Parasitol Res (2012) 110:2167–2174
DOI 10.1007/s00436-011-2743-3

ORIGINAL PAPER

Blastocystis hominis as a contributing risk factor for development of iron deficiency anemia in pregnant women

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Received: 4 October 2011 / Accepted: 29 November 2011 / Published online: 14 December 2011

Abstract Intestinal parasitic infection increases the risk of developing iron deficiency anemia (IDA) during pregnancy. The objective of this study was to assess *Blastocystis hominis* as a contributing risk factor for development of IDA in pregnant women. A total of 200 fecal specimens from 120 pregnant women with IDA (mean Hb=9.6 g/dl), and 80 non-anemic controls were examined for *Blastocystis*. Fecal specimens were examined by the formalin/ethyl-acetate concentration technique, iron hematoxylin staining, modified Ziehl–Neelsen acid-fast staining, and by the in vitro cultivation technique for *Blastocystis*. Frequency of *Blastocystis* infection, detected microscopically and by the in vitro culture technique, was significantly higher in IDA study group (n=48; 40%) compared to non-anemic controls (n=5; 6.3%; P<0.0001), and 26.5% (n=53) in all study subjects. Among the 48 cases, *Blastocystis* without other intestinal parasitic infections was detected in 41 cases (34.2%), while seven cases (5.8%) with *Blastocystis* were coinfected with other intestinal parasites which included *Giardia* and *Cryptosporidium* (1.7% each), and *Entamoeba* sp., *Ascaris*, and *Trichuris* (0.8% each). The mean Hb level of the 48 *Blastocystis*-infected cases was 9.2 g/dl (mild anemia). While the other 72 IDA cases with no infection had mean Hb of 10.0 g/dl (mild anemia), with a significant difference in mean Hb level between *Blastocystis*-infected and the non-infected IDA cases (P<0.0001). Furthermore, among the 48 *Blastocystis*-infected IDA cases, the mean Hb of the 41 *Blastocystis*-infected cases without other intestinal parasitic co-infection was 9.1 g/dl (mild anemia), while the mean Hb level of the 7 *Blastocystis*-infected cases with other intestinal parasitic co-infection was 8.7 g/dl (moderate anemia). Findings of the current study showed that *B. hominis* infection contributes to the development of IDA in pregnant women. Hence, parasitological diagnostic tests are recommended in routine examination at all antenatal clinics.

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Introduction

Blastocystis hominis (*B. hominis*) is an obligate anaerobic protozoan found in the human large intestine, and is the most common eukaryotic organism reported in human fecal samples (Tan 2004; Li et al. 2007; Dogruman-Al et al. 2008; Souppart et al. 2009). It has a worldwide distribution, mainly in developing countries where the prevalence is higher (approximately 30% to 50%) than those observed in developed countries (Nascimento and Moitinho 2005). This protozoan can be transmitted as a cyst by the fecal–oral route, especially in areas with poor hygiene and sanitation (Stenzel and Boreham 1996). In fact, the pathogenicity of *B. hominis* is still in debate, because this parasite is very common in many healthy people without showing any symptoms (Tan et al. 2002, 2010; Dogruman-Al et al. 2009; Eroglu et al. 2009). However, numerous recent in vivo and in vitro studies demonstrated the pathogenic power of this parasite (Hussein et

al. 2008; Abdel Hameed and Hassanin 2011). Among the symptomatic individuals, the clinical consequences of *B. hominis* infection are mainly diarrhea or abdominal pain with nonspecific gastrointestinal symptoms (Su et al. 2009); it can remain in the human gastrointestinal tract for several weeks to many years, until appropriately treated (Stenzel and Boreham 1996; Arribas et al. 2001). Recently, an association between *Blastocystis* infection and iron deficiency anemia (IDA) was reported by Yavasoglu et al. in 2008. A few years earlier, *B. hominis* was shown to elicit hematological changes; with a significantly reduced leukocyte count, hemoglobin (Hb) and hematocrit levels (Cheng et al. 2003).

Anemia in pregnancy remains a major problem in nearly all developing countries (Idowu et al. 2005; Rasheed et al. 2008) and prevalence rate of 58% has been reported by the WHO (Galloway et al. 2002). The most common cause of anemia in pregnancy worldwide is iron deficiency (Broek and Letsky 2000; Nyuke and Letsky 2000). In fact, iron losses due to various parasitic infections were reported to increase anemia in pregnant women (Dreyfuss et al. 2000; Rodríguez-Morales et al. 2006; Ayoya et al. 2006; Fuseini et al. 2010). Pregnant women are particularly vulnerable to develop IDA (Ansari et al. 2009) because iron needs during pregnancy are very high; three to four times the iron needs of non-pregnant women, to meet the requirements for the fetus, placenta, and maternal red cell expansion (Hallberg 1988; Zavaleta et al. 2000). This is in addition to inadequate dietary iron intake, particularly in developing countries where the diets are usually low in bio-available iron (Zavaleta et al. 2000; Baidoo et al. 2010). Prevention of IDA is a high priority in most health systems (Ansari et al. 2009), as it is associated with increased risk of maternal mortality, obstetric hemorrhage, preterm delivery, low birth weight, fetal growth retardation, and poorer labor and delivery performance (Weigel et al. 1996; Nyuke and Letsky 2000). Furthermore, IDA during pregnancy is associated with lower iron stores in the fetus (Dreyfuss et al. 2000) and is a risk factor for infant IDA that, if left uncorrected, can be associated with adverse behavioral and cognitive development (Broek and Letsky 2000). Since anemia in pregnancy is an important preventable cause of maternal and perinatal morbidity and mortality (Shulman et al. 2001), the current study aiming to assess *B. hominis* as a contributing risk factor for development of iron deficiency anemia in pregnant women would be of crucial importance for control of this global problem.

Materials and methods

Sampling

Pregnant women included in this study were recruited on attendance to the antenatal clinic of the Obstetrics and Gynecology Department, Faculty of Medicine, Menoufia University, between January 2010 and December 2010. Informed consents for participation in the study and the testing procedure were obtained from all subjects. According to the hematological data, 200 pregnant women were enrolled. These included: (a) 120 pregnant women with the diagnosis of IDA (aged 19–36 years, 11–37 weeks of gestation and mean Hb±SD=9.6±0.565), and (b) 80 non-anemic pregnant women (aged 19–38 years, 11–36 weeks of gestation and mean Hb±SD=11.4±0.267) as a control. Hematological parameters included hemoglobin (Hb), hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red blood cell count (RBC), white blood cell count, and platelets. The hematological diagnostic inclusion criteria for IDA group were (a) Hb of <11 g/dl (complete blood counts were obtained by using Coulter Hmx hematology analyzer—Beckman, USA) with a blood film that showed microcytic hypochromic anemia, and (b), serum ferritin level of <15 μg/L (serum ferritin concentrations were analyzed using the Microparticle Enzyme Immunoassay—cobas e 411 analyzer—Roche/Hitachi). According to WHO definition, pregnant women with Hb≥11 g/dl were considered normal. Hb between 9 and 10.9 g/dl was considered to have mild anemia, between 7 and 8.9 g/dl moderate anemia, and Hb<7 g/dl was severe anemia (WHO 1998).

All selected cases went through a thorough clinical assessment that included history taking and physical examination. Stool specimens were examined for all participants. History taking included demographic and socioeconomic information (maternal age, education, occupation, income, family size, residence, standards of hygiene and sanitary conditions, and contact with animals); qualitative dietary information (amount of weekly meat consumption, tea consumption soon after meals, vegetables, bread and bean intake, and regularity of iron supplementation); menstrual and obstetric history (menarche, history of polymenorrhea or menorrhagia prior to the current pregnancy, age at first conception, parity, birth spacing, history of any blood loss, and level of Hb last recorded); history of recent febrile illnesses, hepatitis, sickle cell disease, and other medical conditions such as chronic inflammatory disorders; history of any parasitic infection (e.g., schistosomiasis, hookworm, trichuriasis, ascariasis, and ameobiasis); symptoms suggestive of *Blastocystis* infection (diarrhea, abdominal pain, nausea, anorexia, vomiting, weight loss, lassitude, dizziness, and flatulence (Yakoob et al. 2004)). Physical examination included general, abdominal, and obstetric examination, in search for any systemic illness. Abdominal ultrasound was performed to determine gestational age, fetal viability, and intrauterine growth restriction. Excluded from the study were women who were on current or had received antibiotics shortly before study enrollment, as this might decrease the chances of finding intestinal protozoa

(John and Petri 2006), any iron supplementation for treatment of current IDA or pregnant women with chronic illness.

Coproparasitologic examination

Stool examination for helminth ova as well as protozoa trophozoites and cysts was carried out in all samples according to Garcia (2001). Prior to collection of stool specimens subjects were asked to avoid enemas, compounds of kaolin and bismuth, milk of magnesia, and antacids as they can interfere with examination for parasites (John and Petri 2006; Su et al. 2009). Three fecal samples were collected from each subject, on non-consecutive days. Stool specimens were first examined macroscopically for mucus, blood and worms. The consistency of each stool specimen was recorded. Stools were then prepared for microscopic examination. Approximately 2 mg of each fecal sample was thoroughly emulsified on a glass slide in one drop of physiologic saline and covered with a cover slip. A similar preparation was made on another slide using Lugol's iodine. These preparations were examined under both the low power (×10) and high dry (×40) objectives. A portion of each sample was preserved in 10% formalin and then processed for the formalin/ethyl-acetate concentration technique. Another portion was fixed in polyvinyl alcohol and consequently iron hematoxylin staining was carried out. For identification of *Cryptosporidium*, modified Ziehl–Neelsen acid-fast staining was used to examine concentrates of stools fixed in formalin.

Culture of feces

In vitro cultivation of stool samples was performed in the medium of Jones (1946), supplemented with 10% horse serum. Cultures were done by inoculating approximately 50 mg of feces into Jones' medium. The cultures were incubated at 37°C for 48–72 h and then examined by light microscopy with ×10 and ×40 objectives.

Statistical analysis

Data were reported as number (percentage) for categorical data and the mean±standard deviation (SD) for continuous variables. Statistical analyses were done using computerized statistical software program IBM SPSS 19.0. The independent sample *t* test was used to assess the statistical significance of the difference between two study group means. The Pearson chi-square test and the Fisher's exact test were used to examine the relationship between two qualitative variables. Statistical significance was defined as p values<0.05.

Results

Selected characteristics of pregnant women included in the current study are shown in Table 1. With exception of parity ($P=0.0001$) and hygiene practices ($P=0.004$), no other differences in characteristics were seen between the IDA and the non-anemic groups. Among pregnant women with IDA ($n=120$) and non-anemic pregnant women ($n=80$), prevalence of *Blastocystis* infection, detected microscopically and by cultivation, were 40% ($n=48$) and 6.3% ($n=5$), respectively, and 26.5% ($n=53$) in all study subjects ($n=200$). Thus, there was a significant difference in frequency of *Blastocystis* infection between the pregnant women with IDA and the non-anemic controls ($p=0.0001$; Table 2).

Furthermore, among the 48 *Blastocystis*-infected cases from IDA group, *Blastocystis* without other intestinal parasitic infections (*Blastocystis*-only infection) were detected in 41 cases (34.2%), while 7 cases (5.8%) with *Blastocystis* were coinfected with other intestinal parasites (*Blastocystis*-mixed infection). On the other hand, *Blastocystis*-only infection was detected in four controls (5%), and one subject (1.3%) had *Blastocystis*-mixed infection (Table 3). Prevalence between symptomatic and asymptomatic cases was similar in both groups (Table 4). Among symptomatic cases with IDA, 60.4% (29 of 48) had abdominal discomfort and 29.2% (14 of 48) had diarrhea with loose consistency of stools and all non-anemic pregnant women complained of diarrhea. Stool microscopy was positive for *Blastocystis* in 29.2% (35 of 120) of the IDA cases, 5% (4 of 80) of the controls and 19.5% (39 of 200) in all study subjects. On the other hand, stool culture was positive for *Blastocystis* in 40% (48 of 120) of the IDA cases, 6.3% (5 of 80) of the controls and 26.5% (53 of 200) in all study subjects. Thus, stool culture for *Blastocystis* was more sensitive than microscopy ($P=0.0001$; Table 5). The mean Hb (in grams per deciliter) of pregnant women with IDA was 9.6±0.565 (mild anemia). Among the 120 IDA cases, the mean Hb of the 48 *Blastocystis*-infected cases was 9.2±0.46 (mild anemia). On the other hand, the other 72 IDA cases with no infection had mean Hb of 10.0±0.328 (mild anemia), with a significant difference in mean Hb level between *Blastocystis*-infected and non-infected IDA pregnant women ($P=0.0001$; Table 6). Furthermore, among the 48 *Blastocystis*-infected IDA cases, the mean Hb of the 41 *Blastocystis*-only infection cases was 9.1±0.298 (mild anemia) and the mean Hb of the 7 *Blastocystis*-mixed infection cases was 8.7±0.127 (moderate anemia; Table 7).

Discussion

Anemia and iron deficiency in pregnancy is a major public health problem in developing countries and is the most

Table 1 Selected characteristics of pregnant women with IDA compared to non-anemic controls

Subject characteristics	Total (n=200)	Pregnant women with IDA (n=120)	Non-anemic pregnant women (n=80)	P value
Age mean (years)±S.D (range)	24.9±4.216 (19–38)	25.1±4.255 (19–36)	24.7±4.172 (19–38)	0.535
Gestation				
1st trimester (%)	42 (21)	28 (23.3)	14 (17.5)	0.578
2nd trimester (%)	91 (45.5)	54 (45)	37 (46.3)	
3rd trimester (%)	67 (33.5)	38 (31.7)	29 (36.3)	
Parity				
Primigravida (%)	88 (44)	27 (22.5)	61 (76.3)	0.0001
Multigravida (%)	112 (56)	93 (77.5)	19 (23.8)	
Settings				
Rural (%)	77 (38.5)	47 (39.2)	30 (37.5)	0.812
Urban (%)	123 (61.5)	73 (60.8)	50 (62.5)	
Literacy				
Literate (%)	54 (27)	36 (30)	18 (22.5)	0.242
Illiterate (%)	146 (73)	84 (70)	62 (77.5)	
Economic status[a]				
Average (%)	34 (17)	25 (20.8)	9 (11.3)	0.077
Low (%)	166 (83)	95 (79.2)	71 (88.8)	
Hygiene practices				
Good (%)	105 (52.5)	53 (44.2)	52 (65)	0.004
Poor (%)	95 (47.5)	67 (55.8)	28 (35)	
Contact with animals				
Yes (%)	69 (34.5)	47 (39.2)	22 (27.5)	0.089
No (%)	131 (65.5)	73 (60.8)	58 (72.5)	

[a] Average; income of ≥500 L.E/month (5 Egyptian pounds "L.E"=1 US $), low; income of <500 L.E/month

frequent maternal complication of pregnancy (Aimaku and Olayemi 2003; Baidoo et al. 2010). Though many helminthic and protozoal infections, including schistosomiasis, hookworm, whipworm, malaria, have been linked with IDA (Brabin and Brabin 1992; Dreyfuss et al. 2000; Baidoo et al. 2010), little is reported in the literature about prevalence of *Blastocystis* infection in pregnant women with IDA. Considering this, the current study, aiming to assess the role of *B. hominis* infection as a contributing risk factor for development of IDA in pregnant women, would be of crucial importance. In the present work, an association was determined with *Blastocystis* infection and pregnant women with IDA; the frequency of *Blastocystis* infection was significantly higher in the pregnant women with IDA (40%) compared to the control group (6.3%; p=0.0001). Though not conducted on pregnant women, findings from other few studies supported the current data. Cheng et al. (2003) studied the hematological effects of *B. hominis* infection in male foreign workers in Taiwan. Among foreign workers, the *B. hominis*-positive workers had significantly decreased hemoglobin and hematocrit levels when compared with the *B. hominis*-negative workers. More recently, Yavasoglu et al. (2008) reported that the frequency of *B. hominis* in patients with IDA (22.6%) was significantly higher than subjects without IDA (5.6%; p<0.001). Among factors contributing to the high frequency of *Blastocystis* infection (26.5%) reported in the current study were the poor hygiene practices and the low socioeconomic conditions of participants. In fact, groups with lower social-economic level and standards of hygiene tend to present a higher prevalence of *B. hominis* infection than other

Table 2 Frequency of *Blastocystis* infection among pregnant women with IDA compared to non-anemic controls

	Total	Pregnant women with IDA	Non-anemic pregnant women	P value
Number of +ve cases (%)	53 (26.5)	48 (40)	5 (6.3)	0.0001
Number of –ve cases (%)	147 (73.5)	72 (60)	75 (93.8)	
Total	200	120	80	

Table 3 Intestinal parasites detected in pregnant women with IDA and non-anemic control group

Parasite detected	Pregnant women with IDA (n=120)		Non-anemic pregnant women (n=80)	
	N	%	N	%
Blastocystis-only	41	34.2	4	5
Blastocystis and *Giardia*	2	1.7	0	0
Blastocystis and *Cryptosporidium*	2	1.7	0	0
Blastocystis and *Entamoeba* sp.[a]	1	0.8	0	0
Blastocystis and *Ascaris*	1	0.8	0	0
Blastocystis and *Trichuris*	1	0.8	0	0
Blastocystis and *E. coli*	0	0	1	1.3
Total	48/120	40	5/80	6.3

[a] *E. histolytica*/*E. dispar*/*E. moshkovskii*

groups in the community (Nascimento and Moitinho 2005). This is especially true for Egypt where high prevalence (33.3%) was formerly shown (Rayan et al. 2007). Similarly, recent studies from Egypt reported 61.1% *Blastocystis*-infected cases among patients with urticaria (Abdel Hameed et al. 2011) and 40% *Blastocystis*-infected cases among patients with various clinical presentations (Abdel Hameed and Hassanin 2011).

Findings from the current study as well as many different studies demonstrated that the in vitro culture was more sensitive than routine microscopy for *Blastocystis* diagnosis (Leelayoova et al. 2002; Suresh et al. 2004; Yakoob et al. 2004; Al-kaissi and Al-Magdi 2009), because similarity of *Blastocystis* to other small intestinal protozoa and variation in its morphology may lead to misdiagnosis (Yaicharoen et al. 2006). Given these results, the importance of cultivation of stool before excluding *Blastocystis* infection at pregnancy, is quietly obvious.

In the present study, IDA was diagnosed by measuring both Hb and serum ferritin concentrations. Maternal iron status cannot be assessed simply from Hb concentration because pregnancy produces increases in plasma volume and the Hb concentration decreases accordingly (Steer 2000) and the serum ferritin value remains the gold standard to detect iron deficiency (Breymann 2001). The high ferritin cut-off point (<15 μg/L) recommended by WHO for developing countries was used to define iron deficiency in order to compensate for the effect of infection, which can lead to elevation of the level of ferritin (Haidar and Pobocik 2009). In the current work the mean Hb level of 48 *Blastocystis*-infected IDA cases was lower (9.2 g/dl) than that reported for female patients (9.5 g/dl) by Yavasoglu et al. (2008). This may signify that the potential effect of *Blastocystis* infection on Hb would be worsened by pregnancy, being one of the most important "risk factors" for IDA (Breymann 2006). Moreover, though the 41 *Blastocystis*-only infection cases had mild anemia, moderate anemia was seen in the seven *Blastocystis*-mixed infection cases which may point to the significant impact of parasitic co-infection on pregnant women. Actually, the extent of both maternal and fetal morbidity and mortality is dependent upon the severity of anemia and the resulting complications (Breymann 2001). Similarly, accumulating evidence in the literature indicate that intestinal parasitic infection increases risk to develop IDA, particularly in pregnant women. In a study in Ghana (Fuseini et al. 2010), 23.0% of mothers, who had mild anemia (mean Hb=10.02), were infected with one or two of the following helminths: *Schistosoma mansoni* (12.3%), *Ascaris lumbricoides* (0.7%), hookworm (7%), *Strongyloides stercoralis* (2.3%) and *Trichostrongylus* (0.7%). Another study in Venezuela (Rodríguez-Morales et al. 2006) showed a significant risk for anemia in those women with intestinal parasitosis (73.9%); namely *A. lumbricoides* (57.0%), *Trichuris trichiura* (36.0%), *Giardia lamblia* (14.1%), *Entamoeba histolytica* (12.0%), *Necator americanus* (8.1%), *Enterobius vermicularis* (6.3%), and *S. stercoralis* (3.3%). Also, a study performed by Weigel et al. (1996), on the lower class urban Ecuadorian population, showed decreased maternal serum Hb, hematocrit levels, and IDA in cases of chronic intestinal parasitic infection

Table 4 *Blastocystis*-infected symptomatic and asymptomatic cases in IDA group compared to non-anemic group

	Total	Pregnant women with IDA	Non-anemic pregnant women	P value
Symptomatic cases (%)	41 (77.4)	36 (75)	5 (100)	0.577
Asymptomatic cases (%)	12 (22.6)	12 (25)	0	
Total	53	48	5	

Table 5 Comparison between stool microscopy and stool culture results in the diagnosis of *Blastocystis* infection among all study subjects

Stool culture	Stool Microscopy		Total	*P* value
	+ve	–ve		
+ve	39	14	53	0.0001
–ve	0	147	147	
Total	39	161	200	

Table 7 Mean hemoglobin levels (g/dl) of *Blastocystis*-infected pregnant women with IDA

Parasites detected	Cases no (%)	Mean Hb ± S.D
Blastocystis-only	41 (34.2)	9.1±0.298[a]
Blastocystis and other intestinal parasites	7 (5.8)	8.7±0.127[b]
All *Blastocystis* infections	48 (40)	9.2±0.46[a]

[a] Mild anemia

[b] Moderate anemia

without acute symptoms or clinical complications, especially *E. histolytica*. Ninety-three percent of pregnant women studied were infected with at least one species of intestinal parasites (*E. histolytica*, *E. coli*, *Giardia*, *Ascaris*, *Trichuris*); with *E. histolytica* the most common parasite encountered.

Although evidence from the current work and others showed an association between *Blastocystis* infection and IDA, the causal mechanism responsible remains to be elucidated. The fact that whether *B. hominis* can invade the intestinal mucosa was a matter of debate. Some studies showed that *B. hominis* does not invade the colonic mucosa (Tsang et al. 1989; Zuckerman et al. 1990; Yavasoglu et al. 2008). On the contrary, other studies revealed that the parasite invades the intestinal mucosa, submucosa, and the muscle layers (Al-Tawil et al. 1994; Moe et al. 1997; Zhang et al. 2006; Elwakil and Hewedi 2010) and its antigens reach systemic circulation to cause a detectable humoral immune response (Zierdt et al. 1995; Hussain et al. 1997; Abou Gamra et al. 2011). Actually, increased intestinal permeability due to damage to the intestinal wall was demonstrated in patients with *B. hominis* and *Giardia intestinalis* (Dagci et al. 2002), and in *B. hominis* symptomatic subtype 1 compared to symptomatic subtypes-3 and -4 infected rats (Hussein et al. 2008). This is in contrast to an earlier study which showed impaired intestinal permeability in patients with *B. hominis* infection (Zuckerman et al. 1990). In fact, infection causes anemia through loss of nutrients, decreasing appetite, decreasing efficiency of absorption of iron and other micronutrients, and use of nutrients (Weigel et al. 1996; Haidar 2010); the negative effect of *Ascaris* infection on the absorption of retinol-binding protein, iron and other micronutrients is well known (Stenzel and Boreham 1996). Added to this, *B. hominis* might cause terminal ileitis (Tsang et al. 1989) and colitis (Russo et al. 1988). Also, rectal bleeding, though not noticed in the present study, was reported to occur in cases infected with *B. hominis* which might cause changes in RBCs and its related indices (Stenzel and Boreham 1996; Cheng et al. 2003). Furthermore, evidence from an ultrastructural study showed that cationized ferritin, which was used as a tracer for endocytosis, was internalized via coated pits and vesicles and accumulated in the central vacuole (Stenzel et al. 1989).

Table 6 Mean hemoglobin level (in grams per deciliter) of *Blastocystis*-infected compared to non-infected pregnant women with IDA

	Pregnant women with IDA (n=120)		*P* value
	Blastocystis +ve (n=48)	*Blastocystis* –ve (n=72)	
Mean Hb ± S.D	9.2±0.46[a]	10.0±0.328[a]	0.0001

[a] Mild anemia

The fact that 12 cases (25%) among the 48 *Blastocystis*-infected IDA patients in the current work were asymptomatic may point to a chronic *Blastocystis* infection prior to the current pregnancy. Similarly, Weigel et al. (1996) suggested that Hb loss can also occur in individuals with chronic asymptomatic or symptomatic noninvasive amoebiasis, albeit to a much lesser extent than acute amoebiasis. Given these results, screening for *Blastocystis* as well as other parasites prior to and during pregnancy is warranted, especially in anemic and/or low socioeconomic communities for preventing negative impact of parasitic infection on public health.

In conclusion, results of the current study showed that blastocystosis in pregnancy is a risk factor for IDA. Hence, parasitological diagnostic tests should be performed in routine follow-up examination at all antenatal clinics for screening of blastocystosis as well as other parasites among pregnant women and prior to pregnancy. In vitro cultivation technique is recommended before excluding *Blastocystis* infections. Further measures to prevent blastocystosis and other parasitic infections, like health education with emphasis on the mode of transmission and prevention as well as treatment, should be promoted at antenatal clinics.

Future fields of research are elucidating the mechanism of IDA associated with *Blastocystis* infection and evaluating the association between *Blastocystis* subtypes and presence of IDA.

Acknowledgments We herewith declare that the execution of the research described in this article complied with the current laws and regulations of the Egyptian Ministry of Higher Education and Scientific Research.

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