INCREASED URINARY 8-OXO-7,8-DIHYDRO-2′-DEOXYGUANOSINE EXCRETION IN A SAMPLE OF EGYPTIAN CHILDREN WITH BETA THALASSEMA MAJOR: MARKER FOR LIPID PEROXIDATION-INDUCED DNA DAMAGE

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INTRODUCTION

Thalassemia is a major health problem in different countries of Mediterranean origin. It’s an autosomal recessive genetic disorder caused by mutations in globin chains or their regulatory regions, between the two types of thalassemia, β-thalassemia is a heterogeneous state resulted from several abnormalities in the β-globin gene [1]. Hemoglobin (Hb) appeared when there is defect in the β-globin chain in the form of glutamic acid replacement by lysine. Reduced synthesis of the βE-globin chain is due to an abnormal gene, resulting in the formulation of a mild β-thalassemia phenotype [2]. Iron overload is a significant secondary complication that happens in this type of patients, defined as an increase of serum iron saturation of transferrin (the iron-carrier protein), and ferritin (an iron-storage protein). Long-term transfusion therapy to correct anemia leads to toxic iron overload [3]. The excess iron is accumulated as ferritin and hemosiderin in many different organs such as liver and spleen. The deposited iron leads to the production of reactive oxygen species (ROS) like superoxide anion (O2−), which via fenton reaction induces oxidative stress state. Super oxide reacts with nitric oxide radical and forms peroxynitrate resulting in oxidative stress and cellular damage in cellular acids, proteins, lipids, and carbohydrates as shown in Fig. 1 [4].

This reaction is resulting in oxidative stress, which leads to destruction in carbohydrates, lipids, proteins, and cellular nucleic acids [5]. The lipid peroxidation (LPO) process is a consequence of hydroxyl radicals, with special concern to polyunsaturated fatty acids, causing the production of many bio molecules as malondialdehyde, trans-4-hydroxy-2-nonenal, which is considered to be a chief LPO-product in vivo. It also can be oxidized by fatty acid hydro peroxides or hydrogen peroxide to originate its epoxide intermediate, it could assault the nitrogen atom in DNA bases and/or deoxyribonucleoside pool to produce the etheno ring in cytidine and adenosine causing 3,N4-ethenedeoxycytidine (εdC) and 1,N6-ethenedeoxyadenosine (εdA), respectively [6,7]. The purpose of our study was to determine the urinary excretion of human 8-oxo-7,8-dihydro-2′-deoxyguanosine, resulting from cellular oxidative stress urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine will be analyzed by enzyme-linked immunosorbent assay (ELISA). Furthermore, we want to investigate serum levels of antioxidant enzymes including glutathione-s-transferase (GST) and catalase (CAT).

MATERIALS AND METHODS

Materials

The present study conducted on 40 children with thalassemia major, of both sexes, aged 5-15 years, with stable clinical state, no acute or chronic infections, with no acute cardiovascular complications (including uncontrolled hypertension and acute heart failure). Congenital heart disease and acquired heart disease not related to the condition. Children were enrolled from the Hematology Clinic, Abo El-Reash Children Hospital, Cairo University. 40 apparently healthy children of similar age and sex recruited from the Outpatient Clinic, Children’s Hospital, Cairo University. The study had been approved by the Medical Research Ethical Committee of National Research Centre.

An informed parental consent was obtained from every case before the study. Each child subjected for full medical history including duration of the disease, treatment parameters for every case including chelation...
therapy (dose, age of start, complications, and compliance). Blood transfusion frequency clinical examination, assessment of growth through measurements of weight and height. Weight for age, height for age, and body mass index (BMI) for age will be calculated according to Z scores software.

Laboratory investigations

Complete blood count, serum ferritin, kidney functions [urea and creatinine], and liver functions [alanine aminotransferase (ALT) and aspartate aminotransferase]. Urinary excretion of 8-oxo-7,8-dihydro-2'-deoxyguanosine will be analyzed by ELISA. Serum levels of antioxidant enzymes including glutathione (GSH), CAT, LAB, tested in Egyptian National Research Center.

Biochemical markers

Venous blood samples after an 8 hrs fasting were collected from each subject in a sterile plain vacutainer tubes, blood on the plain tubes was allowed to clot for 30 minutes and then centrifuged at 3000×g for 10 minutes at 4°C, then the serum was separated and assayed for biochemical markers. For ELISA assay, aliquots were frozen at −20°C till the time of assay to avoid erroneous results from repeated freeze/ thaw cycles.

Early morning urine samples were collected, centrifuged at 3000×g for 10 minutes and then the supernatant was stored at −20°C till creatinine measurement. Creatinine in urine was measured on spectrophotometer (Bio Systems BTS-302) using BIODIAGNOSTIC kit (colorimetric kinetic method).

Human 8-oxo-7,8-dihydro-2'-deoxyguanosine was assayed in urine using human 8-oxo-7,8-dihydro-2'-deoxyguanosine ELISA kit, Kono Biotech Co., Ltd. The result was expressed as a ratio to urinary creatinine to correct the variation in urinary flow.

Serum CAT was assayed using bio diagnostic colorimetric method (Cat. No. CA 25 17), the reading was on spectrophotometer (Bio Systems BTS-302).

Serum GST was performed on spectrophotometer (BioSystems BTS-302) using biodiagnostic ultraviolet method (Cat. No. GT 25 19).

Statistical analysis

SPSS 16.0 software was used in the performance of all statistical analysis. Normality of variables was evaluated by the Shapiro-Wilk test. Non-normal variables were compared with non-parametric ways that are the Mann-Whitney U. Normal variables were analyzed using parametric methods, that is the Student’s t-test. Simple linear correlation (Pearson correlation) for normal variables and Spearman’s correlation for non-normal variables were also done. p value was considered statistically significant when p<0.05 and considered statistically highly significant when its value was <0.01.

RESULTS

Our study subjects mean age for thalassemia patients is mean±standard deviation (10±3.8) and for control group (11±2.2), there is a significant difference between patients and control in Hb level and hematocrit (%), also for control group (11±2.2), there is a significant elevation of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine and serum levels of antioxidant enzymes including glutathione (GSH), CAT, LAB, tested in Egyptian National Research Center.

controls p=0.05, 0.03, respectively. We found also a significant negative correlation between urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine and CAT level - r=-0.378, p=0.016, HB - r=-0.610, p=0.001, hematocrit (%) - r=-0.478, p=0.002, and significant positive correlation between urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine and ALT - r=0.547, p=0.001, and serum ferritin - r=0.391, p=0.013 as shown in Fig. 4. Furthermore, there is a significant negative correlation between CAT and serum ferritin - r=-0.320, p=0.04.

DISCUSSION

In this study, β-thalassemia major patients with oxidative stress were investigated through evaluation of the urinary excretion of human 8-oxo-7,8-dihydro-2'-deoxyguanosine and serum levels of antioxidant enzymes including CAT and GST. As expected in our study, we found significant growth retardation in patients with β-thalassemia major compared to control (p<0.05), this result is in agreement with many studies [8-10], chronic tissue hypoxia and iron toxicity from transfusion hemosiderosis have been implicated as major causes of retardation [11], also short stature and skeletal dysplasia can be induced by injudicious use of desferroxamine [12,13]. In our study, we found significant reduction of serum GST and CAT in beta thalassemia patients than control where GST level was (773±73.5), CAT level (410±16.1) in patients while GST was (1265±517.5) and CAT was (482±23.9) in control with p=0.05, 0.03, respectively, this result is in agreement with Mahdi, 2014 who found that serum level of GSH reductase, superoxide dismutase (SOD), CAT, and GSH peroxidase (GPX) decreased in thalassemia patients as compared to normal control [14].

Antioxidants are preservative agents which inactivate ROS and protect the cells from the oxidative damage, they are, for example, enzymes (SOD, GPX, and CAT) [8]. Zohaib, 2016 found that the enzymatic antioxidants activities for GSH reductase , GPX, and CAT were extremely reduced in untreated β-thalassemia patients [15]. On the other hand
when it compared to normal subjects the activity of SOD was increased in untreated thalassemia patients. Severe oxidative stress condition in the erythrocytes of the untreated transfusion dependent β-thalassemia patients is known through increase in SOD and decrease in CAT activity [16]. However, our result differs from Shazia et al., 2015 who found that serum CAT and GST are increased in β-thalassemia patients than control (5.84±2.17 nmol/mg protein and (6.25±15.1) while in healthy control (4.76±5.2) and (31.97±7.12 nmol/mg protein) p<0.05. As a compensatory mechanism in response to oxidative stress, there’s increased CAT activity while cellular damage is indicated from increased GST and LPO levels [17]. LPO end products produce miscoding etheno adducts in DNA which are excreted in urine after their repair. In our study, the urinary levels of 8-oxo-7,8-dihydro-2’-deoxyguanisin were significantly increased in β-thalassemia patients. edA and edC levels were assayed in collected urine samples by immune precipitation - high-performance liquid chromatography - fluorescence and 32P-postlabeling thin layer chromatography, respectively. Mean edA (fmol/μmol creatinine) levels in the urine of β-Thal/Hb E patients were found to be 8.7 times higher compared to controls. edC levels were increased 13 times over [18]. In our study, there is a significant positive correlation between urinary 8-oxo-7,8-dihydro-2’-deoxyguanosine and serum ferritin r=0.391 p=0.013 and ferritin levels correlated inversely with CAT – r=−0.320, p=0.44, showing the role of excess iron in producing free radicals and the consequent tissue injury and DNA damage, this result is agreement with study by El-Gindi et al. 2015 [18]. Meerang et al., 2008 found that urinary edC levels were correlated positively with non-transferrin bound iron (NTBI) levels (r=0.517; p=0.002), while there is no significant positive correlation between urinary edA and serum NTBI (r=0.257; p=0.124) [19]. There was no correlation between serum ferritin levels and either with urinary edA and edC or with serum NTBI. Recent studies by the magnetic resonance imaging showed that the total tissue iron does not reflected by serum ferritin alone [20,21]. Total body DNA adducts which are formed in any tissue in the body are excreted in urine after DNA repair. In addition, it is better to know the whole body iron overload than serum ferritin [22].

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