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# A Case Control Study of Vitiligo Patients with Seropositive Helicobacter pylori

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**Abstract:** Background : Vitiligo is an autoimmune skin disorder that affects people of all ages, regardless of gender. The etiology of vitiligo includes both hereditary and environmental factors. During the progression of vitiligo, Helicobacter pylori (H. pylori) may play a significant role. Therefore, this study evaluated the role of H. pylori seropositivity in patients having vitiligo compared to normal individuals. Method: H. pylori infection was tested by Enzyme Linked Immunosorbent Assay (ELISA) test for 72 Vitiligo patients and 68 healthy controls, which matched by sex and age for both groups. SPSS software version 20.0 was used to analyze the collected data. T-tests and ANOVA tests were used to compare the two groups. $P < 0.05$ was considered statistically significant. Results Vitiligo patients had higher mean levels of IgG (29.4 ± 28.21) RU/mL) than (18.6 ± 20.18 RU/mL) in healthy controls ($P < 0.000$). Moreover, there was no significant difference between groups based on the level of IgM ($P < 0.203$). In the vitiligo group, IgG or IgM means were different compared to age ($P < 0.35$)/ ($P < 0.014$), respectively. Unlike IgG, there was a significant difference between the mean levels of IgM, the onset age of vitiligo ($P < 0.024$). Moreover, males and females with vitiligo had a higher seropositivity to H. pylori antibodies than the control group. Conclusion Vitiligo was associated with H. pylori in patients in Baghdad. Consequently, it was likely that H. pylori played an important role in the onset or progression of disease activity in vitiligo.

**Keywords:** Autoimmune, Vitiligo, skin. Helicobacter pylori, Seropositivity, enzyme-linked immunosorbent assay (ELISA).

## INTRODUCTION

Vitiligo is a well-known dermatological disease characterized by skin depigmentation due to a lack of functional melanocytes in the epidermis, leading to clear borders of asymptomatic white spots (Lotti, T., & D'Erme, A. M. 2014). It affects 0.1-2% of the global population, with no notable differences according to gender, race, or geographic region (Krüger, C., & Schallreuter, K. U. 2012; Dermatol, 2012). Vitiligo generally manifests in early life, specifically between the ages of 10 and 30, with most individuals exhibiting symptoms before age 40 and half before age 20 (Zhang, Y. *et al.*, 2016).

The exact aetiology of vitiligo remains unknown. However, numerous prominent theories suggest the disease is caused by a multifactorial manner (Nicolaidou, E. *et al.*, 2019). Vitiligo is currently classified as an autoimmune disease (Wu, J. *et al.*, 2013).

The disease's development (vitiligo) is influenced by the interaction between genetic and environmental agents (Rodrigues, M. *et al.*, 2017).

Various environmental factors, including infectious agents, were also found to be linked to vitiligo. Microbial agents contribute to the propagation of autoimmunity through their interactions with the environment and the human immune system (Metwalley, K. A., & Farghaly, H. S. 2012).

Over the last 25 years, the relationship between infection and autoimmunity has become more clearly recognized (Kundu, R. V. *et al.*, 2019). Infectious agents commonly involved in the pathogenesis of vitiligo include HIV, hepatitis C virus, and cytomegalovirus (Crowe S. E. 2019; Xuan, L. *et al.*, 2014; Fawzy, M. M. *et al.*, 2022) H. pylori is believed to initiate an inflammatory response through the release of various cytotoxic substances, which may modify the body's immunological reactions, including both humoral and cell-mediated responses (Zhuang, T. *et al.*, 2020). Since the identification of Helicobacter pylori in 1983, an increasing quantity of knowledge has emerged, with this pathogen being directly implicated in the pathogenesis of various dermatological diseases (Kundu, R. V. *et al.*, 2019).

These factors contribute not only to gastrointestinal pathologies but are also associated with extra-gastric immune-mediated conditions, including dermatological disorders such as atopic dermatitis, psoriasis, chronic spontaneous urticaria, rosacea, and lichen planus (Ghotaslou, R. *et al.*, 2018). Helicobacter pylori is a recently identified bacterium associated with the pathogenicity of vitiligo. 80- 50 % percent of people worldwide have H. pylori, a spiral-shaped, microaerophilic, gram-negative bacterium, in gastric mucosal tissue (Kutlu, Ö. *Et al.*, 2020)





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H. pylori triggers the response of Th1 cells and IL-2 and IFN-γ production which lead to a chronic inflammatory response and this chronic infection may cause higher levels of pre-inflammatory cytokines such as TNF-α, IL-6, IL-8, and IL-10, leading to CD5 cell proliferation, which produces Ig3 and self-activating IgM (Marshall, B., & Warren, J. R. 1984) .

Helicobacter antigens induce autoantibody synthesis via T cell activation. Moreover, H. pylori possesses thermal shock protein (HSP), which plays a significant role in the aetiology of autoimmune diseases due to its high similarity to human HSP 17. Consequently, our findings show that the presence or absence of H. pylori infection may contribute to the onset of many autoimmune disorders, including vitiligo (Moyat, M., & Velin, D. 2014; Doğan, Z. *et al.*, 2014). Hence, the current study examined H. pylori seropositivity in vitiligo patients with a healthy control group.

## METHODS

From February 2023 to September 2023, a case-control study was conducted at the dermatology department of AL Yarmouk Teaching Hospital in Baghdad. The study included 72 patients presenting with vitiligo, who had less than 30% of their body surface affected, and were treated in the dermatology clinic, associated with dermatological and systemic conditions. Patients were randomly designated from a list that had been compiled, while those unwilling to provide consent were excluded. The questionnaire included questions concerning sex, age, family history of vitiligo, clinical type, duration of disease, age of onset, autoimmune diseases (including Hypothyroidism, Alopecia Areata, Multiple Sclerosis), digestive problems, 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 (Çakmak, S. K. *et al.*, 2015) .A healthy control 68 persons from Baghdad were matched in sex and age. The control group did not have any family members or relatives with vitiligo.

In addition, the control group did not have autoimmune disease, malignant or allergic diseases.

The inclusion criteria are:

1. Age range of 17-54
2. Not taking the antibiotics (Bismuth subsalicylate)
3. Not taking proton pump inhibitors for one month before the beginning of the study.

**The Exclusion Criteria are:**

1. Gastrointestinal symptoms (bloating, nausea, high intestinal upset, premature satiety, heartburn, etc.)
2. Pregnancy or breastfeeding was considered an exclusion criterion for the study.
3. Unwillingness to donate blood samples or complete the questionnaires was considered the exclusion criterion.

Enzyme-Linked Immunosorbent Assay (ELISA) was carried out through a 5 mL blood sample collected and centrifuged for both controls and patients, and the isolated sera were stored at -20 °C. Then, the ELISA method is used to determine the serum levels of IgG and IgM to H. pylori. The manufacturer's instructions were followed to conduct the tests, and seropositivity was defined as an H. pylori IgM titer exceeding 12 RU/mL and an H. pylori IgG titer exceeding 20 RU/mL.

### Statistical analysis

Serum concentrations of IgM and IgG to H. pylori were presented as mean ± SD. The concentrations of IgG and IgM against H. pylori in the two cohorts of vitiligo patients and the control group were analysed using Student's t-test. Additionally, IgG and IgM serum concentrations in each group were analyzed against other factors using non-parametric ANCOVA and Chi-Square tests. A P-value less than 0.05 was deemed statistically significant.

### Ethical standards

The current study received ethical approval from the Ethics Committee for Clinical Research at Ibin Sina University of Medical and Pharmaceutical Sciences. Following the explanation of the study's objective and protocol to the participants, written informed consent was gained from them.

## RESULT

The study included 72 patients with vitiligo (48 women and 24 men). The mean age of the participants was 31.7 ± 12.16 years. The control group included healthy controls (51 women and 17 men). The mean age of the participants was 34.6 ± 15.14 years, as shown in figure 1.





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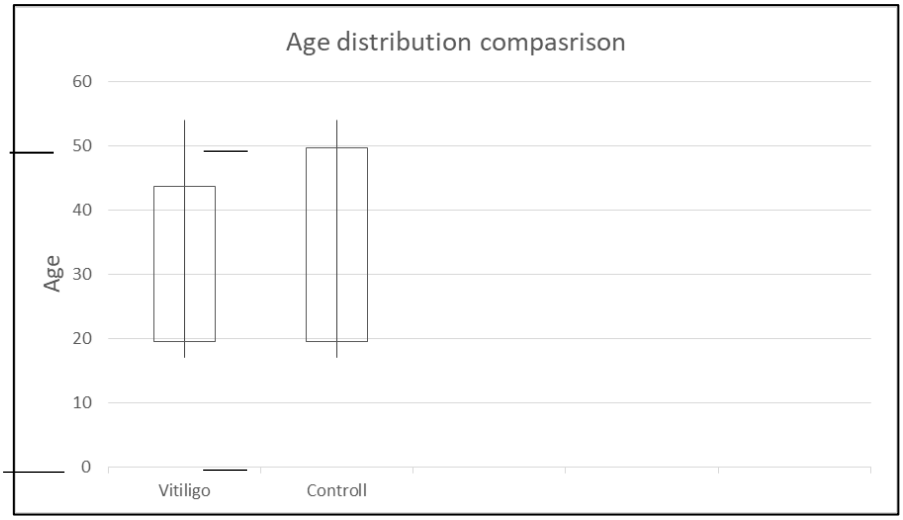


**Figure 1.** Age distribution comparison.

**Table 1.**Demographic characteristics gender of vitiligo (patients and healthy controls)

| Sex | Vitiligo | Control |
|---|---|---|
| Female (%) | 68.57% | 75% |
| Male (%) | 31.43% | 25% |
| N- Number | | |

Seropositive H. pylori related to clinical types of vitiligo: a fifty two patients had generalized vitiligo (34females and 18 females) and 20 patients had localized vitiligo (14 females and 6 males) Figure 2.

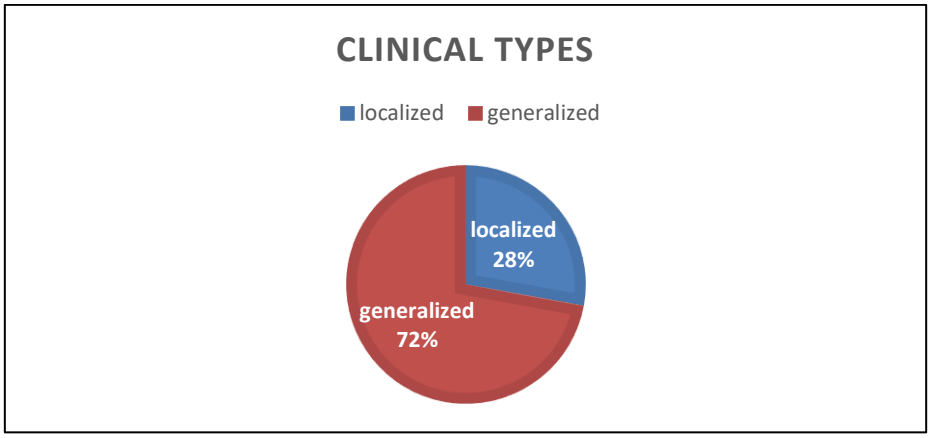


**Figure 2**. Percent of patients related to clinical type of vitiligo

All participants were tested using the ELISA test, and a statistically significant correlation was found between H. pylori positivity in patients with vitiligo and seropositivity in healthy controls. Compared to healthy controls, vitiligo patients exhibited a greater median level of IgG against H. pylori ($P < 0.00$), as shown in Table 2.

**Table 2.**Baseline data vitiligo (mean ± SD) and serum IgG/IgM concentrations of patients with vitiligo and the control group.

| Patient with vitiligo | (n = 72) | Control (n = 64) | P-value |
|---|---|---|---|
| H.pylori IgG | (29.4 ± 28.21) | (18.6 ± 20.18) | 0.000 |
| H.pylori IgM | (0.6 ± 0.23) | (0.68 ± 0. 64) | 0.203 |
| **N- Number** | | | |

In addition, females show a significant difference in the median level of IgG and IgM in opposition to H. pylori between the females and males in both groups.





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

| | Patient's | Healthy control | P-value |
|---|---|---|---|
| | (n = 72) | (n- 64) | |
| H. pylori IgG (RU/ML) | | | |
| Female | 30.8 ± 26.51 | 10.64± 20.29 | 0.000 |
| Male | 29.5 ± 27.11 | 18.86 ± 14.68 | 0.001 |
| H. pylori IgM (RU/ML) | | | |
| Female | 0.72 ± 0.84 | 0.62 ± 0.31 | 0.000 |
| Male | 0.56 ± 0.34 | 0.52 ± 0.26 | 0.007 |
| N, number; F, female; M, male; | | | |

A significant relationship exists between the mean levels of IgG/ IgM to H. pylori and age. The mean IgG/IgM levels were not significantly related to sex, self-autoimmune illnesses, disease duration, smoking, or vitiligo progression. Unlike IgG, a significant correlation was observed between mean IgM levels and both the age of onset and the duration of the disease in patients with vitiligo (Table 4).

**Table 4.** The percentage of patient features

| Characteristic | Patients (n = 72) |
|---|---|
| Family history of vitiligo | %33.4 |
| History of self-autoimmune diseases | %21.9 |
| Family history of autoimmune diseases | %39.4% |
| Smoking history | %28.2 (male) |

## DISCUSSION

The current study obviously demonstrated seropositivity of H. pylori in the vitiligo patients' sample. The vitiligo patients had a much higher mean amount of IgG to H. pylori than the controls. Numerous investigations have been undertaken on the association between H. pylori and vitiligo, yielding various outcomes. 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%) (Godbole, G. *et al.*, 2020). 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 (Bakry, O. A. *et al.*, 2018).

Moreover, in contrast, Seray Külcü Çakmak carried out research and reported that the prevalence of H. pylori infection was not rising in patients with vitiligo (Doğan, Z. *et al.*, 2014). Vitiligo patients had a significant prevalence of H. pylori, according to Tamara A. A. Abdelmoneim's study (Rifaioğlu, E. N. *et al.*, 2014).

Vitiligo is acknowledged to impact individuals of all genders and ages (Bdaiwi, S. A. *et al.*, 2020). The current study found a significantly higher prevalence of vitiligo among middle-aged individuals (30-55 years), with an equal female-to-male ratio. Additionally, an age-related increase in the frequency of H. pylori infection was observed in conjunction with vitiligo. A positive correlation was identified between IgM (but not IgG) and both the age of onset of vitiligo and the duration of the disease. The median titer of H. pylori IgG/IgM was not statistically different between males and females with vitiligo, despite the fact that it was statistically higher than that of the healthy control group. Past research by Arýcan et al. demonstrated that vitiligo was prevalent in both genders (Mahajan, V. K. *et al.*, 2019), which was in accordance with the results of the current investigation; conversely, other research indicated that sex influenced the etiology of the disease (Castanet, J., & Ortonne, J. P. 2997; Arýcan, O. *et al.*, 2008).

Moreover, studies indicated that vitiligo is associated with other autoimmune conditions, particularly alopecia areata, pernicious anemia, diabetes mellitus, thyroid disease and atopic dermatitis (Arýcan, O. *et al.*, 2008).

The current study also included vitiligo patients with a history of autoimmune diseases such as alopecia, lupus, hypothyroidism, type 1 diabetes, and so on. A recent study has shown that infections may have a substantial impact on the severe forms of vitiligo (Dahir, A. M., & Thomsen, S. F. 2018).



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This study found no significant correlation between active vitiligo and H. pylori. Thus, the median concentration of H. pylori IgG/IgM in individuals with inactive vitiligo was negligible in comparison to those with active vitiligo.Dugan et al. also observed no correlation between positive H. pylori and the vitiligo activity score (Bakry, O. A. *et al.*, 2018).

Çakmak et al. additionally observed no significant correlation between vitiligo activity and H. Pylori SAT positive (Doğan, Z. *et al.*, 2014).

On the contrary, Ola Ahmed Bakry's study revealed a link between H. pylori and active vitiligo (Moyat, M., & Velin, D. 2014).

In addition, research demonstrated that the progression of vitiligo varied among individuals, contingent upon elements such as inheritance, infections, allergies, alcohol consumption, and smoking. These risk factors were thought to have the potential to worsen vitiligo , but it could usually be effectively managed with the right care and by avoiding triggers (Dahir, A. M., & Thomsen, S. F. 2018). To get more specific proof about the link between H. pylori and vitiligo, more research needs to be done in other groups of people using a number of exact diagnostic tests.

## CONCLUSION

Regular testing of vitiligo patients for H. pylori infection was essential in relation to the frequency of H. pylori infection. As a result, clearance therapy could be taken as an initial step, and other therapies could be available for these patients, especially in regions with a significant incidence of H. pylori infection.

## RECOMMENDATIONS

For better results, using more than one method to detect H. Pylori infection, especially in vitiligo patients, and assessing the remission rates of vitiligo should be after H. pylori eradication therapy, and more exploring the role of H. pylori in other immunodermatological diseases.

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