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RESEARCH ARTICLE
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Immunological Evaluation of Transforming Growth Factor beta In Patients With Bloody Diarrheal Infection In AL Najaf AL Ashraf Provency

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ABSTRACT

Amoebiasis causes up to 100.000 deaths annually all over the world, E. histolytica known as the main agent of intestinal amoebiasis causing amebic colitis. Amoebiasis is an infection caused by Entamoeba histolytica with or without symptoms. Replacements include amoebiosis, amoebic dysentery or bloody flux. This study comprised 150 blood sample from both gender (75 blood sample from patient bloody diarrhea with E.histolytica and 75 blood sample from patient with diarrhea but no diagnosis E.histolytica) The result showed Serum level of TGF-beta in patient bloody diarrhea with E.histolytica and patient with diarrhea but no diagnosis E.histolytica.

Keywords: *bloody diarrhea, Entamoeba histolytica, diagnosis*

INTRODUCTION

Amoebiasis causes up to 100.000 deaths annually all over the world, E. histolytica known as the main agent of intestinal amoebiasis causing amebic colitis (Al, N.A. H.A.B &AL-Ouqaili.,2017). Amoebiasis is an infection caused by Entamoeba histolytica with or without symptoms. Replacements include entamoebiasis, amoebiosis, amoebic dysentery or bloody flux (Alberta Health, and Wellness .,2011). Entamoeba histolytica is an enteric protozoan parasite that infects 500 million people, causes amoebiasis in 50 million and kills 100 000 individuals annually, thus constituting a serious health public problem (Tankyuksel &William.,2005).

The high prevalence of infection is due to fecal contamination of food and water supply(Jaeffer .,2011). Symptoms of amoebic colitis in humans typically have a onset subacute and include symptoms such as stomach pain, diarrhoea that is watery or bloody diarrhea (Nagaraja and Ankri, 2019).According to Wang and Zuo (2019), TGF- is a pleiotropic cytokine that The inhibits immunological response, cell proliferation, and oncogenesis. This substance is made by many different kinds of cells, such as immune cells and non-hematopoietic cells.The production of TGF- is aided by cytokines, luminal microorganisms, and other stimuli. Integrins trigger latent TGF-, which targets the intestine (Bertolini et al., 2021).

MATERIALS AND METHODS

Patients Group

Samples collection (75 Stool sample and 75 blood sample) were collected in this study and during the period from (1/10/2022) to (30/2/2023) from all ages of patient from both sex (Males and Females). Every patient was reported though a specifically prepared questionnaire which included name ,gender, age ,living , Previouse and address for every patient at (Al-Sader Medical City / AL-Haidarya general hospital / AL-Hakeem general hospital) and all aged taked All of the patients in this study filled out a direct questionnaire.

Control Group

which control group were 75 blood sample from Patient with diarrhea but not diagnosis E.histolytica . The control group was used only for comparing parameter. The control samples were approximately similar with the samples patients in terms of number, ratio of age , in addition to the place of living also country side and city. Also, ask a special question sheet for the control samples. Where blood was drawn from a vein to measure immunological parameters (TGF-beta).

Samples Collection (stool and blood)

Collecting Stool Samples

Stool specimens were collected in a sterile, dry Container and transferred to the parasitology department for direct examination macroscopically and microscopically.

Blood Samples Collection

Patients who were diagnosed with having intestinal parasites had venous blood samples taken and Patient with diarrhea but without parasitic infection With a 3-ml medical syringe, the sample was transferred to clean plastic tubes and let to sit for a set amount of time before being centrifuged (3000 rpm) takes 20 minutes to extract serum from blood and put in eppendroff and then placed in the freezer at -20 degrees Celsius to freeze within 4 hours of collection for immunological tests by ELISA.

Test (TGF-β) ELISA Kit

Standard Curve of transforming growth factor-beta

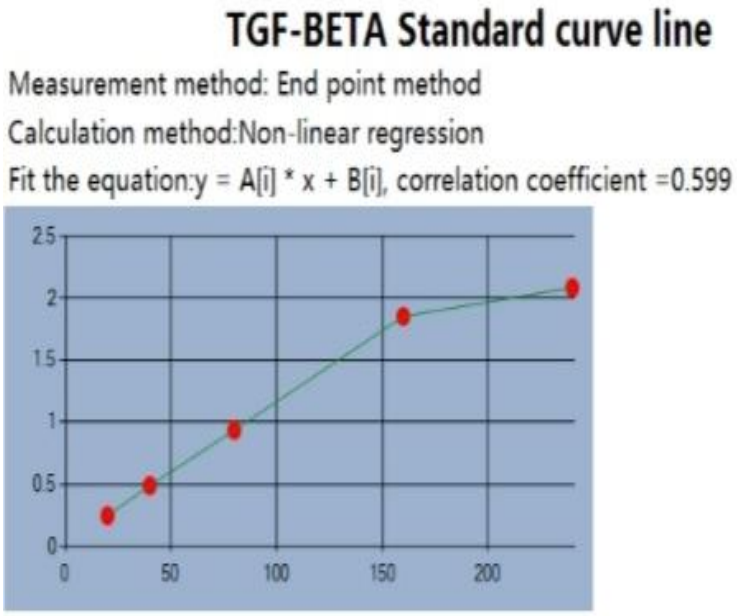

FIGURE 1: Standard Curve of transforming growth factor-beta

J Popul Ther Clin Pharmacol Vol 30(12):e73–e78; 10 May 2023.

RESULT

TABLE 1: sociodemographic characteristics of respondents for both groups

Age mean ± Std		30.633± 12.286		28.666 ± 11.984		*P.value
Variables		Cases		Control		
		Freq.	Perc.	Freq.	Perc.	
Age Groups (Years)						
	7-16	9	12.0	8	10.7	0.0514
	17-26	21	28.0	25	33.3	
	27-36	22	29.3	29	38.7	
	37-46	12	16.0	6	8.0	
	47-56	11	14.7	5	6.7	
	57-66	0	0	2	2.7	
	Total	75	100.0	75	100.0	
Gender						**0.142
	Male	44	58.7	35	46.7	
	Female	31	41.3	40	53.3	
	Total	75	100.0	75	100.0	
Living areas						**0.027
	Urban	49	65.3	61	81.3	
	Rural	26	34.7	14	18.7	
	Total	75	100.0	75	100.0	
Previous Infection						
	Positive	22	29.3	2	2.7	**0.001
	Negative	53	70.7	73	97.3	
	Total	75	100.0	75	100.0	

*t -test, significant at 0.05.

** Mann-Whitney

TABLE 2: T-test comparison between study groups according to the TGF beta

	Groups	N	Mean	Std. Deviation	Std. Error Mean
TGF Beta	Cases	75	52.7397	22.10429	2.55238
	Control	75	71.2359	61.15405	7.06146

Levene's Test for Equality of Variances						
		F	Sig.	t	df	Significant p.value
TGF Beta	Equal variances assumed	12.484	<.001	-2.463-	148	.015
	Equal variances not assumed			-2.463-	93.011	.016

TABLE 3: Regression Coefficients for the relationship between TGF beta and the previous infection for both groups

Coefficients[a]							
TGF beta		Unstandardized Coefficients		Standardized Coefficients			
Model		B	Std. Error	Beta	t	*Sig.	Adjusted R Square
Cases	(Constant)	34.786	9.730		3.575	0.001	0.73

	Previous Infection	10.520	5.508	.218	1.910	.035	
Control	(Constant)	16.826	87.139		.193	.847	0.35
	Previous Infection	27.572	44.012	.073	.626	.533	

Dependent Variable: TGF beta
Predictor
*Significant at level 0.05.

DISCUSSION

The current study result in table 1 shows that most of the respondents (29.3%) were in the, age group (27-36 years) for the study cases group, this agree with result mentioned in the studies that were recently published, as it proved that Amoebiasis most commonly affects young to middle-aged adults (Kumanan et al., 2020 ; Chou and Austin, 2020 ; Ngobeni et al., 2022).

The effect of spreading of E.histolytica in young to middle-aged adults occurs for travellers to countries with poor sanitation (developing countries) (Chou and Austin, 2020).

For the study control group if also be found the same result in the same condition. At the same time, there isn't a statistically significant difference between groups (0.051); this design confirms this result.

As for gender, the males in the study group (cases group) were more than females (58.7%); however, in the study group (control group), the females (53.3%) were more than males. Also, there isn't a statistically significant difference between groups (0.14) this result was confirmed by Saafa and Al-Kaeebi, (2017) who reported that 58.3% of males and 41.6% of females in Al-Qadisiyah provinc were infected.

The result mention by Mohammed et al., (2022) in Sulaymaniyah Governorate , and they also mentioned that , that males recorded a higher infection rate was (17.7%), while the female was (14.3%), (p >0.05) and this result was not a statistically significant difference thus this distribution was compatiple with our distribution of respondents according gender.

The differences in infection rates between males and females might be caused by the different according social behavior and working time between the two gender , as males normally are the working sex, in the society .which made them in contact with the environment .(Al-Hilfi et al., 2021).

According to the living area, the respondents from urban were more than the rural area for both groups. The groups do, however, differ significantly from one another (0.026). For the previous infected history, this result shows that the fact more respondents in the cases group were negative (70.7%), and there is a the existence of a statistically significant distinction between groups (0. 001).

Mohammed et al., (2022) mentioned that Prevalence in rural (20.3%), in,urban (13.8%) , (p < 0.05). and this un compatiple with our distribution of respondents according of residence .

The result of this table 2 shows ,that there is a statistically significant difference between groups for comparison of means for TGF beta (0.015). Also, according to Levene's Test, this result proves that this finding is real, not by chance (0.001).

Recently, a study was conducted by, Mohammed et al., (2022) in Sulaymaniyah Governorate and they mentioned that Increased levels of IL-17 were observed, however there were no statistically significant ,differences (P = 0.282) between the two groups, in the patient with amoebic bloody diarrhea group Mean St. Dev = 15.24 2.60; minimum serum level = 10 pg/ml; maximum = 21 pg/ml; compared to mean St. Dev = 14.78 2.84; minimum = 10.8 pg/ml; maximum = 21 pg/ml; in control individuals, Thus this results do not match with our results of current study.

several of the earlier studies which are not similar , to the results of our current study, also

mentioned that there TGF- serum levels were significantly elevated in E. histolytica-infected patients, in comparision with control group (Sánchez-Alemán et al., 2015).

While Fukata, (2012), Mentioned that the concentration level of TGF-b in healthy people was higher than intestinal amoebiasis patients and this results was compatiple with our present study.

The result of this table 3 shows, that there is a regression relationship, a strong positive, relationship between TGF beta and the previous infection (0.73), and 34.7% of cases can predict them using TGF beta for cases. However, there is a fair regression relationship between TGF beta and the previous infection (0.35), and 16.8% of the control group can predict them using TGF beta.

Because TGF-β plays a crucial role in stimulating the cellular immune response, its presence was linked to the progression of E.histolytica infection , causing symptoms to appear (Verma et al., 2022).

During acute amoebic infection, TGF-β production triggers the synthesis of dormant (latent) TGF-β, whose presence is correlated with immunological responses that are protective against disease. This action slows parasite development early in infection and reduces pathogenesis late in infection (Melby et al., 2019).

Higher parasite growth rates are also associated with TGF-β mediated stimulation of Treg-cell differentiation after intestinal amoebic infection in humans and Stronger CD8+ T-cell responses and host protection from reinfection are achieved by downregulating TGF-β activity (Samanta, 2023).

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