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Immunological tolerance induced by parasitic Infections in Type-1 Diabetes Mellitus children

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Abstract

Background: Insulin-dependent diabetes mellitus (IDDM), commonly referred to as Type 1 diabetes (T1D), is an irreversible, multifactorial auto-immune condition. The onset of diabetes has been linked to a lack of self-tolerance caused by beta-cell autoantigens., which causes the eradication of generating-insulin beta-cells by Th1 cytokines. Parasites and their derivatives significantly alter the immune system by creating anti-inflammatory cytokines, which reduce excessive inflammation and allow the parasite to continue living within the host. Objective: The investigation's objective is to identifyimmunological tolerance by parasite infections in type -1 diabetic children. Materials and Methods: A current review was carried out on 145 children (78males and 57females,1–12 years) grouped into (70 DM1,50 intestinal protozoa infections and 25mixed [DM1infected by intestinal protozoa]) and 35 volunteers control showing the normal result of FBS and HBA1c (20 male and 15 female, 1-12 years). Collected (5) ml of venous blood samples were to measure fasting blood sugar, and glycated hemoglobin and Immunological tests were carried out to establish the quantitative amount of IL-2, IL-10, similarly Prostaglandin -E2 (PG-E2) in a manner (ELISA). and A stool sample was collected from (50 intestinal protozoa infections and 25 Mixed with 35 controls) in a sterile screw cap. For General Stool Examination to detect the causative agents and confirm the diagnosis by Rapid strips of Cryptosporidium +Giardia +Entamoeba, by Immuno- Chromatography (IC). Results: The results were analyzed by SPSS version 24 independent-sample T-test and Pearson correlation analysis. showed the level of IL-2 was high concentration &highly significant differences (p-value = o.ooo) in diabetic patients ,and mixed (89.5±37.8, 48.5±29 ng/l)respectively compared with protozoal infection and control (19.2±4.2 and 25.4±7.6 ng/l,), IL-10 was high concentration &highly significant differences(p-value = o.ooo) in patients with protozoal infections and in mixed groups was (93.4±64.6, 82±59 ng/ml) respectively than control(25.4 ± 6 ng/ml),While Levels of the (IL-10) are decreased in T1DM (21.5±7 ng/ml) and PGE2 concentration was high concentration in parasitic infected patients and mixed groups (94.5±77, 89±37pg/ml) respectively as compared with controls (15.9 ± 6.8 pg/ml) and statistically significantly increased at (P=0.000, P ≤0.05) and low level (14.5±6.3 pg/ml) in DM1 patients as compared with controls (15.9±6.8 pg/ml) at (p=0.58) Conclusions: The conclusion of this study was the level of IL-2 in the mixed group was lower than DM1 that reflects the anti-inflammatory effect of protozoal to decrease the severity of Th1-cytokines on islets cells also increased level of IL-10 and PG-E2 in mixed group clarify its anti-inflammatory Th2 cytokines roles .

Keywords:

Type-1 diabetes (T1D), is an irreversible, multifactorial autoimmune condition., a multifactorial autoimmune disease. Beta-cell death was a consequence of an ongoing inflammatory process in the pancreatic islets of Langerhans (insulitis). Beta cells are finally killed by T lymphocytes that invade pancreatic islets [1].

As autoimmunity in T1DM progresses from an early activation to a chronic disease, several autoantigens discovered in the pancreatic -cells may be significant in the commencement or progression of autoimmune islet destruction. Strong evidence implies that islet autoantibody responses against several islet autoantigens are associated with the emergence of overt disease. Islet autoantigens typically interact with T cells and autoantibodies more frequently. This phenomenon is referred to as epitope spreading. The onset of the disease has been related to a diminish self-tolerance to autoantigens β - cells, which causes T-helper1 mediated death of generating beta - cells for insulin and the production of Th1 cytokines[2].

Between parasites and their hosts, there were immunological interactions of parasites that direct the defense- system toward a strong type-2 immunological reaction that is linked to tissue healing and immune protection. This presents a chance for intestinal protozoa and helminths to induce a more severe type of morbidity in DM patients [3, 4].

In addition, patients with chronic diseases are also at high risk of intestinal parasite infestation. The clinical occurrence and risk factors are more related to children because they are considered to be more highly infected than the other population groups. the infection of endoparasites in diabetes type 1 patient a robust Th2 response to parasites may be connected to Giardia lamblia observed in TID 1-patients [5].

Children with TID experienced an autoimmune reaction that was characterized by the release of Th1 cytokines. Additionally, the development of a regulatory network might help limit inflammation that could otherwise result in pathology while assisting in the regulation of overt immune responses to prolong the parasite's ability to survive [6].

Other concurrent disorders may have an impact on and be impacted by these changes in the host's immunological status. By preventing the Th1-mediated death of β-cells which produce insulin through processes associated with the host's ability to develop a Th2- response to parasites, parasites may prevent type 1 diabetes [7].

It has been hypothesized that parasite infections might either decrease or increase the occurrence of diabetes. Experimental evidence points to the potential for chronic inflammatory disorders like T1DM and other immune-mediated illnesses to be prevented by exposure to parasite infections [6]. The immune-conversion from Th1 to Th2 mechanisms used by parasites, and their clinical significance in human disease [8].

Since the beginning of time, parasites have accompanied humans by generating immune-evasion chemicals that might block an abnormal immunological response in the host. Parasitic diseases have decreased in frequency during the past few decades, probably as a result of improved sanitation. Meanwhile, autoimmune illnesses are becoming more common, which cannot just be attributed to variations in susceptibility genes. While the hygiene hypothesis sheds insight on its shortcomings,"[9].

The hygiene hypothesis is said that increased atopy, asthma, and autoimmune illnesses like DM1 might result from decreased exposure to pathogens throughout childhood. [4] Immunomodulation caused by parasites may enhance insulin sensitivity, trigger immunological tolerance, and assist avoid diabetes. It is thought that parasite infections affect and control human immunity, suppressing autoimmune disorders as a result. Consequently, the promise for treating or curing autoimmune disorders lies in parasite infections. [8]. Numerous epidemiological studies in experimental animal models and people attest to the anti-autoimmune diabetic benefits of parasites...[10].

Materialsand Methods

Patients and Controls

35 healthy volunteers as controls and 145 children with type - 1 diabetes (78 men and 57 women, ages 1 to 12) grouped into four groups as shown in table 1 participated in the current study (20 male and 15 female, 1– 12 years). None of the patients had any immunosuppressive medicine, and it was proven that

none of them had a chronic infectious illness, an allergy, or cancer. Based on World Health Organization (WHO) standards, T1D was identified.[11] All T1D patients required rapid insulin replacement medication, and the diagnosis of the condition was made based on the start of severe ketosis or ketoacidosis.

Collected feces samples and blood samples from healthy volunteers and patients with Giardia lamblia and Entamoebahistolytica infections (70 DM1,50 Parasitic,25 DM1& Parasitic infections, and 35 healthy controls) From diabetes patients who had abdominal pain and diarrhea and were thought to have intestinal parasite infections, as well as from non-diabetic kids in the same age range (1 to 12 years), a stool sample was taken in a sterile screw cap. To examine stools under a microscope and under a macroscopic microscope to identify the etiologic agents and confirm the diagnosis using Rapid CERTEST strips for Cryptosporidium +Giardia + Entamoeba, Immuno-Chromatography (IC). Several tests were included in this study especially immunological tests involving IL-2, IL-10, and Prostaglandin -E2 (PG-E2) concentrations

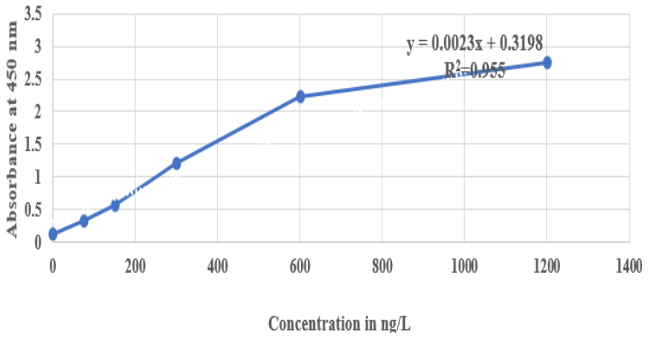

Figure (1) Standard Curve for IL-2

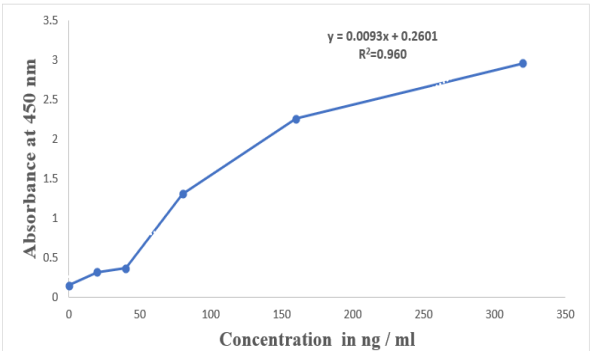

Figure (2) Standard Curve for IL-10

Methods

Assessment of interleukin-2,interleukin-10 and Prostaglandin-E2.

Using the ELISA method, the levels of interleukin-2, interleukin-10, and prostaglandin in the serum of controls and patients were determined. The IL-2, IL-10, and PG-E2 kits all employ the same test techniques and share the same components, and all reagents are ready to use. The procedure was strictly done according to the instructions of the manufacturer's.

Interpretation of results:

Drawing of the standard curve of IL-2, IL-10, and PG-E2 by blotting the mean of drawing a line through the spots on the graph and standardizing the optical density (OD) on the vertical (Y) axis against the concentration on the horizontal (X) axis. With the aid of computerized curve-fitting tools, these calculations can be best - fit, and regression - analysis can be used to determine the best - fit line. The curves are shown in figures (1), (2), and (3).

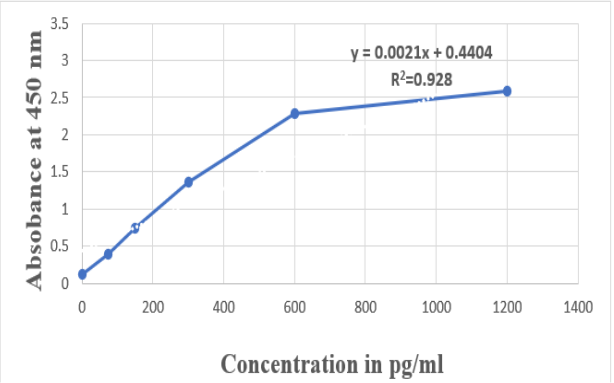

Figure (3) Standard Curve for PG-E2

CERTEST Crypto sporidium +Giardia +Entamoeba COMBO CARD TEST, Zaragoza (Spain) Immuno-Chromatography (IC)

The parasite Triage screen is a new qualitative-Enzyme Immuno-Assay (EIA) screen for the identification of Giardialamblia (α1-giardin), Entamoeba (histolytica / E. dispar), and Crypto sporidium parvumin frozen or fresh, un-fixed human fecal specimens. to examine the parasite, a triage immunochromatography assay was used to identify the three types of the parasite by the technique is called A

lateral flow assay (LFA) test procedure was done according to manufacture instruction.

Results

Clinical Characteristics and Children's Demographic

180 children were enrolled, and Table 2 lists the characteristics of the subjects in each group. There were 33 men and 37 women among the T1D patients. In T1D, both the HbA1c and fasting blood glucose levels were elevated. Likewise, typical in healthy controls. No statistically significant gender difference existed. Additionally, there were notable statistical disparities in age among the four groups.

Sex and Age distribution of Study groups

Of the total of 180 cases (70 DM1, 50 parasitic infections, 25 diabetes with parasites, and 35 normal cases as control groups), 98 (54%) were male cases and the remaining 82 (46%) were female cases as illustrated in Table (1).

Table (1): Sex & Age distribution among study groups

Age groups (Years)	Male				Female				P value
	Diabetes (%)	Parasitic (%)	Mixed (%)	Controls. (%)	Diabetes (%)	Parasitic (%)	Mixed (%)	Controls. (%)	
< 5	4 (12%)	14 (48%)	2 (13%)	11 (52%)	4 (11%)	11 (52%)	3 (30%)	6 (42%)	0.000 HS ₸ †
6-10	20 (60%)	14 (48%)	12 (80%)	9 (42%)	19 (48%)	5 (24%)	6 (60%)	7 (50%)	
>10	9 (28%)	1 (2%)	1 (7%)	1 (6%)	14 (41%)	5 (24%)	1 (10%)	1 (8%)	
N.	33 48%	29 58%	15 60%	21 60%	37 52%	21 42%	10 40%	14 40%	
No. of Males: 98(54 %)					No. of Females: 82(46%)				
0.45 ¥ NS between male &female									
Total 180 (100%)									

(No: numbers of subjects; ₸: independent sample T-test; NS: Non-Significant at p ⩾0.05; HS: Highly Significant at p ⩽ 0.05;¥ (Chi -Square) ,NS: Non Significant at p ⩾ 0.05)

Age of study groups was classified into three groups: less than 5 years (55,30%),6-10 years (92,51%), and more than 10 years (33,19%) there are high- substantial variations between age categories (P = 0.000 , P ⩽ 0.05) in independent sample T-Test .

So there were no obvious differences between male and female distributions by Chi-square test for variables at (P = 0.45, P ⩾ 0.05). In Diabetic children, a percentage of a male under 5 years was 12%, while it was 60% in 6-10 years and 28% in ages more than 10 years In Mixed patients (DM1 infected by intestinal parasites) the percentage of a male under 5 years was 13%, while it was 80% in 6-10 years and 7% in ages more than 10 years.

There was a highly obvious difference (P = 0.000, P value ⩽ 0.05) in age distribution between study groups .there was an increase in the number of females than males in DM1 groupsalso, and there are no significant differences in males & females

In Parasitic patients, the percentage of a male under 5 years was 48%, and in 6-10 years was 48%, while it was 2% in ages more than 10 years

The obtained results were 58% of males were infected with intestinal parasitic infection and 42% of females were.

The study included a sample of 50 patients infected by intestinal parasites. Overall, the study found that the patients possessed a single gut parasite infection by Entamoebahistolytica/disparwas the parasite that was found most frequently in this research 36 (72%) then Giardia lamblia 14 (28%) as in figure (4). The occurrence proportion of male intestinal parasitic infection was 29 (58%), while females were 21(42 %). A linkage between Intestinal Parasitic-Infections (IPIs) with sex was statistically non-significant. The research s results revealed that the occurrence rate was highly obviously differ according to the children's age (P = 0.000) with the most frequently occurring in the age category 0-5 years was 50%, whereas the low-frequently was 11 % in categories of age 10 to 15 years[12].

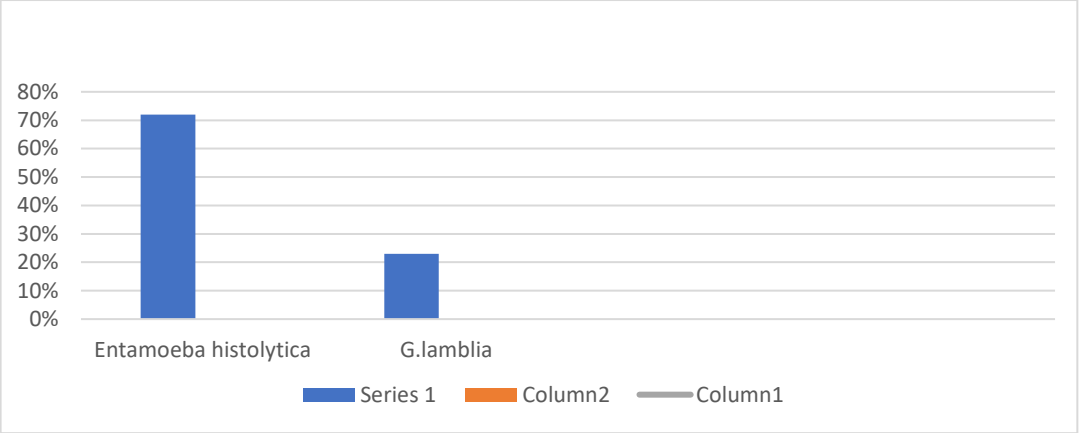

Figure (4) the percentage of parasitic infections in parasitic groups

In the present study, the investigation of the interleukins in children with T1D. showed that the concentration of interleukin - 2, interleukin -10, and PG-E2 in (diabetic patients, mixed patients [DM1 infected by intestinal protozoa], patients infected by intestinal protozoa, and healthy controls)

Interleukin -2 Concentration:

Interleukin -2 (IL-2) was high concentration &highly significant differences (p-value = o.ooo) in diabetic patients and diabetic patients with intestinal parasitic infections (89.5±37.8 ng/l and 48.5±29 ng/l respectively compared with control 25.4±7.6 ng/l,), While the concentration. is low in parasitic infections at (19.2±4.2 ng/l), as shown in table (2).

Table (2) Interleukin-2 concentration in study groups

Studied groups		IL-2	P value
A-Diabetes mellitus DM1	M*± SD**	89.5±37.8	∓
B-Parasitic Infections	M*± SD**	19.2±4.2	
	Significance between A, B	significant (P ≥0.05)	0.000 HS
C- Mixed (DM1 infected by parasites)	M*± SD**	48.5±29	
	Significance between A, C	Not significant (P ≥0.05)	0.209 NS
D- Controls	M*± SD**	25.4±7.6	
	Significance between A, D	Significant (P≤0.05)	0.000 HS
	Significance between B, D	Significant (P≤0.05)	0.000 HS
	Significance between C, D	Significant (P≤0.05)	0.000 HS
	(∓: independent - sample T- test ;NS: Not- Significant at p ≥0.05;HS: High Significant at p ≤ 0.05;* Mean; ** Standard deviation)		

There were no obvious differences in IL-2 concentration between Entamoeba histolytica(28.7± 22 ng/l) and Giardia lambliainfections (29.6±22.2 ng/l) (P= 0.829, P⩾0.05) in parasitic and mixed groups as shown in table (3).

Table (3) Interleukin-2 concentration in Entamoeba histolytica and Giardia lambliainfections

IL-2		N	Mean	Std. Deviation	Sig
	Entamoeba histolytica	53	28.77	22.040	0.829
	Giardia lamblia	22	29.65	22.200	

There were no obvious differences (P=0.463, P⩾0.05) in mean IL-2 levels between 98 males and 82 females as shown in table (4).

Table (4) Interleukin-2 concentration in Male and Female

IL-2		N	Mean	Std. Deviation	sig
	MALE	98	51.98	42.024	0.463
	FEMALE	82	51.71	39.700	

Regarding the age distribution of IL-2, there were high obvious differences among age groups of patients 0 - 5 years,6-10 years (P = 0.000), highly obvious differences (P = 0.000) in 0 - 5 years and 10-15 years, and no significant differences 6-10 years and 10-15 years at (P=0.495),

There was a highly obvious correlation between IL - 2 and IL - 10 (0.745) in mixed groups and no significant correlation with other parameters in study groups as shown in table (5)

Also, the IL-2 amounts and (IL-10, and PG-E2) are negatively (inverse) correlated; i.e., as serum amounts of IL-2 increased,amounts of (IL-10 and PG-E2) tend to decrease as illustrated in table (6)

Table (5): The correlation of IL-2 with other parameters in study groups.

Characteristic	Study's groups			
	Control	Parasitic	Mixed	DM1
Gender	- 0.339	- 0.054	- 0.023	- 0.122
Age	- 0.282	- 0.117	- 0.095	- 0.023
IL-10	- 0.032	- 0.120	0.745 **	0.098
PG-E2	0.144	- 0.108	0.320	- 0.030

** means high significance correlation

Table (6): Correlation of IL-2 with other study's parameters

IL2		IL-10	PG-E2
	Pearson Correlation	-.247**	-.312**
	Sig. (P-value)	.001	.000

Interleukin -10 Level

The level of interleukin IL-10 in children with parasitic infections was (93.4±64.6 ng/ml), which is higher than those of healthy control (25.4±6 ng/ml) And in mixed groups (82±59 ng/ml) While the concentration of the interleukin-10 (IL-10) is decreased in T1DM (21.5±7 ng/ml), as shown in table (7)

Table (7) Interleukin-10 level in study groups

Studied groups		IL-10	P value
A-Diabetes mellitus DM1	M*± SD**	21.5±7.1	∓
B-Parasitic Infections	M*± SD**	93.4±64.6	0.000 HS
	Significance between A, B	significant (P ≥0.05)	
C- Mixed (DM1 infected by parasites)	M*± SD**	82±59	0.000 HS
	Significance between A, C	Significant (P<0.05)	
D- Controls	M*± SD**	25.4±6	0.424 NS
	Significance between A, D	Not significant (P ≥0.05)	
	Significance between B, D	Significant (P ≤0.05)	0.000 HS
	Significance between C, D	Significant (P≤0.05)	0.000 HS
	(∓: independent sample T- test ;NS: Not- Significant at p ≥0.05;HS: High -Significant at p ≤ 0.05;* Mean; ** Standard deviation)		

There are no significant differences in IL-10 concentration between Entamoeba histolytica(89.8± 65.5 ng/ml) and Giardia lambliainfections (89.4±56.6 ng/ml) (P= 0.54, P≥0.05) in parasitic and mixed groups as illustrated in table (8).

Table (8) Interleukin-10 concentration in Entamoeba histolytica and Giardia lambliainfections

IL-10		N	Mean	Std. Deviation	sig
	Entamoeba histolytica	53	89.88	65.531	0.558
	Giardia lamblia	22	89.43	56.670	

The Sex distribution There were no obvious differences (P=0.445 ,P⩾0.05) in mean IL-10 levels between 98 males & 82 females as shown in table (9).

Table (9) Interleukin-10 concentration in Males and Female

IL-10		N	Mean	Std. Deviation	sig
	MALE	98	50.24	51.379	0.445
	FEMALE	82	51.24	53.955	

In the age distribution of IL-10, there were obvious differences among age groups of patients 0-5 years,6-10 years (P=0.007), no obvious differences (P=0.287) in 0-5 years and 10-15 years, and no obvious differences 6-10 years and 10-15 years at (P=0.446)

A high obvious positive- correlation between interleukin -10 and IL-2 concentration in mixed groups and a non-significant correlation between IL-10 and other parameters in study groups as in table (10)

Also,the present study found that serum concentration of IL-10 and PG-E2 were a high significantly positive correlation i.e., asserum concentration of IL-10 increased,PG-E2 levelstend to increase and negatively correlated with IL-2;i.e.,whenthe serum level of IL-10 increased, a level of IL-2 tended to decrease as illustrated in the table (11).

Table (10): The correlation of IL-10 with other parameters in study s groups.

Characteristic	Study's groups			
	Control	Parasitic	Mixed	DM1
Gender	0.009	0.117	0.084	0.128
Age	0.459	0.133	- 0.332	- 0.019
IL-2	- 0.032	- 0.120	0.745 **	0.098
PG-E2	- 0.160	0.379	0.470	0.037

** means high significance correlation

Table (11): The correlation of IL-10 with other parameters

IL10		IL-2	PG-E2
	Pearson Correlation R	-.247**	.640**
	P value(sig)	.001	.000

** means high significance correlation

Prostaglandin PG -E2 Concentration

Prostaglandin PGE2 concentration was not statistically significantly lower (14.5±6.3 pg/ml) in DM1 patients as compared with controls (15.9±6.8 pg/ml) (p=0.58), with Higher concentration. In parasitic infected patients and mixed groups (94.5±77, 89±37pg/ml) respectively as compared with controls (15.9 ±6.8 pg/ml) PGE2 concentration was statistically obviously increased (P=0.000, P ⩽0.05) (89±37 pg/ml) in patients infected with the parasite if compared with controls (15.9 ±6.8 pg/ml) and diabetic (P=0.000, P ⩽0.05)((14.5±6.3 pg/ml),as shown in table (12)

Table (12) Prostaglandin PG-E2 concentration in study groups

Studied groups		PG-E2	P value
A- Diabetes mellitus DM1	M*± SD**	14.5±6.3	∓
B- Parasitic Infections	M*± SD**	94.5±77	0.000 HS
	Significance between A, B	significant (P ≤0.05)	
C-Mixed (DM1 infected by parasites)	M*± SD**	89±37	0.000HS
	Significance between A, C	significant (P ≤0.05)	
D- Controls	M*± SD**	15.9 ±6.8	
	Significance between A, D	Not significant (P ≥0.05)	0.584 NS
	Significance between B, D	Significant 0.000 (P ≤0.05)	0.000 HS
	Significance between C, D	Significant 0.000 (P≤0.05)	0.000 HS
	(∓: independent - sample T- test ;NS: Not-Significant at p ≥0.05;HS: High - Significant at p ≤ 0.05;* Mean; ** Standard deviation)		

There were no obvious differences in PG-E2 concentration between Entamoeba histolytica (101±53.6 pg/ml) and Giardia lambliainfections (88.9± 43.7 pg/ml) (P= 0.066, P≥0.05) in parasitic and mixed groups as illustrated in table (13).

Table (13) Prostaglandin PG-E2 concentration in Entamoeba histolytica and Giardia lambliainfections

PG-E2		N	Mean	Std. Deviation	sig
	Entamoeba histolytica	53	88.91	43.721	0.066

	Giardia lamblia	22	101.91	103.499	

There were no obvious differences (P=0.375, P⩾0.05) in mean PG-E2 levels between 98 males & 82 females as shown in table (14).

Table (14) Prostaglandin PG-E2 Concentration in Male and Female

		N	Mean	Std. Deviation	sig
PG-E2	MALE	98	51.42	65.604	0.375
	FEMALE	82	42.56	46.446	

Regarding the age distribution of PG-E2, there were nonobvious differences with age groups of patients 0-5 years,6-10 years (P=0.490), highly obvious differences (P=0.000) in 0-5 years and 10-15 years, and significant differences (P =0.004) between 6-10 years and 10-15 years.

There were positive obvious correlation among PG-E2 and (IL-10 and IL-2) in mixed groups and significant correlation between PG-E2 and interleukin -10in parasitic groups ;i.e., as serum levels of PG-E2 increased ,IL-10 and IL-2 level tend to increase as in table (15)

Also, the present study found that serum concentration of PG-E2 and interleukin -10 were highly positively correlated;i.e.,as serum concentration of PG-E2 increased,IL-10 levels tend to increase and negativelycorrelated with IL-2,i.e.,when serum levels of PG-E2 increased, the level of IL-2 tended to decrease as shown in table (16).

Table (15): The correlation of PG-E2 with other parameters in the study's groups.

Characteristic	Study's groups			
	Control	Parasitic	Mixed	DM1
Gender	- 0.085	- 0.122	0.235	0.035
Age	- 0.166	0.050	- 0.450	0.221
IL-2	0.144	- 0.108	0.320	0.030
IL-10	- 0.160	0.379	0.470	0.037

Table (16): The correlation of PG-E2 with other parameters.

		IL10	IL2
PGE2	Pearson Correlation	.640**	-.312**
	Sig. (2-tailed)	.000	.000

** means high significance correlation

Discussion

In Diabetic patients, the percentage of the age presentation agreed with [13, 14] found that The predominant age of children of DM1 is between 4 and 6 and early- puberty (10 -14 years).the distribution between study groups this result was also certified by [15] who mentioned that The occurrence of TID has increased rapidly over recent decades, especially in young children, and also [16]who mentioned the occurrence of type-1 and type-2 diabetes has increased obviously in young - children but at a low - rate in current years. While there are non-significant differences in Sex among study groups (p=0.45, P⩾0.05)

Also [17, 18] proved the same age groups The elevated occurrence early life is the time when islet- autoantibodies are first developed (4 to 6 years) .,[19]the occurrence of T1DM in children aging between 0 - 14 years. And [20] The younger children (aged 0.5 – 4 years).

As seen in relatives with type 1 diabetes, this early - peak also suggests that the risk of developing islet autoantibodies decreases with age and that age affects the child's risk for developing islet autoantibodies.

Also, there are no significant differences in males & females that disagree with [21] who certify The immunological aggressiveness of the disease is variable and varied with sexes at a higher occurrence in girls. But the results of percent agree with this study (37, 52%) in diabetic females.

Other results mentioned that higher DM was found in females, and high-risk HLA-DR and HLA-DQ subtypes were prevalent; however, it was inversely related to the

earliest age at which positive autoantibodies first appeared. [22, 23].

Early in the diagnosis of the disease, girls appeared to have worse glycemic control and signs of a severe metabolic disorder. Only GADA positivity was more prevalent in girls, while boys tested more positively for three of the four biochemical auto-antibodies (IAA, IA-2A, and ZnT8A). [20].

The age distribution In Parasitic patients agreed with [24] who mentioned that (Intestinal parasitic-infections IPI) are a global health issue, particularly in developing nations. 450 million people, mostly children, are affected by intestinal parasites, which affect 3.5 billion people worldwide. The age range from 1 to 9 years sees the highest incidence of intestinal parasites. [25] Children aged 6 to 18 reported an increased incidence rate. [26]All age groups were infected with intestinal parasites, but children under the age of 2 and those between the ages of 2 and 3 were, respectively, 4.7 and 2.6 times more likely than children aged 3-5 to contract an intestinal parasite.

Also, the sex distribution of Intestinal Parasitic Infections was higher in a male which matched with [27] and found that the male gender was significantly associated with intestinal parasiticinfectio n at 56.9%. and age less than 10 years.

The type of parasite predominant in the present study was Entamoeba histolytica infections more than Giardia lambliaThese results matched with [28] who found nearly the same results prevalence of entamoeba histolytica (73%).

Interleukin IL-2 Concentrations:

Interleukin IL-2 Concentration was elevated in diabetic patients and diabetic patients with intestinal parasitic infections the obtained results agreed with [29, 30] It was founded that Children with long-term T1D had higher concentrations of IL-2 than healthy children. While other studies show opposite results which found that interleukin IL-2 levels revealed an obvious decrease in all diabetic children [31]. While the concentration. is low in parasitic infections this result was agreed withthat agreement with [32, 33] they mentioned that A temporary decrease in frequency and an elevated in the absolute amount of T-reg cells were brought on by the acute reactions to the pathogens. The start of strong host protection and Th1- responses against the pathogens depended on the infection-induced partial decrease of T-reg cells. The expansion of CD4+ T cells with specificity for pathogens with a constrained ability to produce IL-2 was the source of the observed decrease of T-reg cells, other results found that strengthen when disease-reversing IL-2 signaling occurs, regulatory T-cell function is primarily what provides protection. [34].

And also [35][36]discovered that little doses of IL-2 therapies Dysregulate the immune system associate with the breaking of immune regulation, leading to auto-immune disorders, such as (T1D).

Interleukin IL-10 Level

The level of interleukin IL-10 in patients with parasitic infections was higher than in healthy control the obtained results agree with the other studies' results that found an obvious elevated in the level of interleukins IL-10 in children, was higher than those of healthy control [37]. also, parasite-infected patients had the highest measured concentration of this cytokine, with 4.24-fold increases in IL-10, compared to the control [38].

And the elevated level of IL-10 in mixed groups matched with [39, 40] who approved the Parasites\ or their products could increase IL-10 levels in vivoin different ways. Increased IL-10 can transform the anti-inflammatory cells, such as T-regs, and stop the development of auto-immune diseases. [41]

B-regs' primary biological function is to suppress inflammatory Th1 and Th17 responses that are mediated by cells' interactions with T- cells and produce the IL-10, [42] An anti-inflammatory effect is produced by regulatory B cells (B-regs), a subset of B cells that produce and secrete the inhibitory factor interleukin-10 (IL-10)While the level of the interleukin-10 (IL-10) are low in T1D agree with [42, 43] they approved that concentration of the (IL-10) are low in T1D.

Also [44] had the same results The primary concept is that certain parasites and their byproducts can be used as immunomodulatory agents to create innovative, hopeful therapeutic medicines for the cure of auto-immune diseases. There is some evidence that suggests parasites control interleukin-10, the modification of IL-10, a protein that T- helper cells regulate, lowers the risk of auto-immune illness and aids in maintaining low levels of inflammation in the gut.

Parasites can reduce the host's immunological reaction to themselves (parasite-specific immuno-regulation). These seem capable of inhibiting immunological reactions to pathogens, antigens, auto-immune, and metabolic - diseases during chronic infection. By the stimulation of regulatory T cells or Th2-type cells, parasites cause immuno-regulation. This is done through secreted or expelled parasite - metabolites, proteins, or extra-cellular vesicles (or a mix of these) by activating signaling pathways in host cells to modify their hosts' immune responses [45] .

There are no significant differences in IL-10 concentration between Entamoeba histolytica and Giardia lambliainfections in parasitic and mixed groups this is not matched with [46, 47]they improved that G. lamblia had elevated levels of serum IL-10, (mean 100 ng/ml)compared to controls.

Prostaglandin PG-E2 Concentration:

Prostaglandin PG-E2 concentration was not statistically significantly low in DM1 patients in comparison with the controls the obtained results were also approved by [48] who mentioned the anti-inflammatory effect of PG-E2.

Higher concentration. In parasitic infected patients and mixed groups (109±98,107±122 pg/ml) respectively as compared with controls that agree with [49].

Diabetes mellitus with poor management causes several comorbidities, including an increased risk of infections. Although hyperglycemia improves phagocyte reactivity, the antibacterial capacity of immune cells from diabetic individuals is suboptimal [50].

Through the inhibition of a Th1 response, PGE2 may also be crucial in tipping the balance toward a Th2 response. Human DCs' control over cytokine production and macrophage responses can both contribute to this.[51] Prostaglandins-E2 are group of vital lipid- mediators that have a key role in influencing the onset, activation, keeping, effector activities, and type 2 inflammation resolution. They are secreted during type 2 inflammation.

PGE2 concentration was statistically obviously elevated in patients with the parasite infection in comparison with controls and diabetics. that agrees with [52] who mentioned that Lipid droplets are also exploited by the pathogen to aid in adhesion, promote pathogenesis, and modify host physiology to evade the immune system. Lipid particles served several purposes for the parasites, including altering the recipient cells' permeability and fragility, making it easier for encouraging growth, invasion, and the organism's most effective form of reproduction by allowing the parasite to penetrate the host cell membrane. The lipid is a vital growth stimulant for anaerobic groups of parasites, boosting virulence, promoting encystation and vesicle formation, as well as promoting immune system activation and dendritic cell maturation. Prostaglandins can control the link between parasite infection and allergies, as well as the possible involvement of PG-E2 generated by parasites in the regulation of host-pathogen reactions. novel treatment options for those suffering from Type 2 inflammatory illnesses, such as allergic rhinitis and asthma, that include a large prostaglandin-driven component [49].

prostaglandins concentration was statistically significantly increased ($P \leqslant 0.05$) in mixed groups if compared with controls and this result clarifies the modulatory effect of parasitic infections & invasion of the immune response to be chronic infections that improved by [52, 53], while in diabetic groups the decrease concentration of PG-E2 and no significant differences with controls that disagree with [54] who found that Diabetics had high levels of both PGE2 and IL-1 β as compared to controls.

The condition known as sterile inflammation, which is characterized by a mild inflammatory response, is considered to be brought on by hyperglycemia. While tissue healing and pathogen clearance are both dependent on inflammation, persistent and long-lasting inflammatory responses harm tissue, which results in immunopathology and poorer disease outcomes [55][56].

However, PG causes a wide spectrum of biochemical reactions linked to inflammation. PGE2 production is increased, and it has the potential to signal via four separate main receptors. To trigger either pro-inflammatory or regulatory immune responses, PGE2 concentrations and particular receptor signaling are essential. PGE2 weakens the ability of phagocytes, making the host more vulnerable to bacteria, fungi, viruses, and protozoan parasites, according to several studies [56].

Conclusion

The finding of this research demonstrated that the level of IL-2 in the mixed group was lower than DM1that reflects

the anti-inflammatory effect of protozoal to decrease the severity of Th1-cytokines on islets cells also increased level of IL-10 and PG-E2 in the mixed group clarify roles of protozoal infection with its anti-inflammatory function by Th2 cytokines. The parasitic infection has been shown to modify the immune- response linked with auto-immune dis-orders. It is obvious to decrease the intensity of type 1 diabetes by changing the mechanisms by which Th1-mediated death of insulin-producing cells occurs. So decrease of Auto-immune Type 1 Diabetes by Gastro-intestinal parasitic Infestation.

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