Antimelanocyte Antibodies: A Possible Role in Patients with Vitiligo

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Abstract
Background: Vitiligo is an acquired depigmenting skin disorder with multifactorial pathogenesis including genetic, autoimmune, and neuronal factors. Both humoral- and cell-mediated immunities are supposed to have a role in the pathogenesis of vitiligo. Patients with vitiligo have an increased concentration of circulating autoantibodies that are specific to melanocyte cytoplasm and surface antigens that related to the extent of the disease.

Aims and Objectives: The aim of the present study was to evaluate the role of antimelanocyte antibodies (AMAs), complement 3 and 4 (C3 and C4), and antinuclear antibodies (ANAs) in the pathogenesis of vitiligo. Materials and Methods: This study included 49 patients with nonsegmental vitiligo and 36 healthy individuals as a control group. All participants were subjected to detailed history, general examination, and detailed dermatological examination of the skin, hair, nails, and oral mucosa. The severity of vitiligo was assessed according to the Vitiligo Area Scoring Index (VASI). AMA, C3 and C4, and ANA serum levels were measured for patients and controls. Results: ANA, AMA, and C4 levels were significantly higher in the sera of patients than in controls. ANA, AMA, and C4 serum levels showed significant positive correlations with VASI score. Conclusion: Our results support the role of AMA in the pathogenesis of nonsegmental vitiligo, correlating with the disease extent and severity. However, a longitudinal study in a large cohort of patients to evaluate the clinical and predictive value of AMA is warranted.

Key Words: Antimelanocyte antibodies, antinuclear antibodies, vitiligo

Introduction
Vitiligo is an acquired depigmenting skin disorder of unknown origin and multifactorial pathogenesis with an incidence of 0.5%–2% worldwide. Genetic, autoimmune, neuronal, and autocyctotoxic factors are supported by several studies; yet, the autoimmune hypothesis remains most accepted.[1,2]

The development of autoimmune-associated vitiligo could be attributed to either a T cell-based pathomechanism in cases of localized disease or natural killer cells, T cells, and antibodies in cases of diffuse vitiligo.[1,4] Most vitiligo susceptibility loci identified through genome-wide association studies encode immunomodulatory proteins, supporting the autoimmune pathogenesis of vitiligo.[5]

In individuals predisposed through inheritance, overexpression of the B lymphocyte-activating factor may activate self-reactive B cells to produce autoantibodies against melanocytes with the interaction of both CD4+ and CD8+ T cells subsequently causing autoimmune vitiligo.[6] Therefore, both humoral and cell-mediated immunities are supposed to have a role in the pathogenesis of vitiligo.[6] Patients with vitiligo have an increased concentration of circulating autoantibodies that are specific to melanocyte cytoplasm and surface antigens compared with normal individuals that related to the activity of the disease.[7,8]

On the other hand, it was reported that melanocyte antigen-specific antibodies cannot be used as markers for recent disease activity in patients with vitiligo.[9]

The distinct distribution of vitiligo patches in the body despite the systemic presence of antibodies in the serum suggested that antibodies were not the entire answer.[10] Thus, alternative theories were sought to identify the true pathogenesis of vitiligo.[11] Circulating antinuclear antibodies (ANAs) were reported in patients with vitiligo with different rates ranging from 2.5% to 33.3%.[12]
The aim of the present study was to evaluate the role of antimelanocyte antibodies (AMAs), complement 3 and 4 (C3 and C4), and ANA in the pathogenesis of vitiligo.

**Materials and Methods**

This study included 49 patients with vitiligo attending the Outpatient Clinic of Dermatology Department, Mansoura University Hospital, Egypt. The control group included 36 healthy age and sex-matched volunteers who had no dermatological or systemic diseases.

The Institutional Research Board of the Faculty of Medicine, Mansoura University, approved this study, and each participant provided informed consent before entering the study. Exclusion criteria were patients receiving treatment including phototherapy and psoralens in the last 3 months, patients with a history of hepatitis B virus infection, and patients with other autoimmune diseases.

All participants were subjected to detailed history taking regarding age, sex, occupation, marital status, special habits, drug intake etc. Specific disease history including onset, course, duration of the disease, progression characteristics, associated psychological disturbances, associated medical or surgical conditions, and family history of vitiligo were evaluated. General examination and detailed dermatological examination of the skin, hair, nails, and oral mucosa were done.

Patients were diagnosed as having vitiligo through clinical examination according to the diagnostic criteria adopted by the Vitiligo European Task Force.[13] The severity of vitiligo was assessed according to the Vitiligo Area Scoring Index (VASI). The VASI for each body region is determined by the product of the vitiligo area in hand units and the extent of depigmentation within each hand unit-measured patch (possible values of 0%, 10%, 25%, 50%, 75%, 90%, or 100%). The degree of depigmentation is estimated to the nearest of one of the following percentages: 100%, complete depigmentation, no pigment is present; 90%, specks of pigment present; 75%, depigmented area exceeds the pigmented area; 50%, pigmented and depigmented areas are equal; 25%, pigmented area exceeds depigmented area; and 10%, only specks of depigmentation present.

The total body VASI score was then calculated using the following formula by considering the contributions of all body regions (possible range, 0–100):

\[
\text{Total body VASI} = \sum \text{All body sites (hand units)} \times (\text{residual depigmentation}).
\]

**Laboratory investigations**

After 12 h of fasting, 5 ml venous blood was withdrawn from each participant. Blood samples were allowed to clot for 15 min, centrifuged at 7000 rpm for 10 min for serum separation, and stored at −20°C until analysis.

C3 and C4 were measured by simple radial immunodiffusion using a kit catalog number RID4490 manufactured by Far, Verona, Italy. The reference values according to the kit used were 50–120 mg/dL for C3 and 20–50 mg/dL for C4.

AMA was measured by human melanocyte antibody enzyme-linked immunosorbent assay (ELISA) Kit, catalog number MBS263351, MyBioSource Company, San Diego, California, USA. It detects Melanocyte protein 17 (premelanosome protein 17). These antibodies are specific for a melanocyte-specific type 1 transmembrane glycoprotein.

In summary, the purified human melanocyte antigens were bound to microwells. AMA bound in the samples was added. Washing the microwells removed unbound serum antibodies. Horseradish peroxidase-conjugated AMA was added, forming a (conjugate–antibody–antigen) sandwich. Washing of the microwells removed unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzed to form a blue color. The reaction was terminated by addition of sulfuric acid. The intensity of the color is measured spectrophotometrically at a wavelength of 450 nm. The concentration of AMA in the samples was then determined by comparing the optic density of the samples to the standard curve. The assay range is 3–100 ng/ml.

ANA was measured by commercially available ELISA kit (product code: 2339000) from Trinity Biotech Capita, Ireland.

**Statistical analysis**

Statistical analysis was performed with the Statistical Package for the Social Sciences version 20 (IBM Corporation, Armonk, New York, USA). Qualitative data were described using number and percentage. Quantitative data were described using minimum and maximum and mean and standard deviation. Comparison between different groups regarding categorical variables was tested using Chi-square test, and regarding continuous parametric data, t-test and ANOVA test were used. When more than 20% of the cells have expected count <5, correction for the Chi-square test was conducted using Fisher’s exact test or Monte Carlo correction. For abnormally distributed data, comparison between the two groups was done using Mann–Whitney test. Correlations between the two quantitative variables were assessed using Spearman’s coefficient. \( P < 0.05 \) was considered statistically significant.

**Results**

The study included 49 patients with vitiligo (31 [63.26%] females and 18 [36.74%] males) with age range of
5–65 years and mean 31.2 ± 16.9. Demographic and clinical criteria of patients with nonsegmental vitiligo compared to controls are presented in Table 1. ANA, AMA, and C4 were significantly higher in the sera of patients than in controls while C3 was insignificantly lower in the sera of patients than in controls [Table 2]. ANA, AMA, and C4 serum levels showed significant positive correlations while C3 levels showed no correlation with VASI score [Table 3].

Discussion
Evaluation of AMA in the blood of patients with vitiligo and observing its association with the disease activity are crucial for understanding the pathogenesis of vitiligo, adding new drugs, and for assessing curative effect, including cultured autologous melanocyte transplantation.[15,16] Antibodies to melanocytes occur at a significantly increased frequency in the sera of patients with vitiligo when compared with healthy individuals.[8]

In the present study, AMA, ANA, and C4 serum levels were significantly higher in patients than in controls while C3 serum level failed to show any relation between the two groups. These results agree, to some extent, with Farrokhi et al.[16] who found that AMA was positive in 30.9% of patients with vitiligo and none of the control. They also reported that ANA was insignificantly higher while C3 and C4 values were significantly lower in patients than in control group. In contrast to this, Lin et al.[17] found no obvious differences in the levels of C3 and C4 in children with nonsegmental vitiligo, suggesting dominance of cellular rather than humoral immunity. However, in segmental vitiligo, complement levels were lower in patients in the active stage, suggesting that humoral immunity may play an active role more than cellular immunity.

Several studies showed that serum AMA could be found in 30.9%, 42.8% up to 100% of patients with vitiligo, while it was not detected in controls.[8,18,19] However, a single study from the Kingdom of Saudi Arabia found their patients with vitiligo to have double AMAs compared to normal controls. However, they used a different antibody (vitiligo autoantibodies [V-immunoglobulin G] that are directed against cell surface antigens).[20]

Both the incidence and serum concentrations of these antibodies increase, with the extent of the disease being detected in 50% of patients with minimal vitiligo (<2% of skin area involved) compared with 93% of patients with greater depigmentation (5%–10% of skin area involved).[21-24] These results support our finding of a significant positive correlation between VASI score (disease severity) and AMA. These autoantibodies often localize to the cytoplasm and membrane of the cells, only occasionally appearing in the nuclear region. In addition to exerting an effect on complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC), these antibodies are likely to play a role in increasing antigen uptake and presentation by dendritic cells.[21] Furthermore, vitiligo antibodies have been demonstrated to destroy melanocytes both in vivo and in vitro.[25] Candidate antigens for these autoantibodies include tyrosine hydroxylase, tyrosinase, proto-oncogene C-kit, lysosomal-associated membrane protein-2, vitiligo (vit)-40, vit-75, and vit-90. However, other several antigens still await definite characterization.[26-28]

Table 1: Demographic and clinical criteria of patients with vitiligo compared with controls

| Parameter                      | Patient group (n=49) | Control group (n=36) | t    | P     |
|--------------------------------|---------------------|----------------------|------|-------|
| Age (years)                    |                     |                      |      |       |
| Range                          | 5-65                | 19-40                |      | >0.05 |
| Means±SD                       | 31.2±16.9           | 26.4±6.4             |      |       |
| Sex, n (%)                     |                     |                      |      |       |
| Female                         | 31 (63.26)          | 24 (66.67)           | >0.05|       |
| Male                           | 18 (36-74)          | 12 (33.33)           |      |       |
| Duration of the disease (years)|                     |                      |      |       |
| Range                          | 0.25-25             |                      |      |       |
| Means±SD                       | 6.9±8.2             |                      |      |       |
| Severity (VASI) score          |                     |                      |      |       |
| Range                          | 0.02-104.63         |                      |      |       |
| Means±SD                       | 8.7±20.2            |                      |      |       |

P<0.05 was considered statistically significant. SD: Standard deviation, VASI: Vitiligo Area Scoring Index

Table 2: Laboratory parameters of patients with vitiligo compared with controls

| Parameter | Patient group (n=49) | Control group (n=36) | t    | P     |
|-----------|----------------------|----------------------|------|-------|
| AMA (mg/ml)|                      |                      |      |       |
| Range     | 7.6-40.1             | 5.1-7.3              | 6.9634| <0.001*|
| Means±SD  | 16.4±10.1            | 6.3±0.8              |      |       |
| C3 (mg/dl)|                      |                      |      |       |
| Range     | 16.3-286             | 50-146               | 0.3782| >0.05 |
| Means±SD  | 111.5±155.8          | 103±30.5             |      |       |
| C4 (mg/dl)|                      |                      |      |       |
| Range     | 16.7-86.6            | 9.2-48               | 5.8357| <0.001*|
| Means±SD  | 35.3±15.3            | 19.6±9.4             |      |       |
| ANA       |                      |                      |      |       |
| Range     | 0.58-13.56           | 0.51-0.9             | 4.9862| <0.001*|
| Means±SD  | 2.1±1.9             | 0.78±0.11            |      |       |

*P<0.05 was considered statistically significant.
AMA: Antimelanocyte antibody, ANA: Antinuclear antibody, C3: Complement 3, C4: Complement 4, SD: Standard deviation
The frequency of AMA increased up to 80% in the development stage.\(^\text{[16]}\)

The level of autoantibodies decreased in patients with vitiligo who respond to photochemotherapy or following systemic steroid treatment.\(^\text{[29]}\) In addition, Li et al.\(^\text{[21]}\) proved that hydroxychloroquine protects melanocytes from autoantibody-induced injury by reducing the binding of antigen–antibody complexes reversing the activities of ADCC and CDC in \(\textit{vitro}\).

On the other hand, Kroon et al.\(^\text{[3]}\) found no correlation between the presence of antibodies and recent disease activity or other clinical characteristics such as age, gender, extension, and duration of vitiligo.

**Conclusion**

Our results support the role of AMA in the pathogenesis of nonsegmental vitiligo, correlating with the disease extent and severity. However, a longitudinal study in a large cohort of patients to evaluate the clinical and the predictive value of AMAs would be advisable.

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**Conflicts of interest**

There are no conflicts of interest.

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