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Original paper

Novel association between blastocystosis and some hemogram parameters in Iraqi people with and without irritable bowel syndrome

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ABSTRACT. Many studies have suggested that *Blastocystis* parasites are pathogenic protozoan parasites and one study proposed that these parasites have contributed to anaemia in children. The primary objective of the present study was to made a comparison between healthy control subjects (group 1), *Blastocystis*-positive subjects (group 2), subjects with irritable bowel syndrome (IBS) who were *Blastocystis*-negative (group 3), and IBS patients who were *Blastocystis*-positive (group 4) regarding the haematological indices. From each participant, blood has been collected and the complete blood count (CBC) has been measured. The current study also was designed to evaluate the correlation between blastocystosis and six selected hemogram parameters [monocyte-to-lymphocyte ratio (MLR), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), red cell distribution width (RDW), platelet distribution width (PDW), and mean platelet volume (MPV)]. The results revealed that the total numbers of lymphocytes and platelets decreased significantly (P<0.0001), while the numbers of monocytes (P<0.0001) and neutrophils (P<0.0001) increased significantly in the bloods of IBS patients and non-IBS subjects who were infected with *Blastocystis* parasites in comparison with the healthy subjects in the control group. In addition, our results revealed for the first time that the levels of NLR, MLR, PLR, RDW, PDW and MPV were significantly higher (P=0.05–0.0001) in the bloods of IBS patients and non-IBS subjects who were infected with *Blastocystis* parasites than in their *Blastocystis*-negative counterparts. In conclusion, we believe that the levels of these hemogram parameters can be used as novel markers of blastocystosis. In addition, this study revealed that the infection with *Blastocystis* parasites had a significant impact on the haematological indices in both IBS patients and non-IBS individuals.

Keywords: *Blastocystis* sp., IBS patients, haematological indices, complete blood count, hemogram parameters

Introduction

Blastocystis sp. was first reported by a Russian scientist in 1870, and in 1912 it was considered as a harmless yeast found in a stool sample and named as *Blastocystis hominis*. After that and based on morphological characteristics, it has been recognized as a eukaryotic unicellular organism [1,2]. Based on phylogenetic analysis of small subunit rRNA gene (SSU rDNA), the parasites that belong to the genus *Blastocystis* have been classified as stramenopiles which are very diverse group of organisms including brown algae and diatoms, but *Blastocystis* parasites are unique among this group in terms of the morphology and pathogenicity as they do not have flagella and they are the only members of this group which are anaerobic and infect humans [3,4]. The molecular studies also identified 22 different subtypes (STs) in humans and animals [5] and ST1–9 and 12 have been reported in humans, but ST1–ST4 are the most common subtypes [6].

Currently, this parasite is recognized as the most common intestinal parasite, both in developed (prevalence up to 10%) and developing (prevalence between 50 and 100%) countries, and this increase in the prevalence worldwide is mainly due to the lack of a good health hygiene [7–9]. The parasite colonizes the large intestines of humans and a wide range of animal hosts and can be transmitted easily

to humans via the oral-faecal route after the ingestion of food and water contaminated with water-resistant cysts (the infective stages) which are either thin-walled (responsible for the autoinfection inside the host) and thick-walled cysts (responsible for the infection which happens outside the host) [7,10].

The life cycle is complicated and still not known very well as some scientists suggest that it has 4 stages or forms and others suggest 6 stages or forms and these are: vacuolar form (with a very large central vacuole which occupies about 90% of the cell volume while the cytoplasm located at the periphery and contains many nuclei), granular form, ameboid form and cyst form, and it has been reported that the vacuolar and the granular forms are seen more commonly in stool samples and cultures while the amoeboid form and the cyst stage are seen less frequently [7].

The pathogenicity of the parasites that belong to the genus *Blastocystis* is still debatable issue due to the fact that these parasites have been reported in both asymptomatic and symptomatic individuals [9,11,12]. However, the lack of symptoms in subjects carrying these parasites does not mean that these parasites are not pathogenic [13,14] because some subtypes (STs) of *Blastocystis* sp. are not pathogenic such as ST2, while ST1 is pathogenic to humans [15–17].

Irritable bowel syndrome (IBS) is a common medical disorder with a wide range of symptoms such as abdominal pain, diarrhea, and constipation, and has a worldwide distribution [9,12]. Sometimes, this syndrome causes significant economic and social costs via restricting the ability to go to work, attend social functions and to attend the school or even traveling for short distances [18]. A wide range of factors such as genetic, physical, mental health problems and environmental factors have been suggested to describe the differences regarding the aetiology and pathogenesis of IBS [19,20].

Although many studies have been published regarding *B. hominis* infection, very few publications on its association with haematological indices have been conducted. In Iraq, only one previous study [21] has been conducted in this regard and the results showed a significant increase in the total count of white blood cells, neutrophils, monocytes, while there was a slight decrease in the count of lymphocytes and haemoglobin ratio. The authors concluded that the infection with *B. hominis* had an impact on the haematological parameters.

The diagnostic significance and prediction of the monocyte-to-lymphocyte ratio (MLR), neutrophil-to-lymphocyte ratio (NLR), and the platelet-to-lymphocyte ratio (PLR) parameters which relate to inflammation have been investigated in various viral, bacterial, and parasitic diseases [22–25]. These haematological parameters can be directly, easily and cheaply obtained from the whole blood cell count [26,27]. Moreover, the red cell distribution width (RDW), platelet distribution width (PDW), and mean platelet volume (MPV) are parameters measured automatically in every complete blood count and they are cost-effective tools used in everyday clinical practice in order to define the causes of anaemia and as indexes of activity and severity of various syndromes and diseases [28–30]. However, and as far as we know, the clinical value of these six haematological parameters in blastocystosis is still not evaluated. Therefore, the current study was designed to evaluate the correlation between the disease (blastocystosis) caused by the parasites that belong to the genus *Blastocystis* and the levels of six selected hemogram parameters (NLR, MLR, PLR, PDW, RDW, and MPV).

Materials and Methods

Participants and complete blood count

This study represents the third part of PhD thesis project of the first author. In a previous study which represented the second part of the thesis, we reported 60% *Blastocystis* sp. positivity rate among patients with irritable bowel syndrome (IBS) and 22% positivity rate among non-IBS healthy subjects and the results have been published recently in the *Annals of Parasitology* [31]. The participants in the previous study were asked about the possibility of giving a sample of blood in order to determine the haematological indices in both IBS patients and non-IBS control group with and without the infection of *Blastocystis* and the majority gave their verbal consent. Accordingly, this study was carried out on 50 healthy individuals (controls, group 1) and 50 *Blastocystis*-positive non-IBS subjects (group 2). In addition, 50 *Blastocystis*-negative IBS patients (group 3) and 50 *Blastocystis*-positive IBS patients (group 4) were also included in this study.

From each participant, about 3 ml of venous blood were collected by a registered nurse at the Al-Shams Private Laboratory, Baquba City, Diyala Province, Iraq, into sterile ethylenediamine-

tetraacetic acid (EDTA) tubes, mixed and processed within one hour using XP-300 fully automated haematology analyser (Sysmex, Japan) in order to determine the following blood parameters: total counts of haematological cells (red blood cells, white blood cells and platelets), leukocyte differential counts (neutrophils, lymphocytes, monocytes), haemoglobin, and haematocrit (HCT). In addition, the neutrophil-to-lymphocyte ration (NLR) was calculated by dividing the neutrophil count by the lymphocyte count, the monocyte-to-lymphocyte ration (MLR) was calculated by dividing the number of monocytes by the number of lymphocytes, while the platelet-to-lymphocyte ratio (PLR) was calculated by dividing the number of platelets by the number of lymphocytes as described previously [25]. The red cell distribution width (RDW), platelet distribution width (PDW) and mean platelet volume (MPV) were obtained directly from the data of the complete blood count.

Each participant was supplied with a standardized questionnaire in order to determine the risk factors and outcomes of *Blastocystis* infection, and containing inquiries regarding age, sex, underlying disease, gastrointestinal symptoms, and contact with animals. Verbal consent was obtained from each participant willing to give blood. In the IBS patients, the exclusion criteria were: any type of blood diseases, any type of cancer, diabetes, liver and kidney diseases, any type of infectious diseases,

Table 1. Haematological parameters and the correlation between neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), platelet distribution width (PDW), red blood cell distribution width (RDW) and mean platelet volume (MPV) in the bloods of apparently healthy control subjects (group 1) in comparison with *Blastocystis*-positive non-IBS subjects (group 2)

Parameters	Mean ± Standard error		*P*-value
	Controls (group 1)	Group 2	
Age (years)	35.3 ± 1.46	34.74 ± 2.03	0.7633
Lymphocyte (cell×10^3/μl)	5.46 ± 0.14	2.19 ± 0.08	<0.0001
Monocyte (cell×10^3/μl)	0.97 ± 0.17	1.94 ± 0.10	<0.0001
Neutrophil (cell×10^3/μl)	4.56 ± 0.17	8.65 ± 0.23	<0.0001
WBCs (cell×10^3/μl)	9.16 ± 0.26	14.88 ± 2.65	<0.0001
Platelets (cell×10^3/μl)	260.7 ± 7.51	204.94 ± 4.76	<0.0001
PCV (haematocrit) (%)	12.91 ± 0.13	13.07 ± 0.20	0.0016
RBCs (cells×10^6/μl)	3.88 ± 0.13	3.49 ± 0.13	0.0498
Haemoglobin (g/dl)	13.30 ± 0.14	13.33 ± 0.22	0.4179
Neutrophil-to-lymphocyte ratio (NLR)	0.86 ± 0.04	4.22 ± 0.20	<0.0001
Monocyte-to-lymphocyte ratio (MLR)	0.19 ± 0.03	0.91 ± 0.05	<0.0001
Platelet-to-lymphocyte ratio (PLR)	49.41 ± 1.91	99.64 ± 4.30	<0.0001
Mean platelet volume (MPV) (fl)	7.77 ± 0.09	8.81 ± 0.11	<0.0001
Platelet distribution width (PDW) (fl)	17.31 ± 0.24	17.61 ± 0.21	0.0017
Platelet distribution width (PDW) (%)	58.92 ±1.29	66.45 ± 1.32	<0.0001
Red cell distribution width (RDW) (fl)	77.45 ± 2.16	81.92 ± 1.25	<0.0001
Red cell distribution width (RDW) (%)	13.49 ± 0.14	14.47 ± 0.18	<0.0001

Explanations: fl, femtolitre (measurement unit)

abnormal haemoglobin level or iron deficiency, while in the control healthy subjects, the exclusion criteria were: IBS symptoms, any type of blood disorders including anaemia, abnormal haemoglobin or any type of cancer. None of the participants had a history of using medications that might have affected platelet functions. In addition, any participant with a history of diabetes mellitus, infectious diseases and chronic inflammatory diseases was excluded.

Statistical analysis

The statistical analyses were performed using Graph Pad Prison version 8 (Graph Pad Software Inc., La Jolla, CA). Student's T-test was used to determine whether group variance was significant or not. Chi-square was used to determine count variance among age groups. Data were expressed as mean±standard error (SE) and statistical differences were considered significant when $P \leq 0.05$.

Results

The results are shown in tables 1–3. Table 1 shows a comparison between healthy control subjects (group 1) and non-IBS subjects but infected with *Blastocystis* (group 2) regarding the haematological parameters, and it can be seen that the numbers of lymphocytes ($P<0.0001$), platelets ($P<0.0001$), and red blood cells (RBCs, $P=0.0498$) decreased significantly, while the numbers of monocytes ($P<0.0001$), neutrophils ($P<0.0001$), white blood cells (WBCs, $P<0.0001$), and

Table 2. Haematological parameters and the correlation between neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), platelet distribution width (PDW), red blood cell distribution width (RDW) and mean platelet volume (MPV) in the bloods of healthy control subjects (group 1) in comparison with *Blastocystis*-positive patients with irritable bowel syndrome (group 4)

Parameters	Mean ± Standard error		*P*-value
	Controls (group 1)	Group 4	
Age (years)	35.3 ± 1.48	35.94 ± 2.23	0.7305
Lymphocyte (cell×10^3/µl)	5.46 ± 0.14	2.01 ± 0.08	<0.0001
Monocyte (cell×10^3/µl)	0.97 ± 0.17	1.52 ± 0.09	<0.0001
Neutrophil (cell×10^3/µl)	4.56 ± 0.17	7.81 ± 0.27	<0.0001
WBCs (cell×10^3/µl)	9.16 ± 0.26	8.64 ± 0.22	<0.0001
Platelets (cell×10^3/µl)	260.7 ± 7.51	247.52 ± 6.36	<0.0001
PCV (haematocrit) (%)	12.91 ± 0.13	13.34 ± 0.16	0.0241
RBCs (cells×10^6/µl)	3.88 ± 0.13	4.79 ± 0.12	<0.0001
Haemoglobin (g/dl)	13.30 ± 0.14	13.98 ± 0.20	0.0536
Neutrophil-to-lymphocyte ratio (NLR)	0.86 ± 0.04	4.26 ±0.23	<0.0001
Monocyte-to-lymphocyte ratio (MLR)	0.19 ± 0.03	0.84 ± 0.07	<0.0001
Platelet-to-lymphocyte ratio (PLR)	49.41 ± 1.91	136.63 ± 8.22	<0.0001
Mean platelet volume (MPV) (fl)	7.77 ± 0.09	8.28 ± 0.12	0.0001
Platelet distribution width (PDW) (fl)	17.31 ± 0.24	14.69 ± 0.19	<0.0001
Platelet distribution width (PDW) (%)	58.92 ± 1.29	54.35 ± 1.20	0.0004
Red cell distribution width (RDW) (fl)	77.45 ± 2.16	73.94 ±1.19	0.0711
Red cell distribution width (RDW) (%)	13.49 ± 0.14	13.12 ± 0.12	0.0652

percentage of packed cell volume (% PCV, P=0.0016) increased significantly in the bloods of subjects infected with *Blastocystis* in comparison with healthy subjects. In contrast, no significant differences were observed between the two groups regarding the concentration of haemoglobin.

In addition and in comparison between healthy subjects in the control group (group 1) and the *Blastocystis*-positive non-IBS subjects (group 2), the results of the current study revealed for the first time that the levels of neutrophil-to-lymphocyte ratio (NLR) (0.86 ± 0.04 vs. 4.22 ± 0.20, P<0.0001), monocyte-to-lymphocyte ratio (MLR) (0.19 ± 0.03 vs. 0.91 ± 0.05, P<0.0001), platelet-to-lymphocyte ratio (PLR) (49.41 ± 1.91 vs. 99.64 ± 4.30, P<0.0001), mean platelet volume (MPV) (7.77 ± 0.09 vs. 8.81 ± 0.11, P<0.0001), platelet distribution width (PDW, fL) (17.31 ± 0.24 vs. 17.61 ± 0.21, P=0.0017), platelet distribution width (PDW, %) (58.92 ± 1.29 vs. 66.45 ± 1.32, P<0.0001), red cell distribution width (RDW, fl) (77.45 ± 2.16 vs. 81.92 ± 1.25, P<0.0001), and red cell distribution width (RDW, %) (13.49 ± 0.14 vs. 14.47 ± 0.18, P<0.0001) in the bloods of *Blastocysitis*-positive non-IBS subjects were significantly higher than n the bloods of healthy control subjects (Tab. 1).

It can be seen from table 2 that the numbers of lymphocytes (P<0.0001), platelets (P<0.0001), and WBCs (P<0.0001) decreased significantly, while the numbers of monocytes (P=<0.0001), neutrophils (P<0.0001), RBCs (P<0.0001), and percentage of packed cell volume (% PCV, P=0.0241) increased

Table 3. Haematological parameters and the correlation between neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), platelet distribution width (PDW), red blood cell distribution width (RDW) and mean platelet volume (MPV) in the bloods of *Blastocystis*-negative IBS patients (group 3) in comparison with *Blastocystis*-positive IBS patients (group 4)

Parameters	Mean ± Standard error		P-value
	Group 3	Group 4	
Age (years)	33.18 ± 1.88	35.94 ± 2.23	0.0561
Lymphocyte (cell×10^3/μl)	4.11 ± 0.15	2.01 ± 0.08	<0.0001
Monocyte (cell×10^3/μl)	0.72 ± 0.04	1.52 ± 0.09	<0.0001
Neutrophil (cell×10^3/μl)	5.64 ± 0.17	7.81 ± 0.27	<0.0001
WBCs (cell×10^3/μl)	9.40 ± 0.1 9	8.64 ± 0.22	0.0005
Platelets (cell×10^3/μl)	178.94 ± 4.15	247.52 ± 6.36	<0.0001
PCV (haematocrit) (%)	12.65 ± 0.18	13.34 ± 0.16	0.0022
RBCs (cells×10^6/μl)	3.97 ± 0.14	4.79 ± 0.12	<0.0001
Haemoglobin (g/dl)	12.64 ± 0.21	13.98 ± 0.20	<0.0001
Neutrophil-to-lymphocyte ratio (NLR)	1.51 ± 1.11	4.26 ± 0.23	<0.0001
Monocyte-to-lymphocyte ratio (MLR)	0.19 ± 0.01	0.84 ± 0.07	<0.0001
Platelet-to-lymphocyte ratio (PLR)	46.92 ± 2.31	136.63 ± 8.22	<0.0001
Mean platelet volume (MPV) (fl)	8.04 ± 0.13	8.29 ± 0.12	0.0052
Platelet distribution width (PDW) (fl)	18.03 ± 0.26	14.69 ± 0.19	<0.0001
Platelet distribution width (PDW) (%)	61.79 ± 1.35	54.35 ± 1.20	<0.0001
Red cell distribution width (RDW) (fl)	98.13 ± 1.14	73.94 ± 1.19	<0.0001
Red cell distribution width (RDW) (%)	13.78 ± 0.14	13.12 ± 0.12	0.0052

significantly in the bloods of *Blastocystis*-positive IBS patients (group 4) in comparison with the healthy control subjects. No significant difference was observed between the two groups regarding the level of haemoglobin.

It can be seen from table 2 that the levels of NLR (0.86 ± 0.04 vs. 4.26 ± 0.23, $P<0.0001$), MLR (0.19 ± 0.03 vs. 0.84 ± 0.07, $P<0.0001$), PLR (49.41 ± 1.91 vs. 136.63 ± 8.22, $P<0.0001$), and MPV (7.77 ± 0.09 vs. 8.28 ± 0.12, $P=0.0001$) increased significantly, while the levels of PDW (fl) (17.31 ± 0.24 vs. 14.69 ±0.19, $P<0.0001$) and PDW (%) (58.92 ±1.29 vs. 54.35 ± 1.20, $P=0.0004$) decreased significantly in the bloods of *Blastocystis*-positive IBS patients (group 4) in comparison with healthy control subjects. However, no significant differences were detected in the levels of RDW (fl) (77.45 ± 2.16 vs. 73.94 ± 1.19, $P=0.0711$) and RDW (%) (13.49 ± 0.14 vs. 13.78 ± 0.14, $P=0.0552$) between the two groups.

It can be seen from table 3 that the numbers of lymphocytes ($P<0.0001$) and WBCs ($P=0.0005$) were significantly lower, while the numbers of monocytes ($P<0.0001$), neutrophils ($P<0.0001$), platelets ($P<0.0001$) and RBCs ($P<0.0001$) were significantly higher in the bloods of *Blastocystis*-positive IBS patients (group 4) than in the bloods of *Blastocystis*-negative IBS patients (group 3). In addition, the % PCV ($P=0.0022$) and the concentration of haemoglobin ($P<0.0001$) were significantly higher in the bloods of IBS patients in group 4 than in the blood of IBS patients in group 3. Moreover, the levels of NLR (1.51 ± 1.11 vs. 4.26 ± 0.23, $P<0.0001$), MLR (0.19 ± 0.01 vs. 0.84 ± 0.07, $P<0.0001$), PLR (46.92 ± 2.31 vs. 136.63 ± 8.22, $P<0.0001$), and MPV (8.04 ± 0.13 vs. 8.29 ± 0.12, $P=0.0052$) increased significantly, while the levels of PDW (fl) (18.03 ± 0.24 vs. 14.69 ±0.19, $P<0.0001$), PDW (%) (61.79 ±1.35 vs. 54.35 ± 1.20, $P<0.0001$), RDW (fl) (98.13 ± 1.14 vs. 73.94 ± 1.19, $P<0.0001$) and RDW (%) (13.78 ± 0.14 vs. 13.12 ± 0.12, $P=0.0052$) decreased significantly in the bloods of *Blastocystis*-positive IBS patients (group 4) in comparison with *Blastocystis*-negative IBS patients (group 3) (Tab. 3).

Discussion

In a previous study, we reported 60% *Blastocystis* sp. positivity rate among patients with irritable bowel syndrome (IBS) and 22% positivity rate among non-IBS healthy subjects and the difference between the two groups was highly significant [31]. The primary objective of the current study was to evaluate the impact of the infection with the parasites that belong to the genus *Blastocystis* on the haematological parameters in Iraqi people with and without irritable bowel syndrome (IBS) in comparison with their *Blastocystis*-negative counterparts. In addition, our study is the first in the literature to investigate the levels of six selected hemogram parameters including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), red cell distribution width (RDW), platelet distribution width (PDW), and mean platelet volume (MPV) in the bloods of IBS patients and non-IBS subjects who were infected with *Blastocystis* parasites in comparison with their *Blastocystis*-negative counterparts.

The results revealed that there was a significant increase in leukocyte count (WBC) in the blood of IBS patients and non-IBS subjects who were infected with *Blastocystis* when compared with their counterparts who were not infected with *Blastocystis* and this increment was mainly due to the increase in the numbers of both monocytes and neutrophils, but not the lymphocytes. This finding is in line with the findings of some previous studies. Andiran et al. [32] reported that there was a significant increase in leukocyte count in the Turkish individuals infected with *Blastocystis* in comparison with the healthy subjects in the control group. Laodim et al. [33] reported that there was a significant increase in the numbers of neutrophils and monocytes in Thai people infected with *Blastocystis* when compared with the healthy subjects in the control group. Javaherizadeh et al. [34] studied the haematological indices among Iranian subjects infected with *Blastocystis hominis* (case group) in comparison with controls and reported that there were significant differences between the two groups regarding platelet count and eosinophil count as they were significantly more frequently in the case group than in the control group. The authors concluded that *B. hominis* is a possible factor in haematological abnormalities.

Recently, Mutlag et al. [21] investigated the impact of *Blastocystis hominis* infection on haematological parameters in Iraqi healthy control subjects and individuals suffering from irritable bowel syndrome (IBS) and the authors reported that the result showed a significant increase in the total count of white blood cells, neutrophils, monocytes, while there was a slight decrease in the count of

lymphocytes and haemoglobin ratio in IBS patients when compared with control subjects. The authors concluded that the infection with *B. hominis* had an impact on the haematological parameters. The results of our study agree well with those reported by Mutlag et al. [21]. More recently, Kim et al. [35] investigated the molecular detection, subtyping of *Blastocystis* together with several laboratory parameters in Korean populations and reported that only the creatinine level differed significantly between the *Blastocystis*-positive and *Blastocystis*-negative groups, while no significant differences found between the two groups regarding the white and red blood cell counts, haemoglobin level, percentages of lymphocytes, monocytes, eosinophils, and basophils. Moreover, Kosik-Bogacka et al. [36] investigated the prevalence of *Blastocystis* spp. in pre- and perimenopausal Polish women, and compared haematological and biochemical parameters in women with and without infection of *Blastocystis* and reported that there were no significant associations between *Blastocystis* spp. and blood parameters (White blood cells, red blood cells, haemoglobin, haematocrit, mean corpuscular volume, platelet, neutrophils, eosinophils, basophils, lymphocytes and monocytes) between the two groups.

In the present study, we did not find significant differences between *Blastocystis*-positive individuals and healthy subjects regarding numbers of RBCs, platelets, concentration of haemoglobin and the % haematocrit (% PCV). This finding is in line with the results of a recent study conducted in South Korea by Kim et al. [35] and the results of a more recent study conducted in Poland by Kosik-Bogacka et al. [36] mentioned above.

The significant increase in the total number of leukocytes in individuals who were infected with *Blastocystis*, especially those who suffer from digestive syndromes, may be due to the fact that the parasites that belong to the genus *Blastocystis* have the capacity to stimulate and modify the production of proinflammatory cytokines in intestinal epithelial cells [37]. Moreover, the significant increment in the number of neutrophils in individuals who were infected with *Blastocystis*, reported in the present study and some previous studies [21,33], may be due to the fact that the neutrophils represent the first line of defence against pathogenic agents via recognition and then ingestion of the invading pathogens. The results of some studies have revealed that the parasites that belong to the genus *Blastocystis* have the ability to stimulate the immune response of the host via the production of T-lymphocyte, macrophages and natural killer cells [38].

The current study also was designed to evaluate the correlation between the infection with *Blastocystis* parasites (blastocystosis) and NLR, MLR, PLR, PDW, RDW, and MPV and the results revealed for the first time that the levels of these hemogram parameters were significantly higher in the bloods of *Blastocystis*-positive IBS patients and *Blastocystis*-positive non-IBS subjects than in their *Blastocystis*-negative counterparts. Very few studies have been conducted regarding the role of these hemogram parameters in parasitic infections. Our finding is in line with the results of the studies conducted on other parasitic diseases. Ali et al. [39] evaluated the RDW, PDW and MPV in patients with uncomplicated *Plasmodium falciparum* and *P. vivax* malaria and reported that patients with malaria had significantly lower haemoglobin, leucocyte and platelet counts, and significantly higher RDW, PDW and MPV in comparison with healthy subjects. The authors concluded that PDW and MPV were the main predictors for *P. falciparum* and *P. vivax* malaria infection. Chen et al. [24] investigated NLR and PLR in patients infected with *Clonorchis sinensis* and their results revealed that the values of these haematological parameters of infected patients were significantly higher than those of healthy individuals. The authors concluded that these blood parameters may act as the valuable supplement in detecting *C. sinensis* infections and diseases. Hu et al. [40] evaluated the relationship between NLR and PLR and ancylostomiasis (infection with hook worms) and the results showed that the levels of both parameters in ancylostomosis patients were significantly higher than those in the healthy controls and the authors concluded that the levels of NLR and PLR may serve as valuable indicators for distinguishing patients with ancylostomosis from healthy controls. Recently, Weijian et al. [25] evaluated the correlation between NLR and PLR with hepatic hydatid diseases and their results revealed that the expressions of PLR and NLR were significantly higher in the alveolar hydatidosis group than in the cystic hydatidosis and control groups. The authors concluded that PLR and NLR have certain diagnostic values for hydatid disease classification.

It is important to mention that the NLR has been accepted as a parameter that presents the negative effects of both high neutrophil levels, which reflect

acute inflammation, and low lymphocyte levels, which reflect physiological stress [41]. Although the mechanisms of the haematological change of NLR and PLR and parasitic infections were unclear, few studies showed that neutrophils, lymphocytes and platelets were involved in the development of the ancylostome infection [42,43]. In the present study, we observed a significant increase in the numbers of neutrophils and monocytes, and a significant decrease in the numbers of lymphocytes and platelets in IBS patients and non-IBS subjects who were infected with *Blastocystis* in comparison with healthy subjects in the control group. Consequently, in the present study the elevation in the levels of NLR, MLR and PLR was caused by the increase in the neutrophil count and monocyte count, and the decrease in the lymphocyte count in peripheral blood just like in other systemic inflammatory diseases [44,45]. Moreover, our findings are in line with the results of Hu et al. [40] who observed elevated neutrophils and decreased lymphocytes in patient infected with ancylostomosis in comparison with the healthy controls. It is interesting to mention that these haematological parameters can be directly, easily and cheaply obtained from the whole blood cell count [26,27].

This study revealed that the infection with the parasites that belong to the genus *Blastocystis* had a significant impact on the haematological indices in subjects with and without irritable bowel syndrome (IBS). In addition, our results revealed for the first time that the levels of six hemogram parameters (NLR, MLR, PLR, RDW, PDW and MPV) were significantly higher in the bloods of IBS patients and non-IBS subjects who were infected with *Blastocystis* parasites than in the healthy subjects in the control group. We believe that the levels of these hemogram parameters can be used as novel markers of blastocystosis. These findings need to be confirmed via conducting more detailed and larger clinical studies.

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