

“Role of Nitric Oxide in Liver Cirrhosis”

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ABSTRACT:

Introduction: Patients of liver cirrhosis suffer from deleterious effects of hyper dynamic circulation like ascites, edema etc. Increased Nitric Oxide levels may be one of the causes of them (potent vasodilator). So we have decided to study the role of Nitric Oxide in liver cirrhosis.

Materials and Methods : The Nitric Oxide levels was found in the form of Total Nitrates in 40 normal controls and 40 known cases of liver cirrhosis by Cortas and Wakid method and compared it with liver functions like serum ALT activity (Kinetic Kit method), serum albumin level (Bromocresol Green method) and prothrombin time (Quick's method) in both groups.

Observations and Results; We found that the serum Nitric Oxide levels in the form of Total Nitrates were significantly high in liver cirrhosis patients ($102 \pm 20.3 \mu\text{mol/L}$) as compared to normal controls ($57 \pm 19.9 \mu\text{mol/L}$), but having no co-relation with liver functions like serum albumin levels, serum total ALT levels and prothrombin time.

Conclusion; This study provides the evidence that increased production of Nitric Oxide in liver cirrhosis may be responsible for the hemodynamic changes seen in patients of liver cirrhosis. So by using Nitric Oxide inhibitors patient's discomfort due to hyper dynamic circulation can be reduced.

KEY WORDS : NO (Nitric Oxide), NOS (Nitric Oxide Synthase), Hyper dynamic Circulation

INTRODUCTION:

Liver cirrhosis can occur at any age and often cause prolonged morbidity. The clinical course of patients with advanced cirrhosis is usually complicated by a number of important sequelae. One of them is the systemic cardiovascular complication which includes generalized vasodilatation manifested as hypotention, decreased vascular resistance and increased cardiac output. This is called as "hyper dynamic circulation". [1] Recent studies emphasize the role of endothelium dependent vasodilators that play a role in vasodilatation and associated hyper dynamic circulation. Now the focus is on Nitric Oxide as a potent endothelium derived vasodilator. [2]

Recent references have shown that inhibition of Nitric Oxide synthesis through inhibition of Nitric Oxide Synthase enzyme (eNOS) should reverse all haemodynamic aberrations and restore the sensitivity of blood vessels to Vasoconstrictors. [3, 4] Other laboratory tests give only a rough guide to prognosis in individual patients. Fall in plasma albumin or prolonged Prothrombin Time is bad prognostic feature. [5] The present study was designed to find out the levels of Nitric Oxide in patients with liver cirrhosis and to see its relationship with other parameters like serum albumin level, Prothrombin Time and Serum Alanine Transaminase level.

MATERIALS AND METHODS:

The present study comprised of 40 male patients of liver cirrhosis who were diagnosed clinically and by laboratory

cirrhosis who were diagnosed clinically and by laboratory investigations. Patients belonged to the age group of 30 to 50 years.

Inclusion criteria- Only diagnosed male patients of liver cirrhosis between the ages of 30 to 50 years were included in our study. Permission of Ethical Committee of Dr. D Y Patil University and written consent of the patients were taken.

Exclusion criteria-

Female patients, patients with doubtful diagnosis of liver cirrhosis, patients above 50 years and below 30 years of age were excluded from our studies.

Collection of Blood Samples : Blood was collected by venepuncture with all aseptic precautions. 6 ml was collected in plain bulb and was allowed to clot and 1.6 ml in Citrate bulb. The serum was used for measurement of Nitric Oxide, Albumin and Alanine Transaminase levels and plasma from Citrate bulb was used for measurement of Prothrombin Time.

Laboratory Investigations-

1. The Nitric Oxide level was estimated in terms of the total nitrites in the patients of liver cirrhosis and controls. Method - Kinetic Cadmium-Reduction method by Kortas and wakid, 1990. Normal Range - $51.2 \pm 26.4 \mu\text{mol/L}$. [6]

2. The following three liver function tests were performed to study the correlation of serum nitric oxide levels with liver function. [7]

- a) Alanine Transaminase. - Kinetic Kit method, International Federation of Clinical Chemistry. Normal Range- 0 to 40 IU/L
- b) Albumin. - B. C. G. Dye method, Doumas et al. Normal Range- 3.5 to 5.3 gm/dl
- c) Prothrombin Time. - Quick's method. Normal Range - 15 to 18 seconds.

OBSERVATIONS AND RESULTS :

Table 1 showed Distribution of subjects selected for study groups.

Table 2. It is observed from Table 2 that increase in the serum Nitric Oxide levels in terms of Total Nitrites of Group A patients were statistically significant as compared to the control subjects that is group B, ($P < 0.05$) [8].

Table 3. Serum ALT activity, Serum Albumin level and Prothrombin Time in the liver cirrhosis patients and the control group. It is observed from Table 3 that the difference between all three parameters of Group A and Group B are statistically significant, ($P < 0.05$).

Table 4. Correlation between Nitric Oxide (in terms of Serum Total Nitrites) and Serum ALT level, Serum Total Nitrites and Serum Albumin, Serum Total Nitrites and Prothrombin Time.

To test the significance "Hypothesis of no correlation" is followed. The values of all the three parameters (ALT, Albumin, PT) being less than 2 is not significant at 5% level; hence the "Hypothesis of no correlation" is accepted. In other words Serum Total Nitrites and Alanine Transaminase, Serum Total Nitrites and Albumin, Serum Total Nitrites and Prothrombin Time have no significant correlation.

DISCUSSION : Liver cirrhosis is a chronic illness that occurs due to continued action of the initial damaging factor. The three serious complications of liver cirrhosis are portal hypertension, hepatocellular failure and liver cell carcinoma. The sequelae of portal hypertension which has occurred due to damage to the portal veins are splanchnic congestion and opening of arteriovenous anastomosis. [9]

The splanchnic congestion will cause formation of ascites, oedema, splenomegaly etc. Three theories have been proposed for the formation of ascites out of which the most viable theory is the "peripheral arterial vasodilatation hypothesis". According to this theory portal hypertension results in splanchnic arteriolar

vasodilatation mediated by Nitric Oxide and leading to under filling of the arteriolar vascular space, with baroreceptor mediated stimulation of renin-angiotensin system and release of antidiuretic hormone, which will cause salt and water retention.

Nitric Oxide which has been identified as an important vasodilator produced by vascular endothelial cells is a reactive diatomic gaseous molecule with an unpaired electron. [10]. NO is generated from one of the terminal nitrogen atoms of guanidine group of arginine which yields citrulline along with NO. Molecular oxygen and NADPH are co-substrates and the reaction is catalyzed by Nitric Oxide Synthase (NOS). [11]

Our study provides the evidence that there is increased production of Nitric Oxide in patients with liver cirrhosis ($102.0 \pm 20.3 \mu\text{mol/L}$) as compared to normal control subjects ($57.2 \pm 19.9 \mu\text{mol/L}$) $P < 0.05$. The results obtained are in contention with several studies conducted in the past. [12, 3]

Many scientists have proved this role of NO in liver cirrhosis indirectly by inhibiting its synthesis from arginine by enzyme NO Synthase. They have shown that chronic administration of Amino-guanidine could reduce systemic NO levels as well as suppress inducible NO Synthase (iNOS) expression and activity in aorta of biliary tract ligated rats. It also improved liver function possibly by correcting the systemic hemodynamic disorders by decreasing vascular NO production. [13, 14]. Similar study was done on human being in 2004. Advanced cirrhosis is characterized by arterial vasodilatation and reduced arterial response to vasoconstrictors. Both these are related to an increase in endothelial and non-endothelial vasodilators like NO and glucagon. [15]. The patients were given Octreotide an inhibitor of the release of endogenous vasodilators. They

found that there was a significant increase in mesenteric artery pressure may be as a result of an inhibition of vasodilator substance.

[16]. The significant effect on renal function in patients with advanced cirrhosis can be a promising therapeutic perspective in the treatment of hepatorenal syndrome. [17, 18, 19].

In this study it was found that in patients with cirrhosis Prothrombin Time is increased as compared to controls, Albumin level is decreased as compared to controls and serum ALT level is raised as compared to controls. We couldn't find any co-relation between NO level in blood and the other parameters (Prothrombin Time, Albumin and ALT) as $P > 0.05$.

CONCLUSION : Our study provides the evidence that there is increased production of Nitric Oxide in liver cirrhosis as compared to normal controls. These raised levels of Nitric Oxide may be responsible for the haemodynamic changes seen in patients of liver cirrhosis.

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Tables :

Sr. No.	Clinical category	No. of patients studied	Study group
1	Liver Cirrhosis	40	A
2	Control subjects	40	B

(Table 1)

Study Group	Total Nitrites in Serum $\mu\text{mol/L}$ Mean \pm S.D.
A	102 \pm 20.3
B	57 \pm 19.9

(Table 2)

Sr. No.	Biochemical parameters	Group A	Group B
1.	ALT (IU/L)	70.02 \pm 27.35	30.5 \pm 7.22
2.	Albumin (g/dl)	2.5 \pm 0.5	4.6 \pm 0.58
3.	Prothrombin Time (sec.)	21.9 \pm 4.9	16.6 \pm 1.08

(Table 3)

Sr. No.	Biochemical Parameters	Group A	Group B
1.	Serum Total Nitrites and ALT	r = 0.61	r = -0.28
2.	Serum Total Nitrites and Albumin	r = 0.47	r = -0.29
3.	Serum Total Nitrites and Prothrombin Time.	r = 0.39	r = 0.021

(Table 4)

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