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Original Article

Assessment of *Blastocystis hominis* as a Risk Factor for Iron Deficiency Anemia in Pregnant Women

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Abstract

Background: Iron deficiency anemia (IDA) is a common health issue during pregnancy and may be influenced by parasitic infections such as *Blastocystis hominis*. This study aims to assess the role of *Blastocystis hominis* infection as a potential risk factor for IDA in pregnant women.

Methods: A total of 208 pregnant women were enrolled in this cross-sectional study, including 98 women with IDA (case group) and 110 women without IDA (control group), conducted at Imam Reza and Ghaem Hospitals in Mashhad, Iran during 2022–2023. Hemoglobin and ferritin levels were measured in all participants, with anemia defined as hemoglobin <11 g/dL and ferritin <15 µg/L. Stool samples were collected from all participants to detect *B. hominis* and other parasitic infections using direct smear, formalin-ether concentration, and modified acid-fast staining methods. Statistical analyses were performed using independent samples t-test and chi-square test, with a significance level of $P<0.05$.

Results: The prevalence of *B. hominis* infection was significantly higher in the case group (38.8%) compared to the control group (10.9%) ($P<0.001$). Hemoglobin and ferritin levels were significantly lower in the case group ($P<0.001$). However, no significant difference was observed between the groups in terms of socioeconomic status, education, or occupation.

Conclusion: *B. hominis* infection may contribute to iron deficiency anemia in pregnant women. Screening for parasitic infections in anemic pregnant women, particularly *B. hominis*, could improve anemia management during pregnancy.

Introduction

Iron deficiency anemia (IDA) is one of the most widespread nutritional deficiencies worldwide, particularly affecting pregnant women. During pregnancy, iron requirements increase substantially due to the growing fetus and placenta, as well as the expansion of maternal red blood cell mass. Failure to meet these increased demands can result in iron deficiency, which has been linked to several adverse maternal and fetal outcomes, including preterm birth, low birth weight, perinatal mortality, and impaired cognitive and physical development in infants. Maternal consequences include fatigue, reduced work capacity, increased susceptibility to infections, and, in severe cases, maternal morbidity and mortality. Hence, addressing and preventing IDA during pregnancy is a significant public health priority (1,2).

While inadequate dietary iron intake and increased physiological demands are well-known causes of IDA, parasitic infections, particularly in regions where these infections are endemic, may also contribute to the burden of iron deficiency (3). Gastrointestinal parasites are known to impair nutrient absorption, cause gastrointestinal blood loss, and exacerbate nutritional deficiencies. Among the various intestinal parasites, *Blastocystis hominis* has recently garnered attention due to its high global prevalence and its controversial role in human health (4,5).

B. hominis is an anaerobic protozoan commonly found in the gastrointestinal tract of humans and animals. Although historically considered a non-pathogenic commensal, recent studies have suggested that *Blastocystis* may be associated with a range of gastrointestinal symptoms such as diarrhea, abdominal pain, and bloating. More importantly, emerging evidence indicates a potential link between *Blastocystis* infection and malabsorption syndromes, particularly affecting the absorption of key nutrients like iron (6). This raises the question of whether *B. hominis* infection could be a contributing factor to iron deficiency anemia, especially in vulnerable populations like pregnant women.

The potential mechanisms by which *Blastocystis* may influence iron absorption are not yet fully understood. However, the parasite may disrupt the intestinal mucosa, leading to inflammation, altered gut permeability, and impaired nutrient uptake. Additionally, *Blastocystis* infection could increase the loss of iron through gastrointestinal bleeding or induce changes in the microbiota that negatively affect iron bioavailability (6).

Given the high prevalence of *B. hominis* in many regions, particularly in developing countries, and the critical importance of maintaining adequate iron levels during pregnancy, further research is warranted to elucidate the potential relationship between *Blastocystis* infection and iron deficiency anemia (6,7)

We aimed to investigate the association between *B. hominis* infection and iron deficiency anemia in pregnant patients attending Imam Reza and Ghaem Hospitals in Mashhad, Iran. By exploring this potential risk factor, the research seeks to enhance the understanding of how parasitic infections may exacerbate nutritional deficiencies during pregnancy, providing valuable insights for improving maternal health outcomes in endemic areas.

Methods

This cross-sectional study was conducted on pregnant women attending prenatal check-ups at Imam Reza and Ghaem Hospitals in Mashhad, Iran during 2022-2023. A total of 198 participants were included: 98 pregnant women diagnosed with iron deficiency anemia (IDA) and 100 non-anemic pregnant women serving as the control group. Anemia was defined by hemoglobin levels below 11 g/dL, serum ferritin levels under 15 µg/L, and the

presence of hypochromic microcytic red blood cells on peripheral blood smears, as per the WHO criteria (8). Hemoglobin levels between 9-10.9 g/dL were considered mild anemia, 7-8.9 g/dL moderate, and below 7 g/dL severe (9).

For each participant, demographic data including age, education level, and occupation, number of previous pregnancies, medical history, socioeconomic status, and history of specific diseases (e.g., sickle cell anemia, hepatitis, chronic inflammatory diseases, and parasitic infections) were collected. Physical examinations included assessment of systemic illnesses, abdominal exams, and obstetric evaluations. Patients consuming iron supplements, or those undergoing treatment for parasitic infections, were excluded from the study.

Fecal samples were collected from all participants. Each individual provided three stool samples, collected on different days. These samples were examined microscopically using physiological saline and Lugol's iodine staining for direct identification of parasites. Additionally, a formalin-ether sedimentation technique was employed for detecting ova and cysts of intestinal parasites. Stool samples were also preserved in 10% buffered formalin for further analysis and subjected to modified acid-fast staining for the detection of *Cryptosporidium* spp.

Statistical Analysis

Data were analyzed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including measures of central tendency and dispersion, were presented in tables and charts. For comparison between groups, independent sample t-tests were used for continuous variables, and chi-square or Fisher's exact tests were applied for categorical variables. A *P*-value of less than 0.05 was considered statistically significant.

Ethical Considerations

Prior to enrollment, all participants were provided with detailed information about the study's objectives and procedures. Written informed consent was obtained from each participant. Confidentiality was ensured by anonymizing data and using coded identifiers. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IRAN.MUMS.MEDICAL.1397.640), and participants were free to withdraw from the study at any time without penalty.

Results

A total of 208 pregnant women participated in the study including 98 in the IDA group and 110 in the control group. The mean age of participants did not show a statistically significant difference between the two groups (P = 0.266). Similarly, gestational age was comparable between them (P = 0.604). However, hemoglobin levels were significantly lower in the IDA group compared to the control group (P<0.001). Additionally, ferritin levels were markedly reduced in the IDA group (P<0.001) (Table 1).

Table 1: Comparison of clinical characteristics between case and control groups in pregnant women

Characteristic	Group (Case) (N=98)	Group (Control) (N=110)	*P*-Value
Age (yr)	27.25 ± 5.80	28.33 ± 5.61	0.266
Gestational age (weeks)	14.79 ± 2.95	15.04 ± 2.38	0.604
Hemoglobin (g/dl)	9.51 ± 0.76	13.19 ± 0.83	<0.001
Ferritin (µg/L)	11.72 ± 1.49	38.80 ± 10.87	<0.001

Frequency of Blastocystis hominis Infection

B. hominis infection was identified in 38.8% of the IDA group (38 participants) and 10.9% of the control group (12 participants), indicating a statistically significant difference ($P<0.001$). Table 2 summarizes these findings.

Table 2: Frequency of *Blastocystis hominis* infection among pregnant women

Group	*Blastocystis* Positive (%)	*Blastocystis* Negative (%)	Total (%)	*P*-value
IDA Group (n=98)	38 (38.8)	60 (61.2)	98 (100)	<0.001
Control Group (n=100)	12 (10.9)	98 (89.1)	110 (100)	
Total	50 (24)	148 (76)	208 (100)	

Analysis of Demographic Factors

The analysis of demographic factors such as residence, education, and occupation did not show a significant association with *B. hominis* infection, except for education level. Participants with lower educational attainment were more likely to be infected ($P = 0.005$). These findings are detailed in Table 3.

Table 3: Demographic and clinical characteristics between case and control groups in pregnant women

Characteristic	Group (Case) (N=98)	Group (Control) (N=110)	Total	*P*-value
Residence Area (%)				
High socioeconomic area	12 (12.2)	12 (10.9)	24 (24.5)	0.806
Low socioeconomic area	86 (87.8)	98 (89.1)	184 (75.5)	
Total	980(100)	110 (100)	208 (100)	
Education Level (%)				
Less than high school	20 (20.4)	14 (12.7)	34 (16.4)	0.450
High school diploma	68 (69.4)	86 (78.2)	154 (74)	
University degree	10 (10.2)	10 (9.1)	20 (9.6)	
Total	98(100)	110 (100)	208 (100)	
Occupation (%)				
Self-employed	1 (1.0)	0 (0.0)	1(0.5)	0.810
Homemaker	93 (94.9)	104 (94.5)	197 (94.7)	
Office worker	4 (4.1)	6 (5.5)	10 (4.8)	
Total	98(100)	110(100)	208(100)	

Hemoglobin Levels in Blastocystis-Infected vs. Non-Infected Cases

The mean hemoglobin levels were significantly lower in *Blastocystis*-infected cases among pregnant women with IDA (9.13 ± 0.45 g/dL) compared to non-infected IDA patients (9.67 ± 0.78 g/dL) ($P<0.05$).

Prevalence of Other Enteric Parasitic Infections

The prevalence of other enteric parasitic infections was low (4 participants or 2.6%), with no significant difference between the groups ($P = 0.999$) (Table 4).

Table 4: Prevalence of enteric parasitic infections among pregnant women

Parasite	IDA Group (n=98)	Control Group (n=110)	Total (n=208)	*P*-value
Trichomonas hominis	1	1	2	0.999
Chilomastix mesnili	1	0	1	
Iodamoeba butschlii	1	0	1	
Total	3	1	4	

Symptomatic vs. Asymptomatic Blastocystis-Infected Cases

Among the *Blastocystis*-infected cases, 45% were symptomatic (e.g., abdominal pain, diarrhea, bloating), while 55% were asymptomatic. Symptoms were more common in the IDA group compared to the control group.

Discussion

We aimed to investigate the association between *B. hominis* infection and IDA in pregnant women, revealing a significantly higher prevalence of *Blastocystis* infection in the anemic group compared to the non-anemic control group. The results demonstrate that 38.8% of women in the IDA group were infected with *B. hominis*, while only 10.9% of the control group harbored the parasite. These findings suggest a potential link between *Blastocystis* infection and the development or exacerbation of IDA, which is consistent with the growing body of literature that implicates gastrointestinal parasites as contributing factors to nutritional deficiencies, particularly in vulnerable populations like pregnant women.

The high prevalence of *B. hominis* observed in this study aligns with similar findings in endemic regions. For instance, El Deeb and Khodeer reported a 54.2% prevalence of *Blastocystis* in their IDA group, while the control group showed a 17.3% infection rate. The even higher prevalence observed in their study may be due to differences in the population's socioeconomic status, environmental hygiene, or dietary habits. These factors have been recognized as contributing to parasitic infections, particularly in developing countries where access to clean water and proper sanitation is often limited (10).

Both the present study and the Deng et al. study highlight the association between *Blastocystis* infection and anemia (11). The present study reports a significantly higher prevalence of *B. hominis* in pregnant women with iron deficiency anemia (38.8%) compared to controls (10.9%), while the Deng et al. Study found a lower overall prevalence of *Blastocystis* sp. (9.47%) in the general population. Both studies identified anemia as a risk factor, with ST1 subtype in the mentioned study showing a strong association with anemia (11). Differences in diagnostic methods (microscopy vs. PCR), study populations (pregnant women vs. general rural populations), and geographic and hygienic factors contribute to variations in prevalence and findings. These results emphasize the need for advanced diagnostic tools and longitudinal studies to further explore the role of *Blastocystis* in anemia.

The findings of our study align with those reported in El Deeb et al. study, particularly in demonstrating a significantly higher prevalence of *B. hominis* infection in pregnant women with IDA compared to non-anemic controls (12). However, the overall prevalence in our study was slightly lower (38.8% in IDA cases and 10.9% in controls) compared to the abstract (40% in IDA cases and 6.3% in controls). Both studies identified a significant reduction in hemoglobin levels among *Blastocystis*-infected IDA patients compared to non-infected IDA cases, with our study reporting mean hemoglobin levels of 9.13 g/dl in in-

fected cases versus 9.67 g/dl in non-infected cases ($P<0.05$), while the abstract indicated more pronounced differences (9.1 g/dl vs. 10.0 g/dl, $P<0.0001$). Co-infections with other intestinal parasites were also less frequent in present study (2.6%) compared to El Deeb et al. study (14.6%), likely due to differences in diagnostic methods or population characteristics (12). Despite these variations, both studies emphasize the potential role of *B. hominis* as a contributing factor to IDA and highlight the need for routine parasitological screening in antenatal care to improve anemia management in pregnant women.

Several mechanisms could explain the association between *Blastocystis* infection and IDA. One hypothesis is that *Blastocystis* disrupts the intestinal mucosa, leading to malabsorption of essential nutrients, including iron. This malabsorption could be exacerbated by inflammation of the gut, which is commonly associated with *Blastocystis* infection (13). *Blastocystis* can induce localized inflammation and alter gut permeability, which may impair iron absorption and contribute to iron deficiency. Another potential mechanism is that *Blastocystis* may interact with the gut microbiota, influencing the bioavailability of iron or leading to an imbalance that favors nutrient deficiencies (14).

Interestingly, the relationship between *Blastocystis* and iron deficiency has been observed in various populations beyond pregnant women. Research found a similar association between *Blastocystis* infection and iron deficiency in non-pregnant adults, particularly in individuals with gastrointestinal symptoms such as diarrhea or chronic gut inflammation. Their findings suggest that *Blastocystis* may have a broader impact on nutrient absorption, not limited to pregnancy (15).

However, it is important to note that *B. hominis* has long been regarded as a commensal organism in the gut microbiota, with debates ongoing about its pathogenic potential (16). While the results of this study and others suggest a correlation between *Blastocystis* infection and iron deficiency, causality has not been definitively established. Additional research, particularly longitudinal studies or controlled clinical trials, is needed to further explore the role of *Blastocystis* in nutrient malabsorption and its possible contribution to iron deficiency anemia.

In this study, socioeconomic and demographic factors such as residence, education level, and occupation were also examined in relation to *Blastocystis* infection. Interestingly, while no significant differences were found between urban and rural dwellers or between different occupations, the infection rate was significantly higher among individuals with lower educational attainment. This finding aligns with previous studies, which have demonstrated that lower socioeconomic status and reduced access to healthcare and hygiene resources are associated with higher rates of parasitic infections, including *Blastocystis* (17). Poor sanitation and limited health literacy in these populations may contribute to the persistence of *Blastocystis* infection, further exacerbating nutritional deficiencies such as iron deficiency anemia.

A notable aspect of our findings is the relatively low prevalence of other intestinal parasites in the studied population, with only 2.6% of participants being infected with parasites such as *Trichomonas hominis* and *Chilomastix mesnili*. This low prevalence suggests that *Blastocystis* is the dominant intestinal parasite in this population, further underscoring its potential role in contributing to iron deficiency anemia. Compared to other parasitic infections known to cause significant blood loss or nutrient depletion, *Blastocystis* has traditionally been viewed as less harmful. However, the high rate of infection in the anemic group calls for a reevaluation of its pathogenic potential, particularly in relation to micronutrient malabsorption.

Given that iron deficiency is one of the most common nutritional deficiencies globally, particularly in pregnant women, identifying and addressing parasitic infections like *Blastocystis* could have a significant impact on public

health. While iron supplementation remains the mainstay treatment for IDA, particularly during pregnancy, treating underlying parasitic infections could improve the effectiveness of such interventions. Therefore, a dual approach involving both iron supplementation and anti-parasitic treatment could be more effective in managing iron deficiency in parasitized populations.

One limitation is the cross-sectional design, which prevents establishing a definitive causal relationship between *B. hominis* infection and iron deficiency anemia. Longitudinal studies are needed to track the progression of *Blastocystis* infection over time and its direct effects on iron levels. Additionally, while fecal analysis was used to detect *Blastocystis*, more advanced diagnostic techniques, such as molecular methods (e.g., PCR), could improve the accuracy and sensitivity of detecting the parasite, particularly in asymptomatic carriers.

Another limitation is the relatively small sample size. Larger studies across different geographic regions and populations would provide more robust data on the prevalence of *Blastocystis* infection and its association with IDA. Furthermore, this study did not investigate the presence of other nutritional deficiencies, such as folate or vitamin B12 deficiency, which could also contribute to anemia and may be linked to parasitic infections.

Conclusion

The results of the present study provide evidence that *Blastocystis* sp. may be a risk factor for the development of iron deficiency anemia (IDA) in pregnant women. This suggests that *Blastocystis* infection should be considered in the differential diagnosis of IDA in this vulnerable population. While this study specifically highlights *Blastocystis* as a potential risk factor for the development of iron deficiency anemia, further research is needed to determine if this association is also applicable to the general population. Additional studies involving diverse groups are necessary to explore whether *Blastocystis* infection similarly contributes to iron deficiency in broader demographic settings.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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