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ORIGINAL ARTICLE

Does human leukocyte antigen influence the risk of development and type of vasculitis in Egyptian patients with chronic hepatitis C virus infection?

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KEYWORDS

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Abstract *Aim of the work:* The aim was to investigate the role of the human leukocyte antigen (HLA) class II alleles in the development of hepatitis C virus (HCV)-related vasculitis.

Patients and methods: Fifty HCV related vasculitis patients (32 females) with a mean age of 46.78 ± 10.17 years (range 23–74 years) and a control group including 30 age and sex matched HCV infected patients without any extra-hepatic autoimmune manifestations were recruited in this study. Patients with vasculitis were classified into small and medium sized vessel vasculitis according to the type of clinical manifestations. Assessment of HLA class II alleles in leukocytes of peripheral blood of all patients and controls was performed at allele level 4 digit high resolution for DRB1 region.

Results: Seventeen patients had medium sized vessel vasculitis (group A) and 33 patients had small sized vessel vasculitis (group B). The development of HCV-related medium sized vessel vasculitis is associated with HLADRB1*3 of the 1st allele and HLADRB1*1301 of the 2nd allele-suballele, and the development of small sized vessel vasculitis is associated with HLADRB1*701 of the 1st allele-suballele.

Conclusion: The results suggest that the development and the type of HCV-related vasculitis can be affected by the host genetic factors.

1. Introduction

Hepatitis C virus (HCV) is a globally prevalent pathogen and a leading cause of death and morbidity [1]. Globally the prevalence and the number of people with anti-HCV has increased

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from 2.3% to 2.8% and >122 million to >185 million between 1990 and 2005 [2]. In Egypt, the epidemiological situation differs from western countries. HCV prevalence among the general population of Egypt is documented to be very high. The 2008- Egypt Demographic and Health Survey (EDHS) reported the HCV prevalence to be 14.7% among a nationally representative sample of 11,126 Egyptians aged 15–59 years [3]. This prevalence appears to increase dramatically with age with the highest rates observed among populations older than 40 years [4].

Chronic HCV infection is associated with multiple extrahepatic manifestations (EHM) affecting various organs in the body. Approximately 40% to 75% of patients with chronic HCV infection experience at least one clinical EHM during the course of the HCV infection [5]. Many of these EHM are autoimmune in nature due to the lymphotropism of the virus. Mixed Cryoglobulinemia (MC) is the most documented disorder among them. The prevalence of HCV-infected patients with coexisting circulating MC (reversibly cold precipitable immunoglobulins) ranging from less than 10% to greater than 50%; however, overt vasculitis manifestations are seen in only 2–3% of these patients [6].

Gene polymorphisms of various cytokines and biomarkers have been implicated in the susceptibility of rheumatic diseases as rheumatoid arthritis (RA) [7–11] and systemic lupus erythematosus (SLE) [11–13] in Egyptian patients, some of which were related to the degree of activity. Interestingly, other gene polymorphisms were not found to contribute to the development of RA in an Argentinean cohort [14], to the risk of arthritis in Turkish psoriatic patients [15] or to the development of SLE in Egyptian patients [16,17].

The human leukocyte antigen (HLA) genomic region at chromosomal position 6p21 encodes many genes, which are important for the immune system [18]. Previous studies have shown that polymorphisms in the classical class-I and -II regions were associated with chronic hepatitis C [19,20]. In addition, expression of HLA class I and II antigens has been shown to affect the susceptibility and persistence of viral infection, and influence the disease progression in patients with viral hepatitis B or C [21].

The aim of the study was to investigate the HLA class II polymorphisms among chronic HCV infected patients with and without vasculitis, and to study its relation to the clinical manifestations throughout the course of the disease.

2. Patients and methods

This cross sectional study included 50 consecutive patients with HCV vasculitis (32 women with male to female ratio 0.56, 23–74 year old with mean age 46.78 ± 10.17 years) and 30 consecutive patients with HCV with no extrahepatic manifestations as controls (14 women with male to female ratio 1.14, 31–60 year old with mean age 49.2 ± 8.02 years). They were recruited from the clinics of Rheumatology and Endemic Medicine, Kasr Al Ainy hospital, Cairo University, over one year starting from March 2012. Patients were identified as HCV-related vasculitis using the preliminary classification criteria for cryoglobulinemic vasculitis proposed by *de Vita* et al. [22], which are also useful for classifying cryoglobulinemic vasculitis in patients with negative cryoglobulins by initial laboratory testing. The protocol of the research has been approved by the institutional review committee of Kasr Al Ainy Hospital, Cairo University, and it conforms to the provisions of the World Medical Association's declaration of Helsinki. Informed consent was obtained from all patients for all investigations and patient anonymity was preserved.

HCV infection was confirmed in all patients by HCV quantitation by real time Polymerase chain reaction (PCR).

The data recorded for every patient was as follows: the presence of rheumatologic manifestations, including arthralgia; arthritis; myalgia; sicca manifestations; skin manifestations (Raynaud's phenomenon, purpura, distal ulcers, gangrene); neurological (peripheral and/or central nervous system (CNS) manifestations, including impaired cognitive function and abnormal findings on magnetic resonance imaging of the brain); cardiac; pulmonary; renal; gastrointestinal (peptic ulcers, mesenteric microaneurysms); hepatic; or ophthalmologic involvement. The laboratory data included the rheumatoid factor (RF), antinuclear antibodies (ANA), and anti-double stranded nucleic acid (anti-DNA) antibodies, if ANA was positive. Antineutrophil cytoplasmic antibodies (ANCA), cryoglobulins, and complement components C3 and C4 were done for all patients.

Patients were divided into two groups according to the revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides [23] into patients with medium-vessel vasculitis and with small-vessel vasculitis.

Exclusion criteria included: (1) Patients with coexistent Hepatitis B virus (HBV); by performing HB surface Antigen (HBsAg) and Hb core Antibody (HBcAb) using Enzyme linked immunosorbent essay (ELISA) technique. (2) Human immunodeficiency virus (HIV); by performing HIV I and HIV II antibody testing by ELISA. (3) Co-existence of autoimmune, proliferative, or other infectious disease except HCV infection. (4) The presence of other causes of vasculitis.

Assessment of HLA class II alleles in leukocytes of peripheral blood was performed at allele level 4 digit high resolution for DRB1 region using LABType® SSO Typing Test, which applies Luminex™ technology for DNA typing as recommended by Luminex Corporation [24]. Measurements were achieved using Luminex 100™ device at Clinilab Company (Egypt). The overall method of HLA genotyping included PCR amplification, denaturation, neutralization, hybridization, streptavidine-phycoerythrine reaction and measurements. Upon HLA typing to the patients, and controls, we detected the 1st and 2nd alleles, in addition to the suballeles of the 1st and 2nd alleles, from which we have selected the 1st 2 numbers to display.

Statistical methods: All patients' data were tabulated, and processed using Statistical package for sciences and society (SPSS 11.0) (SPSS Inc., Chicago, USA). Quantitative variables were expressed by mean and standard deviation (SD) and then compared using Student's t-test for two independent variables. We expressed Qualitative variables by frequency and percentage and compared them using chi-square test or Fischer's exact test. Odds ratio (OR) and their 95% confidence interval (CI) were calculated for the presence of each HLA class II alleles in patients and controls. P value was considered significant if equal or less than 0.05.

3. Results

Our HCV-vasculitis group was formed of 32/50 (64%) females, with male to female ratio 0.56. Their ages ranged from 23 to 74 years with a mean of 46.78 ± 10.17 years, with male to female ratio 0.56. The duration of HCV diagnosis ranged from 2 months to 14 years with mean 3.04 ± 3.28 years, whereas, the duration of vasculitis ranged from 3 months to 12 years with a mean of 2.61 ± 3.01 years. The clinical characteristics are shown in Table 1. All HCV-vasculitis patients were negative for anti-DNA and ANCA. On the other hand, among the 30 control subjects, 14 were females (46.7%), with male to female ratio 1.14. Their ages ranged from 31 to 60 years with a mean of 49.2 ± 8.02 years. The duration of HCV in this group ranged from 5 months to 15 years with a mean of 4.21 ± 3.98 years. The difference between patients and controls concerning age, sex and disease duration was non-significant.

Patients were classified into two groups according to the size of the vessel involved: group A; patients with medium vessel vasculitis (17 patients), and group B; patients with small-vessel vasculitis (33 patients).

On analyzing each of the 1st and the 2nd HLA class II alleles, and comparing HCV-controls to all HCV-vasculitis patients, group A and group B; DRB1*3 on the 1st allele was detected significantly more frequently in group A compared to the HCV-controls (p = 0.027), and HLA DRB1*4 on the 1st allele was detected significantly more frequently in the HCV-control group (p = 0.05) (Table 2).

HLA DRB1*15 on the 2nd allele was detected significantly more in the HCV-control group (p = 0.048) compared to all HCV-vasculitis patients (Table 3).

We have chosen from the vast sequence of suballeles the 1stsuballele (the 1st 2 digits). The 1st allele-suballele DRB1*701 was significantly more frequently detected in group B when compared to the HCV-control group (p = 0.05). The most frequent 1st allele-suballeles detected in patient and control population are shown in Table 4.

Table 1 Clinical and laboratory characteristics of the HCV-vasculitis group.

Characteristic *n* (%)	HCV-vasculitis patients (*n* = 50)	
Fever	7	(14)
Fatigue	10	(20)
Fibromyalgia	6	(12)
Arthralgia	26	(52)
Arthritis	11	(22)
Purpura	28	(56)
Skin ulcers	6	(12)
Digital gangrene	9	(18)
Livedo reticularis	1	(2)
Raynaud's phenomenon	5	(10)
Cranial nerve involvement	4	(8)
Peripheral neuropathy	18	(36)
Low C4	17	(34)
Low C3	12	(24)
Positive RF	43	(86)
Positive cryoglobulins	15	(30)
Positive ANA	13	(26)

HCV: hepatitis C virus, C: complement, RF: rheumatoid factor, ANA: antinuclear antibody.

Similarly, we compared the frequency of the 1stsuballele (1st 2 digits) of the 2nd allele among HCV patient with and without vasculitis. The 2nd allele-suballele DRB1*1301 was significantly detected more in group A when compared to the HCV-control group (p = 0.05) (Table 5).

No significant relation was found between the distribution of the different alleles and the clinical manifestations of patients with vasculitis except for the prevalence of mononeuritis multiplex, which was present in 10 patients (9 of them were in group A) and none of them had DRB1*4 on the 1st allele (x^2 = 0.06). They had different alleles including; DR B1*1,*3,*7,*10,*11,*13,*14. DRB1*4 was present in 11 HCV vasculitis patients, none of them had mononeuritis multiplex.

4. Discussion

Although the pathogenic basis for the development of HCV related vasculitis is not fully understood, it is clear that it is complex and multifactorial. Many factors have been postulated for the development of MC, such as the virus genotype. For example, the prevalence of MC in Egyptian patients infected with HCV-genotype 4 was 14%, and that was significantly lower than its prevalence in Japanese patients infected with genotype 1b (40%) [25].

Other studies suggest that not only the genotype of the virus affects the disease, but it is believed that HCV is able to trigger such a disorder only in the presence of genetic factors that are yet to be identified. The host's genetic background forms a complex relationship with HCV thus leading to development of the disease and perhaps leads to different patterns of presentation. Some of these genetic factors include HLA polymorphisms (as HLA-A9, HLA-B8-DR3, HLA-DR3, and HLA-DR5-DQ3) [26–29].

We aimed in this work to study HLA class II alleles in leukocytes of peripheral blood, which are the commonest alleles found in those patients, and to study its relation to the clinical manifestations throughout the course of the disease. We studied it at allele level 4 digit high resolution for DRB1 region.

On comparing the HCV control group to the group of patients with HCV vasculitis, we failed to correlate the HCV vasculitis to a specific gene in our patients, but we found that HLADRB1*15 of the 2nd allele was statistically higher in the control group (p = 0.048, OR = 4.18, [95%CI1.14-15.4]). This can suggest it as a protective gene in our study population.

Two main forms of vasculitis have been identified in relation to the chronic HCV infection; the more common cryoglobulinemic vasculitis, which is a chronic small vessel vasculitis, and the less common polyarteritis nodosa like vasculitis, which is a medium vessel vasculitis occurs in the setting of the chronic HCV infection [30].

We subdivided the HCV vasculitis patients into 2 groups according to the type of the vessel involved (group A; medium size vessel and group B; small size vessel). HLADRB1*3 of the 1st allele was statistically significantly more frequent in group A when compared to the control group (p = 0.027, OR = 0.23, [95% CI 0.06–0.87]). HLADRB1*1301 of the 2nd allele-suballele was statistically significantly higher in group A when compared to the control group (p = 0.05, OR = 0.11, [95% CI 0.01–1.1), while HLADRB1*4 of the

Table 2 Comparison of the 1st allele prevalence between HCV-controls with the HCV-vasculitis patients, patients with medium-sized (group A) and small-sized (group B) vasculitis.

1st allele	HCV patients				HCV-vasculitis (vessel size)					
	Control (n = 30)	Vasculitis (n = 50)			Medium-sized (n = 17)			Small-sized (n = 33)		
	n (%)	n (%)	p	OR 95% (CI)	n (%)	p	OR 95% (CI)	n (%)	p	OR 95% (CI)
DRB1*1	4 (13.3)	5 (10)	0.72	1.39 (0.34–5.62)	1 (5.9)	0.64	2.46 (0.25–24.02)	4 (12.1)	1	1.12 (0.25–4.92)
DRB1*3	5 (16.7)	14 (28)	0.29	0.51 (0.16–1.61)	8 (47.1)	**0.027**	0.23 (0.06–0.87)	6 (18.2)	1	0.9 (0.24–3.32)
DRB1*4	9 (30)	11 (22)	0.6	1.52 (0.54–4.25)	1 (5.9)	**0.05**	6.86 (0.79–59.81)	10 (30.3)	1	0.99 (0.34–2.99)
DRB1*7	4 (13.3)	8 (16)	1	0.81 (0.22–2.95)	3 (17.6)	1	0.72 (0.14–3.67)	5 (15.2)	1	0.86 (0.21–3.56)
DRB1*13	2 (6.7)	7 (14)	0.47	0.44 (0.09–2.29)	2 (11.8)	0.61	0.54 (0.07–4.19)	5 (15.2)	0.43	0.4 (0.07–2.24)

Bold values are significant at $p \leqslant 0.05$, OR; Odds Ratio, CI; confidence interval.

Table 3 Comparison of the 2nd allele prevalence between HCV-controls with the HCV-vasculitis patients, patients with medium-sized (group A) and small-sized (group B) vasculitis.

2nd allele	HCV patients				HCV-vasculitis (vessel size)					
	Control (n = 30)	Vasculitis (n = 50)			Medium-sized (n = 17)			Small-sized (n = 33)		
	n (%)	n (%)	p	OR 95% (CI)	n (%)	P	OR 95% (CI)	n (%)	p	OR 95% (CI)
DRB1*4	4 (13.3)	10 (20)	0.55	0.62 (0.18–2.17)	4 (23.5)	0.44	0.5 (0.11–2.33)	6 (18.2)	0.74	0.69 (0.18–2.74)
DRB1*13	7 (23.3)	16 (32)	0.46	0.65 (0.23–1.82)	5 (29.4)	0.73	2.73 (0.19–2.8)	11 (33.3)	0.15	0.7 (0.31–1.57)
DRB1*14	5 (16.7)	10 (20)	0.78	0.8 (0.25–2.61)	4 (23.5)	0.7	0.65 (0.15–2.84)	6 (18.2)	0.42	0.61 (0.2–1.85)
DRB1*15	8 (26.6)	4 (8)	**0.048**	4.18 (1.14–15.4)	1 (5.9)	0.13	5.82 (0.66–51.28)	3 (9.1)	0.1	3.64 (0.87–15.29)

Bold values are significant at $p \leqslant 0.05$, OR: Odds Ratio, CI: confidence interval.

Table 4 Comparison of the 1st allele-suballele prevalence between HCV-controls with the HCV-vasculitis patients, patients with medium-sized (group A) and small-sized (group B) vasculitis.

1stallele-Suballele	HCV patients				HCV-vasculitis (vessel size)					
	Control (n = 30)	Vasculitis (n = 50)			Medium-sized (n = 17)			Small-sized (n = 33)		
	n (%)	n (%)	p	OR 95% (CI)	n (%)	P	OR 95% (CI)	n (%)	p	OR 95% (CI)
DRB1*102	5 (16.7)	4 (8)	0.28	2.3 (0.57–9.35)	1 (5.9)	0.4	3.2 (0.34–29.96)	3 (9.1)	0.46	2.0 (0.44–9.21)
DRB1*301	4 (13.3)	10 (20)	0.55	0.62 (0.18–2.17)	1 (5.9)	0.64	2.46 (0.25–24.02)	7 (21.2)	0.52	0.57 (0.15–2.19)
DRB1*402	4 (13.3)	7 (14)	1	0.95 (0.25–3.54)	1 (5.9)	0.64	2.46 (0.25–24.02)	6 (18.2)	0.74	0.69 (0.18–2.74)
DRB1*701	1 (3.3)	8 (16)	0.14	0.18 (0.02–1.53)	1 (5.9)	1	0.55 (0.03–9.43)	7 (21.2)	**0.05**	0.13 (0.02–1.11)

Bold values are significant at $p \leqslant 0.05$, OR: Odds Ratio, CI: confidence interval.

Table 5 Comparison of the 2nd allele-suballele prevalence between HCV-controls with the HCV-vasculitis patients, patients with medium-sized (group A) and small-sized (group B) vasculitis.

1stallele-Suballele	HCV patients				HCV-vasculitis (vessel size)					
	Control (n = 30)	Vasculitis (n = 50)			Medium-sized (n = 17)			Small-sized (n = 33)		
	n (%)	n (%)	p	OR 95% (CI)	n (%)	P	OR 95% (CI)	n (%)	p	OR 95% (CI)
DRB1*404	1 (3.3)	4 (8)	0.65	0.4 (0.04–3.73)	3 (17.6)	0.11	0.19 (0.02–1.68)	1 (3)	1	1.1 (0.07–18.46)
DRB1*1301	1 (3.3)	7 (14)	0.25	0.21 (0.03–1.81)	4 (23.5)	**0.05**	0.11 (0.01–1.1)	3 (9.1)	0.61	0.35 (0.03–3.51)
DRB1*1302	2 (6.7)	6 (12)	0.7	0.52 (0.1–2.78)	3 (17.6)	0.34	0.33 (0.05–2.23)	3 (9.1)	1	0.71 (0.11–4.6)
DRB1*1401	4 (13.3)	7 (14)	1	0.95 (0.25–3.54)	1 (5.9)	0.64	2.46 (0.25–24.02)	6 (18.2)	0.74	0.69 (0.18-2.74)

Bold values are significant at $p \leqslant 0.05$, OR: Odds Ratio, CI: confidence interval.

1st allele was statistically significantly higher in control group when compared to group A (p = 0.05, OR = 6.86, [95% CI 0.79–59.81]). Although the p value was significant, their CI was not précised.

HLADRB1*701 of the 1st allele-suballele was statistically significantly higher in group B when compared to control group (p = 0.05, OR = 0.13 [95% CI 0.02–1.11]). The CI was not précised, but still the p value was significant.

This is the first study to our knowledge that suggests a genetic basis for the different forms of the HCV associated vasculitis, either medium or small vessel affected presentations.

In conclusion, the results suggest that the development of HCV-related medium sized vessel vasculitis is associated with HLADRB1*3 of the 1st allele and HLADRB1*1301 of the 2nd allele-suballele, and the development of small sized vessel vasculitis is associated with HLADRB1*701 of the 1st allele-suballele. HLADRB1*15 of the 2nd allele may be protective against the development of vasculitis in HCV patients. HLADRB1*4 of the 1st allele was absent in patients with mononeuritis multiplex. A bigger study is recommended to verify these findings.

Conflict of interest

We have no conflict of interest to declare.

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