RESEARCH PAPER

Estimation of MDA, CRP and Some hematological parameters in the mature Cypriot Thalassemia patients

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Abstract:
Thalassemia is a blood disorder has been known to passes down by parent to their offspring. The root of this disorder mainly returns to the Mediterranean Countries. Degradation of erythrocytes results in a thalassemia disease as a result of producing defeated Hemoglobin Molecules, due to changes occurring in their DNA structure like insertion or deletion. For decades the only medical treatment those patients had is getting blood gave way by healthy volunteers. As a result of this process, they receive lots of Iron minerals possibly causing oxidative Stress, Inflammation and splenomegaly. The aim of the present work aimed to estimate the C-Reactive Proteins (CRP) as Biomarker of Inflammation, Serum Ferritin as Biomarker of Iron overload and MDA as A biomarker of oxidative Stress in patients B- thalassemia then comparing their data to healthy normal volunteers. The study group consisted of 24 thalassemia patients and 24 control groups. The blood samples of β-thalassemia cases were collected in thalassemia center In Dr.Burhan Nalbantoğlu Government Hospital/ Cyprus. After centrifugation, Aliquots of serum and plasma has been stored for the later estimation CRP, MDA and serum ferritin. Our data shows that the levels of C-reactive protein (1.350 ± 1.142 vs 0.325 ± 0.398; p<0.001), MDA (7.734 ± 1.557 vs 5.638 ± 1.219; p<0.001) and serum ferritin (617.92 ± 238.63 vs 433.82 ± 228.61; p=0.016) were significantly greater in contrast to same parameters of healthy controls. overall, our study proved again that examining levels of CRP, MDA and serum ferritin could serve as good indicators of the risks possibly faces those patients, knowledge about the status of those parameters could offer Some Helpful Knowledge assisting health professionals in better management patients with β-Thalassemia.

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1. INTRODUCTION:
Thalassemia is a group of inherited disorders of hemoglobin (Hb) synthesis. In -thalassemia the absent of chain synthesis is resulted from several causes such as a complete block at transcription or RNA processing, leading to lacking the globin mRNA production. In some case it is caused by a point mutation in the DNA sequence, for instance, a nonsense mutation providing production of an incomplete globin chain (Fucharoen & Winichagoon, 1997).

Beta thalassemia occurs when the gene that controls the production of beta globin is defective. Beta thalassemia can be mild to severe and is more common in people of Mediterranean, African, and Southeast Asian descent. A child can only get beta thalassemia by inheriting it from his or her parents. The homozygous or compound heterozygous states for thalassemia also run a variable course, although without transfusion, death usually occurs in the first few years. In Beta-thalassemia cases, there is a mutation in chromosome 11. Which causes lesser production by the chromosomal 11, a source of Beta and
alpha globin chains of hemoglobin constituents. (Weatherall, et al., 2006).

Previously (Chin et al., 2008) have explained this mutation as single base pair changes progressing into frameshift mutations or changes in canonical sequences that influences mRNA stability and processing. As a result hemoglobin will be damaged and lowers the activities of red blood cells to transport oxygen around the body (Winichagoon, Fucharoen, Chen, & Wasi, 2000). Oxidative stress is a body state occurs when the formation of reactive oxygen and the biological system's ability to readily detoxify the reactive intermediates becomes imbalanced. Severe oxidative stress occurs as a result of various disease such as thalassemia which can cause cell death, and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis. Oxidative stress is responsible for reactive oxygen species production, such as free radicals and peroxides. The major portion of long-term effects is afflicted by damage on DNA. (Fiers, et al., 1999), (Nicholls and Budd, 2000) and (Hayes and McLellan, 1999)). Because of continuous blood transfusion by beta-thalassemia patients, the will face more complications if these accumulated iron not removed. Damaging of many organs ma occur as a result of too much accumulations of iron. Thus, iron chelator become a necessary treatment to excrete of these accumulated iron. Although iron chelators may change iron homeostasis (absorption, distribution, and utilization) and in some cases, other metals such as zinc, copper, and calcium from metabolic pools may be removed by iron chelators (ribonucleotide reductase, lipoxygenase)(Breiterman-White, 2006).

Malondialdehyde (MDA) is the organic compound with the formula CH₃(CHO)₂. Produced As a result of enzymatic and oxygen radical-induced lipid peroxidation. The root of these genotoxic protein are located in the human DNA. (Niedernhofer, et al., 2003). Malondialdehyde (MDA) was found to react with normal hemoglobin A (Hb A), forming a number of less cationic components which were detected by cellulose acetate electrophoresis and gel electrofocusing. The nonspecific reaction has occurred between MDA and lysine to make aminoacrolin, dihydropyridine and dicarbaldehyde. As results changed hemoglobin will have more affinity toward the oxygen, and it will directly be oxidized. These outcomes propose that the structural changes in the Hb A was enhanced by the MDA interference. Because MDA is produced in erythrocytes as a yeilds of liquid peroxidation, MDA may react with intracellular Hb A, yields Hb A will be influenced functionally and in the stability term (Kikugawa, Kosugi, & Asakura, 1984).

Anemia is a common feature of many inflammatory, infectious, and malignant disorders (anemia of inflammation, also called anemia of chronic disease). Lynch S¹. (2012). The reaction of body toward injury or infection posses inflammation at acute or chronic stage. Usually Acute inflammation is the initial inflammatory response. Immediately happens after minor injuries like burns and cuts as well as major trauma such as myocardial infarction (MI). Acute inflammation will be curred when an injury or infection is completely removed. Symptoms include redness, swelling, heat, pain and stiffness in the affected area. The publications by New England Journal of Medicine declaered that CRP can serve as indicators and alerts for a patient to have or face future diseases like arthritis, heart attack and many other series health issues.(Nesto R1., 2004).

Previous works have shown that healthcare can predict and expect a patient’s risk for heart disease by estimating and knowing CRP levels of that patients. (Hage, McCrory, & Szalai, 2009). Our aim in these present study will be a comparison between Beta-thalassemia and healthy individuals based on the MDA, CRP, and Some hematological parameters.

2. MATERIALS AND METHODS

This prospective study examines patients who attended the outpatients clinic of Thalassemia Department in Dr. Burhan Nalbantoğlu Government Hospital in Cyprus between October 2012 to June 2013. The study population consisted of 50 subjects (25 males and 25 females with average age 20 to 30 years old). divided into two groups: 24 Thalassemia patients and 26 healthy control subjects. The control group consisted of healthy subjects without any history of thalassemia disease. All subjects provided written informed consent before the study, and the study was approved by our Local Research Ethics

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Committee. General health characteristics such as age, sex, smoking status, menopausal status and alcohol consumption were investigated by a self administered questionnaire.

The height (m), weight (kg), and waist circumference (cm) of each subject were recorded and body mass index (BMI) was calculated (kg / m^2).

Blood samples were drawn from the antecubital vein, after overnight fasting and centrifuged at 4000 rpm for 10 minutes and separated. The serum samples were stored at -20°C until they were analyzed for MDA and CRP.

**2.1 Malondialdehyde (MDA) Detection**

Serum MDA was measured according to the method of Buege and Aust (1978) and was expressed as nmol/l.

**2.2 C-reactive proteins (CRP) detection**

The level of CRP is determined in serum samples by manually CRP agglutination test, in which we mix drops of serum with CRP latex to see the degree of agglutination by which we can decide the level of CRP in patients and control groups.

**2.3 Other biochemical parameters**

The level of Serum ferritin, serum glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), ferritin, Aspartate Aminotransferase (AST), ALT (Alanine Aminotransferase), uric acid and low-density lipoprotein cholesterol (LDL-C) were determined using a fully automated clinical chemistry analyzer (Abbott Architect C8000).

**2.4 Hematological tests**

Several hematological tests including white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hb), pack cells volume (PCV), platelets, lymphocyte, red blood cell indices (MCV, MCH & MCHC), hematocrite, Red blood cell distribution width and Platelet Distribution Width (PDW) were measured for both groups using a routine automated method.

**Statistical analysis**

The Statistical Package for the Social Sciences (SPSS version 15.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All results were expressed as a mean ± standard deviation. The laboratory characteristics in both groups were compared by Student’s t-test. Differences were considered significant when P < 0.05.

**3. RESULTS AND DISCUSSION**

Descriptive statistics of a metabolic characteristics of the study population are presented in Table 1. There was no significant differences between the mean value of fasting glucose, triglycerides, urea and creatinine in both β-thalassemia and control groups. Thalassemia patients had significantly higher AST (p<0.001), ALT (p=0.007), uric acid (p<0.001), lactate dehydrogenase (p<0.001), ferritin (p=0.016), C-reactive protein (p<0.001) and MDA (p<0.001) levels compared to control groups. Total cholesterol (p<0.001) and HDL-cholesterol levels (p<0.001) were significantly higher in control subjects.

| Parameter               | Controls     | Thalassemia patients | p-value |
|-------------------------|--------------|----------------------|---------|
| Fasting glucose (mg/dL) | 92.54 ± 9.14 | 92.25 ± 34.92        | 0.76    |
| Urea (mg/dL)            | 13.91 ± 3.05 | 15.00 ± 2.96         | 0.160   |
| Total cholesterol (mg/dL)| 206.92 ± 21.64| 128.08 ± 18.46     | < 0.001 |
| HDL-cholesterol (mg/dL) | 56.00 ± 11.99| 28.12 ± 7.26         | < 0.001 |
| Triglycerides (mg/dL)   | 107.67 ± 60.04| 132.12 ± 66.11      | 0.205   |
Data are expressed as means ± SD and were compared by t-test.

Blood count results of patients and control subjects are present in Table 4.2. No significant differences in MCV, MCH, PDW and MCHC levels were detected between β-thalassemia and control groups (p>0.05). WBC (p<0.001), RBC (p<0.001), HGB (p<0.001), HCT (p<0.001), RDW (p<0.001), PCT (p=0.012) and PLT (p<0.001) levels were significantly higher in patients with β-thalassemic as compared to control group.

Table 2: Mean± S.D. of some hematological test in patients and control group

| Parameter            | Controls       | Thalasemia patients | p-value |
|----------------------|----------------|---------------------|---------|
| WBC x10^9/L          | 6.40 ± 1.25    | 13.98 ± 5.12        | < 0.001 |
| RBC x10^12/L         | 5.37 ± 0.499   | 3.66 ± 0.531        | < 0.001 |
| LYM x10^9/L          | 1.99 ± 0.88    | 6.81 ± 3.61         | < 0.001 |
| HGB g/L              | 13.46 ± 1.232  | 10.42 ± 1.422       | < 0.001 |
| HCT %                | 40.99 ± 3.241  | 30.65 ± 4.588       | < 0.001 |
| MCV fl               | 82.50 ± 4.32   | 83.70 ± 3.64        | 0.286   |
| MCH fmol             | 28.58 ± 1.526  | 27.39 ± 5.402       | 0.314   |
| MCHC mmol/L          | 33.67 ± 1.260  | 34.07 ± 0.829       | 0.245   |
| RDW %                | 14.037 ± 1.402 | 18.654 ± 4.059      | < 0.001 |
| PLT x10^9/L          | 303.62 ± 33.538| 494.62 ± 244.43     | < 0.001 |
| PCT %                | 0.354 ± 0.106  | 0.511 ± 0.230       | 0.012   |
| PDW 10 xGSD          | 18.654 ± 4.919 | 16.987 ± 0.953      | 0.121   |

As we mentioned before that MDA and c-reactive protein levels of patients higher than control subjects which can we clearly preserved in figure1.
4. Discussion

In beta-thalassemia major, impaired biosynthesis of beta globin leads to accumulation of unpaired alpha globin chain. An iron overload, usually observed, generates oxygen-free radicals and peroxidative tissue injury. This study takes us through the mechanics and tests that need to be done for better managing β-thalassemic patients and yet for preventing further complications that may also occur in those patients. For this purpose in the study malondialdehyde (MDA), a marker of lipid peroxidation was measured as a biomarker of oxidative stress, CRP as a biomarker of inflammation and several hematological parameters are taken and compared between β-thalassemic patients and control groups.

MDA level was higher in β-thalassemic patients (5.638 ± 1.219) than in control groups (7.734 ± 1.557) in our study. (Jokhio et al., 2009). found that MDA can serve as a marker of oxidation to circulating proteins have been found increased in patients with β-thalassaemia with iron overload. Mona Ramadan Nasr et al. 2012, showed that the oxidative capacity of blood and along with excess serum iron and ferritin may underly the highly significant increase of MDA in patients with β-thalassemia. Associated study have been done by Patrick B. Walter, et al. 2006, who showed that mean plasma malondialdehyde concentration levels of the two treatment groups (56 nmol/L) was significantly higher than that of the control group and showed that MDA levels can be controlled by both Deferoxamine and Deferasirox and MDA level can be reduced by chelation. Also, similar results were obtained by (Gunarsih, Amalia, & Boediman, 2012) who found significantly increased levels of serum ferritin and MDA in β-thalassemia.

The value in the control group was in the range found for healthy controls in other studies. There is extensive of in vivo oxidative damage as well as enhanced sensitivity to exogenous oxidant stress in red cells of β-thalassemia ( (Cappellini, 2006)). Sesmek. Et al. 2005, has been postulated that the biochemical and metabolic changes of β-thalassemia red blood cells are associated with constant oxidative stress within the cells caused by the precipitation of excess alpha-globin chains, iron decompartmentalization, and release of free iron. Several studies reported that plasma MDA is increased in β-thalassemia ((Meral et al., 2000) , (Aziz, Al-Kataan, and Ali, 2009) and (Livrea et al., 1996)

Awadalla et al., (2012) concluded that MDA, a product of lipid peroxidation, significantly is elevated in thalassemic patients through different mechanisms including an excess amount of iron binding to erythrocytes and free α-globin chain precipitation and the consequent generation of intracellular ROS. It has also been suggested that increased liver lipid peroxidation as a result of ferritin accumulation could raise the rate of leakage of MDA into the circulation .The levels of IMA (Ischemia modified albumin) are significantly higher in thalassemic patients as compared with healthy controls. High levels of IMA in thalassemic patients significantly correlate with ferritin, MDA, and ferroxidase. While ferritin

Figure 1: Showing differences in serum level of MDA and CRP between control and β-thalassemic patients.
was found to be the only predictor of MDA status in thalassemic patients, both ferritin and ferroxidase were found to be the predictors of the IMA status in such patients.

MDA is a measure of oxidative damage, and it has been found regularly transfused thalassemia major patients (Cighetti et al., 2002). Multifactorial theories have postulated to explain the high levels of serum MDA in β-thalassemic patients. We used CRP as a biomarker of various inflammatory conditions. Our study showed an increased CRP level in β-thalassemic patients (1.350 ± 1.142) than in control groups (0.325 ± 0.398) which are in accordance with the results of similar studies. A research done in Pakistan by Jokhio et al., 2009 concluded that CRP can serve as a biomarker of inflammatory conditions, the progression of cardiovascular diseases and as indicator of morbidity and mortality. High C-reactive proteins in these patients indicate ongoing iron overload toxicity related damage in these patients. (Archararit et al., 20000, has found that interestingly there was a trend towards increasing C-reactive protein levels in beta thal/HbE postsplenec patients with higher platelet count, although no correlation was observed. Besides the inflammatory process, platelet and/or factor(s) that control(s) thrombopoiesis seem(s) to play a role in the high serum C-reactive protein levels in the studied population. C-reactive proteins and cytokines have been used by a number of workers as a biomarker of inflammation in thalassaemia patients as well for another disease as pyogenic infection including pneumonia, infective pulmonary exacerbation in cystic fibrosis, diabetes, hepatitis and as marker for the development of cardiovascular diseases which is a major morbid complication of thalassaemia. (Kanavaki et al., 2009) they found that all endothelial adhesion molecules and CRP were significantly increased in β-thalassemia intermedia patients (p<0.001) and not influenced by treatment. These results agree with the study published earlier where CRP levels were found elevated in β-thalassemia patients compared to healthy individuals, especially in splenectomized patients. Throughout the world, several regions have initiated universal prenatal screening programs to address homozygous α-thalassemia. Although, the prognosis for thalassemia disorders is improving, but still prenatal diagnosis and neonatal screenings are needed. Comprehensive services that address language and social barriers as well as access to Hb F-enhancing agents and transfusions are needed.

5. CONCLUSIONS
Our study showed that MDA and CRP and ferritin can serve as good indicators for the future risks which may those patients may face causing more complicated damages ranging form splenomegaly to heart failure. Thus finding those parameters probably aids health professionals in better managing and delaying the upcoming organ complications may occur in thalassemia patients . Further studies need to be done for more provable evidence showing most beneficial tests may be helpful for localizing inflammation and iron damaging sited in patients with transfusion dependent β-thalassemia. For sure new methods of identification of inflammations sited aids the healthcare professionals in better management of thalassemia patients.
REFERENCES

Awadallah, S. M., Atoum, M. F., Nimer, N. A., & Saleh, S. A. (2012). Ischemia modified albumin: An oxidative stress marker in β-thalassemia major. *Clinica Chimica Acta, 413*(9–10), 907–910.

Aziz, B. N., Al-Kataan, M. A., & Ali, W. K. (2009). Lipid peroxidation and antioxidant status in B-Thalassemic patients: effect of iron overload. *Iraqi Journal of Pharmaceutical Sciences, 18*(2), 8–14.

Breiterman-White, R. (2006). C-reactive protein and anemia: implications for patients on dialysis. *Nephrol Nurs J, 33*(5), 555–558.

Cappellini, M. D. (2006). A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. *Blood, 107*(9), 3455–3462.

Chin, J. Y., Kuan, J. Y., Lonkar, P. S., Krause, D. S., Seidman, M. M., Peterson, K. R., ... Glazer, P. M. (2008). Correction of a splice-site mutation in the beta-globin gene stimulated by triplex-forming peptide nucleic acids. *Proceedings of the National Academy of Sciences, 105*(36), 13514–13519.

Cighetti, G., Duca, L., Bortone, L., Sala, S., Nava, I., Fiorelli, G., & Cappellini, M. D. (2002). Oxidative status and malondialdehyde in beta-thalassaemia patients. *European Journal of Clinical Investigation, 32 Suppl 1*, 55–60.

Fiers, W., Beyaert, R., Declercq, W., & Vandenaeele, P. (1999). More than one way to die: apoptosis, necrosis and reactive oxygen damage. *Oncogene, 18*, 7719–7730.

Fucharoen, S., & Winichagoon, P. (1997). Hemoglobinopathies in Southeast Asia: Molecular Biology and Clinical Medicine. *Hemoglobin.*

Gunarsih, A., Amalia, P., & Boediman, I. (2012). Paediatrica Indonesiana. *Paediatrica Indonesiana, 52*(3), 125–131.

Hage, F. G., McCrory, M. A., & Szalai, A. J. (2009). C-reactive protein and cardiovascular disease: Lessons learned from studying genetically engineered mice. *C-Reactive Protein: New Research.*

Hayes, J. D., & McLellan, L. I. (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. In *Free Radical Research* (Vol. 31, pp. 273–300).

Jokhio, R., Khan, Y., Chughtai, L. A., & Mughal, Z. (2009). C-Reactive (Crp) Protein in Transfusion Dependent Thalassaemic Patients. * Pak J Physiol, 5*(2), 20–23.

Kanavaki, I., Makrythanasis, P., Lazaropoulos, C., Tsironi, M., Kattamis, A., Rombos, I., & Papassotiriou, I. (2009). Soluble endothelial adhesion molecules and inflammation markers in patients with β-thalassemia intermedia. *Blood Cells, Molecules, and Diseases, 43*(3).

Kikugawa, K., Kosugi, H., & Asakura, T. (1984). Effect of malondialdehyde, a product of lipid peroxidation, on the function and stability of hemoglobin. *Archives of Biochemistry and Biophysics, 229*(1), 7–14.

Livrea, M. a, Tesoriere, L., Pintaudi, a M., Calabrese, a, Maggio, a, Freisleben, H. J., ... Bongiorno, a. (1996). Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood, 88*(9), 3608–3614.

Lynch S1. (2012). Influence of infection/inflammation, thalassemia and nutritional status on iron absorption. *Int J Vitam Nutr Res. 77*(3):217-23.

Meral, A., Tuncel, P., Surmen-Gur, E., Ozterk, E., & Gunay, U. (2000). Lipid peroxidation and antioxidant status in beta-thalassemia. *Pediatr Hematol Oncol, 17*(8), 687–93.

Nesto R1. (2004). C-reactive protein, its role in inflammation, Type 2 diabetes and cardiovascular disease, and the effects of insulin-sensitizing treatment with thiazolidinediones. *Diabet Med., 21*(8), 810–7.

Nasr, M. R., Ebrahim, N. A., & Salahedin, O. (2012). Growth pattern in children with beta-thalassemia major and its relation with serum ferritin, IGF1 and IGFBP3. *Journal of Clinical and Experimental Investigations.*

Nicholls, D. G., & Budd, S. L. (2000). Mitochondria and Neuronal Survival. *Physiological Reviews, 80*(1), 315–360.

Niedernhofer, L. J., Daniels, J. S., Rouzer, C. A., Greene, R. E., & Marnett, L. J. (2003). Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *Journal of Biological Chemistry, 278*(33), 31426–31433.

Weatherall, D. J., Akinyanju, O., Fucharoen, S., Oliveri, N., & Musgrove, P. (2006). Inherited disorders of hemoglobin. In *Disease Control Priorities in Developing Countries* (pp. 663–680).

Winichagoon, P., Fucharoen, S., Chen, P., & Wasi, P. (2000). Genetic factors affecting clinical severity in beta-thalassemia syndromes. *J Pediatr Hematol Oncol, 22*(6), 573–580.

Walter, P. B., Fung, E. B., Killilea, D. W., Jiang, Q., Hudes, M., Madden, J., … Harmatz, P. (2006). Oxidative stress and inflammation in iron-overloaded patients with β-thalassemia or sickle cell disease. *British Journal of Haematology.*