Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Opportunistic intestinal parasites, *Helicobacter pylori*, and co-infection in patients living with HIV: Prevalence and risk factors

HIV pozitif bireylerde fırsatçı bağırsak parazitleri, *Helicobacter pylori* ve koenfeksiyon: Prevalans ve risk faktörleri

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ABSTRACT

Objective: In immunocompromised populations, such as patients living with HIV (PLWH), parasitic infections and *Helicobacter pylori* (*H. pylori*) are a major public health concern. Each of them infects the gastrointestinal tract and causes symptoms that are similar. The goal of this study was to establish the prevalence of *H. pylori* and its relationship with opportunistic parasites in PLWH, as well as to estimate co-infection risk and predictors.

Methods: In Tehran, Iran, single fecal samples were collected from 70 PLWH ranging in age from 13 to 60 years. To detect ova, cysts, and coccidian, all stool samples were analyzed microscopically with Iodine and acid-fast stain. Using nested-PCR tests, *H. pylori* and *Cryptosporidium* copro-DNAs were detected.

Results: Molecularly, *H. pylori* was detected in 21.4 % of PLWH; opportunistic and intestinal parasites were found in 74.3 %, with *Cryptosporidium* spp, *Entameba. histolytica*, and *Giardia intestinalis* predominating (28.5 %, 27.1 %, and 22.8 % respectively). *H. pylori* co-infection with *Cryptosporidium* spp. was detected in

ÖZET

Amaç: AIDS olguları gibi bağışıklığı baskılanmış popülasyonlarda paraziter enfeksiyonlar ve *Helicobacter pylori* (*H. pylori*) önemli bir halk sağlığı sorunudur. Her biri gastrointestinal sistemi enfekte eder ve benzer semptomlara neden olur. Bu çalışmanın amacı, HIV hastalarında *H. pylori* prevalansını ve fırsatçı parazitlerle ilişkisini saptamanın yanı sıra koenfeksiyon riskini ve öngörücülerini tahmin etmektir.

Yöntem: İran, Tahran'da, yaşları 13 ile 60 arasında değişen 70 HIV hastasından tek dışkı örneği toplandı. Ova, kistler ve koksidiyen tespiti için tüm dışkı örnekleri iyot ve aside dayanıklı boyama ile mikroskobik olarak analiz edildi. Yuvalanmış PCR testleri kullanılarak *H. pylori* ve *Cryptosporidium* copro-DNA'ları tespit edildi.

Bulgular: Moleküler olarak *H. pylori*, HIV hastalarının %21.4'ünde tespit edilmiştir; fırsatçı ve bağırsak parazitleri %74,3 oranında bulunurken *Cryptosporidium*, *Entameba. histolytica* ve *Giardia intestinalis* baskındır (sırasıyla %28,5, %27,1 ve %22,8). *H. pylori*, *Cryptosporidium* spp. (%53.85) ve *Isospora*

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Geliş Tarihi / Received : 10.06.2021
Kabul Tarihi / Accepted : 09.11.2024

DOI ID : 10.5505/TurkHijyen.2025.90688

Ibrahim A, Raissi V, Gadalla M. Opportunistic intestinal parasites, *Helicobacter pylori*, and co-infection in patients living with HIV: Prevalence and risk factors. Turk Hij Den Biyol Derg, 2025; 82(3): 441 - 450

(53.85%) and *Isospora* spp (15, 38 %). The co-infection of *Cryptosporidium* spp., *Isospora belli*, various parasites, and *H. pylori* was reported to be linked with abdominal pain and diarrhoea.

Conclusion: Our findings shed light on the prevalence of *H. pylori* infection in PLWH who also have opportunistic and intestinal parasites. The co-occurrence of *H. pylori* and *Cryptosporidium* could support the theory of co-infection. More research is needed to confirm the link between gut microbiomes and opportunistic and intestinal parasites, whether *H. pylori* provides appropriate conditions for them or vice versa.

Key Words: Opportunistic intestinal parasites, *Helicobacter pylori*, HIV+ patients, nested-PCR, co-infection

spp (15, %38)'de tespit edilmiştir. *Cryptosporidium*, Isospora, çeşitli parazitler ve *H. pylori*'nin birlikte enfeksiyonunun karın ağrısı ve ishal ile bağlantılı olduğu bildirildi.

Sonuç: Bulgularımız, fırsatçı ve bağırsak parazitleri olan HIV hastalarında *H. pylori* enfeksiyonunun prevalansına ışık tuttu. *H. pylori* ve *Cryptosporidium*'un birlikte ortaya çıkması, birlikte enfeksiyon teorisini destekleyebilir. *H. pylori*'nin onlar için uygun koşulları sağlayıp sağlamadığına bakılmaksızın, bağırsak mikrobiyomları ile fırsatçı ve bağırsak parazitleri arasındaki bağlantıyı doğrulamak için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Fırsatçı bağırsak parazitleri, *Helicobacter pylori*, HIV hastaları, iç içe PCR, koenfeksiyon

INTRODUCTION

HIV continues to be a serious worldwide health concern with an estimated 37.9 million people living with HIV (PLWH) and 1.7 million new infections in 2018 (1). According to the UNAIDS spectrum and modelling (2), 59,000 (95 % CI 33,000 to 130,000) persons in Iran are living with HIV in 2019, with roughly 4,100 (95 % CI 1,200 to 12,000) new infections and 2,500 (95 % CI 1,200 to 5,600) AIDS-related deaths occurring each year.

One of the most common health issues in HIV/AIDS patients is opportunistic parasite infections. In HIV patients, the occurrence of opportunistic parasitic infections indicates that they are in the AIDS phase. The majority of these illnesses are severe, and they frequently result in the death of affected people. According to several studies, the most frequent intestinal protozoan parasites associated with AIDS are *Cryptosporidium parvum*, *Isospora belli*, and *Blastocystis hominis*. In an era of rising HIV/AIDS infections, parasite-caused diarrhoea is increasingly suspected in cases of chronic and persistent diarrhoea in adults (3).

Helicobacter pylori (*H. pylori*) is one of the most prevalent human bacterial infections, with prevalence rates ranging from 10 to 80 percent depending on geography, age, and socioeconomic level. *H. pylori* is a prominent cause of gastritis and is commonly discovered in patients suffering from dyspepsia. People living with HIV (PLWH) frequently experience gastrointestinal symptoms, particularly dyspepsia, during the course of their infection, and while prior studies have shown HIV and *H. pylori* co-infection, there has been little data elucidating the factors that influence this (4).

Co-infections with many pathogens are frequent in low-income countries. *H. pylori* and intestinal parasites both inhabit the human gastrointestinal system. *H. pylori* co-existence with *G. intestinals* and *Cryptosporidium* may suggest the association of *H. pylori* infection with markers of fecal exposure.

Whether *H. pylori* provides favorable conditions for intestinal parasitosis or vice versa (5). The goal of this study was to determine the prevalence of *H. pylori* infection and co-infection with opportunistic parasites among HIV patients. We also looked at risk variables that could increase the prevalence of this co-infection.

MATERIAL and METHOD

In Tehran, Iran, a cross-sectional pilot study was undertaken on 70 PLWH who were selected from total of 132 HIV/AIDS patient. From February 2018 to August 2019, fecal samples were obtained from various hospitals and health institutions, with ages ranging from 13 to 60 years. The inclusion criteria were: Patients with confirmed HIV/AIDS, with or without signs of diarrhea. The exclusion criteria were: those on certain anthelminthic, anti-protozoan, or anti-parasitic medications in the two weeks before to specimen collection, as well as those who had antacid in the two weeks prior to specimen collection. Antacid use can cause protozoan shape to be distorted, making it more difficult to identify the organism.

The sample size was determined as 92 individuals by using previous study prevalence (25.4%) in Isfahan province, Iran, 95% confidence interval (CI), and 5.0% precision (6).

Sample Size n = N * [Z^2 * p * (1-p)/e^2] / [N - 1 + (Z^2 * p * (1-p)/e^2].

Stool specimen processing

Each patient had a single fecal sample (~20-30gm) taken from them. Study patients were interviewed usingthe structured questionnaire and information was obtained on demographic characteristics, present and past history of diarrhea, antibiotic treatment and clinical data including CD4 count, details of antiretroviral therapy (if any), and known concurrent infections. Each sample was inspected microscopically for opportunistic parasites and *H. pylori* and *Cryptosporidium* spp. were detected using nested PCR.

Copro-parasitological examination

By using a direct wet mount with Lugol's iodine staining; all obtained fecal samples were microscopically inspected for opportunistic parasites and related elements such as pus, RBCs, and Charcot-Leyden crystals (7). A microscopic analysis of fecal samples after formalin-ether treatment was also carried out (8). For coccidian protozoa detection, fecal smears were stained with Kinyoun modified acid-fast stain (9).

Copro-PCR assay

Genomic DNA extraction

The oocyst wall was disrupted by thermal shocking each fecal specimen, and genomic copro-DNA was extracted from each sample using the Favor Stool DNA Spin Columns Isolation Kit (cat. no. FAST1; Favorgen Biotech Corporation, Taiwan) according the manufacturer's instructions.

H. pylori nested polymerase chain reaction (nPCR) assay

Using two sequential PCR reactions, *H. pylori* extracted DNA was amplified using nested PCR targeting the *H. pylori* UreA gene. Using the external primers set, the first response amplified result was a 293 bp fragment; 2F2 5'-ATATTATGGAAGAAGCGAGAGC-3' and 2R2 5'-ATGGAAGTGTGAGCCGATTTG-3'. The second reaction amplified product was 200 bp fragment by internal primers set; 2F3 5'-CATGAAGTGGGTATTGAAGC -3' and 2R3 5'-AAGTGTTGAGCCGATTTGAACCG -3'. Amplification in both reactions was carried out according to Sasaki et al., (10). The ethidium bromide-stained nested PCR products were electrophoresed on a 1.5 % agarose gel in TAE buffer and viewed with a UV transilluminator.

Cryptosporidium spp. nPCR assay

Cryptosporidium spp. extracted DNA amplification was conducted by nested PCR that targeted the COWP gene, which involved two sequential PCR reactions. The primary reaction

amplified the 769-bp fragment by using BCOWPF:5 -ACCGCTTCTCAACAACCATCTTGTCCTC-3;and BCOWPR:5´- CGCACCTGTTCCCACTCAATGTAAACCC-3. The secondary reaction amplified the 553-bp fragment by internal sets - Cry-15: 5´-GTAGATAATGGAAGAGATTGTG-3´ and Cry-9:5´-GGACTGAAATACAGGCATTATCTTG-3´. Amplification in each reaction was done following Spano et al., (11) and Pedraza-Díaz et al., (12). Ethidium bromide was used to stain the amplified products, which were then electrophoresed on a 1.5 % agarose gel in TAE buffer and observed using a UV transilluminator. In terms of genotype, Analysis of Restriction-fragment length polymorphism (RFLP) was performed using RsaI to fragment *Cryptosporidium* PCR products according to the manufacturer's instructions (product no. ER1121; Thermo Scientific). After staining with ethidium bromide, fragmented PCR products were electrophoresed in a Metaphor agarose gel (3%) and gels were examined by UV transillumination.

Data were coded and entered using the statistical package of social science (IMB SPSS) version 21 (Chicago, IL, USA) for statistical analysis. The qualitative and quantitative data were presented, and the chi-squared test and Fisher's exact test were used to compare groups when applicable. P-value was statistically significant if the P <0.05

The study was approved by the University of Tehran Ethics Committee (Number: IR.TUMS.SPH.REC.1398.096). All subjects gave their verbally informed consent. Participants were informed that they could stop at any time while completing the study, that all data were kept confidential and secure and with no participant identities.

RESULTS

Socio-demographic characteristics

During the study period, a total of 70 HIV-positive patients were collected. Twenty one (30%) participants were female and 49 (70%) were male. Their age ranged from 13 to 60 years old, with mean ± SD of 36 ±12 years, and predominance of age groups 46-60 and 31-45 (42.8% & 41.4%, respectively). According to stool consistency of the patients was recorded as follows: 35 (50%) diarrheic patients and 35 (50%) being non-diarrheic. Out of all study populations 42 (60%) were literate and animals were contacted by 36 (51.43%).

Clinical and laboratory characteristics

CD4+ T counts (200 cells/mm3) and viral loads (between 10,000 and 100,000) both increased the likelihood of diarrhoea, although not in a way that was statistically significant (p > 0.05), according to an analysis of the clinical and laboratory characteristics of patients with diarrhoea. The strongest associations with diarrhoea were seen in an analysis of the prevalence of intestinal parasites stratified by HAART (highly active antiretroviral therapy) and non-HAART (OR: 2.64; IC95 0.21 to 24.9) with significant differences (p=0.0089)).

Intestinal and opportunistic parasites prevalence

Intestinal parasites (IPs) were found in 52 (74.3%) of the individuals, with *Entameba histolytica* 8 (11.4%), *Giardia intestinalis* 6 (8.6%), *Hymenolepis nana* 4 (5.7%), *Schistosoma mansoni* and *Taenia saginata* 2 (2.9%) being the most common. In terms of opportunistic parasites, *Isospora belli* was found microscopically in 10 (14.3 %) of cases and *Cryptosporidium* spp was found in 20 (28.5%) of cases (Figure1). When the COWP gene and RFLP for *Cryptosporidium* were examined, the prevalence of *C. parvum* was discovered 13 (65%) (Figure2). Polyparasitism was found in 18 (25.8%) of the cases, while a single parasitic agent was found in the remaining cases 34 (48.5 %). Statistically, a strongly significant association was found between the presence of parasites and diarrhea (p= 0.000, OR= 4.22, 95% CI= 1.91-9.68), also between opportunistic parasites and diarrhea (p= 0.000, OR= 4.20, 95% CI= 2.11-8.45).

The frequency of *H. pylori*

The UreA gene for *H. pylori* was found in 15 (21.4 %) of the study populations, with a significant prevalence

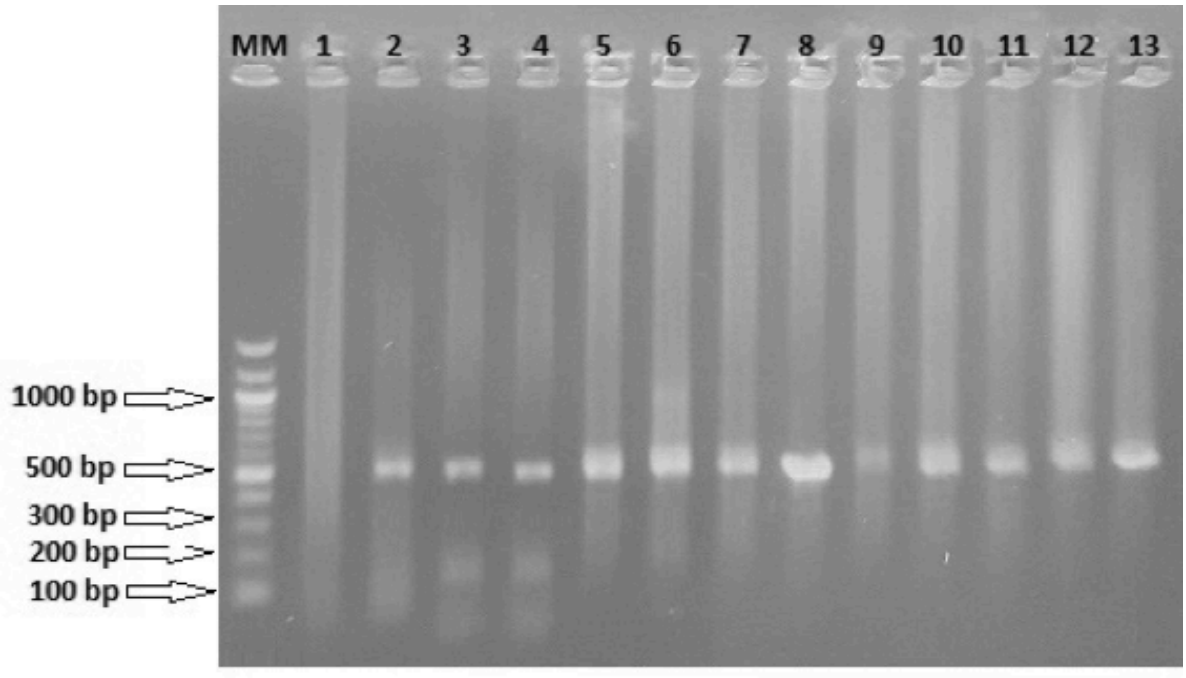

Figure 1. Agarose gel electrophoresis for the products of the nPCR targeting COWP gene of *Cryptosporidium* spp.at 553bp. LaneMM: 50 bp DNA molecular weight marker "ladder". Lane 1: Negative control. Lane 2: Positive control Lanes 3-13: Positive samples.

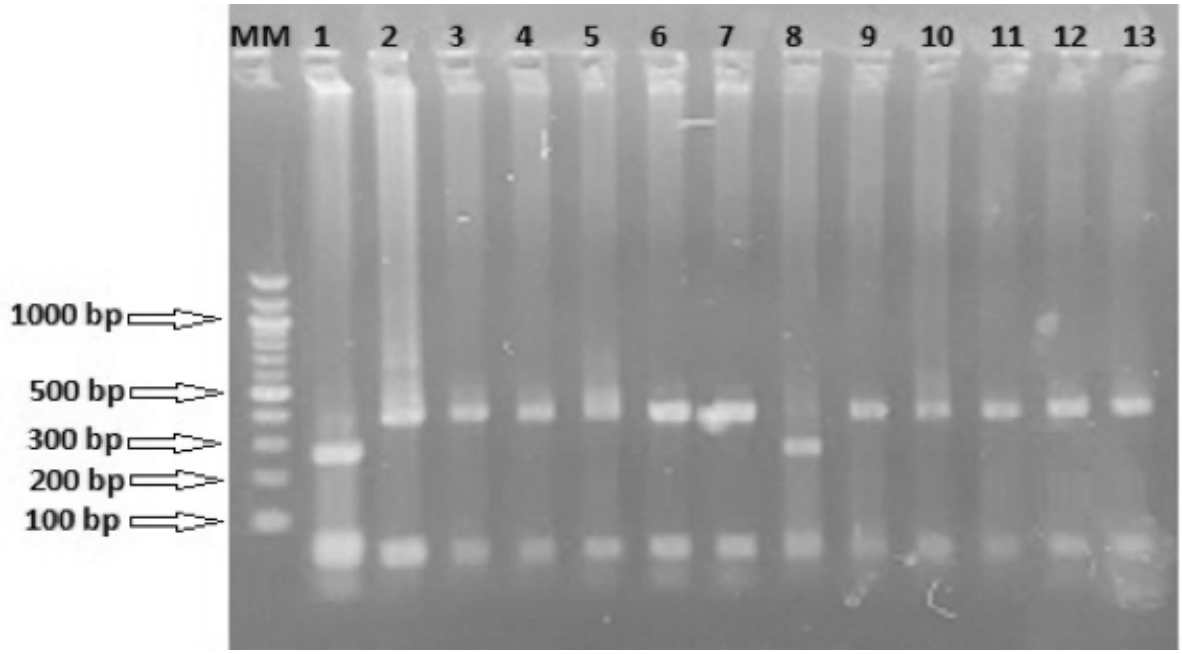

Figure 2. Agarose gel electrophoresis for the products of the nPCR targeting COWP gene of *Cryptosporidium* spp. after digestion by RsaI. Lane MM: 100 bp DNA molecular weight marker "ladder". Lanes 1, 8: Positive *C. hominis* samples (285, 125, 106, and 34 bp). Lanes 2-7, 9-13: Positive *C. parvum* samples (410, 106, and 34 bp).

in the 45-60 age group 8 (11.4 %) and the 31-45 age group 5 (7.1 %). Table (1) shows how the rate of *H. pylori* infection changed according to socio-demographic data without showing any significant correlation. Diarrhea and abdominal pain were reported as the main symptoms for each infection agent Table (2).

H. pylori co-infection with opportunistic and intestinal parasites

The presence of *H. pylori* and parasites was found in 13/52 (25%) of the total participants. *Cryptosporidium* spp. were the most common co-infected agent, accounting for 7/13 (53.85%), *E. histolytica* 3/13 (23.07%), *G. intestinalis* 2/13 (15.38%), and *Isospora belli* 1/13 (7.7 %). Table (3) summarizes the co-infection rate and socio-demographic data association. Statistically, a strongly significant association was found between the presence of opportunistic parasites (*Cryptosporidium* spp. & *Isospora belli*) and *H. pylori* (OR=3.90, 95% CI=1.72-8.24).

Table 1. Socio-demographic data and infection rate

Variables Age goups	Total N (%)	*Cryptosporidium spp.* Positivity N (%)	*p*-value	*Isospora belli* Positivity N (%)	*p*-value	*H. pylori* Positivity N (%)	*p*-value
15-30	11(15.80%)	06(54.50%)		00 (0.00%)		02(13.00%)	0.8029
31-45	29(41.40%)	00(0.00%)	**0.00032***	09 (31.00%)	**0.0187***	05(33.00%)	
46-60	30(42.80%)	14(46.60%)		01 (3.00%)		08(54.00%)	
Sex			**0.062**		**0.0556**		1.00
Male	49(70.00%)	09(18.70%)		10(20.41%)		11(73.34%)	
Female	21(30.00%)	11(52.30%)		00(0.00%)		04(26.66%)	
Education level			**0.797**		**0.0046***		0.774
Literate	42(60.00%)	13(31.00%)		01(2.40%)		08(54.00%)	
Illiterate	28(40.00%)	07(25.00%)		09(32.10%)		07(46.00%)	
Animal house			**0.00003***		**0.0173***		0.582
Yes	36 (51.40%)	20(55.60%)		01(2.80%)		09(25.00%)	
No	34 (48.60%)	00(0.00%)		09(26.50%)		06(11.80%)	
Total	**70(100.00%)**	**20(28.60%)**		**10 (14.30%)**		**15(21.42%)**	

Data presented as n and % age with (*) *p*-value < 0.05 is significant.

Table 2. Clinical symptoms and infection association

Variables	*Cryptosporidium spp.* Positivity N (%)	*p* value	*Isospora belli* Positivity N (%)	*p*-value	*H. pylori* Positivity N (%)	*p* value
Abdominal pain		**0.000***		**0.198**		0.190
Yes	20 (100.00%)		04 (40.00%)		11 (73.34%)	
No	00 (0.00%)		06 (60.00%)		04 (26.66%)	
Diarrhea		**0.000***		**0.006***		0.382
Yes	20 (100.00%)		01 (10.00%)		09 (60.00%)	
No	00 (0.00%)		09 (90.00%)		06 (40.00%)	

Data presented as n and percentage with (*) *p*-value < 0.05 is significant.

Several symptoms were noted, with diarrhoea being the most common gastrointestinal infection symptom and abdominal discomfort being the most common parasite infection symptoms (Table 4).

Table 3. Socio-demographic data and co-infection association

Variables	*Coinfection of H.pylori & Cryptosporidium spp.* Positivity N (%)		*p*-value	*Co-infection of H. pylori & Isospora belli* Positivity N (%)		*p*-value
Age			**0.054**			0.620
15-30	02	(28.55%)		00	(0.00%)	
31-45	00	(0.00%)		01	(100.00%)	
46-60	05	(71.45%)		00	(0.00%)	
Total	**07**	(100.00%)		**01**	(100.00%)	
Sex			**0.099**			0.246
Male	03	(42.85%)		01	(100.00%)	
Female	04	(57.15%)		00	(0.00%)	
Education level			**0.089**			0.771
Literate	05	(71.45%)		00	(0.00%)	
Illiterate	02	(28.55%)		01	(100.00%)	
Animal house			**0.007***			0.522
Yes	07	(100.00%)		01	(33.33%)	
No	00	(0.00%)		00	(66.67%)	

Data presented as n and percentage with (*) *p*-value < 0.05 is significant.

Table 4. Co-infection and associated symptoms

Variables	*Coinfection of H.pylori & Cryptosporidium spp.* Positivity N (%)		*p*-value	*Co-infection of H. pylori & Isospora* Positivity N (%)		*p*-value
Abdominal pain			**0.019***			0.771
Yes	07	(100.00%)		01	(100.00%)	
No	00	(0.00%)		00	(0.00%)	
Diarrhea			**0.005***			0.555
Yes	07	(100.00%)		00	(0.00%)	
No	00	(0.00%)		01	(100.00%)	

Data presented as n and %age with (*) *p*-value < 0.05 is significant.

Risk factors for *H. pylori* co-infection with opportunistic and intestinal parasites

In order to reveal potential shared risk factors that could explain the link between *H.pylori* and certain opportunistic and intestinal parasites, researchers conducted a study. Several environmental markers that indicate fecal-oral transmission pathway exposure have been studied. Variables like animal interaction and co-infection with *H.pylori* by *Isospora belli* raise the risk by more than double (p= 0.52, OR= 2.72, 95% CI=0.86-8.69). Gender was found to be a common risk factor for *H.pylori* co-infection with *Cryptosporidium* spp. and *Isospora belli*. (p= 0.099, OR=1.97, 95% CI=0.45-8.55 & p= 0.246, OR=1.93 95%

CI=0.49-7.66)Co-infection causes abdominal pain and diarrhoea as a main symptom. Statistically, a strongly significant association was found between coinfection of (*Cryptosporidium* spp. & *H.pylori*) and abdominal pain (p= 0.019, OR=4.11, 95% CI=1.81-8.35) & diarrhea (p= 0.005, OR=3.70, 95% CI=1.69-8.41).

DISCUSSION

Parasitic infections are ubiquitous throughout the world, especially in areas where HIV/AIDS is widespread (13). In this study, 52 of 70 HIV-positive patients (74.3 %) tested positive for parasite infection. The finding was higher than previous studies reported from Ethiopia (13.9%) (14), Nigeria (11.4%) (15), Cameroon (14.64%) (14, 16) and Iran (67.7%) (17). The disparity in prevalence could be explained by differences in sampling size, study population, and socioeconomic position. With regard to the species of intestinal parasites, the most common parasite detected in this study were *Cryptosporidium* spp 30 (42.8%), *Entameba histolytica* 8 (11.4%), *Giardia intestinalis* 6 (8.6%), *Hymenolepis nana* 4 (5.7%), *Schistosoma mansoni* and *Taenia saginata* 2 (2.9%). The finding was higher than previous studies reported from Iran, which showed that the prevalence of cryptosporidiosis among HIV/AIDS patients was 10.8% (18).

In HIV patients with diarrhoea, the parasite *Isospora belli* is the most common. *Isospora belli* was found in 10/70 (14.3 %) cases in this investigation. This finding is in the line of Certad et al., (19) who reported *Isospora belli* in 14 % of the cases. In contrast to other studies this reported high prevalence in Nigeria (24.3%) (20), and a low prevalence has been shown in Italy (0.6%) (21). Statistically, a strongly significant association was found between the presence of parasites and diarrhea, also between opportunistic parasites and diarrhea. In agreement with Wang et al., (22), who reported that the estimated pooled random effects ORs of *Cryptosporidium*, microsporidia and *Isospora* infection in HIV people with diarrhea compared with their controls were 4.09 (95% CI: 2.32–7.20), 4.72 (95% CI: 3.47–6.42), and 4.93 (95% CI: 3.33–7.29), respectively. The strongest associations with diarrhoea were seen in an analysis of the prevalence of intestinal parasites stratified by HAART (highly active antiretroviral therapy) and non-HAART with significant differences. These findings are in the line of previous study of Barcelos et al.,(23), who reported a significant positive correlation between intestinal parasites and the clinical status and the use of antiretroviral therapy (ART) and CD4+ lymphocyte counts.

The total prevalence of *H. pylori* was 15/70 in this study (21.4 %). This study's results are lower than earlier published study in Iran (24) which recorded 69.7%. Furthermore, it is greater than the 8.3 % rate reported by Perry et al., (25) from Romania. *H. pylori* co-existence with intestinal and opportunistic parasites is more common in low-income developing countries, and it may be connected mechanically or pathologically in HIV patients due to immune defects. This hypothesis may be supported by our findings of a statistically significant association between the presence of *H. pylori* and polyparasitism of intestinal and opportunistic parasites in HIV patients. Polyparasitism can increase the susceptibility of humans to *H. pylori* and other intestinal microbial infections. The estimated risk factors for *H. pylori* and gastrointestinal parasites are the same (26). Co-infection with *H. pylori* and intestinal parasites may have a synergistic effect, resulting in catastrophic health implications. Cryptosporidiosis co-infection with *H. pylori* was found to be (53.85 %), followed by *E. histolytica* (23.07 %), *G. intestinalis* (15.38 %), and *Isospora belli* (7.7%) with statistical significance (27). The prevalence of cryptosporidiosis co-infection with *H. pylori* (60%) and giardiasis (58%) in children was reported by Ibrahim et al., (5). The variance in prevalence rates could be attributable to variance in the study population.

Furthermore, *H. pylori* may support *Cryptosporidium* spp. and *Giardia intestinalis* colonization in the human gastrointestinal tract, which occurs when the urease enzyme produced by *H. pylori* overcomes stomach acidity (28, 29). Statistically, a strongly significant association was found between the presence of opportunistic parasites (*Cryptosporidium* spp. & *Isospora belli*) and *H. pylori*. This high level of co-infection could indicate that *H. pylori* is a

risk factor for intestinal and opportunistic parasite infection, or vice versa, but more research is needed.

Many socio-behavioral and demographic variables have been linked to *H. pylori* in the past, with conflicting results (30, 31). Animal (cow, goat, and sheep) contact was connected as one of the major sources of *H. pylori* infection in our investigation, however there was no significant relation between age, gender, education level, or animal (cow, goat, and sheep) contact (32). The results of our research revealed an association between *Isospora belli* and *Cryptosporidium* spp. and gender, age group, education level, and animal exposure. The small sample size of our study, which could be attributed to a lack of financing, is a significant limitation. Another constraint was the lack of precise data. A sample for estimation will adequate for the capture and recapture model.

In conclusion; this study found a substantial association between *Cryptosporidium*, *Giardia intestinalis*, and *H. pylori* co-infection in 74.3 % of IPs, 21.4 % of IPs, and *H. pylori* infection, respectively. In HIV patients, *H. pylori* was found in 53.85% of those with cryptosporidiosis, 15.38 % with giardiasis, and 7.7% with isosporiasis. Our study highlighted that *Cryptosporidium parvum* was more prevalent in patients living with HIV. For efficient therapy of gastrointestinal infectious agents, HIV positive patients should be tested for cryptosporidiosis, isosporiasis, and giardiasis in addition to *H. pylori* studies. Gender, diarrhoea, abdominal pain, and polyparasitism have all been identified as risk factors for co-infection with *H. pylori*. This research sheds further light on their co-infection and the subsequent development of medications to help patients living with HIV.

ETHICS COMMITTEE APPROVAL

* The study was approved by the University of Tehran Ethics Committee (Number: IR.TUMS.SPH.REC.1398.096).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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