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BRIEF COMMUNICATION

TOTAL ANTIOXIDANT CAPACITY AND LIPID PEROXIDATION IN LIVER TRANSPLANTATION

Vaishali N Thorat , Adinath N Suryakar*, Pradeep Naik and Bipin M Tiwale**

*Department of Biochemistry, Dr. D. Y. Patil Medical College, Kolhapur, *RCSM Govt. Medical College, Kolhapur and **Global Hospitals, Hyderabad*

ABSTRACT

Liver transplantation is an accepted therapy for chronic liver disease patients. These patients generally have low levels of fat soluble vitamins, which have important antioxidant roles. Therefore, this study was undertaken to investigate whether such patients had evidence of antioxidant depletion and increased lipid peroxidation before transplant and whether the subsequent ischemia and reperfusion encountered during liver transplantation have any effect on antioxidant levels and lipid peroxidation. We assessed plasma total antioxidant capacity and serum lipid peroxide in 12 patients undergoing liver transplantation and equal numbers of healthy subjects. We found that before reperfusion, antioxidant levels were significantly decreased along with significantly elevated lipid peroxidation levels as compared with healthy controls (P<0.001). On reperfusion of the liver graft, further declined values of total antioxidant accompanied with highly elevated lipid peroxidation were seen than those of pre-reperfusion samples (P<0.001). This data shows that patients undergoing liver transplant have lowered antioxidant defenses and evidence of free radical damage, which compound the additional insult of reperfusion injury. Therefore antioxidant therapy in these patients before transplantation may ameliorate the effects of reperfusion.

KEY WORDS

Ischemia/reperfusion injury, Total antioxidant, Lipid peroxidation.

INTRODUCTION

Liver transplantation has firmly evolved as a viable option for a condemned patient with end stage liver disease (ESLD) (1). Even with major improvements in the logistics of organ transplantation, every transplantation starts with an inevitable insult on the graft: Ischemia and Reperfusion (2). During ischemia reperfusion injury, generation of reactive oxygen species (ROS) is carried out. These species may interact with essential cellular targets, including proteins, lipids and DNA, compromising cell viability and function (3, 4). However, liver cells, mainly hepatocytes have developed a comprehensive array of antioxidant defenses to prevent formation of ROS or limit their damaging effects.

In many acute and chronic disease processes, free radical activity is increased, often associated with antioxidant depletion (5). Patients with chronic liver damage undergoing elective transplantation may have inadequate antioxidant defenses in the face of increased free radical production. This study was undertaken to investigate whether such patients had evidence of total antioxidant depletion and increased lipid peroxidation before transplant and whether the subsequent ischemia and reperfusion encountered during liver transplantation have any effect on total antioxidant level and lipid peroxidation.

Although it is possible to measure the individual antioxidant concentration directly, the combined effect due to cooperativity between the antioxidants, for example vitamin C and E (6) and any compensation mechanism due to relative deficiency

Address for Correspondence :

Prof. A. N. Suryakar,
Department of Biochemistry,
RCSM Govt. Medical College, Kolhapur-416410
E-mail: shali_s_patil@yahoo.co.in

in any one antioxidant is better examined by measurement of the total antioxidant capacity.

MATERIALS AND METHODS

The present work was carried out in the Department of Biochemistry, Global Hospitals, Hyderabad and Dr. V. M. Govt. Medical College, Solapur. In the present study 12 patients between 23-62 years of age undergoing elective liver transplantation and 12 normal healthy age and sex matched controls were included. Blood samples were collected from arterial lines before reperfusion of new liver, 15 minutes after reperfusion and at the end of the transplantation. 2ml of blood sample was collected in the vacutainer. Out of this, 1 ml blood was taken in heparin containing vacutainer for estimation of plasma total antioxidant capacity by the FRAP (Ferric Reducing Ability of Plasma) assay (7). Remaining blood sample was used for the determination of serum lipid peroxidation as malondialdehyde (MDA) by Satoh method (8). All samples were immediately placed at 4°C until they were processed (within 2 hours) and were stored at -70°C in separate aliquots for each assay until analysis.

Statistical analysis was performed by using students paired and unpaired 't'-test. P value less than 0.05 ($P < 0.05$) was considered as significant.

RESULTS AND DISCUSSION

In Table 1, increased levels of serum lipid peroxide (MDA) were observed in all stages of liver transplant patients as compared to that of controls ($P< 0.001$). Further it was noted that significantly increased serum lipid peroxide levels in reperfused phase than those of pre-reperfusion ($P<0.001$) and post-reperfusion ($P<0.001$) phases of liver transplant (Fig. A). Cellular damage by free radicals associated with lipid peroxidation is thought to be a significant factor in a wide variety of disease conditions including reperfusion injury during organ transplantation.

Table 1: Plasma total antioxidant capacity and serum lipid peroxidation (MDA) in patients undergoing liver transplantation and normal healthy control subjects

Sr. No.	Groups	Serum Lipid peroxide (n mol MDA/ml)	Plasma Total antioxidant (n mol /L)
1	Controls (n=12)	3.43±1.23	5320± 120
2a	Pre-reperfusion (n=12)	6.24±0.7*	3220± 0.7*
2b	Reperfusion (n=12)	8.10±0.4**	1433±0.18**
2c	Post-reperfusion (n=12)	7.1±0.5[a]	3012± 0.36*

n=number of subjects studied; Values are expressed as Mean±SD
** P<0.001 as compared to controls; * P<0.001 group 2b versus 2a and 2c; [a] P<0.05 group 2b versus 2c.

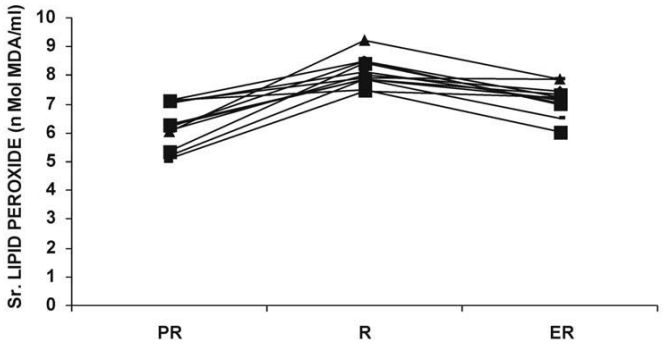

Fig. A : Serum Lipid Peroxidation in Different Stages of Liver Transplantation

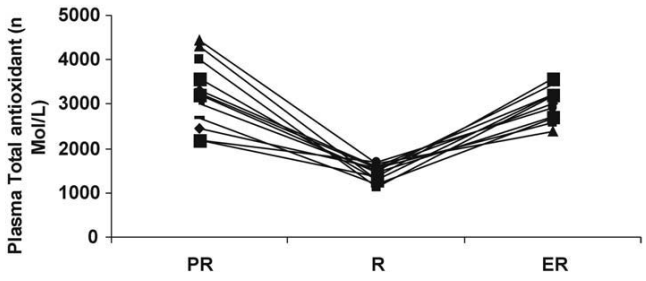

Fig. B : Plasma Total Antioxidant Capacity in Different Stages of Liver Transplantation (PR - Pre-reperfusion, R-reperfusion, ER- end of reperfusion)

The most commonly implicated mechanism for generation of free radicals in liver transplant patient is hypoxanthine/xanthine oxidase system. During hypoxia, hypoxanthine accumulates in the tissues. When a high concentration of oxygen is administered via blood during reperfusion in ischemic explants, large amount of oxygen free radicals are produced that cause lipid peroxidation. As a consequence of lipid peroxidation and their action, there is not only tissue damage but also pronounced consumption of the serum antioxidant capacity in organ recipients.

Depletion in the level of plasma total antioxidants was seen in

all liver transplant patients as compared to controls (P<0.001) (Table 1). In our study there was significant fall in total antioxidant level at the time of reperfusion than those of pre-reperfusion (P<0.001) and post-reperfusion (P<0.001) phases of liver transplant (Fig. B), which is in agreement with Goode et al (9), who stated that total antioxidant utilization is associated with increased lipid peroxidation.

Production of free radicals during reperfusion is inevitable and whenever they are produced they are always damaging. Defenses against the deleterious actions of such free radicals have evolved. These are known as antioxidant defenses and the two main categories are those, whose role is to prevent the generation of free radicals and those which intercept the radicals generated.

The present study demonstrated that patients undergoing liver transplant have lowered antioxidant defenses and evidence of free radical damage. The results of the present study are in agreement with earlier reports (5, 9).

Mechanism behind the significant reduction in total ferric reducing ability of plasma is due to increased oxidative stress by both, pre-existing cirrhosis and ischemic-reperfusion injury that results in imbalance between antioxidant capacity and pro-oxidants. Therefore antioxidants can be used in liver transplantation to effectively inhibit the severity of reperfusion injury and to improve short term allograft function and survival. Antioxidant therapy in these patients before transplantation may ameliorate the effect of reperfusion.

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