Selenium and Vitamin E as antioxidants in chronic hemolytic anemia: Are they deficient? A case-control study in a group of Egyptian children

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ABSTRACT
Accelerated oxidative damage is one of the hallmarks in both sickle cell disease (SCD) and thalassemia major (TM). A decreased antioxidant level is found in both diseases. Our study was carried out to evaluate the variation in serum levels of Selenium and Vitamin E among a group of transfusion dependant Egyptian SCD and TM patients, further more to correlate these levels with iron overload status or transfusion requirements. A case-control study was conducted at the Cairo University Pediatric Hospital to assess the serum levels of Selenium using Atomic Absorption Spectrometer and Vitamin E using commercially available ELISA Kit in transfusion dependent children, 30 with beta thalassemia and 30 with SCD in a steady state aged from 6 to 18 years, these findings were compared to 30 age/sex matched healthy controls. Our results revealed a depleted antioxidants level in the studied group of Egyptian children with TM and SCD relative to healthy controls (P < 0.05). A significant positive correlation was found between Vitamin E levels and ferritin (r = 0.26, p = 0.047) in SCD and TM patients. Non-significant correlation was detected between serum Selenium and Vitamin E. Moreover, values of these antioxidants did not correlate with indices of hemolysis nor with those of inflammation in chronically transfused TM and SCD patients.

Introduction
Vitamins and trace minerals represent key buffers against oxidative damage [1]. Chronic hemoglobinopathies are characterized by oxidant damage due to increased resting oxygen consumption and circulating prooxidative free hemoglobin [2]. In sickle cell disease (SCD), Hgb S is unstable and generates free radicals which damage cellular enzymes and membrane lipids, production of reactive oxygen species and hyperhemolysis has been postulated to be the dominant mechanisms for the consumption of these compounds [3]. Patients with SCD have been demonstrated to have reduced levels of zinc, selenium, and glutathione as well as vitamins A, C, riboflavin, D, and E [4]. Oxidative stress biomarkers are also elevated in chronically transfused SCD and thalassemia major (TM) patients and correlate most strongly with non-transferrin bound iron (NTBI) levels [5]. A study by Nur

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et al. [6] had shown that N-acetylcysteine as an important antioxidant with pleiotropic effects on inflammation treatment of sickle cell patients seems to reduce erythrocyte phosphatidylserine (PS) expression, as a direct indicator of erythrocyte membrane (oxidative) damage. In view of all of the above mentioned facts the present study was initiated to evaluate the role of Vitamin E and Selenium levels as antioxidants in multi-transfused Egyptian β-thalassemia and sickle cell anemia patients and its relation to their iron overload, hemolytic rate, and inflammatory markers.

Patients and methods

This case control study was carried out in the Department of the Chemical pathology; it included 60 patients; 30 cases with β-thalassemia and 30 cases with sickle cell disease who were attending the Hematology Clinic of Cairo University Pediatric Hospital. All recruited patients were in a steady state attending routine follow-up during the study period (from December, 2012 to June, 2013). Patients with acute febrile illness within 72 h, acute vaso-occlusive crisis (VOC) within three months prior to enrollment, or serious concurrent illness were excluded. None of recruited subjects received supplemental antioxidants or vitamins e.g. vitamin E. The study protocol was approved by the local ethics committee as to be in accordance with Helsinki Declaration II, Finland. Consent forms were obtained from the patients or their legal guardians after they were informed about the study to be conducted and its expected outcomes. Further 30 apparently healthy subjects with matching age and sex were included and served as a control group, none of these children had history of anemia, abnormal complete blood counts or abnormal hemoglobin electrophoresis results. Detailed history-taking and thorough clinical examinations were performed for all patients and controls. All TM patients received simple transfusions of 10–15 cc/kg every 3 or 4 week. Three patients with SCD have blood transfusion twice per month, 6 patients once per month and the rest transfused every two or three years. In sickle cell disease patients the blood transfusion is not regular depending upon hydroxyurea (HU) treatment, compliance and stop treatment due to HU side effects. The number of vaso-occlusive crisis (VOC) in sickle cell disease patients was variable; 20 times per year in 4 patients, 12 times per year in 7 patients, 6 times in 7 patients, twice per year in 6 patients and once yearly in 6 patients, twenty-four of them were on hydroxyurea treatment, twenty-eight patients had undergone splenectomy. Blood samples from thalassemia and SCD patients were collected just before the transfusion.

Sampling

All patients were instructed to fast for at least 12 h, 10 ml of venous blood was collected from the subjects under aseptic condition, 6 ml of this blood was collected in plain vacutainer and the remaining 4 ml of blood was poured in EDTA anticoagulated vacutainer. Serum was separated by centrifugation at 3000 rpm for 10 min at room temperature.

Biochemical, hematologic and immunologic analysis

Analysis of all the biochemical parameters including ALT, AST, total Cholesterol, Triglycerides (TG), LDL and HDL- cholesterol were analyzed by chemistry auto analyzers on Dimension EXL® (Siemens Healthcare, Germany). Serum ferritin was measured using chemiluminescent immunoassay on AsSYM (Abbott Laboratories, Chicago, IL, USA). CBC was done by fully automated hematology analyzer Sysmex (Sysmex Asia Pacific, Japan); CRP was done by latex slide agglutination technique.

Measurement of serum Vitamin E and Selenium

Serum Vitamin E was determined using Vitamin E ELISA Kit (Catalog No: E0922h, www.eiaab.com).

Selenium determination had been carried out using Atomic Absorption Spectrometer on Varian SpectrAA 220 (Labexchange, Germany).

Statistical analysis

All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows. Numerical Data were expressed in terms of mean ± standard deviation (SD); comparison of the three groups was done using one way analysis of variance (ANOVA) test with Bonferroni’s post hoc test. Student’s t-test was used for comparisons between two groups. Categorical data were expressed as numbers (frequency) and percentages, and compared between groups using the chi-squared test. Correlation between various variables was done using Pearson r correlation coefficient. P-values less than 0.05 were considered statistically significant.

Results

β-Thalassemia group composed of 17 (56.7%) males and 13 (43.3%) females while SCD group composed of 14 (46.7%) males and 16 (53.3%) females (P > 0.05). The mean age of β-thalassemia group was 12.9 ± 3.2 years and this was comparable with that of SCD group 11.8 ± 2.9 years (P > 0.05). β-Thalassemia group showed a statistically significant higher prevalence of positive consanguinity (P = 0.01), splenectomy (P < 0.001), and frequency of blood transfusion/year (P = 0.014) compared to SCD group, Table 1 summarizes our patient demographics. As for weight, children below 5th percentile represented 86.7% of TM group versus 33.3% in SCD cases (P < 0.05).

Regarding the laboratory findings, thalassemia group showed significantly lower Hb, MCV, MCH, MCHC, platelets, LDH and higher HCT, ferritin and AST levels compared to SCD cases (P < 0.05). We set a cut-off limit of 1000 ng/ml for serum ferritin to differentiate between adequately chelated and poorly chelated patients, we found that only 3.3% of TM patients were chelated adequately versus 26.7% SCD patients and this difference was statistically significant (P = 0.01). SCD patients showed significantly higher surrogate values for hemolysis and inflammation when compared to thalassemia group (P < 0.05). LDH was nearly five times larger than normal values in SCD patients, indicating that transfusions were incompletely effective in suppressing endogenous RBC production. C-reactive protein (CRP) was also almost 3-fold larger in SCD patients. Twenty-three percent of thalassemics had more than 2-fold rise in their transaminases.
versus none of SCD group. Nonsignificant difference was
detected in the mean levels of WBC, Neutrophils percentage,
Reticulocyte count and ALT between the two groups
\((P > 0.05)\), as shown in Table 2.

Table 2 illustrates a comparison of mean levels of lipid pro-
file of our patients; total cholesterol, LDL-cholesterol, as well
as triglyceride (TG) were significantly lower in patients with
beta thalassemia and sickle cell anemia than relevant controls
\((P < 0.05)\), which is peculiar of these diseases. However,
nonsignificant differences were detected in their mean levels
of \((P > 0.05)\) between beta thalassemia and sickle cell anemia
cases.

Mean Selenium level of TM and SCD cases was significant-
ly lower when compared to the control group \((P < 0.05)\).
However, mean Selenium level showed no significant differ-
ence between thalassemia and SCD groups \((P > 0.05)\).
Similarly mean Vitamin E level of TM and SCD cases was
significantly lower when compared to the control group

Table 1  Patients’ demographics.

| Variables                  | TM \((n = 30)\) | SCD \((n = 30)\) | \(P\)-value  |
|----------------------------|----------------|-----------------|-------------|
| Age (years)                | 12.9 ± 3.2     | 11.8 ± 2.9      | 0.243       |
| Gender \((n, \%)\)         |                |                 |             |
| Female                     | 13 (43.3)      | 16 (53.3)       | 0.425       |
| Male                       | 17 (56.7)      | 14 (46.7)       |             |
| Family history \((n, \%)\) |                |                 |             |
| Yes                        | 11 (36.7)      | 16 (53.3)       | 0.194       |
| No                         | 19 (63.3)      | 14 (46.7)       |             |
| Consanguinity \((n, \%)\)  |                |                 |             |
| Yes                        | 20 (66.7)      | 10 (33.3)       |             |
| No                         | 10 (33.3)      | 20 (66.7)       | 0.010*      |
| Splenectomy \((n, \%)\)    | 23 (76.7)      | 5 (16.7)        |             |
| Weight (kg)                | 34.2 ± 13      | 36.1 ± 12.4     | 0.788       |
| Height (cm)                | 140.5 ± 12.7   | 133.8 ± 13.4    | 0.091       |
| Transfusion frequency/year | 13.8 ± 5.0     | 9.8 ± 5.5       | 0.014*      |

* Statistically significant value, \(n\); number of individuals.

Table 2  Comparison of biochemical, hematologic, and immunologic variables between thalassemia and SCD groups (mean ± SD).

| Variables                  | Thalassemia \((n = 30)\) | SCD \((n = 30)\) | \(P\)-value  |
|----------------------------|--------------------------|-----------------|-------------|
| HB (g/dl)                  | 7.1 ± 1.4                | 8.2 ± 1.1       | <0.001*     |
| HCT (%)                    | 26 ± 5.5                 | 23 ± 3.8        | 0.028*      |
| MCV (fl)                   | 63.7 ± 4.8               | 89.4 ± 15.3     | <0.001*     |
| MCH (pg)                   | 24.8 ± 3.8               | 30.4 ± 6.1      | <0.001*     |
| MCHC (g/dl)                | 32.7 ± 1.7               | 33.8 ± 2.5      | 0.034*      |
| Platelets \((10^3/cm m)\) | 350 ± 201.7              | 449.4 ± 189.8   | <0.001*     |
| WBC (10^3/cm m)            | 13.1 ± 9.8               | 10.0 ± 4.0      | 0.275       |
| Neutrophils (%)            | 53.0 ± 12.4              | 45.5 ± 18.0     | 0.068       |
| Reticulocyte (%)           | 9.9 ± 3.8                | 9.6 ± 6.6       | 1.000       |
| LDH (U/L)                  | 803.9 ± 231.3            | 1070.3 ± 251.2  | <0.001*     |
| Ferritin (ng/ml)           | 3855.4 ± 2207.8          | 1464.9 ± 993.4  | <0.001*     |
| CRP (mg/dl)                | 24.1 ± 23.9              | 38.6 ± 22.6     | 0.003*      |
| ALT (U/L)                  | 68.5 ± 33.5              | 54.8 ± 20.1     | 0.164       |
| AST (U/L)                  | 71.7 ± 33.6              | 53.6 ± 15.4     | 0.048*      |

* Statistically significant value.

Table 3  Comparison of lipid profile between thalassemia, SCD and control groups (mean ± SD).

| Variables                  | Thalassemia \((n = 30)\) | SCD \((n = 30)\) | Control \((n = 30)\) | \(P\)-value  |
|----------------------------|--------------------------|-----------------|----------------------|-------------|
| Cholesterol (mg/dl)        | 94.7 ± 20.0 \(b\)       | 90.1 ± 25.2 \(b\) | 182.3 ± 7.3 \(a\)   | <0.001*     |
| TG (mg/dl)                 | 67.7 ± 35.8 \(b\)       | 57.6 ± 38.9 \(b\) | 161.5 ± 1.9 \(a\)   | <0.001*     |
| HDL (mg/dl)                | 28.2 ± 5.7               | 28.8 ± 5.5      | 28.5 ± 3.6           | 0.876       |
| LDL (mg/dl)                | 50.1 ± 19.2 \(b\)       | 48.9 ± 20.5 \(b\) | 121.5 ± 1.9 \(a\)   | <0.001*     |

* Statistically significant value.

\(a,b\) Groups in the same row sharing same initials are not statistically significant, while those with different initials are statistically significant.
Table 4  Selenium levels and vitamin E in the three groups (mean ± SD).

| Variables          | Thalassemia (n = 30) | SCD (n = 30) | Control (n = 30) | P-value |
|--------------------|----------------------|--------------|-----------------|---------|
| Selenium (µg/L)    | 30.6 ± 23.6          | 29.8 ± 20.8  | 109.9 ± 8.3     | <0.001* |
| Vitamin E (mg/L)   | 3.1 ± 1.0            | 3.1 ± 0.7    | 14.6 ± 2.2      | <0.001* |

* Statistically significant value.

a,b Groups in the same row sharing same initials are not statistically significant, while those with different initials are statistically significant.

(P < 0.05). However, mean Vitamin E level showed no significant difference between thalassemia and SCD groups (P > 0.05) as summarized in Table 4.

A significant positive correlation was found between Vitamin E levels and ferritin. However nonsignificant correlations were detected between any of the studied antioxidants and any other laboratory variables, including the other antioxidant among the two groups of patients recruited in our study as revealed in Table 5.

Discussion

Our study was designed to investigate the antioxidant (Vitamin E and Selenium) levels and lipid profile in transfusion dependent Egyptian children with β-thalassemia and sickle cell disease and to correlate these levels with iron overload status or transfusion requirements.

Highly significant depletion (P < 0.001) in serum vitamin E was observed in our study. Vitamin E plays a key role in protecting cells against oxidative damage. The antioxidant role of Vitamin E is attributed to its ability in quenching highly reactive lipid peroxide intermediate by donating hydrogen and this prevents extraction of hydrogen from Polyunsaturated fatty acids (PUFAs), this assists in restricting self perpetuated lipid peroxidation chain reaction [7]. To the authors’ knowledge; a single previous study was carried out [8] and examined the oxidant-antioxidant status in 40 children with SCD. Most of the previous studies concerned with the oxidative stress in chronic hemoglobinopathies were done mainly on adults and examined previous studies concerned with the oxidative stress in chronic dant-antioxidant status in 40 children with SCD. Most of the studies reported that vitamin E deficiency might not be condu- cive for VOCs and recommended using more specific antioxidants such as total antioxidant capacity (TAO) or nitric oxide [5,8]. However, inverse correlation between Vitamin E level and transfusion frequency was reported by Marwah et al. [5].

Among our SCD cases, vitamin E did not correlate with any of the tested variables including transfusion frequency, VOC frequency, and indices of hemolysis, selenium, serum cholesterol, HDL or LDL-cholesterol.

Selenium plays a significant role in the prevention of the oxidative modification of lipids, reducing inflammation and preventing platelets from aggregating [12]. Its supplementation in patients with cardiovascular disease was found to lower the levels of total plasma cholesterol and low-density-lipoprotein (LDL) plasma cholesterol and doses as high as 300 mcg/day significantly increased HDL levels [13–15].

The most commonly used measures of Selenium status are plasma and serum Selenium concentrations [16]. But concentrations in blood reflect recent Selenium intake and not the long term intake. This may explain why normal subjects had low Selenium levels. Mean Selenium level of TM was 30.6 ± 23.6 µg/L and SCD cases was 29.8 ± 20.8 µg/L and both were significantly lower when compared to the control group 109.9 ± 8.3 µg/L (p < 0.05). However, mean Selenium level showed no significant difference between thalassemia and SCD groups (p > 0.05). Our data were in accordance with previous studies [17–19]. This state of apparent selenium defi- ciency in thalassemia and SCD groups may be explained by the state of chronic oxidative stress with subsequent depletion of all antioxidants [19]. Whether Selenium supplementation might be helpful or not in such group of patients warrants fur- ther studies.

In the current work 30 patients (50%) had history of positive consanguinity and 27 (45%) had similar conditions in the family this can be explained that the prevalence of beta-tha- lassemia is very high in the Mediterranean region [20] and SCD is very high in central Africa, Mediterranean region, eastern countries [21].

Twenty-eight patients (46.7%) had been splenectomized because of frequent transfusions. Among SCD patients, the median frequency of VOCs that necessitate hospital admission over last year was 5 (IQR 2–12) which can be explained as a complication of the SCD [5]. β-Thalassemia group showed a sig- nificant higher prevalence of positive consangiuinity (p = 0.01) and splenectomy compared to SCD group (P < 0.001). All β-thalassemia patients were transfusion dependant and received blood at a frequency ranging from 4 to 24 times per year with a mean 13.8 ± 5.0 and this was significantly higher when com- pared to SCD group where only 21 were transfusion dependant and received blood at a frequency ranging from 2 to 24 times per year with a mean 9.8 ± 5.5 (P = 0.014). The treatment of thalassemia major is blood transfusions to maintain the hemoglobin level [22]. Proper Iron chelation is an essential component of β-thalassemia and SCD therapy that considerably delay the tissue injuries from iron overload and improves life expectancy [23].

Table 5  Correlations of selenium and vitamin E levels and patients’ laboratory variables.

| Variables       | Selenium | Vitamin E | r-Coefficient | P-value | r-Coefficient | P-value |
|-----------------|----------|----------|---------------|---------|---------------|---------|
| Ferritin (ng/ml)| −0.026   | 0.257    | 0.047*        |         |               |         |
| CRP (mg/dl)     | −0.065   | 0.016    | 0.906         |         |               |         |
| Reticulocyte (%)| −0.083   | 0.044    | 0.737         |         |               |         |
| LDH (U/L)       | 0.098    | −0.159   | 0.225         |         |               |         |

* Statistically significant value.
Thalassemia group showed prevalence of children below 5th percentile in weight 86.7% versus 33.3% in SCD cases and 0% in the control group and height for age percentiles 80% versus 46.7% of SCD cases and 0% in the control group. These differences were significant \( (P < 0.05) \) and in agreement with previous reports of increased prevalence of retarded growth among transfusion dependant thalassemia and SCD patients secondary to iron-overload associated endocrinopathy [24,23].

Chronically anemic patients maintain increased cardiac output to maintain oxygen delivery [25]. This produces a mildly hypercatabolic state, increased resting energy expenditure, and chronic oxidative stress [26,27].

Our data showed that all our patients were chronically anemic and both thalassemia and SCD groups showed hemoglobin and red cell indices, below the normal physiologic levels. However, beta thalassemia patients showed significantly lower mean hemoglobin, MCV, MCH and MCHC and higher AST bin and red cell indices, below the normal physiologic levels. This was in line with a previous study [28] on a large cohort of \( \beta \)-thalassemia major patients following up at the same center and reported a mean pre-transfusion hemoglobin level as low as 5.7 ± 1.16 g/dl which is lower than similar study in which median baseline hemoglobin reached 10.0 g/dl [29]. This may be explained by the restrictive transfusion regimen adopted by our center and actually reflecting our financial backgrounds with a limited availability of blood as well as proper chelating. However, other indices of hemolytic including reticulocytic count were comparable between both groups. On the other hand; SCD patients showed statistically higher surrogate values for hemolysis and inflammation when compared to thalassemia group \( (P < 0.05) \); LDH was nearly five times larger than normal values in SCD patients, indicating that transfusions were incompletely effective in suppressing endogenous RBC production and mean C-reactive protein (CRP) was also almost 3-fold larger in SCD patients, consistent with the state of chronic inflammation. Four of 30 TM patients exhibited abnormal values of aspartate and alanine transaminases exceeding 2 folds versus none of SCD group.

SCD patients showed significant increase in platelet counts compared to thalassemia group which can be explained by the state of functional asplenia and secondary thrombocytosis among those patients [30,31].

When proper chelation is applied, it is expected that serum ferritin levels be maintained within normal limits regardless the total number of transfusions. This might be related to the improper chelation practices or variable response to chelation therapy among patients [23]. However such a uniform maintenance of serum ferritin levels is still lacking among our population. Both groups had elevated serum ferritin levels where thalassemia group had serum ferritin values between 898 and 12,128 ng/ml and SCD patients showed levels ranging from 560 to 6000 ng/ml. This state of iron overload suggested decreased chelation compliance in our population [28]. Reported a compliance rate 26.3% among those who received deferoxamine and 58.6% among those who received oral chelators, however, it is not only the poor compliance but also the availability of such chelators. Nevertheless, the level among thalassemia group was significantly higher when compared SCD patients and confirmed the higher frequency of transfusion dependency as well as excessive iron absorption secondary to increased rate of ineffective erythropoiesis among thalassemics [19]. When we set the cut-off limit for serum ferritin of below 1000 ng/ml between adequately chelated and poorly chelated patients according to the Thalassaemia International Federation’s guidelines [32,33] we found that only 3.3% of TM patients were chelated adequately versus 26.7% SCD patients and this difference was significant \( (p = 0.01) \). This rate among TM patients was lower than the rate reported by Ragab et al. [28] in their TM cohort which was 30% but nearly similar to Shah et al. [23] who reported a prevalence rate of 6.3% among their studied group.

Among thalassemia group; serum ferritin and selenium levels did not correlate with any of the tested variables including other antioxidants. This was in agreement with Claster et al. [19] who studied group of chronically transfused patients we studied 43 patients with SCD (17 male, 26 female) and 24 patients with TM (13 male and 11 female). They aged from 1.5 to 31.4 years and found that Levels of vitamin E as well as selenium were low and showed little association with iron overload, hemolysis, or inflammation. Similarly, SCD group showed no correlations of serum ferritin and selenium levels with any of the tested variables including other antioxidants.

Many factors such as iron overload, liver injury, and hormonal disturbances affect lipids pattern among patients with major form of beta-thalassemia. Some authors suggested that accelerated erythropoiesis and increased uptake of LDL by macrophages and histiocytes of the reticuloendothelial system are the main determinants of low plasma cholesterol levels in beta thalassemia major [34,35].

Our data showed that total cholesterol and LDL-cholesterol, as well as TG were significantly lower in thalassemia group than relevant controls \( (P < 0.001) \) this was in agreement with the previous reports [36,37]. On the other hand, Chrysohoou et al. [35] reported higher TG levels among thalassemia patients. This is different from our results and can be explained by the younger age group included in our study. Similarly, SCD group had significantly lower total cholesterol, LDL-cholesterol, and TG than controls \( (P < 0.001) \), which is peculiar of this disease and this was in agreement with VanderJagt et al. [38]. However, Total cholesterol, LDL-cholesterol, as well as TG were within the normal range and were comparable in patients with beta thalassemia and sickle cell anemia \( (P > 0.05) \). In addition, there were no significant differences between mean HDL-cholesterol levels in the three groups \( (P > 0.05) \).

Limitations of the study

One of the limitations of our study that we tested for individual antioxidants like Vitamin E and Selenium which may be less informative than other tests like total antioxidant capacity which reflects the collective contribution to the reducing property of no protein individual antioxidant or electron donating components and nitric oxide which is currently en vogue accounting for vascular injury and thrombosis in the context of hemolysis but also an important interacter with Selenium and Vitamin E, nevertheless, due to limited funding status of our work, we were not able to evaluate these parameters and their correlation with other indices, however we are aiming to complete this work in another study in the near future. Another limiting factor was the small sample size which might affect our conclusions, and this was because of the limited financial resources of our work as it is not funded by any of the funding agencies.
Conclusions

Based on the results of our study, patients with β-thalassemia and SCD have depleted antioxidants and subsequently increased oxidative stress relative to healthy controls. This is an indication that thalassemic and SCD patients produced greater quantities of reactive oxygen species which are less likely to be removed effectively with the endogenous mechanism. However, levels of these antioxidants did not correlate with indices of hemolysis nor of inflammation in chronically transfused patients.

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Authors’ contribution

All authors had substantially contributed to the intellectual content of this paper. Mona Hamdy, MD: Study design, revising the article for intellectual content and final approval of the version to be published.

Dalia Mosallam, MD: Study design, Conception and acquisition of clinical data and clinical application of results.

Alaa Jamal MSc: Acquisition of data, samples and clinical application of results.

Walaa Rabie, MD: Study design, laboratory work, interpretation of results and data analysis, manuscript writing. All authors had read and approved the final manuscript.

Conflict of Interest

The authors have declared no conflict of interest.

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