RESEARCH
O&G Forum 2024; 34 – 3s: 2441-2451

EXPLORING THE ROLE OF IL-10 IN DIABETES MELLITUS PATIENTS WITH ENTAMOEBA HISTOLYTICA INDUCED DIARRHEA

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Abstract
Background: A key immunoregulatory cytokine that predisposes amebic invasion to occur is interleukin-10. Intestinal amebiasis resistance depends on IL-10, and a lack of IL-10 increases a person's susceptibility to amebic infection. Because interleukin (IL-10) is crucial for preserving the mucosal barrier, it provided protection against infections caused by E. histolytica.
Objective: The goal of this study was to detection of *E. histolytica* in Diabetes patients and compare immunological parameter IL-10 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 IL-10 by ELISA technique.
Results: When comparing the means of IL-10 with a 95% confidence interval, the results showed that a statistically significant difference (P-value <0.0001) between the groups, with the mean concentration level of IL-10 in the patient groups being lower than in the control groups (29.808 ± 1.658 pg/ml and 43.529 ± 1.330 pg/ml, respectively). The average rank concentration of interleukin 10 peaked at its highest level (57.68 pg/ml) among a group of 28 diabetic patients who were infected with the parasite E.histolytica. Among the group of 48 diabetic patients not infected with parasite E.histolytica, the average level of interleukin 10 was at its lowest point (27.31 pg/ml). This variation had a p-value of 0.000, indicating statistical significance. The oldest age group (63-72 years) had the highest average rank for interleukin 10, reaching 55.00. Conversely, the youngest age group (3-12 years) had the lowest average rank for interleukin 10, reaching 10.29. There is no significant difference between males and females, or between urban and rural patients in the study group, as indicated by the Asymp. Sig. The (2-tailed) probabilities are 0.597 and 0.503, respectively. The average rank for IL-10 concentration levels in females (40.11) was slightly greater than in males (37.39). The average rank for IL-10 concentration levels was slightly higher in rural patients (40.38) compared to urban patients (36.98).
Conclusions: The IL-10 levels were found to be considerably lower in the patient group compared to the control group. The noticeable disparity in interleukin 10 concentration levels between diabetic patients with and without E.histolytica parasitic infection indicates a possible connection between parasite infection and elevated interleukin 10 levels in diabetic patients.

Key words: Interleukin-10, *Entamoeba histolitica*, Diabetes mellitus.

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. (Manich et al., 2018). 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).

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).

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. (Elandi et al., 2015; Sabah and Temshah 2015).

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).

Strongly anti-inflammatory cytokine interleukin 10 (IL-10) is essential for controlling the host immune response to infections, minimizing host injury, and preserving healthy tissue homeostasis (Steen *et al.*, 2020).

By selectively inhibiting the CD28 co-stimulatory pathway, IL-10 induces tolerance in T cells and subsequently regulates the establishment and suppression of antigen-specific immunity (Boonpiyathad *et al.*, 2019). A host defensive mechanism called tolerance lessens the detrimental effects of infection on host fitness. Tolerance does not influence pathogen load directly, in contrast to resistance mechanisms. Instead, tolerance lessens the host's vulnerability to immune system or pathogen-induced tissue damage or others (Singh and Best, 2021).

T cells regulatory in Colon arise in response to *E. histolytica*. Anti-inflammatory cytokines, such as TGF- β and IL-10, are produced by Treg cells, which are believed to play role in self-tolerance because of their ability to decrease inflammatory responses (Horst *et al.*, 2021).

The host tissues may sustain direct injury from E. histolytica. Through the resistance mechanism, the host's immune system lowers the burden of the E. histolytica pathogen. Host tissue damage might also result from the immunological response. Through tolerance mechanisms that lessen immunopathology as well as direct tissue damage caused by E. histolytica, the host can lessen these damages by tolerance (Guillén, 2023).

The mucosal barrier is maintained in part by interleukin (IL-10). The IL10 level is protective of E. histolytica (Shimokawa *et al.*, 2018).

The ability to fend against intestinal amebiasis depends on IL-10, and a lack of this protein makes people very susceptible to amebic infections. On the other hand, it is unknown how IL-10 production affects amebiasis-DM coinfection (Uddin *et al.*, 2021).

According to some research, those who generate higher amounts of IL-10 than those who produce lower levels are probably more likely to have a lower prevalence of amebiasis. The patient's increased IL-10 levels indicate that inflammation is already present in their body and that their body is making an effort to reduce it. This suggests that IL-10 may have a protective function in those who may have had an E. histolytica infection. Therefore, the development of amebiasis may be significantly influenced by high blood levels of IL-10 (Oshiba *et al.*, 2023).

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 thousands 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.

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 IL-10

IL-10 concentration level was measured by ELISA technique according to data of manufacturing of MELSIN/ China origin.

Results and Discussion

1. 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				

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 .

he 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.

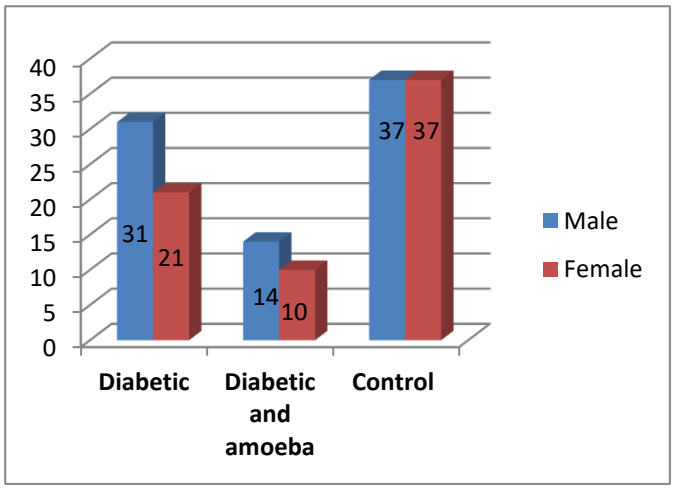

Figure (4-1): Study group distribution based on gender

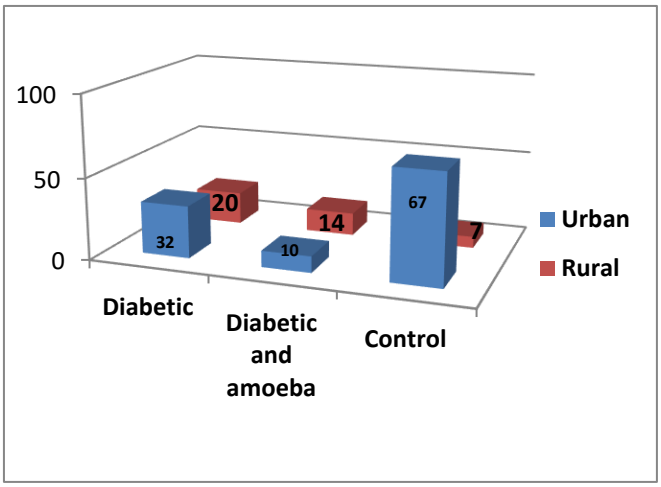

Figure (4-2): Study group distribution based on residence

2. Interleukin-10 Levels in Patients and the Control Group

When comparing the means of IL-10 with a 95% confidence interval, the results in Table (2) demonstrated a statistically significant difference (P-value <0.0001) between the groups, with the mean concentration level of IL-10 in the patient groups being lower than in the control groups (29.808 ± 1.658 pg/ml and 43.529 ± 1.330 pg/ml, respectively).

Our explanation for this decrease may be due to:

One of the distinguishing features of diabetes is the presence of chronic, mild inflammation that may lead to a defect in the process of synthesis of anti- and pro-inflammatory cytokines. The first reason could be persistent inflammation: Chronic low-grade inflammation is a hallmark of diabetes, and it can cause an imbalance in the synthesis of cytokines that are pro- and anti-inflammatory. Diabetic patients have a decrease in anti-inflammatory cytokines, such as IL-10, and this leads to a systemic inflammatory state.

Also, the reason for the decrease in IL-10 can be attributed to the interference of diabetes with the work of the immune system, and this in turn may cause a defect in the regulation and production of immune cytokines. Therefore, the level of IL-10 decreases in diabetics, as it plays an important role in reducing inflammation by controlling responses.

Also, insulin resistance can be considered one of the reasons for this decrease in anti-inflammatory cytokines, such as IL-10, as they are an important aspect of diabetes and cause an imbalance in the regulation and increase of immune responses.

In addition to what was mentioned, the lack of production of anti-inflammatory cytokines such as IL-10 results from a weak immune system that occurs perhaps due to oxidative stress, which is associated with diabetes, and this in turn causes a weakness in the production of this cytokine.

Not to mention the complications of diabetes, such as nephropathy, retinopathy, and neuropathy. All of these complications may be related to an increase

in pro-inflammatory cytokines and a decrease in anti-inflammatory cytokines, and this results in an exacerbation of immune responses in diabetic patients.

Therefore, all of these reasons are most likely the reason for the lower concentration of IL-10 in diabetic patients compared to healthy individuals.

People with low IL-10 levels had a higher incidence of *Entamoeba histolytica* infection (68.1%), while people with high IL-10 levels had a lower incidence of infection (58.2%). Thus, IL-10 protects against infections caused by *Entamoeba histolytica* (Ngobeni et al., 2022) .

There are few studies linking IL-10 levels to amebiasis. According to Bernin's et al., (2014) study, patients had much higher levels of IL-10 in their blood, suggesting that *Entamoeba histolytica's* invasion of liver tissue triggers an anti-inflammatory immune response.

A previous study by Hamano and colleagues showed that IL10 protects patients against amebiasis (Hamano et al., 2006). Elevated levels of IL-10 in patients indicate that inflammation is already present in their bodies and that their bodies are working to reduce it. This suggests that IL-10 may have a protective function in those who may have an *E. histolytica* infection. Therefore, high levels of interleukin-10 in the blood may significantly influence the development of amebiasis (Nakada-Tsukui, and Nozaki, 2016).

A study by Saad Dahhaam and Mohammed, (2022) showed that there was a significant difference of 0.01 in serum level of IL-10 between the infected amoebiasis group and the control group, and high concentration recorded in patients group. This dis-agreed with our results.

Table (2): The Level of Interleukin-10 in The Patients and Control Groups

IL-10	Mean ± SEM	t	DF	95% confidence interval	P value
Patient	29.808 ± 1.658	6.436	148	9.508 to 17.93	<0.0001 ****
Control	43.529 ± 1.330				
SEM: Standard error of the mean, DF: Degree freedom. * P-value ≤ 0.05 considered as significant.					

3. Level of Concentration of Interleukin-10 in Patient Groups

Mann-Whitney U test, which is a non-parametric statistical test used to compare two independent, non-normally distributed groups of data, as shown in Table (3). The difference in the sum of the ranks of two groups is represented by the Mann-Whitney “U” test statistic. The smaller the group size (considering the number of participants in each group), the lower the probability that it happened by random chance. The Wilcoxon W test is a non-parametric analysis often used to compare two related samples.

Table (4-6) demonstrates that the average rank concentration of interleukin 10 peaked at its highest level (57.68 pg/ml) among a group of 28 diabetic patients who were infected with the parasite *E.histolytica*. Among the group of 48 diabetic patients not infected with parasite *E.histolytica*, the average level of interleukin 10 was at its lowest point (27.31 pg/ml). This variation had a p-value of 0.000, indicating statistical significance.

We believe that there are several possible reasons for the increased IL-10 secretion seen in diabetic patients with *Entamoeba histolytica* infection.

Reduced production of anti-inflammatory cytokines, such IL-10, may result from imbalance in immune system. because it is known that the functioning of immune cells, such as macrophage and T cells, is compromised by high glucose levels, which may lead to an unbalanced immune response. This may occur as a result of high blood sugar.

Diabetes makes the functioning of the immune system weak, which in turn causes an increase in the production of IL-10 and thus makes diabetics more susceptible to infections such as *Entamoeba histolytica*.

Diabetes is linked to low-intensity, chronic inflammation, which may lead to an imbalance between pro- and anti-inflammatory cytokines. It is well recognized that IL-10 plays a significant function in reducing inflammation and shielding tissues from harm. In an effort to control the immune response, there may be an increase in IL-10 production in diabetic individuals infected with *Entamoeba histolytica* as a result of chronic inflammation. Changes in the gut microbiota: Diabetes can impact the makeup of the gut microbiota, which may result in dysbiosis and a higher risk of infections. Alterations in the bacteria living in the gut can impact the generation of cytokines, such as IL-10.

Changes in the intestinal bacteria of diabetic patients infected with *Entamoeba histolytica* could lead to higher production of IL-10 as a component of the body's immune reaction.

In general, the intricate relationship between diabetes, immune system dysfunction, long-lasting inflammation, and changes in the gut microbiota may result in diabetic patients infected with *Entamoeba histolytica* producing more IL-10.

In the intestinal epithelial layer, IL-10 promotes mucin secretion by goblet cells, leading to their inhibition. Thus, by trophozoite attachment to

the epithelial layer, studies have shown that in animals lacking IL-10, epithelial cells are unable to form mucus, a substance necessary to reduce amoebic adhesion to the gut (Hamano et al., 2006).

A study by Saad Dahhaam and Mohammed, 2022) showed that the mean concentrations of IL-10 in diabetic and Entamoeba histolytica reached 46.00±9.32 pg/ml in compared with DM patients without infection the mean concentrations was 6.48±1.21 pg/ml.

The results of IL-10 cytokinesis are inconsistent with Garcia-Zepeda et al., (2007), but consistent with Isabel et al., (2010). Some studies showing that dysregulation of the innate immune response depends on pro- and anti-inflammatory cytokines produced by macrophages and monocytes balance. Any disease's host is harmed by the innate immune response. Thus, IL-10 is a cytokine that maintains immune stability and homeostasis by modulating macrophage function and inhibiting NF-KB, a gene expression factor that drives cytokine activity. It prevents the development of tissue damage and inflammation by inhibiting TLR (cell surface receptor) synthesis, phagocytic activity, and exacerbating immune responses (Rigosi et al., 2014).

Table (3): The level of Interleukin-10 in patient groups categorized based on PCR test outcomes

	Groups	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed
IL-10	Diabetic and amoeba	28	57.68	1615.00	135.000	1311.000	-5.783	0.000***
	Diabetic only	48	27.31	1311.00				
	Total	76						

4. The concentration of IL-10 levels according to age groups of patients.

The findings presented in Table (4) indicated that the oldest age group (63-72 years) had the highest average rank for interleukin 10, reaching 55.00. Conversely, the youngest age group (3-12 years) had the lowest average rank for interleukin 10, reaching 10.29.

The average rank of this interleukin was 22.05 in the age group of 13-22 years, 37.85 in the age group of 23-32 years, 51.53 in the age group of 33-42 years, 54.60 in the age group of 43-52 years, and 48.20 in the age group of 53-62 years. The discrepancy in age groups was found to be statistically significant, as indicated by the p value (0.000) and chi-square value (46.348).

There might be various reasons for the drop in IL10 secretion in diabetic patients aged 3-12 years, and the rise in those aged 63-72 years.

Our explanation for these results may be one of the following reasons:

Aging is usually accompanied by hormonal changes starting from childhood until the elderly, and therefore the production and secretion of cytokines such as IL10 can be affected by these changes.

Also, the increase in the cytokine IL10 in the elderly can be attributed to the decreased performance of the immune system as a result of aging, and this in turn causes increased secretion of this cytokine as a means of compensating for weakness in the immune system.

Changes in dietary patterns, exercise routines, and stress levels can potentially affect IL10 secretion .

Diabetes is linked to chronic inflammation, which may have an impact on the synthesis of IL10. older people may have higher levels owing to persistent inflammation.

The findings of Olaniyan et al., (2024) indicated that young adults infected with E. histolytica had a significantly lower level of IL-10 compared to non-infected control volunteers.

When the age of the study participants was compared with the expression of IL-10, patients aged 46 to 80 years (35.3%) were found to produce the highest levels of this cytokine, followed by the 0 to 25 age group (27.9%). %), age group 26. 45 (27.3%) produced the lowest amount of this cytokine (Ngobeni et al., 2022).

Rodriguez et al., (2014) conducted a study that was useful in understanding how IL-10 levels in patients with amebiasis relate to age. His results indicated differences in IL-10 responses between *Entamoeba histolytica* infection in people of different ages, and he said that age has an impact on how the body responds to this parasitic amoeba infection, through significant age-related changes in IL-10 levels.

A study conducted by Garcia et al. (2017) revealed a noteworthy rise in interleukin 10 concentration levels across several age cohorts, corroborating the impact of age on interleukin levels in amebiasis patients.

Another study provided insight into the relationship between age, IL-10 levels, and clinical outcomes in patients with amebiasis and found significant increases in IL-10 levels that may be related to the severity of the disease (Flores Huerta et al., 2024).

Table (4): shows the concentration of IL-10 levels according to age groups of patients.

	Age group	N	Mean Rank	Chi-Square	df	Asymp. Sig.
IL-10	3-12	14	10.29	46.348	6	0.000***
	13-22	11	22.05			
	23-32	10	37.85			
	33-42	15	51.53			
	43-52	20	54.60			
	53-62	5	48.20			
	63-72	1	55.00			
	Total	76				

5. The levels of Interleukin-10 in patients vary based on their gender and where they live.

Based on the results in Table (5) in the "Asymp. Sig. (2-tailed)" column, there is no significant difference between males and females, or between urban and rural patients in the study group, as indicated by the Asymp. Sig. The (2-tailed) probabilities are 0.597 and 0.503, respectively.

The Mean Rank column shows the average position of IL-10 in each group. The total of all the ranks for each parameter within each category is shown in the column labeled "Sum of Ranks".

The mean rank of IL-10 concentration levels in females (40.11) was slightly greater than in males (37.39).

Our explanation for these results may be due to: different hormone levels

Between males and females, it may affect how the female immune system responds and produces cytokines.

According to Fan et al. (2019), estrogen can improve the body's capacity to combat inflammation by raising IL-10 production, for example. Thus, in females with diabetes, this contributes to an increase in interleukin 10 production.

Furthermore, IL-10 secretion may be impacted by variations in metabolic variables between male and female diabetes patients. For example, differences in insulin sensitivity, glucose metabolism, or adipokine levels between males and females can affect IL-10 formation.

External influences can also affect the control of IL-10 secretion in people with diabetes, such as physical activity, stress, and diet.

Genetic factors may differ between genders, resulting in different levels of interleukin 10 secretion in response to diabetes.

Not to mention, men and women have natural differences in how their immune systems react. For example, women usually show stronger immune responses than men. In females with DM illness, this may result in increased interleukin 10 secretion levels, which is thought to be a defense mechanism against tissue damage and inflammatory regulation.

Patients in rural areas had an average rank for IL-10 concentration levels that was somewhat higher (40.38) than for patients in urban areas (36.98).

According to our interpretation, there are several reasons for this results:

Lifestyles in rural communities are often different from those in metropolitan regions; they tend to be less stressful, include more physical exercise, and eat better. These components may improve immune regulation and function, which may lead to higher levels of IL-10 in individuals from rural settings.

For individuals living in rural areas, fresh air may reduce inflammations and immune activation, which may raise levels of anti-inflammatory cytokines such as IL-10. Because rural community have lower levels of pollution and industrial chemicals, may have an effect on the immune system. Rural areas also have a greater diversity of microorganisms.

city areas mostly have increased contamination , overcrowding, and stress, all of which may lead to chronic inflammation and immune system problems due to socioeconomic factors. Patients living in rural areas may have better access to fresh air, natural environments, and lower stress levels, all of which may lead to increased IL-10 levels.

Both genetic variations and epigenetic modifications can contribute to cytokine production and immune responses. Variations in genetic makeup and how genes are activated may impact levels of IL-10 in rural and urban populations.

Furthermore, variations in lifestyle, diet, genetics, and environmental factors among individuals may also impact cytokine levels, and thus should be taken into account when analyzing these results.

Important mediators of the host immune response are cytokines. They can serve as indicators to identify immune processes during amebic infection. Although intestinal amebiasis is very common, little

is known about how the host innate immune system influences the clinical consequences of amoebic infection in humans (Hemphill et al., 2019).

According to Burrello et al. (2018), exposure to diverse microbial communities has been shown to augment the synthesis of IL-10 and other cytokines that mitigate inflammation.

According results of Van Eijk et al., (2007), there was no discernible difference in IL-10 levels between males and females. This uncompatible with our current findings.

Our results also contradicted with results of Smith et al., (2019), which used established techniques to quantify IL-10 and had a sizable sample size and discovered no discernible variation between males and females.

Women produced more IL-10 (30.3%) compared to men (25.9%) (Ngobeni et al., 2022). This compatible with our current findings.

According to Da Pozzo et al., (2018), there was the concentration of IL-10 in females was slightly more than that in men. The statistical significance of this difference points to a possible gender variance in IL-10 levels. This agreement with our current results.

Areas with limited water supplies, poor sanitation, and low levels of socioeconomic development, especially in less developed countries, are thought to have higher E. histolytica seroprevalence. Study of Ngobeni et al., (2022) showed that patients in rural areas had higher seroprevalence (66.0%) than patients in urban areas (63.9%).

Agricultural background and inadequate hygiene practices may contribute to higher infection rates in rural areas (Zaglool et al., 2011).

Table (5): Shows the concentration of interleukin-10 levels in patients group according to their gender and residence

	Gender	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)
IL-10	Male	45	37.39	1682.50	647.500	1682.500	- 0.529	0.597
	Female	31	40.11	1243.50				
	Total	76						
	Living							
	Urban	42	36.98	1553.00	650.000	1553.000	- 0.669	0.503
	Rural	34	40.38	1373.00				
	Total	76						

Conclusions

1. The IL-10 levels were found to be considerably lower in the patient group compared to the control group.
2. The noticeable disparity in interleukin 10 concentration levels between diabetic patients with and without *E.histolytica* parasitic infection indicates a possible connection between parasite infection and elevated interleukin 10 levels in diabetic patients.
3. There is a notable disparity in interleukin 10 concentration levels among different age groups, with the older age group having the highest average rank and the youngest age group having the lowest.
4. The average rank for IL-10 concentration levels in rural females patients was slightly greater than in urban males.

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