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Thrombophilia Gene Screen in Cirrhotic Patients with Portal Vein Thrombosis While Awaiting Liver Transplant

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ABSTRACT

Background: Portal vein thrombosis (PVT) is a recognized complication of liver cirrhosis. The pathogenesis of PVT is multifactorial. The extent to which thrombophilia promotes PVT and whether patients with PVT should be routinely tested for thrombophilia remain uncertain.

Objective: This retrospective research aimed to assess the frequency of thrombophilia in cirrhotic patients with non-malignant PVT to clarify the relevance of thrombophilia testing in this particular context.

Patients and methods: This study involved 40 cirrhotic patients with protein C & S deficiency prior to liver transplantation, where 20 patients had PVT and the other group (20) had no PVT. Thrombophilia testing included gene analysis for beta-fibrinogen -455G>A, factor XIII V34L, factor V H1299R (R2), prothrombin G20210A, factor V Leiden, methylene tetrahydrofolate reductase (MTHFR) C677T, and MTHFR A1298C.

Results: Regarding the thrombophilia gene screen, no statistically significant differences were detected between the two groups. In addition, the inherited thrombophilia gene status was not a risk factor for PVT. However, factor V Leiden showed a high odds ratio of 11.457 and 95% CI of 0.885 - 148.27.

Conclusion: Inherited thrombophilia is considered to be a low-risk factor with low prevalence in patients with cirrhotic non-malignant PVT. Therefore, routine testing for these conditions does not seem to be indicated in this setting.

Keywords: Liver cirrhosis, Protein C, Protein S, Thrombophilia, PVT.

INTRODUCTION

PVT is defined as the presence of a thrombus in the portal vein main trunk and intrahepatic portal branches, which results in either full or partial obstruction of hepatic blood flow [1].

It is a known complication of hepatic cirrhosis. The pathology of PVT in liver cirrhosis is complicated and multifactorial. The liver has a crucial function in the homeostasis of coagulation process. Consequently, liver cirrhosis results in significant bleeding or thrombosis [2]. Furthermore, in the case of cirrhosis, the most important factors appear to be the reduced portal flow velocity and increased vascular resistance in the portal circulation brought on by portal hypertension [3]. Other potential mechanisms include endotoxemia especially in severe stages of the disease [4, 5].

The risk of PVT is increased by more than 7-folds in cirrhotic patients compared to the general population [6]. According to reports, the frequency of PVT is only 1% in compensated cirrhosis. However, in patients awaiting liver transplantation, this number rises to 8–25%, and in cases of hepatocellular carcinoma (HCC), it reaches 40% [3].

Nevertheless, around 20% of patients with PVT and cirrhosis or HCC exhibited either inherited or acquired thrombophilia. These conditions included myeloproliferative neoplasms, antiphospholipid antibody syndrome, factor V Leiden mutation, paroxysmal nocturnal hemoglobinuria, prothrombin G20210A mutation, Janus kinase 2 (JAK2) V617F mutation, protein C, protein S, and antithrombin deficiency [7].

There might be a potential involvement of thrombophilia to be revealed. Therefore, we conducted this retrospective analysis among a group of cirrhotic patients who had protein C and S deficiency and non-malignant PVT to determine the prevalence of inherited thrombophilia to evaluate the relevance of thrombophilia testing in this setting.

PATIENTS AND METHODS

This retrospective research examined 40 cirrhotic patients diagnosed with protein C & S deficiency who were awaiting liver transplantation. Among these patients, 20 had PVT.

Exclusion criteria: HCC or any other malignancy, known thrombophilia or other blood disorders, Budd-Chiari syndrome, autoimmune diseases, ongoing anticoagulant or antiplatelet therapy, current sepsis, recent gastrointestinal endoscopic interventions, severe dehydration, splenectomy, recent surgery, and hormonal replacement therapy within 3 months prior to diagnosis or pregnancy.

Doppler ultrasound with colour and pulsed mode was utilized to identify PVT. Then, abdominal computed tomography with contrast was used to confirm the location, the extent, and the nature of PVT [1].

The thrombophilia screening tests included the assessment of serum protein C and protein S levels, as well as conducting a gene study for factor V Leiden, prothrombin G20210A, factor V H1299R (R2), beta-

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fibrinogen -455G>A, MTHFR C677T, factor XIII V34L, and MTHFR A1298C mutation.

Ethical approval: The study procedure adhered to the ethical standards of the 1964 Declaration of Helsinki and got authorization from The Ethics Committee for the scientific research of Faculty of Medicine, Ain Shams University (FMASU MS 478/2023). Every patient participating in this research provided written informed permission.

Statistical analysis

The SPSS version 25.0 was utilized for the statistical analysis processes. Numbers and proportions were provided for qualitative data, while mean ± SD was used to describe quantitative data. The Shapiro-Wilk test was used to test the distribution of the quantitative data. Fischer's exact test, the X^2-test, and the t-test were used to evaluate the correlations between the variables. The threshold for statistical significance was a P value of 5%.

RESULTS

This research retrospectively analyzed a group of 40 patients who had liver cirrhosis and were diagnosed with protein C & S deficiency. These patients were waiting for liver transplantation. The patients were evenly distributed into two groups based on the diagnosis of PVT. No statistically significant variations were detected in age and cause of cirrhosis between the two groups. However, the PVT group had a considerably larger proportion of men (Table 1).

Table (1): Demographic data of study participants

		PVT		P-value
		PVT	No PVT	
Age (Years)		50.950 ± 10.855	45.350 ± 13.956	0.165
Etiology of liver cirrhosis	HCV	13 (65%)	9 (45%)	0.373
	HBV	1 (5%)	2 (10%)	
	Cryptogenic	3 (15%)	5 (25%)	
	Autoimmune hepatitis	1 (5%)	4 (20%)	
	Wilson disease	1 (5%)	0 (0%)	
	Primary biliary cirrhosis	1 (5%)	0 (0%)	
Sex	Male	19 (95%)	12 (60%)	0.008
	Female	1 (5%)	8 (40%)	

Regarding laboratory investigations, no statistically significant variations were observed between the two groups (Table 2).

Table (2): Laboratory investigations of study participants

	PVT		P-value
	PVT	No PVT	
Hemoglobin (g/dL)	11.26 ± 1.61	10.75 ± 1.99	0.384
Platelets (cells/L)	101.000 ± 24.86	91.050 ± 21.74	0.501
White blood cells (10^9/L)	4.45 ± 1.10	4.56 ± 1.11	0.899
International normalized ratio	1.47 ± 0.35	1.57 ± 0.39	0.413
Alanine aminotransferase (IU/L)	31.30 ± 7.69	37.02 ± 8.79	0.415
Aspartate aminotransferase (IU/L)	46.80 ± 11.58	49.55 ± 12.10	0.741
Total bilirubin (mg/dL)	2.79 ± 0.69	2.72 ± 0.66	0.906
Direct bilirubin (mg/dL)	1.13 ± 0.27	1.41 ± 0.34	0.318
Urea (mg/dL)	27.50 ± 6.79	34.05 ± 8.41	0.187
Creatinine (mg/dL)	0.89 ± 0.21	0.76 ± 0.18	0.153
Albumin (g/dL)	3.06 ± 0.29	3.09 ± 0.62	0.872

Concerning the thrombophilia gene screen, no statistically significant differences were detected between the two groups (Table 3 & figure 1).

Table (3): Thrombophilia gene screen in study participants

		PVT		P-value
		PVT	No PVT	
Factor V Leiden	No mutation	14 (70%)	18 (90%)	0.114
	Mutation	6 (30%)	2 (10%)	
Factor V H1299R (R2)	No mutation	16 (80%)	17 (85%)	0.677
	Mutation	4 (20%)	3 (15%)	
Prothrombin G20210A	No mutation	19 (95%)	19 (95%)	1.000
	Mutation	1 (5%)	1 (5%)	
Factor XIII V34L	No mutation	19 (95%)	16 (80%)	0.151
	Mutation	1 (5%)	4 (20%)	
Beta-Fibrinogen -455G>A	No mutation	15 (75%)	10 (50%)	0.102
	Mutation	5 (25%)	10 (50%)	
MTHFR C677T	No mutation	10 (50%)	11 (55%)	0.752
	Mutation	10 (50%)	9 (45%)	
MTHFR A1298C	No mutation	7 (35%)	8 (40%)	0.744
	Mutation	13 (65%)	12 (60%)	

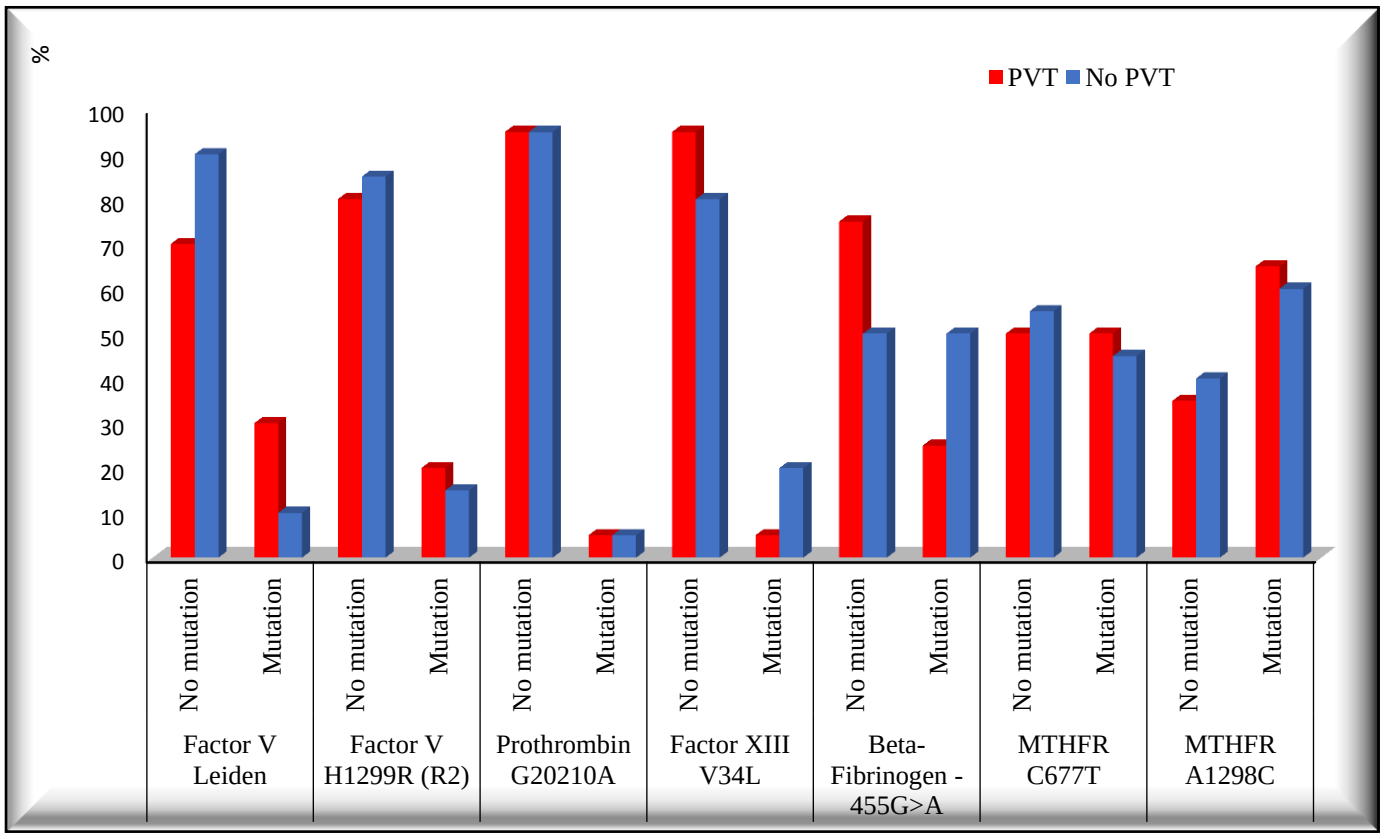

Figure (1): Thrombophilia gene screen of study participants.

In addition, the inherited thrombophilia gene status was not a significant risk factor for PVT. However, factor V Leiden showed a high odds ratio of 11.457 and a 95% CI of 0.885 - 148.27 (Table 4).

Table (4): Risk factors for PVT

	Odd ratio	95% CI	P-value
Factor V Leiden	11.457	0.885 - 148.27	0.062
Factor V H1299R (R2)	1.210	0.166 - 8.817	0.851
Prothrombin G20210A	0.222	0.002 - 19.99	0.512
Factor XIII V34L	0.040	0.001 - 1.212	0.064
Beta-Fibrinogen -455G>A	0.260	0.048 - 1.400	0.117
MTHFR C677T	0.927	0.175 - 4.905	0.929
MTHFR A1298C	5.039	0.711 - 35.71	0.106

DISCUSSION

PVT is an uncommon but potentially fatal disease, with a 5-year mortality of 27% that varies according to the underlying disease [8]. The impact of acquired and inherited thrombophilia on the development of PVT in cirrhotic patients remains undefined. These conflicting data are mainly due to the small sample size and heterogeneity of the studies regarding included thrombophilia and patient selection criteria, especially concerning cirrhosis [9, 10].

This research found no statistically significant differences between the two groups regarding the thrombophilia gene screen. In addition, the inherited thrombophilia gene status was not a risk factor for PVT. However, factor V Leiden showed a high odds ratio of 11.457 and a 95% CI of 0.885 - 148.27.

Similarly, amid the different prothrombotic genetic abnormalities, prothrombin G20210A and factor V Leiden mutations have been the most extensively investigated. Prior meta-analyses showed that they promote the risk of PVT in cirrhotic patients [11, 12]. However, one of these analyses did not find this correlation for prothrombin G20210A mutations, as reported in the present research [12].

The detection of inherited protein C, protein S, or antithrombin III deficiencies is challenging because of the existence of concurrent liver synthetic dysfunction. Consistent with the current study, it has been shown that patients with cirrhosis have reduced concentrations of antithrombin III, proteins C, and S. However, it remains uncertain whether this is a result of genetic thrombophilia or acquired due to liver illness [3, 13, 14]. Contrary to these results, a study by **Qi *et al.*** [15] found no significant relationship between antithrombin III and proteins C and S levels, and the development of PVT in cirrhosis patients. MTHFR C677T mutations were also found as a risk factor for PVT [16]. However, according to the findings [17], these mutations are not associated with an increased thrombotic risk.

In agreement with the current study, in previous **Rabie *et al.*** research [18], 100 patients with PVT (76 with HCC and 24 with liver cirrhosis) and 100 healthy controls were examined. The distribution of inherited thrombophilia in PVT patients was JAK2 mutation in 28/100 (28%), protein C deficiency in 28 (28%), protein S deficiency in 10 (10%), antithrombin III deficiency in 52 (52%), MTHFR mutation in 42 (42%), factor V Leiden mutation in 12 (12%), prothrombin G20210A mutation in 4 (4%), and antiphospholipid syndrome in 18 (18%) patients. In addition, there was no significant difference in the frequency of MTHFR mutation among PVT patients and controls ($p<0.24$).

In accordance with the present study, 394 non-cirrhotic, non-malignant PVT cases were screened for prothrombotic factors. The frequency of prothrombotic factors in PVT cases was myeloproliferative neoplasms in 102/361 (28.3%), antiphospholipid antibody syndrome in 22/296 (7.4%), protein C deficiency in 16/362 (4.4%), protein S deficiency in 65/362 (18%), and antithrombin deficiency in 12/362 (3.3%) patients. In contrast, factor V Leiden mutation was detected in a single patient out of 266 (0.4%), whereas the prothrombin G20210A mutation was not recognized in any of the 266 cases with PVT (0.0%). These findings indicate that it may not be necessary to routinely screen for factor V Leiden and prothrombin G20210A mutations in individuals with PVT. Other thrombophilic conditions, such as MTHFR C677T mutation, was not detected because earlier studies have shown that these conditions may not be linked to PVT [19].

Similarly, **Fortea *et al.*** study [13] examined a group of 77 cirrhotic patients and non-malignant PVT. Out of these patients, six individuals (7.8%) had thrombophilia. Specifically, four cases had antiphospholipid syndrome, one had a prothrombin gene mutation, and one had a factor V Leiden mutation. Antiphospholipid syndrome was the most frequent disorder observed in their cohort (5.3%). The levels of antithrombin III, protein S, and protein C were found to be reduced in 70 (90.9%), 72 (93.5%), and 44 (57.1%) cases respectively. These deficits were attributed to hepatic cirrhosis rather than being considered as an inherited thrombophilia. Similar findings have been observed in the present study and others [9], but not all studies [20]. These discrepancies might be attributed to differences in the study design and target population. Furthermore, it emphasizes the challenges in accurately evaluating the hypercoagulable panel in patients of cirrhosis.

As a result of the contradicting evidence discussed above, current clinical guidelines do not provide any clear advice regarding testing for thrombophilia either as a screening measure before diagnosing PVT or as a confirming test once PVT has occurred [3, 21, 22].

In summary, the present results indicated a low occurrence of inherited thrombophilia, which aligns with earlier studies that found no correlation between the presence of thrombophilia and the risk of PVT in cirrhotic patients [9, 13]. However, it should be stated that earlier studies often included a relatively small sample size, making it hard to ascertain whether the previously undetected correlations were related to the limited sample size or truly low prevalence of thrombophilia in patients with PVT. Therefore, it is crucial to conduct comprehensive investigations to provide stronger evidence. Till further evidence, the current data support thrombophilia testing on a personalized basis. Further prospective studies with the integration of hepatic disease severity and genetic factors are needed to provide personalized criteria for conducting these tests and to assess the effect of thrombophilia on the severity of PVT and response to therapy [13].

CONCLUSION

Inherited thrombophilia is considered to be a low-risk factor with low prevalence in patients with cirrhotic non-malignant PVT. Therefore, routine testing for these conditions does not seem to be indicated in this setting.

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REFERENCES

1. **European Association for the Study of the Liver (2016):** EASL Clinical Practice Guidelines: Vascular diseases of the liver. J Hepatol., 64 (1): 179-202.
2. **Safdar T, Nazir M, Iqbal S *et al.* (2021):** Determine the frequency of portal vein thrombosis in patients with liver cirrhosis. Pak J Med Health Sci., 15: 1617-1619.
3. **Intagliata N, Argo C, Stine J *et al.* (2018):** Concepts and Controversies in Haemostasis and Thrombosis Associated with Liver Disease: Proceedings of the 7th International Coagulation in Liver Disease Conference. Thromb Haemost., 118 (8): 1491-1506.
4. **Intagliata N, Caldwell S, Tripodi A (2019):** Diagnosis, Development, and Treatment of Portal Vein Thrombosis in Patients With and Without Cirrhosis. Gastroenterology, 156 (6): 1582-1599.
5. **Huang X, Fan X, Zhang R (2020):** Systemic inflammation and portal vein thrombosis in cirrhotic patients with gastroesophageal varices. Eur J Gastroenterol Hepatol., 32 (3): 401-405.
6. **Ogren M, Bergqvist D, Björck M (2006):** Portal vein thrombosis: prevalence, patient characteristics and lifetime risk: a population study based on 23,796 consecutive autopsies. World J Gastroenterol., 12 (13): 2115-2119.
7. **Poisson J, Plessier A, Kiladjian J *et al.* (2017):** Selective testing for calreticulin gene mutations in patients with splanchnic vein thrombosis: A prospective cohort study. J Hepatol., 67 (3): 501-507.
8. **Søgaard K, Darvalics B, Horváth-Puhó E (2016):** Survival after splanchnic vein thrombosis: A 20-year nationwide cohort study. Thromb Res., 141: 1. doi:10.1016/j.thromres.2016.02.024
9. **Tremblay D, Naymagon L, Troy K *et al.* (2020):** The utility of thrombophilia testing in patients with newly diagnosed portal vein thrombosis. Blood Coagul Fibrinolysis, 31 (3): 213-218.
10. **Zanetto A, Garcia-Tsao G (2020):** Some Answers and More Questions About Portal Vein Thrombosis in Patients With Decompensated Cirrhosis. Clin Gastroenterol Hepatol., 18(11):2432-2434.
11. **Ma S, Wang J, Bezinover D *et al.* (2019):** Inherited thrombophilia and portal vein thrombosis in cirrhosis: A systematic review and meta-analysis. Res Pract Thromb Haemost., 3 (4): 658-667.
12. **Qi X, Ren W, De Stefano V *et al.* (2014):** Associations of coagulation factor V Leiden and prothrombin G20210A mutations with Budd-Chiari syndrome and portal vein thrombosis: a systematic review and meta-analysis. Clin Gastroenterol Hepatol., 12 (11): 1801-12.
13. **Fortea J, Carrera I, Puente Á *et al.* (2020):** Portal Thrombosis in Cirrhosis: Role of Thrombophilic Disorders. J Clin Med., 9 (9): 2822. doi:10.3390/jcm9092822
14. **Metawea M, El Wazzan D, El-Shendidi A (2022):** Significance of altered anticoagulant proteins and D-dimer in cirrhotic portal vein thrombosis: relation to the degree of liver dysfunction. Clin Exp Hepatol., 8 (3): 233-242.
15. **Qi X, Chen H, Han G (2013):** Effect of antithrombin, protein C and protein S on portal vein thrombosis in liver cirrhosis: a meta-analysis. Am J Med Sci., 346 (1): 38-44.
16. **Pasta L, Pasta F, D'Amico M (2016):** PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q, and Prothrombin 20210A in Splanchnic Vein Thrombosis: Analysis of Individual Patient Data From Three Prospective Studies. J Clin Exp Hepatol., 6 (1): 10-14.
17. **Colucci G, Tsakiris D (2020):** Thrombophilia screening revisited: an issue of personalized medicine. J Thromb Thrombolysis, 49 (4): 618-629.
18. **Rabie H, Othman W, Elsabaawy D *et al.* (2021):** Janus Kinase-2 Mutation Associated Portal Vein Thrombosis Complicating Liver Cirrhosis and Hepatocellular Carcinoma. Asian Pac J Cancer Prev., 22 (1): 267-275.
19. **Fan J, Wang Q, Luo B *et al.* (2020):** Prevalence of prothrombotic factors in patients with Budd-Chiari syndrome or non-cirrhotic nonmalignant portal vein thrombosis: A hospital-based observational study. J Gastroenterol Hepatol., 35 (7): 1215-1222.
20. **Werner K, Sando S, Carey E *et al.* (2013):** Portal vein thrombosis in patients with end stage liver disease awaiting liver transplantation: outcome of anticoagulation. Dig Dis Sci., 58 (6): 1776-1780.
21. **O'Leary J, Greenberg C, Patton H *et al.* (2019):** AGA Clinical Practice Update: Coagulation in Cirrhosis. Gastroenterology, 157 (1): 34-43.
22. **Kirkeby M, Larsen J, Grønbaek H *et al.* (2020):** Thrombophilia testing in patients with portal vein thrombosis. Scand J Clin Lab Invest., 80 (8): 694-698.