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THE LEVEL OF MYELOPEROXIDASE (MPO) AND ANTI- PROTEINASE 3 IN PATIENTS WITH *ENTAMOEBA HISTOLYTICA*

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SUMMARY

The study was carried out during the period from the beginning of (November/2013 - November/2014) for detection of *Entamoeba histolytica* in patients with age range from (3-60) year who attended to AL-Yarmouk teaching hospital and AL-Tifil central hospital. The diagnosis done by microscopic examination. A total of 200 suspected patient there was120 infected with the parasite diagnosed by the direct examination method, a blood sample was taken from each one, as well as (60) healthy controls were involved. The study included the measurement of Anti-neutrophil cytoplasmic antibodies c-ANCA (Anti PR3) and anti- myeloperoxidase p-ANCA (MPO) levels by Enzyme linked immuno sorbent assay (ELISA). The results indicated: The prevalence of *Entamoeba histolytica* by using microscopic examination was 145 (72.5%) in comparison to 120(82.75%) by using Triage (Micro parasite panel test). The level of Myeloperoxidase (MPO) and Anti- proteinase- 3increased significantly ($P<0.05$) in sera patients in comparison to healthy control, but there is no-significant ($P>0.05$) differences between the gender in both groups.

KEYWORDS: *Entamoeba histolytica*, Anti-pr3, MPO.

INTRODUCTION

Entamoeba histolytica is a protozoan parasite that causes amoebic dysentery and liver abscess. The disease is still one of the major health problems and predominantly affects individuals of lower socioeconomic status who live in developing countries.[1]

Infections can be intestinal, extra intestinal, or both. Most cases are intestinal and asymptomatic. Symptoms, when occur, are multiple and varied, ranging from mild abdominal discomfort and diarrhea (often with blood and mucus) alternating with periods of remission or constipation, to severe illness with fever, chills and significant bloody or mucoid diarrhea (”amoebic dysentery”). Amoebic colitis may be confused with inflammatory bowel disease such as ulcerative colitis.[2]

Anti-neutrophil cytoplasmic antibodies c-ANCA (Anti PR3) and anti myeloperoxidase p-ANCA(MPO) are group of auto antibodies, mainly of the IgG type, against antigens in the cytoplasm of neutrophil granulocytes (the most common type of white blood cell) and monocytes. They are detected as a blood test in a number of autoimmune disorders, but are particularly associated with systemic vasculitis, so called ANCA-associated vasculitides.[3] Anti-neutrophil cytiplasmic antibodies (ANCAs) are associated with small vessel vasculitides including granulomatosis with polyngitis (previously known as Wegener’s granulomatosis), microscopic polyangiitis, primary pauci- immune necrotizing crescentic glomerulonephritis (a type of renal-limited microscopic polyangitis), Churg-Strauss syndrome and drug induced vasculitides. Proteinase -3 directed c-ANCA (atypical) is present in granulomatosis with polyngitis, microscopic polyangiitis, pauci-immune crescentic glomerulonephritis and Churg-Strauss syndrome. c-ANCA (atypical) is present in cystic fibrosis (with BPI as the target antigen) and also in inflammatory bowel disease , primary sclerosingitis and rheumatoid arthritis (with antibodies to multiple antigenic targets).

Peri-nuclear (p-ANCA) with MPO specificity is found in microscopic polyangiitis, primary pauci-immune necrotizing crescentic glomerulo-nephritis and Churg-Strauss syndrome. p-ANCA with specificity to other antigens are associated with inflammatory bowel disease, Rheumatoid arthritis, drug-induced vasculitis, autoimmune liver disease, drug induced syndromes and parasitic infections. Atypical ANCA is associated with drug-induced systemic vasculitis, inflammatory bowel disease and Rheumatoid arthritis.[4,5]

The presence or absence of ANCA cannot indicate presence or absence of disease and results are correlated with clinical features. The association of ANCA and disease activity remains controversial; however, the reappearance of ANCA after treatment can indicate a relapse.[6,7]

MATERIALS AND METHODS

Studied groups

The study carried out during the period from (November 2013- November 2014), the age of patients extended from (3 – 60) years, two studied groups were involved.

- Suspected patients: Blood and stool samples were obtained from a total of 200 patients clinically suspected with amoebic dysentery that had been examined and defined as suspected cases by specialized physician; the samples were collected from (Al-Yarmouk teaching hospital & Al-Tifil central hospital) in Baghdad.
- Healthy Control: Blood and stool samples from a total 60 healthy control group were involved from Al-Yarmouk teaching hospital staff and from different places in Baghdad; they were examined and defined as healthy, with no history of amoebic dysentery.

Samples collection

Stool sample from each patient was collected in a clean, dry tight cover container and examined with a half an hour. The samples were examined for the presence of *E. histolytica.*

Stool sample examination

Macroscopic examination

It was performed by observing the consistency of stool, presence of blood, mucous and other substances.

Microscopic examination

For each stool sample, wet mount preparation slide was examined by clean, dry slides by obtaining one drop of normal saline and small amount of stool from different places of stool by using clean wooden stick, especially when blood or mucous were noticed, then mixed gently with normal saline and covered with cover slip, the slide was examined under the low (10x) and high power (40x) of microscope.[8]

Blood samples

Five mL of Venus blood was obtained from each patient and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in Eppendorf tubes and stored in deep freeze at -20°C until the time for using. The current study included some Immunological aspects: One hundred twenty clinical patients of *E.histolytica* and (60) healthy control involved in the study. The level of Anti protienase-3; Cyclic -anti neutrophil cytoplasmic antibodies (c-ANCA) are examined by Enzyme Linked Immunosorbant Assay (ELISA).According to.[9]

Statistical analysis

The statistical Analysis (T–test) was used to compare between means in studied groups according to.[10]

RESULTS AND DISCUSSION

The level of anti-PR3 in the serum of patients with *E.histolytica* shows a significant increasing ($P< 0.05$) in comparison to healthy control, while, the results shows no-significant difference ($P>0.05$) between the gender in both groups.

The serum level of anti-PR3 was (21.79 ± 0.15) mg/mL, (21.89 ± 0.15) mg/mL in males and females of patients group respectively. In comparison to (17.08 ± 0.21) mg/mL, (15.85 ±0.66) mg/mL in males and females of healthy control group. The result is agree with another studies[11,4] they found that Anti PR3 is present in inflammatory bowel diseases Table (1).

There are many explanations for the presence of the antibodies in amoebiasis, one of them is the disruptive effect of *E.histolytica* on polymorph nuclear PMN expose, change intracellular proteins and rendering them antigenicity and result in the production of antibodies for epitope of anti - PR3[12], also the other explanation is the antibody produced as a response to amoebic antigen, this antigen is cross-react with PMNL cytoplasmic components like anti PR3.[12]

Table (1): The level of anti PR3 (IU/mL) in sera of *E.histolytica* patients and healthy control group.

Group	Mean ± S.D Gender	
	Male	Female
Patients	21.79 ± 0.15	21.89 ± 0.15
Control	17.08 ± 0.21	15.85 ±0.66
T-test value	0.49 *	0.96 *

*($P<0.05$).

The level of anti-MPO shows a significant increasing ($P<0.05$) in serum of patients with *E.histolytica* in comparison with healthy control, while the results shows no significant differences ($P>0.05$) between the gender in both groups.The level was (21.48 ± 0.15) mg/mL, (21.64 ± 0.18) mg/mL in males and females of patients groups respectively in comparison with (17.35 ± 0.24) mg/mL, (17.80 ±0.26) mg/mL in males and females of healthy control respectively Table (2).

The results are in agree with other studies which proved that PMNL granulocytes have an important role in innate immunity and their programmed cell death & removal are effective for acute inflammation resolution. MPO which is a heme protein generally associated with killing bacteria and tissue injury which oxidative, this property is expressed in neutrophils, MPO binds to neutrophil.[13] The pathogenesis of ANCA disease is multifactorial, with genetic and environmental factors influencing onset

of the autoimmune response, the mediation of acute injury and the induction of the chronic response to injury.[14]

Table (2): The level of anti MPO (IU/mL) in sera of *E.histolytica* patients and healthy control group.

Group	Mean ± S.D Gender	
	Male	Female
Patients	21.48 ± 0.15	21.64 ± 0.18
Control	17.35 ± 0.24	17.80 ±0.26
T-test value	0.54 *	0.64 *

*(P<0.05)

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