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The relationship between nitric oxide and age with entamoeba histolytica

Teebah T. Abdulridha and Rasha A. Noori

University of Kufa, Faculty of Science, *Dept. of pathological Analyses* , Najaf,

Teebaht.alhchaimi@student.uokufa.edu.iq

rashaa.altufaili@uokufa.edu.iq

Abstract

Amebiasis, caused by Entamoeba histolytica, is a significant global health issue, particularly in regions with poor sanitation. Nitric oxide, an adaptable signaling molecule, plays a complex role in this infection. While NO can contribute to tissue destruction and inflammation, it also exhibits protective properties by aiding in wound healing and inhibiting parasite proliferation. Thus, NO's impact on amebiasis involves a delicate balance of harmful and useful effects. This study aimed to determine the relationships between age, *Entamoeba histolytica* infection, and nitric oxide levels in infected patients. This case-control study, conducted in Al-Najaf, Iraq, from September 2024 to January 2025, investigated nitric oxide levels in 50 confirmed *Entamoeba histolytica* infection cases by examining serum samples collected from patients at multiple hospitals. Serum samples were obtained and stored at -80°C for nitric oxide analysis. The study indicated that *E. histolytica* infection was most frequent among individuals aged 21-40 years. Additionally, a notable rise in nitric oxide levels was observed in infected patients between 31 and 49 years old. In conclusion, this study suggests Infection was most common in 21-40 year olds, while NO levels were significantly elevated in infected individuals aged 31-49.

Key words: Entamoeba histolytica ; Nitric oxide ; Age ; serum

Introduction

Amebiasis is a bowel infection caused by the parasitic amoeba *E. histolytica*, which results in amoebic diarrhea marked by blood and mucus in the stool. Although three types of Entamoeba look alike, only *E. histolytica* causes illness. This disease is a major global health issue, causing millions of new

infections and thousands of deaths a year. It is particularly common in tropical and subtropical regions with poor sanitation and congestion. In contrast, in developed nations, the infection is more often seen in older adults, male who have sex with males, and those in institutional care. However, in tropical areas, it affects a broader range of people, particularly those who seek medical attention for diarrhea [1]. NO is a straightforward molecule that is made by both simple and complex organisms. It is produced from the amino acids L-arginine and L-citrulline with the help of NO synthase enzymes. Also, it can be formed by a process that turns nitrate into nitrite, which is then converted into NO. [2,3]. NO is an adjustable signaling molecule that performs important roles in managing how our bodies function. It aids in regulating our blood vessels, nerves, and metabolism, which in turn can lead to lower blood pressure, better physical performance, and protection against metabolic disorders [4]. Within the immune system, nitric oxide is released by cells to fight infections and regulate inflammation. In amebiasis, studies show that higher NO levels can aggravate intestinal tissue damage and inflammation caused by the parasitic infection. Yet, NO also has protective effects, as it helps with wound healing and stops the parasite from multiplying. So, NO's role in amebiasis is complex, with both harmful and helpful properties [5].

Material and method

This case-control study investigated NO levels in amebiasis patients. At the Advanced Research Laboratory in the Al-Amin Center for Advanced Biotechnology, the research was done between September 2024 and January 2025. 110 samples had been collected, with 50 confirmed cases of amebiasis infection. Blood samples were taken from patients at different hospitals in Al-Najaf, including Al-Manathera General Hospital, Al-Hakeem General Hospital, Al-Sader Medical City, Al-Zahraa Teaching Hospital, and Al-Najaf Al-Ashraf Teaching Hospital. From these 110 samples, 50 were confirmed positive for the parasite. Three milliliters of venous blood were collected from each of the 50 confirmed positive cases. The blood was processed by centrifuging to separate the serum, which was then stored at -80°C for later analysis of nitric oxide levels**.**

Commercial kit and its components

As shown in table 1

procedure

1. The spectrophotometer was prepared by preheated for 30 minutes. The wavelength was adjusted to 550 nm. Zero was set with distilled water.
2. Sampling table 2

Table 1 Commercial kit and their components

Commercial kits	**Components**	**Manufacturer / Country**
Nitric oxide (NO)Assay Kit	Extract solution, Reagent I, Reagent II, Reagent II working solution, Reagent III, Reagent IV, Reagent V, Developer A, Developer B, Developer solution, Clarifier, Standard	Solarbio, China, Beijing, Fengtai District, Room 8101, Sihai Qianglong Office Building, No. 1, Beidajie Beili Jia

Table 2 Sampling table

Reagent name（μL）	Test tube (AT)	Standard tube (AS)	Blank tube (AB)
Sample	240	-	-
0.025μmol/mL Standard	-	240	-
Distilled water	-	160	400
Reagent I	20	-	-
Reagent II working solution	40	-	-
Reagent III	20	-	-
Mix well, react at 37 °C for 120 min			
Reagent IV	40	-	-
Reagent V	40	-	-
Mix well, react at 37 °C for 30 min			
Developer solution	400	400	400
After mixing, the reaction was allowed to proceed at room temperature for 10 minutes. The absorbance values of each tube were measured at 550 nm, and were recorded as AT, AS, and AB, respectively. ΔAT was calculated as AT-AB, and ΔAS was calculated as AS-AB. The blank tube and standard tube only needed to be measured 1-2 times.			

Calculations

Concentration of NO = absorbance of standard/ absorbance of sample × Concentration of standard

Result and discussion

Distribution of patients according to age

According to the study, the age groups with the highest Entamoeba histolytica infection rates were those between 21 and 40 years old, with a prevalence of 66%. This was a statistically significant difference compared to the control group, which had a 34% infection rate. The infection was less common in elderly individuals (24%) and had the lowest prevalence among children (10%), with these differences also being statistically significant, as shown in Figure 1.

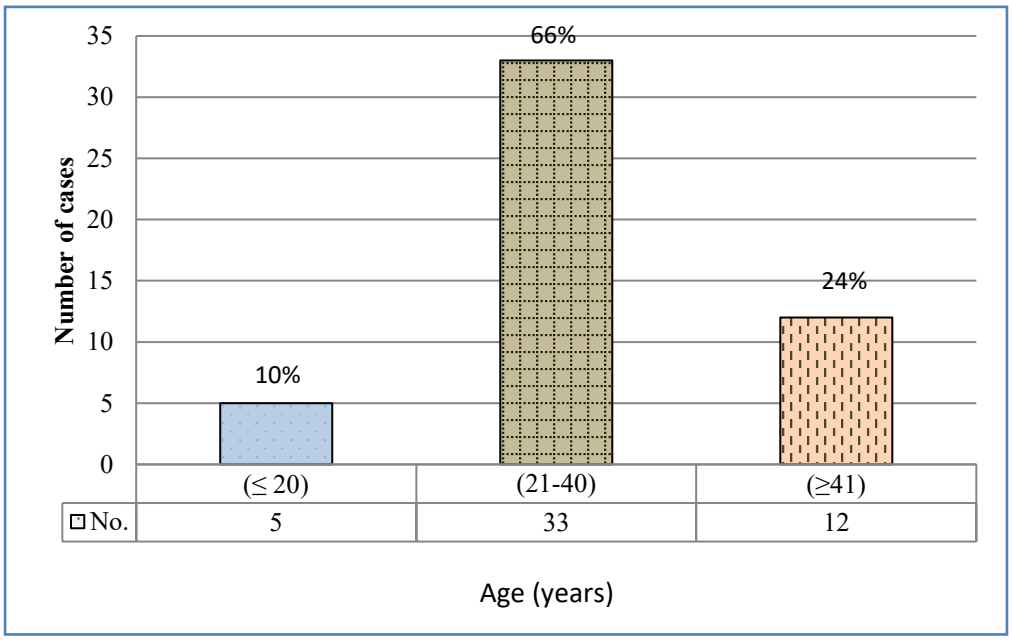

Figure (1) : Distribution of patients according to age.

A study categorizing patients by age revealed a significant link between age and Entamoeba histolytica infection rates. The highest prevalence was found in individuals aged 21–40, with 66% of cases compared to 34% in the control group. Middle-aged individuals had a moderate prevalence of 24%, while children had the lowest at just 10%. These age-related differences in infection prevalence were statistically significant ($P = 0.004$, $\chi^2 = 10.96$). According to a 2021 study by Begum et al., several factors contribute to E. histolytica infection, including eating contaminated street food, poor handwashing habits, and exposure

to unhygienic workplaces [6]. The high rate of infection among individuals aged 21–40 is likely because they make up a larger part of the workforce. This greater exposure in occupational settings and more frequent consumption of street food increases their risk of contracting the parasite. However, other studies show different age-related trends. For example, research by Tasawar et al. (2010) and Zahida et al. (2010) found the highest rates of E. histolytica infection in young children (1–15 years), at 30.82%, while the lowest rates were in middle-aged adults (31–45 years), at 17.34%. These differences were statistically significant (P<0.05) [7,8]. Roro et al. (2022) reported that the Children's increased vulnerability is attributed to less developed hygiene practices, close contact in childcare and school settings, and exposure to contaminated soil and water in play areas, facilitating fecal-oral transmission.

Nitric Oxide between the control and patients

Infected patients with Entamoeba histolytica showed a statistically significant increase in nitric oxide (NO) concentration compared to the control group. Figure 2 shows that the average of the NO level in infected individuals was 0.037±0.002, which was higher than the control group's average of 0.02762±0.001, a difference that was significant at P ≤ 0.05.

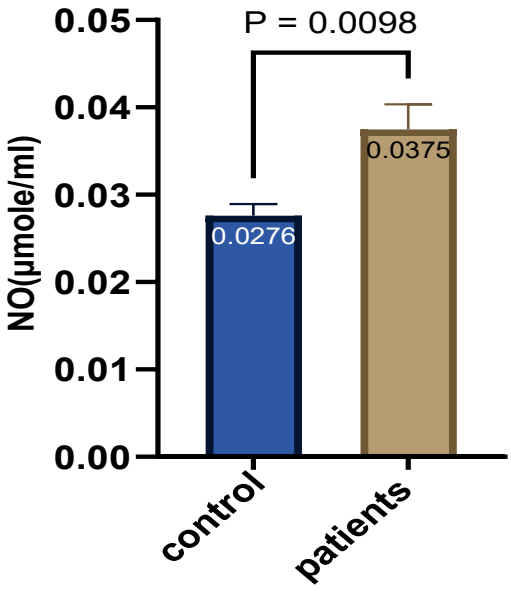

Figure (2) Comparison of NO(µmole/ml) between control and patients.

Within the immune system, nitric oxide is a signaling molecule produced by immune cells, such as macrophages, to protect the body against pathogens. The observed rise in NO concentration suggests that the body was mounting an active immune response to the amebiasis infection. Throughout this process, NO displays a dual nature, with both beneficial and harmful effects. It can either directly kill pathogens or inhibit their growth [10]. A 1992 study by Lin and Chadee showed that nitric oxide, which is produced from the amino acid L-arginine, is essential for macrophages to kill Entamoeba histolytica trophozoites. When these mouse macrophages were activated with IFN-γ, they became very effective at killing the parasites and produced more NO. On the other hand, blocking NO production or removing L-arginine decreased the macrophages' ability to kill the amoebae. This killing ability was restored when L-arginine was added back to the macrophages. [10].

Comparison of biomarker according to age between control and patient group

A significant rise in NO levels was shown in patients aged 31-49 years by this study as seen in table 3 (P-value <0.001).

Table 3 Comparison of biomarkers according to age between control and patient group

Biomarker	Mean±S.E		p-value*
	Control group	Patient group	
NO(µmole/ml)			
(≤30) years	0.033 ± 0.003	0.393 ± 0.007	0.667
(31-49) years	0.025 ± 0.001	0.041 ± 0.004	<0.001
(≥50) years	0.028 ± 0.002	0.034 ± 0.005	0.252
p-value#	0.205	0.558	-

*Independent samples- T test, #One-Way ANOVA test, S.E: standard error, P≤0.05.

The 31-49 age range may have specific physiological or immune properties that affect NO production during the studied condition. It's possible that this age group had the most active immune response to the amebiasis. The increased NO levels could serve as a possible biomarker for this condition in this age range, reflecting either

the body's attempt to fight the infection or a contribution to the disease's progression [11]. Nitric oxide is an essential signaling molecule involved in many bodily functions, including those of the cardiovascular and nervous systems. It's created by nitric oxide synthases (NOSs), and its activity is carefully controlled. With age, NO production is often reduced due to endothelial dysfunction. According to a 2024 study by Mazuryk et al., genetic variations in NOSs are linked to aging and longevity, influencing our cognitive and physical abilities. This reduction in NO contributes to key signs of aging, such as cellular weakness, increased cell death, and mitochondrial dysfunction [12].

Conclusion

The study found that amebiasis infection was most common in individuals between 21 and 40 years old. In addition, a notable elevation in nitric oxide levels was observed in infected patients who were slightly older, especially those aged 31 to 49 years.

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conflict of interest

The authors declare no conflict of interest**.**

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دراسة العلاقة بين أكسيد النيتريك والعمر وتأثيرها على الأميبا الحالة للنسج

طيبه طالب عبدالرضا و رشا عامر نوري

جامعة الكوفة ,كلية العلوم , قسم التحليلات المرضية ,النجف, العراق

المُلخَّص

داء الأميبات، الذي تسببه الأميبا الحالة للنسج، يمثل مشكلة صحية عالمية هامة، خاصة في المناطق ذات الصرف الصحي السيئ. يلعب أكسيد النيتريك ، وهو جزيء إشارة متعدد الاستخدامات، دورًا معقدًا في هذه العدوى. فبينما يمكن أن يساهم أكسيد النيتريك في تلف الأنسجة والالتهابات، فإنه يظهر أيضًا خصائص وقائية عن طريق المساعدة في التئام الجروح وتثبيط تكاثر الطفيليات. وبالتالي، فإن تأثير أكسيد النيتريك على داء الأميبات ينطوي على توازن دقيق بين الآثار الضارة والمفيدة. هدفت هذه الدراسة إلى تحديد العلاقات بين العمر، وعدوى الأميبا الحالة للنسج، ومستويات أكسيد النيتريك في المرضى المصابين. هذه الدراسة، التي أجريت في النجف، العراق، من سبتمبر 2024 إلى يناير 2025، بحثت مستويات أكسيد النيتريك في 50 حالة مؤكدة للإصابة بالأميبا الحالة للنسج عن طريق تحليل عينات مصل الدم التي تم جمعها من المرضى في عدة مستشفيات. تم الحصول على عينات مصل الدم وتخزينها عند -80 درجة مئوية لتحليل أكسيد النيتريك. أشارت الدراسة إلى أن عدوى الأميبا الحالة للنسج كانت الأكثر انتشارًا بين الأفراد الذين تتراوح أعمارهم بين 21-40 عامًا. بالإضافة إلى ذلك، لوحظ ارتفاع ملحوظ في مستويات أكسيد النيتريك في المرضى المصابين الذين تتراوح أعمارهم بين 31-49 عامًا. في الختام، تشير هذه الدراسة إلى أن العدوى كانت الأكثر شيوعًا في الفئة العمرية 21-40 عامًا، بينما زادت مستويات أكسيد النيتريك بشكل ملحوظ في الأفراد المصابين الذين تتراوح أعمارهم بين 31-49 عامًا .

الكلمات المفتاحية: الأميبا الحالة للنسج؛ أكسيد النيتريك؛ العمر؛ المصل