

Human leukocyte antigen among Egyptian patients with Hepatitis C virus vasculitis

Submitted for the partial fulfillment of the M.D of Rheumatology and
Rehabilitation

By

Basma Mohammed Medhat Ali Mohammed

M.B; M.Ch, MSc

Supervised by

Prof. Dr. Amira Abd El Sabour Shahin

Professor of Rheumatology and Rehabiliatation

Faculty of Medicine, Cairo University

Prof.Dr.Olfat Gamal Shaker

Professor of Biochemistry

Faculty of Medicine, Cairo University

Assistant Prof.Dr.Hanan El Sayed Ali Darweesh

Assistant Professor of Rheumatology and Rehabiliatation

Faculty of Medicine, Cairo University

Dr.Mohamed El Sayed Soliman

Lecturer of Tropical medicine

Faculty of Medicine, Cairo University

Faculty of Medicine

Cairo University

2014

Acknowledgment

I would like to express my deepest gratitude; first to Allah for giving me the will and strength to finish this work; then to my family, especially my dearest parents for their continuous support, help, and encouragement.

I would like to express my deepest appreciation to **Dr. Amira Shahin, professor of Rheumatology and Rehabilitation, Cairo University**, for her keen interest in the progress of this work. She was very generous with her time, her knowledge, and patience.

Many thanks to **Dr. Olfat Shaker, professor of Biochemistry, Cairo University**, for her great assistance in the detailed clinical part of this work.

Special thanks to **Dr. Hanan Darweesh, assistant professor of Rheumatology and Rehabilitation, Cairo University**, for her great patience in reading and revising manuscripts, and for her great help and valuable ideas throughout this work.

I would like to thank **Dr. Mohammed Soliman, lecturer of Tropical medicine, Cairo University**, for his support and guidance through the recruitment of patients from the Tropical department.

Finally, I thank my colleagues in our department for their help and sincere encouragement.

Abstract

Aim of the work:

The aim of this work was to study the different Human leukocyte antigen (HLA) alleles in patients with Hepatitis C virus (HCV) vasculitis and compare them to patients with HCV with no extrahepatic manifestations, in order to detect susceptibility to development of HCV.

Patients and methods:

Fifty consecutive HCV vasculitis patients seeking medical advice at the Rheumatology department of EL Kasr EL Ainy were included in this study. 30 HCV patients with no extrahepatic manifestations seeking medical advice at the Tropical outpatient clinic and the Tropical department were involved in this study. All patients were subjected to detailed history taking, clinical examination, and laboratory investigations. In addition to quantitative Polymerase chain reaction measurement and HLA genotyping. Abdominal ultrasonography was done to both cases and controls. Cryoglobulins and complement 3 and 4 were done to the HCV vasculitis group.

Results:

The group of 1st alleles more common in patients with HCV vasculitis were alleles DRB1*3, DRB1*4, DRB1*7, DRB1*10, DRB1*13, this group was statistically higher in the HCV control group ($p=0.002$, OR=5.250, [95% CI 1.854-14.869]). The group of 2nd alleles more common in patients with HCV vasculitis were alleles DRB1*1, DRB1*4, DRB1*8, DRB1*13, DRB1*14, and this group was also statistically higher than in the HCV control group ($p=0.022$, OR=3.500,

[95% CI 1.291-9.489]). On the other hand, the group of 1st alleles more common in the HCV control group were alleles DRB1*1, DRB1*9, DRB1*11, DRB1*14, and this group was statistically higher than in patients with HCV vasculitis (p=0.002, OR 0.19, [95% CI 0.67-0.539]). Similarly, the group of 2nd alleles more common in the HCV control group were alleles DRB1*3, DRB1*7, DRB1*11, DRB1*15, and this group was statistically higher than in patients with HCV vasculitis (p=0.01, OR=0.251, [95% CI 0.91-0.694])

DRB1*3 was the most common 1st allele in patients with HCV vasculitis, but the difference in frequency between the 2 groups did not reach statistical significance (p=0.053, OR=1.467, [95% CI 0.398-3.243]). DRB1*9 of the 1st allele and DRB1*15 of the 2nd allele were the most common alleles in the HCV control group with a statistical significance (p=0.002, OR=0.800, 95% CI 0.178-2.931 and p=0.005, OR=0.115, [95% CI 0.22-0.585] respectively)

Conclusion:

Human leukocyte antigen, could play a role in the pathogenesis and presentation of patients with HCV vasculitis, which increase the susceptibility of certain patients according to their genetic background and certain HLA polymorphisms to the development of vasculitis. On the other hand, other HLA alleles could be protective and decrease the incidence of development of vasculitis despite the infection with HCV.

Keywords: HCV vasculitis – Human Leukocyte Antigen

Table of contents

	Page
List of Abbreviations	I
List of Tables	III
List of Figures	VI
Introduction and aim of work	1
Review Of Literature	3
Chapter 1: Hepatitis C virus	
Chapter 2: Extrahepatic Manifestations of Hepatistis C virus	8
- Hepatitis C virus associated vasculitis	12
- Mixed Cryoglobulinemia	12
- Treatment of Mixed Cryoglobulinemia	25
- Therapy for Hepatitis C Virus infection	42
- Polyarteritis Nodosa	55
- Other Rheumatic manifestations with HCV	60
Chapter 3: Human Leukocyte Antigen	70
- The Human Leukocyte Antigen	70
- Human Leukocyte Antigen and Hepatitis C Virus	84
- Human Leukocyte Antigen and Rheumatic manifestations	91
Patients and Methods	113

Results	128
Discussion	171
References	193
Arabic Summary	1

List of Abbreviations

- ACPA: Anti-citrullinated peptide antibodies
- ALT: Alanine transaminase
- AS: Ankylosing spondylitis
- AST: Aspartate transaminase
- BVAS: Birmingham Vasculitis Activity Score
- C3: Complement 3
- C4: Complement 4
- CBC: Complete blood picture
- CG: Cryoglobulins
- CI: Confidence interval
- EHM: Extrahepatic manifestations
- ELISA: enzyme linked immunosorbent assay
- EMG: Electromyography
- ESR: Erythrocyte Sedimentation rate
- EULAR: European League Against Rheumatism
- GC: Glucocorticoids
- HCQ: Hydroxychloroquine
- HCV: Hepatitis C virus
- HLA: human leukocyte antigen
- HSP: Heat shock protein gene
- Mb: megabases
- MC: Mixed cryoglobulinemia
- MHC: Major histocompatibility complex
- MIC: MHC class I polypeptide related
- MTX: Methotrexate

- OR: Odds ratio
- P value: Probability value
- PCR: Polymerase chain reaction
- RA: Rheumatoid arthritis
- RF: Rheumatoid factor
- RM: Rheumatic manifestations
- SD: standard deviation
- SE: Shared epitope
- SLE: Systemic lupus erythematosus
- SS: Sjögren's syndrome
- SSZ: Sulfasalazine
- TAP: Transporter associated with antigen processing genes
- TNF: Tumor necrosis factor- α
- VDI: Vasculitis Damage index
- WBC: White Blood cells

List of Tables

Table		Page
1	Demographic data of cases and control	129
2	Routine laboratory investigations done to cases and controls	136
3	Various degrees of viremia	137
4	Mean levels of PCR done to cases and controls	138
5	Number of percentages with consumed complement and mean values	138
6	1 st alleles that showed higher frequency in patients with HCV vasculitis	145
7	2 nd alleles that were more common in patients with HCV vasculitis	146
8	1 st alleles more common in HCV control group	147
9	2 nd alleles more common in HCV control group	148
10	Group of 1 st alleles more common in patients with HCV vasculitis	149
11	Group of 2 nd alleles more common in patients with HCV vasculitis	150

12	Group of 1 st alleles more common in the HCV control group	151
13	Group of 2 nd alleles more common in the HCV control group	152
14	Group of 1 st allele's suballeles more common in the HCV vasculitis group	153
15	Group of 2 nd allele's suballeles more common in the patients with HCV vasculitis	154
16	Group of 1 st allele's suballeles more common in the control HCV group	155
17	Group of 2 nd allele's suballeles more common in the HCV control group	155
18	1 st alleles more common in patients with small vessel vasculitis	156
19	2 nd alleles more common in patients with small vessel vasculitis	157
20	1 st alleles more common in patients with medium vessel vasculitis	158
21	2 nd alleles more common in patients with HCV vasculitis	159
22	Group of 1 st alleles more common in patients with small vessel vasculitis	160
23	Group of 2 nd alleles more common in patients with small	160

	vessel vasculitis	
24	Group of 1 st alleles more common in patients with medium vessel vasculitis	161
25	Group of 2 nd alleles more common in patients with medium vessel vasculitis	162
26	Frequency of 1 st alleles in patients with and without purpura	163
27	Frequency of 2 nd alleles in patients with and without purpura	164
28	Frequency of 1 st alleles in patients with gangrene	165
29	Frequency of 2 nd alleles in patients with gangrene	166
30	Frequency of 1 st alleles in patients with neurological symptoms	167
31	Frequency of 2 nd alleles in patients with neurological symptoms	168
32	The frequency of the 1 st alleles in patients with renal manifestations	168
33	The frequency of the 2 nd alleles in patients with renal manifestations	169

List of Figures

1	Extrahepatic manifestations of HCV	10
2	Stimulation of B lymphocytes	17
3	Production of monoclonal and polyclonal immunoglobulins due to HCV infection	17
4	Palpable purpura and skin biopsy showing leukocytoclastic vasculitis	18
5	Various skin lesions including palpable purpura, and skin ulcers	19
6	Normal glomerulus	20
7	Light microscopy showing membranoproliferative pattern	21
8	Intraluminal thrombi on LM	21
9	Immunofluorescence in capillary loops	22
10	EM of a normal glomerular capillary loop	23
11	High power electron micrograph of a cryoprecipitate in the mesangium, showing the characteristic substructure which often has a "fingerprint" appearance	23
12	High power electron micrograph of a cryoprecipitate in the mesangium, showing the characteristic substructure which	23

	often has a "fingerprint" appearance	
13	Molecular structure of cyclophosphamide	29
14	Mechanism action of Rituximab	33
15	Rituximab and the B cell	33
16	Hepatic osteosclerosis	69
17	Schematic diagram of site of the MHC	70
18	Alpha and Beta chains of class I and II MHC	72
19	Gene map of the human leukocyte antigen (HLA) region	73
20	Antigen Presentation	78
21	The three-dimensional structure of the alpha1 and alpha 2 domains of HLA-B*2705. Numbers identify polymorphic residues among B27 subtypes. A peptide (green) intimately interacts with the groove through hydrogen bonds. Each of six pockets (A-F) can bind the side chain of an individual amino acid	104
22	Schematic diagram showing binding of antigenic peptides to HLA-B27 and recognition by the T-cell receptor. The side-chains of peptide "anchor" residues P2, P3 and P9 (C-terminal) are bound in pockets. The side-chains of P1, P4 and P8, which extend out of the peptide-binding groove, are critical for T-cell recognition	104

23	Birhingam activity score	124
24	Vasculitis damage index	126
25	Multiple ulcers on lower limb	130
26	Gangrene of toes bilaterally	131
27	Pictures of dorsal and palmar aspects showing gangrene of fingers.	131
28	Livedo reticularis on lower limbs	132
29	Ptosis of right eye	133
30	Graph chart of 1 st alleles more common in patients with HCV vasculitis	145
31	Graph chart showing 2 nd alleles more common in HCV vasculitis group	146
32	Graph chart of 1 st alleles more common in the HCV control group	147
33	Graph chart of 2 nd alleles more common in the HCV control	148
34	Graph chart of 1 st alleles more common in patients with small vessel vasculitis	156
35	Graph chart of 2 nd alleles more common in patients with small vessel vasculitis	157
36	Graph chart of 1 st alleles more common in patients with medium vessel vasculitis	158

37	Graph chart of 2 nd alleles more common in patients with medium sized vasculitis	159