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Helicobacter pylori seropositivity in Iranian patients with vitiligo: a case-control study

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Background: Vitiligo is an autoimmune disease of the skin that affects both sexes and people of any age. The genetic and environmental factors are involved in the vitiligo etiology. *Helicobacter pylori* (*H. pylori*) has an important role in vitiligo progression. Therefore, the present study evaluated *H. pylori* seropositivity in vitiligo patients compared to healthy individuals.

Method: *H. pylori* infection was investigated in 210 vitiligo patients and 127 sex- and age-matched healthy controls using Enzyme-Linked Immunosorbent Assay (ELISA) test. The data was analyzed using SPSS software version 20.0, and the groups were compared using T-test and ANOVA tests. $P < 0.05$ was considered statistically significant.

Results: Vitiligo patients had higher median levels of IgG (29.68 ± 28.28 RU/mL) than (19.08 ± 20.12 RU/mL) in healthy controls ($P < 0.000$). Moreover, there was no significant difference between groups based on the level of IgM ($P < 0.207$). In the vitiligo group, IgG or IgM means were different compared to age ($P < 0.33$)/ ($P < 0.017$) and early symptoms ($P < 0.00$) ($P < 0.02$), respectively. Unlike IgG, there was a significant difference between the mean level of IgM, the onset age of vitiligo ($P < 0.022$), and the duration of the disease ($P < 0.05$). Moreover, males and females with vitiligo had a higher seropositivity to *H. pylori* antibodies than the control group.

Conclusion: Vitiligo was found to be significantly associated with *H. pylori* in Iranian patients. Therefore, it seemed probable that *H. pylori* had an important role in the initiation or progression of disease activity in vitiligo.

Keywords: vitiligo, *helicobacter pylori,* seropositivity, enzyme-linked immunosorbent assay (ELISA)

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INTRODUCTION

Vitiligo is a depigmentation skin disease characterized by the presence of asymptomatic white spots with clear borders caused by a lack of functional melanocytes in the epidermis [1]. The disease affects areas of the skin that are exposed to the sun, as well as the eyes and hair [2]. The disease affects 0.06 to 2.28% of all races (about 56 million people) and both sexes equally [3]. Vitiligo typically develops early in life (between the ages of 10 and 30), with the majority of patients showing symptoms before the age of 40 and half before the age of 20 [4]. The disease has two clinical types: A and B. Type A is more prevalent, with a somewhat symmetrical pattern of involvement characterized by white and depigmented macules and colorless spots measuring 0.5-0.5 cm with perfectly clear margins. Common sites of type A involvement include the back of the hands and fingers, the face, body folds, armpits, and genitals. Type A mostly affects the pores of the body, such as the eyes, nostrils, mouth, nipples, navel, and anus [5]. Type B is a type of vitiligo that is confined to a specific part of the body, such as an organ, and is more common in childhood [6]. The cause of vitiligo is unknown. However, there are several prominent theories regarding the multifactorial cause of the disease [7]. The interaction of environmental and genetic agents contributes to the development of the disease [8]. Several potential environmental factors, including infectious agents, also appeared to be associated with vitiligo. Microbial agents are involved in the spread of autoimmunity due to their interactions with the environment and the human immune system [9]. The most common infectious agents in the pathophysiology of vitiligo are acquired immunodeficiency virus (HIV) [10], hepatitis C virus (HCV) [11], and cytomegalovirus (CMV) [12]. *Helicobacter pylori* is one of the most recent bacteria which is implicated in the pathogenicity of Vitiligo.

H. pylori is a Gram-negative, microaerophilic, and spiral-shaped bacteria that colonizes the gastric mucus of 80-50% of the world's population [13], with different degrees of prevalence (7% in the Czech Republic and 87% in South Africa) [14]. *H. pylori* is classified into two types (I and II), type I expresses the Vacuolating cytotoxin (VacA) and the Cytotoxin-associated gene (CagA), whereas type II does not [15].

H. pylori infection is related to gastric disorders pathogenesis such as gastritis, gastric cancer, duodenal and gastric ulcers, and a variety of extra-digestive disorders [16]. Since the risk of contact with bacteria is related to socioeconomic status and poor living conditions, infection is more common in developing countries, particularly in children under the age of 10 [17]. In terms of the potential course of intestinal diseases, *H. pylori* infection stimulates strong immune responses by secreting a variety of host factors and bacterial cytotoxic agents that lead to neutrophil and monocyte infiltration into intestinal mucosa and mucosal inflammation [18]. It also produces enzymes such as catalase, urease, lipase, protease, and phospholipase, which can be involved in the pathogenesis of intestinal inflammation [16]. In addition to *H. pylori's* known role in intestinal disorders, some researchers proposed an essential role of *H. pylori* in several extra-intestinal diseases such as blood, nervous, metabolic, autoimmune, and dermatological diseases. As a result, some studies suggested a relationship between *H. pylori* infection and several distinct immune and non-immune disorders with skin manifestations, including Behçet's disease, psoriasis, rosacea, sweet syndrome, chronic itching, burning, systemic sclerosis, and atopic skin inflammation [19,20]. *H. pylori* infection can be diagnosed through various methods including histology, rapid urease test (RUT), PCR, stool antigen test, and serology [21]. The key mechanism of physiological damage includes the onset and continuation of a chronic inflammatory response, as the *H. pylori* abdominal migration triggers the response of Th1 cells and IL-2 and IFN-γ production. Furthermore, this chronic infection leads to the production of higher levels of pre-inflammatory cytokines such as TNF-α, IL-6, IL-8, and IL-10, leading to uncontrollable growth and CD5 cell proliferation, which produces self-activating IgM and IgG3 antibodies [22].

Helicobacter antigens also stimulate the production of autoantibodies by activating T cells. Furthermore, the thermal shock protein (HSP) of *H. pylori* has an important potential in the pathogenesis of autoimmune disorders, due to its high similarity with human HSP [23]. Therefore, according to these findings, it is widely known that the presence or absence of *H. pylori* infection might lead to the development of several autoimmune diseases, such as vitiligo [24-26]. Hence, the present study compared *H. pylori* seropositivity in vitiligo patients and the healthy control group.

METHODS

Study area

The present case-control study was carried out from September 2022 to February 2023 in Isfahan (Iran), (latitude 30-34°N and longitude 49-55°E), with moderate and dry weather ranging between 10-40 °C at a distance of around 450 Km from the Persian Gulf. Isfahan had a population of more than 4 million people who were ethnically Persian/Caucasian. According to a study in 2000, vitiligo outbreaks were high in Isfahan, particularly among women, and could be attributed to hereditary or racial factors [27].

Patients

210 confirmed vitiligo patients (146 active and 64 inactive) with less than 20% body coverage, who were being treated by dermatologists of Isfahan University's Skin Diseases Clinics of the Isfahan University of Medical Sciences, with or without significant improvement, were selected randomly. To complete the sample size, the random sampling method was used with the following formula:

$N = (Z_{1-\alpha/2}+Z_{1-\beta})^2[\ P_1(1-P_1)+ (P_2(1-P_2)\ /d^2$.

$\alpha = 0.05$

$\beta = 0.2$

$d = 0.3$

Patients were randomly assigned based on a list prepared in advance by a computer program of the subjects attending skin centers. Vitiligo was divided clinically into six types: Vulgaris, Acrofacial, Focal, Piece, Global, and Mucous. Then, basic data was collected using a researcher-made questionnaire. The questionnaire consisted of questions about age, sex, family history of vitiligo, age of onset, duration of disease, digestive problems, autoimmune diseases (including Hypothyroidism, Alopecia Areata, Multiple Sclerosis, and so on), and disease course (the disease was defined as "active" if there were alternating phases of worsening and improvement in the previous year, and "inactive" otherwise.) [28].

Controls

There were 127 healthy controls from Isfahan who were matched in age and sex. The control group had no family members or relatives with vitiligo. In addition, none of the control group had malignant, allergic, or autoimmune disease.

The inclusion criteria

The inclusion criteria were the age range of 17-56, not taking the antibiotics (Bismuth subsalicylate), and proton pump inhibitors (Nexium and Prilosec) for one month before the initiation of the study.

The exclusion criteria

Gastrointestinal symptoms (bloating, nausea, high intestinal upset, premature satiety, heartburn, etc.), pregnancy, or breastfeeding were considered as exclusion criteria for the study. Moreover, unwillingness to donate blood samples or complete the questionnaires was considered as the exclusion criterion.

Enzyme-Linked Immunosorbent Assay (ELISA) Test

A 5 mL peripheral blood sample was taken from patients and controls. All the samples were centrifuged at 5000 rpm for 5 minutes, and the isolated sera were stored at -20 °C. Then, the serum levels of IgM and IgG to *H. pylori* were determined using the ELISA method, and the *H. pylori* IgG and IgM kit (Order no: E1 2080-9601 G, Euoimmun olfactory, Germany). Tests were performed according to the manufacturer's instructions, and *H. pylori* IgM titer > 12 RU/mL and *H. pylori* IgG titer > 20 RU/mL were regarded as seropositive.

Statistical analysis

Serum levels of IgG and IgM to *H. pylori* were expressed as mean ± SD. The levels of IgM and IgG against *H. pylori* in the two groups of vitiligo patients and control were compared using Student's t-test. Moreover, IgM and IgG serum levels in each group were compared to other variables with non-parametric ANCOVA and Chi-Square tests. *P*-value < 0.05 was considered statistically significant.

Ethical standards

The present study was ethically approved by the Ethics Committee for Clinical Research of Isfahan University of Medical Sciences, with code number IR.MUI.REC.1395.2.239. After the purpose and protocol of the study were explained to the participants, written informed consent was obtained from them.

RESULTS

Patients and controls

The study included 210 vitiligo patients (106 females and 104 males). The female-to-male ratio was 1/1.01. The mean age of the participants was 39.7 ± 13.16 years. The control group included 127 healthy controls (75 females and 52 males). The female-to-male ratio was 1/1.4. The mean age of the participants was 40.6 ± 15.14 years. Among patients with vitiligo, 64 patients (30.5%) had an inactive course, and 146 patients (69.5%) had an active course of vitiligo. The most common symptoms of vitiligo were flat white spots or patches on the hands, fingertips, wrists, around the eyes or mouth, or on the feet. The baseline demographic in the vitiligo patients is shown in Table 1.

ELISA

There was a statistically significant correlation between *H. pylori* positivity in vitiligo patients and seropositivity in healthy controls. In contrast to IgM, vitiligo patients had a higher median level of IgG to *H. pylori* than the healthy controls ($P < 0.00$) (Table 2).

In addition, sex-based data analysis revealed a significant difference in the median level of IgG and IgM against *H. pylori* between the females and males in the vitiligo and control groups. The median titer of either *H. pylori* IgG / IgM in females and males of the vitiligo group was statistically higher than the females and males of the healthy control group (Table 3).

Statistically, there was a significant relationship between the mean levels of IgG or IgM to *H. pylori* with age and early symptoms in the vitiligo patients compared to the controls. However, there was no significant relationship between the mean of IgG/IgM levels with sex, self-autoimmune diseases, duration of disease, smoking, and course of vitiligo.

In contrast to IgG, there was a significant relationship between the mean IgM levels and the onset age and duration of the disease in the vitiligo patients. Unlike IgM, there was a significant relationship between the mean of IgG levels with digestive problems.

Moreover, there was no significant relationship between the duration of the disease with digestive problems and the course of the disease.

Regarding digestion problems, there was a significant relationship between self-autoimmune diseases (but not early symptoms) and gastrointestinal issues.

In terms of the course of the disease, no correlation was found between the onset age, autoimmune

Table 1. The percentage of patients' characteristics

Characterization	Patients (n = 210)
Nationality (Iranian)	%96.7
Family history of vitiligo	%39.0
History of travel to the sea	%31.4
History of self-autoimmune diseases	%26.7
Family history of autoimmune diseases	%36.7
Smoking history	%16.2 (male)
Drinking history	%9.0 (male)

N, number

Table 3. The mean serum level of IgG/IgM to *H. pylori* in the males and females with vitiligo and controls

	Patients (n = 210)	Healthy Control (n = 127)	*P*-value
H. pylori IgG (RU/ML)			
Female	29.8 ± 29.51	19.23 ± 22.19	0.000
Male	29.5 ± 27.11	18.86 ± 16.88	0.001
H. pylori IgM (RU/ML)			
Female	0.85 ± 0.72	0.52 ± 0.29	0.000
Male	0.51 ± 0.43	0.47 ± 0.24	0.008

F, female; M, male; n, number

Table 2. Baseline data (Demographic and clinical characteristics of vitiligo patients and healthy controls) (mean ± SD) and serum IgG/IgM concentrations of patients with vitiligo and the control group

	Patient with vitiligo (n = 210)	Control (n = 127)	*P*-value
Age, years, mean ± SD	(39.72 ± 13.16)	(40.64 ± 15.14)	0.55
Sex			
Women (%)	50.5	59.1	
Men (%)	49.5	40.9	
Disease duration, years, mean ± SD	(12.8 ± 10.7)	----------	
H. Pylori IgG (RU/mL)	(29.68 ± 28.28)	(19.08 ± 20.12)	0.000
H. Pylori IgM (RU/mL)	(0.50 ± 0.27)	(0.68 ± 0.62)	0.207

N, number

diseases, gastrointestinal issues or early symptoms, and the course of disease.

DISCUSSION

The present study clearly showed seropositivity of *H. pylori* in the vitiligo patients' sample. Thus, the mean level of IgG to *H. pylori* in the vitiligo patients was significantly higher than the controls. Regarding the relationship between *H. pylori* and vitiligo, several studies were conducted, and different findings were reported.

In 2014, Emine Nur RİFAİOĞLU *et al.* found that the prevalence of *H. pylori* in 34 patients with vitiligo (64.7%) was significantly higher than in 30 patients in the control group (33.3%) [29]. Furthermore, Doğan *et al.* examined 79 people with vitiligo and 72 with telogen effluvium (TE) for the presence of IgG and Cag A Helicobacter pylori. Their findings indicated higher levels of serum IgG and *Helicobacter* CagA in people with vitiligo than in the TE group [25]. Moreover, similar results and conclusions were revealed in another study performed on an Iraqi population using the *H. pylori* stool antigen test [30]. Tamara A A Abdelmoneim's study also found a high prevalence of *H. pylori* among vitiligo patients [31]. In contrast, Seray Külcü Çakmak conducted a study and reported that *H. pylori* infection did not increase in vitiligo patients [26].

Vitiligo is also widely recognized to affect both sexes and people of all ages [32]. In the present study, vitiligo was significantly more prevalent in middle-aged (30-60), and the female-to-male ratio was equal. Furthermore, there was an age-related increased frequency of *H. pylori* infection as that of vitiligo, and we found a positive relationship between IgM (not IgG) with age of onset of vitiligo and duration of disease. The median titer of *H. pylori* IgG/IgM was not different between males and females with vitiligo; although it was statistically higher than the females and males of the healthy control group. Previous reports of Arýcan *et al.* indicated that vitiligo was common in both sexes [33], which was consistent with the findings of the present study. In contrast, other studies revealed that sex played a role in the etiology of the disease [27,34].

Furthermore, research showed that vitiligo was related to several autoimmune disorders, especially pernicious anemia, thyroid disease, diabetes mellitus, atopic dermatitis, and alopecia areata [35]. The present study also included vitiligo patients with a history of autoimmune diseases such as lupus, alopecia, type 1 diabetes, hypothyroidism, and so on. However, there was no significant relationship among the mean of IgG or IgM levels with autoimmune diseases in vitiligo patients.

A recent study demonstrated that infections might play a significant role in the severe forms of vitiligo [36]. Nevertheless, this study found no significant relationship between active vitiligo and *H. pylori*. Consequently, the median level of *H. pylori* IgG/IgM in patients with inactive vitiligo was insignificant compared to active vitiligo. Similarly, Dugan *et al.* found no association between positive *H. pylori* and vitiligo activity score [25]. Çakmak *et al.* also reported no significant relationship between vitiligo activity and *H. Pylori* SAT positivity [26]. On the contrary, Ola Ahmed Bakry's study showed that there was an association between *H. pylori* and active vitiligo [24].

Research also showed that the progression of vitiligo varied between individuals, depending on factors such as genetics, infections, skin sensitivity, consumption of alcohol, smoking, and so on. These risk factors were believed to potentially exacerbate the condition, although, with appropriate treatment and avoidance of triggers, vitiligo generally could be well controlled [37]. Despite this matter, no relationship between smoking and alcohol consumption with the progression of vitiligo was found in the present research. Similarly, Rashidi reported that smoking could not influence vitiligo [38]. However, Young Bok Lee reported that the risk of vitiligo in current smokers was reduced in relation to the dose of smoking [39].

Moreover, research showed that apart from accelerating leaky gut symptoms, alcohol can exacerbate the vitiligo symptoms for a variety of reasons, including increased levels of toxins in the blood, liver damage, nutritional deficiencies, depression, and infections [40]. In the present study, few patients with vitiligo consumed alcohol, and there was no significant association between alcohol consumption with *H. pylori* infection and the activity of vitiligo.

Although this study was conducted in a large cohort population, the possible involvement of *H. pylori* in vitiligo requires further investigation in other populations using multiple exact diagnostic tests to obtain more detailed evidence about the

relationship between *H. pylori* and vitiligo.

CONCLUSION

With regard to the *H. pylori* infection frequency in vitiligo patients, routine screening of vitiligo patients for *H. pylori* infection was critical. Consequently, eradication treatment could be pursued as an initial step, and additional treatment options could be available for these patients, particularly in areas with a high prevalence of *H. pylori* infection. Thus, further studies are required to determine the significance of these results.

Author's Contribution

M.A. contributed to the drafting and revising of the draft and performed the project. SH.A. edited the paper. F.I. contributed to the drafting and revising of the draft. MA.N drafted and revised the draft. SM.H analyzed the data. ZA.SH conceived and designed the experiments. SH.H approved the final version of the manuscript.

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Compliance with Ethical Standards

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The ethical approval number of the study was IR.MUI.REC.1395.2.239.

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Conflicts of Interest: None declared.

REFERENCES

1. Lotti T, D'Erme AM. Vitiligo as a systemic disease. Clin Dermatol. 2014;32(3):430-4.
2. Goldsmith LA, Katz SI, Gilchrest BA, et al. Fitzpatrick's Dermatology in General Medicine. 8th edition. USA: McGrawHill Medical; 2012.
3. Krüger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. Int J Dermatol. 2012;51(10):1206-12.
4. Nicolaidou E, Mastraftsi S, Tzanetakou V, et al. Childhood vitiligo. Am J Clin Dermatol 2019;20:515-26.
5. van Geel N, Speeckaert R. Segmental vitiligo. Dermatol Clin. 2017;35(2):145-50.
6. van Geel N, Mollet I, Brochez L, et al. New insights in segmental vitiligo: case report and review of theories. Br J Dermatol. 2012;166(2):240-6.
7. Wu J, Zhou M, Wan Y, et al. CD8+ T cells from vitiligo perilesional margins induce autologous melanocyte apoptosis. Mol Med Rep. 2013;7(1):237-41.
8. Metwalley KA, Farghaly HS. Hepatitis C virus infection in a child with autoimmune polyendocrine syndrome type 2: a case report. J Med Case Rep. 2012;6:1-4.
9. Kundu RV, Mhlaba JM, Rangel SM, et al. The convergence theory for vitiligo: a reappraisal. Exp Dermatol. 2019;28(6):647-55.
10. Xuan L, Baohua Y, Lan B. Alopecia areata and vitiligo as primary presentations in a young male with human immunodeficiency virus. Indian J Dermatol. 2014;59(2):209.
11. Fawzy MM, Hammad NM, Sharaf AL, et al. Hepatitis C virus infection could be a risk factor for adult-onset vitiligo in Egyptian patients: a cross-sectional study. J Cosmet Dermatol. 2022;21(10):4983-9.
12. Zhuang T, Yi X, Chen J, et al. Intracellular virus sensor MDA5 exacerbates vitiligo by inducing the secretion of chemokines in keratinocytes under virus invasion. Cell Death Dis. 2020;11(6):453.
13. Crowe SE. *Helicobacter pylori* infection. New Eng J Med. 2019;380(12):1158-65.
14. Muhammad JS, Zaidi SF, Sugiyama T. Epidemiological ins and outs of *helicobacter pylori*: a review. J Pak Med Assoc. 2012;62(9):955.
15. Chen Y-L, Mo X-Q, Huang G-R, et al. Gene polymorphisms of pathogenic *Helicobacter pylori* in patients with different types of gastrointestinal diseases. World J Gastroenterol. 2016;22(44):9718.
16. Charitos IA, D'Agostino D, Topi S, et al. 40 years of *Helicobacter pylori*: a revolution in biomedical thought. Gastroenterol Insights. 2021;12(2):111-35.
17. Benajah DA, Lahbabi M, Alaoui S, et al. Prevalence of *Helicobacter pylori* and its recurrence after successful eradication in a developing nation (Morocco). Clin Res Hepatol Gastroenterol. 2013;37(5):519-26.
18. White JR, Winter JA, Robinson K. Differential inflammatory response to *Helicobacter pylori* infection: etiology and clinical outcomes. J Inflamm Res. 2015:137-47.
19. Mărginean CD, Mărginean CO, Meliț LE. *Helicobacter pylori*-Related Extraintestinal Manifestations—Myth or Reality. Children. 2022;9(9):1352.
20. Wedi B, Kapp A. *Helicobacter pylori* infection in skin diseases: a critical appraisal. Am J Clin Dermatol. 2002;3:273-82.

21. Godbole G, Mégraud F, Bessède E. Diagnosis of *Helicobacter pylori* infection. Helicobacter. 2020;25:e12735.
22. Magen E, Delgado J-S. *Helicobacter pylori* and skin autoimmune diseases. World J Gastroenterol. 2014;20(6):1510.
23. Moyat M, Velin D. Immune responses to *Helicobacter pylori* infection. World J Gastroenterol. 2014;20(19):5583.
24. Bakry OA, Basha M, El Hefnawy S, et al. Relationship between disease activity and *Helicobacter pylori* infection in patients with vitiligo. Indian Dermatol Online J. 2018;9(1):59-61.
25. Doğan Z, Özdemir P, Ekşioğlu M, et al. Relationship between *Helicobacter pylori* infection and vitiligo: a prospective study. Am J Clin Dermatol. 2014;15:457-62.
26. Çakmak S, Tantoglu B, Onan D, et al. The frequency of *Helicobacter pylori* infection in vitiligo patients. Pigment Int. 2015;2(2).
27. Momeni A, Kharazi M, Amin Javaheri M. Vitiligo in Isfahan, Iran: clinical study of 437 cases. Iran J Dermatol. 2000;3(3):29-34.
28. Aghaei M, Aghaei S, Nilforoushzadeh M-A, et al. Chlamydia pneumoniae seropositivity in the Iranian patients with the skin inflammatory disorder of rosacea. Adv Biomed Res. 2023;12(1):72.
29. Rifaioğlu EN, Aydoğan F, Şen BB, et al. Investigation into the frequency of *Helicobacter pylori* infection with carbon 14 urea breath test in patients with vitiligo. Turk J Med Sci. 2014;44(6):1051-4.
30. Bdaiwi SA, AL-Hattab MK, AL-Nafakh MS. The frequency of *Helicobacter pylori* stool antigen in patients with different types of vitiligo in Iraqi Patients. Int J Pharm Res. 2020;12(2).
31. Abdelmoneim TA, Aljarbou AN, Alfarouk KO, et al. The prevalence of vitiligo variants in association with *helicobacter pylori* infection. Am J Dermatol Venereol. 2018;7(1):1-5.
32. Mahajan VK, Vashist S, Chauhan PS, et al. Clinico-epidemiological profile of patients with vitiligo: a retrospective study from a tertiary care center of North India. Indian Dermatol Online J. 2019;10(1):38.
33. Arýcan O, Koç K, Ersoy L. Clinical characteristics in 113 Turkish vitiligo patients. Acta Dermatovenerol Alp Pannonica Adriat. 2008;17(3):129-32.
34. de Barros JC, Filho CDASM, Abreu LC, et al. A study of clinical profiles of vitiligo in different ages: an analysis of 669 outpatients. Int J Dermatol. 2014;53(7):842-8.
35. Dahir AM, Thomsen SF. Comorbidities in vitiligo: comprehensive review. Int J Dermatol. 2018;57(10):1157-64.
36. Castanet J, Ortonne J-P. Pathophysiology of vitiligo. Clin Dermatol. 1997;15(6):845-51.
37. Aghamajidi A, Raoufi E, Parsamanesh G, et al. The attentive focus on T cell-mediated autoimmune pathogenesis of psoriasis, lichen planus and vitiligo. Scand J Immunol. 2021;93(4):e13000.
38. Rashidi T, Ghasemi-rad M, Dehghan M, et al. Comparison of the smoking prevalence in patients with vitiligo, other skin diseases and general population. Stu Med Sci. 2015;26(4):290-5.
39. Lee YB, Lee JH, Lee SY, et al. Association between vitiligo and smoking: a nationwide population-based study in Korea. Sci Rep. 2020;10(1):1-9.
40. Serdaroğlu S, Parlak AH, Engin B, et al. The prevalence of psoriasis and vitiligo in a rural area in Turkey. J Turk Acad Dermatol. 2012;6(1).