Using Anti-Nuclear Antibody and Rheumatoid Factor to Evaluation of Immune- Pathogenic Link of Rheumatoid Arthritis and Hepatitis C Virus in Iraqi Patients

Mohammed A. Saleh

Department of Biology, College of Science, Diyala University, Diyala-Iraq.

E-mail: Mohammed mostafa 78@yahoo.com.

Abstract

This study was aimed to assess immune- pathogenic association of HCV infection among RA patients. A cross sectional study was conducted on the following groups: patient group includes forty patients with RA. Their age range was between (20-70) years with (7) (17.5%) males and (33) (82.5%) females. Healthy Group: thirteen serum samples were collected from apparently healthy individuals they served as a control group.Their age range was between (23-59) years. Results of this study reveals that the majority of RA patients are females (82,5 %) with females to males ratio of 4.7: 1. Moreover, the mean of age for RA and control groups are (46.83 ± 12.06) and (45.23 ± 12.58), respectively. The prevalence of RA among RA females was (82.5%) and (17.5%) for male and the frequency of rheumatoid Factor positivity among RA patients was (70%) in comparison with (0.0%) for apparently healthy control group with significant differences ($P \leq 0.001$). Also, ANA positivity was higher (27,5%) than that for healthy control group (0.0%) with significant differences ($P \leq 0.005$). Results showed that, the frequency of positivity anti-HCV Ab was (5%) among RA patients in comparison with apparently healthy control group (0 %). Our results sustain the possible contribution of HCV infection in the pathogenesis of RA.

Keywords: Rheumatoid Arthritis, Hepatitis C Virus, Anti-Nuclear Antibody.

Introduction

Hepatitis C virus (HCV) infection has become a major public health problem, with 170 million people are infected worldwide. The disease progresses slowly and a chronic infection develops in 85% of the cases. Among patients with chronic hepatitis, 20 to 30% develop cirrhosis that, once established, carries a poor prognosis, with a high risk of developing hepatocarcinoma [1].

Autoimmune manifestations are common in patients chronically infected by HCV [2]. These manifestations can be dominant, while the hepatic disease can be quiescent or mild. There has been growing interest in the relationship between HCV and Sjogren's syndrome (SS), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [3].

Rheumatoid arthritis usually presents as an unresolving polyarthritis of small joints with or without large joint involvement. RA is an autoimmune disease of multifactorial etiology. Although genetic predisposition (including the presence of particular HLA-DR4 and/or DR1 alleles) is significant in the development, clinical course, severity, and expression of extraarticular manifestations, the cause of RA remains elusive [4].

Many viruses and bacteria have been implicated as causative agents. These include the Epstein-Barr virus, parvovirus B19, hepatitis C and Rubella and bacterial organism such as Mycoplasma, mycobacterium and Yersina [5]. It has been suggested that these organism infect host cell and alter the reactivity or responsiveness of the T- cells of the immune system sufficiently to trigger the disease [6].

The worldwide prevalence of arthritis presumed to be due to hepatitis C virus (HCV) infection has been reported between 2.4 million and 45.9 million people [7]. Finally, serum anti-HCV antibodies were detected in 5.2% and 7.6% of patients with RA[8,9], thus a causal link might exist between HCV and RA in some patients. The objective of this study was to determine if there is a possible relation between RA and HCV infection by evaluating the immune- pathogenic link of this infection among patients with RA.

Materials and Methods
Subjects

A cross sectional study was conducted on the following groups in the period between January 2010 to August 2010.

Patient Study Group

This group included forty patients with RA. Those patients had RA as defined by the American Rheumatism Association (ACR). These patients attended the Rheumatology Department of Baghdad Teaching Hospital during the study period between January 2010 to August 2010. The diagnosis of those patients was performed under the supervision of rheumatic disease Consultant Rheumatologist. Their age range was between (20-70) years with (7) (17.5%) males and (33) (82.5%) females. Those patient who had history or clinical evidence of RA which includes: insidious pain, stiffness, erythema warmth and symmetric joint swelling. This work was done in Baghdad teaching hospital / medical city ,immunological departments in teaching laboratories/ medical city and viral hepatitis reference laboratory.

Healthy Control Group

Thirteen apparently healthy volunteers included in this study as a control group. Their age range was between (23-59) years with (1) male and (12) females. Those healthy who have no history or clinical evidence of RA or any other chronic disease, and no obvious abnormalities.

Sample Collection

From each individual included in this study, 5-10 ml of blood was drawn by vein puncture using disposable syringes. The blood was placed in plastic disposable plane tubes, it was left to stand at room temperature (20-25°C) to allow it to clot, then the sera was separated by centrifugation for 5 minutes, and divided into aliquots (250 µl) and stored at -20°C till examination. Each aliquot of the serum was used once to avoid thawing and freezing. All sera and reagents were allowed to stand at room temperature before use in the test.

Methods
Immunological markers
A.Enzyme linked immunoassay for Rheumatoid Factor detection

Diluted serum sample (1: 20) was incubated with the specific antigen which coated micro titer plate's wells. Patient's antibodies, if present in the specimen, combined with the antigen. Un-bound fraction was washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horse radish Peroxidase (HRP-conjugate) are incubated and reacted with the Antigen-Antibody complex of the sample in the micro wells. Un-bound conjugate was washed off by washing solution. Addition of Tetra-Methyl Benzydine (TMB-substrate) generates an enzymatic colorimetric (blue) reaction, which was stopped by diluted acid solution to yield yellow discoloration. The rate of color formation from the chromogen was proportional to the initial concentration of the respective Abs in the patient's sample. Microtiter plate reader at 450 nm was the reading filter and optional 620 nm [10]. The detailed procedure was carried out as has been suggested in the leaflet supplied with the test kit (Omega, UK). This method was done in immunological departments in teaching laboratories/ medical city.

B.Indirect Immunoflurescent Antibody Test for Antinuclear Antibody (ANA) Detection

In indirect immunoflurescent technique, the patient serum was incubated with a suitable substrate to allow specific binding and formation of a stable antigen-antibody complex in the presence of specific antibody. After washing, the substrate is incubated with fluorescein conjugated antihuman globulin reagent to give three–part complex which emits in the fluorescent microscope [11]. Substrate slide: HEP-2 cell line for ANA detection was used, 25 µL of dilution of patient serum (1/40) was layered over the substrate and allowed to incubate at room temperature in a moist chamber for 20 min, the slide was then washed with a stream of phosphate buffer saline (P.B.S) pH 7.4 and left in a staining dish filled with PBS for 1 minute with a magnetic stirrer. After that, the slide was removed, excess PBS was removed by blotting strips and placed in moisture chamber

to add 25 μL of fluoresce in conjugated antisera to human Immunoglobulins to each well and washed for mounted using mount media (buffered polyvinyl alcohol) to be examined under fluorescent microscope in a dark room[11].

Virological Marker
A.ELISA for Detection of Antibodies to HCV (Screening Test) :

This is an immune- enzymatic method in which the wells of a micro titer plate were coated with recombinant antigens (Ags) representing epitopes of HCV. Serum or plasma samples were added to these wells. If the Abs specific for HCV are present in the sample, they form stable complexes with the HCV Ag in the well. After washing to remove the unbound material a rabbit anti-human IgG labeled with Horse Radish Peroxidase HRP (as conjugate) was added and, if the Ag / Ab complex is present, the conjugate will bind to the complex. After a second wash, an enzyme substrate solution containing a chromogen was added. This solution develops a blue color if the sample is positive. The blue color changes to yellow after blocking the reaction with sulphuric acid. The intensity of color is proportional to the anti-HCV Abs concentration in the sample. Wells containing negative samples remain colorless [12].

B. Immunoblot Anti-HCV (Confirmatory Test) :

This method was done in viral hepatitis reference laboratory /Baghdad.

The present immunoblot makes use of gene technology produced virus antigen. Four recombinant HCV antigens are used into the test strips:
- Core, Capsid antigen.
- NS-3, encoding the viral protease and helicase.
- NS-4, N-terminal part of NS.

The detailed procedure was carried out as has been suggested in the leaflet supplied with the test kit (Bayer health care, U.S.A) [13].

Results and Discussion

Forty patients (33 women, 7 men) who age range (20-70 years) were studied. The demographical picture of studied group (RA patients and healthy control group) are listed in the Table (1). This table reveals that the majority of RA patients are females (82,5 %) with females to males ratio of 4.7: 1. Moreover, the mean of age for RA and control groups are (46.83 ± 12.06) and (45.23 ± 12.58), respectively.

Table (1)
Distribution of the study groups according to gender and age groups.

Variables	Rheumatoid arthritis		Healthy control	
	No	%	No	%
Sex Male	7	17.5	1	7.7
Female	33	82.5	12	92.3
Age (years) 20—29	4	10.0	2	15.4
30—39	5	12.5	2	15.4
40—49	13	32.5	2	15.4
50—59	12	30.0	5	38.5
60—69	4	10.0	2	15.4
=>70	2	5.0	-	-
Mean±SD (Range)	46.83±12.06 (20-70)		45.23±12.58 (26-61)	

Rheumatoid arthritis is one of the most common autoimmune diseases, affecting 0.5% of the population [14]. In this current study, the mean age for RA patients was at the fourth decade (46.83±12.06 years) which is in agreement with previous studies [15,20].

Moreover, the prevalence of RA among RA females was (82.5%) and (17.5%) for male. This frequency is higher to some extent, with that of local previous studies in Iraq which are mentioned by [16,17] who noticed the lower percentage which reached to (70.7) and (79.7) % respectively.These results may be due to the environmental conditions beside the psychological situation of Iraqi people which results in high stress that enhance RA development. Also higher prevalence was noticed in North American families (76.8%) [18]. This result denoted a high frequency among females rather than males which may be due to the hormonal differences between them and in turn, their effects on the immune responses [19]. Furthermore, the frequency of rheumatoid Factor (RF) positivity rate among RA patients is (70%) in comparison with (0.0%) for apparently healthy control group

[4] Buch, M. and Emery, P. "The Etiology and Pathogenesis of rheumatoid arthritis". J. Hospital Pharmacist. 9, 5-10, 2002.

[5] Stransky, G.; Vernon, J. and Aicher, W. "Virus-like particles in synovial fluid from patients with rheumatoid arthritis"; J. Rheumatol. 32, 1044, 1993.

[6] Olivieri, I.; Palazzi, C. and Padula, A. "Hepatitis C virus and arthritis". Rheum. Dis. Clin. North Amer. 29,111–122, 2003.

[7] Rosner, I.; Rozenbaum, M.; Toubi, E.; Kessel, A.; Naschitz, J.E.; Zuckerman, E. "The case for hepatitis C arthritis"; Semin. Arthr. Rheum. 33,375–387, 2004.

[8] Taglione, E.; Vatteroni, M.L.;Martini,P.; Galluzzo, E. and Lombardini, F. "Hepatitis C virus infection: prevalence in psoriasis and psoriatic arthritis" ; J. Rheumatol. 26,370–372,1999.

[9] Rivera,J.; Garcia-Monforte, A.; Pineda, A. and Millan, J. "Arthritis in patients with chronic hepatitis C virus infection" ; J. Rheumatol. 26,420–424,1999.

[10] Bennett,R.H. and Burch TA. "New York symposium on population studies in the rheumatic disease: new diagnostic criteria"; Ann. Intern. Med. 17, 453-458, 2004.

[11] Van-Boekel,M."Autoantibody system in Rheumatoid arthritis: specificity, sensitivitiy and diagnostic"; Arthri. Res. 4,87-93, 2002.

[12] Countreras,M.and Barbara,J." Screening for hepatitis C antibodies"; The Lancet. 2,505, 1989.

[13] Gretch, D.R." Diagnostic test for hepatitis C". Hepatol., 26, 43-47,1997.

[14] Shankar, S. and Handa, R. (2009). Biological agents in Rheumatoid arthritis. Postgrade Med. 50, 293-299, 2009.

[15] El-saffar, J. "Role of Anti-Cyclic citrullinated peptide antibodies and interleukin -2,6 in diagnosis of rheumatoid arthritis in Iraqi patients", PhD thesis. College of Science, University of Baghdad, 2008.

[16] Abdul-Abbas, H. "Correlation between rheumatoid arthritis and some cytokines among Iraqi rheumatoid arthritis patients", Msc thesis,College of Health and Medical Technology, Foundation of Medical Education, 2007.

[17] Al-Haidary, B. "HLA typing for rheumatoid arthritis patients (familial profile)", PhD thesis, College of Medicine, University of Baghdad, 2003.

[18] Jawaheer, D.; Raymond, L.; Gregersen, P. and Criswell, L. "Influence of male sex on disease phenotype in familial rheumatoid arthritis"; Arth. and Rheum. 5, 3087-3094, 2006.

[19] Koopmans, W. "Prospects for Autoimmune disease"; JAMA. 285, 648-649, 2001.

[20] Al-Rawi, Z.S.; Azzawi, A.J.; Al-Ajili, F.M. and Al-Wakil, R. "RA in Population Samples in Iraq"; Ann. Rheum. Dis. 37, 73-75, 1998.

[21] Anaya, J.; Correa, P.; Mantilla, R. and Arcos-Burgos, M. "Rheumatoid arthritis association in Colombian population is restricted to HLA-DRB1"; Gene Immun. 3, 56-58, 2002.

[22] Al-naqdy, A.; Al-Busaidy, J. and Hassan, B. "Evaluation of Anti-dsDNA antibodies in anti-nuclear antibody positive Omani patients"; Pak. J. Med. Sci. 23, 211-215, 2007.

[23] Hsu, F.C.;Starkebaum, G.; Boyko, E.J. and Dominitz,J.A." Prevalence of rheumatoid arthritis and hepatitis C in those age 60 and older in a US population based study"; J. Rheumatol. 30, 455-458, 2003.

[24] Maillefert, J. F.; Muller, G .; Falgarone, G. and Bour, J.B."Prevalence of hepatitis C virus infection in patients with rheumatoid arthritis"; Ann. Rheum. Dis. 61,635-637, 2002.

[25] Lovy, M.R.; Starkebaum, G.and Uberoi, S."Hepatitis C Infection Presenting with Rheumatic Manifestations/ A Mimic of Rheumatoid Arthritis"; J. Rheumato. 23, 979-983, 1996.

[26] Sorlie, D.E. "Medical biostatistics and Epidemiology: Examination and board review". First ed. Appleton and Lange, Norwalk, Connecticut. PP : 47-88 ; 1995.

الخلاصة

هدفت هذه الدراسة إلى أثبات العلاقة مابين التهاب الكبد الفيروسي نمط (ج) ومرض الحمى الرثوانية وذلك من خلال قياس العلاقة المناعية–الأمراضية مابين التهاب الكبد الفيروسي نمط (ج) والحمى الرثوانية.تم أجراء هذه الدراسة على المجموعات التالية: مجموعة المرضى والتي تضمنت أربعين مريض مصابين بمرض الحمى الرثوانية وكان مدى أعمار المرضى مابين (20–70) سنة وعدد الذكور (7) (17.5 %) والإناث (33) (82.5%). مجموعة الأصحاء: جمعت ثلاثة عشر عينة مصل من أفراد أصحاء ظاهريا وكان مدى أعمار لأصحاء مابين (23–59) سنة. أظهرت نتائج الدراسة بان أغلبية المرضى من الإناث (82.5 %) ونسبة الذكور إلى الإناث 4.7:1، علاوة على ذلك كان متوسط العمر لمجموعتين المرضى والسيطرة (46.38 ± 12.06),(45.23 ± 12.58) على التوالي. أكدت الدراسة بان نسبة انتشار مرض الحمى الرثوانية في الإناث (82.5%) وفي الذكور (17.5%) وبينما معدل موجبيه العامل الرثواني في مرضى الحمى الرثواني كان (70%) بالمقارنة مع مجموعة السيطرة (0.0%) مع وجود فرق معنوي ، من ناحية أخرى أوضحت الدراسة بان نسبة موجبيه الأجسام المضادة ضد النووية عالية (27%) بالمقارنة مع مجموعة السيطرة (0.0%) مع وجود فرق معنوي ، فضلا على ذلك أكدت الدراسة بان نسبة الايجابية للإصابة بفيروس التهاب الكبد الفيروسي نمط (ج) في مرضى الحمى الرثوانية كانت (5%) بالمقارنة مع مجموعة السيطرة. نتائج الدراسة أثبتت احتمالية مساهمة فيروس التهاب الكبد نمط (ج) في أمراضية الحمى الرثوانية.