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Saif Mohammed Algebory

Zainab Ali Hussein

Sarah Suliman Mohammed

Alaa Ihsan Saymeh

Hayder Kareem Algabri

ORIGINAL STUDY

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The Function of Di-Methyl Fumarate in Immunobiochemical and Hematological Changes in Individuals Infected with *Entamoeba histolytica*

Saif Mohammed Algebory [a,*], Zainab Ali Hussein [b], Sarah Suliman Mohammed [c], Alaa Ihsan Saymeh [d], Hayder Kareem Algabri [e]

[a] Department of Medical Laboratory Technology, College of Health and Medical Technology, University of Gezira, Khartoum, Sudan
[b] Department of Medical Laboratory Technology, College of Health and Medical Technology, Al bath University, Damascus, Syria
[c] College of Radiography and Medical Imaging Sciences, National University- Sudan, Khartoum, Sudan
[d] CV Raman Global University, Bhubaneswar, India
[e] Department of Cybersecurity Engineering Technologies, University of Hilla, Babylon, Iraq

Abstract

Background: Amebiasis can cause a number of symptoms, from infections that are passed on when someone touches something infected to serious illness.

Objectives: role of DMF on reducing *Entamoeba histolytica* (E. histolytica) infection.

Materials and Methods: The study involved patients supplying E. histolytica-related symptoms. Diagnoses were confirmed using stool samples, and 10 mL of venous blood was obtained for antioxidant and immunological investigation. Groups: Control (uninfected patients), Normal medication (infected patients on normal medication), and Combination (infected patients getting standard treatment with DMF).

Results: Group 2 and 3 had lower red blood cell and haemoglobin levels than group 1. Group 2 was different from groups 1 and 3 ($P < 0.05$), which shows that DMF reduces red blood cell and haemoglobin loss. Group 1 had higher levels of different types of white blood cells, including neutrophils, monocytes, and eosinophils. Group 1 had lower amounts of lymphocytes compared to groups 2 and 3. Statistical analysis showed a significant decrease in SOD, GSH, and CAT levels in patients receiving E. histolytica standard treatment compared to the healthy control group, while malondialdehyde levels increased a lot ($P \leq 0.05$) in the same group. Group 3 showed a significant increase ($P \leq 0.05$) in SOD, GSH, and CAT levels compared to the standard therapy group, while malondialdehyde levels increased significantly in patients treated with E. histolytica standard therapy plus DMF.

Conclusion: E. histolytica infection has been found to generate leukocytosis, lower antioxidant levels, and boost MDA. It is advised that a potent antioxidant be added to conventional treatment.

Keywords: Amebiasis, Di-Methyl fumarate, Diarrhea, Inflammatory, *Entamoeba histolytica*

1. Introduction

The unicellular protozoan parasite *Entamoeba histolytica* infects 45–50 million people annually, resulting in 40,000–100,000 fatalities. Infection can cause life-threatening hemorrhagic colitis and extraintestinal abscesses [1]. Amebiases demonstrated a number of clinical signs, ranging from touching infections to serious illness. After exposure, clinical symptoms may appear today or week's period. Visible symptoms include mucus and/or blood, and usually characterize cramps, abdominal pain and diarrhea [2].

The interpretation of geographical location, historical statement or clinical symptoms has little effect

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* Corresponding author.
E-mail addresses: drsaifalgebory13@gmail.com (S. M. Algebory), zainabalmubarak2018@gmail.com (Z. A. Hussein), Saras8521@gmail.com (S. S. Mohammed), alaasaymeh12@gmail.com (A. I. Saymeh), hayder_kareem_algabri@hilla-unc.edu.iq (H. K. Algabri).

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on the delicate process of parasite infection diagnosis. Each of these strategies has unique benefits and resistance [3]. For example, the presence of diarrhea with the presence of blood or secret stools (i.e. stools that do not appear for naked eyes) with diarrhea, *Salmonella*, *Campylobacter* and *E coli*. It can be a sign of many different diagnosis, including different forms of *E. coli* [4]. In addition, a complete assessment should be made to identify the presence of non-infectious etiology, covering, but not limited to inflammatory bowel disease, ischemic colitis, diverticulitis, and distortion of the arteries [5].

In addition, a comprehensive assessment should be made to detect the presence of non-infectious etiology, which includes, but not limited to inflammatory bowel disease, ischemic colitis, diverticulitis and distortion of the arteries [6]. In addition, a complete evaluation should be done to identify the presence of non-infectious etiology, cover, but not limited to inflammatory bowel disease, ischemic colitis, diversulite and distortion of arteries [7]. Metronidazole is accepted as the selected therapy, with a treated rate of more than 90%. Given that about 10% of touch-oriented cyst carrier risks developing aggressive disease, e.g. The drug is suggested for all patients harassing histolytica [8].

The parasite lives within and reproduces the low oxygen environment of the human intestine. During the process of tissue invasion, e.g. Histoltica is exposed to the amount of height of reactive oxygen species (ROS), which includes supexidions (O_2^-) and hydrogen peroxide (H_2O_2) [9]. In addition, it has been proven that the parasite causes a host-immunity response, causing cytokines TNF-A and IFN-A, which promotes ROS formation in phagocytes [10]. Malondialdehyde, a by-product of reactive oxygen species (ROS), is often used as one in vivo-diagnostic tool for oxidative stress [11]. Cellular immune response provides adequate and important work to treat the disease. Over the past decade, it was popularly believed that free oxygen radical cellular immune response was the most important agent responsible for killing the parasite [12]. However, recent studies have shown that reactive nitrogen radicals, in fact, important mechanisms such as E. histolica are dried from the body [13].

2. Materials and methods

2.1. Patients and study design

This retrospective study was carried out from December 1, 2012, to June 30, 2013. A total of 391 individuals participated in the study, with samples gathered from government healthcare facilities and private clinics. Participants were requested to furnish information regarding their age, address, and clinical symptoms associated with E. histolytica. Stool samples were collected from all patients, The samples were examined macroscopically, according to stool consistency, presence of blood, and mucous, and microscopic examination, also done to detect trophozoites stage, RBCs, and pus cells. Positive results were used to confirm the diagnosis. Furthermore, 10 milliliters of venous blood were drawn from the same individuals for antioxidant and immunological analysis.

2.2. Study groups

1. Group 1 (control) is included: Patients without E. histolytica infection.
2. Group 2 (stander treatment): Patients with E. histolytica who received standard treatment.
3. Group 3 (Combination): Patients with E. histolytica who received standard treatment in addition to DMF.

Exclusion criteria encompassed conditions such as pregnancy, cardiovascular diseases, anemia, autoimmune disorders, and other conditions with the potential to disrupt hematological markers.

2.3. Statistical analysis

The data were analyzed using using Statistical Package for the Social Sciences (SPSS) version 26.0 (SPSS, IBM Company, Chicago, IL 60606, USA), with the mean ± standard error being reported. For qualitative data, the chi-square test was employed. GraphPad v. 8.1 was utilized to set a significance level at $p < 0.05$, and the graphs were generated.

3. Results

3.1. Basic clinical characteristics

In the present study, 391 stool samples were analyzed; 300 of these were found to be indicative of a sudden onset of diarrhea with inflammatory characteristics. These samples were subsequently determined to be related with *E. histolytica*. Subsequent analysis of these samples revealed patterns of infection and potential channels for transmission, yielding valuable insights into the epidemiology and toxicity of the bacterium (Table 1).

3.2. Biochemical parameters

The statistical analysis of the results revealed a significant decrease ($P \leq 0.05$) in the concentrations

Table 1. Basic characteristics of the studied sample.

Parameters	Group 1 Mean ± SEM	Group 2 Mean ± SEM	Group 3 Mean ± SEM
Age	30.18 ± 4.19	29.98 ± 3.11	28.48 ± 5.17
BMI (kg/m^2)	22.11 ± 2.31	24.42 ± 3.17	24.42 ± 3.17
Duration of Disease (days)	0	3.22 ± 1.05	2.62 ± 0.93
Once Treatment (days)	0	3.60 ± 3.58	3.42 ± 3.05

of superoxide dismutase (SOD), glutathione peroxidase (GSH), and catalase (CAT) in individuals with *E. histolytica* standard treatment compared to the healthy control group, (Table 2 and Fig. 1). Conversely, the concentration of malondialdehyde in the patient group with *E. histolytica* infection showed a significant increase ($P \leq 0.05$) when compared to the healthy control group, as detailed in Table 1.

In another hand, Group 3 demonstrated a significant increase ($P \leq 0.05$) in the concentrations of superoxide dismutase (SOD), glutathione peroxidase (GSH), and catalase (CAT) in individuals when compared to the *E. histolytica* standard treatment group. Conversely, the patient group receiving E. histolytica standard treatment plus DMF exhibited a substantial increase ($P \leq 0.05$) in the concentration of malondialdehyde when compared to the E. histolytica standard treatment group.

3.3. Hematological parameters

The statistical analysis of the results has shown a significant decrease ($P \leq 0.05$) in the count of red blood corpuscles and the haemoglobin concentration of both groups 2 and 3, in comparison with group 1. Nevertheless, it has been demonstrated by our results

Table 2. Biochemical parameters of control and patients undergoing treatment for E. histolytica, and patients receiving treatment for E. histolytica combined with DMF.

parameters	Group 1	Group 2	Group 3	P value
GSH μmol/L	359.6 ± 11.8	208.7 ± 15.2*	286.7 ± 25.5*#	0.0001
SOD μmol/L	229.6 ± 1.9	119.6 ± 12.9*	153.6 ± 15.2*#	0.0001
CAT μmol/L	219.6 ± 1.10	109.6 ± 10.12*	143.2 ± 13.32*#	0.0001
MDA μmol/L	77.17 ± 11.13	27.37 ± 12.15*	44.13 ± 10.21*#	0.0001

* vs group 1, # vs Group 2. Mean, SEM stander error of mean.

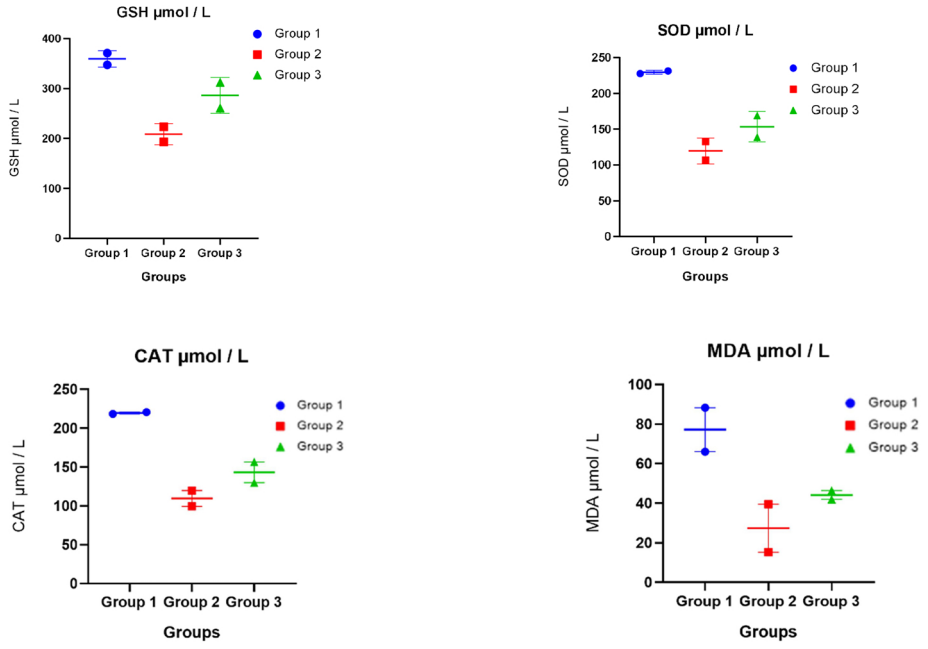

Fig. 1. The biochemical parameters of healthy control and patients undergoing standard treatment for E. histolytica, and patients receiving standard treatment for E. histolytica combined with DMF.

Table 3. The frequency of RBCs and HB among groups.

Group	Group 1	Group 2	Group 3	P-value
RBCs X10^6 / mm^3	4.55 ± 0.08	3.15 ± 0.19*	4.05 ± 0.10*#	0.001
Hb g/dl	12.15 ± 0.25	8.15 ± 0.26*	9.65 ± 0.27*#	0.000

* vs group 1, # vs Group 2. Mean, SEM stander error of mean.

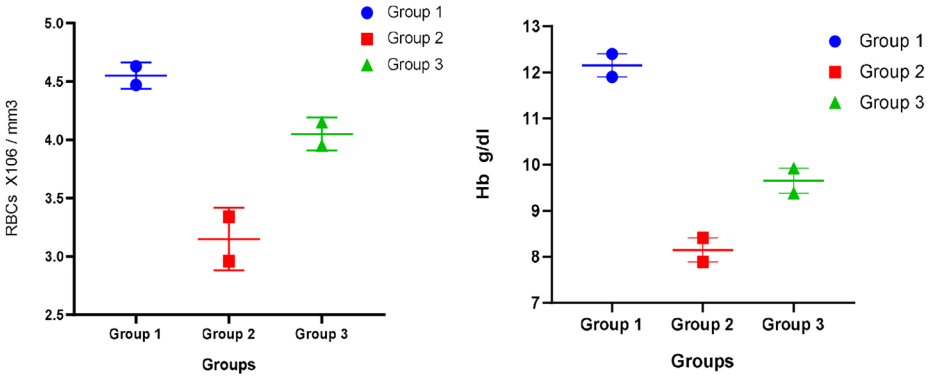

Fig. 2. A comparative analysis was conducted of red blood cells (RBCs) and haemoglobin (Hb) levels in the control group with those observed in patients infected with E. histolytica.

that group 2 has a significant difference ($P < 0.05$) compared to group 1 and group 2, indicating that DMF lowers the loss of red blood cells and haemoglobin (Table 3 and Fig. 2).

The results of this analysis revealed significant differences. Patients infected with Entamoeba histolytica exhibited a marked reduction in both RBC count and Hb concentration. The results demonstrated a notable increase in the total leukocyte count in groups 2 and 3. Furthermore, the differential leukocyte count exhibited a significant rise ($P \leq 0.05$) in the percentages of neutrophils, monocytes, and eosinophils, alongside a marked decrease in the percentage of lymphocytes. Conversely, the basophil percentage exhibited a non-significant increase ($P > 0.05$) compared to patients in group 1, as outlined in Table 4. Furthermore, a significant difference was observed between groups 2 and 3, with both groups showing increased percentages of neutrophils, monocytes, eosinophils, and lymphocytes when compared to each other.

4. Discussion

Compared to the control group, the current study e. In patients with hysterical treatment group, hemoglobin (HB) and red blood cells (RBC) showed a significant reduction in concentration, while the DMF was added to the DMF. This behavior in RBC have been associated with the processes of hemolysis and phagocytosis of *E. histolytica* parasites. Repetition of bleeding with bloody stools can help explain the defect reported in RBC calculations. The reduction in hemoglobin (HB) content is associated with RBC hemolysis, while the use of DMF is displayed to reduce these processes by reducing free radicals and increasing antioxidant agents [14, 15].

Anemia diet in individuals infected with amoeba may be responsible for iron deficiency [16]. It is a well-established fact that due to the common effects of illiteracy and poverty, in developing countries, many individuals and diets of homes are missing

Table 4. The hematological parameters of healthy control and patients undergoing standard treatment for E. histolytica, and patients receiving standard treatment for E. histolytica combined with DMF.

parameters	Group 1 M ± SEM	Group 2 M ± SEM	Group 3 M ± SEM	P value
TLC X10^3 / mm^3	6.50 ± 0.5	12.16 ± 0.22*	10.36 ± 0.12*#	0.0001
Neutrophil %	55.13 ± 1.3	86.13 ± 0.4*	67.53 ± 0.85*#	0.0001
Lymphocyte %	38.18 ± 0.8	22.11 ± 0.4*	31.21 ± 0.3*#	0.0001
Monocyte %	6.31 ± 0.2	8.87 ± 0.18*	7.17 ± 0.23*#	0.0001
Eosinophil %	2.85 ± 0.18	5.11 ± 0.03*	3.41 ± 0.09*#	0.0001
Basophil %	0.31 ± 0.07	0.38 ± 0.05	0.35 ± 0.06	0.0001

* vs group 1, # vs Group 2. Mean, SEM stander error of mean.

usually important elements required for blood formation, including iron. As a result, the high prevalence of anemia is seen in the studio sector responsible for these underlying factors [17].

The procedure involving trophozoites can depend on red blood cells many virus factors, such as hemoglobinase. This enzyme is originally released to divide hemoglobin into its component parts - iron and globin. Subsequently, Globin has been broken down into amino acids in hemolycin, and thus further expanded to this process [18, 19]. The findings from this study compared to the control group. Histolica standard treatment demonstrated sufficient increase in total WBC of patients infected with patients. This growth can be attributed to stimulation of cellular and comic immunity reactions caused by infection with this parasite. Infection with intestinal parasites is established to increase a major impact on blood component values, white blood cells (WBCS), eosinophils and hemoglobin (Hb). The decline in hemoglobin levels, along with an increase in eosinophils and total WBC, matches data reported by other researchers [20, 21].

The study found that lymphocyte levels in patient groups treated for E. histolytica infection were considerably lower compared to the control group. This study demonstrates that T-cell proliferation is reduced during E. histolytica infection. Furthermore, the study reveals that blood from human patients and animal models harboring amoebas can restrict T-cell proliferation by reducing the production of interleukin-2 (IL-2), a crucial growth factor generated by T-helper cells [21].

In assessments with control groups, patients undergoing routine *E. histolytica* treatment show dramatically reduced serum GSH, SOD, and CAT levels. Conversely, these levels were greatly raised in the DMF-treated group. The lower antioxidant levels seen in the latter group point to the occurrence of oxidative damage, which could be ascribed to several elements, including enhanced catabolism, poor synthesis, higher GSSG conversion, and free radical generation resulting from parasite resistance to phagocytosis [22, 23].

Reactive oxygen species (ROS) are highly reactive chemicals that promote oxidative stress and lipid peroxidation, particularly in proximity to cell membranes, such as those of intestinal epithelial cells [24]. This process has been found to break down polyunsaturated fatty acids in biomembranes, thereby lowering membrane integrity and causing cellular harm [25].

The study discovered significantly more malondialdehyde (MDA), a lipid peroxidation marker, in patients with acute intestinal hemibiasis compared to control (PU digit (0.05). As a result, the level of blood in the perobias function is a result of blood in Hemibias [26–28].

5. Conclusion

Entamoeba histolytica infection has been found to generate leukocytosis, which is marked by an increase in monocytes, eosinophils, and neutrophils. Furthermore, the illness has been observed to lower antioxidant levels while boosting malondialdehyde (MDA) concentrations in patients. It is therefore recommended that a potent antioxidant be added to E. histolytica standard treatment.

Ethical issue

Ethics Committee has confirmed that no ethical approval is required.

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Conflict of interest

The authors declare no conflict of interest.

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