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IMPACT OF TNF-A CYTOKINE ON ENTAMOEBA HISTOLITICA PARASITE INFECTION IN THE CONTEXT OF DIABETES

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ABSTRACT

*One Background: TNF-α, a proinflammatory cytokine, is a factor secreted by macrophages and plays an important role in enhancing the immune-inflammatory response to Entamoeba histolytica. Objective: The goal of this study was to detection of E. histolytica in Diabetes patients and compare immunological parameter TNF-α between Type 2 Diabetes mellitus patients and controls. Methodology: 150 of participants (75 diabetic patients suffered from with diarrhea + 75 controls) were involved in this study and during the period from beginning of March 2023 to the end of Nov, 2023. Including people of two sexes as well as every generation. Each individual's identity, sex, date of birth, and place of residency were recorded using an individualized survey. At (Najaf Diabetes and Endocrinology Center, AL Hakeem Public Medical institution, AL Furat AL Awsat Medical institution and AL Sader Medical City) and all ages took, whole participants in this examination topped up an individualized survey. Stool and blood samples were collected from all participants for detection of E.histolytica and measure TNF-α by ELISA technique. Results: The mean concentration of TNF-α in patients (72.563 ± 6.165 pg/ml) was higher than in control groups (23.314 ± 0.775 pg/ml) with statistically significant difference (P-value <0.0001) between groups with 95% confidence interval ranges from -61.69 to -36.81. The average rank concentration of TNF-α peaked at 63.67 pg/ml in diabetic patients infected with the parasite E.histolytica, a group consisting of 28 patients. The average level of TNF-α was at its lowest point (26.52 pg/ml) in a group of 48 diabetic patients who did not have the parasite E.histolytica. This variation had a p-value of 0.000 (significantly significant statistically). The age group of 63-72 years had the highest average rank for TNFα, with a measurement of 75.00 pg/ml. The age group of 3-12 years had the lowest average rank for TNFα, measuring at 14.29 pg/ml. Mean rank for awareness stage of TNf-α in ladies (39.08) was once barely greater than in men (38.10). Conclusions: The average concentration level of TNF-α cytokine differs significantly between the patient and control groups, with the patient group exhibiting greater levels***.** *When compared to diabetic individuals without the parasite E. histolytica, the presence of the parasite caused a significant increase in TNF-α levels.*

INTRODUCTION

Entamoeba-histolytica is an ameba, which lives anaerobically and influences intestinal tract of individual, it is linked to individual amebiasis, it comes at the 3rd stage worldwide as greatest common fatal parasite illness (Carrero et al., 2020). In 90 % of all cases, infections with amoebas are asymptomatic and self-healing but diarrhea, colitis, as well as diarrhea contain analytic signs related to amebiasis (Uribe-Querol and Rosales, 2020).

Many crucial levels into the pathophysiology related to E-histolytica, containing ruin barrier of the mucosa, destruction into epithelial cells, binding to the intestinal epithelium, and spreading through another part of the body. When amoebas get into fibers and organs, immune defense line begins with respond to the organism (Uribe-Querol and Rosales, 2020).

A collection of metabolism-related conditions known as diabetes-mellitus cause elevated blood sugar levels in patients due to insufficient insulin secretion by the pancreas or to the tissues' inability to respond to generated insulin (Krishnamoorthi et al., 2022). Diabetes is a predisposition disease that can lead to an opportunistic infection like parasites causing high mortality and morbidity. It is well-known that diabetics are more likely to be affected by intestinal parasitic infections such as Entamoeba histolytica, fragility, with neurological disorder, as a result of an intensified immunological reaction. (Hussein and Neamah, 2021).

Amongst the parasitic organisms that attack humans on a regular basis around the entire globe are gastrointestinal microorganisms or IPs. The kinds nevertheless varied from place to place due to a variety of reasons, including geography, cultural as well as ecological variations. (Wong et al., 2020). The gastrointestinal parasitic organism, Entamoeba histolytica, has gained prominence recently being a significant advantageous disease-causing organism that is medically accountable for diseases in immunocompromised people. (Waly et al., 2021).

Regular poo examination ought to be taken into consideration for the recognition of people with diabetes since they are more susceptible to some illnesses, especially those caused by the gastrointestinal pathogen Entamoeba histolytica. Diabetic individuals have compromised adapting immunological reactions. As a result, amoeba infections leading to sickness are readily capable to influence people suffering from diabetes. E. histolytica takes advantage of this to make people with diabetes become increasingly virulent and chronically ill. (Siciliano et al., 2020).

The blood sugar level can rise to dangerously high levels. As a part of a protection manner in which human-being system defense to combat amoeba, more glucose is released into the bloodstream. This can happen even if you stop eating or eat less than usual. White blood cells cannot fight infections effectively when blood glucose levels are high, so any amoeba infection tends to be more severe and take time in people with diabetes (Sabaa and Mohammad, 2021).

Several studies have reported that TNF-α, a pro inflammatory cytokine, is a factor secreted by macrophages and plays an important role in enhancing the immune-inflammatory response to Entamoeba histolytica (Chen et al., 2022; Al-sabawy and JM, 2020). E. histolytica impacted negatively via nitricoxide, as well as laboratory experiments has demonstrated how Tumor necrosis factor αlpha stimulates phage to generate Nitricoxide. (Sharma et al., 2021).

E. histolytica and amebic proteins in human stimulate the production of TNF-α and nitric oxide in macrophages primed by gamma interferon, thus E. histolytica is being poisoned when macrophages produce nitric oxide (NO) in response to TNF-α (Chulanetra and Chaicumpa, 2021). TNF-α-stimulated macrophages are more efficient in eliminating amebic trophozoites (Uddin et al., 2021).

METHODOLOGY

Patients and controls groups

Seventy-five of diabetic patients suffered from with diarrhea were involved in this study and during the period from beginning of March 2023 to the end of Nov, two thousand and twenty-three. Including people of two sexes as well as every generation. Each individual's identity, sex, date of birth, and place of residency were recorded using an individualized survey. at (Najaf Diabetes and Endocrinology Center, AL Hakeem Public Medical institution, AL Furat AL Awsat

Medical institution and AL Sader Medical City) and all ages took, whole participants in this examination topped up an individualized survey.

The control group were 75 of participants, they had no history of diabetes and did not suffer from diarrhea at the time the samples for the current study were collected. The comparison band served exclusively like a means of standard comparison. The comparison specimens matched the individual's specimens quite well with respect to quantity, distribution of generations, and residential location (both town as well as rural). In order to test immune markers, three milliliters of plasma was collected through a blood vessel to supply experimental specimens. Additionally, invite them to fill out a specific section of the form. (TNF-α).

Inclusion and Exclusion Criteria

Inclusion Criteria

All diabetic patients who had a confirmed history of the disease, whether they suffered from type 1 or type 2 diabetes and suffered from diarrhea, were included. Provided that they do not take any medication or antibiotics. All ages are included without exception.

Exclusion Criteria

Diabetic patients who took antibiotics or treatments with the aim of reducing or eliminating diarrhea, were excluded also pregnant women who suffer from diabetes were excluded due to violent hormonal changes that affect the woman's body at the time of pregnancy, which increases the rate of digestive system work and causes diarrhea.

Samples Collection

Blood Samples Collection

One hundred fifty of blood samples (3ml) were collected from both diabetic patients with diarrhea and control subjects by the material was placed from a three-milliliter medicinal needle into cleaned containers and allowed to rest until a certain period of duration until having been spun at three thousand revolutions per minute. Plasma was extracted from bloodstream in a twenty-minute period. Deposited in an eppen droff container, and frozen at -20°C during a four-hour period of collecting on behalf of Enzyme-linked immunosorbent assay immune-mediated testing.

Gathering of Poo Specimen

Samples of poo were gathered into a dried, sterilized jar plus sent to the parasitic biology division further in-depth large-scale as well as microscopic analysis.

Ethical Standards

Department of biology Services Division of the College of Education for Girls, Najaf Health Department, and the Training and Development Center all gave their stamp of approval to the current study, each member of the study's subjects (both groups) gave their informed written consent.

Macroscopic Examination

Color, amount, consistency, odor, shape and mucus content are only few of the macroscopic characteristics that should be considered while analyzing stool samples. Some mucous in the feces is to be expected. Nonetheless, excessive mucus production or mucus that is bloody should raise suspicions (Kasirga, 2019).

Microscopic Examination

Direct Wet Mount Method

Direct wet mount microscopic analysis of feces samples to analyze the infectious phases of the parasite, a wet direct swab was prepared from a number of feces collected with special wooden sticks from various parts of the sample and mixing them thoroughly. After placing one drop of Logule solution Iodin 1% and one collyrium from standard sodium chloride water solution on opposite ends of an immaculate glazier surface. After that, you cover the slide with a cover slide and look at your samples under a light microscope at magnifications of 10x and 40x (Oliewi and Al-Hamairy, 2016).

Approximately one to two grams for every feces specimen were obtained in order to determine and recognize parasitic ova. These grams were then analyzed via immediate blot production utilizing an iodine dye plus concentrations approach. as described by (Bahrami et al., 2019). A light microscope (Olympus) was used to examine the smears, first at a magnification of 10x and then at a magnification of 40x.

Enzyme Linked Immunosorbent Assay (ELISA) for TNFα

TNF-α concentration level was measured by ELISA technique according to data of manufacturing of MELSIN/ China origin.

RESULTS AND DISCUSSION

Basic Patient and Control Subject Demographic Information

The findings presented in Table (4-1) indicate that the average age group value among the 76 diabetic patients who took part in the study (3.4605 ± 1.66085) was greater than the average age group value among the 74 participants in the control group (2.7432 ± 0.81191).

As the table also demonstrates, in the diabetes group, the age group with the highest participation rate—20 patients—was 43–52 years old, while the age group with the lowest percentage—1 patient—was 63–72 years old.

In contrast, the age group 23–32 years old had the highest participation rate in the control group, with 30 patients, while the age groups 53–62 years old and 63–72 years old had the lowest percentage, with 0 patients in each group.

Table (1): Distribution of Subjects: Patients and Controls based on Mean Age

Age group	DM Patient (N)	Mean ±Std. Deviation	Control (N)	Mean ±Std. Deviation	Chi-Square	df	Asymp. Sig.
3-12	14	3.4605 ± 1.66085	2	2.7432 ± 0.81191	43.198	2	0.000***
13-22	11		29				
23-32	10		30				
33-42	15		12				
43-52	20		1				
53-62	5		0				
63-72	1		0				
Total	76		74				

As shown in Table (2), the diabetic patients in our study were split into two groups: the first group, which consisted of 52 diabetic patients without amoeba parasite infection, and the second group, which consisted of 24 diabetic patients with parasite infection.

With respect to the cohort of diabetic patients infected with the amoeba histolytica parasite, age-specific data indicated that 11 of the patients fell into the 43–52 age group, which was the highest age group. In contrast, there was no participation for the age groups of 3–12 and 13–22 years.

Regarding the diabetic patients who were not infected with the amoeba parasite, the age groups showed that 14 of them belonged to the 3–12 age group, while the age group that had the lowest participation rate, which was 63–72 years, had zero participants.
There were statistically significant differences between the age groups based on the p-value (0.000) and Chi-square (85.409).

Depending on sex: The number of female participants was less than the number of male participants in both groups of DM patients with and those without parasitic infection. In the first group (the diabetes group only), they numbered 31 males and 21 females, whereas in the group of patients with the parasite infection, there were 10 males and 14 females. In contrast, there seemed to be an equal number of men and women in the control group—37 men and 37 women—and this distribution was unbiased and random. The p value (o.524) and chi-square (1.294) indicated that there was no statistically significant difference between the groups according to sex.

Based on the p value (0.000) and the chi-square (26.757), there was a statistically significant difference according to the residence region, with 32 individuals from the urban and 20 from the rural areas appearing in the diabetes group exclusively who were not parasite-infected. Among the group of diabetic patients with parasitic infection were 14 individuals from rural areas and 10 individuals from urban areas. While there were 67 participants from urban areas and only 7 participants from rural areas in the control group.

Our findings support the findings of (Chou and Austin, 2020; Tharmegan et al., 2020; Ngobeni et al., 2022) that young adults are more susceptible to amoebiasis infection in general .

Our findings in this study are consistent with those of Ahmed and Sayel (2023), who reported that, depending on gender, the number of women (53.3%) was smaller than the number of men (587%) but the number of women was greater (587%). in the study group (control group). In addition, the difference between the two groups was not statistically significant ($P < 0.14$).

Challoop, (2023), recorded that the age group under 50 had the highest prevalence of E. histolytica infection (46%) followed by age group 41–50 (27%), age group 31–40 (20%), and age group 20–30 (7%). This nearly agreed with our results .

Ngobeni et al., (2022), recorded that patients aged 0 to 25 years old have the highest incidence rate (68.9%), followed by patients aged 46 to 80 years old, with the lowest prevalence rate (67.6%). Patients aged 26 to 45 years old had the lowest prevalence (64.0%).

According to a study on risk factors contributing to the high prevalence of Entamoeba histolytica infection in children, conducted in Jeddah, 60.8% of children between one month and six years old are infected with Entamoeba histolytica. However, their study had a sample size of 300 and was limited to children (Jamila, 2014).

Our results are almost identical to those of Sami et al., (2010), Taswar et al., (2010) who found the highest incidence of E. histolytica infection in the 33-48 years age group (16.67%).

Muhammad et al. (2022) in the city of Sulaymaniyah reported results whose differences were statistically significant ($p > 0.05$) showing that the infection rate among women (14.3%) was lower than that of men (17.7%). This distribution is consistent with the gender distribution of participants in the current study. The gender differences observed in the study group can be attributed to differences in social behavior and working hours between the genders. Since men are generally the working gender in society, they are more likely than women to eat outside their comfort zones, which puts them in contact with the environment (Al-Hilfi et al., 2021).

Young and middle-aged persons are susceptible to contracting Entamoeba histolytica from tourists visiting developing nations lacking proper sanitary facilities (Chou and Austin, 2020).

Furthermore, our findings are consistent with those of Saafa and Kaeebi (2017), who found that 41.6% of women and 58.3% of men in Qadisiya Governorate were infected with the disease.

Un-similar results for current study, regarding the sex distribution of E. histolytica infections were also observed in a study conducted by Sami et al., (2010), showing a higher prevalence of E. histolytica infection in women compared with men. This suggests that women are more likely than men to develop asymptomatic infection rather than invasive disease .

The results of current study also conflicted those of Jamila's, (2014) study, which found that women were more likely to be infected with Entamoeba histolytica (48.7 percent) than men (47.8 percent).

In Ngobeni's et al., (2022) study, women (66.1%) were more affected than men (63.5%), although the difference was not statistically significant. This difference in male-to-female ratio can be explained by some results which suggest that most families prefer maids to men for customary, religious and cultural reasons. This results uncompatible with our results.

Our results deviate slightly from those of Ahmed and Sayel's 2023 research since the majority of participants (29.3%) fall into the age range of study patients and healthy controls, which is 27 to 36.

While Al-Zayyadi and Alkhuzaia, (2023) explained that the percentage of women participating in the study (51.43%) was slightly higher than the percentage of males participating in the study (48.57%), but he did not clarify the prevalence of the parasite between the sexes, knowing that the prevalence of the parasite in his study was only 13.6%.

study of Ahmed and sayel, (2023) showed that for both groups (controls and patients), there were fewer responses with a significant difference between the groups (0.026) from rural areas than from urban areas. Regarding the history of previous infection, with a statistically significant difference between the two groups (P < .001), the results showed that the percentage of negative participants was higher in the patient group (70.7%).

According to Muhammad et al., (2022) the prevalence was 20.3% in rural areas and 13.8% in urban areas (P < 0.05). Furthermore, this is not consistent with the residential distribution of respondents in this study.

Our data did not align with Rahi et al., (2021), which found a significant incidence of amoebic dysentery infection was noted in many rural areas of Wasit. These disparities between urban and rural locations might be attributed to the high consumption of prepared foods in metropolitan areas.

Table (2): Basic Patient and Control Subject Demographic Information

			Groups			Total	Chi-Square	Asymp. Sig.
			Diabetic	Diabetic and amoeba	Control			
Age	3-12y	Count	14	0	2	16	85.409	0.000***
	13-22y	Count	11	0	29	40		
	23-32y	Count	8	2	30	40		
	33-42y	Count	6	9	12	27		
	43-52y	Count	9	11	1	21		
	53-62y	Count	4	1	0	5		

	63-72y	Count	0	1	0	1		
Total		Count	52	24	74	150		
Sex	male	Count	31	14	37	82	1.294	0.524 ns
	female	Count	21	10	37	68		
Total		Count	52	24	74	150		
Living	urban	Count	32	10	67	109	26.757	0.000***
	rural	Count	20	14	7	41		
Total		Count	52	24	74	150		

The TNF-α levels in both the patients and the control group.

The results displayed in table (3) indicate a statistically significant difference (P-value <0.0001) between groups when comparing the means for TNF-α. The 95% confidence interval ranges from -61.69 to -36.81. This implies that the average TNF-α concentration in the sick population (72.563 ± 6.165 pg/ml) was greater than that of the control subjects (23.314 ± 0.775 pg/ml).

The pathogenesis of diabetes and its effect on the immune system is linked to several variables, including increased levels of TNF-α observed in diabetic patients compared to healthy individuals. Therefore, the results of our current study indicate an imbalance between anti- and pro-inflammatory cytokines, and thus the presence of persistent, mild-intensity inflammation associated with diabetes causes increased production of TNF-α is one of the important inflammatory molecules that is elevated in chronic inflammation.

One feature of type 2 diabetes is insulin resistance. High blood levels of free fatty acids and dysfunction of adipose tissue may be associated with insulin resistance. This leads to increased production of TNF-α by adipose and immune cells in diabetic patients.

In diabetic patients, inflammation is common in adipose tissue, where macrophages and other immune cells release pro-inflammatory cytokines such as TNF-α. The inflammation in adipose tissue is a factor in causing overall inflammation and higher levels of TNF-α in individuals with diabetes.

Diabetes is linked to higher oxidative stress levels, which can trigger the generation of TNF-α. Oxidative stress and reactive oxygen species have the ability to trigger inflammatory pathways and stimulate the release of pro-inflammatory cytokines, such as TNF-α.

Diabetes can result in endothelial dysfunction, which causes endothelial cells to produce higher levels of TNF-α. TNF-α has the potential to worsen issues related to diabetes by causing endothelial dysfunction and inflammation within the blood vessels.

High levels of blood glucose in diabetic patients can also lead to production of TNF-α, a cytokine. High levels of sugar in the blood can trigger pathways that cause inflammation and lead to an increased release of molecules that produce inflammation, ultimately raising the levels of TNF-α in individuals with diabetes.

In general, the increased levels of TNF-α in diabetic individuals indicate the inflammation and immune system imbalance linked to diabetes. TNF-α and other pro-inflammatory cytokines represent a promising target for diabetes patients' therapy, since they may help lower inflammation and improve patient outcomes.

According to study of Zhao et al., (2022), macrophages cells release TNFα, which can activate other phagocytes, neutrophil accumulation, elevation of other inflammatory mediators and cause tissue inflammation. It also causes edema and decreased barrier function due to increased cell permeability (Liu et al., 2020). Individuals with inflammatory colitis have elevated intestinal levels of TNF-α, which plays a crucial role in mucosal inflammation. For example, this cytokine is critical for the development of inflammatory bowel disease (IBD), and anti-TNF drug treatment has been shown to be effective in some individuals (Kaur and Goggolidou, 2020).

Many in vitro experiments have been performed to study the effects of TNF-α on Entamoeba histolytica but with inconsistent results. Increased tissue damage and increased death of Entamoeba histolytica (Al-Attar and Ali, 2020; Khalaf et al., 2022). Therefore, it is unclear how TNF-α affects the progression of the disease. TNF-α promotes diarrhea in Entamoeba histolytica (Ghosh et al., 2019).

According to Mortimer et al. (2014), macrophages produce TNF-α in response to *Entamoeba histolytica* adhesion lectin or galectin.

Whole blood and colon biopsy samples from patients with acute and convalescent diarrhea from Entamoeba histolytica also showed elevated TNF-α mRNA expression, but this was not statistically significant. According to Peterson et al. (2010), these results also suggest a role for TNF-α in pathophysiology and susceptibility to diarrhea in *Entamoeba histolytica*.

These findings are supported by studies using human colon xenografts, which found that TNF-α activating genes (IL-1β, IL-6, or IL-8) were expressed during infection compared with infection-free control colon samples. produce higher expressions. (Lim et al., 2014).

Researchers have demonstrated a link between Entamoeba histolytica diarrhea and increased TNF-α production in multiple studies (Ghosh et al., 2019; Al-Ubaydi et al., 2019).

TNF-α has been shown to exacerbate tissue damage in patients with amoebic colitis (Ghosh et al., 2019). Furthermore, Entamoeba histolytica trophozoites are attracted to TNF-α via chemotaxis, which may contribute to the parasite's tissue invasion and colitis (Castellanos-Castro et al., 2020).

The highly aggressive immune response induced by TNF-α may lead to increased inflammation, leading to disease (Garbarino et al., 2021).

Resistance to amoebae infection occurs upon depletion of TNF-α (Noor et al., 2017).

According to Peterson et al. (2010), there is no evidence that TNF-α prevents diarrhea caused by Entamoeba histolytica and may increase susceptibility. Therefore, TNF-α is considered a risk factor for Entamoeba histolytica, and the results of this study are consistent with those of Peterson et al. (2010), who reported that higher TNF-α levels were associated with higher risk factors for *Entamoeba histolytica* infection ($P = 0.01$) .

Consistent with the results of the current study, Khalaf et al. (2022) showed an association between higher TNF-α production in patients and increased prevalence of *Entamoeba histolytica* diarrhea.

In the current study, we found that patients with amoebic colitis had higher TNF- α levels from the control group. while the study of Yu and Li (2019), found the mean TNF-α value of the control group was higher than that of the cases, which makes the results of this study inconsistent with our finding.

Children with bloody diarrhea caused by *Entamoeba histolytica* have higher TNF-α protein levels compared with children without symptoms or disease (Ndiabamoh et al., 2020).

The results of the current study are consistent with those of Muhammad et al. (2022), who reported that a mean ST = 12.03 ± 5.09, a minimum serum concentration = 5.4 pg/mL, and a maximum serum concentration = 33 pg/mL were found in amoebic patients; this difference for TNF-α was statistically significant ($P < 0.001$), whereas serum levels in control subjects ranged between the mean ± standard deviation. Dev = 9.54 ± 1.88, lowest serum level was 7.4 pg/ml, highest = 99 pg/ml.

TNF-α concentrations are elevated and overexpressed in *Entamoeba histolytica* patients compared with controls (Gonzalez Rivas et al., 2018). These results are consistent with the current study. However, this study contradicts a study by Ahmed and Sayl (2022), which found that TNF-α concentration in amebic patients (mean ± SD 44.27881 ± 19.601535) was lower than that in controls (mean ± SD Difference 60.18145) .±) 64.100002).

The results of the current study contradict those presented by Abdel Hafeez et al. (2013), found no significant difference in TNF-α levels in serum samples from patients with *Entamoeba histolytica* and controls.

Also the current findings contrast with those of a study published by Grover et al., 2014, which found no changes in TNF-ɑ concentrations between controls and patients with amoebic dysentery.

A study by Saad Dahhaam and Mohammed, (2022) showed that there was a significant difference of 0.01 in serum level of TNF-ɑ between the infected amoebiasis group and the control group, and high concentration recorded in patients' group. This agreed with our results.

Table (3): The TNF-α levels in both the patients and the control group.

TNF-a	Mean ± SEM (Pg/ml)	T test	DF	95% confidence interval	P value
Patient	72.563 ± 6.165	7.823	148	-61.69 to -36.81	<0.0001 ****
Control	23.314 ± 0.775				

The level of TNF-α concentration in patients varies depending on the results of the diagnosis.

Table (4) reveals that the average rank concentration of TNF-α peaked at 63.67 pg/ml in diabetic patients infected with the parasite *E.histolytica*, a group consisting of 28 patients. The average level of TNF-α was at its lowest point (26.52 pg/ml) in a group of 48 diabetic patients who did not have the parasite E.histolytica. This variation had a p-value of 0.000 (significantly significant statistically).

The increased level of TNF-α seen in diabetic patients with an *E. histolytica* infection compared to those without can be attributed to an extra immune response stimulated by the presence of the parasite. *E.histolytica* is a parasite that can cause amoebiasis, an intestinal infection that can result in inflammation and damage to the gut tissue.

When the immune system recognizes the parasite's existence, it initiates a reaction to eradicate the intruder, resulting in the activation of pro-inflammatory cytokines like TNFα. The relationship between the parasite and immune system of the host leads to an elevation of production of TNFα, as the body works to fight off the infection.

In patients with diabetes, who already have an immune system that does not function properly and experience ongoing inflammation, getting infected with *E.histolytica* can worsen the inflammatory response. Compared to diabetic patients who are not infected, individuals who are also infected with a parasite have a greater peak concentration of TNFα because of a combination of diabetes-related inflammation and the immune system's reaction to the parasite.

The presence of *Entamoeba histolytica* can directly cause the inflammatory pathways and immune cells to release TNFα in reaction to the parasite infection. For diabetic individuals infected with *E. histolytica*, this may lead to a greater increase in TNFα levels.

Overall, the elevated levels of TNF-α in diabetic patients infected with *Entamoeba histolytica* indicate a complex relationship between the immune response to the parasite, diabetes-induced inflammation, and stimulation of pro-inflammatory pathways during infection.

When amoeba disrupts the intestinal mucosal barrier, it becomes easier for the parasite to penetrate and destroy tissue. Therefore, parasites induce the production of multiple inflammatory stimuli at the site of infection, including TNF-α, which is associated with an increased risk of diarrhea in dysentery patients (Nour et al., 2017).

TNF-α causes blood vessels to become more permeable, allowing red blood cells to leak from damaged capillaries into tissues. Erythrocytes are the preferred food source for parasites (Ankri, 2015; Noor et al., 2017).

Studies have found that people with diabetes, especially older adults, have weaker immune systems. In addition, a large part of the body's defense against parasitic infection is derived from genetic factors (Daryabor et al., 2020).

The current findings are consistent with those of Khudair et al. (2017) in Amara's city and Ahmed, Hoda Mounir (2018) in Tikrit's city suggesting that TNFα is associated with a elevated risk of developing *Entamoeba histolytica* diarrhea as the parasite degrades mucosal barrier of intestine, which makes it easier for parasites to penetrate tissue and cause infection destroy it. Therefore, at the site of infection, parasites promote the production of multiple inflammatory stimuli, including TNF-α (Noor et al., 2017).

A study by Saad Dahhaam and Mohammed, 2022) showed that the mean concentrations of TNF-α in diabetic and *Entamoeba histolytica* reached 92.9±31.8 pg/ml in compared with DM patients without infection the mean concentrations was 19.60±7.18 pg/ml. This similar to our results.

The presented study was compatible with results of Sabaa and Mohammad, (2021), which found the concentration of cytokine TNFα was the highest in patients suffered from diabetes and infected with *E.histolytica* infection (28.98 ±5.65 pg/ml) in comparison with diabetes patients non infected with parasite (11.77 ± 7.46pg/ml).

Table (4): The level of TNF-α concentration in patients varies depending on the results of the diagnosis.

	Groups	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed
TNF-α	Diabetic and amoeba	28	63.67	1593.00	157.000	1333.000	-5.546	0.000***
	Diabetic only	48	26.52	1333.00				
	Total	76						

The concentration of TNFα, levels according to age groups of patients.

The findings presented in Table (5) indicated that the age group of 63-72 years had the highest average rank for TNFα, with a measurement of 75.00 pg/ml. The age group of 3-12 years had the lowest average rank for TNFα, measuring at 14.29 pg/ml.

According to our interpretation these results may be due to:

Insulin resistance, which is often seen in diabetes, can lead to higher levels of inflammatory cytokines such as TNFa. Individuals in the age group of 63-72 are more likely to develop insulin resistance, which may be the reason for higher TNF-a levels in this age group.

Furthermore, diabetes is accompanied by persistent inflammation, which can lead to increased levels of inflammatory cytokines such as TNFa. As people age, the effect of long-term inflammation may become more pronounced, leading to increased TNF-a levels in older individuals.

As people age, their immune system may become weaker compared to younger age groups, leading to decreased production of TNFa (an inflammatory cytokine released by the immune system in response to inflammation or infection). This may be a reason for lower TNFa levels in age group 3. 12 years.

Thus a combination of changes in the immune system associated with insulin resistance, aging and persistent inflammation could be a factor in the different levels of TNF-a observed in diabetic patients of different ages.The average level of this interleukin was 23.82 pg/ml in one age group, and 32.90 pg/ml, 48.67 pg/ml, 53.70 pg/ml, and 51.20 pg/ml in four other age groups spanning from 13 to 62 years old. A statistically significant difference was observed between the age groups, as demonstrated by the chi-square test (39.378) and p value (0.000). This suggests that as diabetes mellitus patients aged, their TNF-a levels increased, maybe due to:

TNFa is a cytokine that has a role in controlling immune responses and inflammation in the body. It also increases inflammation. Chronic inflammations are mostly seen in diabetics patients due to high levels of blood sugar and insulin resistance.

People's immune systems may change as they age, which may increase the production of cytokines that promote inflammation, such as TNF-a. Elevated levels of TNF-a can be caused by the combination of chronic inflammation that persists in diabetes individuals and an age-related increase in TNF-a production.

In diabetic patients, insulin resistance and elevated blood glucose levels can promote the production of pro-inflammatory cytokines such as TNFa .

thus, a combination of diabetes and aging may increase TNFa levels, which may cause chronic infection and other problems associated with these illnesses. TNFα is a powerful inflammatory substance performs a quintessential function in controlling more than a few factors of macrophage pastime (Siouti and Andreakos, 2019). TNFα is rapidly launched following amoebic contamination and regarded one of principal early mediators in tissue infection.

As people age, their ability to fight off infections decreases while their bodies continually produce pro-inflammatory compounds due to the constant stimulation of the innate immune system. According to Rea et al. (2018), chronic, mild inflammation plays a significant role in the development of various age-related health issues and illnesses.

TNFα is key controller of the production of pro-inflammatory cytokines (Malkov et al., 2021). The inflammatory cytokine TNF-α is linked to age-related diseases and rises with age. It is a pro-inflammatory mediator that, although it may be advantageous when acting locally inside the tissues, when released systemically, may be extremely detrimental (Rea et al., 2018).

Research on the aging process within cells in the elderly has revealed that increased TNFa is a mediator of changes in metabolism; high TNF-α levels have been associated with decreased levels of strength and muscle mass in older persons with type 2 diabetes (Bastos et al., 2024).

TNF-α triggers the activation of macrophages, which then migrate to areas of inflammation and destroy *E.histolytica*. This is achieved by generating more harmful oxygen molecules and activating inducible nitric oxide synthase (iNOS) to generate nitric oxide (NO) (Uribe-Querol and Rosales, 2020).

Alberro et al., (2021) revealed that there was significance difference regarding TNFα concentration level between patients according to age groups.

While Lampropoulou (2020) et al., referred to no significance difference between age categories appeared in TNFα in patients group.

Olaniyan et al., (2024) indicated that young adults infected with E. histolytica had a notably higher level of TNF-α in their plasma compared to non-infected control volunteers.

Table (5): Explain the concentration of TNFα, levels according to age groups of patients.

	Age group	N	Mean Rank	Chi-Square	df	Asymp. Sig.
TNF-α	3-12	14	14.29	39.378	6	0.000***
	13-22	11	23.82			
	23-32	10	32.90			
	33-42	15	48.67			
	43-52	20	53.70			
	53-62	5	51.20			
	63-72	1	75.00			
	Total	76				

5. The Concentration Level of Tumor necrosis aspect in Patients According to Gender and Residence

According to findings that recorded in Table (6), which are displayed in the "Asymp. Sig. (2-tailed)" column, there is a statistically non-significant distinction between adult males and females, and between city and rural in sufferers' group, as proven through the Asymp. Sig. (2-tailed) price of (0.849 and 0.372) respectively.

Mean rank for awareness stage of TNf-α in ladies (39.08) was once barely greater than in men (38.10).

The lack of substantial distinction in TNF-a attention degrees between male and girl diabetic sufferers may also be due to various factors:

Similar underlying inflammatory processes: Both male and woman diabetic sufferers can also trip comparable ranges of continual irritation due to insulin resistance and improved blood glucose levels. This shared inflammatory response can lead to similar TNF-a levels in both sexes.

Although sex hormones can influence inflammatory reactions, it's possible that their influence on TNF-a levels in diabetes individuals isn't significant enough to explain the gender difference. Furthermore, many factors that influence the production of TNF-a may also serve to balance out the hormonal differences between men and women. Variability in person responses: There is inherent variability in man or woman responses to inflammatory stimuli, together with TNF-a. This variability can also make contributions to the lack of extensive distinction in TNF-a tiers between male and girl diabetic patients.

Overall, the non-significant distinction in TNF-a attention degrees between adult males and women of diabetic sufferers may want to be influenced by way of a mixture of shared inflammatory processes, hormonal influences, pattern dimension limitations, and man or woman variability in response to inflammation.

Mean rank for attention stage of IL-10 in rural sufferers (41.01) was once barely greater than in city sufferers (36.46).

The lack of vast distinction in TNF-a attention tiers between rural and city diabetic sufferers may want to be due to countless factors:

Similar way of life and environmental factors: Rural and city settings may additionally have comparable life and environmental exposures that may want to have an impact on infection tiers in diabetic patients. For example, if each rural and city areas have comparable ranges of pollution, diet, stress, and get right of entry to to healthcare, then there might also now not be a widespread distinction in TNF-a tiers between the two groups.

Genetic variants among people may be more important in determining TNF-a levels than their place of residence. Any reasonable differences based only on residential location should be outweighed by differences in genetic susceptibilities to infection and cytokine production.

Small pattern size: the contemporary finds out about evaluating TNF-a ranges between rural and city diabetic sufferers can also now not have been massive sufficient to notice a giant difference. A large pattern dimension would amplify the electricity of the find out about and enhance the capability to observe any actual variations between the groups.

Variability in person responses: Individual variability in response to infection and cytokine manufacturing ought to masks any manageable variations between rural and city diabetic patients. Factors such as age, sickness severity, medicinal drug use, and comorbidities may want to all affect TNF-a ranges and affect the consequences of the study.

Thus, the lack of sizable distinction in TNF-a attention tiers between rural and city diabetic sufferers can also be influenced with the aid of a mixture of way of life factors, genetic variations, pattern dimension limitations, and character variability in inflammatory responses.

Sexual differences in the immune system involve the generation of cytokines that facilitate communication between immune cells. According to Klein and Flanagan's research from 2016, women tend to produce more type 2 cytokines, whereas men tend to produce more type 1 cytokines like TNFa.

Several proteins in *Entamoeba histolytica* regulate host immune responses. According to Singh et al. (2016), the amebicidal effect of Gal/GalNAc-lectin leads to TNF-α production by macrophages.

To help establish infection, the amoeba must maintain a balance of TGF-β and pro-inflammatory cytokines such as TNF-α and IL-17A. After exposure to lipopeptide phosphoglycans (LPPG), peritoneal monocytes and macrophages secrete TNF-α, IL-6, IL-8, IL-12, and IL-10 via Toll-like receptor 2. Therefore, LPPG-dependent signaling may initiate a negative feedback loop that reduces the inflammatory response (Gonzalez Rivas et al., 2018).

Host defense requires humoral and cellular responses; however, it is unclear how Entamoeba histolytica evades the immune system. One such approach might include the parasite's ability to control the production of inflammatory cytokines. This begins with the release of pro-inflammatory cytokines such as TNF-α (Botwright et al., 2021).

Popko et al., (2010) mentioned that no significance difference between male and female in TNFa serum level patients. Also Islam et al., (2022) found no discernible variations in plasma TNF-α levels across genders. These studies nearly compatible with our results.

The significance of inflammatory mediators in the aetiology of diabetes has been verified by both genders, and there is a notable increase in TNF-α levels in both genders (Bashir et al., 2020).

While Bernardi et al., (2020) referred that in men's serum circulation TNF-α levels are greater than women's, and this difference was significant. This un compatible with our results.

Table (6): Concentration Level of TNf-α In Patients Accordance to Gender and Residence

TNF-α	Gender	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)
	Male	45	38.10	1714.50	679.500	1714.500	- 0.190	0.849
	Female	31	39.08	1211.50				

	Total	76						
	Living							
	Urban	42	36.46	1531.50	628.500	1531.500	- 0.893	0.372
	Rural	34	41.01	1394.50				
	Total	76						

CONCLUSION

The The average concentration level of TNF-α cytokine differs significantly between the patient and control groups, with the patient group exhibiting greater levels. When compared to diabetic individuals without the parasite E. histolytica, the presence of the parasite caused a significant increase in TNF-α levels. The individuals between the ages of 63 and 72 had the highest amount of TNFα, while those aged between 3 and 12 had the lowest level of interleukin-10. There is positive correlation between TNF-a and IL-10 levels in Entamoeba histolytica -infected individuals.

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