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Study of Plasma Cyclic Guanosine Monophosphate as a New Marker in Patients with Portal Hypertension

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ABSTRACT

Background: Clinically significant portal hypertension (CSPH), causes a number of problems that is linked to decreased survival. Its diagnosis done by measuring the hepatic venous pressure gradient (HVPG). Although being invasive but gold standard one. In addition to fibrotic liver tissue remodeling, poor vasotonus control plays a role in the pathophysiology of portal hypertension (PH) in liver cirrhosis. Research on PH-affected animal models has shown that hepatic cGMP activity is reduced while systemic and splanchnic cGMP activity are reflectively elevated. These changes are part of what causes cirrhotic PH, which is characterised by hyperdynamic systemic and splanchnic circulation as well as profuse hepatic vascular resistance. This pathophysiological context implies that cGMP may serve as a PH marker. **Objective:** The aim of the current study was for evaluation of plasma level of cyclic guanosine monophosphate (cGMP) as a surrogate non-invasive biomarker of portal hypertension.

Patients and methods: This case control study were performed at Ain Shams University Hospitals Inpatients and Outpatients' settings for one year. It included 40 cirrhotic patients with portal hypertension (Group I), 40 cirrhotic patients without PH (Group II), and 20 healthy controls (Group III). The following investigations were assessed for all study subjects: plasma cGMP, and abdominal US with portal vein duplex. Upper endoscopy was performed only for group I. **Results:** This study showed high statistically significant difference between the studied groups as regards plasma cGMP. Also, there was a statistically significant correlation between cGMP and portal vein diameter and splenic diameter in group I, but there was no statistically significant relation between plasma cGMP and presence of esophageal varices in group I. ROC curve for plasma cGMP to differentiate between cirrhosis cases with and without PH showed at cut off point > 53.6 plasma cGMP had a sensitivity of 95%, and specificity of 100% to detect portal hypertension in patient with liver cirrhosis.

Conclusion: cGMP can be used as a noninvasive biomarker of portal hypertension. Also, cGMP at a cut off value >53.6 (with 99.4% accuracy) had 95% sensitivity, and 100% specificity. However, cGMP couldn't be used as a screening for esophageal varices.

Keywords: Plasma cyclic guanosine monophosphate, Clinically significant portal hypertension.

INTRODUCTION

Even in the early stages of cirrhosis, no specific symptoms are associated with chronic hepatitis until the development of clinically significant portal hypertension [(1)]. Ascites and gastric varices are the most prevalent presentations for clinically significant portal hypertension. Prognostic factors that are associated with the severity of PH include hepatic encephalopathy (HE), spontaneous bacterial peritonitis (SBP), variceal haemorrhage, infections other than SBP, and hepato-renal syndrome (HRS) [(2)].The difference between the portal pressure and the inferior cava vein pressure, or portal pressure gradient, is known as PH [(3)]. It can be more than 5 mmHg. In the majority of cirrhosis aetiologies, the portal pressure gradient is accurately reflected by the HVPG, or the difference between wedged and free hepatic venous pressure values [(4)].In cirrhotic liver, there is an overexpression of the major enzymes phosphodiesterase-5 (PDE-5), soluble guanylate cyclase (sGC), and endothelial NO synthase (eNOS), leading to a decrease in cyclic guanosine monophosphate (cGMP). The hepatic sinusoids constrict as a result, increasing portal pressure by approximately thirty percent. Conversely, peripheral artery dilatation is more common because of high levels of cGMP and low PDE-5. The conventional concept of the pathophysiology of PH is defined by the "NO-paradox" that is defined as increasing NO synthesis in the peripheral circulatory system and decreased NO availability within the liver. However, recent studies suggest that rather than concentrating solely on NO availability, cGMP availability may be more pertinent in understanding the paradoxical outcomes of peripheral vasodilation in the body and intrahepatic vasoconstriction [(5)]. The aim of this study was for evaluation of the plasma level of cyclic guanosine monophosphate (cGMP) as a new non-invasive biomarker for portal hypertension.

PATIENTS AND METHODS

This prospective cross-sectional case control study included a total of one hundred subjects consist of twenty healthy controls and eighty cirrhotic patients from Inpatients or Outpatients at El-Demerdash Hospital, Ain Shams University Hepatology

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Outpatient Clinic, Endoscopy Unit, and Radiodiagnosis Department. This research was carried out between June 2022 and June 2023.

Patients were classified into three groups: Group I included forty cirrhotic patients with PH, group II consisted of forty cirrhotic patients without portal hypertension, and group III that consisted of twenty healthy controls.

Diagnosis of liver cirrhosis:
If two or all three of the following are present [6].
(1) Clinical manifestations (ascites or splenomegaly, clubbing, gynecomastia and hepatic encephalopathy).
(2) Impaired laboratory tests congruent with cirrhosis (high INR, high total bilirubin, and low serum alb).
(3) Abdominal ultrasonography featuring cirrhosis-like features.

Diagnosis of portal hypertension [7]:
1) Clinical signs (ascites and splenomegaly).
2) Low platelet count [< 150 [103/μl] found by laboratory test.
3) Large spleen and portal vein diameter (PVD).
4) Endoscopic findings consistent with portal hypertension, such as esophageal varices, fundal varices, and portal hypertensive gastropathy (PHG).

The following were the exclusion criteria:
(1) Individuals suffering from portal vein thrombosis. (2) Individuals with substantial cardiovascular, pulmonary, renal, or metabolic problems preclude them from receiving an esophago-gastro-duodenoscopy (EGD). (3) Refusing to give permission or have an esophagogastroduodenoscopy.

Method:
Study design: Every patient was subjected to complete history taken, and clinical information was gathered. Comprehensive basic lab testing. Aspartate transaminase to platelet ratio index (APRI) and the Child Pugh score were computed. Cyclic guanosine monophosphate (cGMP) levels in plasma were assessed for each of the three study groups. Additionally, all research participants underwent a portal vein duplex abdominal US scan. Only patients in group I who met the previously stated diagnostic criteria for portal hypertension underwent esophagogastroduodenoscopy (EGD).

Laboratory testing including ALT, AST, CBC involving platelet count, PT, INR, and PTT.

Calculation of Child Pugh Score (CPS): was used to evaluate liver cirrhosis (CPS) severity. Clinical and laboratory factors, such as ascites, the degree of encephalopathy, serum albumin, bilirubin, and prothrombin time, all affect this score [8] (Table 1).

Table (1): Child-Pugh score (CPS)

Parameter	Assign one point	Assign two points	Assign three points
Ascites	Absent	Slight	Moderate
Alb (g/dL)	>3.5	2.8-3.5	<2.8
PT (second over control) Or INR	<four <1.7	Four-six 1.7-2.3	>six 2.3
Bilirubin	< two	Two-three	>three
Encephalopathy	None	Grade 1-2 (Mild to Moderate)	Grade 3-4 (Sever)

Calculation of APRI score: The AST to APRI tool is useful as a non-invasive index that correlates with liver biopsy findings of fibrosis and cirrhosis. AST to Platelet Ratio Index (APRI) by Wai's formula [9] = (AST / ULN of AST) / ((platelet count$\times 10^9$/L) × 100).

Plasma cyclic guanosine monophosphate (cGMP) level of both patients and healthy control: cGMP levels were determined in plasma using an ELISA by DL Sci & Tech Development Co; Ltd.

Reagents and materials provided:
1- Pre-coated, ready to use 96-well strip plate.
2- Standard.
3- Detection Solution A.
4- Detection Solution B.
5- Wash Buffer (30 × concentrate).

Sample collection and storage: Use heparin or EDTA as an anticoagulant when collecting plasma. Samples should be centrifuged for 15 minutes and then stored in aliquots at -20°C (≤1 month) or -80°C (≤ 2 months) until needed.

Upper gastrointestinal (UGI) endoscopy: Following an overnight fast, a gastrointestinal endoscopy was performed under general anaesthesia using an Olympus GIF type 1TQ160 gastrointestinal videoscope in order to confirm or deny the existence of endoscopic manifestations of portal hypertension. Patients in group I who met the previously stated criteria for a diagnosis of PH underwent UGI endoscopy.

Radiological Parameters
Abdominal US evaluation and portal vein duplex (PVD): After an overnight fasting, all subjects were undergone real time abdominal ultrasonography using Hitachi EUB 515 or Toshiba SSA-340A machine with a 3.5 MHZ convex linear transducer examination with comment on hepatic and splenic size and texture, and presence of ascites. Then portal vein duplex to detect PV dilatation.
To identify PV dilatation, a portal vein duplex was performed. The anatomy of the portal vein (PV) was evaluated with B-mode imaging. The PV is situated in the hepatoduodenal ligament, post to the bile duct and

post to the hepatic artery. To identify the splenic vein, continue it straight until it meets the superior mesenteric vein. The PV and the bile duct or IVC are not confused while using this technique. On the other hand, if the patient is supine and the PV is difficult to see, the patient should be evaluated in the left lateral position. In 97% of cases, the examination in this position successfully demonstrates the PV; if it is not visualized, there is a possibility that the patient has portal vein thrombosis [(10)].

On the B-mode picture, the PV diameter was measured between the inner ant and inner post walls. PV diameter often increases by 20–30 mm when food and perspiration are consumed. The PV is dilated (>13 mm) and either non-existent or varies by less than 20% with respiration in portal hypertension.

Hepatopetal, or the liver, is the target of the low-velocity, breathing-induced oscillations in blood flow in the PV. It is important to differentiate this from the flow of blood in the liver. Duplex scanning has an eighty-three percent accuracy rate in determining the direction of portal blood flow. The presence of hepatofugal blood flow in the PV, or blood flow that is directed away from the liver, may be a sign of portal hypertension. Normally, breathing and eating cause the portal vein's blood flow to rise. Absence of these outcomes likewise suggests portal hypertension [(10)].

Ethical Consideration: Helsinki Declaration and its subsequent revisions was followed all through conduction of the study. Ain Shams University Research Committee (whose reference number is 000017585) approved all procedures used in this investigation. Before being enrolled in the study, each subject gave written, informed consent.

Statistical analysis: The collected data were analysed, coded, and loaded and SPSS Statistics V. 23.0 for Windows was used. The analysis of variance (ANOVA), linear correlation coefficient, student t-test, mean, standard deviation, and Chi-square tests. In the case of non-parametric data, the data were presented as the median and interquartile range (IQR), with figures and percentages denoting qualitative factors. The Post Hoc test was used to compare any and all possible pairs of group means. The statistical significance was ascertained using the Unpaired Student T-test, and the relationship between two qualitative variables was examined using the Chi-square test. The Receiver Operating Characteristic (ROC) curve is a useful tool for evaluating the specificity and sensitivity of quantitative diagnostic tests that split cases into two groups. The degree of a linear link between two variables was assessed using the Pearson correlation coefficient. The significance level of the p-value was determined by applying the following criteria: $p > 0.05$ indicated a non-significant (NS) value, $p \leq 0.05$ a significant (S) value, and $p \leq 0.01$ a very significant value. Additionally, the level of significance for the r-value was found to fall into three categories: decent (between 0.3 and 0.6), strong ($r > 0.6$), and week correlation (between 0.01 and 0.3).

RESULTS

In all, one hundred subjects (twenty healthy controls and eighty patients with cirrhosis) were involved in this investigation. Table (2) displayed the study individuals' basic laboratory results as well as the results of an abdominal ultrasonography with portal vein duplex findings.

Table (2): Basic lab investigations and radiological parameter of the subjects

		Group I (portal hypertension cirrhotic patients) (no = 40)	Group II (cirrhotic patients without PH) (no = 40)	Group III (healthy controls) (no = 20)	Test of Sig.	p
ALT (U/l)	Mean ± SD	30.88 ± 7.71	45.9 ± 8.17	25.7 ± 4.05	F= 65.701	**<0.001***
	Post-hoc	**p1<0.001*, p2=0.012*, p3<0.001***				
AST (U/l)	Mean ± SD	50.43 ± 10.68	47.23 ± 7.54	27.05 ± 4.7	F= 53.329	**<0.001***
	Post-hoc	p1=0.097, **p2<0.001*, p3<0.001***				
Platelets [10^3/µl]	Mean ± SD	108.65 ± 25.79	22.83 ± 23.94	242.45 ± 14.47	F= 242.107	**<0.001***
	Post-hoc	**p1=0.008*, p2<0.001*, p3<0.001***				
INR	Mean ± SD	1.19 ± 0.09	1.01 ± 0.08	0.99 ± 0.07	F= 65.527	**<0.001***
	Post-hoc	**p1<0.001*, p2<0.001*,** p3=0.310				
Bilirubin [mg/dl]	Mean ± SD	1.28 ± 0.31	0.68 ± 0.14	0.52 ± 0.09	F= 45.810	**<0.001***
	Post-hoc	**p1<0.001*, p2<0.001*,** p3=0.090				
Albumin [g/dl]	Mean ± SD	3.37 ± 0.48	4.55 ± 0.18	4.56 ± 0.11	F= 154.035	**<0.001***
	Post-hoc	**p1<0.001*, p2<0.001*,** p3=0.912				
PVD [mm]	Mean ± SD	16.52 ± 1.99	11.11 ± 1.16	10.86 ± 1.25	F= 148.125	**<0.001***
	Post-hoc	**p1<0.001*, p2<0.001*,** p3=0.557				
Spleen diameter [mm]	Mean ± SD.	145.83 ± 34.91	121.65 ± 29.97	110.75 ± 11.32	F= 6.853	**0.002***
	Post-hoc	**p1=0.006*, p2=0.001*,** p3=0.302				

Both the Child Pugh and APRI scores were examined. We found that in group I, there were 9 patients (22.5%) who were classified as class A, 23 patients (57.5%) who were classed as class B, and 8 patients (20%) who were classified as class C, according to the Child-Pugh Classification, which measures the liver disease severity. Group II included 33 (82.5%) patients identified as class A, 7 (17.5%) patients classified as class B, and no patients with a validated class C. After calculating the APRI score for each individual, we found that the mean for group I was 1.24 ± 0.45 and for group II it was 0.99 ± 0.24. While, the mean of group III was 0.28 ± 0.05 (Table 3).

Table (3): Comparing studied cases according to Child Pugh classification and APRI score

	Group I (cirrhotic patients with PH) (no = forty)		**Group II (cirrhotic patients without PH) (no = forty)**		**Group III (healthy controls) (no = 20)**		**Test of Sig.**	**p**
Child-Pugh classification	N	%	N	%	—	—		
A	9	22.5	33	82.5	—	—	χ^2= 30.250	**<0.001***
B	23	57.5	7	17.5	—	—		
C	8	20.0	0	0.0	—	—		
APRI								
Range.	0.66 – 2.68		0.62 – 1.58		0.18 – 0.38		F= 59.185	**<0.001***
Mean ± SD.	1.24 ± 0.45		0.99 ± 0.24		0.28 ± 0.05			
Post-hoc	**p1=0.001*, p2<0.001*, p3<0.001***							

According to **plasma level of cGMP,** which was measured for all the study subjects, our results revealed that in group I, the mean was 83.97 ± 17.09, in group II the mean was 38.43 ± 7.21. While the mean of group III was 41.44 ± 3.53 (Table 4).

Table (4): Comparison between studied cases according to plasma cGMP.

	Group I (liver cirrhosis with PH) (n = 40)	**Group II (liver cirrhosis without PH) (n = 40)**	**Group III (healthy controls) (n = 20)**	**Test of Sig.**	**p**
Plasma cGMP					
Range	46.4 – 111.4	25.1 – 53.6	35.7 – 47.1	F= 169.520	**<0.001***
Mean ± SD.	83.97 ± 17.09	38.43 ± 7.21	41.44 ± 3.53		
Post-hoc	**p1<0.001*, p2<0.001*,** p3=0.357				

Our results showed that fourteen individuals in group I had esophageal varices (35%), thirty participants had portal hypertensive gastropathy (PHG) (75%), and no patient had fundal varices based on endoscopic findings from esophagogastroduodenoscopy (EGD) (Table 5).

Table (5): Endoscopic findings of EGD in group I

	Group I (cirrhotic patients with PH) (n = 40)
Endoscopic findings of EGD	N (%)
Esophageal varices	14 (35%)
Fundal varices	No (0%)
Portal hypertensive gastropathy (PHG)	30 (75%)

The current study showed that the plasma level of cGMP at a cut off value more than 53.6 (with 99.4% accuracy) has 100% specificity and 95% sensitivity for predicting the presence of portal hypertension in patients with liver cirrhosis (Table 6 & figure 1).

Table (6): Using plasma cGMP cutoffs, sensitivity, specificity, (PPV and NPV), as well as diagnostic accuracy, for PH prediction in patients with cirrhosis

ROC curve					
Cut off point	**AUC**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
>53.6	0.994	95.00	100.00	100.0	95.2

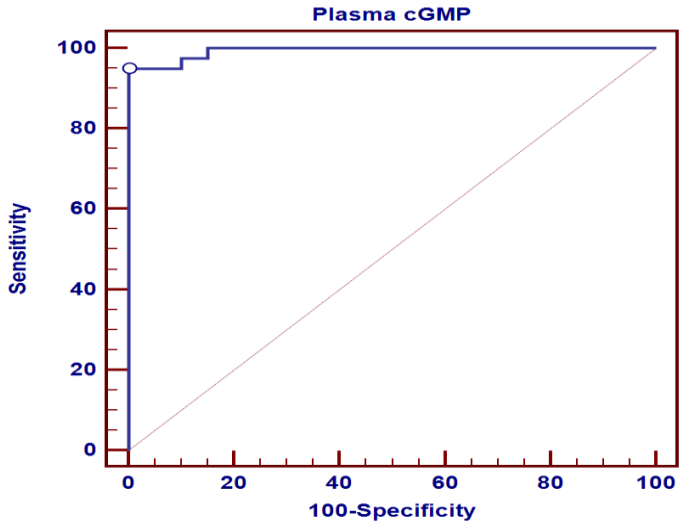

Figure (1): ROC curve.

Our results showed no statistical significance relation between plasma cGMP and presence of esophageal varices in group I (Table 7 & Figure 2).

Table (7): Plasma cGMP and esophageal varices correlation in **Group I**

	Presence of Esophageal Varices		Test of Sig.	p
	No (n = 26)	Yes (n = 14)		
Plasma cGMP				
Range.	46.4 – 111.3	62.5 – 111.3	t= 0.517	0.608
Mean ± SD.	85 ± 14.96	82.05 ± 20.96		

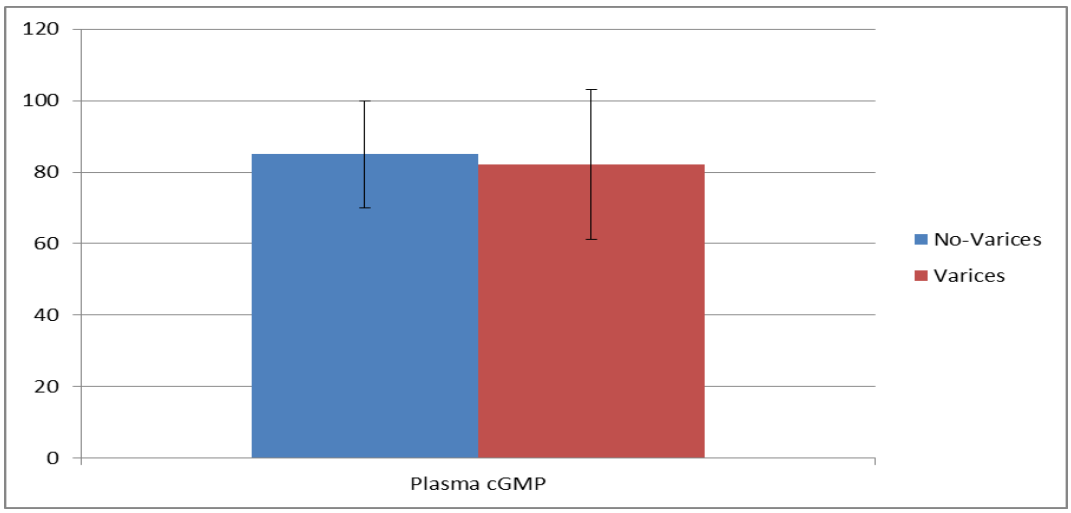

Figure (2): Relation between Plasma cGMP and esophageal varices in Group I.

Our result also showed that there was statistical significant correlation between cGMP and portal vein diameter and splenic diameter in group I (Table 8).

Table (8): serum cGMP and PVD and Splenic diameter correlation in Group I

Variables	cGMP	
	R	P
PVD	**0.736****	<0.001
Splenic diameter	**0.349****	0.002

DISCUSSION

Individuals suffering from liver disease display a notably disrupted equilibrium of cyclic GMP (cGMP), as seen by notably elevated levels of cGMP in plasma. It's unclear why liver illness causes a notable rise in plasma cGMP. Additionally, it's possible that some of the clinical modifications seen in these patients are connected to the higher-than-normal concentration of cGMP in their plasma. Though its precise role is unknown, extracellular cGMP has been demonstrated lately to regulate Na^+ absorption in human renal tubular cells. Thus, the altered Na^+ homeostasis seen in liver cirrhosis patients may be related to the altered cGMP homeostasis [(11)].

These changes in cGMP homeostasis are caused by unknown causes. Enzymes that synthesize cGMP are classified into two categories: soluble and particulate guanylate cyclases. Nitric oxide activates soluble guanylate cyclase, which is mostly found in the cytoplasm. Nitric oxide is produced by several NO synthases. The other kind of guanylate cyclase is linked to membrane receptors and is mostly triggered by atrial natriuretic peptides. These findings imply that decreased plasma cGMP levels in individuals with cirrhosis may indicate portal hypertension [(12)].

The present investigation showed a statistical significance discrepancy in Child-Pugh scores between groups I and II. Specifically, the majority of cases in group I (liver cirrhosis with portal hypertension) were classified as class B, whereas the majority of cases in group II (liver cirrhosis without portal hypertension) were classified as class A. As per the findings of **Gabr *et al.*** [(13)] it can be inferred that patients with portal hypertension accelerate the progression of liver cirrhosis, as patients without portal hypertension represented Child–Pugh class A in sixty percent of cases and those with portal hypertension represented Child–Pugh class C in 42.9 % (severe) trials.

According to our study, group I platelet count was lower (108.65 ± 25.79, range: 56-150) than in group II (122.83 ± 23.94, range: 82-163). This difference was statistically significant. This finding is similar to a research by **Gamaleldin *et al.*** [(15)], which showed that the PHT group with cirrhosis had the lowest platelet count (86.9 ± 23.6x10^3/uL), and that there was a statistically significant correlation between the groups.

Patients with PH had a higher portal vein diameter (16.52 ± 1.99, range: 13.9–20.2) than patients without portal hypertension (11.11 ± 1.16, range: 9–12.8), according to a statistically significant difference in spleen and portal vein diameters between the study groups. Additionally, group I had larger spleens (145.83 ± 34.91, range: 59-237) than group II (121.65 ± 29.97, range: 54-214). Additionally, our research is supported by the findings of **Mathiesen *et al.*** [(16)], who reported a significant increase in splenic diameter in individuals with PH and cirrhotic liver.

The results of the current investigation demonstrated that there was a statistical significant variation in APRI between the groups under examination. Additionally, there was a statistically significance discrepancy between the groups under study when it came to the amount of plasma cGMP, with patients with portal hypertension having a higher level than those without.

According to the current investigation, there was no statistically significant correlation (p-value = 0.608) between plasma cGMP and esophageal varices in individuals with portal hypertension (Group I). On the other hand, **Sturm *et al.*** [(17)] observed that patients with esophageal varices had cGMP levels that were considerably higher than those with liver cirrhosis without PH.

CONCLUSION

Given that the plasma level of cGMP was greater in group I (83.97 ± 17.09) than in group II (38.43 ± 7.21). It is possible to draw the conclusion that cGMP can be employed as a blood-derived noninvasive biomarker to detect PH in cirrhotic patients. Also, cGMP had a specificity of one hundred percent and 95% sensitivity at a cutoff value >53.6 (with 99.4% accuracy). As a result, it can lessen the difficulty and risk associated with utilising invasive methods to identify portal hypertension. It was not possible to screen for esophageal varices using cGMP yet.

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