Original Article

Hepatic Expression of Nitric Oxide Isoforms and Serum Nitrites/ Nitrates in Chronic Hepatitis C With or Without Schistosomiasis

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ABSTRACT

Background: Role of nitric oxide (NO) in pathogenesis of chronic viral hepatitis is not fully understood but it seems that its overproduction is responsible for the pathological changes under inflammatory conditions.

Methods: This study was undertaken to evaluate hepatic expression of inducible NO synthase (iNOS) and endothelial NO synthase (eNOS) as well as assessment of serum nitrates/nitrites representative to NO release in Egyptian chronic hepatitis C (CHC) with or without schistosomiasis and their relation to histopathology in 72 core liver biopsies from CHC patients. Hepatic sections were immunohistochemically stained by antibodies of iNOS and eNOS.

Results: In control livers, iNOS was detected in hepatocytes and localized mainly in periportal zone of liver acinus, while eNOS was uniformly distributed in hepatocytes as well as in sinusoidal and vascular endothelium. In diseased livers, both isoforms overexpressed in a diffuse distribution pattern, eNOS translocated to hepatocytic nuclei, and iNOS consistently labeled portal tract inflammatory cells. Over expression of iNOS and serum level of NO correlated with hepatitis activity and fibrosis, while that of eNOS correlated with Schistosomal coinfection.

Conclusions: Chronic hepatitis C is accompanied by significant iNOS up-regulated expression and increased serum level of NO that reflects disease severity. Schistosomal coinfection can be considered as a risk factor for haemodynamic disturbance. Further studies are required to determine whether iNOS inhibitors could be useful in reducing liver disease severity and improve the benefits of antiviral therapies.

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Introduction

World Health Organization (WHO), 2015[1]reported that 130-150 million people globally have chronic hepatitis C infection and approximately 500 000 people die each year from hepatitis C-related diseases.Egypt has the highest prevalence of hepatitis C virus (HCV) in the world, estimated nationally at 14.7%,[2]especially genotype 4a.[3]

Schistosomiasis is a parasitic disease caused by blood flukes (Trematodes) of the genus Schistosoma.[4]According to WHO, 2012[5],the disease affects at least 240 million of people in the world. In Egypt viral hepatitis along with Schistosoma mansoni infection is the major cause of chronic liver disease and liver cirrhosis.[6]

Hepatic inflammation, steatosis and fibrosis are either direct consequences of hepatitis B and C virus infection or indirect damage determined by the subsequent inflammation and oxidative stress.[7]An important mediator of chronic inflammatory processes in liver cells could be the nitric oxide(NO).[8]This free radical is produced from L-arginin during the conversion of citrulin in a reaction catalyzed by nitric oxide synthase (NOS).[9]NO is produced by 2 constitutive isoforms of the enzyme NOS (endothelial or type 3 NOS/eNOS and neuronal or type 1NOS/nNOS) and 1 inducible isoform (iNOS or type 2NOS).[10]

The results of reports investigating NOS isoforms in hepatic tissue are somewhat controversial.[11]In healthy livers, iNOS is not thought to be expressed constitutively. However, it is readily up-regulated in the liver under a number of disease conditions, including ischemia-reperfusion injury, hepatic fibrosis, cirrhosis and regeneration.iNOS is also up-regulated *in vitro* in hepatocytes and Kupffer cells in response to endotoxins and cytokines alone or in combination.[12]The availability of specific antibodies directed against iNOS has prompted attempts to understand their cellular distribution in the liver, and how that may affect the pathogenesis of liver dysfunction.[12]

In normal liver, small quantities of NO are generated by eNOS in order to maintain perfusion in liver sinusoids by influencing vascular tonus or permeability. NO could also regulate leukocytes adhesion to liver sinusoids endothelium and inhibits platelets adhesion and aggregation.[13]

Nitric oxide is considered to exert a hepato-protective action against tissue injury and cytotoxic effects of invading microorganisms, parasites and tumor cells.[14, 15]Nitric oxide is involved in the control of programmed cell death. Its effects on apoptosis depend on its concentration in one hand and the cell types in the other. Low concentrations block apoptosis via inhibition of the main mediators of cell death-caspases (caspase-3 and -8), while higher concentrations are toxic via the formation of reactive products like peroxynitrite and dinitrogen trioxide.[16]However any situation that causes uncontrolled, prolonged and/or massive production of NO by iNOS may result in liver damage; leading to inflammation and even tumor development.[17]Nitric oxide plays an active role in the progression of liver fibrosis and hepatocellular damage in chronic viral hepatitis.[18, 19]

In cirrhosis, the primary factor leading to portal hypertension is an increase in intra-hepatic resistance to blood flow. Many vasoactive substances contribute to the development of portal hypertension. Among these, nitric oxide is the key mediator that paradoxically regulates the sinusoidal (intrahepatic) and systemic/splanchnic circulation.[20] NO is considered a powerful endogenous vasodilator,[21]and the NOS isoform involved in this seems to be mainly eNOS.[22]

The intracellular NO quickly forms nitrate and nitrites which are the stable end product that can be estimated in plasma, urine, and other body fluids as ascitic fluid.[23]

This study aims to reveal the role of NO in pathogenesis of chronic liver diseases through demonstration of hepatic immunoexpression of iNOS and eNOS in Egyptian CHC with or without schistosomiasisas well as estimation of circulating NO in these disease entities.

Material and Methods

Patients and Controls

This study was conducted on 72 patients with chronic liver disease admitted to Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute (TBRI), Giza, Egypt.Patients were subjected to thorough clinical examination and abdominal ultrasound (Hitachi EuB-515A).Core liver biopsies were obtained from patients by percutaneous ultrasound-guided Menghini needle for histopathological and immunohistochemical studies.

Serological Investigations

1. **Liver Function Tests:** Asparate aminotransferase (AST), Alanine aminotransferase (ALT) activities, and bilirubin were determined in serum according to the method described by Reitman &Frankel.[24]
2. **Circulating schistosomal antigen and anti:** schistosomal antibodies were done according to Demerdash et al[25]and Engvall & Perlman[26] respectively.
3. **Hepatitis viral Markers were Detected Including**: Hepatitis B surface antigen, anti-HBs antibodies, total and IgM class antibodies against hepatitis B core antigen using enzyme immunoassay kits (Murex Diagnostics, Dartford, England). Anti-HCV antibodies were detected using Version V anti-HCV ELISA kit

(Murex Diagnostics, Dartford, England). Circulating HCV-RNA was performed to confirm the presence of HCV antigenemia by nested RT-PCR using a set of primers within the 5' non-translated region according to Saber et al[27]

4. **Nitric Oxide Level:** Quantitative determination of nitric oxide concentration in serum was done using total NO/Nitrite/Nitrate assay (R&D systems, Inc., Code no. KGE001, Minneapolis, Minnesota, USA).

Patients were included in this study if they had: (a) Clinical and laboratory evidence of CHC (b) Circulating anti-HCV antibodies by enzyme-linked immunosorbent assay (ELISA) (c) HCV-RNA viraemia (d) Histological evidence of chronic hepatitis consistent with HCV disease.

The study also included 5 controls(needle liver biopsies taken from donors for liver transplantation). They had normal clinical, biochemical and ultrasonographic findings. Hepatitis B surface antigen and anti-HCV antibodies were negative.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Patients were informed and signed the study informed consent following instructions of TBRI ethical committee.

Histological Assessment

Liver biopsies were fixed in formalin and embedded in paraffin. The slides were stained by Hematoxylin-eosin and Masson trichrome for routine histopathological evaluation and assessment of fibrosis.Degree of CHC disease necro-inflammation (activity) and stage of fibrosis were scored according to METAVIR scoring system using grade and stage system as follow:[28]

- Grade of hepatitis activity; based on amount of inflammation:

  A0 = no activity.
  A1 = mild activity.
  A2 = moderate activity.
  A3 = severe activity.

- Stage of fibrosis; representing amount of fibrosis or scarring:

  F0 = no fibrosis.
  F1 = portal fibrosis without septa.
  F2 = portal fibrosis with few septa.
  F3 = numerous septa without cirrhosis.
  F4 = cirrhosis.

A1 included 34 cases; A2:26 cases and A3:12 cases.
F0/F1 included 44 cases; F2:14 cases; F3:8 cases and F4:6 cases.

Schistosomal granulomatous lesions and steatosis were also assessed.

Immunohistochemical Technique

The paraffin embedded liver biopsies were used by applying the Avidin-Biotin peroxidase complex (ABC) method. Sections were dewaxed and rehydrated. Endogenous peroxidase activity was quenched by incubation of the slides in a solution of 0.3 % hydrogen peroxide in methanol for 10 minutes. Antigen retrieval was performed to unmask the antigens by microwaving the slides in citrate buffer solution (pH 6.0) for 15 minutes at 700w. Duplicate liver sections were incubated overnight at 4^{o}C withrabbit anti-human polyclonal antibodiesfor iNOS(Santa Cruz Biotechnology Inc., Catalog no. SC-65, Dallas, Texas, USA) and eNOS (NeoMarkers-Lab vision corp., Code no.RB-1711, Cheshire, UK). Both antibodies were used in the appropriate dilutions (1:50) using antibody diluent.Sections were incubated at room temperature for 10 minutes with biotinylated secondary anti-rabbit antibody (Universal detection kit, DAKO, Code no.K0673, Glostrup, Denmark). Then streptavidin horseradish peroxidase conjugate was applied on the slides for 10 minutes. The antigen was visualized by the addition of diaminobenzidine substrate solution for 10 minutes. Sections were counterstained with Mayers Hematoxylin then mounted. For each antibody tested, we performed a negativecontrol in which the primary antibody was replaced byphosphate buffer saline.

Immunostaining Interpretation

Immunstained sections were examined under light microscopy at 400X to assess type of cells expressing the studied antibodies, type of expression either nuclear or cytoplasmic as brown deposits in labeled structures, and the extent of expression by semi-quantitative evaluation of the mean percentage of positive cells in 10 consecutive microscopic fields.

Statistical Analysis

All data were analyzed by SPSS software version 18 (IBM corporation, Armonk, New York,USA) and expressed as mean ± SD.

Comparisons were performed by using ANOVA test. Spearman correlation coefficient served to clarify the relationship between variables. A "p" value less than 0.05 was considered statistically significant.

Results

The study included 72 patients (50 men and 22 women) with a mean age of 38.67± 8.38 (age range from 20-50). All patients were categorized as Child-Pugh class A of chronic liver disease.

All cases were positive for HCV-RNA with a viral load ranged from low (<200 000 genome/ml) to high (500 000-

1000 000 genome/ml). No significant difference was found in viraemia among grades of hepatitis activity (p>0.05).

AST, ALT, as well as bilirubin levels were steadily elevated along grades of hepatitis activity (Table 1).

Table 2 showed that most cases were in METAVIR grades A1 and A2 of hepatitis activity (47.2% and 36.1% respectively) and F0/F1 METAVIR stage of fibrosis (61.2%).

Schistosomiasis was diagnosed as co-infection with CHC in 33.3% of the examined cases (24/72). Associated schistosomal affection was diagnosed upon presence of hepatic schistosomal granulomatous lesion or serological positivity for anti-schistosomal antibodies ± circulating schistosomal antigen. All cirrhotic cases (6 cases) were found to be associated with schistosomiasis (Table 2, Fig.1).

Steatosis was present in 41.7% of examined cases (30/72) (Table 2), without significant difference between male and female cases. Steatosis correlated significantly with grades of hepatitis activity (r=0.446, p<0.001), stages of fibrosis (r= 0.256, p<0.05), bilirubin (r=0.541, p<0.001) and ALT levels (r=0.290, p<0.001).

In control hepatic tissue, the mean immunoexpression value of iNOS was 17.5±4.63 appeared as faint cytoplasmic staining localized in periportal hepatocytes (zone 1) with a sharp transition between iNOS-rich and iNOS-poor regions of the acinus. Endothelial NOS was expressed as widespread cytoplasmic staining in hepatocytes, sinusoidal and vascular endothelium (throughout all zones) with a mean value of 56.25±20.48 (Table 3).

In CHC, extent of iNOS expression was expanded to involve zones 2 and 3 of hepatic acini, inflammatory cells and endothelial lining of bile ducts and sinusoids with a mean value of 43.19 ± 18.21. The immunoexpression of iNOS was significantly up-regulated in higher grades of activity (p<0.05) compared to either lower grades or controls (Table 3). Liver biopsies ofF0/F1 and F2 patients revealed a discrete and isolated iNOS positive immunoreactions in the cytoplasm of hepatocytes, mainly surrounding areas of portal fibrosis (Fig.2A).while in advanced stages of liver fibrosis and cirrhotic patients (F4), there was a uniform distribution of iNOS in the hepatocytes of cirrhotic regions with a significant increase of iNOS immnoexpression compared to other stages(p<0.05) (Table4&Fig.2B). No difference was found in iNOS expression in liver biopsies with or without hepatic schistosomal infection (Table 5).

The pattern of eNOS immunoreactivity in CHC patients was similar to that of iNOS with additional translocation to hepatocytic nuclei. Extent of tissue expression of eNOS showed significant down-regulation in A3 grade compared to other ones as well as over-expression in F4stage compared to other stages (Tables3&4). In addition, it was found that eNOS was significantly over-expressed in cases associated with schistosomal infection compared to negative ones (p<0.01) (Table 5; Fig.3).

Measurement of serum nitrite/nitrate levels (serum NO) showed a significant increase in CHC patients group (8.86±7.42) compared to controls (6.95±0.33) (Tables 3,4) without significant difference between schistosomal and non schistosomal patients (Table 5). Serum NO level significantly correlated to hepatitis activity, fibrotic stage, steatosis extent, and serum bilirubin level (Table 6).

Statistical evaluation of data showed that extent of iNOS expression correlated significantly with hepatic activity, fibrosis, steatosis extent, bilirubin, ALT and nitrite/nitrate levels (Table 6).

Spearmen correlation test proved that eNOS extent of expression inversely correlated with upgrading of hepatitis activity (r=-0.301, p<0.01) and positively correlated with presence of schistosomal affection (r=0.253, p<0.01) (Table 6).

Discussion

Nitric oxide (NO), an important nitrogen reactive species, is a free radical with controversial roles which can be

Table 1: Serological parameters in different grades of hepatitis activity.

Items	Normal value	METAVIR grades of activity		
		A1	A2	A3
		Mean ± SD	Mean ± SD	Mean ± SD
AST (IU/L)	≤12	41.82±26.46	59.30±34.48	93.0±61.3*#
ALT (IU/L)	≤12	49.52±41.92	65.30±33.41	117.0±97.59*#
Bilirubin (mg/dL)	0-1	0.68±0.23	0.9±0.32	1.23±0.36*#

** p<0.01 versus grade A2 activity.*
 # p<0.001 versus grade A1 activity.
AST: Asparate aminotransferase, ALT: Alanine aminotransferase
SD: Standard Deviation

Table 2: Incidence of schistosomiasis, steatosis, and fibrosis in different grades of hepatitis activity

ItemsN of cases (%)	METAVIR grades of activity		
	A1	A2	A3
	N (%)	N (%)	N (%)
Schistosomiasis			
Positive cases, N: 24 (33.3%)	16 (47.1)	6 (23.1)	2 (16.7)
Negative cases, N: 48 (66.7%)	18 (52.9)	20 (76.9)	10 (83.3)
Steatosis			
Positive cases, N: 30 (41.7%)	6 (17.6)	16 (61.5)	8 (66.7)
Negative cases, N: 42 (58.3%)	28 (82.4)	10 (35.5)	4 (33.3)
METAVIR stages of fibrosis			
F0/ F1 N: 44 (61.2%)	30 (68.2)	12 (27.3)	2 (4.5)
F2 N: 14 (19.4%)	4 (28.6)	8 (57.1)	2 (14.3)
F3 N: 8 (11.1%)	-	3 (37.5)	5 (62.5)
F4 N: 6 (8.3%)	-	3 (50.0)	3 (50.0)
Total N of cases: 72 (100%)	**34 (47.2)**	**26 (36.1)**	**12 (16.7)**

Table 3: Immunoexpressions of iNOS and eNOS ,and serum level of nitrite/nitrate in different grades of hepatitis activity

Items	Control (N=5)	CHC (N=72)	METAVIR grades of activity		
			A1 (N=34)	A2 (N=26)	A3 (N=12)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
iNOS	17.5±4.63	43.19±18.21*	33.17±15.18*	50.15±13.59$^{*\#}$	65.5±11.65$^{*\#}$
eNOS	56.25±20.48	58.53 ±18.91	64.41±21.80	57.69±15.95	50.83±14.89$^{\#}$
Nitrite/ nitrate	6.95±0.33	8.86±7.42*	6.91±7.62	8.21±4.49	15.82±8.36$^{*\#}$

* p<0.05 compared to control.$^{\#}$ p<0.05compared to other grades
iNOS: indicible nitric oxide synthase; eNOS: endothelial nitric oxide synthase.
SD: Standard Deviation; CHC: chronic hepatitis C

Table 4: Immunoexpressions of iNOS and eNOS, and serum level of nitrite/nitrate in different stages of hepatic fibrosis

Items	Control (N=5)	CHC (N=72)	METAVIR stages of fibrosis			
			F0/F1 (N=44)	F2 (N=14)	F3 (N=8)	F4 (N=6)
	Mean ±SD	Mean ± SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
iNOS	17.5±4.63	43.19±18.21*	35.20±14.20*	57.14±12.09*$^{\Delta}$	58.33±16.93*$^{\Delta}$	80.1±0.20*$^{\#}$
eNOS	56.25±20.48	58.53 ±18.91	60.65±20.56	50.71±14.26$^{\Delta}$	56.67±13.66	70.2±0.30$^{\#}$
Nitrite/nitrate	6.95±0.33	8.86±7.42	5.82±5.13$^{\#}$	9.94±3.74*	12.18±6.23*	20.45±10.86^{*o}

*p<0.01 compared to control. $^{\#}$ p<0.05 compared to other stages.
^{o}p<0.05 compared to F2 stage. $^{\Delta}$ p<0.05 compared to F0/F1 stage
iNOS: indicible nitric oxide synthase; eNOS: endothelial nitric oxide synthase.
SD: Standard Deviation; CHC: chronic hepatitis C

Table 5: Immunoexpressions of iNOS and eNOS, and serum level of nitrite/nitrate in relation to schistosomiasis

Schistosomiasis	iNOS	eNOS	Nitrite/nitrate
	Mean ± SD	Mean ± SD	Mean ± SD
Positive (N=24)	40.58±21.21	66.67±14.50*	8.53±8.49
Negative (N= 48)	46.25±16.77	56.25±20.49	9.03±6.92

** p<0.01 compared to negative group.*

iNOS: indicible nitric oxide synthase; eNOS: endothelial nitric oxide synthase.

Table 6: Statistical correlation for studied parameters.

Studied parameters	Correlation co efficient (r)	p value
iNOS immunoexpression VS grades of hepatitis activity	0. 648	p<0.001
iNOS immunoexpression VS stages of hepatic fibrosis.	0.625	p<0.001
iNOS immunoexpression VS steatosis.	0.337	p<0.01
iNOS immunoexpression VS bilirubin level.	0.405	p<0.01
iNOSimmunoexpression VS ALT level.	0.294	p<0.001
iNOS immunoexpression VS nitrite/nitrate level.	0.755	p<0.001
eNOS immunoexpression VS grades of hepatitis activity	-0.301	p<0.01
eNOS immunoexpression VS Schistosomal affection.	0.253	p<0.01
Nitrite/nitrate level VS grades of hepatitis activity.	0.484	p<0.001
Nitrite/nitrate level VS stages of hepatic fibrosis.	0.596	p<0.001
Nitrite/nitrate level VS extent of steatosis.	0.300	p<0.05
Nitrite/nitrate level VS bilirubin level	0.306	p<0.01

VS = versus.
iNOS: indicible nitric oxide synthase; eNOS: endothelial nitric oxide synthase.

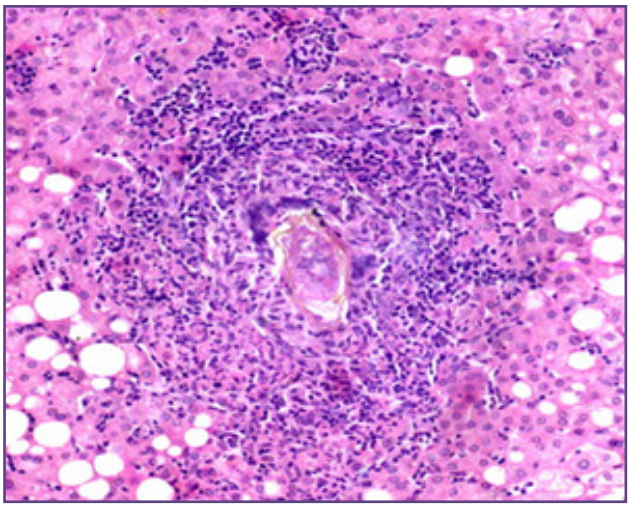

Fig. 1: Granulomatous lesion developed encircling schistosomal ova in a case of HCV infected liver with evident steatosis (Hx and Eosin stain X400).

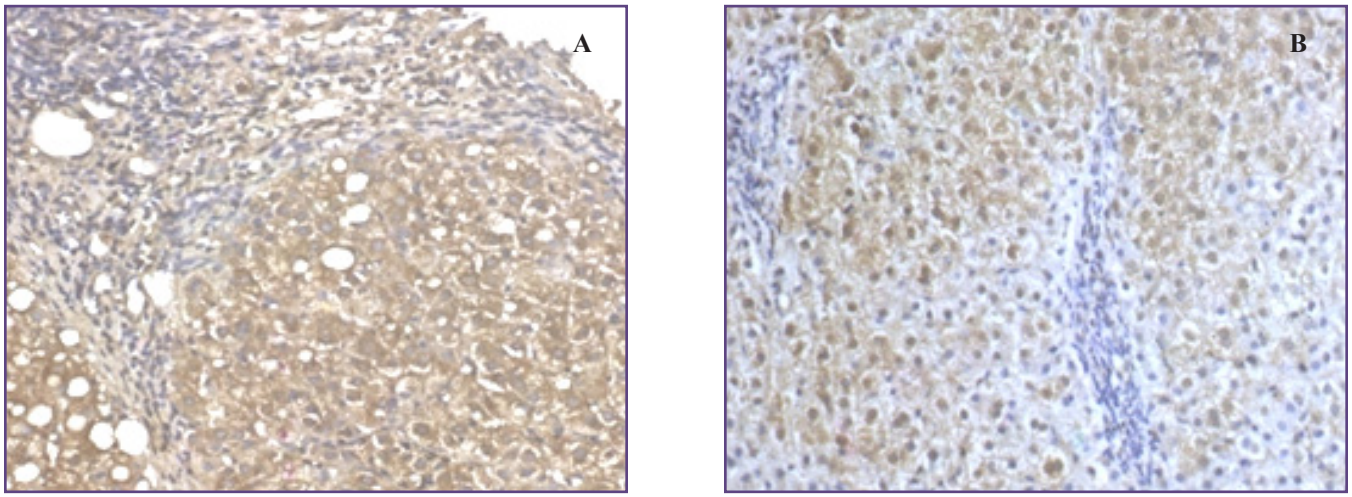

Fig. 2: Immunohistochemistry for iNOS in liver sections expressed as brown cytoplasmic staining in hepatocytes, (A) a case of CHC with mild activity and fibrosis (A1F0/F1) showing zonal distribution of staining mainly periportal; (B) wide marked iNOS expression in hepatocytes and portal inflammatory cells in a cirrhotic liver on top of CHC . iNOS: inducible nitric oxide synthase; CHC: chronic hepatitis C (X400).

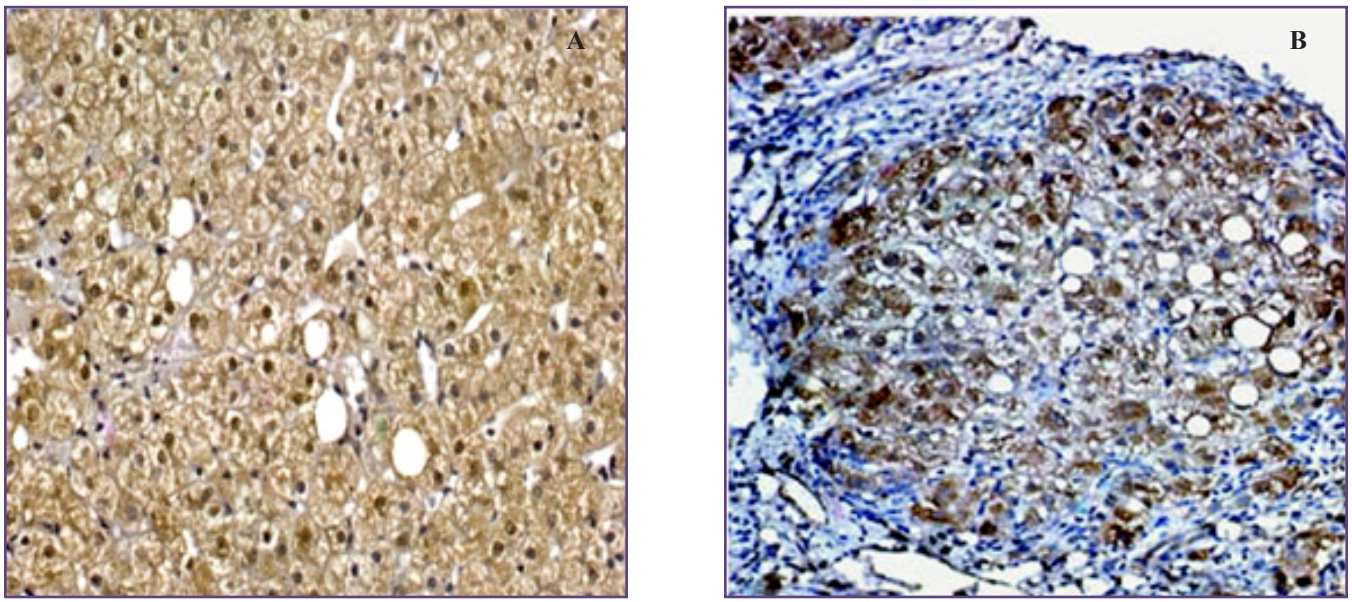

Fig. 3: Immunohistochemistry for eNOS in liver sections expressed as brown cytoplasmic and additional nuclear staining in hepatocytes (A) wide cytoplasmic expression of eNOS in hepatocytes in a case of CHC with mild activity and fibrosis (A1F0/F1). (B) Marked cytoplasmic expression of eNOS in regenerating nodule of cirrhotic liver as well as in endothelium of portal vasculature (X400).

generated in almost all hepatic cells (hepatocytes, kupffer, endothelial and hepatic stellate cells).[8]NO is an important mediator of liver physiology and pathophysiology.[29]It is conceivable that, in the intra-hepatic microenvironment of CHC, an important source of NO exists. Since a marked induction of iNOS expression is observed in the majority of hepatocytes from patients with CHC, it is likely that these liver cells are a powerful cellular source of large quantities of NO.[30]

In CHC, it has been suggested that the viral infection itself, by mechanisms not yet fully understood, may be a triggering factor. It could be suggested that this nitration process represents a non-specific pathogenic mechanism, common to different chronic inflammatory diseases and secondary to the elicitation of inflammation rather than its cause.[15] Machida et al. concluded that HCV infection can stimulate production of NO through activation of the gene for iNOS by the viral core.[31]

Hepatitis C virus infection induces the production of total nitric oxide (NO), i.e., NO_X which includes both nitrites (NO_2^-) and nitrates (NO_3^-).[31]

Serum NO level in CHC patient was studied through estimation of nitrate or nitrite/nitrate level in multiple researches. In chronic active hepatitis patients, Parvu et al did not found difference in nitrite/nitrate levels of the patients and healthy control,[32]multiple studies recorded increased nitric oxide serum level in advanced stages of cirrhosis (Child class B&C),[23, 33-35]while McNaughton et al were unable to detect significant changes in serum NO of their patients with viral hepatitis, cirrhosis, and cholestasis when compared with controls.[36]In our study, serum nitrite/nitrate level variably increased in diseased cases with definite correlation to grade of hepatitis activity, stage of fibrosis, and extent of tissue expression of iNOS. No significant difference was recorded in NO serum level or hepatic expression of iNOS between our schistosomal and non schistosomal patients. Although Hassan et al estimated enhanced NO levels in cirrhotic patients with HCV infection and schistosomiasis compared to non-schistosomal HCV infected patients.[23]

We found that iNOS was constitutively expressed in a zonal pattern in the normal hepatic acinus. The distribution of iNOS showed the strongest expression in the periportal region, with diminution of intensity toward the perivenous regions of the hepatic acinus. These results agree with those of McNaughton et al.[36]Our study also provides the evidence that there was increased production of NO in CHC patients, appeared as over-expression of iNOS with an almost diffuse distribution pattern throughout the hepatic lobules mainly in hepatocytic cytoplasm but also in nuclei. These results were in contention with several studies conducted before.[8,10,11,15,18]Furthermore iNOS consistently labeled mononuclear cells infiltrating portal tracts in all our hepatitis samples. Also,this over-expression of iNOS correlated with disease severity in the form of hepatitis

activity and fibrosis which is consistent with the results of Atik et al.[15]

In this study, eNOS immunostaining was uniformly distributed in hepatocytes, present in the endothelium of hepatic arteries, terminal hepatic venules and sinusoids. Interestingly, the epithelium of biliary ducts showed strong expression of eNOS. Thus, in addition to endothelial cells, both hepatocytes and biliary epithelium express eNOS. There are very few studies that have examined the role of NO in biliary epithelial cell function. The functional significance of eNOS expression in the endothelium as a regulator of blood flow and cell–cell interactions has been proposed.[36]

The immunohistochemical analysis in our study showed profound changes in the cellular distribution of eNOS in CHC tissue samples, leading to its translocation to hepatocytic nuclei. Interestingly, growth factors such as vascular-endothelial growth factor are known to cause nuclear translocation of eNOS in vascular endothelium.[37]The significance of this observation is unclear. Again, nuclear translocation of eNOS may merely reflect "growth factor storm" that is characteristic of chronic liver inflammation and cirrhosis.[38]Alternatively, the eNOS translocation may be a part of a liver defense mechanism aimed at limiting the effects of growth factors by decreasing the rate of cellular proliferation or apoptosis that is known to be regulated by NO.[36]

Schistosomiasis is an intravascular disease associated with inflammation, fibrosis and venous intrahepatic portal lesions which are the main pathogenic factors in production of portal hypertension leading to perisinusoidal block and increased resistance to portal blood flow.[39] Increased NO metabolism is associated with the hemodynamic alteration induced by portal hypertension,[33, 35] as endothelial cells control vascular tonus and permeability [21] via NO generation by eNOS.[13] Chang et al suggested that eNOS rather than iNOS involved in vascular response to vasoconstrictors in portal-systemic collaterals of portal hypertension.[22]This may explain the significant over-expression of eNOS in our cases associated with Schistosomal affection compared to negative ones.

Conclusion

Both iNOS and eNOS proteins are differentially expressed in healthy human liver, and this expression is altered in chronic hepatitis C with or without schistosomiasis. Similarly, serum nitrite/nitrate level is elevated in these disease entities. Schistosomal coinfection can be considered as a risk factor for haemodynamic disturbance in CHC patients. So, our findings triggered us to hypothesize the possible use of NO markers as an independent predictor of virological response in HCV genotype 4 infected patients. Further studies are required to determine whether iNOS inhibitors could be useful in reducing liver disease severity and improve the benefits of antiviral therapies.

Abbreviations

World Health Organization, WHO; hepatitis C virus, HCV; nitric oxide, NO; nitric oxide synthase, NOS; endothelial NOS, eNOS; inducible NOS, iNOS; chronic hepatitis C, CHC; Asparate aminotransferase, AST; Alanine aminotransferase, ALT; enzyme-linked immunosorbent assay, ELISA.

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Competing Interests

None Declared

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