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Beneficial role of antioxidants during liver transplantation

Vaishali S. Patil[1*], Adinath N. Suryakar[2] and Pradeep Naik[3]

[1]Department of Biochemistry, Dr. D. Y. Patil Medical College, Vidyanagar, Kasba Bawda, Kolhapur, Maharashtra, India, [2]Registrar, Maharashtra University of Health Sciences, Mhasrul, Vani-Dindori Road, Nashik, Maharashtra, India and [3]Department of Biochemistry, Global Hospitals, Khairatabad Road, Lakdikapul, Hyderabad, Andhra Pradesh, India

Abstract: *Background & Objectives:* Initial graft dysfunction, an event mainly due to the unavoidable ischemia-reperfusion (I/R) injury of the transplanted organ, is one of the most important early post-operative problems in liver transplantation. It is well known that antioxidants significantly improves early allograft function and both graft and patient survival. Thus, the present study was carried out to evaluate the exact role of antioxidants during liver transplantation. *Method:* We assessed serum lipid peroxide (as oxidant), serum superoxide dismutase (SOD) and serum vitamin E (as antioxidant) in 30 patients undergoing liver transplantation and equal numbers of healthy subjects. *Results:* We found that the concentration of serum lipid peroxide (MDA) was significantly increased and antioxidants were significantly decreased in all stages of liver transplant patients as compared with healthy controls ($P<0.001$). On reperfusion there was pronounced consumption of antioxidants and highly elevated levels of serum lipid peroxide was seen than in those of pre-reperfusion and post-reperfusion stages ($P<0.0001$) of liver transplantations. *Conclusion:* Antioxidants can be used in liver transplantation patients to effectively reduce the severity of reperfusion injury and to improve short-term allograft function and patient survival.

Keywords: Hepatic ischemia/reperfusion injury; Lipid peroxide (MDA); Superoxide dismutase (SOD); Vitamin-E.

Introduction

Transplantation of a solid organ from an organ donor has emerged as a treatment option for many diseases that otherwise mean certain death or long-term dependency on life support systems for the patient [1]. Despite the shortage of organs for transplantation, careful attention is paid to the fact that only functionally intact undamaged organs are used for transplantation [2]. However, even with major improvements in the logistics of organ transplantation, every transplantation starts with an inevitable insult on the graft: ischemia and reperfusion (I/R) [3]. Initial graft dysfunction, an event mainly due to the unavoidable ischemia-reperfusion injury of the transplanted organ, is one of the most important early post-operative problems in liver transplantation [4].

A major source of liver graft injury results from the over generation of reactive oxygen species (ROS) during the reperfusion phase [5-6]. This over generation of ROS stimulated by the I/R is thought to play an important role in mediating cell injury as these species may interact with essential cellular targets, including proteins ,lipids and DNA, compromising cell viability and function [7].

A variety of oxygen derived free radicals are produced during I/R injury. Superoxide (O_2^-) is generated by the activation of xanthine oxidase and in the presence of free iron, forms the damaging hydroxyl radical. Nitric oxide ($NO^·$), itself a free radical is also liberated and reacts with superoxide (O_2^-) to form peroxynitrite, eventually decomposing to the hydroxyl radical (OH^-). Thus, reperfusion injury is characterized by loss of endothelial cell viability, which occurs after cold ischemic storage and reperfusion of liver at transplantation [6].

Liver cells, mainly hepatocytes, contain a number of mechanisms designed to counterbalance the potential damaging effects exerted by ROS, superoxide dismutase (SOD) and vitamin-E are the most important of these

defenses. Superoxide dismutase is a naturally occurring enzymatic antioxidant that specifically scavenges the superoxide anion (O_2^-) thereby inhibiting the formation of hydroxyl radical(OH^-). Vitamin-E is the most important lipid-soluble chain-breaking antioxidant and protects membranes from free radical mediated lipid peroxidation [8].

Recent experimental studies indicate that antioxidants significantly improve early allograft function and graft survival [9-11]. Despite ample knowledge about these interactions, little attention is paid to the antioxidant status in patients undergoing liver transplantation. Therefore the present study was carried out to evaluate the role of antioxidants during liver transplantation.

Material and Methods

The present work was carried out in the Department of Biochemistry, Global Hospitals Hyderabad. Thirty patients of different age groups from 23-62 years, scheduled for orthotopic liver transplantation were enrolled in the present study. Blood samples were collected at different stages of liver transplantation such as-

- Pre-reperfusion stage- In this stage blood samples were collected one day before liver transplantation.
- Reperfusion stage- It is the stage when oxygenated blood is introduced in the ischemic explants upon completion of portal vein anastomosis and attempts were made to collect the blood samples at this stage.
- Post- reperfusion- From all the transplanted patients samples were collected on the next day of transplantation.

Total 3 ml blood was collected in non-heparinised vacutainer for estimation of serum lipid peroxide as malondialdehyde (MDA) by Satoh K method [12], serum SOD by Markland and Markland's method [13] and serum vitamin-E by Baker & Frank method [14]. Results obtained were compared with age and sex matched healthy control subjects. All the samples were immediately placed at 4^0C until they were processed (within 2 hours) and were stored at -70^0C in separate aliquots for each assay until analysis.

Statistical analysis was carried out using student's paired and unpaired't' test. P value less than ($P<0.05$) was considered as significant.

Results

Increased levels of serum lipid peroxide (MDA) was observed in all stages of liver transplant patients as compared to that of healthy controls ($P<0.001$). Further it was noted that significantly increased serum lipid peroxide levels in reperfusion stage than those of pre-reperfusion ($P<0.001$) and post-reperfusion ($P<0.001$) stages of liver transplantation (Table-1).

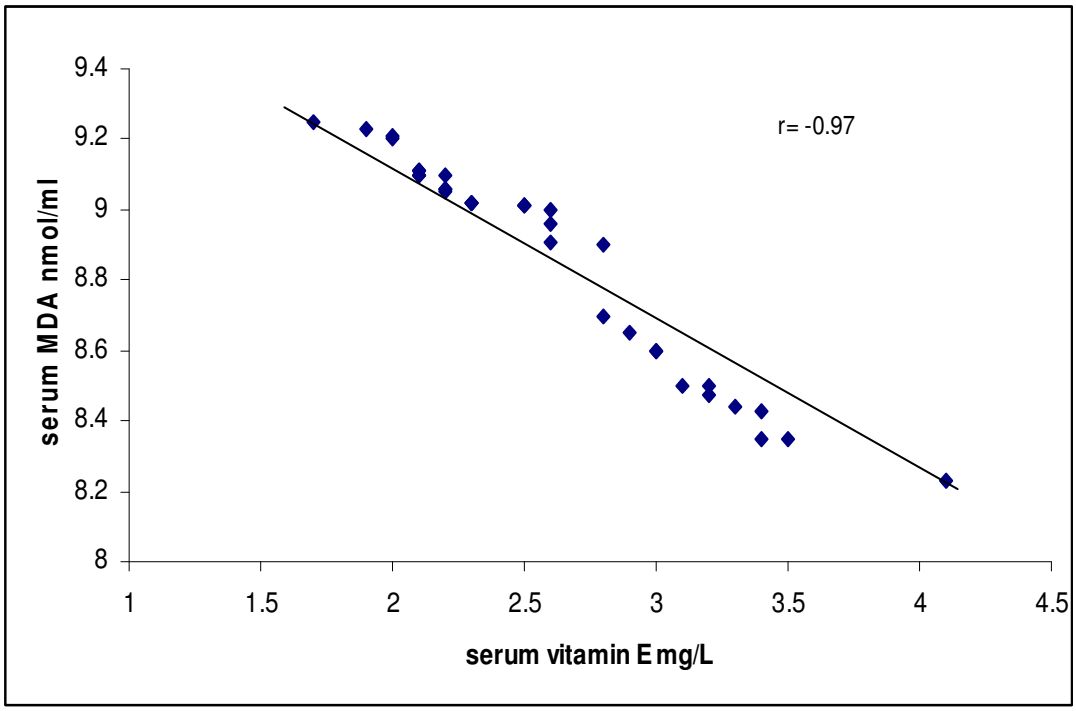
Decreased concentrations of serum SOD and serum vitamin E were seen in all liver transplantation patients as compared to controls ($P<0.001$) (Table-1).

Table-1: Levels of serum Lipid peroxide (MDA), serum superoxide dismutase (SOD) and serum vitamin E in healthy controls and Liver Transplantation patients.

Sr. No.	Study subjects	Number of subjects (n)	Serum MDA (nmol/ml)	Serum SOD (U/L)	Serum Vitamin E (mg/l)
1	Control	30	3.43±1.23	12.8±0.68	11.2±2.82
2	Pre-reperfusion	30	6.06±0.6	8.3±0.36	4.4±0.4
3	Reperfusion	30	8.8±0.3**	5.9±0.4**	2.5±0.5**
4	Post-reperfusion	30	8.3±0.4	7.09±0.7	4.9±0.6

Values were expressed as Mean ±SD, **-P<0.001- Highly significant

Figure-1: Correlation between serum MDA and serum vitamin E. A negative correlation was observed during reperfusion between serum vitamin E and serum lipid peroxide (r = -0.97), suggesting utilization of antioxidants during ischemia reperfusion injury



Discussion

Following reperfusion, a condition of oxidative stress occurs in the transplanted liver of all patients. The marked change towards oxidation of the tissue redox equilibrium was shown indirectly in the peripheral blood by the increase in the level of lipid peroxidation [15]. In particular, significant difference in the levels of lipid peroxides before reperfusion and after an end of reperfusion confirms that re-oxygenation is the triggering event in the pathogenesis of oxidative damage consequent on liver transplantation [16].

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 high concentrations of oxygen is administered to such patients during reperfusion, large amount of oxygen free radicals are produced that causes lipid peroxidation. As a consequence of lipid peroxidation and their action, there is not only tissue damage but also pronounced consumption of the antioxidant serum capacity in organ recipients [17].

Patients with chronic liver damage undergoing liver transplantation had already low levels of antioxidant defenses and increased lipid peroxidation [18]. In the present study, it was noted that the activity of serum SOD were decreased significantly in all stages of transplantation as compared with control subjects ($P<0.001$). Further it was observed that the activity of serum SOD was highly decreased at the time of reperfusion than that of pre-reperfusion and post-reperfusion levels.

Ischemia followed by reperfusion in organ transplantation is associated with oxygen free radical formation. The liver is a very rich source of xanthine oxidase and it is therefore likely that the liver graft is damaged by oxygen free radicals during reperfusion after the ischemic period of organ preservation. Superoxide dismutase is a naturally occurring enzyme that specifically scavenges the superoxide anion (O_2^-) thereby inhibiting the formation of hydroxyl radical (OH^-). Thus utilization of SOD is depending upon the amount of superoxide radicals (O_2^-) produced during reperfusion [19- 20].

In the present study, decreased activity of SOD was observed due to I/R injury. Study by Minor et al suggested that pre-ischemic treatment with SOD significantly reduce hepatic vascular resistance and loss of parenchymal enzymes in a comparable manner. Its therapy resulted in a significant increase of hepatic tissue content of ATP at the end of reperfusion. Superoxide dismutase prevents the leakage of purine nucleoside phosphorylase and significantly reduces tissue levels of lipid peroxide [21]. In contrast Dev A. et al. observed no correlation between SOD activity with I/R injury [22].

Depletion in the level of serum vitamin E were seen in all liver transplant patients as compared to controls ($P<0.001$). In our study there was significant fall in serum vitamin E level at the time of reperfusion than those of pre-reperfusion and post- reperfusion stages ($P<0.001$) of liver transplantation which is in agreement with Helen F Goode et al, who stated that total antioxidant utilization is associated with increased lipid peroxidation [6].

Significant reduction in the levels of serum vitamin E in liver transplant patients in the present study is attributed to the following mechanism. Peroxyl radicals are generated in the chain reaction of lipid peroxidation which is self perpetuating, particularly in the liver with high concentration of poly-unsaturated fatty acids, unless the reaction is quenched by a chain breaker. α-tocopherol (vitamin-E) is the most effective chain breaker in cellular membranes and in lipid phases and thus plays a significant role as an effective antioxidant by protecting membranes from free radical-mediated lipid peroxidation [23]. Therefore, antioxidants can be used in liver transplantation to effectively reduce the severity of reperfusion injury and to improve short-term allograft function and patient's survival.

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*All correspondences to: Dr. Mrs. Vaishali Santosh Patil, Associate Professor, Department of Biochemistry, Dr. D. Y. Patil Medical College, Vidyanagar, Kasba Bawda, Kolhapur-416006, Maharashtra, India. E-mail: shali_s_patil@yahoo.co.in