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Original Research Article

Study of Serum Nitrate, Albumin and Ascitic Fluid Nitrate, Albumin Levels in Patients with Liver Cirrhosis.

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ABSTRACT

Nitric Oxide (NO^0), a potent vasodilator has been proposed to play a role in pathogenesis of ascites and hepatic disorders. Hence, this study was aimed to study synthesis of nitric oxide in liver cirrhosis patients with ascites and without ascites. The study showed highly significant increase in nitric oxide synthesis in liver cirrhosis patients with ascites as compared to controls as well as compared to liver cirrhosis patients without ascites.
In addition, serum albumin level was highly significantly decreased in both ascitic as well as non-ascitic liver cirrhosis patients. Thus the study differentiates the expression of isoforms of nitric oxide synthase in healthy human liver and cirrhotic liver.
Key words: - Nitric Oxide (NO^0), liver cirrhosis, albumin, nitric oxide synthase.

INTRODUCTION

Chronic active hepatitis and cirrhosis are classified as chronic liver diseases. Cirrhotic patients exhibit characteristic hemodynamic dysfunction manifested by tendency to arterial hypotension. They show systemic and splanchnic vascular resistance. These changes have been attributed to the excessive production or the decreased metabolism of as yet undetermined endogenous vasodilator substances.

Nitric oxide (NO^0) is a highly reactive, diffusible gas that is produced by many tissues, and it exerts a range of physiological & pathophysiological effects. The liver is the one organ clearly influenced by nitric oxide, and acute versus chronic exposure to this substance has been associated with distinct patterns of liver disease. [1]

Nitric oxide is short living biological mediator generated from L-arginine by Nitric oxide synthase (NOS). The family of NOS includes constitutively expressed

endothelial NOS (eNOS or type 3 NOS) and neuronal NOS (n NOS or type 1 NOS) as well as inducible NOS (iNOS or type 2 NOS). Nitric oxide exerts a broad spectrum of physiological functions including regulation of vascular reactivity, platelet and leukocyte activation, neurotransmission, regulation of cellular proliferation and nonspecific immunity reactions. An increased release of NO^0 has been proposed to play a role in the pathogenesis of vasodilation and vascular hypercontractility associated with portal hypertension. While peripheral arterial vasodilation is an important event in the pathophysiology of ascites formation in patients with cirrhosis**.** [2] [3]

Aims and Objectives:

- To study association of nitric oxide synthesis in cirrhotic patients with ascites and without ascites with normal healthy controls.
- To study albumin synthesis in cirrhotic patients with ascites and without ascites.

MATERIAL AND METHODS

The study was carried out at Sassoon General Hospitals, Pune. About 5 ml of blood sample was collected by vene puncture in plain bulb & was allowed to clot. Serum was separated after centrifugation. Ascitic fluid from patients of liver cirrhosis with ascites was collected by ascitic tap in plain autoclaved bulb with all aseptic precautions. The serum and ascitic fluid samples were preserved at -70^0c until analysis. The samples were thawed and used for following analysis:-

A. Estimation of nitrite and nitrate (NOx) by Moshage method [4] to assess nitric oxide synthesis in cirrhotic patients with ascites and without ascites.
B. Estimation of serum albumin and ascitic fluid albumin levels by Bromocresol Green method (BCG) [5] to assess liver function.

Patients with liver cirrhosis were grouped as:-

1) Group I – 15 clinically diagnosed patients of liver cirrhosis with ascites.
2) Group II – 25 clinically diagnosed patients of liver cirrhosis without ascites.
3) Group III - 25 age & sex matched healthy controls.

After analysis, all the parameters were subjected to statistical analysis.

RESULTS

Levels of nitric oxide in serum and ascitic fluid were compared.

TABLE – I: Comparison of NO^0 levels in serum and ascitic fluid in Groups

STUDY GROUP	NO^o IN SERUM AND ASCITIC FLUID (umol/L)	
	SERUM (MEAN ± SD)	ASCITIC FLUID (MEAN ± SD)
Group III (n = 25)	37.96 ± 11.04	--
Group I (n = 15)	253.4 ± 34.82*	303.48 ± 41.76
Group II (n = 25)	93.71 ± 17.81*	

P < 0.05 = Significant

The levels of serum nitric oxide were increased in Group I as well as Group II patients as compared to controls and this increase was statistically significant. (P<0.05). The levels were also significantly increased in Group I patients as compared to Group II patients suggesting the hemodynamic dysfunction in patients with ascites. The levels of nitric oxide were also found to be increased in ascitic fluid in group I patients.

TABLE – II: Comparison of serum and ascitic fluid albumin levels in Groups.

STUDY GROUP	ALBUMIN (gm/dL)	
	Serum	Ascitic Fluid
	MEAN ± SD	MEAN ± SD
Group III (n = 25)	3.84 ± 0.26	--
Group I (n = 15)	3.16 ± 0.50**	0.89 ± 0.42
Group II (n = 25)	3.05 ± 0.54**	--

P < 0.001 = Highly Significant

The decrease in serum albumin level was highly significant in Group I and Group II patients as compared to control Group. In Group II, the levels were significantly low

as compared to Group I. The levels were also significantly lower in ascitic fluid.

DISCUSSION

Cirrhosis patients have some characteristic symptoms such as hypotension, low systemic vascular resistance and a reduced sensitivity to vasoconstrictors. During the progress of cirrhosis, vascular resistance continues to decrease and the low arterial pressure may lead to secondary disturbances in renal and hepatic blood flow and to ascites. [6]

More recently, attention has turned to nitric oxide (NO^0), a secretory product of mammalian cells with a powerful relaxant effect on vascular wall. Evidence suggest that increased nitric oxide synthesis leads to the pathogenesis of arterial vasodilation in cirrhosis. [7] Endogenous NO stimulates soluble guanylate cyclase in smooth muscle and thus induces increased levels of the vasodilator cyclic GMP. This explains the hyperkinetic circulation in cirrhosis. [8]

Endotoxemia is a common feature of cirrhosis. Endotoxins released by the bacteria contain lipopolysaccharides. They are responsible for induction of L-arginine dependent nitric oxide pathway in a number of cell types. The neutrophils, macrophages, smooth muscle cells, fibroblasts are some cell types which are activated to produce nitric oxide. [9, 10]

In present study, the nitric oxide level is increased in liver cirrhosis patients. This increase is found to be highly significant in ascites patients as compared to non ascitic cirrhosis. This result is in accordance with the study done by Coskun et al who showed that spontaneous bacterial peritonitis (SBP) in cirrhotic patients was accompanied by an increased nitric oxide production as detected by increase in concentration of nitrate in serum and ascetic fluid. [6]

The increase in nitric oxide production in patients of cirrhosis and ascites is also studied by Tajiri et al. [11] and Laffi et al. [12] Our results are also in accordance with Arkenau HT et al., [13] Guldal KIRKALI et al. [14] Such J et al. [15] and Mohammed NA et al. [16]

In present study, albumin synthesis by hepatocytes is also significantly decreased in study groups as compared to the controls. This is mainly due to the defective hepatic cell functions. This result is in agreement with the study done by Hiller G I et al. [17]

CONCLUSION

Our study reveals that there is marked increase in serum nitrate levels in liver cirrhosis patients. The increase may be related with circulating endotoxins in cirrhosis that lead to hemodynamic dysfunction in patients.

The nitric oxide may not be having diagnostic or prognostic value in liver cirrhosis, but in future the appropriate inhibition of nitric oxide activity by using active agents may provide a novel strategy for the treatment of patients with liver cirrhosis

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