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RESEARCH ARTICLE

POSSIBLE ROLE OF HUMAN GLUTATHIONE PEROXIDASE-2 LEVEL IN PATIENTS SUFFERING FROM AMEBIASIS.

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Abstract

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Entamoeba histolytica, glutathione peroxidase, ELISA technique, diarrhea.

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Background: Entamoeba histolytica, a microaerophilic protozoan parasite has to build up mechanisms to protect themselves against oxidative stress within the human gut to establish its pathogenesis, role of glutathione metabolism so important in amoebiasis but very little is known about mechanisms so this study aim to evaluate the level of human glutathione peroxidase-2 in the sera of patients suffering from amoebiasis.

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Methods: Blood samples were collected from 120 volunteer patients suffering from amoebiasis (62 females, 58 males) and compared with 50 healthy looking controls (25 females, 25 males). Attended to the central teaching hospital for pediatric in Baghdad city - Iraq, during the period between 1st March 2015 till 1st July 2015. The diagnosis was established based on direct microscopic examination of stool and the human glutathione peroxidase-2 concentaration has been evaluated based on quantitative sandwich enzyme immuno assay (Hcusabio- Cat. No. CSB-EL009867AU)

Results: We examined 120 children infected with Entamoeba histolytica, the infection in females 62 (55.66%) was higher than in males 58(48.33%). Highest prevalence of 57.50% (69/120) of Entamoeba histolytica infection was found in the age group of 10-16 years. The level of human glutathione peroxidase-2 was significantly lower in the patients than in control group. There were significant associations between the serum level of human glutathione peroxidase-2 and Entamoeba histolytica infection.

Conclusion: Entamoeba histolytica infection had significant decreased on level of glutathione this related with utilizes by Entamoeba histolytica and used in detoxification processes.

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Introduction:-

Amebiasis is a disease caused by Entamoeba histolytica, a parasite protozoan that infects humans and is responsible for 40,000 to 110,000 deaths per year (1).

Entamoeba histolytica that colonizes human intestines and presents two evolutionary forms: the cyst and trophozoite (2). Infections are usually asymptomatic, but in approximately 10 % of cases, the trophozoites penetrate the gut tissue, initiate hemorrhagic colitis and induce amoebic liver abscess (3). Food and drink contaminated with faeces containing the cysts is a common source of infection. Most cases arise from human carriers, or cyst passers, who pass cysts in formed or semiformed stools (4).Traditional microscopy approaches are neither sensitive nor specific for Entamoeba histolytica. Antigen assays are more specific and although polymerase chain reaction (PCR) is effective (5).

Glutathione is synthesized by the successive action of Y-glutamylcysteinyl synthetase (GGCSS) and glutathione synthetizes. Both require ATP. Reduced glutathione inhibits GGCS by non-allosteric feedback (6). Glutathione-dependent enzymes significantly accelerate most of these chemical reactions in numerous metabolic pathways. Accordingly, tens of thousands of articles on glutathione-dependent enzymes and pathways have been published since the disputed discovery of glutathione by Hopkins as well as Hunter and Eagles in the 1920s (7).

Glutathione is low molecular weight thiol that is found in highest concentration in mammalian cells. It protects against toxicity from highly electrophilic compounds or from their metobites and against free radicals (8). The defense mechanism used by mammalian cell to eliminated free radicals are multiple and diverse (9).

Many of the parasitic protozoa, such as Entamoeba histolytica, Giardia, Trypanosoma, Leishmania, and Plasmodium, are considered to be anaerobes because they can be grown in vitro only under conditions of reduced oxygen tension. However, these parasitic protozoa have been found to be aerotolerant and also to consume oxygen to a certain extent. Furthermore, these organisms are highly susceptible to exogenous reactive oxygen species, such as hydrogen peroxide (10).

In Iraq, Al.Hadraawy (2014) study role of glutathione in males infected with giardiasis in Al-Najaf city also other study done by Al-Jalal who show decrease in the level of glutathione in women infected with Toxoplasma gondii.So this study design to evaluate the level of human glutathione peroxidase-2 in the sera of patients suffering from amoebiasis.

Materials and Methods:-

Subjects: Participants were recruited from1st March 2015 till 1st July 2015 as part of the cross-sectional study. All volunteer patients suffering from amoebiasis (62 females, 58 males) and 50 healthy looking controls (25 females, 25 males), aged 3-16 years were collected from the central teaching hospital for pediatric in Baghdad city - Iraq.

Sample collection:- Stool samples were collected in screw-capped stool containers and stored in the cold room before examination, five ml of venous blood was collected from each patient. The collected sample was transferred immediately in a plain plastic tube and left to clot at room temperature, then spun at 3500 rpm using ordinary centrifuge for 10 minutes, finally the sera were collect and dispensed in plastic appendorf tube, and stored at -20° C until used for test.

Parasitological Examination:- Fresh faecal samples were examined by direct wet mount (normal saline and Lugol's-iodine stained).

Serological examination:- The patient serum had been tested for human glutathione peroxidase-2 by using quantitative sandwich enzyme immuno assay (Hcusabio- Cat. No. CSB-EL009867AU) .

Statistical analysis: The Statistical Analysis System- SAS (2012) was used to compare between patients and control in concentration of Glutathione. T-Test was used to significant compare between means and in this study (13).

Results:-

The total number of patients infected with amebiasis included in this study was 120. The number of females was 62 and of the males were 58 as shown in table (1). The results showed that more females (55.66%) were found to be infected than males (48.33%), When comparing with healthy control group, but there was no significant differences (P>0.05) noticed between both of them.

Gender	Patients	Control	Comparison of Significance
			P-value
Female	62(55.66%)	25(50%)	Non-Sig. (P>0.05)
Male	58(48.33%)	25(50%)	
Total	120(99.99%)	100%	

Table (1): Distribution of patients according to their gender:-

The ages of patients studied were between 3 to 16 years. The majority of cases which were found to be infected 69 (57.50%) were the age group between 10 and 16 years. Table (2)

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Age	Females	Males	Total number	
3-9 year	26(41.93%)	25(43.10%)	51 (42.50%)	
10-16 year	36(58.06%)	33(56.89%)	69(57.50%)	
Total	62	58	120(100%)	

Table (2): Age distribution in studied groups:-

Sig. (P<0.05)

In our study, decrease in concentration of human glutathione peroxidase-2 was observed in patients compared with healthy control group as shown in table (3). A statistically significant differences was found between the patients and healthy control group (p<0.01).

. Concentration of numan glutatinone peroxidase-2 in studied						
Groups	Number	Mean ± SE				
Patients	120	37.52 ± 1.19				
Control	50	58.92 ± 3.02				
T-test value	-	5.335 **				

 Table 3: Concentration of human glutathione peroxidase-2 in studied groups

** (P<0.01).

Discussion:-

Intestinal parasitic infections are endemic worldwide and remain a major public health concern in many tropical and subtropical countries. About one third of the world (more than two billion people) is infected with intestinal parasites. Poverty, illiteracy, poor hygiene, lack of access to potable water, and a hot and humid tropical parasitic infection (IPI). Approximately 500 million people in the world are infected with E. histolytica (14). The World Health Organization and the Pan-American Health Organization recommend the treatment of all patients with confirmed E. histolytica infection, regardless of the presence of symptoms (15).

According to the gender distribution, the percent study revealed that the prevalence of E. histolytica infections was slightly higher in females than males, which is comparable with study done by (16) who demonstrated that female patients a higher prevalence of intestinal parasitic infections (42.7%) than males (39.0%). While our result is differ from those of (17) who found that infection rates of amebiasis were 54.58% and 45.42% in males and females respectively. Also this results agreement with result of (18) who found no significant difference was found between males and females (P>0.05). Studies from other countries reported difference in the prevalence of infections between males and females (19, 20).

The present study observed a higher prevalence of amebiasis among the 10-16 year age groups, this result is agreed with that of (21) who found that infection rates of amebiasis were high in this age group. The reason for this finding could be that the possibility of parasitic infections could be higher in primary school children than in the upper and lower age groups because of lack of information about the prevention of parasitosis among those children.

The present study design to measuring concentration of human glutathione peroxidase-2 in studied groups, Entamoeba histolytica is a human pathogen that lacks the capacity to synthesize glutathione but can incorporate it from the growth media or presumably from human host to form tryptothione [N(1), N(8)-bis(glutathionyl)-spermidine conjugate] (22).

Also agreement with study of (23) who showed that increased levels of reactive oxygen species (ROS) and reactive nitric oxide (RNI) which led to reduced activities of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase and also with (24) who found significant decrease in the levels of human glutathione in patients infected with Entamoeba coli compared to healthy group group.

Other study done by (25) who studied glutathione transferase (GST) enzymes in nine protozoans and who showed that glutathione transferase are toxicologically important from many points of view and six aerobic amoebae had

very different specific GST activities, but an anaerobic amoeba and two anaerobic flagellates did not have any GST activity.

The decrease in the concentration of human glutathione peroxidase-2 may be due to oxidative damage, decrease in the glutathione synthesis, it's the catabolism increasing or due to increase transformation it to disulfide form glutathione disulfide (GSSG) (26).

The low level of glutathione may be attributed to the low level of the substrate which necessary for building it during oxidative stress such as NAD pH which promise important stimulation material for action of glutathione reductase which act to restatement the active form of glutathione from inactive form (27).

Furthermore. It may be due to the resistance of parasite to phagocytosis by increasing the free radical and this leads to decrease in level of glutathione in serum of patients (28).

In conclusion, Entamoeba histolytica infection had significant decreased on level of human glutathione peroxidase-2 this related with utilizes by Entamoeba histolytica and used in detoxification processes.

Further studies with large sample size are needed. Studying the role of glutathione in serum of patients infected with other protozoa, study the role of this enzyme as a key drug target during protozoa infection.

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