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Evaluation of autoimmune biomarkers in vitiligo patients' serum

Ekhlas A. K. Kanani[1], Awatif H. Issa[2], Khalil I. Al-Hamdi[3]
[1] Biology Department, College of Science, University of Basra, Iraq,
[2] Department of pathological analyses, College of Sciences, University of Basra, Iraq,
[3] Department of Dermatology, College of Medicine, University of Basra,

Corresponding author: Awatif Hameed Issa, Biology Department, College of Science, University of Basra, Iraq,
E-mail: [2]awatifhissa@uobasrah.edu.iq, [1]ekhlasimmuno2019@yahoo.com, [3]khalil.ismail@uobasrah.edu.iq.

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ABSTRACT

Background: Vitiligo is one of the diseases which its pathogenesis has not yet been clearly understood, however many studies underline this disease as an autoimmune. The majority of authority preferred the autoimmune origins due to the substantial correlations between vitiligo and other autoimmune disorders, as well as having autoantibodies. The presence of antinuclear antibodies in vitiligo patients appear to support an autoimmune involvement in the disease's etiology.

Aim of the study: investigation the prevalence and significance of antinuclear antibodies ANAs, high sensitivity C-reactive protein (hsCRP) and C-reactive protein (CRP) in vitiligo patients compared with healthy subjects.

Methods: Ninety sera specimens from (45 vitiligo patients with matched 45 healthy controls) were analyzed in this study for Euroline ANA profile 23 (IgG) , hsCRP and CRP tests.

Results: There were statistically significant positive findings of ANA in vitiligo patients in comparsion with controls subjects. Anti-RP-155 was the most prevalent in patients. In addition to high positive findings of hsCRP and CRP tests related to patients than controls.

Conclusion: The study supports a significant association between vitiligo and other autoimmune diseases. Identification and characterization of anti-nuclear autoantibodies in vitiligo patients considered vital indicators support the findings of the most other researches indicating this disease is an autoimmune disease, and can pave the way for identifying the extent possible incidence of other autoimmune diseases, to take the appropriate medical procedure to avoid the exacerbation of diseases. ANAs findings supported with high positive findings of hsCRP and CRP proteins that might have a role in vitiligo pathogenesis.

INTRODUCTION

Vitiligo is a chronic skin condition characterized by progressively melanocytes destruction, manifests as hypochromic or achromic macules and patches on the skin, which enlarge and increase over time (1). The visibility of the lesions of many people affected with vitiligo disease is perceived disfiguring and inconvenient (2,3). According to an updated study based on screening of more than 50 global studies, vitiligo affects between 0.5 and 2% of the global population (4). Vitiligo can be clinically classified as non-segmental vitiligo form (NSV) a frequently symmetrical distribution of macules and patches, affecting any part of the body, and segmental vitiligo (SV) present unilaterally as an asymmetric distribution of macules and patches, generally around the midline (5).

A multitude of plausible hypotheses have been proposed to explain the pathogenesis of vitiligo. A genetic predisposition, autoimmune destruction of melanocytes, oxidative stress, neural hypothesis, and impaired melanocyte adhesion, are among the

important ones. The combination of all of these effectively explains the pathogenesis of vitiligo (the combination theory). The autoimmune theory is one of the most plausible theories that have been proposed to understand the pathogenesis of vitiligo (6).

Most authorities favored the autoimmune hypothesis due to the strong relationship of vitiligo disease with other multiple autoimmune diseases and the presence of autoantibodies (7,8). Vitiligo pathogenesis is a complex pathogenic mechanism involving cellular and humoral immunity (9,10). The release of proinflammatory cytokines triggers an inflammatory response. These cytokines also cause the production of acute-phase reactant proteins such high-sensitivity C-reactive proteins (hs-CRPs), lectin proteins, hepcidin, and others, which are used as inflammatory indicators (11). CRP is one of the primary acute phase proteins, mainly synthesized by hepatocytes in response to proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and IL-1 βeta (IL-1 β). Raised hs-CRP levels have been reported in response to a variety of cellular abnormalities such as tissue inflammation, infection, cardiovascular disease, and thromboembolism (12,13).

Autoantibodies that attack self-proteins within cell nucleus structures are known as antinuclear antibodies (ANAs); their presence in serum may indicate an autoimmune disease (14). ANAs react against various intracellular components that are essential for transcription, splicing, and cell division, resulting in the expression of a specific autoantibody (15), and have the ability to influence gene expression, cell growth, and apoptosis (16,17,18). ANAs are crucial for the identification of systemic autoimmune rheumatic disorders (SARDs). These antibodies have also been found in patients suffering from organ-specific autoimmune diseases (19,20). In the case of dermatologic disorders like vitiligo and alopecia areata, a positive ANA could be a sign of an underlying, clinically silent autoimmune condition such as Addison's disease, pernicious anemia, or autoimmune thyroiditis (21). Early detection of autoimmune diseases is essential, but due to the lack of distinct symptoms in the early stages of these diseases in many individuals, the process can take a long period (22,23). This aspect indicates the importance of looking for laboratory tests that may be used to diagnose and screen for autoimmune diseases early on, so that proper therapy can be prescribed. This review was to conduct some autoantigens profile, and their identification and characterization defined by their antinuclear antibodies IgG in vitiligo patients' serum comparing with their corresponding healthy individuals, as potential biomarkers for laboratory diagnosis, monitoring and assessment vitiligo disease condition and predict related autoimmune diseases.

MATERIALS AND METHODS

The study enrolled 45 patients with vitiligo, (27 female and 18 male), Age range was (7-65) years old, and 45 corresponding matched healthy individuals as control subjects (had no any evidence of vitiligo or any other diseases, whether autoimmune or not), conducted in (pathological analyses department /College Sciences / Basrah University) of Basrah province during period from February 2020 to July 2020. The patients were diagnosed clinically by dermatologist. There were non-segmental vitiligo patients at different stages and patients with segmental vitiligo.

Some of patients were in active vitiligo status defined on the basis of progression or appearance of lesions in the last 3 months, and some were in stable disease (The course of vitiligo was defined as stable when new lesions had not appeared within 1 year). All patients that had received phototherapy or any specific topical and systemic steroids therapy were excluded, they were discontinued from treatment at least three months. A detailed history was taken from all patients, related to age, sex, age at onset disease, family history of vitiligo, disease duration and association of other diseases recorded. No one of them had presented any additional other autoimmune diseases. Informed consent of patients was obtained before being included in this study.

Venous blood samples (5ml) were collected in vacuum tubes under sterile conditions from both patients and controls. In this study, (Euroline ANA profile 23 (IgG), hsCRP, and CRP tests) was investigated in all subjects.

Euroline ANA Profile 23 (IgG) Test: For screening and semi-quantitative determination of ANA in serum specimens using the Euroline ANA profile 23 test kit (EUROIMMUN/a PerkinElmer company/Germany) that provides a qualitative in vitro assay for human antinuclear-antibodies of the IgG class to 23 different antigens **(**dsDNA, nucleosomes, histones, SS-A, Ro-52, SS-B,

nRNP/Sm, Sm, Mi-2α, Mi $-$ 2 β, Ku, CENP A, CENP B, sp100, PML, Scl-70, PM-Sc100, PM-Scl75, RP11, RP-155, gp210, PCNA, and DFS70**)**, offers a multiplex approach for the determination of these antibodies in a single reaction, with optimal, fully automated processing and objective evaluation of the test results using the Euroline Scan software.

The test kit contains test strips coated with parallel lines of highly purified antigens. In the first reaction step, diluted patient serum samples are incubated with the immunoblot strips. In the case of positive samples, the specific IgG antibodies will bind to the corresponding antigenic site. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

CRP and hsCRP Test: CRP and hsCRP proteins investigated using a fluorescence immunoassay used along with Finecare™ FIA System (model no:FS-112/FS-113/FS-205) for quantitative determination of hsCRP and CRP in serums. Test uses a sandwich immunodetection method.

RESULTS

TABLE 1 Clinical characteristics of vitiligo patients.

Age of vitiligo patients' range	7-65 years
Mean of patients ages	23.7±15.6
Range of onset age	4-60 years
Mean of onset range	19.8±15.7
Men patients	18 (40%)
Men age range	8-52 years
Women patients	27 (60%)
Women age range	7-65 years
Active vitiligo	38 (84.4%)
Stable vitiligo	7 (15.5%)
Non-segmental vitiligo	34 (75.55%)
Segmental vitiligo	11 (24.44%)
Patients have vitiligo relatives	19 (42.22%)

Table-1 illustrated the clinical characteristics and age distribution of the study included vitiligo patients; they were from Basrah province/Iraq. Age was ranged from 7-65 years old, the mean age (23.7±15.6 SD) years. Range of disease onset age was (4-60) years old, mean of onset age range (19.8±15.7 SD).

The largest proportion of patients were females, they were 27(60%) while the males 18(40%), were less than females. The patients categorized into (non-segmental and segmental vitiligo), 34 (75.55%) were with non-segmental vitiligo, they represented the largest proportion of patients, while 11 patients were segmental vitiligo (24.44%). Disease was active in 38 (84.4%) of total patients, and only 7 (15.5%) were in stable status of disease. 19 patients at 42.2% percent had family history (1st and 2nd degree relative) affected with vitiligo.

TABLE 2 Total results of ANA profile 23 IgG antinuclear antibodies test related with total hsCRP and CRP test results for vitiligo patients with matched healthy controls.

	Tests					
Group	+ ANA	- ANA	+hsCRP	-hsCRP	+CRP	-CRP
Vitiligo	21 (46.6%)	24 (53.3%)	28(62.2%)	17(37.7%)	20(44.4%)	25(55.5%)
Control	0 (0%)	45 (100%)	11(24.4%)	34(75.5%)	4(8.8%)	41(91.1 %)
Total	21 (23.3%)	69 (76.6%)	39(43.3%)	51(56.6%)	24(26.6%)	66(73.3%)
	(X^2)=27.4, P-value<0.001		(X^2)=12.06,P-value<0.001		(X^2)=14.55,P-value<0.001	

The total serum specimens were subjected to Euroline ANA Profile 23(IgG), hsCRP and CRP test. It was analyzed the differences in the results of subjects with ANAs profile between two groups, Table-2. In the vitiligo group there were 21(46.6%) patients with positive findings of (Chi square) test. Statistical significance was set at p<0.05. The calculated (X^2=27.4, P-value<0.001), indicate of higher statistical significance of ANAs association with vitiligo patients in comparison with control subjects. ANAs in general, and no one of controls group 0(0%) tested positive ANAs. 24(53.3%) of vitiligo patients have negative findings of ANAs. The frequencies of positive or negative ANA test values were compared between the two groups using X^2

Concerning hsCRP test, it was statistically significant (X^2=12.06, P-value < 0.001) associated with vitiligo patients 28(62.2%) in comparison with controls 11(24.4%).

The positive hsCRP+ findings of patients 28(62.2%) were higher than their negative findings 11(24.4%).

Values of positive CRP test findings 20(44.4%) in vitiligo patients were lower than negative findings 25(55.5%), but it was statistically significance (X^2=14.55, P-value <0.001) compared with control subjects 4(8.8%), as illustrated in Table-2.

TABLE 3 The frequencies of ANAs in vitiligo patients.

ANAs	Vitiligo patients
RP-155	12 (26.6%)
Mi-2***α***	8 (17.7%)
RP11	5 (11.11%)
Mi-2β	4 (8.8%)
gp210	4 (8.8%)
Ku	3 (6.6%)
DFS70	3 (6.6%)
dsDNA	2 (4.4%)
PM-Scl100	1 (2.2%)
SS-A	1 (2.2%)
Scl-70	1 (2.2%)
PM-Scl75	1 (2.2%)
PML	1 (2.2%)
CENP A	0
CENP B	0
Ro-52	0
Sm	0
Sp 100	0
histones	0
nucleosomes	0
SS-B	0
PCNA	0
nRNP/Sm	0

According to the study observation, out of 21 patients with positive findings ANAs, 12(26.6%) patients have antibodies against RP-155 (anti recombinant subunit POLR3A of human RNA polymerase III antigen)
with highest percentage and was most prevalent among vitiligo patients.

Anti Mi-2α was detected in 8 (17.7%) patients as the second frequent antinuclear antibodies, (Table-3).

Anti-RP-11 detected in 5(11.11%) patients, followed by 4(8.8%) patients were positive for anti-Mi-2β and 4(8.8%) for anti-gp210. While only 3(6.6%) patients were detected positive for each anti-DFS70, and anti-ku. 2(4.4%) patients had positive test results for anti-dsDNA. The lowest detection results were 1(2.2%) for anti-(SS-A, PM-Scl100, PM-Scl75, Scl-70, and PML). No one of patients was detected to have immune-reactivity corresponding the following antigens (nucleosomes, histones, Ro-52, SS-B, nRNP/Sm, Sm, CENP A, CENP B, sp100, and PCNA).

TABLE 4. Intersection results of hsCRP and CRP with ANA results for vitiligo patients.

	+ANA	- ANA		+ANA	-ANA
+hsCRP	14(31.1%)	14(31.1%)	**+CRP**	10(22.2%)	10(22.2%)
-hsCRP	7(15.5%)	10(22.2%)	**-CRP**	11(24.4%)	14(31.1%)
X2=0.33, P-value=0.56			X2=0.16, P-value=0.68		

It was observed that among 21(+ANA) vitiligo patients, 14 patients at (31.1%) percentage were positive also for hsCRP, while 7 patients (15.5%) were negative for hsCRP, as illustrated in Table-4.

But in general, there were 14 (31.3%) of (+ANA +hsCRP) vitiligo patients' group that have positive findings for each hsCRP and ANA tests, were equal regarding (-ANA+hsCRP) vitiligo patients' group 14(31.1%). There was no statistically significant correlation (X^2=0.33, P-value=0.56) were found to associate ANA with hsCRP findings.

The same condition was observed for CRP test findings association with ANA profile test findings. It was observed that there is no statistically significant relationship (X2=0.16, P-value=0.68) between the results of ANA and CRP in patients in general. The (+ANA +CRP) patients group results and the (-ANA+CRP) group results were equal 10(22.2%), and CRP test positive findings 10(22.2%) among (+ANA) vitiligo patients were slightly lower than negative findings 11(24.4%).

DISCUSSION

It would be tried in this study to find out the relationship of serum level antinuclear antibodies (ANAs) and hsCRP with CRP (as a biomarker for systemic inflammation) in vitiligo patients to the pathogenesis of vitiligo. According to this study results, it was revealed the prevalence of antinuclear antibodies against different types of

autoantigens were highly statistically significant (X^2=27.4, P-value<0.001) when compared with controls subjects, Table-2, especially the patients included in this study had no symptoms or clinical signs of other autoimmune diseases, that the absence of specific symptoms in the early stages of these autoimmune diseases in many patients makes early diagnosis of it desirable, but the process often takes a long time (22,24). There is some evidences confirming that ANAs can be detected in patients with rheumatic diseases long before their clinical manifestations (25), that it can potentially serve as predictive diagnostic biomarkers for autoimmune diseases. As most studies reported (ANAs), is important in the diagnosis of systemic autoimmune rheumatic diseases (SARDs). Therefore, when a systemic autoimmune etiology is suspected, testing for ANAs is an appropriate initial step in the differential evaluation of patients (26).

At the same time, various studies reported that ANA-positive patients do not necessarily develop autoimmune diseases in the follow-up, at least for those diseases which are generally recognized as autoimmune ones (27,28). Moreover, ANAs can be seen in a broad range of autoimmune, viral, and oncological disorders (29,30). The detection of such autoantibodies in the vitiligo patients of this study may considered one of the supporting factors to the autoimmune theories that explain vitiligo developing.

The most elevated anti-nuclear antibodies were observed in this study in vitiligo patients were the autoantibodies against RP-155 (26.6%), anti-Mi-2α (17.7%), and anti-RP11 (11.11%) as illustrated in Table-3.

Anti-RNA polymerase III (RP-155 and RP-11) are the most systemic sclerosis SSc specific antibodies, found in over 50% of patients with the disease (31). Up to 50% of people with systemic sclerosis (SSc) possess anti-RP-155 antibodies, that are the most prevalent (32). In addition, anti-RP-11 are reported to be more associated and prevalent with SSc disease patients (33). In virtually, the occurrence of (ANAs) are relatively frequent finding in SSc patients, with important clinical correlations. This is what studies support that the presence of (ANAs) is reported in 90% of SSc patients a as relevant feature of immune system activation (15).

Anti-Mi-2 autoantibodies (Mi-2α and Mi-2β) are frequently found in Dermatomyositis (DM) patients (34), and were the first Dermatomyositis (DM) -specific autoantibodies identified (35), it is indicated that the proportion of Anti-Mi-2 has been found in 2 to 45% of adult DM patients (36). In the vitiligo patients enrolled in this study, anti-Mi-2α and Mi-2β were found at (17.7%) and (8.8%) respectively.

The other autoantibodies detected with lower percentages, as demonstrated in Table-3 such as anti-(gp210, ku, DFS70, dsDNA, SS-A, Scl-70, PM/Scl 100, PM/Scl 75, and PML), that reported with different frequencies in relation to various autoimmune diseases. ANAs antibodies directed against nuclear envelope proteins, such as anti-gp210 antibodies, are uncommon; however, they appear to be very specific for Primary biliary cholangitis PBC (37,38), also antibodies directed against PML are reported to be associated with (PBC) disease (39,40).

Anti-ku may be involved at the onset or during the development of autoimmunity in certain systemic autoimmune rheumatic diseases. Autoantibodies directed against the Ku autoantigen have been associated to systemic sclerosis (SSc), and related with myositis overlap and interstitial lung disease (ILD) (41).

Recent research has also highlighted the significance of DFS70 antibody as a crucial biological marker for identifying people with positive ANA who do not develop systemic autoimmune diseases (SID). This is based on what was reported that the prevalence of these antibodies is higher in healthy people than in SID patients (42).

Anti-double stranded DNA (dsDNA) antibodies play an important role in the diagnosis, classification and management of systemic lupus erythematosus (SLE) (43).

The specificity of anti-SS-A autoantibodies is controversial because they have been described in a variety of autoimmune and even non autoimmune diseases (44). It is often observed as anti-syndrome-related Sjögren's antigen A. It is also found with congenital heart block (CHB) and neonatal lupus syndrome (NLS) to be correlated with maternal levels of anti-SSA autoantibodies, with CHB occurring in 1%–2% of fetuses exposed to anti-SSA (45).

Sometimes referred to as anti-Scl-70 antibodies, are associated to the severity and progression of SSc-Interstitial Lung Disease (46).

Anti-(PM/Scl 100 and PM/Scl 75) are associated with the clinical course of systemic sclerosis (47). There are reports of a-PM/Scl antibodies to appear in a variety of systemic autoimmune disorders,

including overlap syndromes, polymyositis (PM), and dermatomyositis (DM) (48).

It is observed through reviewing above mentioned studies in this field, that there are various anti-nuclear antibodies with different correlations and explanations with specific diseases, that may serve a primary or secondary role in the possible progression of these diseases in vitiligo patients, and patho-mechanisms of vitiligo. The positive ANA results were reinforced with significantly increased positive results of hsCRP (X^2)=12.06, P-value<0.001 and CRP (X^2)=14.55, P-value<0.001 in vitiligo patients compared with controls subjects, as most applied inflammatory markers.

CONCLUSION

The detection of anti-nuclear autoantibodies in vitiligo patients considered vital indicators support the findings of the most investigations indicating this disease is an autoimmune disease, and can predict the possibility of other autoimmune diseases incidence in the future, supported with high positive findings of hsCRP and CRP that might have a role in vitiligo pathogenesis.

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