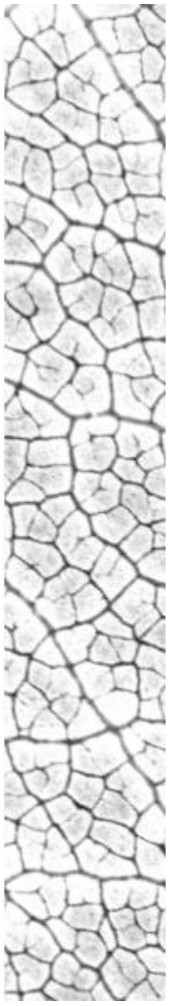

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Evaluation of oxidative stress and growth status in molecular diagnostic patients with entamoeba histolytica

Rasha Amer Nouri Al-Tufaili [1*], Rasha Shakir Nima [2]

[1] Department of Laboratory Investigation, Faculty of Science, University of Kufa, IRAQ
*Corresponding author: rashaa.altufaili@uokufa.edu.iq

Abstract
In the developing world, amoebiasis is a widespread parasitic disease in which Entameoba histolytica is the causative agent for a human. The aim of this study is to evaluate the end products of oxidative stress include; serum levels of malondialdehyde (MDA), and serum levels of total protein carbonyl (POC) in Entameoba histolytica patients. In addition to estimate the growth retardation of children infected with this parasite. In the current study the sera and stool of 37 patients with Entameoba histolytica infection and 37 healthy children in the control group were diagnosed by Molecular detection techniques. Growth status were calculated and oxidative stress were analyzed. The mean age of the patient was 5.16 ± 4.811 years. There were significant lower differences between the mean of z-score for high and mean z-score for weight in patient than that of controls. T-test demonstrated markedly higher MDA levels, and POC levels were spotted in serum of patients as matched to the patients with control group. It is concluded that the Entameoba histolytica infection was associated with significant oxidative stress and growth retardation.

Keywords: Entameoba histolytica, malondialdehyde, total protein carbonyl, growth status

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INTRODUCTION

Amoebiasis is still a big problem in human development at the beginning of the 21st century, and all research in this area are thus helpful. The third explanation is attributed to parasite infections in humans following plasmdiumasis and schistosomiasis; between 40,000-100,000 people passed each year (Martinez 2019). In Iraq, the prevalence of disease varies between regions. Iraq has recoeded Basra with the elevated prevalence rates (59.98%); trailed to Sulaimani (26.28 percent); Najaf (24.89%); Duhuk (20.10%); Nassriyah (12.02%) and Anbar (7.08%) as demonstrated in The Communicable Disease Report Control Center (Al-Taie 2009). The genus of Entamoeba comprises a variety of species, of which (6) are to be found in the human intestinal tract: Entamoeba dispar, Entamoeba histolytica, Entamoeba coli, Entamoeba moshkovskii, Entamoeba hartmanni, and Entamoeba polecki. Only E histolytica of these species is linked with the disease; others are considered nonpathogenic (Cui et al. 2019). The host immune system supplies cell defense toward parasites. Entamoeba histolytica is always risky to stress conditions during invasion of human tissues or during its life cycle (Kumari et al. 2019; Girma, et al. 2017). In vitro, the parasite can survive up to 50% oxygen; The parasite must respond with a rising content of oxygen that have been found in tissues and blood during the invasion, in addition to reactive oxygen and nitrogen species (ROS and NOS, respectively), derived both from host (the defense first line versus infection) or from oxygen and NOs detoxification systems within p (Olivos-García et al. 2020).

MATERIALS AND METHODS

This research was conducted in Al-Sadder Medical City, Najaf, Iraq from June 2018 through February 2019, from June 2019 through February 2020. The research topic has been authorized by Kufa University's Ethics Committee. Before participating in the study, each subject also signed an informed approval. The patients received written informed consent. All the patients were Iraqi. In the Faculty of Science, University of Kufa, Iraq, the samples of blood and stool were tested. Study Groups are included; Patient Group: including 37 patients aged 1-5 years, admitted to Hospital. Medical symptoms (dysentery, tenesm, stomach pain, and tenderness, flatulence, diarrhea, vomiting or fever) often occurred. A pediatrician from each hospital supervises the diagnosis. In addition to Control group: consists from 37 healthy children all were with no history of parasitic

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infection, and without clinical manifestation of any disease .

Blood withdrawals were performed for each child. Part of data was collected by using close-ended questionnaire. In the serum tubes for sera obtained all patients had approximately 5 ml of the venous blood collected. From each patient, a fresh stool specimen was collected and stored in deep freeze (-800C) till to use for extraction DNA. Genomic DNA extraction for frozen human blood sample, accrue by using Stool DNA extraction kit (Biomeer, France). The total serum protein was assisted by an assay kit (Biolabo, France) using a biuret method. For evaluating MAD in the serum sample malondialdehyde test kit (Elabscience,) has been used. Colorimetric approach was used in the serum protein carbonyl content research kit (Elabscience,) Protein Carbonyl content. Colorimetric method (Gee et al. 2017) was used to determined serum total protein, by using total protein biuret method ready for use kit (Elabscience,). The following two set of specific primers from a non-coding short tandem repeat (STR) have been checked by PCR for the molecular diagnosis for E. histolytica and exclusion of E. dispar in locus DA, also known as locus 1-2: Hsp1, and Hsp2: (GAG TTC TCT TTT TAT ACT TTT ATA TGT T) and (ATT AAC AAT AAA GAG GGA GGT) (respectively), for E.histolytica and Dsp1, and Dsp2 :(TTG AAG AGT TCA CTT TTT ATACTA TA) and (TAA CAA TAA AGG GGA GGG) (respectively) for E. dispar (Zaki et al. 2002, Pestehchian et al. 2011). The thermocycling conditions consisted of an initial denaturation of 5 min at 940C, 40 cycles of 1 min at 940C, 1 min at 58 0C, and 30 sec at 72 0C, and a final extension of 5 min at 72 0C. Finally, the amplicons were detected by electrophoresis on 2% agarose gels. And the results were visualized under UV illumination (Biometra, Germany).

Statistical analysis: The results of the study have been analyzed statistically using SPSS (version 20, IBM Corporation, Somers, NY). Comparisons were made with the independent t-test analysis between the mean values of the various parameters. The statistically significant result of any two-tailed $P < 0.05$ has been considered. The Z- scores for height and weight were evaluated using the WHO standard equation (WHO 2006).

Median reference value in this formula M that calculates the mean population. L = power required to transform the data for skewnes removal (i.e., to normalize the data).

S = coefficient of variation (or equivalent). The reference values for z-score for height and BMI that taken from the WHO standards (Chiang et al. 2020).

RESULTS

In the current research, E histolytica molecular diagnosis and E dispar exclusion is used in feces and the serum specimen collected by patients and a control group used for biochemical studies. In addition to the growth status were evaluated. The patient's average age was 5,16 ± 4,811 years. 12 males and 15 females were in the patient group. All 15 subjects were male, on the other hand, and their average age was 4,52 ± 3,081years. Between the mean of z-score for high and mean z-score for weight, there were significant lower differences in patient group than that of controls group (**Table 1**). The levels of lipid peroxides (MDA) and protein carbonyl (POC) in patients serum have been assessed by the oxidation harm to lipid and protein biomolecules in patients. The mean values are as seen in **Table 2**. T-test display substantially higher MDA levels, and POC levels have been noted in serum of patients as In comparison with the patients with control group.

Table 1. The demographic data and growth status of patient and control groups

Parameters	Patient group (37)	Control group (37)	P value
Age (year)	5.16 ± 4.811	4.52 ± 3.081	NS
Gender (F/M)	15/12	13/15	NS
Height for age Z-score	-3.01± 1.16393	-0.078 ± 0.11471	S
Weight for age Z-score	-1.8262 ± 0.682	-0.052 ±0.17284	S

Table 2. Oxidative Stress parameters of patient and control groups

Parameters	Patient group (37)	Control group (37)	P value
MDA (µmol/L)	12.493 ± 2.658	6.13664 ± 5.535	S
POC (nmol/mg Protein)	2.879 ± 0.805	0.649 ± 0.428	S

MDA: Malondialdehyde, total protein carbonyl.

$$\text{Z-score}(\text{height}) = \frac{(\text{Observed value}) - (\text{Median reference value})}{\text{Z-score value for reference population}}$$

$$\text{Z-score}(\text{BMI}) = \frac{(\text{Observed value} \div \text{M})^{L} - 1}{L \times S}$$

DISCUSSION

Previous studies have demonstrated a serum MDA rise in parasite diseases (Al-Azzauy 2016, Kiran et al. 2019, Amaral et al. 2019). The important lipid peroxidation product is MDA, which is obtained through the peroxidation of unsaturated, that including three or more double bonds fatty acids (Tsikas 2017). The MDA product can trigger cross-connection of membrane elements by influencing the exchange of ions from cell membranes, resulting in changes in the diversity of the enzymes and ion permeability (Dai et al. 2017). As a result, MDA can be mutagenic, genotoxic, and carcinogenic by reacting with nitrogen bases in DNA molecules (Kopanska et al. 2017). Prior study shows that a raised MDA activity in dust mite positive skin test

erythrocytes and lymphocytes for positivity shows an oxidative stress in patients infested with dust mite (Lee et al. 2019). In current data, serum MDA level was high significantly when matched with control group, this data proposing that of Entamoeba hisolytica presence cause production of oxidative stress in the hosts. To our recognition, in Entamoeba hisolytica patients, there has been no earlier research examining serum MDA and serum POC levels. The increase of serum MDA levels as a result of lipid peroxidation in patients with Entamoeba hisolytica is proof of a detrimental effect on the host. The total carbohydrate protein is the bio-marker most widely used to cause oxidative protein damage and demonstrates the damage caused by several forms of ROS. Indeed, total levels of carbonyl protein significantly increase with age and in many disease conditions including diabetes, neurodegenerative conditions and obesity increase (Seyyedebrahimi et al. 2018)

The rise in protein carbonyl groups is associated to the progression and severity of the infection in several pathological conditions (Yazici et al. 2016). The higher levels of protein carbonyl measured in Entamoeba hisolytica patients could thus not only be a symptom of oxidative stress, but also of protein dysfunction derived from diseases that complicates Entamoeba hisolytica's pathophysiology.

The serum POC level elevation can be related with the cell-mediated immune system stimulation. These results showed that the parasite can be pathogenic (Sabzevari-Zadeh et al. 2016).

CONCLUSIONS

All the results of the current study suggest a possible pathogen Entamoeba hisolytica .

In addition, the condition of parasitisation (Entamoeba hisolytica) may have had huge implications for the growth status of the children involved. Interestingly, Polymerase Chain Reaction was involved in molecular techniques to enhance diagnosis.

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