IGM Publication
Journal of Medical Science and Clinical Research
Volume||1||Issue||4||Pages165-171||2013
Website: www.jmscr.igmpublication.org ISSN (e): 2347-176X

Antioxidant and Hematological Study among Egyptian Thalassemic Children

[1]Samah A. Bastawy, [2]Mahmoud B. Abd El wahab and [3]Maha A. Fahmy

1 Clinical Pathology Department, 2 Biochemistry Department, Ain shams University Hospitals, 3 Pediatric Department-National Hepatology and Tropical Medicine Research Institute
Ain Shams University Hospitals- Emtedad Ramses- Abbassia-cardiosurathic surgery institute

e-mail: bastawysamah@yahoo.com

Abstract:

Patients with beta thalassemia need repeated blood transfusion for survival which may cause oxidative stres s and tissue injury due to iron overload and altered antioxidant enzymes. Our review aiming to study the anti oxidant levels changes in Egyptian thalassemic children and recognize either the antioxidant changes is due to associated repeated blood transfusion or due to the disease itself. We also monitor the damage occur by a ntioxidant on liver functions.

INTRODUCTION:

The patients with β-thalassemia major usually suffer from iron overload as a consequence of recurrent transfusion and ineffective erythropoiesis. Iron has a catalytic role to produce powerful reactive oxidant species (ROS) and free radicals, which lead to oxidative damage [1].

β-thalassemia major, the iron load and antioxidant capacity fluctuates with the trans-fusion treatment and enhances products of per oxidative damage. As a result, iron has been shown in serum and the intracellular transit pool, which can result in producing toxic oxygen as well as nitrogen-free radicals that can affect host antioxidant defenses. This induced oxidative status causes damage to cells and organelle membranes in organs that accumulate excess metal [2].

Malondialdehyde (MDA) a product of lipid per oxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassaemic erythrocytes. Glutathione and its redox enzyme system are an essential element of erythrocyte's antioxidant defense mechanism against free radical accumulations and functions by scavenging free radicals and detoxifying lipid peroxides via glutathione peroxidase (GPX) [3].

Antioxidants play an essential role in protection of the cells from oxidative damage. They include several agents such as enzymes (glutathione peroxidase, superoxide dismutase, malonaldehyde and ferroxidase), large molecules (ferritin and albumin), and small molecules (bilirubin, AST and ALT). Their defense mechanism in biological system involves chain breaking and preventive mechanisms [4]. Antioxidant defense can be evaluated by measurement of either individual antioxidants levels in cells or plasma [5].

Aim of the work:

The present review article is a comparative study aiming to study the antioxidant levels changes in Egyptian thalassemic children and recognize either the antioxidant changes is due to associated repeated blood transfusion or due to the disease itself. We also monitor the damage occur by antioxidant on liver functions.

Material and methods:

This is a case control study comprised 50 Beta-thalassemia male patients attending (the Hematology childre n clinic, Mataria Teaching Hospital. They were classified into 2 Groups: Group (G1) 25 newly diagnosed (n on-transfusion dependent) B-thalassemia patients, Group (G2) 25 transfusion-dependent B-thalassemia patie nts. Mean age of patients and participants was in range between 10 to 15 years.

The study is approved by the ethical committee of the Faculty of Medicine, Ain Shams University and infor med written consent is taken from all participants. Exclusion criteria include receiving antioxidants, diabetes mellitus, hypothyroidism, hyperthyroidism and renal failure.

Both G1 and G2 are compared with 20 healthy normal participants on the basis of age, sex, dietary conditions and life styles. Patients (G1, G2) as well as controls are subjected to detailed medical history taking, full clinical examination and laboratory investigations including (CBC, Ferritin, Ferroxidase, SOD, GPX, MDA, Albumin, T. Billirubin, AST, and ALT).

A complete blood profile is done using ABX Micros 60 Automated Hematology Analyzer. Serum albumin is measured using bromocresol green method) [6]. Bilirubin level is measured using dichloroaniline method [7]. Alanine transaminase (ALT), and aspartate transaminase (AST) [8]. Serum ferritin levels are determined by (ferritin ELISA coated microstrips) immunoassay analyzer (Elecsys, Roche, Germany). Ferroxidase activity is determined by spectrophotometry using O-dianisidinedihydrochloride method [9]. Malondialdehyde (MDA) is determined by a colorimetric reaction with thiobarbituric acid (TBA) according to method of [10]. Superoxide dismutase (SOD) Erythrocyte SOD activity is measured by method of [11]. Glutathione peroxidase (GPx) Erythrocyte GX is measured by method of [12]. All kits are supplied by commercial analytical kits from Sigma (St Louis, MO). And analyzed by auto analyzer express II.

The statistical analysis is carried out using the SPSS (Statistical Package for Social Sciences) software, version 16.0 for Windows. Results are expressed in mean ± SD. The two tail ANOVAs p values which are <0.001 where considered as highly significant.

Results :

Table (1) Mean values of hematological parameters among the studied groups

Parameter	Control	G1	G2	Anova	P	Sig.
Hb (gm/dl)	12±0.4	7.7±1.8	6.3±1.7	43.6	0.001	H.S.
PCV (%)	33.4±1.2	26.5±6.5	24.7±3.4	9.6	0.001	H.S.
RBCs (10^6/mm^3)	5.2±0.4	3.7±0.9	3.3±1.1	18.72	0.001	H.S.
Retics (%)	0.5±0.3	4±4.8	3.3±2	5.25	0.001	H.S.
MCV (FL)	85.1±3.8	71.9±8.5	75.5±10.4	4.83	0.001	H.S.
MCH(Pg)	28.4±1.1	22.6±3.4	23±2.6	12.41	0.001	H.S.
MCHC	31.4±1.7	30.9±2.7	30.2±1.9	1.76	0.001	H.S.
TLC (10^3/mm^3)	6.5±2.4	20.8±30.9	14.2±4.9	6.22	0.001	H.S.
Plts (10^3/mm^3)	315.5±87.4	243.8±44.9	555.4±201.9	7.49	0.001	H.S.

Table (2) Mean values of Malonaldehyde, Ferririn, Ferroxidase, superoxid dismutase,Glutathion peroxidase among studied groups

Parameter	Control	G1	G2	Anova	P	Sig.
MDA (µmol/l)	1.3±0.42	1.9±0.91	2.1±0.95	5.717	0.005	H.S.
Ferritin (µg/l)	39±25.5	717.3±581.1	2419±2109	21.78	0.001	H.S.
Ferroxidase(u/l)	106.5±29.8	136.2±35.8	163.6±29.6	19.053	0.001	H.S.
SOD (U/ML)	202.57±5.59	121±3.77	99.5±6.6	2.286	0.001	H.S.
GPX (U/L)	7538±135.30	4083.95±181.69	3789.55±155.7	3.754	0.001	H.S.

Table (3) Mean values of Albumin, T.billirubin, AST, ALT among the studied groups

Parameter	Control	G1	G2	Anova	P	Sig.
Albumin(g/dl)	4.63±0.39	4.51±0.4	4.55±0.38	1.227	0.296	N.S.
T.Billi.(mg/dl)	0.69±0.39	1.2±0.83	1.6±0.78	21.997	0.001	H.S.
AST(U/l)	13.5±6.5	33.5±12.8	39.8±14.8	66.631	0.001	H.S.
ALT(U/l)	11.7±7.8	35.9±17.9	36.4±11.7	57.705	0.001	H.S.

Discussion:

Serum ferritin, Ferroxidase and MDA were high significant increase among studied groups compared to control ($p<0.001$), which are in agreement with [13]. Our findings are in agreement with [14] that iron indices were markedly increased and the mean concentration of serum ferritin was elevated more than 20 times than healthy controls (table 2). Also in agreement with [15] who stated that thalassemic patients are also under significant oxidative stress as indicated by the high levels of ferritin, MDA, and ferroxidase. The mean serum levels of all the three parameters were significantly higher in patients than in controls.

Many studies suggested that increased levels of malondialdehyde may be due iron overload through repeated blood transfusions and subsequent oxidative stress produced by reactive oxygen species. The rise in superoxide dismutase and glutathione peroxidase may occur as a result of compensatory mechanisms in response to oxidative stress [16]. Erythrocyte superoxide dismutase (SOD) and glutathione peroxides activity (GPX) were high significant decreased among studied groups compared to control ($p<0.001$), which are in agreement with [17] who stated that, it is important to measure the decrease in activity of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPX) in thalassemia and examine its relation to severity of the disease.

Repeated blood transfusions and increased gastrointestinal iron absorption lead to an iron over-load in the body, which induces a vicious circle and results in chronic oxidative stress. The induced oxygen free radicals and a peroxidative tissue injury accompany the severe anemia (range 2-7gm/dl Hb) and the unavoidable complications depletion of the endogenous antioxidants [18]. Glutathione peroxidase levels in the body are in close relation with the glutathione which is the most important antioxidant present in the cytoplasm of the cells. The stability of the cellular and subcellular membranes depends mainly on glutathione peroxidase and the protective antioxidant effect of glutathione peroxidase depends on the presence of selenium. Glutathione peroxidase (GPX) also protects the heart from damage by oxidative stress due to oxygen free radicals through its antioxidant effect [19].

Among the studied groups there was no significant change in albumin which in accordance with [15] who stated that there is no significant difference was found between patients and controls in terms of total protein and albumin, (table 3).There was a significant increase in AST, ALT and T.Billirubin among the studied group as compared with the control group (table3) which in agreement with [20] who stated that Serum total and direct bilirubin, AST and ALT were significantly elevated in thalassemic subjects indicating liver cell damage.

The present studied groups show that Hb. PCV, RBCs, MCV and MCH were significantly decreased ($p<0.001$), in comparison to controls. This was in agreement with [21] who stated that marked decreased Hb. Level ranged from 2-8 g/dl. He also found significant decrease in MCV and MCH .This is in accordance

with the study of [22], who found low Hb. And low PCV in both the newly diagnosed group and the regular transfused group also compared to control group, (table 1). Among the studied groups (table1) there were significant increase in Retics, TLC, and platelets while no difference regarding MCHC [23] agreed with our results regarding TLC, Retics, and also increased circulating platelets in thalassemic patients, while in another study there was no significant change in retics count between thalassemics and control as stated by [24].

Conclusion:

Melondialdehyde, Ferroxidase, Glutathione, and Peroxidase, levels were significantly raised while superoxide dismutase enzyme was markedly decreased, these markers significantly correlated with serum ferritin levels which significantly rose in our study so the administration of selective antioxidants along with essential trace elements and minerals to reduce the extent of oxidative damage and related complications in beta thalassemia major still need further evaluation. Beta thalassemia major manifests itself with severe anemia (range 3-7 gm/dl of Hb) and lifelong depends on blood transfusion to sustain life. In patients with iron overload (blood transfusion) exceeds total iron binding capacity of transferrin and non- transferrin bound iron which causes tissue toxicity leading to increased lipid peroxidation with subsequent consumption of antioxidants.

References:

1. R. A. Ghone, K. M. Kumbar, A. N. Suryakar, "Oxidative stress and disturbance in antioxidant balance in beta thalassemia major", Ind J CliniBiochem, vol. 23, pp. 337-40, 2008.
2. R. Naithani, J. Chandra, J. Bhattacharjee, P. Verma, S. Narayan, "Per-oxidative stress and antioxidant enzymes in children with beta-thalassemia major", Pediatr Blood Cancer, vol. 46, pp. 780-5, 2006.
3. A.B. Patne, P. J. Hisalkar, S. B. Gaikwad, S. V. Patil, "Alterations in antioxidant enzyme status with lipid peroxidation in thalassemia major patients", Int. J. of Pharm. & Life Sci. (IJPLS), vol. 3, pp. 10, 2012.
4. V. Dhawan, K. Ratan Kumar, R. K. Marwaha, "Antioxidant status in children with homozygous β-thalassemia", IndPediatr, vol. 42, pp. 1141-5, 2005.
5. A. Cakmak, M. Soker, A. Koc, N. Aksoy, "Prolidase activity and oxidative status in patients with thalassemia major", J Clin Lab Analysis, vol. 24, pp. 6–11, 2010.
6. B. T. Dumas, W. A. Watson, H. G. Biggs, "Albumin standards and the measurement of serum albumin with bromcresol green", Clin.Chim, Acta, vol. 31, pp. 87, 1971.
7. R. W. Rand, A. Pasqua, "A new diazo method for the determination of bilirubin:, Clin. Chem, vol. 8, pp. 570, 1962.
8. R. Rej, "Aspartate aminotransferase activity and isoenzyme preparation", Clin. Chem., vol. 24, pp. 555-559, 1978.
9. J. Winkles, A. F Jones, P. Winyard, D. R. Blake, J. Lunec, "An automated method for the kinetic

measurement of ferroxidase activity", Ann ClinBiochem, vol. 25, pp. 250-254, 1988.

10. J. A. Buege, S. D. Aust, "Microsomal lipid peroxidation, in Fleischer S, L. Packer (eds): Methods in Enzymology, New York, NY, Academic, vol. 52, pp. 302, 1978.

11. Kajari Das, "A modified spectrophotometric assay of superoxide dismutase using nitrate formation by superoxide radicals", Indian J of Biochem and Biophys, vol. 57, pp. 201-4, 2000.

12. D. E. Paglia, W. N. Valentine, "Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase", Journal of Laboratory and Clinical Medicine, vol. 70, pp. 158-169, 1967.

13. S. Shalitin, D. Carmi, N. Weitrob, M. Philip, H. Miskin, L. Kornreich, R. Zilber, I. Yaniv, H. Tamary, "Serum frritin level as a predictor of impaired growth and puberty in thalassemia major patients", Eur J Haematol, vol. 47, pp. 93-100, 2005.

14. S. Filiz, O. Gulyuz, K. Sabri, E. Deniz, H. Alev, "Oxidant and antioxidant status in beta thalassemia major patients", Ankara Universitesi Tip FakultesiMecmuasi, vol. 58, pp. 34–38, 2005.

15. M. Samir, Awadallah, F. Manar, Atoum, A. Nisreen. Nimer, A. Suleiman. S. M. Saleh, "Ischemia modified albumin: An oxidative stress marker in β-thalassemia major", Clinica Chimica Acta, vol. 413, pp. 907–910, 2012.

16. Q. Shazia, Z. H. Mohammad, I. TaiburRahman, and HossainUddinShekhar - Correlation of Oxidative Stress with SerumTrace ElementLevels and Antioxidant Enzyme Status in Beta Thalassemia Major Patients: A Review of the Literature -Hindawi Publishing Corporation Anemia Volume 2012, Article ID 2709231

17. P. B. Walter, E. B. Fung, D. W. Killilea, Q. Jiang, M. Hudes, J. Madden, "Oxidative stress and inflammation in iron-overloaded patients with â-thalassemia or sickle cell disease", Br J Haematol, vol. 135, pp. 254-63, 2006.

18. K. K. Gupta, A. Mishra, A. Tiwari, "The production of the reactive oxygen species,their effects and the drugs and the plant extracts which are used as antioxidants and chelators on thalassemic patients: A Review:, International J of Pharmaceutical Sciences and Research, vol. 2, pp. 2278-85, 2011.

19. Kassab-Chekir, S. Laradi, S. Ferchichi, "Oxidant, antioxi-dant status and metabolic data in patients with beta-thalassemia", **ClinChimActa, vol. 338, pp. 79-86, 2003.**

20. F. Waseem, K. A. Khemomal, R. Sajid, "Antioxidant status in beta thalassemia major: A single-center study", Indian J PatholMicrobiol, vol. 54, pp. 761-3, 2011.

21. M. Y. Hassan, "Thalassemia", E medicine Journal, vol. 2, pp. 8, 2001.

22. M. Bravo-Urquiola, A. Arends, S. Montilla, Gevara-ijm, G. Garcia, M. Alvarez, O. Castillo < "Phenotyping and genotyping studies in a family with the compound heterozygositydeltabeta Thalassemia/beta(IVSII-849) Thalassemia", Invest Clin. Jun, vol. 47, pp. 179-84, 2006.

23. Eldor, Y. Krausz, H. Atlan, D. Snyder, A. Goldfrab, E. Hy-Am, E. A. Rachmilewitz,H. F. Kotze, P.

Heyns Du, "Platelets survival in patients with beta- thalassemia", American Journal of Hematology, vol. 32, pp. 94-99, 2006.

24. F. Krugner, T. R. Zaccariotto, E. T. Rosim, F. F. Costa, H. Z. Grotto, M. F. Sonati, "Reticulocyte evaluation in ∞ thalassemia", Am J Hematol, vol. 81, pp. 68-70, 2006.