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ORIGINAL ARTICLE

Inflammatory biomarker alterations and *Caspase-3* gene expression in patients with *Entamoeba* spp. infection

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Abstract

Background Microscopically diagnosed *Entamoeba* spp. infection is associated with inflammatory and cellular responses that may contribute to disease pathogenesis. Caspase-3 is a key apoptosis-related gene involved in the execution phase of programmed cell death. This study aimed to evaluate *Caspase-3* gene expression in peripheral blood samples obtained from patients with microscopically diagnosed Entamoeba spp. infection.

Methods and Results Peripheral blood samples were collected from 31 infected patients and 14 healthy controls. Relative Caspase-3 mRNA expression was quantified using SYBR Green–based quantitative real-time PCR (qPCR) and normalized against β-actin. Relative expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method, while statistical analyses were performed on ΔCt values. The mean ΔCt value was significantly lower in infected patients (6.306 ± 0.398) than in controls (7.658 ± 0.221), indicating increased *Caspase-3* gene expression in the infected group. The mean difference between groups was 1.352 cycles (95% CI: 1.19–1.51; $p < 0.0001$). Relative expression analysis demonstrated a 2.65 ± 0.75-fold increase in Caspase-3 expression in infected patients compared with controls. Melt curve analysis confirmed specific amplification of both Caspase-3 and β-actin.

Conclusions These findings suggest the possible involvement of apoptosis-associated transcriptional changes during Entamoeba spp. infection; however, functional apoptotic activation was not directly assessed.

Keywords *Entamoeba* spp. infection · *Entamoeba spp.* · *Caspase-3* · Apoptosis · Gene expression · Quantitative real-time PCR (qPCR)

Introduction

Intestinal protozoan infections continue to represent a major group of parasitic diseases affecting populations worldwide, particularly in developing regions where sanitation is inadequate and access to clean water is limited [1–4]. Among these parasites, *Entamoeba histolytica* is one of the most clinically important pathogenic species, causing amoebiasis, which may range from asymptomatic intestinal colonization to severe invasive disease, including amoebic colitis and liver abscess [5]. This infection remains a significant cause of morbidity, particularly in endemic areas [2, 3, 6]. The pathogenesis of *Entamoeba* spp. infection involves complex interactions between the parasite and the host immune system. Following ingestion of infective cysts, trophozoites colonize the intestinal mucosa and may invade host tissues through virulence mechanisms such as cysteine proteases, amoebapores, and adhesion molecules, leading to epithelial disruption and activation of host immune responses [7]. Although these immune responses contribute to parasite control, excessive inflammatory activity may result in tissue damage and increased cellular stress [8].

Apoptosis represents a key cellular process involved in host–parasite interactions, functioning to eliminate damaged or infected cells while maintaining tissue homeostasis. In parasitic infections, apoptosis may act as a protective mechanism; however, it may also contribute to disease progression depending on the balance between host defense and parasite-induced cytotoxicity [9]. Caspase-3 is a central executioner enzyme in the apoptotic pathway, responsible for the terminal stages of programmed cell death through cleavage of intracellular substrates and induction of DNA

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