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# Identification of the Frequency and Prominence of Helicobacter pylori infection in Patients with Polycystic Ovary Syndrome referred to Several Tehran Private Hospitals, Iran

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**Abstract**

Polycystic ovary syndrome (PCOS) ranks among the most pervasive endocrine disorders experienced by premenopausal women. *Helicobacter pylori* is a prevalent infection in human populations. The present study was designed to investigate the prevalence of *Helicobacter pylori* infection in PCOS patients. It was a case-control investigation comprising 100 female participants who sought medical care at the Tehran Army Hospital in Iran. Initially, fifty individuals diagnosed with polycystic ovary syndrome, based on the Rotterdam criteria and verified by two experienced physicians, were selected for inclusion in the study. Afterward, an equal number of women without said syndrome were included in a one-to-one comparison. Subsequent to acquiring blood samples of uniform volume from all donors, serum separation was performed, and the resulting samples were subsequently stored in a freezer at a temperature of -20 °C. The study involved quantifying IgG-antibody titer employing an ELISA reader in adherence to the protocol established by the kit manufacturer, namely Trinity Biotech. The study participants were found to have a mean age of 20-45 years with a standard deviation of 3.71. The age and body mass index (BMI) variables were found to be statistically insignificant between the two groups under investigation, indicating that the groups were comparable in terms of these factors. The Ig G serology results indicated a positive outcome among 64% (n=32) of the case group and 32% (n=16) of the control group. Statistical analysis using the chi-square test revealed a significantly different proportion between the two groups, as evidenced by a P-value of less than 0.05. The present study's findings indicate a significant disparity in the incidence of *H. pylori* infection between individuals diagnosed with polycystic ovary syndrome and their counterparts without the condition. Specifically, a higher prevalence of the infection was observed among patients with polycystic ovary syndrome. Ultimately, the proposition concerning the function of *H. pylori* was evaluated. It is posited that *H. pylori* may represent a potential risk factor for the acquisition of the mentioned condition. Therefore, carefully designed analytical and controlled investigations should be performed to substantiate this supposition.

## 1. Introduction

Polycystic ovary syndrome (PCOS) is a frequently encountered endocrine disorder, having a global prevalence ranging from 4% to 12% (Deswal et al., 2020; Jabeenet al., 2022; Farhadi-Azar et al., 2022; Ranathunga et al., 2022). Polycystic Ovary Syndrome is a medical condition characterized by a collection of symptoms that negatively impact an individual's

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ovulatory function and ovarian morphology (Witchel et al., 2019; Singh et al., 2023). The present condition was initially characterized in 1935 (Buggs & Rosenfield, 2005), and it is typified by persistent anovulation, a polycystic ovary, female sterility, menstrual irregularities, hirsutism, hyperandrogenism, and excessive weight gain (Singh et al., 2023). The emergence of pathological or atypical cysts, including polycystic ovary syndrome (PCOS) cysts, may lead to distressing and uncomfortable symptoms. A diagnosis of Polycystic Ovary Syndrome requires that a woman manifest at least two of the previously delineated symptoms (Ndefo et al., 2013; El Hayek et al., 2016). These symptoms encompass irregular menstrual cycles due to ovulation disorder, high levels of testosterone (hyperandrogenism), and polycystic ovaries identified through ultrasonography. Females diagnosed with PCOS exhibit a 100% increased likelihood of delivering offspring with congenital disabilities. Individuals in this demographic exhibit a higher incidence of spontaneous abortion, elevated blood pressure, and gestational diabetes than other populations (Yang et al., 2023; Pattnaik et al., 2022; Foroozanfard et al., 2014). Nevertheless, females with Polycystic Ovary Syndrome are able to augment their ovulatory frequency and achieve conception through the administration of fertility therapies (Sawant & Bhide, 2019; Melo et al., 2015). Due to its metabolic impact, polycystic ovary syndrome elevates the likelihood of encountering gestational diabetes, preeclampsia, preterm delivery, low birth weight or macrosomia, and admission to the Neonatal Intensive Care Unit (NICU) (Manoharan & Wong, 2020). Polycystic ovary syndrome is a significant phenomenon due to its adverse impact on fertility, in addition to being the primary cause of ovarian dysfunction among women (Kshetrimayum et al., 2019; Rojas et al., 2014). *Helicobacter pylori* (*H. pylori*) is a Gram-negative bacillus with a small, curved morphology (Krzyżek & Gościniak, 2018; Faghri et al., 2014; Vinette et al., 2002). *H. pylori* are a prevalent pathogen in human populations, such that it is estimated that approximately 50% of individuals have incurred an infection from this bacterial species (Bravo et al., 2018; Alexander et al., 2021). *H. pylori* is a prevalent bacterial infection among the human population, with an estimated incidence rate of approximately 50% (Salih et al., 2009). Various diseases have been linked to the presence of the bacterium *H. pylori*, such as lung cancer, cardiovascular diseases, inflammatory bowel diseases, chronic gastritis, and inflammatory bowel diseases (Campuzano-Maya et al., 2014; Roussos et al., 2003). For example, recent research has documented the transmission of bacteria via the oral-genital route (Eslick 2000). Additionally, the similarity between the stomach and vagina, as well as the cells in the female reproductive system, has been demonstrated to facilitate the implantation of the bacterium. Consequently, an asymptomatic infection may develop over a prolonged period (Amabebe & Anumba, 2020; Kazemi and Khazaei, 2018; Darvishi et al., 2017). The presence of *H. pylori* in women may stem from the direct transmission of the bacterium to the vaginal region or through an immunoglobulin-mediated response with the tissues that comprise the genital tract (Sánchez-Alonzo et al., 2021; Zhang et al., 2022). The colonization of *H. pylori* species may occur due to anatomical similarities between the stomach and vagina, as well as shared cellular characteristics of the female reproductive system (Sánchez-Alonzo et al., 2021; Hernandez et al., 2020). The present study examined the incidence of both *H. pylori* infection and ovarian cyst syndrome (OCS) in pregnant women diagnosed with gestational diabetes and referred to the hospital. The primary objective of this research was to ascertain the adverse pregnancy outcomes among expectant females with PCOS residing in Tehran, Iran, and juxtapose the findings with a control group. The present study was designed to consider the prevalence of *H. pylori* infection in PCOS patients and to explore the antibody titration of *H.*

*pylori*. The association between *H. pylori* and infertility has been investigated in two cohorts of individuals, namely cases and controls. Furthermore, this research examined the correlation between the duration of marriage and positive antibody titer among infertile couples, alongside key infertility factors such as polycystic ovary.

## 2. Material and Methods

### 2.1. Specifications of the tested sample

The present investigation was a case-control study. The research population included female subjects diagnosed with polycystic ovary syndrome and those not afflicted with the condition (control), who were referred to several private hospitals. The sampling process entailed a non-random selection approach. For the purpose of this study, we initially incorporated a cohort of 50 female subjects referred to the women's clinic who subsequently received a definitive diagnosis of polycystic ovary disease that was validated by the informed evaluation of two specialized physicians. Another cohort of fifty additional female patients who did not present with polycystic ovaries but sought screening examinations at the women's clinic was also included. The patients gave informed consent only if they fulfilled the criteria for study enrollment and received a comprehensive briefing regarding all the intervention phases. Subsequently, a survey was conducted to elicit vital demographic data, such as age, weight, and height, from the participating patients.

### 2.2. Inclusion criteria

Females within the reproductive age range of 20 to 45 years who underwent examination by two gynecologists were diagnosed with polycystic ovary syndrome based on the Rotterdam criteria of 2003. These criteria include clinical symptoms such as amenorrhea, oligomenorrhea, or infertility, as well as laboratory or clinical manifestations of hyperandrogenism such as acne and hirsutism. Treatment options have been prescribed for this patient population (Darvishi et al., 2017; Torabi et al., 2022)

### 2.3. Exclusion criteria

The patients with previous infection of Helicobacter pylori were excluded.

### 2.4. Serological test

A volume of 5 mL of blood was extracted from each patient, and the serum component was subsequently isolated. The IgG antibody of *H. pylori* analyzed by the Pishtaz Teb kit and ELISA method had a sensitivity of higher than 98%. This method interpreted a titer higher than 10 U/ml as positive and less than 5 U/ml as negative (Sohrabvand et al., 2014). Following serum isolation, the IgG antibody titration analysis was conducted on all serum samples utilizing the enzyme-linked immunosorbent assay (ELISA) method outlined in the manufacturer's (Trinity Biotech) protocol.

Finally, the antibody titration levels were duly noted and recorded in the patients' respective records. The body mass index (BMI) was computed as the quotient obtained by dividing an individual's weight (measured in kilograms) by the square of their height (measured in square meters). To obtain the cohort data, the subjects' body mass was measured via a seca 0 lever scale with the ability to gauge a weight with an exactitude of 0.1 kg while minimizing clothing coverage and footwear. Additionally, the subjects' height was measured with a calibrated height meter affixed to the aforementioned scale, providing an accuracy of 0.1 cm in a standing orientation (Torabi et al., 2022; Zargham et al., 2015; Nilnam et al., 2018; Khalili et al., 2007).

### 2.5. Statistical analysis

After completing the tests and recording the antibody titer of each person along with their biographical information, Kolmogorov-Smirnov (KS) and t-test data analysis were done using SPSS 22 software. A chi-square statistical test was used to compare qualitative variables

## 3. Results and Discussion

### 3.1. Examining the demographic characteristics of the studied patients

This study involved the examination of 100 female cases referred to several hospitals in Tehran, Iran. The cases were separated into two groups consisting of 50 women, namely the case and control groups. Initially, the study population's demographic variables (age and BMI) were analyzed, as delineated in (Table 1). Subsequently, a separate evaluation was conducted on these variables within the case and control groups. Among the 100 samples, the BMI2 peak was reported as 27.03, and the age range of 26 to 29 had the highest frequency (Fig 1).

The present investigation used the variables of age and BMI from the two distinct study groups. A comparison of means t-test was utilized to gauge differences between the two independent groups. The results obtained from this test are outlined in (Table 2). The findings of this study indicate that the obtained P-value was greater than 0.05, indicating no statistically significant difference between the two investigated groups concerning age and BMI variables. These outcomes suggest that the two groups were essentially equivalent.

**Table 1:** Variable distribution of age and BMI in the Study and Control Groups

| Variable (Age and BMI) | Number | The maximum amount | The lowest amount | Standard deviation | Mean |
|---|---|---|---|---|---|
| Age (Case group) year | 50 | 37 | 19 | 4.23 | 27.18 |
| BMI (Case group) kg/m$^2$ | 50 | 35.42 | 20.66 | 3.51 | 27.67 |
| Age (Control group) year | 50 | 34 | 21 | 3.14 | 27.04 |
| BMI (Control group) kg/m$^2$ | 50 | 35.61 | 20.24 | 4.01 | 26.38 |

Note: All values reported are the mean ± SEM of 3 determinations, P<0.05 values are significantly different.

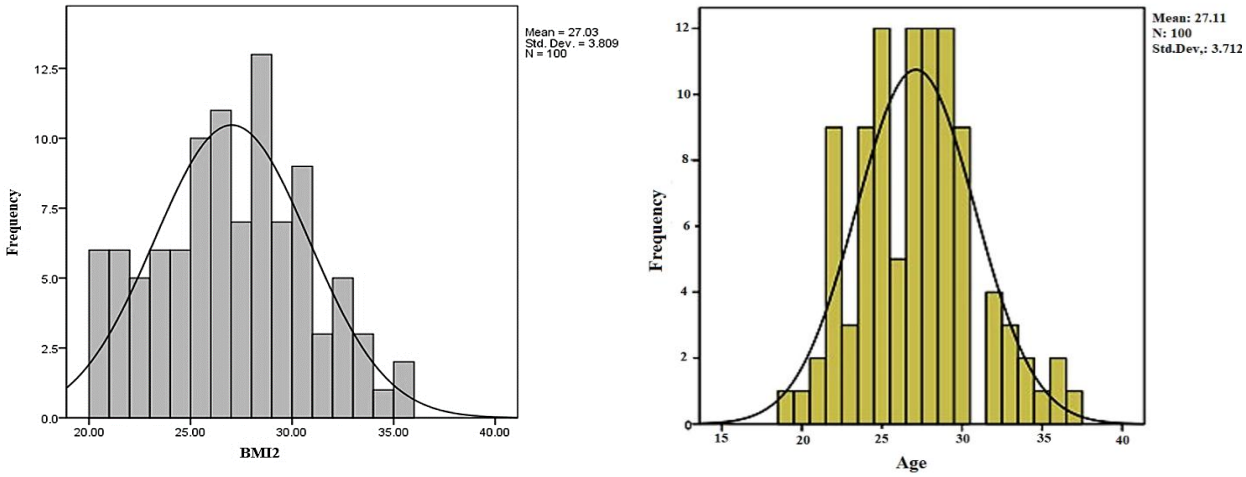


**Figure 1**: BMI and age variable in all studied cases; note: There was no statistically significant difference between the two investigated groups, P>0.05.

**Table 2:** Comparison of the Variable Mean of Age and BMI in the Case and Control Groups using a T-test

| Independent Samples Test | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
| | | F | Sig. | t | Df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| **Age** | **Equal variances assumed** | 2.705 | .103 | .188 | 98 | .852 | .140 | .746 | -1.340 | 1.620 |
| | **Equal variances not assumed** | | | .188 | 90.535 | .852 | .140 | .746 | -1.342 | 1.622 |
| **BMI2** | **Equal variances assumed** | .763 | .385 | 1.710 | 98 | .090 | 1.29011 | .75452 | -.20721 | 2.78743 |
| | **Equal variances not assumed** | | | 1.710 | 96.297 | .091 | 1.29011 | .75452 | -.20754 | 2.78776 |

Note: There was no statistically significant difference between the two investigated groups, P>0.05.

The study's findings provided evidential support for the notion that bacterial transmission occurs via the oral-genital route. Multiple studies have validated the research findings through the isolation of *H. pylori* (Dimitriadi, 2014; Zhang et al., 2022), with the bacteria known as *H. pylori* having been found in both the oral and vaginal cavities of individuals (Yee 2017; Zhang et al., 2022; Chen et al., 2022). Many research endeavors have postulated that *H. pylori* infection may correlate with various pathologies, such as the co-occurrence of *H. pylori* positivity and PCOS in gestational diabetic women during pregnancy (Li et al., 2021; Xia et al., 2020). However, the potential of *H. pylori* to serve as a novel etiological agent for PCOS remains ambiguous. Undoubtedly, *H. pylori* infection presents a certain degree of correlation with impaired fertility, and polycystic ovary syndrome represents a frequently encountered etiological factor for infertility. Further investigations are required to assess this matter comprehensively and conclusively. One viable intervention for addressing infertility among individuals diagnosed with polycystic ovary syndrome entails a course of medication aimed at stimulating ovulation. Polycystic ovary syndrome holds significant implications in terms of reproductive health as it is known to impact fertility. Moreover, it is regarded as the most prevalent cause of ovarian dysfunction. Infertility exhibits a significant prevalence within the familial domain and is deemed a salient concern in relation to reproductive well-being (Dennett & Simon, 2015).

### 3.2. Serological results (Ig G)

The serology results showed that 36% of the case group had negative Ig G, and 64% had positive Ig G antibodies. In the control group, 68% had negative IgG antibodies, and 32% positive IgG antibodies were observed. The present study utilized the chi-square test in order to analyze the serological results pertaining to Ig G in the two groups under investigation. The findings of the test are presented in (Tables 3) and 4. The findings of this inquiry evinced a statistical significance with a P-value of less than 0.05, indicating a notable dissimilarity between the two scrutinized cohorts. The study's findings demonstrated a statistically significant association between the prevalence of *H. pylori* in patients diagnosed with polycystic ovary syndrome (Fig. 2).

Table 3: Comparison of Serology Results (Ig G) in Case and Control Groups

| Studied groups | Serology results (Ig G) | | |
|---|---|---|---|
| | Total number No. (%) | Negative cases No. (%) | Positive cases No. (%) |
| **Case Group** | 50 (100) | 18 (36%) | 32 (64%) |
| **Control Group** | 50 (100) | 34 (68%) | 16 (32%) |

Note: The values are significantly different (P<0.05).

**Table 4:** Chi-square Test and Variable Comparison of Serology (Ig G) Test Results in Case and Control Groups

| | Value | Df | Asymp. Sig. (2-sided) | Exact Sig. (2-sided) | Exact Sig. (1-sided) |
|---|---|---|---|---|---|
| **Pearson Chi-Square** | 10.256[a] | 1 | .001 | | |
| **Continuity Correction[b]** | 9.014 | 1 | .003 | | |
| **Likelihood Ratio** | 10.441 | 1 | .001 | | |
| **Fisher's Exact Test** | | | | .003 | .001 |
| **Linear-by-Linear Association** | 10.154 | 1 | .001 | | |
| **N of Valid Cases** | 100 | | | | |

a: 0 cells (.0%) have an expected count of less than 5. The minimum expected count is 24.00.
b: Computed only for a 2x2 table

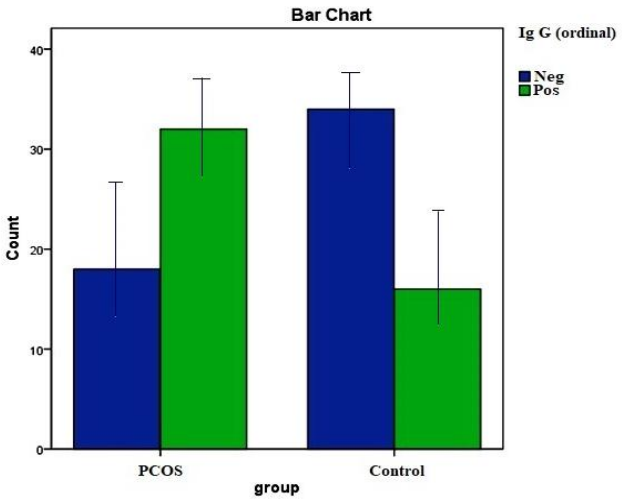


Figure 2: Comparison of serological results (Ig G) in both PCOS patients and control groups.

## 3.3. Gastrointestinal symptoms

According to the findings, out of 50 samples, 18% of the case group (50 samples) had no gastrointestinal symptoms, and 82% had gastrointestinal symptoms. Also, 14% of the control group had no gastrointestinal symptoms, and 86% had gastrointestinal symptoms. The current study employed the Chi-square statistical test to examine the variable of gastrointestinal symptoms in both the case and control groups. The findings revealed a statistically significant difference (P<0.05) in the incidence of digestive symptoms between the two investigated cohorts (Table 5).

| Studied groups | Gastrointestinal symptoms | | |
|---|---|---|---|
| | Total number No. (%) | Negative cases No. (%) | Positive cases No. (%) |
| **Case Group** | 50 (100) | 9 (18%) | 41 (82%) |
| **Control Group** | 50 (100) | 7 (14%) | 43 (86%) |

Table 5: Comparison of Gastrointestinal Symptoms in Case and Control Groups
Note: The values are significantly different (P<0.05).

Sohrabvand et al. determined *H. Pylori* antibody IgG serum levels were positive in 78% and 76.5% of the PCOS and non-PCOS cases (2014). Similar to this study, they also found there was no significant difference between the *H. Pylori* antibody levels in the two groups (P>0.05). Kiani et al. (2015) found the positive titers of *H. pylori* antibodies IgG in the PCOS group were 79 (62%), while the non-PCOS group

was 76 (60%). The sera positive for IgG antibody in spouses of the non-PCOS group were 84 (66%), but in spouses of the PCOS group was 83 (66%); these results revealed that *H. pylori* infection probably did not affect infertility or reproduction (Kiani et al., 2015). The incidence of *H. pylori* infection has been observed to be notably higher among individuals diagnosed with polycystic ovary syndrome (Moretti et al., 2014).

In other research, Pakniat et al. (2022) investigated 150 pregnant women with PCOS (18-35 years). Their results revealed that no statistically significant differences were observed between the two cohorts regarding demographic characteristics, specifically age, weight, height, and BMI. However, in the group diagnosed with polycystic ovary syndrome (PCOS), the incidence of gestational diabetes was confirmed in 33 instances (64.7%), preeclampsia in 44 instances (67.7%), and preterm delivery in 70 instances (75.3%). Comparing the outcomes, the case and control groups displayed a noteworthy dissimilarity ($P<0.0001$) (Pakniat et al., 2022). Elsick et al. posited that this phenomenon might have been perpetuated over an extended period of time (2000), with participants 10, 9, and 8 successfully demonstrating the presence of a virus within the device, despite the absence of visible indications of infection. They concluded that the bacterium in question is transmitted via the path of the genital-oral route. Simultaneously, various academic inquiries substantiated the findings of the abovementioned scholar via the isolation of *H. pylori*. *H. pylori* was isolated from the oral and vaginal specimens of the patients under scrutiny.

Despite attempts by the mentioned researchers to establish a correlation between the infertility of female subjects and inflammation of their genital organs, Figura and colleagues (2002) posited that infertility may be attributable to alternative factors. They credited the manifestation of the disease in eight immune partners to the generation of complex formations between antibodies and antigens or a linear homology between the primary protein of the pathogen, *H. pylori*. The bacterium *H. pylori*, commonly referred to as Ag, introduces itself by binding to the human tubulin proteins. Figura et al. substantiated their hypothesis by examining 167 women who suffered from infertility, whereby they ascertained the presence of anti-*H. pylori* and analyzed the *H. pylori* antibody titer levels in comparison to the control group (2002). Conversely, in two studies conducted by Yavasoglu and colleagues in 2009 and 2012, a significant correlation was observed between the incidence of polycystic ovary syndrome and the positivity of the *H. pylori* antigen antibody titer in patients. They found the antibody titer was significantly higher in patients than in control subjects using the *H. pylori* serology test as a diagnostic tool to detect *H. pylori* antibodies in biological specimens. Their findings were consistent with our preliminary research, suggesting that the identified infection may play a significant role in the development of polycystic ovary syndrome.

Overall, our investigation reveals a heightened incidence of *H. pylori* in the population under examination. This study compared the incidence of *H. pylori* infection among women with polycystic ovaries to a control group. The dearth of research on the subject of infertility in both global and Iranian contexts, as evidenced by the scarcity of published scientific articles, underscores the potential significance of conducting extensive investigations in this sphere. It is conceivable that unearthing viable solutions to this challenge could serve as a key step towards addressing one of the root causes of infertility in our cherished nation, Iran. It is recommended that additional analytical and controlled research studies be undertaken in order to substantiate this hypothesis. It is postulated that the colonization of the female reproductive system by *H. pylori* species may have occurred as

a result of the analogous anatomical features shared by the stomach and vagina.

## 4. Conclusion

It has been theorized that colonization of the *H. pylori* species in the female reproductive system may occur as a result of anatomical similarities between the stomach and vagina, as well as between the cells in the female reproductive system. Such colonization may provoke an asymptomatic infection that develops gradually over time. Therefore, since very few studies or scientific articles are related to this issue worldwide, this issue is an important topic for further investigation.

## Conflict of interest

The authors have declared that there is no conflict of interest.

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## Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.



## References

[1] Alexander, S.M., Retnakumar, R.J., Chouhan, D., Devi, T.N.B., Dharmaseelan, S., Devadas, K., Thapa, N., Tamang, J.P., Lamtha, S.C. and Chattopadhyay, S. (2021) *Helicobacter pylori* in Human Stomach: The Inconsistencies in Clinical Outcomes and the Probable Causes. Front Microbiol 12:713955. doi: 10.3389/fmicb.2021.713955.

[2] Amabebe, E. and Anumba, DOC. (2020) Female Gut and Genital Tract Microbiota-Induced Crosstalk and Differential Effects of Short-Chain Fatty Acids on Immune Sequelae. Front Immunol 11:2184. doi: 10.3389/fimmu.2020.02184.

[3] Bravo, D., Hoare, A., Soto, C., Valenzuela, M.A. and Quest, A.F. (2018) *Helicobacter pylori* in human health and disease: Mechanisms for local gastric and systemic effects. World J Gastroenterol 24(28):3071-3089. doi: 10.3748/wjg.v24.i28.3071.

[4] Buggs, C. and Rosenfield, R.L. (2005) Polycystic ovary syndrome in adolescence. Endocrinol Metab Clin North Am 34(3):677-705, x. doi: 10.1016/j.ecl.2005.04.005.

[5] Campuzano-Maya, G. (2014) Hematologic manifestations of *Helicobacter pylori* infection. World J Gastroenterol 20(36):12818-38. doi: 10.3748/wjg.v20.i36.12818.

[6] Chen, X., Wang, N., Wang, J., Liao, B., Cheng, L. and Ren, B. (2022) The interactions between oral-gut axis microbiota and *Helicobacter pylori*. Front. Cell. Infect. Microbiol 12:914418. doi: 10.3389/fcimb.2022.914418

[7] Darvishi, M., Forootan, M., Azmodeh, O., Forootan, M. and Matinfar, H. (2017) Studying the Frequency of *Helicobacter Pylori* Infection Among Women Suffering from Polycystic ovary Resorting to Hospital Infertility Center. Biomed Pharmacol J 10(1).

[8] Dennett, C.C. and Simon, J. (2015) The role of polycystic ovary syndrome in reproductive and metabolic health: overview and approaches for treatment. Diabetes Spectr 28(2):116-20. doi: 10.2337/diaspect.28.2.116.

[9] Deswal, R., Narwal, V., Dang, A. and Pundir, C.S. (2020) The Prevalence of Polycystic Ovary Syndrome: A Brief Systematic Review. J Hum Reprod Sci 13(4):261-271. doi: 10.4103/jhrs.JHRS_95_18.

[10] Dimitriadi, D. (2014) *Helicobacter pylori*: a sexually transmitted bacterium? Cent European J Urol 67(4):407-9. doi: 10.5173/ceju.2014.04.art18.

[11] El Hayek, S., Bitar, L., Hamdar, L.H., Mirza, F.G. and Daoud, G. (2016) Poly Cystic Ovarian Syndrome: An Updated Overview. *Front. Physiol.* 7:124. doi: 10.3389/fphys.2016.00124

[12] Eslick, G.D. (2000) *Helicobacter pylori* infection transmitted sexually via oral-genital contact: a hypothetical model. Sex Transm Infect 76(6):489-92. doi: 10.1136/sti.76.6.489.

[13] Faghri, J., Poursina, F., Moghim, S., Zarkesh Esfahani, H., Nasr Esfahani B., et al. (2014) Morphological and Bactericidal Effects of Different Antibiotics on *Helicobacter pylori*. Jundishapur J Microbiol 7(1):e8704.

[14] Farhadi-Azar, M., Behboudi-Gandevani, S., Rahmati, M., Mahboobifard F., Khalili Pouya E., Ramezani Tehrani F. and Azizi F. (2022) The Prevalence of Polycystic Ovary Syndrome, Its Phenotypes and Cardio-Metabolic Features in a Community Sample of Iranian Population: Tehran Lipid and Glucose Study. Front. Endocrinol 13:825528. doi: 10.3389/fendo.2022.825528

[15] Figura, N., Piomboni, P., Ponzetto, A., Gambera, L., Lenzi, C., Vaira, D., et al. (2002) *Helicobacter pylori* infection and infertility. Eur J Gastroenterol Hepatol 14: 663-9.

[16] Foroozanfard, F., Moosavi, S.G., Mansouri, F. and Bazarganipour, F. (2014) Obstetric and Neonatal Outcome in PCOS with Gestational Diabetes Mellitus. J Family Reprod Health 8(1):7-12.

[17] Hernandez, C.D., Shin, H., Troncoso, P.A., Vera, M.H., Villagran, A.A., Rodriguez-Rivera, S.M., Ortiz, M.A., Serrano, C.A., Borzutzky, A., Dominguez-Bello, M.G. and Harris, P.R. (2020) Maternal *H. pylori* is associated with differential fecal microbiota in infants born by vaginal delivery. Sci Rep 10(1):7305. doi: 10.1038/s41598-020-64296-7.

[18] Jabeen, A., Yamini, V., Rahman, Amberina, A., Dinesh Eshwar, M., Vadakedath, S., Begum, G.S. and Kandi, V. (2022) Polycystic Ovarian Syndrome: Prevalence, Predisposing Factors, and Awareness Among Adolescent and Young Girls of South India. Cureus 14(8):e27943. doi: 10.7759/cureus.27943.

[19] Kazemi, E. and Khazaei, M. (2018). 'A review of the effects of *Helicobacter pylori* infection on reproduction, pregnancy and gynecologic diseases', The Iranian Journal of Obstetrics, Gynecology and Infertility, 21(Supplement), 67-75. doi: 10.22038/ijogi.2018.11622

[20] Khalili, Sharifi yazdi, Sadeh. (2007) Correlation of *H. pylori* infection and infertility; a survey in Yazd infertility clinic.Tehran University Medical Journal; Vol. 65, No. 3, Jun 2007: 72-77.

[21] Kiani, A.H., Asadbeik, E., Bibalan, M.H., Sedighi, M., Eshaghi, M., Gholami, M. and Pournajaf, A. (2015) Serological diagnosis of *Helicobacter pylori* infection in patients with a polycystic ovary syndrome. Archives of Clinical Infectious Diseases, 10 (2).

[22] Krzyżek, P. and Gościniak, G. (2018) Morphology of *Helicobacter pylori* as a result of peptidoglycan and cytoskeleton rearrangements. Prz Gastroenterol 13(3):182-195. doi: 10.5114/pg.2018.78284.

[23] Kshetrimayum, C., Sharma, A., Mishra, V.V. and Kumar, S. (2019) Polycystic ovarian syndrome: Environmental/occupational, lifestyle factors; an overview. J Turk Ger Gynecol Assoc 20(4):255-263. doi: 10.4274/jtgga.galenos.2019.2018.0142.

[24] Li, J., Fan, M., Ma, F., Zhang, S. and Li, Q. (2021) The effects of *Helicobacter pylori* infection on pregnancy-related diseases and fetal development in diabetes in pregnancy. Ann Transl Med 9(8):686. doi: 10.21037/atm-21-1209.

[25] Manoharan, V. and Wong, VW. (2020) Impact of comorbid polycystic ovarian syndrome and gestational diabetes mellitus on pregnancy outcomes: a retrospective cohort study. BMC Pregnancy Childbirth 20(1):484. doi: 10.1186/s12884-020-03175-5.

[26] Melo, A.S., Ferriani, R.A. and Navarro P.A. (2015) Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. Clinics (Sao Paulo) 70(11):765-9. doi: 10.6061/clinics/2015(11)09.

[27] Moretti, E., Figura, N., Collodel, G. and Ponzetto, A. (2014) Can *Helicobacter pylori* infection influence human reproduction? World J Gastroenterol 20(19):5567-74. doi: 10.3748/wjg.v20.i19.5567.

[28] Ndefo, U.A., Eaton, A. and Green, MR. (2013) Polycystic ovary syndrome: a review of treatment options with a focus on pharmacological approaches. PT 38(6):336-55.

[29] Niknam, R., Fattahi, M.R., Sepehrimanesh, M. and Safarpour, A. (2018) Prevalence of *Helicobacter pylori* in Southern Part of Iran. Jundishapur J Microbiol 11(6): e62379.

[30] Pakniat H., Kazemi F., Movahed F., Haji Seyed Abotorabi, SH.S., Soufizadeh, N. and Yaghobi, F. (2022) Evaluation of Pregnancy consequences in Women with Polycystic Ovary Syndrome: A Case-Control Study in Qazvin, Iran. SJKU 27(1):74-83.

[31] Pattnaik, L., Naaz, S.A., Das, B., Dash, P., Pattanaik, M. (2022) Adverse Pregnancy Outcome in Polycystic Ovarian Syndrome: A Comparative Study. Cureus 14(6): e25790. doi: 10.7759/cureus.25790.

[32] Ranathunga, I., Athukorala, T.G., Sumanatilleke, M.R. *et al.* (2022) Evaluation of socio-demographic and clinical characteristics of PCOS patients attending a tertiary care institute in Colombo. BMC Endocr Disord 22, 289.

[33] Rojas, J., Chávez, M., Olivar, L., Rojas, M., Morillo, J., Mejías, J., Calvo, M. and Bermúdez, V. (2014) Polycystic ovary syndrome, insulin resistance, and obesity: navigating the pathophysiologic labyrinth. Int J Reprod Med 719050. doi: 10.1155/2014/719050.

[34] Roussos, A., Philippou, N. and Gourgoulianis, K.I. (2003) *Helicobacter pylori* infection and respiratory diseases: a review. World J Gastroenterol 9(1):5-8. doi: 10.3748/wjg.v9.i1.5.

[35] Salih, B.A. (2009) *Helicobacter pylori* infection in developing countries: the burden for how long? Saudi J Gastroenterol 15(3):201-7. doi: 10.4103/1319-3767.54743.

[36] Sánchez-Alonzo, K., Matamala-Valdés, L., Parra-Sepúlveda, C., Bernasconi, H., Campos, V.L., Smith, C.T., Sáez, K. and García-Cancino, A. (2021) Intracellular Presence of *Helicobacter pylori* and Its Virulence-Associated Genotypes within the Vaginal Yeast of Term Pregnant Women. Microorganisms 9(1):131. doi: 10.3390/microorganisms9010131.

[37] Sawant, S. and Bhide, P. (2023) Fertility Treatment Options for Women with Polycystic Ovary Syndrome. Clin

Med Insights Reprod Health. 2019. doi: 10.1177/1179558119890867.
[38] Singh, S, Pal, N, Shubham, S, Sarma, DK, Verma, V, Marotta, F, Kumar, M. (2023) Polycystic Ovary Syndrome: Etiology, Current Management, and Future Therapeutics. Journal of Clinical Medicine 12(4):1454.
[39] Sohrabvand, F., Shariat, M., Farahvash, M.J., Haghollahi, F., Khosravi, M., Maasomi, M., et al. (2014) Serological study of *Helicobacter pylori* infection in patients with Polycystic Ovary Syndrome. Tehran Univ Med J 71 (10) :660-664
[40] Torabi, R, Tebyanian, H, Heiat, M. and Choopani, A. (2022) Seroepidemiological Prevalence of *Helicobacter pylori* in the south of Tehran, Iran. Novelty in Clinical Medicine 1(1): 55-58. doi: 10.22034/ncm.2022.140810
[41] Vinette, K.M., Gibney, K.M., Proujansky, R. *et al.* (2002) Growth of *Helicobacter pylori* in a long spiral form does not alter expression of immunodominant proteins. BMC Microbiol 2, 24.
[42] Witchel, S.F., Oberfield, S.E., and Peña A.S. (2019) Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment with Emphasis on Adolescent Girls. J Endocr Soc 3(8):1545-1573. doi: 10.1210/js.2019-00078.
[43] Xia, B., Wang, W., Lu, Y. and Chen, C. (2020) *Helicobacter pylori* infection increases the risk of metabolic syndrome in pregnancy: a cohort study. Ann Transl Med 8(14):875. doi: 10.21037/atm-20-4863.
[44] Yang, S.W., Yoon, S.H., Kim, M., Seo, Y.S. and Yuk, J.S. (2023) Risk of Gestational Diabetes and Pregnancy-Induced Hypertension with a History of Polycystic Ovary Syndrome: A Nationwide Population-Based Cohort Study. Journal of Clinical Medicine *12*(5):1738.
[45] Yavasoglu, I., Kucuk, M., Cildag, B., Arslan, E., Gok, M., and Kafkas, S. (2009) A novel association between polycystic ovary syndrome and *Helicobacter pylori*. Am J Med Sci 338(3):174–177. doi: 10.1097/MAJ.0b013e3181a63c8a
[46] Yavaşoglu, I. and Küçük, M. (2012) Anti-*Helicobacter pylori* antibodies and polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol
[47] Yee, J.K.C. (2017) Are the view of *Helicobacter pylori* colonized in the oral cavity an illusion? Exp Mol Med 49(11): e397. doi: 10.1038/emm.2017.225.
[48] Zargham, R., Moteshakker-Arani, M. and Soleimani-Meigooni, S. (2015) Prevalence of *Helicobacter pylori* infection on polycystic ovary syndrome in the women visiting Shariati hospital in 2013. NPWJM 3 (6) :61-65.
[49] Zhang, L., Chen, X., Ren, B., Zhou, X. and Cheng, L. (2022) *Helicobacter pylori* in the Oral Cavity: Current Evidence and Potential Survival Strategies. Int. J. Mol. Sci 23, 13646.