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Hepatitis C Virus Antibodies and Vitiligo Disease

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Abstract

Vitiligo is a common skin disorder, characterized by depigmented patches due to selective destruction of melanocytes. The etiology of this disease is unknown. A number of hypotheses including viral theory have been proposed to explain the etiology. To determine the prevalence of antibody to hepatitis C virus infection in vitiligo patients, the present study was performed. Third generation ELISA test was used for detection of antibodies to HCV in human sera. All normal controls were anti-HCV negative whereas only one patient was positive for anti-HCV and there was no significant difference in the prevalence of anti-HCV between patients and controls. These results indicate that hepatitis C virus has not a direct causal role in the pathogenesis of vitiligo, however, this does not rul out a "hit and run" virus induced disease.

Keywords: *Hepatitis C virus, Vitiligo,* ELISA*, Iran*

Introduction

Hepatitis C virus (HCV) is a linear, single stranded virus of the flaviviridae family, with genomic variability associated with different autoimmune manifestations (1). Vitiligo is an acquired idiopathic hypomelanotic disorder resulting from the loss of melanocytes (2). The pathogenesis of viruses in vitiligo is still to be fully clarified even if several factors such as immunological, neural and biochemical ones have been proposed as an etiologic determinant. It has been proposed that autoimmunity might arise as a secondary phenomenon in response to melanocyte damage by other mechanisms (3, 4). It seems that vitiligo and HCV infection share common features including their association with autoimmunity. Despite several studies in different countries (5, 6), the relationship between vitiligo and HCV infection is not yet fully understood. Since different strains of HCV have been identified and the distribution of strains of these viruses in geographical regions is different (7, 8), the lack of association of HCV with vitiligo in certain countries can not be generalized, thus the present investigation was undertaken to find out the frequency and significance of HCV infection in Iranian vitiligo patients.

Materials and Methods

Patients Sera from 65 vitiligo patients (23 males; 42 females; mean age 31.4±14.5 years) were collected in dermatology clinics. The clinical diagnosis of patients was accomplished

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by consultant dermatologist. Sera from 44 healthy individuals (23 male; 21 female; mean age 26.8±7.2 years), with no history of either vitiligo or autoimmune disorders, such as Hashimoto's thyroiditis, Graves' disease, insulin -dependent diabetes mellitus, and alopecia areata were used as controls. All sera were kept frozen at -70˚ C until use.

ELISA Third generation ELISA test was used for the detection of antibodies to HCV in human sera (bioelisa HCV, Spain). Briefly, microtiter plates were coated with recombinant antigens representing epitopes of HCV: Core, NS3, NS4 and NS5. Serum samples were diluted 1:20 in diluent (Tris buffer) and were added (200µl/well) to microtiter plates, mixed gently and incubated for one hour at 37˚ C. In each plate, 200µl of negative and positive control samples were added to 3 and 2 wells, respectively. After washing with phosphate buffer containing 1% Tween 20, plates were incubated with a horseradish peroxidase labelled rabbit anti-human IgG (100µl/ well) diluted 1:51 in conjugate diluent (Tris buffer) for 30 min at 37˚C. Following a second wash, a solution of enzyme substrate (citrate-acetate buffer together with hydrogen peroxid) containing a chromogen tetramethylbenzidine (TMB) dissolved in dimethyl sulphoxide (DMSO) was added (100µl/ well). After 30 min incubation at room temperature and addition of stopping reagent (1N sulphuric acid), the absorbance was read at 450nm. The presence or absence of anti-HCV antibodies in the sample was determined by relating the absorbance value of each sample to the cut-off value. This value was the mean value obtained for negative controls plus 0.300.

Western blotting HCV Blot 3.0 kit (Genelabs diagnostics SA, Switzerland) was used as confirmatory assay for positive results. Briefly, the nitrocellulose strips which contain four recombinant HCV proteins from the capsid, NS3, NS4, and NS5 regions of the HCV genome were incubated with washing buffer (Tris buffer with tween 20) at room temperature for two min. The strips were then incubated with patients' sera or controls diluted 1:100 with blotting buffer (5% non-fat dry milk in Tris buffer) at room temperature for one hour. After washing, the strips were incubated with goat anti-human IgG conjugated with alkaline phosphatase, diluted 1:1000 in blotting buffer for one hour at room temperature. Finally, another washing step, specific binding was revealed by the addition of substrate solution, 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium.

Liver function tests Hepatic function was estimated with serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase and total bilirubin using conventional methods (Ziestchem, Iran).

Statistical analysis Chi square test was used to calculate the significance of differences in the frequencies of ELISA reactivity between patients and normal controls. P-values lower than 0.05 were considered statistically significant.

Results

Sixty five patients with vitiligo and fourty four normal controls were included in the study. Patients were characterized with respect to the presence of associated autoimmune disorders: 29 had no family history of autoimmune disease and no other disorders, 28 had a family history of autoimmune disease but had no other disease, and 8 had an autoimmune disorder. One case of alopecia areata, six hypothyroidism and one hyperthyroidism were detected as autoimmune disease. The mean duration of vitiligo was 9.92±8.95 years (female patients, 9.34±7.50 years; male patients 11±11.04 years). ELISA results among typical vitiligo patients indicated, lack of HCV virus infection. Only one out of 65 patients was anti-HCV positive, but all 44 controls were negative. Anti-HCV immunoreactivity by enzyme immunoassay was confirmed by Western blot test. The positive patient was a 27-year-old man without a family

history of vitiligo. This patient with normal liver function tests had a generalized form of vitiligo, which is more frequently associated with autoimmune disorders.

Discussion

A large number of potential etiologies have been put forth to explain autoimmune diseases. It seems unlikely that a single explanation is adequate to account for the diverse phenomena that are observed in autoimmunity. A description of the factors that may contribute to induce or development of autoimmunity is difficult, but there are several evidences that viruses have been proposed as possible etiologic or triggering agents in the pathogenesis of autoimmune diseases (9-13). The mechanism by which the viruses induce vitiligo is not known. It is possible that viruses kill melanocyte by attacking cellular DNA directly or during melanocyte formation. Moreover melanocytes may be abberently damaged by the immune system when this system attempt to eliminate ejected viruses from nerve ending (14). A variety of viruses such as cytomegalovirus, Epstein-Barr virus, hepatitis B and C viruses have been surveyed in vitiligo, but there are no definitive data to validate or disprove the viral hypothesis (6, 15, 16). In the present study the relationship between HCV infection and vitiligo was investigated because there are a number of reports indicating the association of HCV infection and vitiligo with autoimmune diseases including thyroiditis, lichen planus, and rheumatoid arthritis (1, 3, 17, 18). In addition some investigators reported the presence of circulating autoantibodies such as anti-smooth muscle antibodies, anti-nuclear antibodies, and rheumatoid factor in both diseases (15, 19-21). Finally extra hepatic manifestations of hepatitis C that are observed in dermatology such as lichen planus, alopecia areata, porphyria cutanea trada and pruritus (1,19) are additional indications to suspect the involvement of this virus in vitiligo.

In the present study, we analyzed a series of vitiligo patients and normal controls for anti-HCV antibodies, and only one patient was seropositive. There was no statistical difference between patients and normal controls. This survey was the first study to investigate the relationship between vitiligo and hepatitis C virus in Iran, although several related reports in the form of case report have previously been published. Yamamoto and Nishioka (5) described five vitiligo patients with HCV seropositivity. Pondanyi et al (22) introduced a patient with vitiligo and prurigo in setting of HCV infection. Adiloglu et al (6) and Nihat et al (23) described a 0 and 1 patient with HCV seropositivity respectively. The results of this study indicated that HCV seropositivities were not related to vitiligo and additional studies must be done to clarify the direct or indirect role of viruses in the pathogenesis of vitiligo.

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