www.abiosci.com

ISSN: 2348-1927

RESEARCH ARTICLE

Annals of Biological Sciences
2014, 2 (4):56-59

Oxidative Stress and Effects of Vitamin Supplementation on Freshly Diagnosed Anemic Patients

[1]Nirjala Laxmi Madhikarmi* and [2]Kora Rudraiah Siddalinga Murthy

[1]Department of Biochemistry, M. B. Kedia Dental, Birgunj, Narayani Zone, Birgunj, Nepal
[2]Department of Biochemistry, Bangalore University, Bangalore, India
Correspondence: nirjala4@gmail.com

(Received: 31/10/14) *(Accepted:15/11/14)*

ABSTRACT

Globally anemia is one of the most common causes of morbidity and mortality, affecting people of all ages in both developed and developing countries and oxidative stress is known to be positive contributor for anemia, giving its effects on lipid peroxidation and DNA damage. A study was carried out to investigate oxidant and antioxidant status in patients with freshly diagnosed anemia and effect of vitamin C and E supplementation. Blood samples obtained from females- anemic patients and healthy controls were analyzed for quantification of serum lipid peroxide, vitamin E, Vitamin C and enzymatic antioxidants namely superoxide dismutase and catalase. The efficiency of vitamins C and E supplementation in anemic patients was assessed by re-evaluating the oxidant and antioxidant status of same patients after supplementation. As compared to controls, the levels of serum lipid peroxides were significantly decreased; activities of erythrocytes SOD, GPx and CAT, levels of serum vitamin E and C were significantly increased in the patients after supplementation. Our results suggest the presence of oxidative stress and the possible preventive role of vitamin C and E therapy in anemic patients.

Keywords: Anemia, Antioxidant, Oxidative stress, Vitamin supplementation, Vitamin C, Vitamin E

INTRODUCTION

Globally anemia is one of the major causes of morbidity and mortality, common hematological problems in children, menstruating age female group and old aged group and is defined as a decrease in amount of red blood cells (RBCs) or the amount of hemoglobin in the blood below 11.5g/dl in females and 13.5g/dl in males [1]. People with anemia are at increased risk of developing complicated diseases and a substantial reduction in life expectancy. Oxidative stress can damage many biological molecules. Proteins and DNA are often more significant targets of injury than lipids. Lipid peroxidation is often a late event, accompanying rather than causing final cell death [1-3]. An increased concentration of end products of lipid peroxidation is the evidence most frequently quoted for the involvement of free radicals in human disease or tissue injury by toxins. Evidences from epidemiological and clinical studies suggest a possible correlation between antioxidant levels and the anemic disease risk [4,5].

MATERIALS AND METHODS

Case and control study of only females were divided into two groups; anemic patients (30) and healthy controls (30). We selected healthy controls from Bangalore University Staff, Ladies Hostel and students while anemic individuals from K. C. General Hospital, Malleshwaram, Bangalore. Informed consent was obtained from all subjects involved in the study. Height and weight was measured to determine respective body mass index and surface area. The age

ranged from 15 to 40 years. Criteria for anemia are hemoglobin concentration <11.5g/dl in women, plasma iron concentration <45µg/dl and total iron binding capacity (TIBC) >60µmol/l.

Vitamin C & E (400mg once daily) was supplemented to anemic as well as healthy controls for 15days. Blood was collected before and after vitamin supplementation in a sterile vial containing ethylene diamine tetraacetic acetate. Plasma was collected by centrifugation at 4000rpm for 15min. The red blood cells (RBC) pellet was thrice washed with chilled saline and was lysed with chilled distilled water in a ratio of 1:4. The lysed RBC was centrifuged to obtain clear RBC hemolysate from cell debris. The hemoglobin (Hb) level was determined by Cyanmethemoglobin method using Beacon Diagnostics kit. Iron was determined by Ferrozine method using Coral Clinical Systems kit. Total iron binding capacity was determined by Ferrozine method using Coral Clinical Systems kit.

The lipid peroxidation product, malondialdehyde (MDA) in plasma determined according to Buege and Aust [6], and lipid hydroperoxide by the method of Jiang et al [7], Catalase (CAT) activity were assayed by the method of Sinha [8], Glutathione peroxidase (GPx) by the method of Rotruck method [9], Superoxide dismutase (SOD) activity in hemolysed RBC was determined by the method of Kakkar et al [10], Vitamin A was estimated by the method of Bessy et al [11], Vitamin C (Ascorbic acid) was estimated by the method of Natelson [12] using DNPH, Vitamin E was measured by the method of Baker and Frank method [13] Total antioxidant activity was determined according to Benzie & Strain [14]. Descriptive statistics are means ± standard deviations (SD). Results were analyzed using Student's *t*-test, with 95% confidence interval. The packaged program SPSS for windows version 13.0 was used for statistical analysis.

RESULTS AND DISCUSSION

The demographic data of our experiment illustrated in table 1 shows mean age, BMI, SA and dietary habit. Neither patients nor healthy control group were on medication. Our cases were freshly diagnosed with anemia. Various biochemical investigations were performed in case and control (table 2) before and after vitamin supplementation. When case were compared with controls before supplementation, all the lipid peroxidation, enzymatic and non-enzymatic antioxidants showed statistically significant relations ($p<0.005$). Likewise on comparing them after supplementation- the lipid peroxidation, enzymatic and non-enzymatic antioxidant parameters showed statistically significant relations at $p<0.005$.

On vitamin supplementation to female cases, BMI and Hb showed no statistical significance as compared to after vitamin supplementation. However, both lipid hydroperoxide and TBARS levels decreased after vitamin supplementation. Likewise enzymatic antioxidants SOD, GPx and Vitamin C and Total antioxidant activity showed statistically significant increase after vitamin supplementation as compared with before supplementation group.

An increased concentration of end products of lipid peroxidation is the evidence most frequently quoted for the involvement of free radicals in human disease. However, it is likely that increased oxidative damage occurs in most, if not all, human diseases and plays a significant pathological role in only some of them. Earlier studies have proved that poor hemoglobin levels (anemia) are due to increased turnover of hemoglobin protein due to increased level of malondialdehyde or reactive oxygen species. Anemia that comes on quickly often has greater symptoms which may include: confusion, feeling like one is going to pass out, and an increased desire to drink fluids. Oxidative equilibrium is disturbed due to decrease antioxidant power to scavenge free radicals. In anemia, circulating vitamins C and E were decreased as compared to healthy subjects. Both vitamin E and MDA showed a significant correlation with hemoglobin levels.

It is evident that vitamin E and vitamin C are important antioxidants that cannot be synthesized by most mammals and humans and is therefore required from the diet. Dietary supplementation of vitamin C and E has been shown to increase tissue resistance to lipid peroxidation in humans. The major lipid soluble antioxidant, vitamin E protects cell membrane from free radical injury [2-5]. Ascorbic acid is a water soluble antioxidant in cytosol and extracellular fluid. Its chemical properties allow it to interact directly with superoxide, hydroxyl and singlet oxygen in the aqueous phase such as plasma thus preventing damage to erythrocyte membrane. Erythrocytes are susceptible to oxidation, but has good capacity for recovery, so that significant decrease in MDA levels in anemic patients after iron supplementation with ferrous sulfate has been reported [3-5]. Two possible factors may lead to increased lipid peroxidation: the increasing production of free radicals and the declining activity of the antioxidant system [15, 16]. Antioxidant enzymes are the major defense systems of cells in normal aerobic reactions. Erythrocytes are equipped

with a highly effective antioxidant defense system. After confirming a positive association between anemia status and plasma MDA levels, an attempt was made to identify factors associated with increased MDA levels [13, 17-19]. Very low erythrocytic SOD and GPx in anemia patients was detected which corroborates with this study [3,5,18-20]. The CAT activity too was decreased in their study group. Interestingly, GPx activity in IDA group was similar to healthy control group. This finding is in accordance with the findings that showed GPx activity was similar to healthy controls [21]. In contrast to our result, positive correlation between GPx activity and serum iron levels were found by few researchers [15-20] In the present study, SOD activity was significantly lower than the controls which are in contrast with some research reported that an assay of lipid peroxidation and activity of antioxidant enzymes in anemic cells showed malondialdehyde production was elevated as an indication of oxidative stress as proved by the present study.

Table 1: Demographic data of anemic case and healthy controls

Parameter	Anemic case	Healthy Control
Number	25	25
Mean Age (yr)	33.61±6.12	38.81±2.83
BMI (kg/m^2)	17.21±1.43	22.64±2.38
Surface area (m^2)	1.48±0.21	1.72±0.38
Family History of anemia	No	No
Fruits	Occasional	Occasional
Vegetables	Daily	Daily
Diet-Veg (%)	47	51
Non-veg occasional (%)	46	29
Non-veg regular (%)	7	20

Note: BMI-Body Mass Index,
Data are expressed in Mean±Standard deviation

Table 2: Biochemical investigation of females before and after Vitamin Suppl

	Suppl	Healthy Control	Anemic (Case)	Sig.
BMI	before	20.48±1.07*	21.361.70	0.628
	after	20.68±1.57*	22.49±1.98	
Hb	before	14.71±0.83*	8.46±0.09	0.576
	after	15.47±0.63*	9.27±0.88	
Lipid peroxidation parameter:				
TBARS	before	3.21±1.46*	6.14±0.79	
	after	3.54±2.42*	5.43±0.62	0.482
LOOH	before	2.51±1.46*	11.86±0.95	**0.000**
	after	2.24±2.42*	7.18±0.02	
Enzymatic antioxidant parameter:				
CAT	before	358.62±19.34*	818.29±53.57	
	after	180.88±26.05	744.02±65.93	0.331
SOD	before	58.41±9.07*	23.74±1.65	**0.000**
	after	64.75±5.14	49.55±1.18	
GPx	before	3.85±1.32*	1.37±0.25	**0.000**
	after	4.21±0.43*	3.77±0.26	
Non-Enzymatic antioxidant parameter:				
VITA	before	75.53±15.65*	54.78±6.89	
	after	82.26±19.63*	54.20±7.60	0.544
VITC	before	0.48±0.26*	0.38±0.03	**0.009**
	after	0.74±0.25*	0.54±0.06	
VITE	before	1.12±0.22*	0.52±0.12	0.333
	after	1.22±0.34*	0.49±0.14	
TAA	before	675.17±12.41*	506.11±8.17	**0.025**
	after	852.22±14.36*	565.50±18.13	

*Note: * denotes significance (p<0.005); case versus control before vitamin supplementation; and case versus control after vitamin supplementation.*
Data are expressed in Mean±Standard deviation; Suppl-Supplementation

Low SOD activity was revealed in anemic patients and was significantly increased after every treatment and supplementation [16-19]. GPx activities in anemic patients were similar to that of the controls but significantly decreased after parental iron and parental iron plus vitamin E treatments. Some researchers showed decreased activities of antioxidant enzymes, such as SOD, GPx and CAT, in patients with anemia [20-22]. In the present study,

SOD activity in anemic patients was lower than that of control group, which might be caused by insufficient nutrition and oxidative stress under hypoxic condition. It is well known that ROS, especially hydrogen peroxide inhibit SOD activity. Furthermore, decreased SOD activity may contribute to free radical production.

Studies also found that supplementation of iron leads to oxidative stress. However, iron is required as a structural and functional component of various compound (catalase, peroxidase, cytochrome oxidase, NADH reductase, iron sulfur complex), it plays a vital role in maintaining the antioxidant defense system of human body. In anemia, the enzymes involved in the antioxidant defense system will be functionally defective. So the balance gets tilted towards free radicals triggering oxidative damage. The correction of anemia with drug and vitamin supplementation leads to rejuvenation of defective antioxidant system and brings back the balance between ROS and antioxidant system [15, 18-20].

CONCLUSION

The outcomes may be an important impact towards the identification of the role played by lipid peroxides in the determination of the anemic condition. Free radical damage was noticeable in anemia which can be well-adjusted by the diet rich in antioxidant and hemoglobin content and further studies has to be performed with more sample size which was lack in our study as there was loss of contact with patients after vitamin supplementation.

Acknowledgement
We would like to thank Central College Campus and Bangalore University, Research Scholars, postgraduate students and Ladies Hostel students who kindly agreed to draw blood for control samples. We would also like to thank K. C. General Hospital and all the anemic patients who kindly permitted us to draw blood to carry out this experiment. We are also indebted to Indian Government for providing Silver Jubilee Scholarship.

REFERENCES

[1] http://en.wikipedia.org/wiki/Anemia
[2] R Hoffman R, EJ Benz, SJ Shanttil, B Furie, HJ Cohen, LE Silberstein, et al. In: Hematology: Basic Principles and Practice. 4th Ed. UK: Elsevier Churchill Livingston. **2005**.
[3] B Lachili, I Hininger, H Faure, J Arnaud, MJ Richard, A Favier, et al. *Biol Trace Elem Res*, **2001**, 83(2): 103-110.
[4] ALA Ferreira, PEA Machado, LS Matsubara. *Braz J Med Biol Res*. **1999**, 32(6): 689-94.
[5] J Acharya, NA Punchard, JA Taylor, RP Thompson, TC Pearson. *Eur J Hematol*. **1991**, 47(4): 287-291.
[6] JA Buege, SD Aust. Microsomal lipid peroxidation. *Methods Enzymol*. **1978**, 52: 302-310.
[7] ZY Jiang, JV Hunt, SP Wolff. *Anal Biochem*. **1992**, 202: 384-389.
[8] KA Sinha. Colorimetric assay of catalase. *J Biochem*. **1972**, 47: 389.
[9] JT Rotruck, L Pope, HE Ganther, AB Swanson. *Science*. **1973**, 179: 588.
[10] P Kakkar, B Das, PN Vishwanathan. *Ind J Biochem Biophys*. **1984**, 21: 130–132.
[11] OA Bessey, OH Lowry, MJ Brock, JA Lopez. *J Biol Chem*. **1946**, 166: 177-188.
[12] S Natelson. Determination of ascorbic acid by 2, 4- dinitrophenyl hydrazine. In: Techniques of clinical chemistry. 3rd Ed.USA: Charles C Thomas Springfield. **1971**, 165-166.
[13] H Baker, O Frank. Determination of Vitamin E. Determination of vitamin E. In: Clinical vitaminology. USA. **1968**, 172.
[14] IFF Benzie, JJ Strain. *Analytical Biochem*. **1996**, 239; 70-76.
[15] B Halliwell. *Lancet* **1994**, 344: 721.
[16] T Grune, O Sommmerburg, WG Siems. *Clin Nephrol*. **2000**, 53(1 Suppl): S18-22.
[17] E Nagababu, ME Fabry, RL Nagel, JM Rifkind. *Blood Cells Mol Dis*. **2008**, 41(1): 60-66.
[18] M Isler, N Delibas, M Guclu, F Gultekin, R Sutcu, M Bahceci, et al. *Crot Med J*. **2002**, 43(1): 16-19.
[19] Y Sevgi, C Gonenc, A Ciodem. *Scand J Hematol*. **1986**, 36(1): 58-60.
[20] A Rehman, CS Collis, M Yang, M Kelly, Diplock AT, B Halliwell, et al. *Biochemical and Biophysical Research Communications* **1998**, 246(1): 293-298.
[21] M Yang, CS Collis, M Kelly, AT Diplock, CR Evans. *Eur J Clin Nutr*. **1999**, 53(5): 367-374.
[22] N Kumar, N Chandhiok, B Dhillon, P Kumar. *Indian J Clin Biochem*. **2009**, 24(1): 5-14.