(U/L)	7.42±1.64	7.48±1.71	10.90±2.22	10.90± 2.22
ALP(U/L)	269.40±63.76	299.80±29.51	302.00±26.05	302.00±26.05
ALT(U/L)	21.4±6.72	17.81±5.47	60.52±99.33*	60.52±99.33*
AST(U/L)	24.6±5.48	21.57±5.37	60.06±57.23*	60.06±57.23*
Albumin(mg/dl)	4.24±0.25	4.23±0.31	4.02±0.56	4.02±0.56
BilirubinTotal(mg/dl)	0.54±0.06	0.78±0.11*	1.60±0.73	1.60±0.73

* Significance at the level ($P < 0.05$)

Both ALT and AST levels in the thalassemia TI and TM groups increased significantly, with respect to the control level, which indicates that an insult occurred in the liver as judged by the increase of bilirubin T and bilirubin D. These biochemical parameters increase as a result of blocking of the hepatic ducts, which causes leakage into the bloodstream, and which occurs mainly in TI and TM.

Ferritin is a universal protein that stores iron and releases it in a controlled manner. In human beings, ferritin acts as a buffer against iron deficiency and iron overload [22]. In the present

work, ferritin is lowered in the blood of T-Minor patients, indicating the presence of anemia. However, ferritin is raised highly in the blood of TI and TM patients, a condition which affects the heart and causes spleen enlargement. So, ferritin must be removed by using deferoxamine, as reported by Brittenham *et al.* [23]. Of course, the elevated levels of ferritin are due mainly to repeated blood transfusions, on which TI and TM patients are dependent. In some cases, patients may be subjected to splenectomy to reduce the frequency of blood transfusion.

3.3 Oxidative stress markers

Super-Oxide Dismutase (SOD) activity decreased significantly in all thalassemia groups with respect to the control and between each two groups, i.e. between T-Minor and both TI and TM but not between TI and TM. Catalase assay decreased significantly in all thalassemia groups with respect to the control and between each two groups, i.e. between T-Minor and both TI and TM, and between TI and TM. Glutathione Peroxidase (GPx) decreased significantly in all thalassemia groups with respect to the control and between each two groups, i.e. between T-Minor and both TI and TM, and between TI and TM. Glutathione S-transferase (GST) decreased significantly in all thalassemia groups with respect to the control and between each two groups, i.e. between T-Minor and both TI and TM, and between TI and TM. Total antioxidant capacity (TAC) decreased significantly in all thalassemia groups with respect to the control and between each two groups, i.e. between T-Minor and both TI and TM, and between TI and TM. Oxidative stress markers are illustrated in Table 4.

Table 4. Variation of the oxidative stress markers in thalassemia groups and control

Element type	Statistic	Groups: n = 20			
		Control	**T-Minor**	**TI**	**TM**
SOD (U/ml)	Range	98.12-161.16	75.47-118.75	48.32-92.96	36.17-86.83
	Mean±SD	129.64±18.27	97.11±18.21*	70.64±17.88*	61.5±16.52*
CAT (U/l)	Range	38.43-81.07	38.85-61.13	17.88-42.88	15.4-24.28
	Mean±SD	59.75±11.53	49.99±9.63*	30.38±10.42*	19.84±3.03*
GPx (U/l)	Range	12.12-21.22	12.44-14.72	9.06-11.66	4.56-8.05
	Mean±SD	16.67±2.91	13.54±1.27*	10.36±0.93*	6.35±1.42*
GRm (mol/l)	Range	11.67-19.25	11.02-13.89	8.18-11.19	4.66-8.26
	Mean±SD	15.46±2.39	12.33±1.29*	9.58±1.31*	6.46±1.61*
GST (U/l)	Range	20.88-28.46	16.01-23.09	11.15-20.17	6.49-13.09
	Mean±SD	24.67±2.71	19.55±2.64*	9.58±15.66*	9.69±2.62*
TAC (mM/l)	Range	2.4-.51	1.75-.85	1.34-1.45	0.73-.87
	Mean±SD	2.45±0.05	1.77±0.07*	1.39±0.03*	0.82±0.04*
MDA (mmol/ml)	Range	7.29-12.99	10.32-13.69	12.87-15.85	16.85-20.25
	Mean±SD	10.09±2.09	12.42±1.49*	14.33±1.33*	18.55±1.25*

* Significance at the level ($P < 0.05$)

Disturbance of the balance between oxidants and reductants in the body leads to what is known as oxidative stress. This condition arises due to the production of peroxides and free radicals and leads to cellular injuries. Oxidative stress occurs due to increased levels of lipid peroxidation and free-radical formation. Oxidative stress occurs in β-thalassemia patients, who are regularly supplied with blood transfusions, as described by Pavlova *et al*. [24] and Ghone *et al*. [25]. In this

work, ferritin increased significantly in both TI and TM, indicating that the children in these two categories were subjected to iron overload, which in turn made the erythrocytes of these patients vulnerable to peroxidative injury, as reported by Naithanj *et al*. [26].

In a typical normal environment, each cell in the body is subjected to invasion by 100 billion superoxide radicals per day [27]. This account is clear evidence of the size of the risk posed by ROS and the effectiveness of the defense of the biological system toward their damaging effects. The defense of the biological antioxidant system is able to reduce the regular flux state of free radicals, either by blocking their production or by correcting their levels with primary antioxidants, e.g., superoxide dismutase (SOD) and glutathione peroxidase (GPx), which hinder the flow of new unbound root types (ROS) either by modifying present unbound roots by dismutation or by blocking the production of unbound roots from other molecules. In the present work, both (SOD) and (GPx) activities decreased significantly in the TI and TM groups. Moreover, secondary antioxidants, e.g., glutathione (GSH) and bilirubin, work by trapping radicals. GSH, similarly, decreased in the TI and TM groups, indicating either a decrease of the oxidants, or a decrease of the gene responsible for the synthesis of the enzyme protein itself, which is the case in the present work with the TI and TM children's groups.

3.4 Dielectric relaxation

3.4.1 Gross conductivity (σ)

The conductivity of the RBCs increases with increasing frequency and the higher level in the control subjects. The result is shown in Figure 1.

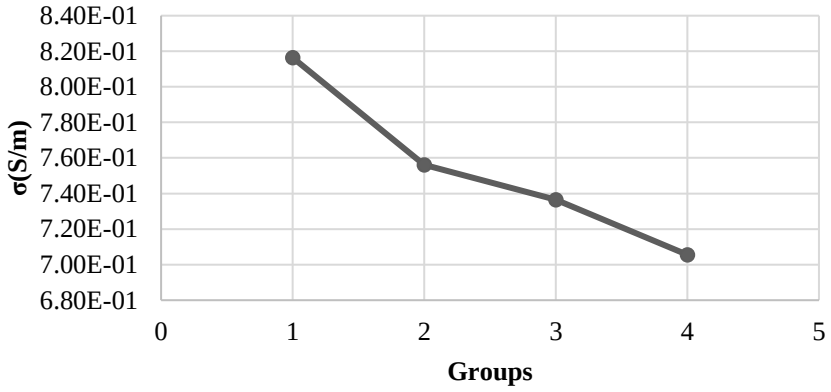

Figure 1. Variation of the conductivity levels in thalassemia and control blood (1: control, 2: T-Minor, 3: TI and 4TM)

3.4.2 Relative permittivity (έ)

The relative permittivity decreases with increasing frequency. The variation of this dielectric parameter reveals that there is a dielectric dispersion occurring at different critical frequencies for each group, as illustrated in Figure 2. The decrease in the relative permittivity at the dispersion point decreases in the order: control >T-Minor > TI > TM.

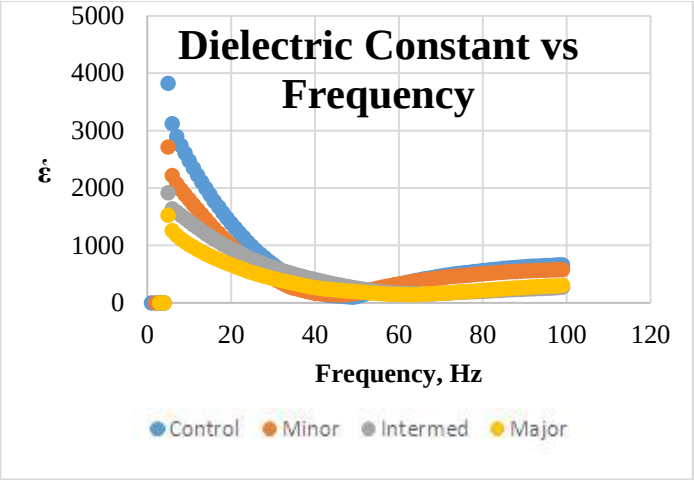

Figure 2. Variation of the relative permittivity (dielectric constant) of the blood groups with frequency

For the impedance Z, the blood impedance decreased with increasing frequency with the minimum level in the case of control blood with a higher value in the case of TM.This variation goes in the reverse direction of the conductivity variation with frequency.

The dielectric strength ($\Delta\dot{\varepsilon} = \dot{\varepsilon} - \dot{\varepsilon}\infty$, the relaxation time, $\tau = 2\pi fc$, where fc is the frequency at the midpoint of dispersion, and the conductivity,σ, at 5 MHz) was calculated and represented in Table 5. The dielectric properties that were used in the comparison between the blood of control children and the blood of the thalassemia patients were numerous. They were namely; the relative permittivity (ε̇), gross conductivity (σ), the dielectric strength (Δε̇), the ac conductivity (σAC) and the relaxation time. Each of these parameters reflects specific differences between the control blood and the thalassemia blood. Importantly, the dielectric properties of the blood are related mainly to the RBCs, which constitute the major component of the blood. Consequently, significant changes in the dielectric properties of the blood are related to the density of the negative charges on RBC membranes. In the present work, increasing frequency was inversely proportional to the relative permittivity of the control and thalassemia blood with gradually reduced levels of (ε̇) from thalassemia minor to thalassemia intermedia and finally to thalassemia major. At the same time, dispersion occurred at different frequencies, as previously described in Table 4. Moreover, the gross conductivity of the control blood was higher than that of the thalassemia blood, and it decreased in the order; σ (control) > σ(T-Minor) >σ (T-intermedia) >σ (T-major), as described in Figure 1. In addition, the blood impedance (z) was lower in the control blood, and increased in an inverse order, i.e. Z (control) < Z (T-Minor) < Z (T-intermedia) <Z (T-major), a result which coincides with the variation in conductivity reported by Haemmerich *et al*. [28]. In our opinion, these variations may be mainly due to the reduction of the sialic acid content on the RBCs. According to Eylar *et al*. [29] and Kahane *et al*. [30], the sialic acid content of glycophorins on thalassemic RBC membranes is about 25% lower than the sialic acid content of glycophorins on healthy RBC membranes. This result means that the carboxyl group, related to sialic acid, is also reduced, resulting in lower conductivity and higher impedance in thalassemia blood. Similarly, there are significant differences between the other dielectric parameters, i.e. the dielectric strength and relaxation time, which are mainly related to the polarization induced in the blood membrane, which differs significantly in the control and thalassemia blood, as described by Desouky *et al*. [31].

Table 5. The dielectric strength, the relaxation time, and the conductivity at 5 MHz

Measure	Control	T-Minor	T-Intermediate	T-Major
Δε	3146.7	2127.2	1633.4	1424.2
τ (S)	7×10^{-7}	7.51×10^{-7}	5×10^{-8}	5.25×10^{-8}
σ(S/m)	0.811	0.742	0.752	0.739

4. Conclusions

The research produced a wide range of data and gave rise to the following conclusions; 1) Most of the measured parameters revealed significant differences between the control blood and the three types of β-thalassemia blood and between each type of thalassemia blood; 2) Patients of thalassemia suffer oxidative stress that strongly affects their activity and quality of life; 3) The β-thalassemia major type shows the maximum differences because patients of this category need regular blood transfusions that slightly improve their quality of life, but increase the toxicity of excess ferritin. Thus, patients within this category needs regular injections of Desferal, which is considered the best agent for the removal of overloaded iron; and 4) Analysis of various dielectric parameters reveal decreased RBC conductivity and related parameters, effects that are mainly due to deformations in RBC membranes due to decreased content of sialic acid in the glycophorins of thalassemic RBC membranes.

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