Indian Journal of Clinical Biochemistry, 2008 / 23 (1) 95-97

ERYTHROCYTE GLUTATHIONE STATUS IN HUMAN VISCERAL LEISHMANIASIS

D P Neupane, S Majhi, L Chandra, S Rijal* and N Baral

*Departments of Biochemistry and *Medicine, B. P. Koirala Institute of Health Sciences, Dharan, Nepal*

ABSTRACT

A glutathione redox cycle is a major antioxidant defense system for the detoxification of reactive oxygen species within erythrocytes. Reactive oxygen species such as superoxide anions, hydrogen peroxide and hydroxyl radicals are generated as a host defense mechanism for killing of engulfed Leishmania donovani*, a causative agent of visceral leishmaniasis, are capable of damaging lipids and other biomolecules when produced in excess. Erythrocytes are most vulnerable to Reactive oxygen species. In present study we aimed to evaluate erythrocyte reduced glutathione (GSH) levels as an antioxidant and erythrocyte malondialdehyde (MDA) as a marker of lipid peroxidation. The study included twenty-five Visceral leishmaniasis patients and they were followed up after their complete chemotherapy with antileishmanial drugs (sodium stibogluconate) for 30 days. Forty six age and sex matched healthy individuals were taken as controls. GSH levels in erythrocytes of visceral leishmaniasis patients were increased in spite of significant increased erythrocyte MDA as compared to controls. Whereas erythrocyte GSH and MDA levels of follow up patients were decreased as compared to patients before treatment groups. We concluded that visceral leishmaniasis patients are in oxidative stress which most likely induces the endogenous antioxidant such as GSH or its poor utilization by cells.*

KEY WORDS

Glutathione, Malondialdehyde, Visceral Leishmaniasis.

INTRODUCTION

Visceral Leishmaniasis is caused by the protozoan parasite *Leishmania donovani* and is transmitted through bite of an insect vector, phlebotomine sand fly. Once parasites inoculated in the skin are phagocytozed by macrophages which in turn produces reactive oxygen species (ROS) such as superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) as a host defense mechanism. But the accumulation of these ROS in the cells are capable of damaging biomolecules in which lipids are probably the most susceptible if not controlled by appropriate antioxidant scavenging system. The oxidative destruction of polyunsaturated fatty acids (PUFAs) is particularly damaging because it proceeds as a self perpetuating chain reaction (1). One of the toxic end products of lipid peroxidation is malondialdehyde (MDA). The accumulation of H_2O_2 decreases half life of erythrocytes by increasing oxidation of PUFAs of membranes and also by oxidizing hemoglobin to methemoglobin. Reduced glutathione (GSH) plays a central role in detoxifying these ROS by direct scavenging and also as a substrate for glutathione peroxidase which removes H_2O_2 accumulated in the cells. Erythrocytes are particularly sensitive to oxidative stress and like other cells are supplied with protective antioxidant mechanism in order to counteract the toxic action of ROS. Erythrocytes reduced GSH is one of the major endogenous antioxidant protecting tissue against ROS. This study was therefore carried out to find out the extent of oxidative stress in erythrocytes by estimating the level of MDA and glutathione status in visceral leishmaniasis patients.

Address for Correspondence :

Prof. Nirmal Baral
Department of Biochemistry,
B. P. Koirala Institute of Health Sciences, Dharan, Nepal
E-mail : nirmalbaral@yahoo.com

MATERIALS AND METHODS

The present study was conducted at B. P. Koirala Institute of Health Sciences, Dharan, Nepal. This was a longitudinal study which included 25 newly diagnosed (n-25) visceral leishmaniasis patients and they were followed-up after complete chemotherapy with Sodium Stibogluconate (SSG) for 30 days. Patients before treatment (n-25) and after

treatment (n = 25) groups were compared separately with forty six (n= 46) age and sex matched healthy individuals as control group. The patient before treatment group was also compared with their follow-up. Informed consent was taken from patients as well as control groups. Three milliliters of blood samples were taken in EDTA vials. The chemicals used were of analytical grade and obtained from E-Merck and Hi-Media laboratory limited. The following estimations were carried out:

- Hemolysate MDA (HMDA): The hemolysate was prepared as described by Laxmi and Rajagopal (2). The breakdown product of Lipid peroxidation like MDA reacting with thiobarbituric acid to form pink chromogen was measured at 535nm by the method of Donnan (3).
- Reduced glutathione (GSH): The method is based on the development of a yellow color when 5, 5'-dithiobis-(2-nitrobenzoic acid) is added to the sulfhydryl compounds as described by Beutler *et al* method (4).
- Hemoglobin (Hb): Hb in whole blood and hemolysate is oxidized to methemoglobin with alkaline cyanide reagents to produce brown color compound as described by Drabkin and Austin (5).

The results were analyzed by student's 't' test to find out level of significance. P value ≤ 0.05 is considered as statistically significant.

RESULTS AND DISCUSSION

The results obtained are shown in Table 1.The result showed an important role of free radicals in etiopathogenesis of visceral leishmaniasis. The extent of Lipid peroxidation (LPO) can be measured by the amount of MDA formed in erythrocytes (6). In present study there was significantly increased HMDA in patients before treatment group which is due to increased production of ROS as host defense mechanism against invaded parasites. This indicates that patients of this group are in oxidative stress which is supported by their decreased level of Hb. Several lines of evidences suggest an important role of enhanced lipid peroxidation in pathogenesis of hemolytic anemia. Increased level of erythrocyte MDA and decreased level of Hb have been described in experimental visceral leishmaniasis in Hamsters by Sen et al (7).The increased MDA in erythrocytes may be seen as a result of tissue catabolism of PUFAs (8). HMDA levels were significantly decreased after the treatment of patient which suggests decrease in the production of ROS due to killing of microorganisms.

In all cell types exposed to oxidative stress, GSH plays a central role in protecting the cell. It has been reported that GSH can transduce its reducing power and serve as sulfhydryl buffer contributing the maintenance of reduced state of many sulfur-rich proteins spanning the erythrocytes membrane (9). A glutathione redox cycle is a major defense system for detoxification of ROS within the erythrocytes (10). In the present study, the significantly decreased level of whole blood GSH in patient before treatment groups as compared to control is due to high oxidative stress and over utilization of GSH by cells. At the same time significantly decreased levels of whole blood GSH in patient after treatment group may be due to the involvement of GSH in drug metabolism. The patients treated with SSG for 30 days may be using GSH for their metabolism. Hence, in our results, its level is decreased in whole blood. This is in agreement with previous study done by Carter et al (11) who has reported that GSH is involved in SSG metabolism.

However there is high GSH concentration in erythrocytes in spite of a significant increase in erythrocyte MDA. There is positive correlation between erythrocyte MDA and GSH levels. Glutathione peroxidase (GPx) is a powerful antioxidant enzyme that removes hydrogen peroxide from erythrocytes and its activity is dependent on GSH concentration. The GSH content in turn is maintained by the NADPH levels via glutathione reductase (GR). Thus it seems that the slight accumulation of GSH in patients before treatment groups may be due to reduction in the activity of GPx in comparison to

Table 1 : Comparison of different parameters among patients before treatment (BT) and after treatment (AT) with control group

Group	Hemolysate MDA (nmol/gHb)	Whole blood GSH (mg/dl)	Hemolysate GSH (mmol/g Hb)	Hb (g/dl)
Control (n=46)	4.79 ± 1.46	35.02 ± 7.8	8.15 ± 1.51	14.19 ± 2.24
Patients_BT (n=25)	7.91 ± 2.97 ^{a***}	21.83 ± 5.87 ^{a***}	9.11 ± 3.31	8.31 ± 2.14 ^{a***}
Patients_AT (n=25)	5.05 ± 2.04$^{a***, b***}$	24.52 ± 5.34 ^{a***}	8.2 ± 1.97	10.10 ± 1.80 $^{a***, b**}$

Comparison of patient groups with control groups (a) and among patient groups (b); The values are expressed in Mean ± SD
At p, a*** ≤ 0.001, b** ≤ 0.01, b*** ≤ 0.001

GR. Moreover, in active visceral leishmaniasis patients there was severe oxidative stress accumulating ROS. It has been shown that in cases of oxidative stress GPx can be inactivated as $O_2^{-\cdot}$ can inhibit peroxide function (12). Decreased activity of GPx decreases the utilization of GSH by erythrocytes and consequently increased level of GSH. The studies done by Erel et al (13) and Kocyigit et al (14) showed the decreased GPx activity in erythrocytes of cutaneous leishmaniasis patients compared to control. There is slight decrease of GSH in erythrocytes of follow-up patients as compared to patients before treatment indicates that the patients were trying to come to normal GSH levels with good prognosis. Our results are supported by the study done by Vural et al (15) who suggested increased MDA and increased GSH level in erythrocytes of cutaneous leishmaniasis.

In conclusion, the patients suffering from visceral leishmaniasis are in oxidative stress. Parasite invading the macrophages causes respiratory burst releasing different reactive oxidative species as a host defense. Increased ROS not only kill the parasites but also damage the cells and release MDA as a secondary marker of tissue damage. The whole blood GSH levels have been found to be decreased significantly due to counteract with ROS but erythrocyte GSH levels have been found to be increased indicating that either GSH is induced endogenously or is not effectively utilized by the erythrocytes to counteract the ROS.

REFERENCES

1. Chessaman KH. Lipid peroxidation in biological systems. In: Halliwell B, Aruoma OI, eds. DNA and Free Radicals. London: Ellis Horwood; 1993: 201-11.
2. Laxmi S, Rajagopal G. Reduced glutathione level and catalase activity in erythrocytes in patients with Diabetes Mellitus. Biomedicine 1998; 18(1): 37-9.
3. Donnan SK. The thiobarbituric acid test applied to tissues from rats treated in various ways. Biochem J 1950; 182: 415.
4. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab and Clin Med 1963; 61(5): 882-8.
5. Drabkin DL, Austin JM. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. Journal of Biological Chemistry 1932; 98: 719-33.
6. Biswas T, Ghose DK, Mukherjee N, Ghosal J. Lipid peroxidation of erythrocytes in visceral leishmaniasis. J Parasitol 1997; 83: 151-2.
7. Sen G, Mukhopadhyay R, Ghosal J, Biswas T. Oxidative damage of erythrocytes: a possible mechanism for premature hemolysis in experimental Visceral Leishmaniasis in hamsters. Ann Hematol 2001; 80: 32-7.
8. Gutteridge JMC, Tickner TR. The characterization of Thiobarbituric acid reactivity in human plasma and urine. Analyte Biochem1978; 91: 250-7.
9. Bozzi A, Parisi M, Strom R. Erythrocyte glutathione determination in the diagnosis of glucose-6-phosphate dehydrogenase deficiency. Biochem Mol Biol Int 1996; 40: 561-9.
10. Saltman P. Oxidative stress: a radical view. Semin Hematol1989; 26: 249-56.
11. Carter KC, Sundar S, Spickett C, Pereiva OC, Mullen AB. The *in vivo* susceptibility of *Leshmania donovani* to sodium stibogluconate is drug specific and can be reversed by inhibiting glutathione biosynthesis. Antimicrobial Agents and Chemotherapy 2003; 47: 1529-35.
12. Blum J, Fridovich I. Inactivation of glutathione peroxidase by superoxide radical. Arch Biochem Biophys 1985; 240: 500-8.
13. Erel O, Kocyigit A, Bulut V. Reactive nitrogen and oxygen intermediates in patients with cutaneous leishmaniasis. Mem Inst Oswaldo Cruz 1999; 94: 179-83.
14. Kocyigit A, Gurel M, Ulukanligil M. Erythrocyte antioxidative enzyme activities and lipid peroxidation levels in patients with cutaneous leishmaniasis. Parasite 2003; 10(3): 277-81.
15. Vural H, Aksoy N, Orbilge H. Alterations of oxidative-antioxidative status in human cutaneous leishmaniasis. Wiley Inter Science 2004; 22: 153-6. .