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NF-KB ACTIVATION BY INFESTATION WITH ENTAMOEBA HISTOLYTICA

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ABSTRACT

Amoebiasis cused by *Entamoeba histolytica* is still considered amajor health problem in developing countries. In This work (70) serum samples of Patients S uffered from diarrhea and infected with *Entamoebahistolytica* were examined to evaluate the NF-KB concentration by Elisa method .Recent study revealed that there was an increasing in concentration of the studied NF-KB in patient serum(49.59) ng/ml in acomparsion with control group (29.30) with asignificant differences($p< 0.05$).

KEYWORDS: Nf-Kb, *Entamoebahistolytica*, Thi-Qar

INTRODUCTION

Entamoebahistolytica is aworld wide parasitic protozoan which caused gastrointestinal infestation called amoebiasis which has been targeted is apart the who (neglected disease Initiative) since 2006 **(Saveoli,et al.,2006)**,which abroad range clinical manifestation from symptomatic carriage,chronic diarrhea to severe malabsorption .The symptoms of *Entamoeba* infection can vary in intensity from person to person,many people may have no symptoms at all while,others showed symptoms of diarrhea that lasts 10 day s or more abdominal pain,nausea,vomiting,fever,chills and weight loss .complications include severe dehydration duce to the lose of fluids and electrolytes which can lead to an electrolyte imbalance and shock,and can even be fatal **(Nash and Patel,2010).**Differences in clinical manifestations may due to number of factors,including host age,immune and nutritional status,concurrent infections,the virulence and pathogenicity of the Entamoeba strain**(Alind& Hill,2003)**.Transmissino of amoebiasis via the feacal –oral route,either directly from person to person or indirectly through contaminated water or food**(Barry,et al .,2013)**.Potent immune response is important for eradication of the parasite during infection and development to protective immunity, both humoral and cellular mechanism are important in the resistance to amoebiasis**(Fanbert,2006).**NF-KB may have role amoebiasis infection .NF-KB are secreted by lymphocyte macrophages. They act on other cells of the immune system to regulate their functions NF-KB **(R.S 1992& P.A 1997)** cause pro and anti-inflammatory response in parasitic disease .the present work was determine the total infection rate in the sera of infection**(N.N:2003)**

MATERIALS & METHODS

A total of 277 human stool were colleScted from patients (insterile plastic cups) during the period of five months,January and guly /2015 from AL-Yarmouk hospital in Baghdad .samples(included 180 male and 80 female age from 2-10 year) were examined under microscope(40xlens) by using saline wet-mount method (1g of stool in 1ml of normal saline) for amoebiasis detection (Alam et al, 2011).serum NF-KB were estimated in 70 patients suffering from amoebiasis and 30 apparently healthy controls,NF-KB were determined by ELISA.Samples of blood were collected (3ml) in plant tube from patients,serum was separated by centrifuged the blood at 3000 rpm for 5 minutes and stored in

Eppendrof tube at -40c until use.

Detection of Nuclear Factor Kappa B (NF-KB) by Elisa Method

Following the instructions of NF-KB kit, catalog number H-1386,frozen serum samples were brought to room temperature and mixed thoroughly with out foaming, one hundred ml of stander and sample per well, the covered plate with afresh sealer was incubated for 90 minutes at room tempature at 37c,removed the liquid of each well but don't wash, added 100 ml of biotin to each well cover with the plate sealer incubate for 1 hour at 37c.The plate was washed by filling each well with wash buffer 350 ml bottle multi-channel pipette, mani fold dispenser, or out washer, and let is stand for 2 minutes .complete removal of liquid at each step is essential to good performance after the last wash, remove any remaining wash buffer by aspirating or decanting invert the plate and blot is against thick clean absorbent paper .

Add 100 ml of Hrp- avidin (1x) to each well .cover the micro titer plate with anew sealer incubate for 30 minutes at 37C. repeat the aspiration wash process for five time added 90 ml of tmb substrate to each well.incubate for 15 minutes at 37 C .protect from light added 50 ml of stop solution, gently tap the plate to ensure mixing., the absorbance of each well was recorded by using ELISA micro plate reader (Olympus /Japan) at 490 nm.

RESULTS

The level of Nuclear factor kappa B(NF-KB) were detected using Elisa method in 70 serum samples of infected patients with another 30 serum samples from uninfected, the results showed significant differences in the mean concentrations between infected and uninfected persons for NF-KB .The mean of normal serum concentrations for NF-KB was (29.30)ng/ml in comparison with the mean concentrations of 70 infected patients showed increased in level for NF-KB which recorded (49.59)ng/ml .The results indicated that NF-KB could be considered as a marker for immunity response to the parasite .

Table 1: It Shows the Concentration Rate of NF-KB in the Serum Blood Children infected with *Entamoebahistolytica* Compared with the Samples Control

Parameter	Subject	N of sample	Mean	S.D	t-Value	D.F	P-Value
NF-KB	Patient	70	49.59	43.34	1.053	98	0.01
	Control	30	29.30	27.96			

P<0.05

DISCUSSIONS

The immune system has multiple weapons which is uses to help control infections .Many infectious result in activation of several of these response mechanisms, but it is not always clear which responses actually contribute to control of the pathogen and which are bystander effects. Data from humans suggest that antibody responses are important in preventing infections, although roles for the cellular responses have not been excluded **(Solaymani-Mohammadi and singer 2010)**.NF-κB (nuclear factor kappa B) family transcription factors are master regulators of immune and inflammatory processes in response to both injury and infection**(Nctaet al., 2000).**Entamoebahistolytica induce NF-KN activation during infestation for do its work in the control of infection and**(Artis,2002)**elimination so this protein NF-kB as increase in patients with amoebiasis has effect on amoebapathogenesis**(Ishi.N 1998&seydel 1998; cooper,2001).** Production of NF-KB can regulate macrophages parasitocidal activity which may results in damage a few tissue gut by necrotizing enzyme this phenomenon helps to limited efforts an invasive Entamoebahistolyticathere fore A moebiasis

suppress by the macrophage with the help of NF-KB **(Virk et al,1990&zaph.2007)** the induction of mucosal inflammation driven by TH2 so NF-KB contain amoebic infection at early stage protection against parasite and preventing colonization of the gut**(Tomzak,2003&kunsch,1993)**

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