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Levels of Some Autoantibodies in Vitiligo Patients: A Case-Control Study

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ABSTRACT

Background: Vitiligo is a type of autoimmune disease (AID) of the skin that is acquired and characterized by loss of melanocytes. The precise cause of vitiligo is still not well-identified. Autoimmune theory is the most supported by reliable laboratory and clinical data, particularly for non-segmental vitiligo (NSV).

Objective: This study aimed to compare the levels of circulating autoantibodies namely anti-thyroperoxidase Ab (anti-TPO), anti-melanocyte Ab (AMA), anti-nuclear Ab (ANA), in the sera of vitiligo patients versus controls.

Patients and Methods: This was a case-control study enrolled 50 vitiligo cases and40 healthy with matched age and sex as control group. The degree of vitiligo was assessed using the vitiligo area severity index (VASI) score. All patients and controls had laboratory test for measurement of anti-thyroperoxidase Ab, anti-melanocyte Ab and anti-nuclear Ab by ELISA kits. **Results:** There were significantly higher median levels of anti-TPO Ab, AMA and ANA compared to the control group. There were no significant differences in anti-TPO Ab levels, AMA levels and ANA levels based on gender, smoking status, and family history. There was a significant positive relationship between levels of anti-TPO Ab, AMA and ANA and the VASI score.

Conclusion: There was a potential relationship between anti-TPO Ab levels, AMA levels & ANA levels and disease severity. These results suggest that higher levels of these antibodies are associated with increased disease severity (assessed by VASI) in vitiligo patients.

Keywords: Vitiligo, Autoimmune, Anti-thyroid peroxidase, Anti-melanocyte Ab, Anti-nuclear Ab, Vitiligo area severity index.

INTRODUCTION

Vitiligo has been considered the commonest cause of acquired skin, depigmentation, and occasionally happens as an inherited disease. In clinical practice, it is characterized by the extensive loss of melanocytes which ultimately ends in well-defined white skin macules. From the histological point of view, it could be detected as basal hypopigmented areas and dermal inflammation comparative to the adjacent normal skin, associated with full or partial loss of melanocytes [1]. Vitiligo pathogenesis could be explained by numerous hypothesis such as neural hypothesis, genetic hypothesis, the autoimmune hypothesis, autocytotoxic, microenvironmental and multivariate theories, melanocytorrhagy hypothesis, viral factors, apoptotic mechanisms in addition to the generation of free radicals and cell adhesion disorders [2].

Even though numerous theories were conducted to clarify vitiligo pathogenesis, the autoimmune hypothesis is the most accepted one, being reliant on multiple epidemiologic, clinical, and experimental outcomes in particular for NSV [3]. Although it appears that segmental vitiligo (SV), has, various pathological mechanisms, there is agreement that in all types of vitiligo there is a particular level of autoimmune components [4].

In addition, vitiligo could be detected in cases with the autoimmune polyendocrine syndrome [5], and it is more often involved in family members of cases affected by AIDs, which include psoriasis, rheumatoid arthritis, lupus erythematosus, and pernicious anaemia. Therefore this study aimed to compare the levels of circulating autoantibodies namely anti-thyroperoxidase Ab (anti-TPO), anti-melanocyte Ab (AMA) and anti-nuclear Ab (ANA) in the sera of vitiligo patients versus controls.

PATIENTS AND METHODS

This study enrolled 90 cases. They were chosen from the Out-patient Clinic of Dermatology, Andrology & STDs Department, Mansoura University Hospitals during period from April 2021 to October 2021. The subjects were divided into, group A including 50 patients with NSV and group B including 40 age- and sex-matched healthy controls.

Inclusion criteria: Patients with non-segmental active vitiligo (occurrence of recent lesions or progression of current lesions either untreated or newly diagnosed within the 60 days before the study based on the patient's observation) and aged from 10 to 60 years old.

Exclusion criteria: Patients who received systemic steroids (within the last 2 weeks), patients who had narrow band sessions, and patients with systemic diseases or autoimmune diseases.

Methods: Every patient was subjected to history taking regarding age, gender, occupation, marital condition, pregnancy and lactation, dietary intake, accompanying psychiatric disturbances, accompanying medical or surgical problems, drug intake, familial history of vitiligo or other AID and duration of vitiligo. Complete general examination was conducted, which included weight, height, body mass index, and thyroid examination. Full dermatologic assessment included skin, hair, nail, oral and genital mucosa to rule out any accompanying diseases.

Examination of vitiligo included site, symmetry, extent, localization, clinical types, activity and stability.

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The percentage of vitiligo involvement was assessed by VASI score that was calculated with regard to hand units. A single hand unit was equal to one percent of the body surface area, one hundred percent is full depigmentation, 90% is dots of pigment present, 75% is depigmented area more than the pigmented one, 50% is pigmented and depigmented areas are identical, 25% is pigmented area more than depigmented area, and 10% is minor dots of depigmented areas present [6].

Laboratory investigations: A 5 cm venous blood sample were collected from patients and control group under complete asepsis precautions. The samples were stored for measurement of anti-thyroperoxidase Ab (anti-TPO) by ELISA kits designed for 96 tests, manufactured by INOVA diagnostics, Inc. (San Diego, United States of America). The sample should be stored at -20 ℃. After clotting, the serum was separated by centrifugation. The kit detects Anti-TPO of IgG class. During the first incubation, anti-TPO from test samples binds to TPO coated onto the inner surface of the microplate well. ANA (anti-nuclear Ab) was measured by ELISA kits designed for 96 tests, manufactured by INOVA diagnostics, Inc. (San Diego, United States of America). The sample was collected by venipuncture, separated after being clotted, the serum stored at -20 ℃. Anti-melanocyte Ab (AMA) was measured by ELISA kits (Sandwich-ELISA approach) designed for 96 tests (Wuhan fine Biotech co, China). The micro-ELISA plate provided in this kit was pre-coated with an antigen (Ag) specific to Human MC-Ab. Standards or specimens were added to the micro-ELISA plate wells and combined with the specialized Ag.

Ethical approval: The Ethics Committee of the Faculty of Medicine, Mansoura University approved the study (Approved No. MS.20. 071202.R1.R2). Each participant received a full summary of the study's aims prior to signing an informed consent form. The Helsinki Declaration was followed at all stages of the inquiry.

Statistical analysis

The collected data were revised, coded, and tabulated using SPSS (IBM Corp. Released 2017. Version 25. Armonk, NY). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). A significant p-value was considered when it is equal or less than 0.05.

RESULTS

Table **(1)** showed that there was no any statistically difference between patient and control concerning age & sex, while there was statistically difference between both groups regarding special habits and family history. There were significantly higher median levels of anti-TPO Ab (0.2 IU/mL), AMA (0.67 mg/ml) and ANA (0.17) compared to the control group (0.13, 0.58, 0.13 respectively) ($p < 0.001$).

Table (1): Comparison between the studied groups regarding socioeconomic data

	Healthy control (N = 40)		Vitiligo (N=50)		Test	*p*
	No.	%	No.	%		
Age (years)						
Mean ± SD	31.87±10.75		36.3±14.8		U=794.5	0.095
Gender						
Male	25	62.5%	15	30.0%	X^2= 0.984	0.321
Female	15	37.5%	35	70.0%		
Special habits						
Non-smoker	40	100.0%	42	84.0%	X^2= 7.024	0.008*
Smoker	0	0.0%	8	16.0%		
Family history						
Negative	40	100.0%	45	90.0%	X^2= 4.235	0.040*
Positive	0	0.0%	5	10.0%		
Anti-TPO Ab (IU/mL)						
Median	0.13		0.2		541.000	<0.001*
Min-max	0.07-1.01		0.08-2.14			
AMA (mg/ml)						
Median	0.58		0.67		536.000	<0.001*
Min-max	0.13-1.23		0.19-3.89			
ANA titre						
Median	0.13		0.17		471.500	<0.001*
Min-max	0.05-0.23		0.07-1.11			

Min-max and Median: Non parametric test. Min, minimum; Max, maximum; U, Mann Whitney test; X^2: Chi Square, *: Significant when p value <0.05.

Table **(2)** showed that the median duration of illness was 2.5 years, with a wide range from 0.08 to 20 years. The median VASI score was 2.5 and ranged from 0.25 to 13.5.

Table (2): Disease duration and disease severity (VASI score)

	Vitiligo N=50
Duration of illness (years)	
Mean ± SD	3.72±4.42
median	2.5
Min-max	0.08-20
VASI	
Mean ± SD	3.75±3.46
median	2.5
Min-max	0.25-13.5

Min, minimum; Max, maximum.

Table **(3)** showed the values that indicate moderate discriminative ability for all three antibodies. The sensitivity of anti-TPO Ab, AMA, and ANA was 76%, 63.3%, and 74% respectively, while the specificity was 62.5%, 70%, and 72.5% respectively. The PPV ranged from 71.7% to 77.1%, and the NPV ranged from 60.4% to 69.0%. The overall accuracy ranged from 66.3% to 73.3%. The p-values for all three antibodies were significant (< 0.001) indicating their potential for distinguishing vitiligo patients from the control group. The cut-off values for anti-TPO Ab, AMA, and ANA were > 0.138, > 0.617, and > 0.143 respectively. No significant differences in discriminatory a

bility between vitiligo cases and healthy subjects regarding anti-TPO, AMA, and ANA (p >0.05 for each).

Table (3): Value of anti-TPO, AMA and ANA as a predictive factor for discrimination between vitiligo cases and the controls.

	anti-TPO	**AMA**	**ANA**
AUC	0.730	0.727	0.764
95% CI	0.626 - 0.818	0.622 - 0.816	0.663 - 0.847
P1	<0.001	<0.001	<0.001
Cut off	>0.138	>0.617	>0.143
Sensitivity (%)	76	63.3	74
Specificity (%)	62.5	70	72.5
PPV (%)	71.7	72.5	77.1
NPV (%)	67.6	60.4	69.0
Accuracy (%)	70.0	66.3	73.3
P2	-	0.942	0.454
P3	-	-	0.458

AUC: Area under ROC curve; CI: Confidence interval, PPV, positive predictive value; NPV, negative predictive value. *: P value Significant <0.05. P1, probability of AUC; p2, comparison between discriminatory ability versus TPO; p3, comparison between discriminatory ability versus AMA.

Table **(4)** showed that there was no significant association between anti-TPO Ab, AMA and ANA with age or disease duration (p>0.05). In contrast, there was a significant positive association between anti-TPO Ab, AMA and ANA levels and the VASI score (p < 0.001), (p = 0.023), (p < 0.001) indicating a potential association between anti-TPO Ab, AMA and ANA levels and disease severity.

Table (4): Correlation of anti-TPO Ab, AMA and ANA with age, duration and VASI score

	anti-TPO Ab		**AMA**		**ANA**	
	Rs	***P***	***rs***	***P***	***Rs***	***P***
Age	0.149	0.301	-0.031	0.832	0.065	0.652
Duration	0.023	0.871	0.068	0.644	0.017	0.905
VASI	0.487	<0.001*	0.323	0.023*	0.998	<0.001*

rs, correlation coefficient, *: Significant when p value <0.05.

Table **(5)** showed the correlation of anti-TPO, AMA, and ANA with each other among all studied groups. As regards the control group, there was no significant correlation between anti-TPO and AMA or between anti-TPO and ANA. In the same line, there was no signifiant association between AMA and ANA (p>0.05 for each). In the vitiligo group, there was a +ve association between anti-TPO and AMA (rs = 0.308, p = 0.031) and a +ve association between anti-TPO and ANA (rs = 0.485, p < 0.001). In addition, there was a positive association between AMA and ANA (rs = 0.317, p = 0.026). After adjusting for other factors in the multivariate analysis, anti-TPO, AMA, and ANA remained significantly associated with vitiligo susceptibility. These findings indicated that higher levels of these antibodies are accompanied by an increased risk of developing vitiligo.

Table (5): Correlation of anti-TPO, AMA and ANA with each other among all studied groups

		AMA		**ANA**	
		rs	***p***	***Rs***	***p***
Control	**anti-TPO**	0.140	0.390	0.111	0.497
	AMA	-	-	-0.035	0.829
Vitiligo	**anti-TPO**	0.308	0.031*	0.485	<0.001*
	AMA	-	-	0.317	0.026*

rs, correlation coefficient, *: Significant when p value <0.05.

DISCUSSION

Vitiligo is an acquired skin lesion affects about 1.5% of subjects globally characterized by depigmented areas [7]. The mechanism of vitiligo is reflected by biomarkers such as histological examination of the skin, comprising more staining for CD4 and CD8 T

lymphocytes, or cytokines such as interleukin-1 β, interferon-γ, antibodies, biomarkers of oxidative stress [8].

Although the actual function of the Abs in vitiligo pathogenesis hasn't been detected, there is an association between their existence and the disease level, degree, and activity [9]. In addition, the sera from vitiligo cases could cause destruction to human melanocytes in vivo as well as in vitro [10].

In the context of melanocyte-specific cellular immune responses, novel researches displayed the existence of plasma MelanA-specific cT lymphocytes in a considerable number of vitiligo cases in comparison with healthy controls. The frequency of the T cells' high expression of the skin-homing receptor cutaneous lymphocyte-accompanying Ag was linked with the degree of depigmentation and the disease activity. In addition, isolated MelanA- specific T cells have the ability to lyse melanoma cells, which indicate their cytotoxic activity [11].

ANAs are essential for the diagnosis of autoimmune rheumatic diseases. In addition, such antibodies could be detected in cases complaining of organ-specific AIDs [12]. With regard to the dermatological diseases such as vitiligo, a positive ANA may be a feature of an underlying, silent autoimmune state, which include pernicious anaemia, or autoimmune thyroiditis (AIT) [13].

Rapid detection of AIDs is of great importance. On the other hand, owing to the lack of distinctive manifestations in the initial phases of such AIDs in several subjects, reaching a proper diagnosis is challenging and often delayed [9]. Such an aspect denotes the significance of searching for laboratory tests that might be utilized for diagnosis and screening for AIDs early for adequate therapy could be prescribed [14].

We aimed to compare the levels of circulating autoantibodies namely anti-thyroperoxidase Ab, anti-melanocyte Ab and anti-nuclear Ab in the sera of vitiligo patients versus controls. In the current study, gender distribution showed that the percentage of females in the vitiligo group was 70.0%. This is in accordance with **Montgomery *et al.*** [15] who found that most patients (66%) identified were females. This could be explained by the fact that the distressing appearance of the disease forced women to seek for dermatological consultation earlier than males as they more concerned about their appearance. Moreover, females are more vulnerable to AID compared to males.

In the instant study, the median duration of illness was 2.5 years, with a wide range from 0.08 to 20 years. This result is similar to **Hou *et al.*** [16] who reported that median disease duration was 2 years. This corroborates its slow progression and asymptomatic nature.

In the current study, the vitiligo group exhibited significantly higher median levels of anti-TPO Ab (0.20 IU/mL) compared to the control group (0.13 IU/mL) ($p < 0.001$). Similarly, the vitiligo group had significantly higher median levels of AMA (0.67 mg/ml) and ANA (0.17) compared to the control group (AMA: 0.58 mg/ml, ANA: 0.13) ($p < 0.001$ for both comparisons). These results indicated a strong association between vitiligo and elevated levels of these antibodies, suggesting an autoimmune component in the pathogenesis of vitiligo.

Regarding levels of anti-TPO Ab among vitiligo patients, this Ab has been confirmed as a sensitive test for the determination of early subclinical AITD (auto immune thyroid disease); follow-up of the responses to immunotherapy and recognition of at-risk cases for AITD [17]. In line with our results a recent study reported that frequency of +ve thyroid peroxidase antibodies (TPOAb) in cases of generalized vitiligo was reported in 32.8%. So, they reported that ratio of positive TPOAb is significant in cases of generalized vitiligo [18]. The prevalence of anti-TPO antibodies ranges from 21% to 40% in cases with vitiligo [19]. Cases with NSV display a higher inheritance of AIDs compared to cases with SV [20].

There was a significant positive relationship between anti-TPO Ab, AMA and ANA levels and the VASI score ($p<0.05$ for all) indicating a potential relationship between anti-TPO Ab, AMA and ANA levels and disease severity. **Fouad *et al.*** [21] found a positive association between values of anti-TPO and the disease duration among vitiligo cases, which also agree with **Gey *et al.*** [22] and **Atallah *et al.*** [23] who revealed that the risk of cases with vitiligo developing AITD is increased by two-fold every five years, hence cases were frequently screened for anti-thyroid antibodies.

Regarding levels of AMA & ANA among vitiligo patients, our study displayed that the vitiligo group had significantly higher median levels of AMA (0.67 mg/ml) and ANA (0.17) compared to the control group (AMA: 0.58 mg/ml, ANA: 0.13) ($p < 0.001$ for both comparisons). In agreement with our results, **El-Gayyar *et al.*** [24] conducted their study to assess the severity of vitiligo and displayed that ANA, AMA, and C4 levels were significantly higher in the sera of vitiligo cases compared to healthy controls. ANA, AMA, and C4 serum levels displayed significant positive associations with VASI score. **Pandit** [25] noticed that there was a significant increase in AMA level in vitiligo cases compared to healthy control.

Li *et al.* [26] confirmed that hydroxychloroquine could protect melanocytes from autoantibody-mediated injury by decreasing the binding of Ag–Ab complexes reversing the activities of ADCC (antibody dependent cellular cytotoxicity) and CDC in vitro. **Chaiyabutr *et al.*** [27] found among 85 vitiligo cases, the ANA prevalence was 35.3%. The speckled ANA pattern was the commonest, and the most of cases (80%) had a titer of ≤ 1:100. Factors accompanied by positive ANA were female sex and positive anti-thyroglobulin (ATG).

A lot of factors were reported to have a significant association with positive ANA in cases with vitiligo, which involved female sex, existence of ATG [27], disease duration more than 12 months [28] and lesions

affecting the hands and arms [29]. Of note, vitiligo cases with AIT were demonstrated to have significantly smaller thyroid volume if ANA was present [30].

CONCLUSION

The vitiligo group exhibited significantly higher median levels of anti-TPO Ab compared to the control group. Similarly, the vitiligo group had significantly higher median levels of AMA and ANA compared to the control group (for both comparisons). These results indicated a strong association between vitiligo and elevated levels of these antibodies suggesting an autoimmune component in the pathogenesis of vitiligo. There was a potential correlation between anti-TPO Ab levels, AMA levels & ANA levels and disease severity. These results suggest that higher levels of these antibodies are associated with increased disease severity (assessed by VASI) in vitiligo patients.

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REFERENCES

1. **Ezzedine K, Lim H, Suzuki T *et al.* (2012):** Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. Pigment Cell Melanoma Res., 25 (3): 1-13.
2. **Xie B, Zhu Y, Shen Y *et al.* (2023):** Treatment update for vitiligo based on autoimmune inhibition and melanocyte protection. Expert Opin Ther Targets, 27 (3): 189-206.
3. **Baldini E, Odorisio T, Sorrenti S *et al.* (2017):** Vitiligo and Autoimmune Thyroid Disorders. Front Endocrinol., 8: 290-95.
4. **Lim H, Bae M, Jeong K *et al.* (2016):** Positivity rates of antithyroid antibody, antinuclear antibody and thyroid peroxidase antibody in different types of vitiligo. Clin Exp Dermatol., 41 (3): 242-247.
5. **Cutolo M (2014):** Autoimmune polyendocrine syndromes. Autoimmun Rev., 13 (2): 85-89.
6. **Komen L, da Graça V, Wolkerstorfer A *et al.* (2015):** Vitiligo Area Scoring Index and Vitiligo European Task Force assessment: reliable and responsive instruments to measure the degree of depigmentation in vitiligo. Br J Dermatol., 172 (2): 437-443.
7. **Abdelhameed M, Mostafa F, Abdelmohsen R *et al.* (2023):** Association of Thyroid Dysfunction and Autoantibody Abnormality with Vitiligo in Pediatric Patients: A Cross-Sectional Study. The Egyptian Journal of Hospital Medicine, 91 (1): 5289-5296.
8. **Premkumar M, Kalarani I, Mohammed V *et al.* (2024):** An Extensive Review of Vitiligo-Associated Conditions. International Journal of Dermatology and Venereology, 7 (01): 44-51.
9. **Marchioro H, Castro C, Fava V *et al.* (2022):** Update on the pathogenesis of vitiligo. Anais Brasileiros de Dermatologia, 97 (4): 478-490.
10. **Chen J, Li S, Li C (2021):** Mechanisms of melanocyte death in vitiligo. Medicinal Research Reviews, 41 (2): 1138-1166.
11. **Diotallevi F, Gioacchini H, De Simoni E *et al.* (2023):** Vitiligo, from pathogenesis to therapeutic advances: state of the art. International Journal of Molecular Sciences, 24 (5): 4910-15.
12. **Chivu A, Bălăşescu E, Pandia L *et al.* (2022):** Vitiligo—Thyroid Disease Association: When, in Whom, and Why Should It Be Suspected? A Systematic Review. Journal of Personalized Medicine, 12 (12): 2048-53.
13. **Martini A, Ravelli A, Avcin T *et al.* (2019):** Toward new classification criteria for juvenile idiopathic arthritis: first steps, pediatric rheumatology international trials organization international consensus. The Journal of Rheumatology, 46 (2): 190-197.
14. **Tarvin S, O'Neil K (2018):** Systemic lupus erythematosus, Sjögren syndrome, and mixed connective tissue disease in children and adolescents. Pediatric Clinics, 65 (4): 711-737.
15. **Montgomery S, Syder N, Barajas G *et al.* (2023):** Vitiligo and Associated Psychological Comorbidities: A Retrospective Cross-sectional Analysis in an Urban Population. Arch Dermatol Res., 316 (1): 14. doi: 10.1007/s00403-023-02771-7.
16. **Hou X, Wu N, Xu M *et al.* (2023):** Demographic and clinical feature disparity between progress and non-progress patients with vitiligo after COVID-19 vaccination: A cross-sectional study. Exp Dermatol., 32 (9): 1344-1349.
17. **Prashant P, Kataria U, Vashist S *et al.* (2024):** Vitiligo and Autoimmune Thyroid Disorders: Is There Any Correlation?. Recent Updates in Disease and Health Research, 1(27): 89-104.
18. **Ashraf A (2021):** Frequency of positive thyroid peroxidase antibody levels in patients of generalized vitiligo. Journal of Pakistan Association of Dermatologists, 31 (4): 583-587.
19. **Gehris R, Herman E (2020):** Pediatric teledermatology: a review. Current Dermatology Reports, 9: 114-122.
20. **Rork J, Rashighi M, Harris J (2016):** Understanding autoimmunity of vitiligo and alopecia areata. Current Opinion in Pediatrics, 28 (4): 463-469.
21. **Fouad S, El Shahawy E, Darwish N *et al.* (2024):** Anti-Thyroid Peroxidase Antibody and Segmental Vitiligo. The Egyptian Journal of Hospital Medicine, 94: 799-804.
22. **Gey A, Diallo A, Seneschal J *et al.* (2013):** Autoimmune thyroid disease in vitiligo: multivariate analysis indicates intricate pathomechanisms. British Journal of Dermatology, 168 (4): 756-761.
23. **Atallah R, Zaky M, Ghanem A (2018):** Thyroid dysfunction in non-segmental versus segmental vitiligo. The Egyptian Journal of Hospital Medicine, 73 (10): 7622-7631.
24. **El-Gayyar M, Helmy M, Amer E *et al.* (2020):** Antimelanocyte Antibodies: A Possible Role in Patients with Vitiligo. Indian J Dermatol., 65 (1): 33-37.
25. **Pandit S (2019):** Assessment of antimelanocyte antibodies in patients of vitiligo. International Journal of Dermatology, Venereology and Leprosy Sciences, 2 (1): 16-18.
26. **Li M, Rudensky A (2016):** T cell receptor signalling in the control of regulatory T cell differentiation and function. Nat Rev Immunol., 16 (4): 220-233.
27. **Chaiyabutr C, Wongpraparut C, Charoenpipatsin N *et al.* (2020):** The necessity of antinuclear antibody investigation in pre-phototherapy vitiligo patients: A retrospective study. Photodermatol Photoimmunol Photomed., 36 (5): 373-377.
28. **Hann S, Im S, Kim H *et al.* (1993)**: Increased incidence of antismooth muscle antibody in Korean vitiligo patients. J Dermatol., 20(11): 679-683.
29. **Vachiramon V, Harnchoowong S, Onprasert W *et al.* (2017):** Prevalence of Thyroid Abnormalities in Thai Patients with Vitiligo. Biomed Res Int., 17: 7502935. doi: 10.1155/2017/7502935.
30. **Zettinig G, Tanew A, Fischer G *et al.* (2003):** Autoimmune diseases in vitiligo: do anti-nuclear antibodies decrease thyroid volume? Clin Exp Immunol., 131 (2): 347-354.