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The Utility of STRIATIN as A Novel Diagnostic Marker in Cirrhosis

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ABSTRACT

Aim: *Endothelial dysfunction and associated endothelial Nitric Oxide Synthase (eNOS) mediated reduction in Nitric Oxide (NO) bioavailability has been implicated in the development of Portal Hypertension (PHT) in cirrhosis. However, the role of striatin, a key protein involved in the activation of the eNOS signaling pathway, remains largely unexplored in cirrhosis. Here, we aimed to study the hepatic expression of striatin and its connotation with eNOS in cirrhosis. Also, the systemic concentration of striatin in cirrhotic patients and to determine its potential as a diagnostic marker for cirrhotic patients with Portal Hypertension (PHT).*

Materials and methods: *This case-control study includes 40 cirrhotic patients and 40 healthy volunteers (control subjects). Hepatic striatin and phosphorylated eNOS (peNOS) expression were studied using western blotting. Systemic striatin and cyclic Guanosine Monophosphate (cGMP) levels were analyzed by ELISA and biochemical parameters were analysed using Beckman Coulter (AU 680) autoanalyzer.*

Results: *Systemic striatin concentration was markedly lower in cirrhotic patients compared to control subjects (6.86 ± 1.7 vs. 10.9 ± 1.7 ng/ml; P<0.0001). By contrast, systemic cGMP concentration was markedly increased in cirrhotic patients compared to control subjects (10.90 ± 2.8 vs. 5.19 ± 2.3 pmol/ml; P<0.0001), however, no association was observed between these parameters. Of note, decreased hepatic striatin protein expression observed in cirrhotic liver was positively associated with reduced peNOS expression. Moreover, Receiver Operator Characteristics (ROC) curve analysis shows that striatin had a better predictive diagnostic capability than cGMP in cirrhotic patients.*

Conclusions: *Our study shows evidence that systemic striatin levels and hepatic expression were markedly lowered in cirrhotic patients, and thus, it could be used as a possible potential diagnostic biomarker for liver cirrhotic patients with PHT. Indeed, our further study will pinpoint the therapeutic role of Striatin-eNOS-NO signaling in advanced cirrhosis.*

Keywords: Cirrhosis, eNOS, Inflammation, Striatin, Nitric oxide

Abbreviations: ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; CCLD: Compensated Liver Disease; cGMP: cyclic Guanosine Monophosphate; DBIL: Direct Bilirubin; DCLD: Decompensated Liver Disease; eNOS: endothelial Nitric Oxide Synthase; GGT: Gamma-Glutamyl Transferase; HSCs: Hepatic Stellate Cells; INR: International Normalized Ratio; LFT: Liver Function Tests; NAFLD: Non-Alcoholic Fatty Liver Disease; NO: Nitric Oxide; PT: Prothrombin Time; TBIL: Total Bilirubin; USG: Ultrasonography

INTRODUCTION

Cirrhosis is an end-stage chronic hepatic ailment characterized by progressive injury of hepatic parenchyma followed by collagenous scarring and fibrous obstruction of the hepatic micro-vasculature [1]. It is the 14th leading cause of fatality worldwide, accounting for nearly 1.03 million deaths globally [2]. The process of fibrogenesis in cirrhosis is driven by the Hepatic Stellate Cells (HSCs) activation and myofibroblasts trans-differentiation [3]. Activated HSCs contract in response to vasoactive substances and modulate the sinusoidal blood flow by decreasing the diameter of the sinusoid, leading to portal hypertension [3,4]. Portal Hypertension (PHT) is a major complication of cirrhosis, described by elevated Intrahepatic Vascular Resistance (IHVR) and splanchnic blood flow [5-7]. PHT is mediated by Endothelial Dysfunction (ED) marked by diminished intrahepatic bioavailability of NO as a consequence of decreased endothelial Nitric Oxide Synthase (eNOS) activity [7,8]. ED implicates the defective functioning of the endothelium, which includes impairment in the synthesis and release of vasodilatory substances, notably Nitric Oxide (NO) [7]. Undoubtedly, the ED of the hepatic vascular bed plays an indispensable role in the advancement of cirrhosis [9]. Reduced NO bioavailability aggravates IHVR and further exacerbates PHT [8]. Cyclic Guanosine Monophosphate (cGMP) is an important downstream regulatory protein of the NO signaling pathway. NO plays a crucial role in sustaining vascular homoeostasis by activating guanylate cyclase enzyme, which converts Guanosine Triphosphate (GTP) into cGMP [3]. cGMP is the functionally active signaling protein that induces vasodilation indirectly *via* activating Protein Kinase G (PKG).

Striatin is a 110 KDa membrane-bound protein found primarily in the dendritic spines of neurons. It possesses 4 protein-protein interaction domains, such as the caveolin binding domain, WD repeat domain, putative coiled-coil structure and Calcium-Calmodulin (CaM)-binding domain. Based on the observations of Castets et al., striatin binds to the calcium-dependent calmodulin-1 and caveolin, thereby regulating signal transduction molecules such as eNOS. Furthermore, striatin acts as a molecular scaffold required for forming (Estrogen receptor-α) ERα-Gαi complex, involved in estrogen-mediated non-genomic activation of eNOS. Heterozygous striatin-deficient mice fed with a liberal salt diet exhibited exaggerated vasoconstriction and declined vasorelaxation, indicating the vital role of striatin in maintaining vascular homoeostasis through its regulatory effect on NO-cGMP pathway. These observations suggest the involvement of striatin in sustaining vascular homoeostasis. Moreover, the investigation of new biomarkers for cirrhosis is essential, particularly in light of the increasing prevalence of liver disease and the limitations associated with existing diagnostic methods. Traditional diagnostic techniques, such as liver biopsy, are invasive and pose risks to patients, underscoring the urgent need for non-invasive alternatives. Consequently, we aimed to study the systemic and hepatic striatin levels in cirrhotic patients and further investigate its potential as a diagnostic marker for cirrhosis.

MATERIALS AND METHODS

Study participants

This pilot case-control study was performed on 40 cirrhotic patients and 40 control subjects. Following the Institute Ethics Committee for Human Studies based on the Ethical Guidelines of the Helinski Declaration of 2013, we recruited cirrhotic patients from the Medical Gastroenterology Department from March 2015 to December 2016. The study was conducted in the Department of Biochemistry at Jawaharlal Institute of Postgraduate and Medical Education and Research (JIPMER). Cirrhotic patients (both males and females) aged between 18 and 65 who demonstrated the combination of clinical, biochemical, endoscopic and ultrasound findings. Clinical examination, radiological (ultrasonography and computed tomography scan), biochemical (prolonged prothrombin time and international normalized ratio, low serum albumin, hyperbilirubinemia and elevated serum transaminases) or histologically confirmed cases of cirrhosis are included in this study. Exclusion criteria apply to individuals who were unable or unwilling to provide informed consent, those with conditions unrelated to cirrhosis decompensation

(such as comorbidities including established cardiovascular disease or diabetes), and those with organ failure or any malignancy (either extrahepatic or hepatocellular carcinoma). Additionally, patients who had undergone major surgery, such as liver resection and pregnancy, were excluded from the study. Control subjects were age and sex-matched healthy volunteers who fit the inclusion and exclusion criteria. The investigation began after obtaining written informed consent from both patients and control subjects. Patients attending Medical Gastroenterology OPD of JIPMER aged between 18 and 65 years diagnosed cirrhosis with PHT (n=40). Hepatic Venous Pressure Gradient (HVPG) >10 mmHg corresponds to clinically significant PHT and is the strongest predictor of decompensation. In cirrhosis, PHT occurs due to the formation of scar tissue and regenerative nodules, increasing IHVR and, thus elevated portal pressure. The patients were diagnosed as cirrhotic, based on histological examination and by Ultrasonography (USG). We did not recruit cirrhotic patients when they had co-morbidities such as renal or cardiovascular dysfunction, neoplasia or any infection. However, cirrhotic patients present with ascites were included. Control subjects (n=40) were age and sex-matched, and they constituted the study population with normal hepatic function and without a history of recent illness.

Sample collection

Immediately after collecting 5 ml blood samples from study participants in pre-cooled with or without EDTA coated tube, samples were centrifuged at 3000 RPM for 10 minutes, and plasma/serum separated was used to measure routine biochemical parameters (AU680 Beckman Coulter auto analyzer, USA). PT and INR were measured using STA Compact Max (France). The remaining stored (-80°C) blood samples were used to estimate striatin and cGMP concentrations. For histology or IHC, tissue blocks were made from the liver tissue samples obtained from cirrhotic patients, while the surrounding unaffected area of the liver tissue was considered as the control liver after histological confirmation or non-cirrhotic patient underwent surgery for any other liver ailments. The exclusion criteria for cases and controls were pregnancy, hepatocellular carcinoma, extrahepatic complications, other malignancies, and inflammatory diseases.

Picrosirius red staining and immunohistochemistry

Cirrhotic liver tissue and the surrounding normal liver tissue of cirrhotic patients obtained were fixed with 10% formalin. Paraffin-embedded liver tissues were sectioned with a thickness of 3 to 5 µm and treated with picrosirius red staining for 10 minutes, followed by washing in 0.5% glacial acetic acid and dehydration with absolute ethanol. For Immunohistochemistry, saline-coated slides were used. Hepatic striatin was localized by immunostaining using rabbit polyclonal antibody (Genetex, Cat: GTX32902, 1:100). Inactivation of endogenous peroxidase activity (3% H_2O_2 in Phosphate-Buffered Saline (PBS) for 10 min) was done in deparaffinized and rehydrated sections followed by antigen unmasking using citrate buffer (110°C for 10 min). Primary striatin antibody (1:100 dilution) was added and then incubated for 1 hour at room temperature. Then, a secondary antibody (Vector lab) was added and incubated for 30 minutes, followed by incubation with 3,3'-Diaminobenzidine (DAB) chromogen (Vector lab) and counter-staining with Mayer's hematoxylin solution. The sections were mounted with Histomount mounting media (Invitrogen). The Evos FLc cell imaging system (Invitrogen, USA) was used to capture the images.

Western blot analysis

Freshly collected cirrhotic and surrounding normal liver tissue were snap-frozen in liquid nitrogen and homogenized in TRIS-EDTA buffer (pH 7.4) containing protease inhibitor cocktail (Sigma-Aldrich, USA), phenylmethanesulfonyl fluoride, and EDTA. Following protein extraction, the protein concentration was measured using BCA assay kit (Thermo Fisher Scientific, USA). 4 to 12% NuPAGE Bis-Tris gels were used to separate the protein and the gels were transferred onto PVDF membrane (Invitrogen, UK), then probed with primary rabbit polyclonal anti-striatin (Genetex, USA Cat. No. GTX32902), anti-peNOS (Ser1177) (1:1000; Sigma, USA) and anti-β-actin (Abcam, USA) followed by incubation with horse radish peroxidase-conjugated secondary antibody. ECL detection kit (Amersham, UK) was used to visualize the bands using the ChemiDoc imaging system (Bio-Rad laboratories), and the bands were quantified by densitometry using Image Lab 6.0 software.

Determination of striatin concentration in plasma

The concentration of striatin in blood samples was analysed by Enzyme-Linked Immunosorbent Assay (ELISA) (Bioassay Cat. No. E2956Hu) Shanghai Korain Biotech CO. LTD, China). Either 50 µl of standard solution or 50 µl of plasma were added into a microplate, precoated with an antibody specific for striatin. The wells were then incubated with 10 µl biotin labelled striatin antibodies at 37°C for 60 minutes. The microplate wells were washed three times then incubated with streptavidin-HRP conjugate for 30 minutes at 37°C. The unbound enzyme was removed by gentle washing, and then substrates A and B were added. The color intensity of the Samples and the Concentration of Human Striatin (STRN) were positively correlated. The results were expressed as ng/mL. The ELISA detection limit was (115 pg/ml), as determined by the assay specifications.

Determination of cyclic Guanosine Monophosphate (cGMP) in plasma

The cGMP concentration in plasma samples was measured using a commercially available quantitative sandwich enzyme-based immunoassay (YH Biosearch Laboratory, China). Either 50 µl of standard solution or 50 µl of plasma or liver tissue homogenate were added into a microplate precoated with an antibody specific for cGMP. The wells were then incubated with 10 µl biotin labelled cGMP antibodies at 37°C for 60 minutes. The microplate wells were washed three times and incubated with streptavidin-HRP conjugate for 30 minutes at 37°C. The unbound enzyme was removed by gentle washing, followed by the addition of substrates A and B. The color intensity of the samples and the concentration of human cGMP were positively correlated. The results were expressed as pmol/mL.

Statistical analysis

Data are expressed as mean ± Standard Deviation (SD) with $p<0.05$ was considered statistically significant. Interquartile Range (IQR) was used for normal and non-normal data distribution. The Mann–Whitney test was performed to compare control subjects and cirrhotic patients. Spearman's correlation was performed to measure the association between ELISA parameters. A Receiver Operator Curve (ROC) analysis was performed for striatin, cGMP, total bilirubin and albumin. Software used includes GraphPad Prism 10.0 (SanDiego, CA) and Microsoft Excel 2023(Microsoft Corp., Redmond, WA).

RESULTS

Baseline clinical characteristics in cirrhotic patients

The mean age was similar, and most of the populations were male in both the control and cirrhotic groups. The clinical characteristics, including ascites, encephalopathy, oesophageal varices, variceal bleeding and portal gastropathy of the cirrhotic patients who underwent endoscopy are listed in Table 1. Seven of 40 cirrhotic patients had stage I-II oesophageal varices (15%), and three had stage II-III oesophageal varices (7.5%). In addition, two patients (5%) had a history of variceal bleeding, four patients (10%) had mild portal gastropathy, and one patient had hepatic encephalopathy. Out of all the cirrhotic patients, 85% of the population had no hepatitis infection, and six patients (15%) were HBs Ag positive. Moreover, ten patients (25%) had compensated liver disease, and 30 patients (75%) had decompensated liver disease.

Table 1 Clinical characteristics observed in cirrhotic patients

Characteristics	**Cirrhotic patients N=40**
Etiology of cirrhosis Alcohol/Viral/ Cryptogenic	33/6/1
Portal hypertension Present/Absent	36/40
Ascites	34/40

Ascites level (1/2/3)	25/7/2
Current encephalopathy	1/40
Oesophageal varices Present/Absent	10/30
Oesophageal varices grade (I-II/II-III)	7/3
History of variceal Bleeding	2/40
Portal gastropathy (Mild / Severe)	4/0
Liver disease stage (Compensated/ Decompensated)	10/30

Biochemical parameters in control subjects and cirrhotic patients

The routine clinical biochemistry in control subjects and cirrhotic patients are depicted in Table 2. The plasma levels of T.BIL, D.BIL, GGT, ALP, AST and ALT were significantly elevated ($p<0.001$), while albumin ($p<0.001$) and total protein ($p<0.01$) levels declined in cirrhotic patients when compared to controls. Moreover, observed concentrations of creatinine, urea, and electrolytes (sodium, potassium and chloride) in cirrhotic patients were not significantly altered compared to controls.

Table 2 Liver function parameters and disease severity in controls and cirrhotic patients

Parameters	**Control subjects N=40**	**Cirrhotic patients N=40**
Age (years) Median (IQR)	49 (28-66)	45 (26-69)
Gender (Male/Female)	36/4	31/9
AST (IU/L)	24.7 ± 7.51	49 ± 19.1***
ALT (IU/L)	24.2 ± 8.22	36.7 ± 18.5***
ALP (IU/L)	209.65 ± 63.9	358.9 ± 168.8***
γGT (IU/L)	21.3 ± 10.99	56.4 ± 53.25**
Total bilirubin (mg/dl)	0.755 ± 0.19	2.10 ± 1.79***
Direct bilirubin (mg/dl)	0.29 ± 0.10	0.80 ± 0.60***
Total protein (gm/dl)	7.04 ± 0.45	6.6 ± 0.72**
Albumin (gm/dl)	4.3 ± 0.44	3.0 ± 0.50***
Urea (mg/dl)	22.5 ± 6.5	23.3 ± 13.5
Creatinine (mg/dl)	0.93 ± 0.2	1.0 ± 0.48
Na^+ (mEq/L)	136.5 ± 5.3	132.5 ± 7.1
K^+ (mEq/L)	4.27 ± 0.4	4.25 ± 0.6

Cl^- (mEq/L)	101.2 ± 5.49	99.9 ± 6.72
PT (mg/dl)	-	22.2 ± 5.3
INR (mg/dl)	-	1.8 ± 0.50
Child-Pugh score	-	8.28 ± 2.39
MELD-Na score	-	19.98 ± 5.77

Note: Data are expressed as mean ± SD and median with interquartile range for 40 cirrhotic patients and controls.

ALT: Alanine Transaminase; AST: Aspartate Transaminase; ALP: Alkaline Phosphatase; Cgmp: cyclic Guanosine Monophosphate; Γgt: gamma-Glutamyl Transpeptidase; INR: International normalized ratio; MELD: Model for End-Stage Liver Disease; Na: Sodium; K: Potassium; Cl: chloride; PT: Prothrombin Time.

$^{***}p<0.001$, $^{**}p<0.01$ – cirrhosis *vs.* control subjects.

Plasma striatin and cGMP levels in control subjects and cirrhotic patients

Plasma striatin levels were decreased ($p<0.0001$) in patients with cirrhosis compared to controls (Figure 1A and Table 2). By contrast, plasma cGMP levels were elevated ($p<0.0001$) in cirrhosis compared to controls (Figure 1B). Moreover, there was no significant association between systemic striatin and cGMP levels (Figure 1C).

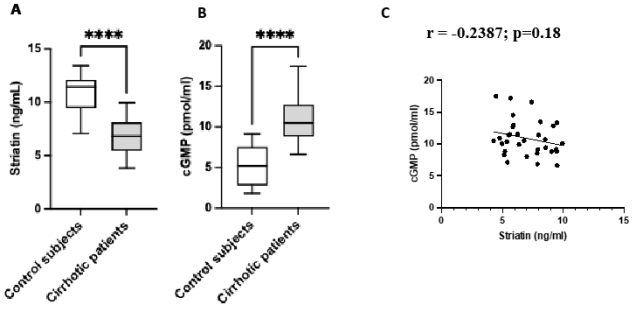

Figure 1 Plasma levels of striatin (A), cGMP (B) and their correlation (C) in cirrhotic patients and control subjects. Data are expressed as mean ± SD and median with interquartile range. The Mann–Whitney test was performed to compare control and cases. P-value<0.05 was considered statistically significant. Note: $^{****}p<0.0001$ cirrhosis *vs.* control subjects. Pearson correlation between striatin and cGMP (C). Values with $p<0.05$ were considered statistically significant. Correlation coefficient (r) and statistical p value are shown. Individual sample numbers are signified as dots

Receiver Operator Characteristics (ROC) curve analysis of striatin and cGMP in control subjects and cirrhotic patients

The ROC curve analysis measuring Area Under the Curve (AUC), sensitivity and specificity of striatin, cGMP, bilirubin and albumin are shown in Figure 2 (A-D) and Table 3. Striatin at cut-off 9.046 (ng/ml) had a sensitivity of 85.00, specificity of 87.50% and AUC of 0.942 with a 95% confidence interval of 0.90-0.99. Moreover, a positive likelihood ratio of 3.39 and a negative likelihood ratio of 0.23 were observed for the population under study (A). Similarly, cGMP at cut off of 6.866 (pmol/ml) had a sensitivity of 71.05%, specificity of 67.50% and AUC of 0.769 with a 95% confidence interval (0.65-0.88). For the population under study, a negative likelihood ratio of 0.42 and a positive likelihood ratio of 2.18 were noted (B). Furthermore, ROC analysis of albumin (C) and total bilirubin (D) with AUC (0.855 and 0.984, respectively) and cut off (3.750 and 0.850, respectively) were observed and compared with striatin (Table 3).

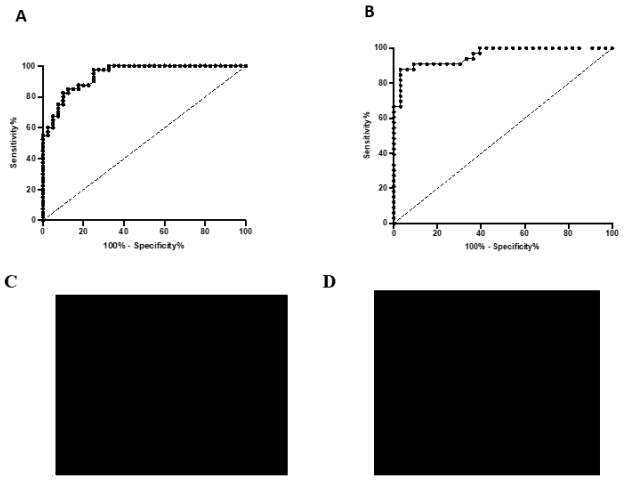

Figure 2 ROC curve analysis of Striatin (A), cGMP (B), albumin (C) and total bilirubin (D) between control subjects and cirrhotic patients

Table 3 ROC curve analysis of striatin, cGMP, albumin and total bilirubin between cirrhotic patients and control subjects

Parameters	**Cut off value**	**Sensitivity (%)**	**Specificity (%)**	**AUC**
Striatin (ng/ml)	<9.046	85	87.5	0. 942
cGMP (pmol/ml)	<6.866	71.05	67.5	0.769
Albumin (gm/dl)	<3.750	90	95	0.984
Total bilirubin (mg/dl)	>0.850	75	82.5	0.855
Note: AUC: Area Under Curve; ROC: Receiver Operator Characteristics				

Expression of striatin and peNOS in cirrhotic and control liver specimens

The human cirrhotic liver tissue and the surrounding normal liver tissue (histologically proven control) were validated by picrosirius red staining for fibrosis (Figure 3A). The presence of broad fibrous septa in cirrhotic liver tissue indicated fibrotic scarring of the liver tissue, while it was absent in control liver. Immunohistochemical analysis showed in the control liver that there is a faint expression of striatin, which were expressed in the sinusoidal endothelial cells whilst it was absent in cirrhotic liver (Figure 3B). Furthermore, striatin and peNOS expressions were assessed by Western blotting in control and cirrhotic liver tissues (Figures 4A and B). Striatin expression was decreased ($p<0.05$) in cirrhotic liver when compared to control liver tissue (Figure 4A). Moreover, peNOS protein hepatic expression was also significantly diminished in cirrhosis when compared to control liver (Figure 3B). We also observed significantly decreased hepatic cGMP concentration in cirrhotic liver compared to control liver (Figure 4C).

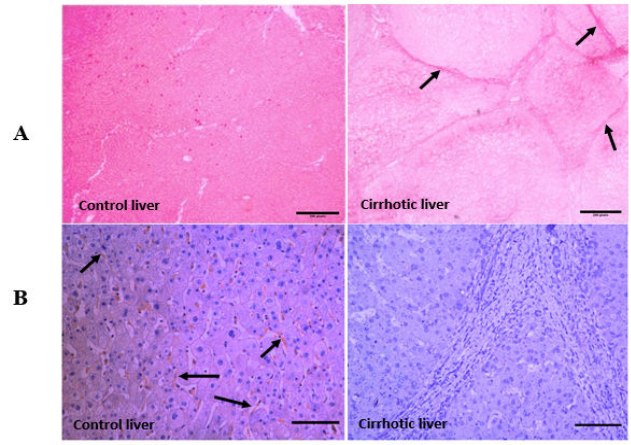

Figure 3 Picrosirius red staining (A) and immunostaining of striatin (B) in cirrhotic and non-cirrhotic (control) liver tissues. Representative images of picrosirius red staining in control and cirrhotic liver tissues at 10X magnification. Picrosirius red staining revealed the presence of broad fibrous septa in the cirrhotic liver, while the fibrotic changes were absent in the control liver tissue. Immunostaining of striatin expression is present in the control liver, but the expression is absent in the cirrhotic liver (20X magnification)

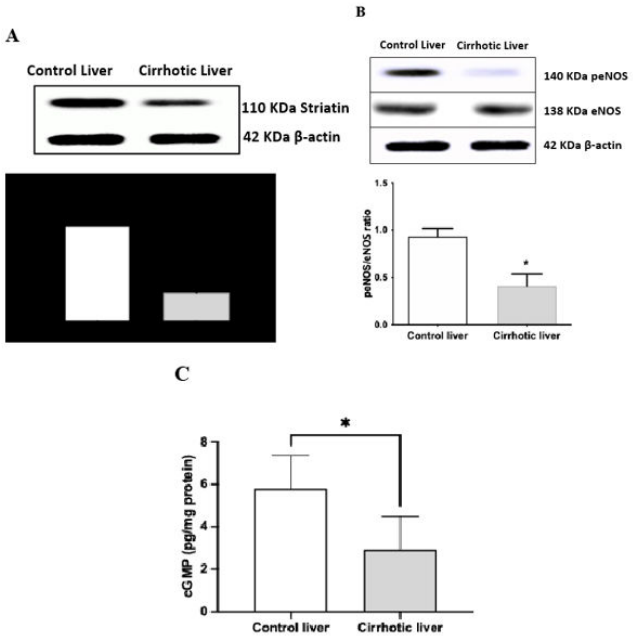

Figure 4 Protein expression of striatin (A) and peNOS (B) and hepatic cGMP levels (C) in cirrhotic and non-cirrhotic (control) liver tissues. Note: $^{*}p<0.05$ – control *vs.* cirrhosis. β-actin was used as an endogenous housekeeping protein.

DISCUSSION

The current study demonstrates that both systemic and hepatic striatin levels were reduced in cirrhotic patients with Portal Hypertension (PHT) compared to the control group. Plasma cGMP levels were elevated in cirrhotic patients relative to controls. ROC curve analysis revealed that striatin outperformed cGMP as a diagnostic biomarker for cirrhosis, exhibiting higher sensitivity and specificity. Furthermore, we found a reduction in hepatic striatin expression in cirrhotic livers, which contributed to impaired eNOS activity, as indicated by lower phosphorylated eNOS levels compared to controls. Immunostaining confirmed the presence of striatin in control liver tissue, whereas its expression was absent in cirrhotic liver tissue.

Endothelial dysfunction is a primary contributor to portal hypertension in cirrhosis, characterized by reduced NO bioavailability in the injured liver [8]. Striatin, a scaffold protein essential for regulating eNOS activity, plays a critical role in maintaining vascular homeostasis. In our study, we observed a significant reduction in hepatic phosphorylated eNOS (peNOS) expression, indicative of diminished eNOS activity in the injured cirrhotic liver compared to control liver tissues. Immunohistochemistry (IHC) results corroborated this finding, revealing that striatin was expressed in the endothelial cells of the control liver but absent in the injured liver. In line with the above findings, a previous study revealed that striatin heterozygous knockout mice exhibited exacerbated vasoconstriction and reduced vasorelaxation in response to sodium intake, suggesting a correlation between striatin deficiency and salt sensitivity in blood pressure regulation. Additionally, striatin has been shown to possess a vasoprotective role through the estrogen receptor-mediated non-genomic activation of eNOS.

This study found a significant downregulation of hepatic striatin protein expression in cirrhotic livers compared to the control liver. Furthermore, cirrhotic patients displayed decreased systemic striatin concentrations, likely reflecting the reduced hepatic expression of this protein in the damaged liver. The mechanism underlying the downregulation of striatin in the cirrhotic liver remains unknown; however, our previous study shows evidence that striatin was expressed in the placental tissues from both normal and preeclamptic cases. Furthermore, blood striatin concentration was observed without any statistical difference between pregnant women with and without preeclampsia. However, in the current study, we observed that blood striatin concentration was significantly increased in healthy volunteers compared to cirrhosis. Moreover, striatin tissue expression was higher in the control liver tissue than in the cirrhotic liver. Our IHC results also show in the control liver that striatin is expressed mainly in the vascular endothelial cells. In this context, a recent study shows evidence that striatin is expressed in the human heart and plays a critical role in the early remodelling processes in the heart induced by angiotensin II. These findings indicate that striatin is expressed in many human tissues, and its levels were detectable in the blood. Indeed, further research is warranted to understand the comprehensive role of striatin in the circulation and liver tissue of healthy volunteers and cirrhotic patients. Moreover, increased inflammation observed during preeclampsia was associated with diminished peNOS and striatin expression. So, we speculate that increased inflammation or infection in cirrhotic patients may be related to lowered striatin levels; therefore, understanding the Striatin-eNOS axis may provide therapeutic options for the management of cirrhotic patients with PHT.

Furthermore, consistent with previous studies, cGMP levels were significantly elevated in the plasma of cirrhotic patients and were correlated with hemodynamic instability and increased oxidative stress. Furthermore, increased plasma endotoxin observed in cirrhotic patients further activates monocytes, neutrophils and other vascular endothelial cells, resulting in increased nitrosative stress-mediated iNOS synthesis and, thus, activated sGC, leading to increased cGMP synthesis from GTP. This is also supported by our previous study in cirrhosis that elevated hepatic iNOS protein expression following endotoxin treatment. Elevated portal pressure in the cirrhotic liver also plays a crucial role in augmenting cGMP. Moreover, increased cGMP-mediated NO synthesis was observed in CCl_4-induced cirrhotic rats, contributing to markedly elevated systemic hemodynamics. Moreover, in line with evidence from previous studies, we also observed increased systemic cGMP levels in cirrhotic patients compared to control subjects. Increased cGMP levels may indicate worsening liver function and vascular homoeostasis in cirrhosis since lowered hepatic nitric oxide concentration was noted in cirrhotic patients. It is known that Phosphodiesterase-5 (PDE-5) inhibition is controlled by cellular Nitric Oxide (NO). However, due to low PDE-5, cGMP concentration remains elevated in peripheral arteries, leading to hyperdynamic circulatory dysfunction. Furthermore, under basal conditions, caveolin-1 (cav-1) binds with eNOS, and an increase in intracellular Ca^{2+} frees eNOS from cav-1. In the cytosol, Mitogen-Activated Protein Kinase (MAPK) and Akt, also called protein kinase B, activate eNOS through phosphorylation, resulting in increased synthesis of NO. This Ca^{2+}-mediated release of eNOS could be through striatin, which has binding sites for both Ca^{2+}-CAM and caveolin. However, it is currently unknown how the striatin family proteins modulate Ca^{2+} signaling. Notably, decreased hepatic striatin expression observed in the current study may modulate eNOS activity, reflecting reduced NO synthesis observed in cirrhotic patients. Indeed, further large-scale cohort studies are warranted to ascertain the causal relationship between the reduction in systemic striatin levels and the worsening of endothelial dysfunction in cirrhosis.

CONCLUSION

In conclusion, our data show evidence that decreased blood striatin levels observed in cirrhotic patients raise the possibility that it could be used as a possible potential diagnostic marker for cirrhotic patients with PHT. Furthermore, decreased hepatic striatin expression was directly proportional to diminished eNOS activity in cirrhosis. This may be associated with decreased hepatic NO synthesis in the cirrhotic liver and lead to endothelial

dysfunction. However, our further study will explore the critical role of striatin in modulating NO-cGMP-eNOS in regulating vascular homeostasis in cirrhotic patients with a larger sample size.

The key limitations are our study is a single-centre study with a limited sample size of 40 cirrhotic patients and 40 healthy controls for studying blood parameters. Furthermore, we used n=4 tissue samples for western blotting and immunostaining. Striatin isoforms are not studied in cirrhotic patients and cirrhotic animal models. Significant reduction of striatin expression and associated eNOS dysfunction observed in cirrhotic patients need further exploration, particularly using bile duct ligation or CCl_4-induced cirrhotic animal models, which may evidently reveal the underlined pathophysiological mechanism associated with striatin/eNOS/NO signaling in cirrhosis.

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AUTHOR CONTRIBUTIONS

VV: Conducted the study and wrote the manuscript. BV: Designed the study, analysed the data, wrote the manuscript, and critically reviewed the manuscript. PM: Critically reviewed the manuscript. All authors have read and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

This study was reviewed and approved by the JIPMER Scientific Advisory Committee (JSAC) and the Institute Ethics Committee (IEC) for Human Studies (No.JIP/IEC/SC/2015/23/841). All patients were provided with informed written consent regarding the data collection and scientific publication.

CONSENT TO PARTICIPATE

Informed consent to participate in the study was obtained from all participants.

CONSENT TO PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Datasets are available on request.

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