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CHRONIC AMEBIASIS [ENTAMOEBA HISTOLYTICA]: DETECTION BY IMMUNODIAGNOSIS METHOD

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ABSTRACT

Enzyme immunoassay (EIA) kits for Entamoeba histolytica antibody detection are commercially available. Antibody detection is most useful in patients with extra intestinal disease (amebic liver abscess) when organisms are not generally found on stool examination. A capture sandwich ELISA using purified antibodies was able to detect 70 ng of amoebae protein, showing a sensitivity of 93 % and specificity of 94%. The combination of microscopic examination and ELISA gave a concordance and discordance of 93.25% and 6.75%, respectively. It was concluded that capture sandwich ELISA is highly specific for the detection of coproantigens of E. histolytica in faeces of infected or chronic patients is quicker to perform, easier and more sensitive than microscopic examination.

KEYWORDS: Entamoeba histolytica; chronic amebiasis, antibody detection (IgG); ELISA.

INTRODUCTION

Entamoeba histolytica is an anaerobe parasite forming cysts which have four small nuclei and measure 10-15micrometer in diameter. The cysts are sturdy and resist adverse environmental conditions. After ingestion by a susceptible host (invertebrates and vertebrates including humans), its wall is disrupted by the formation of a small opening through which an amoeba emerge. The amoeba divides serially through three cycles giving rise to eight uninucleated trophozoites from one cyst which are motile and measure 20-30 micrometer in diameter. Some of the trophozoites then invade the tissues of the large intestine and may erode them so extensively that they gain entrance into the bloodstream. Thus, amoeba can reach all parts of the body. Infection with Entamoeba histolytica has worldwide distribution. It is the causative agent of amoebiasis and amoebic dysentery and inhabits the lumen and mucosa of the large intestine, predominantly the transverse colon and cecum. Extra intestinal amoebiasis can afflict any organ or tissue. The majority of infected individuals are free of symptoms; this high incidence of asymptomatic carriers complicates matter. Those who are symptomatic experience a wide range of manifestations. Members of all age groups and both sexes are infected. The risk of infection increases with inadequate sanitary conditions. An increased prevalence of amoebiasis is found among people, who have an increased risk of exposure in the agricultural occupations and in male homosexuals.[1]

PATHOLOGY

In the vast majority of cases, infection is asymptomatic and the carrier is unaware they are infected. However, in an estimated 10% of cases E. histolytica causes disease. Once the trophozoites are excysted they colonize the large bowel, remaining on the surface of the mucus layer and feeding on bacteria and food particles. Occasionally, and in response to unknown stimuli, trophozoites move through the mucus layer where they come in contact with the epithelial cell layer and start the pathological process. E. histolytica has a lectin that binds to galactose and N-acetylgalactosamine sugars on the surface of the epithelial cells; the lectin normally is used to bind bacteria for ingestion. The parasite has several enzymes such as pore forming proteins, lipases and cysteine proteases, which are normally used to digest bacteria in food vacuoles but which can cause lysis of the epithelial cells by inducing cellular necrosis and apoptosis when the trophozoite comes in contact with them and binds via the lectin. The trophozoites will then ingest these dead cells. This damage to the epithelial cell layer attract human immune cells and these in turn can be lysed by the trophozoite, which releases the immune cell's own lytic enzymes into the surrounding tissue, creating a type of chain reaction and leading to tissue destruction. This destruction manifests itself in the form of an 'ulcer 'in the tissue, typically described as flask-shaped because of its appearance in transverse section. This tissue destruction can also involve blood vessels leading to

bloody diarrhea, amebic dysentery. Occasionally, trophozoites enter the bloodstream where they are transported typically to the liver via the portal the portal system. In the liver a similar pathological sequence ensures, leading to amoebic liver abscesses. The trophozoites can also end up in other organs, sometimes via the bloodstream, sometimes via liver abscess rupture or fistulas. In all locations, similar pathology can occur. [1-2]

DIAGNOSIS

It can be diagnosed by stool samples, but it is important to note that certain other species are impossible to distinguish by microscopy alone. Trophozoites may be seen in a fresh faecal smear and cysts in an ordinary stool sample. Increase chronic amebiasis, IgG detection of Entamoeba histolytica by ELISA method is useful. Serological tests exist and most individuals (whether with symptoms or not) will test positive for the presence of antibodies. The levels of antibody are much higher in individuals with liver abscesses. Serology only becomes positive about two weeks after infection. The Entamoeba histolytica IgG ELISA is a microtiter strip enzyme immunoassay for the detection of IgG class antibodies to Entamoeba histolytica antigens in human serum. Microtiter strip wells as a solid phase are coated with monoclonal anti-Entamoeba histolytica antibodies. Diluted patient specimens, ready for use controls antibodies are pipette into these wells. During incubation Entamoeba histolytica antigen-specific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample, control material horseradish peroxidase conjugated Protein-A is dispensed into the wells. During a second incubation this Protein-A conjugate binds specifically to IgG antibodies resulting in the formation of enzyme-linked immune complexes. After a second washing step to remove unbound conjugate, any immune complexes forms are detected by incubation with TMB substrate, and development of a blue colour. The blue colour is turned into yellow by stopping the enzymatic indicator reaction with sulphuric acid. The intensity of this colour is directly proportional to the amount of Entamoeba histolytica specific antibodies in the patient specimen. Absorbance at 450nm is read using ELISA microtiter plate reader.[3-6]

CONCLUSION

Entamoeba histolytica, the causative agent of intestinal and extra intestinal amebiasis, is a common parasitic cause of significant morbidity and mortality in the developing countries. It was concluded that ELISA is highly specific for the detection of antibody (IgG) of E. histolytica in serum of asymptomatic patients or chronic amebiasis is quicker to perform, easier and more sensitive than microscopic examination.

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