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ORIGINAL ARTICLE

Brain-Derived Neurotrophic Factor and Interleukin-1 Beta as Novel Biomarkers in Patients Infected With *E.Coli* O157:H7 And *Entamoeba Histolytica*

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ABSTRACT

Background: *The immune response to E. coli O157:H7 and Entamoeba histolytica (E.histolytica) includes both humoral and cellular components; however, despite the immune response, these organisms are able to evade host defenses through production of anti-phagocytic factors and toxins which hinder immune cell functions, making resolution of disease more difficult.* ***Objective:*** *The aim was to study the immunological role and correlation of Brain-derived neurotrophic factor (BDNF) and Interleukin-1 beta (IL-1β) in patients with diarrhea due to E. coli O157:H7 and E. histolytica.* ***Methodology:*** *This study enrolled 60 healthy participants as a control group, 30 patients infected with E. histolytica, 30 patients infected with E. coli O157:H7 and E. histolytica were the case group. PCR was used to diagnose E. coli O157:H7. E. histolytica was diagnosed by using wet mount examination with 0.85% saline and Lugol's iodine. BDNF and IL-1β have been measured in patients' blood using the ELISA technique.* ***Results:*** *The patients who had E. histolytica infection or co-infections showed significantly heightened IL-1β concentrations above normal values and co-infected patients demonstrated the most extreme IL-1β response pattern. Patients infected with E. histolytica exhibited a positive yet insignificant correlation pattern between BDNF and IL-1β levels that also applied to patients with co-infections.* ***Conclusion:*** *Patients co-infected with E. coli O157:H7 and E. histolytica, as well as E. histolytica alone, had markedly elevated levels of IL-1β in the blood profile, whereas BDNF levels were unaffected by the intestinal infection.*

INTRODUCTION

Organisms such as *Escherichia coli* O157:H7 and *Entamoeba histolytica* are pathogenic microorganisms responsible for a large burden of human disease worldwide, particularly in regions with low sanitation coverage and/or poor food safety[1]. These pathogens induce different immune responses in infected patients that affect the course of disease and its possible sequelae. Whereas *E. coli* O157:H7 is a bacterial pathogen that primarily causes bloody diarrhea and hemolytic uremic syndrome (HUS), *Entamoeba histolytica* is a protozoan parasite that causes amebiasis, which can result in an invasive disease with extraintestinal manifestations[2]. A better understanding of the host immune response to these diseases is critical to the development of effective diagnostic, therapeutic, and preventive measures[3].

The immune reactivation against *E. coli* O157:H7 has both innate and adaptive pathways. Bacterial infection activates the innate immune system through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), that recognize bacterial lipopolysaccharides (LPS) during infection[4]. Although neutrophils are essential for bacterial clearance, they can be implicated in intestinal injury.

When macrophages, dendritic cells, and neutrophils recognize the parasite through pattern recognition receptors (PRRs), the innate immune response is activated[5]. Besides directly killing the parasite, neutrophils also release reactive oxygen species (ROS) which can cause tissue damage. Macrophages also secrete nitric oxide (NO), which is cytotoxic for the parasite. Innate adaptive immunity is similar to active Th1, known for the secretion of interferon-gamma (IFN-γ) and IL-12, both intracellular macrophage activators[6]. However, *E. histolytica* possesses immune evasion mechanisms that allow its perception by the host and facilitates its survival within the host, such as cysteine proteases that inhibit the action of host antibodies and complement proteins similarly it manipulates apoptosis in infected cells to enable its persistence and spread[7].

Neurotrophins (e.g. brain derived neurotrophic factor: BDNF) and pro-inflammtory cytokines (e.g. interleukin-1 beta: IL-1β) enable neuroimmune and inflammatory responses[8]. BDNF (brain-derived neurotrophic factor) is a critical neurotrophin involved

with neuronal survival and the neural plasticity associated with learning and memory. IL-1β is a key pro-inflammatory cytokine responsible for regulating inflammation in response to tissue damage and infection[9]. Such molecules interact after infection with enteric pathogens (e.g., *Escherichia coli* O157:H7 and *Entamoeba histolytica*) that induce systemic inflammatory and neuroimmune responses.

Escherichia coli O157: H7 is a Shiga toxin-producing enterohemorrhagic bacteria that cause gastrointestinal infection, which often leads to hemorrhagic colitis and haemolytic uremic syndrome[10]. The infection induces a strong inflammatory response in the gut, which is accompanied by increased levels of IL-1β, tumour necrosis factor-alpha (TNF-α) and multiple cytokines. IL-1β is a key regulator of the innate immune response through the promotion of neutrophil recruitment and the enhancement of epithelial barrier function, as well as through the regulation of the synthesis of other inflammatory mediators[11]. This study provides an immunological perspective on the roles of BDNF and IL-1β, documenting their immune functions in the clinical setting of patients suffering from *Escherichia coli* O157:H7 and *Entamoeba histolytica* infections.

METHODOLOGY

Participants in the study

A case-control design performed in the Gastroenterology Department of Al-Najaf Teaching Hospital in Iraq. This is from the period between 1 October 2023 and 1 March 2025. Sixty healthy volunteers served as the control group for this study. Thirty patients were infected by *E. histolytica* and thirty patients infected with co-infection by *E. coli* O157:H7 and *E. histolytica.*

Ethical Considerations

Approval for the study was obtained from the Institutional Ethics Committee of the Faculty of Science, University of Kufa, College of Medicine.

***E. coli* O157:H7 and *E. histolytica* detection**

Polymerase Chain Reaction (PCR) and chromogenic agar were used to diagnose *E. coli* O157:H7. *E. histolytica* has been diagnosed by using wet mount examination with 0.85% saline and Lugol's iodine[12].

Quantitative ELISA Technique for measurement of BDNF and IL-1β levels in patients' blood

Five ml of blood sample were collected from all the individuals and 2 ml of serum for each individual has been obtained by centrifugation at 8000 rpm/10 min. The concentrations of immune parameters BDNF and IL-1β in peripheral blood were quantified using the enzyme-linked immunosorbent assay (ELISA) technique (Accubiotech– China), with Kits from (Bioassay Technology Laboratory. Shanghai, China), and concentration standards were established to facilitate the extraction of absorbance. This procedure facilitated the deriving the concentrations from absorbance measurements by applying the Beer-Lambert law for both groups. Plates are coated with human BDNF monoclonal antibodies, which bind to the sample's BDNF protein. Streptavidin-HRP binds to biotinylated BDNF antibody, and absorbance is measured at 450 nm. The same method applies to IL-1β concentration[13].

Statistical analysis

The mean and standard error (SE) were calculated for each value using GraphPad Prism. A p-value of less than 0.05 was considered statistically significant in the analysis.

RESULTS

BDNF

Patients infected with *E. histolytica* exhibited a non-significant increase (P = 0.4108) in serum BDNF levels (919.7 ± 29.04 ng/ml) when compared to the control group (930.5 ± 38.93 ng/ml). In addition, blood BDNF levels (941.7 ± 26.27 ng/ml) in *E. coli* O157:H7 and *E. histolytica* co-infected patients did not differ significantly (P = 0.4042) from the control group. Similarly, serum BDNF levels did not show significant difference (P = 0.2887) between patients infected with *E. coli* O157:H7 and co-infected patients with *E. coli* O157:H7 and *E. histolytica* (Figure 1).

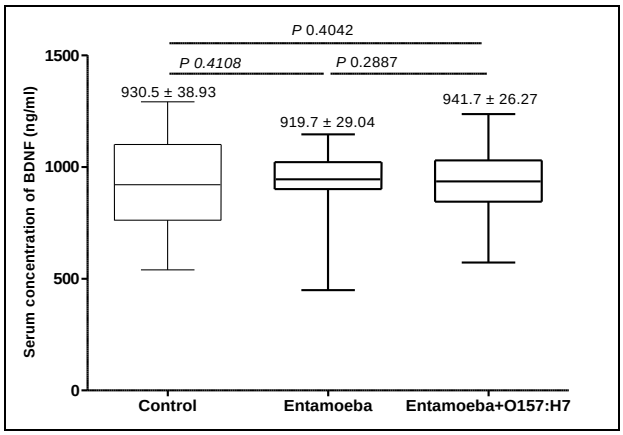

Fig. 1: BDNF serum levels in *E. coli* O157:H7 and *E. histolytica* co-infected patients compared to control

In the case of *E. histolytica* affected patients, male subjects (986.6 ± 26.35 ng/ml) had a slightly elevated total blood BDNF level when compared to their female counterparts (1006 ± 17.99 ng/ml) but results were not significant (P = 0.2710). An analogous pattern was observed for BDNF serum levels with no differences (P = 0.1549) being detected between male (1008 ± 27.50 ng/ml) and female (953.0 ± 30.41 ng/ml) *E. coli* O157:H7/ *E. histolytica* co-infected patients (Figure 2).

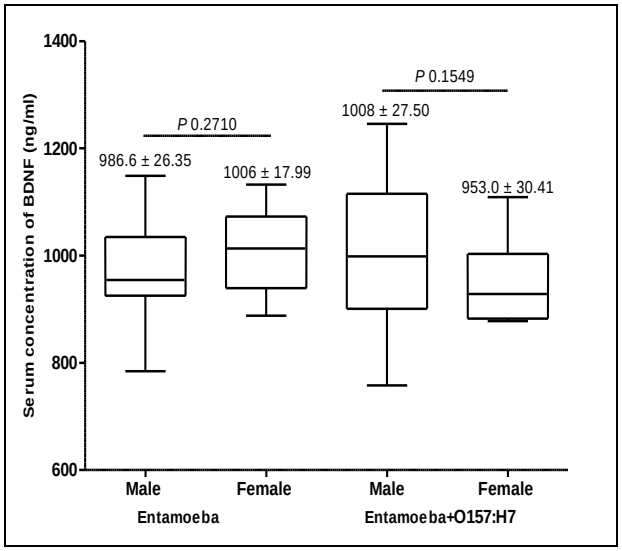

Fig. 2: BDNF serum levels in male and female infected with *E. coli* O157:H7 and *E. coli* O157:H7 and *E. histolytica* co-infected

IL-1β

Our results showed that the blood levels of IL-1β were significantly higher (P < 0.0001) in all *E. histolytica* infected patients (3.482 ± 0.053 ng/ml) in relation to the control group (0.7359 ± 0.056 ng/ml). Moreover, serum IL-1β were markedly (P < 0.0001) increased in (4.416 ± 0.072 ng/ml) co-infected patients with *E. histolytica* and *E. coli* O157:H7 than control. In contrast, serum IL-1β levels were also significantly different (P < 0.0001) between patients co-infected with *E. histolytica* and E. coli O157:H7 and those that were infected with *E. histolytica* alone (Figure 3).

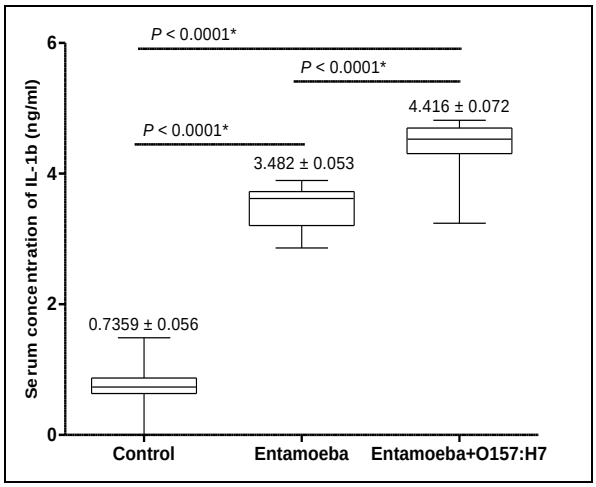

Fig. 3: IL-1β serum levels in *E. coli* O157:H7 and *E. histolytica* co-infected patients compared to control

The total blood IL-1β level of female participants was singly increased (3.485 ± 0.084 ng/ml) as compared to the male participant (3.480 ± 0.067 ng/ml) in exacerbated *E. histolytica* infection in patients, however, the difference was statistically not significant (P = 0.4825). A similar binding was also observed for IL-1β serum levels, but with no significant differences (P = 0.1979) between male (4.451 ± 0.070 ng/ml) and female (4.303 ± 0.212 ng/ml) patients provocated by co-infection *of E. coli* O157:H7 and *E. histolytica* (Figure 4).

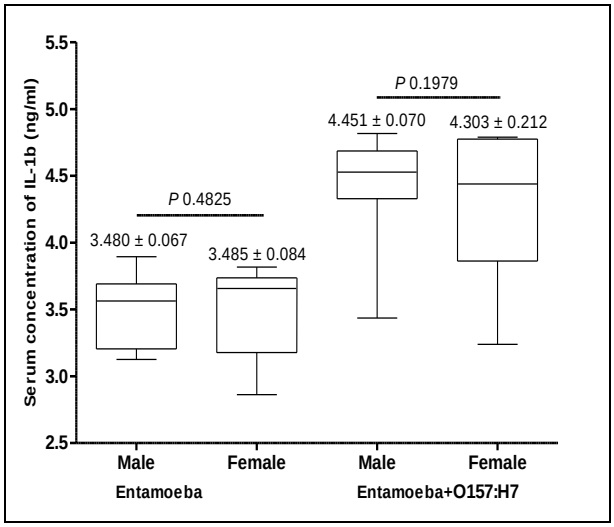

Fig. 4: IL-1β serum levels in male and female infected with *E. coli* O157:H7 and *E. coli* O157:H7 and *E. histolytica* co-infected

Our results revealed that the levels of BDNF and IL-1β in the blood of patients infected with *E. histolytica* were positively correlated with each other, with no statistically significant difference P=0.2163, R=0.05406 (Figure 5). A similar positive correlation was observed between the two markers in case of *E. coli* O157:H7 and *E. histolytica* co-infected patients P=0.3389, R=0.03271 (Figure 6)

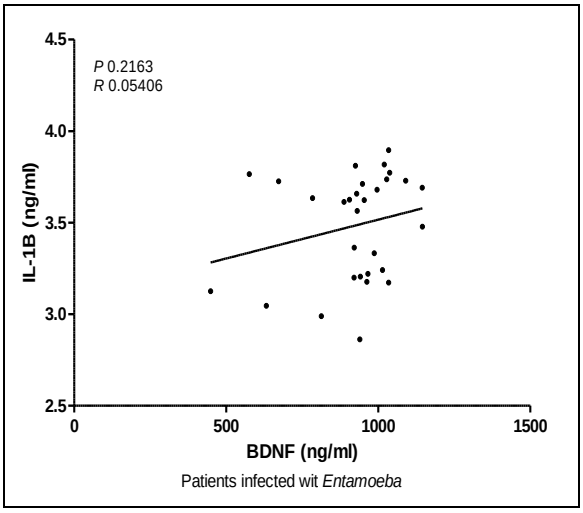

Fig. 5: Correlation between total serum's levels of BDNF and IL-1β in patients infected with *E. histolytica*

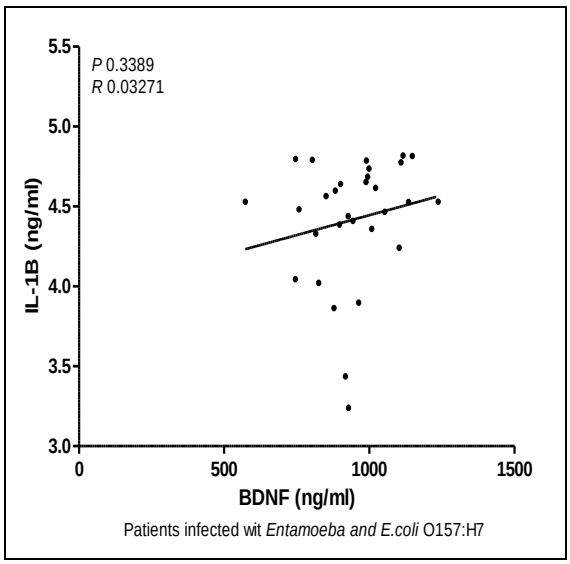

Fig. 6: Correlation between total serum's levels of BDNF and IL-1β of *E. coli* O157:H7 and *E. histolytica* co-infected

DISCUSSION

Our results showed no significant difference in BDNF serum levels of patients infected by *E. histolytica* and co-infected by *E. coli* O157:H7 and *E. histolytica* to control values, similarly, there was no significant difference found among *E. coli* O157:H7-infected patients and co-infected patients.

Brain-derived neurotrophic factor (BDNF), a type of neurotrophin mainly produced by neurons involved in neuronal survival and synaptic plasticity, has recently attracted attention because of its potential involvement in immune regulation[14]. BDNF is expressed but also upregulated in response to inflammatory stimuli in multiple immune cells e.g. macrophages, dendritic cells, during infection. In the case of *E. coli* O157:H7, an enteropathogen that causes severe hemorrhagic colitis and hemolytic uremic syndrome (HUS), BDNF has been shown to influence gut epithelial barrier integrity[15]. BDNF has also been implicated in epithelial repair and survival, in part via its ability to promote tight junction formation, which can help to limit bacterial translocation and systemic spread[16]. Moreover, within the CNS, BDNF serves to regulate neuroinflammation induced by bacterial endotoxins, with some evidence suggesting it may confer protection from cognitive impairment associated with systemic infections[17].

During *E. histolytica* infection, the role of brain-derived neuron factor (BDNF) is not well-defined but has been reported to be involved in neural-immune interactions regulating the inflammatory response[18]. The amoebic dysentery and liver abscesses caused by the protozoan parasite *E. histolytica* result from its potent cytotoxic activity. New findings suggest that BDNF could impact macrophage polarization towards an M2 anti-inflammatory phenotype, which could paradoxically promote the persistence of pathogens by repressing the host ability to clear the infection effectively[19]. These neuro-phenomena underline the ambivalent role of neurotrophic factors in immunity.

In our study, the level of IL-1β was found to be significantly higher in patients infected with *E. histolytica* and co-infected patients than in the control group. Moreover, levels of IL-1β were markedly increased in co-infected patients when compared to patients with *E. histolytica* alone.

IL-1β, an archetypal cytokine of the innate immune response, plays an essential role in pathogen recognition and removal. The inflammasome mediates most of its activation by recognizing pathogen-associated molecular patterns (PAMPs) and inducing pyroptosis, a proinflammatory programmed cell death[20]. When factoring in the Th9/nemesis interaction, Shiga toxin-driven IL-1β secretion in *E. coli* O157:H7 infections makes sense, as IL-1β is upregulated by Shiga toxin and lipopolysaccharide (LPS) and induced in conjunction with neutrophil recruitment and cytokine storm that can worsen disease severity by increasing vascular damage and thrombosis[21].

Likewise, IL-1β is essential in initiating inflammatory pathways in *E. histolytica* infections leading to tissue destruction[22]. The infected macrophages release factors such as IL-1β, which promote neutrophilic infiltration, critical for pathogen clearance, but also leading to excessive inflammatory response and collateral tissue damage[23]. Adjacent to BDNF, which may have both protective and damaging effects, IL-1β mainly amplifies inflammation, a response that is required for clearing pathogens but can also be deleterious to the host when aberrantly activated[24].

A better understanding of the interplay between BDNF suppression and IL-1β upregulation during *E. coli* O157:H7 infection might shed light on potential neuroprotective strategies aimed at mitigating long-term neurological sequelae[25]. BDNF protects gut homeostasis and maintains neuronal function, however, its link with IL-1β in *E. histolytica* infections is relatively unexplored. Since neuroinflammation and oxidative stress may downregulate BDNF expression, severe *E. histolytica* infections are likely to promote cognitive dysfunction via the gut-brain axis. Examining the interplay of BDNF and IL-1β in amoebiasis might reveal new treatment targets aimed to curb both gastrointestinal and neurological manifestations[26].

Our results proved that no significant difference of positive correlation were observed between blood BDNF and IL-1β levels in *E. histolytica*-infected patients as well as in co-infected patients with *E. coli* O157:H7. The weak positive correlation between BDNF and IL-1β is not statistically significant in Entamoeba-

infected patients. This means that changes in BDNF are unlikely to affect IL-1β levels in these patients.

Immunity to *E. coli O157:H7* and *E. histolytica* is characterized by complex and interplaying responses between components of innate and adaptive immunity; however, each pathogen has developed unique evasion mechanisms that enable them to survive within the host, and contribute to virulence[27]. *E. coli* O157:H7 mainly causes a neutrophil-mediated inflammatory response causing intestinal and systemic complications, while *E. histolytica* evokes a macrophage- and T cell-mediated response which can cause chronic infection and tissue destruction. Further studies of host-pathogen interactions and immune modulation mechanisms are needed to develop vaccines and therapeutic approaches to these infections[28].

Some studies further emphasize the differing roles of BDNF and IL-1β during infection and their opposing contributions to immune regulation. Although IL-1β provides the inflammation and pathogen clearance, its hyperproduction can damage the host tissues, indicating the need of active regulatory mechanisms to limit the effects[29].

BDNF, in particular, can promote tissue healing and neuroprotection, but paradoxically is an easier clearer synonym, counter intuitively; it can also inhibit effective immune responses when overexpressed. These opposing functions indicate that specific modulation of these molecules could be a potential target for therapeutic applications. For example, IL-1 receptor antagonists (IL-1RAs) may help to alleviate excessive inflammation as a result of E. coli infections, while BDNF mimetics or inhibitors may be investigated to fine-tune the immune response to *E. histolytica* infections[30].

CONCLUSION

IL-1β promotes infection responses through inflammation, but excessive levels could impair neurons and epithelial cells via lower BDNF. Associations between BDNF and IL-1β were poorly positively correlated in *Entamoeba* infected patients. This indicates that alterations in BDNF levels are unlikely to significantly impact the levels of IL-1β. Developing a better understanding of how these two things interact with each other could help with therapy.

Conflict of interest

The authors insured there was no conflict of interest in this study

Patient Declarations

All patients in the study (including controls for blood sampling from healthy individuals) provided written consent. This research was completed with great cooperation between them.

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