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RESEARCH ARTICLE

Assessment of Factor V Gene G1691A Mutation (Factor V Leiden) among Chronic Hepatitis C Patients with Thrombocytopenia

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ABSTRACT

Background: Factor V plays a crucial role in the coagulation process. Factor V Leiden (FVL) is considered a mutant form of factor V, which may contribute to blood vessel thrombosis. Also, Hepatitis C Virus (HCV) infection may exert some coagulation changes leading to Portal Vein Thrombosis (PVT). Thrombocytopenia in patients with chronic HCV infection constitutes a major challenge.

Objective: This study aimed to investigate the incidence of FVL (factor V gene G1691A mutation) and its influence on coagulation among HCV chronically infected patients suffering from thrombocytopenia. Besides, it was designed to demonstrate the association of chronic HCV infection with coagulation disturbances in such patients.

Methods: This study was conducted on blood samples of a total of 54 subjects (33 HCV chronically infected patients and 21 healthy controls). HCV chronically infected patients with thrombocytopenia (Platelet Count [PLC] below 150 thousand/microliter) were selected. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPPT) were previously determined as coagulation parameters. Protein C and protein S antigens were determined by ELISA as coagulation-regulating (anticoagulants) parameters. All samples were investigated for the presence of FVL by real-time PCR.

Results: PLC, PT, and protein C antigen showed highly significant differences ($p < 0.001$) between controls (group 1) and HCV chronically infected patients (group 2). Likewise, a highly significant change ($p = 0.006$) was achieved for the protein S antigen. A non-significant change was detected for the incidence of FVL with a heterozygous pattern between controls (group 1) and HCV chronically infected patients (group 2). Additionally, non-significant changes were detected for all of the investigated coagulation and coagulation-regulating (anticoagulants) parameters between control subjects with negative FVL (subgroup 1) and others with positive FVL (subgroup 2). Similarly, non-significant changes for such parameters were recoded for HCV chronically infected patients with negative FVL (subgroup 3) and others with positive FVL (subgroup 4).

Conclusion: Chronic HCV infection in the presence of thrombocytopenia is associated with coagulation disturbances. The incidence of FVL with its heterozygous pattern is unaffected by chronic HCV infection, and no association is shown with worsening of coagulation in chronic hepatitis C patients suffering from thrombocytopenia.

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Introduction

Infection with the Hepatitis C Virus (HCV) constitutes a global health problem. It has been estimated that 71 million individuals are chronically infected with HCV worldwide [1,2]. There is no doubt that patients with chronic HCV infection fail to clear this virus during the disease's acute phase. Consequently, they turn into chronic carriers. Chronic humoral immunity activation against HCV and the inability to clear this viral infection can result in recurrent exposure to a self-reactive antibody and, as a result, organ damage [3,4]. Also, a strong T-cell reaction is induced by HCV, generating different cytokines including interleukin 2 (IL-2), Tumor Necrosis Factor-Alpha (TNF alpha), and interferon-gamma (IFN-gamma), which in particular correlate with liver inflammation and fibrosis [3,4] and cause hepatic dysfunction [4]. Thrombocytopenia results from a decreased level of platelets and is a fundamental health problem in chronic HCV infection. It is of multifactorial pathophysiology, which may involve auto-immunogenicity, hypersplenism, the decline in thrombopoietin production, direct bone marrow suppression, and therapeutic adverse effects. It is a significant challenge in the management of chronic HCV infection and the decision to initiate interferon-containing antiviral therapy in chronic hepatitis C patients with thrombocytopenia. Although it is possible to avoid this challenge by using Direct Antiviral Agents (DAAs) as the major treatment for chronic HCV infection [5], thrombocytopenia remains a chief health problem, especially in chronic hepatitis C patients with advanced liver disease [5]. Those patients experienced a high risk of serious bleeding with severe thrombocytopenia, which can prevent liver biopsies for staging as invasive procedures [5,6]. Complications from bleeding manifestations, such as variceal bleeding, are caused by thrombocytopenia. It may counteract the initiation and continuation of antiviral therapy, resulting in a decline in the success of HCV treatment [5,7].

Generally, chronic viral hepatitis may lead to thromboembolism, especially Portal Vein Thrombosis (PVT) [4], which refers to portal vein obstruction by a blood clot [8,9]. Therefore, special attention has been given to the Hepatitis C Virus (HCV) infection due to its ability to activate hemostais in several ways during its course of pathogenicity [10], which involves a serious liver injury that leads to Chronic Liver Disease (CLD). CLD starts with liver inflammation that may progress to different degrees of liver fibrosis, cirrhosis, and Hepatocellular Carcinoma (HCC) [11,12]. Indeed, CLD and liver cancer can cause disturbances in the coagulation system [13]. The imbalance in the coagulation factors, including procoagulants and anticoagulants, in CLD may have clinical implications, with PVT as a possible complication in hepatic cirrhosis [14]. Chronic hepatitis C patients with cirrhosis are likely to have an increased risk for thromboembolic manifestations, even though it is uncertain whether HCV itself causes PVT or not [14].

There is no doubt that factor V is one of the most important components of the coagulation system. It is a glycoprotein that can contribute to the coagulation process through both procoagulant and anticoagulant functions [15,16]. It is needed for normal blood clotting to take place in response to the injury to the blood vessel [17]. Factor V is mainly produced in the liver by hepatocytes and megakaryocytes. The plasma-derived factor V is produced by hepatocytes. The contribution of platelet-derived factor V can increase factor V locally at the site of injury [16,18,19]. During the day, the blood coagulation process can be activated several times to repair the damage [17]. Such a process can be promoted by the modification of factor V through Activated Protein C (APC), activated factor X, and thrombin. Factor V plays an important role in the coagulation cascade, the common pathway. This cascade consists of intrinsic, extrinsic, and common pathways. In the coagulation cascade, the prothrombinase complex is formed from factor V with the contribution of factor X. Such a complex helps in the development of the blood clot, leading to the stopping of bleeding [15,16]. Then, coagulation was initiated either on the intrinsic pathway, which is revealed by Activated Partial Thromboplastin Time (aPTT), or on the extrinsic pathway, revealed by Prothrombin Time (PT), or by directly activating factor X [20]. Generally, the coagulation process involves clotting factors being activated through serine proteases' action. Some of these clotting factors can bind to form complexes, which can act as serine proteases, in turn activating more downstream clotting factors. Finally, the coagulation cascade produces a fibrin clot, which contributes to hemostasis as a normal body

function for producing a clot as a response to injury [15,16]. Platelets play a key role in the hemostasis process, which involves mechanisms of adhesion, activation, and aggregation of platelets as the primary hemostasis, as well as the formation of a fibrin clot as the end product of the coagulation process, which is initiated by the damaged endothelium [10,21]. The coagulation process results in an imbalance that results in thrombosis or hemorrhage, which refers to a pathological condition where a blood clot can be formed where it was not required, so this can block the passage of blood flow [15,16].

The blood coagulation mechanism involves the action of activated factor X on prothrombin to produce thrombin. This can promote the activation and aggregation of platelets as well as the activation of factors V, VIII, and XI, forming a positive feedback loop that emphasizes the need for a counter-regulatory mechanism. Antithrombin, tissue factor pathway inhibitors, thrombomodulin, protein C (a vitamin-K dependent protein synthesized by the liver), and protein S are all part of this [10,21]. Once the binding of thrombin to protein C is established, protein C is activated quickly by thrombin. Once activated, protein C can bind to protein S, forming a complex that prohibits activated factor V and factor VIII, leading to a decrease in the generation of thrombin [10,21]. Therefore, the normal function of factor V can contribute to appropriate homeostasis. Disorders in factor V may lead to an increase in bleeding or the formation of thrombosis [15,16]. The G1691A mutation in the factor V gene gives rise to Factor V Leiden (FVL). Such mutation refers to the change of arginine to glutamine at the site (Arg506Gln) [16,22]. This gene is located on chromosome 1q23 [16,18,23]. It is an inherited genetic disorder that causes an increased risk of thrombosis throughout life. It is inherited as an autosomal dominant trait [24]. In individuals with FVL, the coagulation process is turned off more slowly than in individuals with normal factor V [17]. The presence of FVL can result in an Activated Protein C (APC) resistance condition. It is also associated with thromboembolic disorders [16,25]. This mutation can slow the modification exerted by APC to convert factor V to activated factor V, which in turn decreases the anticoagulation activity of APC and leads to coagulation [16,25]. The decrease in APC activity or the increase in APC resistance, can contribute to the increase in the coagulation process, which leads to the thromboembolic phenotype related to FVL [16,25]. So, diseases such as deep vein thrombosis are more likely to be associated with FVL [16,25]. Pathologically, FVL is the most prevalent inherited form of inherited thrombophilia [26]. Thrombophilia or hypercoagulability can describe the disorders associated with the hereditary and acquired conditions characterized by the development of thrombi in the arteries, veins, or both [27]. The coagulation system plays a critical role in liver injury due to the chronic HCV infection, and the presence of FVL significantly increases the risk for liver disease progression [28].

Materials and Methods

Study population

This is a cross-sectional study conducted on preserved samples of Egyptian subjects. The blood samples of a total of fifty-four subjects were enrolled in this study. Subjects were divided into two groups: a group of thirty-three patients chronically infected with Hepatitis C Virus (HCV) and another group of twenty-one control individuals. Those adult patients with Chronic Liver Disease (CLD) of both sexes and different ages who were confirmed to have HCV infection and were free of Hepatitis B Virus (HBV) infection met the inclusion criteria for HCV chronically infected patients, whereas those adult healthy individuals of both sexes and various ages who were free of any other aetiology met the inclusion criteria for controls. Samples from HCV chronically infected patients were distinguished by the presence of both HCV antibody (HCV-Ab) and HCV-RNA, as well as the absence of both Hepatitis B Surface Antigen (HBs-Ag) and human immunodeficiency virus antibody (HIV-Ab), whereas the samples from controls were characterized by the absence of HCV-Ab, HIV-Ab, and HBs-Ag. Control subjects and patients had previously undergone routine investigations for coagulation markers, including Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPPT), and Platelet Count (PLC). HCV chronically infected patients with thrombocytopenia were identified by PLC < 150 thousands/cmm. Coagulation-regulating (anticoagulants) parameters including protein C and protein S antigens assays, were determined by an Enzyme-Linked Immunosorbent Assay (ELISA).

DNA was extracted from EDTA blood samples and investigated for the presence of the G1691A mutation in the factor V gene (Factor V Leiden [FVL]) among the entire study cohort. The statistical data analysis was performed using SPSS version 20. The study was conducted according to the World Medical Association's Declaration of Helsinki guidelines.

Detection of Factor V Leiden (FVL)

Extraction of DNA: Genomic DNA was extracted from whole blood (blood samples collected on EDTA-coated tubes) according to the manufacturer's instructions of the Qiagen DNA extraction kit (Qiagen, Santa Clarita, CA). After digestion with proteinase K, QIAamp Mini spin columns were used for subsequent salting out of cellular proteins by centrifugation steps at 8000 rpm, which allowed optimal binding of the DNA to the QIAamp membrane followed by DNA elution and final storage at -20˚C until required.

Factor V Leiden (FVL) assay: Regarding the extracted DNA, real-time Polymerase Chain Reaction (PCR) amplification was applied, especially the quantitative real-time PCR (qPCR) method using fluorescent probes according to Higuchi R, **et al.** [29]. The genotyping of samples was carried out using real-time hydrolysis probes. Two labelled probes corresponding to the two genotypes under investigation were contained in the genotyping primer/probe mixture [30,31]. The probes involved were specified as the following: one specific to the wild-type G1691 allele, labelled with fluorescein amidites (FAM-labeled), and the other specific to the mutant A1691 allele, labelled with Victoria dye [2′-chloro-7′-phenyl-1,4-dichloro-6-carboxy-fluorescein] (VIC-labeled) [30,31]. The reaction mixture was placed in a 96-well PCR plate employing a 7500 Fast Real-Time PCR system (Applied Biosystems, CA, USA). The overall PCR run involved three steps: first, a pre-read run to record the background signal (at 60˚C for 2 minutes); second, an amplification run, which started with one hold (at 50˚C for 2 minutes), followed by another hold (at 95˚C for 10 minutes), and proceeded with 40 cycles at alternative temperatures: 95˚C for 15 seconds, causing denaturation, and 60˚C for 1 minute, causing annealing and extension; finally, a post-read run to subtract amplified signals from the background signal at 60˚C for 2 minutes [30]. During the 40 cycles of alternating temperature qPCR amplification of the target DNA, the probes competed for binding across the variant region. The probe, which was 100% homologous to the DNA binding site, specifically bonded, producing a fluorescent signal by proceeding with PCR. Then, the instrument software could analyze the results to determine the presence of one of the following patterns in each DNA specimen: wild-type alleles (normal), mutant alleles (homozygous FVL), or both wild-type and mutant alleles (heterozygous FVL) [31].

Determination of protein C and protein S antigens

The protein C antigen was determined quantitatively from citrated blood plasma according to the manufacturer's instructions for the REAADS® protein C antigen test kit (Corgenix, Inc., USA). It referred to a sandwich type of Enzyme-Linked Immunosorbent Assay (ELISA). In such an assay, an antibody specific for capturing human protein C is coated onto 96-microwell polystyrene plates. This permitted any available protein C to bind to the antihuman protein S antibody on the surface of the microwell when diluted patient plasma was incubated in such wells. After the washing step of the plates to remove unbound proteins, quantitative determination for bound protein S was performed using Horseradish Peroxidase (HRP)-conjugated anti-human protein S detection antibody. After incubation and washing to remove unbound conjugate, the chromogenic substrate containing Tetramethylbenzidine (TMB) and Hydrogen Peroxide (H_2O_2) was added to develop a colored reaction. Finally, the color intensity was measured in Optical Density (OD) units at 450 nm with a spectrophotometer using a multi-well plate reader (Tecan; Sunrise, Austria GmbH), and protein C relative percent concentrations in patient plasma were determined against a curve prepared from the reference plasma provided with the kit.

The protein S antigen was determined quantitatively by ELISA from citrated blood plasma according to the manufacturer's instructions for the REAADS® protein S antigen test kit (Corgenix, Inc., USA). The same as mentioned previously in the determination of protein C antigen, except that an antibody specific for capturing human protein S antigen was coated onto 96- microwell polystyrene plates rather than protein C antigen.

Results

Demographic information and coagulation parameters for the study cohort

The study cohort included groups 1 (controls, *n* = 21 [18 males and 3 females]) and 2 (HCV chronically infected patients, *n* = 33 [25 males and 8 females]). The following data represent demographic information, including gender and age parameters, and coagulation parameters, including PLC, PT, and aPTT. Also, such data represent coagulation-regulating (anticoagulants) parameters ([protein C antigen and protein S antigen]). These data are reported in table 1. For parameters such as age, PLC, PT, and protein C, a highly significant difference ($p < 0.001$) is observed between group 1 (controls) and group 2 (HCV chronically infected patients). Likewise, a highly significant change ($p = 0.006$) was obtained for the protein S antigen.

Incidence of Factor V Leiden (FVL) in the study cohort

The incidence of Factor V Leiden (FVL) [factor V gene G1691A mutation] among group 1 (controls) and group 2 (HCV chronically infected patients) is reported in table 2. The absence of FVL is indicated as a negative, while the presence of such a mutation is noted as a positive; all positive cases were heterozygous, and none of the tested samples were homozygous. The positive cases of FVL are recorded in 6 of 21 (28.67%) of group 1 (controls, *n* = 21), while it is recorded in 8 of 33 (24.24%) of group 2 (HCV chronically infected patients, *n* = 33). A non-significant change was observed for the incidence of FVL between group 1 and group 2.

Influence of Factor V Leiden (FVL) Incidence on Coagulation Parameters in the Study Cohort

Effect of Factor V Leiden (FVL) incidence in controls: Furthermore, group 1 (controls) is subdivided into 2 subgroups according to the results of the incidence of the FVL. Subgroup 1 is characterized by negative results of the FVL (*n* = 15), and subgroup 2 is distinguished by positive results of the FVL (*n* = 6). The comparison of such subgroups regarding the coagulation parameters, including PLC, PT, and aPTT, as well as anticoagulants (protein C and protein S antigens), is reported in table 3. Non-significant changes are shown for all measured parameters between subgroup 1 (negative FVL) and subgroup 2 (positive FVL) in controls.

Effect of Factor V Leiden (FVL) incidence in HCV chronically infected patients: Similarly, group 2 (HCV chronically infected patients) is subdivided into 2 subgroups according to the results for the incidence of FVL. Subgroup 3 is distinguished by the

Table 1: Demographic data and coagulation parameters among chronic HCV patients and controls.

	Subjects of Study		
Parameter	Group 1 (Controls)	Group 2 (HCV Chronically Infected Patients)	*p*-value
Gender (M/F)	18/3	25/8	0.376
Age (years)	27.952 ± 6.644	52.710 ± 6.881	< 0.001*
PLC (thousands/cmm)	240.000 ± 86.208	100.257 ± 67.179	< 0.001*
Patient PT (seconds)	12.669 ± 1.774	18.048 ± 4.716	< 0.001*
Control PT (seconds)	12.012 ± 0.950	11.768 ± 0.947	0.364
PC (%)	91.500 ± 14.270	51.768 ± 20.435	< 0.001*
INR	1.070 ± 0.158	1.794 ± 0.614	< 0.001*
aPTT (seconds)	32.948 ± 13.018	37.618 ± 8.744	0.214
Protein C Antigen (%)	104.009 ± 16.299	67.242 ± 37.292	< 0.001*
Protein S Antigen (%)	103.968 ± 10.791	79.650 ± 36.665	0.006*

Where: M: Male; F: Female; PLC: Platelets Count; PT: Prothrombin Time; PC: Prothrombin Concentration; INR: International Normalized Ratio; aPPT: Activated Partial Thromboplastin Time; and * = significant value. Normal ranges are as follows: PLC, from 150,000 to 450,000 platelets per microliter of blood, or 150 to 450 thousands/cmm; the PT test (from 11 to 13.5 seconds for PT; from 70 to 120 for PC; from 0.8 to 1.1 for INR); aPPT, from 30 to 40 seconds; protein C antigen from 72 to 160%; and protein S antigen from 60 to 150%. The chi-square test was applied to analyze the data for the gender parameter, whereas the T-test was used to analyze the data for all of the other parameters, which were expressed as means and standard deviation values (M ± SD). The significant changes were determined at $p \leq 0.05$.

Table 2: Incidence of Factor V Leiden (FVL) among HCV chronically infected patients and controls.

Factor V Leiden	Subjects of Study				*p*-value
	Group 1 (Controls)		Group 2 (HCV Chronically Infected Patients)		
	N	%	N	%	
Negative	15	71.43	25	75.76	0.723
Positive	6	28.57	8	24.24	
Total	21	100	33	100	

Where: N = Number. The chi-Square test was applied to analyze the data. The significant change was determined at $p \leq 0.05$.

Table 3: Comparison of coagulation parameters among subgroups of controls based on the incidence of FVL.

	Parameter	Subgroup 1 (Negative) Mean SD	Subgroup 2 (Positive) Mean SD	*p*-value
Group 1 (Controls)	PLC (thousands/cmm)	235.571 ± 100.413	249.833 ± 40.192	0.743
	Patient PT (seconds)	12.373 ± 1.153	12.033 ± 1.490	0.608
	Control PT (seconds)	12.009 ± 0.984	11.683 ± 0.968	0.522
	PC (%)	94.318 ± 10.371	93.817 ± 8.275	0.920
	INR	1.037 ± 0.069	1.037 ± 0.066	0.986
	aPTT (seconds)	37.400 ± 19.324	28.067 ± 1.815	0.265
	Protein C Antigen (%)	102.846 ± 15.061	115.317 ± 11.450	0.091
	Protein S Antigen (%)	102.585 ± 11.072	106.850 ± 6.823	0.400

Where: FVL: Factor V Leiden; PLC: Platelets Count; PT: Prothrombin Time; PC: Prothrombin Concentration; INR: International-Normalized Ratio; aPPT: Activated Partial Thromboplastin Time; SD: Standard Deviation. The T- test was applied to analyze the data. The significant changes were determined at $p \leq 0.05$.

negative results of the FVL (n = 25), and subgroup 4 is characterized by the positive results of the FVL (n = 6). The comparison of such subgroups regarding the HCV-RNA and coagulation parameters, including PLC, PT, and aPTT, as well as anticoagulants (protein C and protein S antigens), is reported in table 4. Unfortunately, aPTT data was available for just one patient in subgroup 4. Non-significant changes are shown for all measured parameters between subgroup 3 (negative FVL) and subgroup 4 (positive FVL) in HCV chronically infected patients.

Discussion

Several studies support the evidence that the Hepatitis C Virus (HCV) can activate hemostasis, mainly due to HCV-induced endothelial damage or activation [10]. In HCV infection, the damaged endothelium is the major source of activated tissue factor, which is the chief requirement for the initiation of the coagulation cascade. This endothelial damage may be exerted through the binding of HCV viral RNA to Toll-Like Receptor (TLR)-3 within endothelial cells, resulting in inflammation [10,32]. Besides, cryoglobulinemia-associated HCV-induced endothelial damage is another endothelial damage process in which a type 3 hypersensitivity reaction has proceeded with the formation of antibodies as immune complexes directed against HCV viral RNA. Such immune complexes in turn can lead to the activation of endothelial cells [10,33]. Consequentially, the inflammation generated can promote the expression of both Tumor Necrosis Factor (TNF)-α and TNF receptor 2. The expressed TNF-α is an inducer of tissue factor expression, thus exerting a prothrombotic effect [10,34]. This can downregulate the expression of thrombomodulin as well [10,35]. In patients with chronic HCV infection, tissue factor is present in large amounts of microparticles [10,36], which may contribute to enhanced coagulation [10]. In the current study, HCV chronically infected patients showed a significant increase in Prothrombin Time (PT) as a coagulation marker. This result is in agreement with Leticia OI, et al. [4], who studied the effect of HCV infection on PT and Activated Partial

Table 4: Comparison of HCV-RNA and coagulation parameters among subgroups of HCV chronically infected patients based on the incidence of FVL.

		FVL		
	Parameter	**Subgroup 3 (Negative) Mean SD**	**Subgroup 4 (positive) Mean SD**	***p*-value**
Group 2 (HCV chronically infected patients)	HCV-RNA (IU/ml)	160793.261 ± 184804.386	532012.429 ± 868471.120	0.68
	PLC (thousands/cmm)	111.238 ± 110.967	79.250 ± 18.006	0.315
	Patient PT (seconds)	15.250 ± 4.296	20.050 ± 6.152	0.448
	Control PT (seconds)	11.425 ± 0.967	12.600 ± 0.424	0.405
	PC (%)	66.850 ± 33.916	45.500 ± 16.263	0.057
	INR	1.440 ± 0.389	1.805 ± 0.742	0.274
	aPTT (seconds)	32.920 ± 3.616	36.600 ± 0.000	0.331
	Protein C Antigen (%)	100.100 ± 44.011	87.800 ± 35.497	0.192
	Protein S Antigen (%)	85.600 ± 39.259	76.550 ± 62.013	0.464

Where: FVL: Factor V Leiden; PLC: Platelets Count; PT: Prothrombin Time; PC: Prothrombin Concentration; INR: International-Normalized Ratio; aPPT: Activated Partial Thromboplastin Time, SD: Standard Deviation. The T- test was applied to analyze the data. The significant changes were determined at $p \leq 0.05$.

Thromboplastin Time (aPTT) in a group of 32 chronic HCV patients. They reported a significant increase in PT in chronic hepatitis C patients when compared to controls. Moreover, the present study showed a biological increase in aPPT in HCV chronically infected patients when compared to controls, but this change did not reach a significant value ($p = 0.214$). By contrast, this result disagrees with the former study conducted by Leticia OI, **et al.** [4], who referred to a significant increase in aPPT in chronic hepatitis C patients when compared to controls. Generally, the prolongation of PT and aPTT as a consequence of viral hepatitis with thrombocytopenia was explained due to the loss of integrity of both the extrinsic and common pathways concerned with the coagulation process [4,37].

Thrombocytopenia is more noticeable in chronic hepatitis C patients than in patients with other types of Chronic Liver Disease (CLD) at a comparable stage of severity [10,38]. In the present study, the marked thrombocytopenia in HCV chronically infected patients may be explained by the impairment in hepatic synthesis of thrombopoietin, which is the major physiological regulator of platelet production in the liver [4,39]. Some reports highlighted the decrease in thrombopoietin levels concerning the progression of liver disease, but others referred to its normal level in chronic hepatitis C patients compared with controls [10,40,41]. In patients with chronic HCV infection, the chemokine [C-X-C motif] ligand (CXCL12) is up-regulated in the endothelium of the blood vessel; this endothelium forms active inflammatory foci. Consequently, CXCL12 binds to chemokine [C-X-C motif] receptor 4 (CXCR4), which is overexpressed by liver-infiltrating lymphocytes [8,42]. Indeed, CXCL12 is a potent promoter of platelet adhesion and aggregation [10,43]. Moreover, increased levels of platelet activation and aggregation are found among patients with chronic HCV infection [10,44,45]. These could explain some of the remarkable platelet count decline features observed in chronic HCV infection [10,38].

The current study refers to a significant decrease in protein S and protein C antigens as coagulation-regulating (anticoagulants) parameters in HCV chronically infected patients compared to controls. This finding falls in agreement with that reported by Gürsoy S, et al. [46], who recorded the significant decrease of protein S and protein C in a cohort of 35 cirrhotic CLD patients compared to controls. Further, this finding agrees with the last report by Saray A, et al. [47], who declared that protein C deficiency is reported in an early stage of CLD. Indeed, in CLD, especially in patients with cirrhosis, there is reduced synthesis of both procoagulant and anticoagulant proteins and decreased endogenous anticoagulants such as protein C, protein S, and antithrombin [48-50]. Moreover, the results in the current study are similar to those reported by Singhal A, et al. [51], who referred to the deficiency of anticoagulant proteins

in an early phase of CLD, even before prolongation of PT. Similarly, Saray A, et al. [47] reported that protein C deficiency is detected in the early stages of liver fibrosis. The severity of such a deficiency is proportional to the extent of liver fibrosis progression. Actually, protein C can play a key role in linking hypercoagulability with fibrogenesis in CLD. Furthermore, in the present study, protein C and protein S antigens levels were significantly lower ($p < 0.001$) in HCV chronically infected patients than controls. This observation referred to the actual risk for PVT development in those patients, in accordance with Chien Hung HC, et al. [52], who identified protein C and protein S deficiency as risk factors for PVT development in cirrhotic patients waiting for transplantation based on univariant data analysis. On the other hand, multivariate analysis for the same results referred to protein S deficiency as the only independent risk factor for PVT development in such patients.

Factor V Leiden (FVL) [factor V gene G1691A mutation] is highly prevalent in the general population [11]. FVL represents a risk factor for thrombosis, thrombophilic genes in the form of FVL are responsible for 40-50% of cases of thrombosis [26,53]. In the present study, the incidence of FVL with its heterozygous pattern was reported as 8/33 (24%) in the group of HCV chronically infected patients. This result is different to some extent to that previously reported by Wright M, et al. [28], who studied a cohort of 352 patients with chronic HCV infection for the frequency of FVL. They only reported 16 cases of heterozygous FVL, which means that one copy of the defective gene was inherited, and the rest of the cases (336 subjects) were reported with wild-type factor V for the entire study cohort. In no case of such a study has the homozygous FVL, which contains inherited two copies of the defective gene, been recorded. They concluded that FVL increases the risk for fibrosis progression in patients with chronic HCV infection. Further, the data in the current study are similar to some extent to those obtained by Arsov T, **et al.** [54], who reported FVL in 21.1% of patients with thromboembolic disease. By contrast, FVL was recorded at 5.5% in the control group of the later study, compared to 28% in the present study. In fact, the incidence of FVL was found to be high in populations of Caucasian origin, but it mostly was absent in non-Caucasians. It was also recorded in populations in North Africa and the Middle East [55]. In the present study, all positive cases for FVL were of a heterozygous pattern for both groups of controls and HCV chronically infected patients. Additionally, significant changes were not achieved for coagulation or coagulation-regulating (anticoagulants) parameters due to the incidence of FVL in each of the two groups separately. This may be explained by the fact that most subjects who have heterozygous cases of FVL still have some functional factor V that is able to initiate Activated Protein C activity (APC) towards anticoagulation [16,22]. Individuals with heterozygous FVL never appear to develop blood clots, whereas others with homozygous FVL have a 25- to 50-fold increased risk of developing blood clots [17].

Conclusion

Hepatitis C Virus (HCV) infection that is chronic and accompanied by thrombocytopenia is associated with coagulation disturbances due to significant deterioration changes in Prothrombin Time (PT), which is a coagulation parameter, as well as protein C and protein S antigens, which are coagulation-regulating (anticoagulants) parameters. The incidence of Factor V Leiden (FVL), with its heterozygous pattern, does not differ significantly between chronic hepatitis C patients suffering from thrombocytopenia and HCV-noninfected healthy individuals. It also does not appear to be associated with an increase in coagulation disturbances in such patients.

Recommendations

Further cross-sectional studies with a larger cohort size referring to different stages of Chronic Liver Disease (CLD) are still needed to support the significant role of Hepatitis C Virus (HCV) infection in the worsening of coagulation disturbances and to evaluate a more detailed and accurate role of Factor V Leiden (FVL) in the coagulation system in each of CLD stages. Additionally, longitudinal studies are recommended to assess the clinical implications of FVL at time intervals in each of the CLD stages separately. The same is recommended for Hepatitis B Virus (HBV) infected patients and HBV/HCV coinfected patients, especially those with occult HBV infection.

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