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Microbiological and Immunological Studies on Type I Diabetes Mellitus Patients

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Abstract: Type 1 diabetes mellitus is the most common chronic condition in children, although it can strike anyone at any age. T1DM has been steadily increasing in incidence and prevalence, accounting for about 5% to 10% of all diabetics. Environmental factors such as dietary variables, endocrine disruptors and other environmental pollution, and gut microbiome composition have all been linked to type 1 and type 2 diabetes. Obesity and insulin resistance, in addition to their well-known involvement in type 2 diabetes, may act as type 1 diabetes accelerators. In contrast, in a fraction of persons diagnosed with type 2 diabetes, islet autoimmunity linked to probable environmental factors (e.g., food, infection) may play a role.

keywords Microbiological, Immunological, type I diabetes mellitus

1.Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune illness caused by the loss of beta cells in the pancreas. T1DM can strike at any age, but it is more common in adolescents, with a peak onset around puberty. T1DM is equally frequent in both sexes during childhood, although males are more likely to develop the condition in early adulthood [1]. Although it was once most frequent among Europeans, it is now spreading to other ethnic groups. According to the 2015 Atlas of the International Diabetes Federation (IDF), 415 million people globally have diabetes. By 2040, this number is expected to rise to 642 million [2].

T1DM accounts for 5-10% of all diabetes cases and is one of the most common autoimmune disorders in childhood. T1DM is becoming more common in all populations. In Europe, it is expected that the incidence of T1DM in children under the age of five will double in less than 20 years [3]. Type 1 diabetes has no recognised cause [4]. However, it is thought to be the result of a mixture of hereditary and environmental influences [5]. Having a family member with the disease is one of the risk factors. The autoimmune death of insulin-producing beta cells in the pancreas is the underlying process [5]. T1D is an autoimmune illness that results in the loss of pancreatic b cells, which is defined histologically by insulitis (i.e., islet cell inflammation) and b-cell damage. It's still a mystery why autoimmunity in T1D is limited to the insulin-producing B cells [6].

Many evidences revealed that environmental factors like diet, physical activities and gut microbiome are considered essential modulators of DM [6]. The gut microbiome is usually considered a functional and measurable organ [7].

The composition of gut microbiota varies along the gut and differs from one to another according to nutritional status and dietary lifestyle [8]. Mammalian gut microbiota mainly involves four main phyla: Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria, which are essential for the host metabolic activity and physiology upregulation [9]. Gut microbiome diversity is very essential for

immune system development and consequently protection from several diseases [10].

Aim of the Work

The work was planned to investigate the immune and microbiological factors of type 1 diabetes patients.

2.Materials and methods

Collection of Blood and Stool

52 Clinical samples of blood and stool were collected from patients admitted to MUHC and 20 healthy groups. Samples were collected under aseptic conditions Samples of stool were collected under aseptic conditions after 3 days of stopping antibiotics if it was taken. The Immunological examinations of blood samples (ICA, ANA, and ADNA) were performed for all type 1 diabetic patients and healthy groups. Part of the stool was inoculated in cooked meat media (Colombia, Blood and MacConkey's agar media) and mixed thoroughly for aerobic and anaerobic cultivation for all type 1 diabetic patients and healthy groups.

Stool culture

Part of the stool was inoculated in cooked meat media (Colombia, Blood and MacConkey's agar media) and mixed thoroughly for aerobic and anaerobic cultivation. For isolation of anaerobic bacteria, processing of primary anaerobic plates was performed as soon as possible and incubated in an anaerobic jar in presence of nitrogen Gas Pack and resazurine indicator strip immediately at 35-37 °C and examined after 3-7 days.

One drop of homogenized stool sample was transferred with a micropipette to blood agar plate for incubation under aerobic condition and Columbia blood agar plate supplemented with vitamin K and hemin (Sigma Aldrich, USA) for incubation under anaerobic condition in an anaerobic Jar.

Detection of autoantibody in the sample

After the blood samples were collected in a test tube and after almost half an hour, the serum was separated by a centrifuge, then the serum was withdrawn by a pipette and stored in an Eppendorf tube in the refrigerator freezer. After all samples were collected, immunological tests were performed on all blood samples using the ELISA device in the immunology laboratory In the Immunology and Microbiology Unit at the Faculty of Medicine, Mansoura University

3. Results and Discussion

Detection of autoantibodies (ANA, anti DNA and ICA)

Out of the 52 patients (Group A) with T1D 26 (50.0%) suffered from autoimmune disease (ICA) and 5 suffered from ANA was found in 52 (9.62 %) patients and two additional autoimmune diseases (Anti-dsDNA) in 52 (3.84 %) patients (Table 1).

While 20 health groups (Group B) did not suffer from any immune disease from the three diseases (ICA, ANA, and Anti-dsDNA).

Table (1): Result of Autoantibodies Positive and Negative

Test	Group A 52 cases	Group B 20 control
ANA	+Ve 5 (9.62 %) -Ve 47 (90.38 %)	+Ve 0 (0 %) -Ve 20 (100 %)
Anti-dsDNA	+Ve 2 (3.84%) -Ve 50 (96.15%)	+Ve 0 (0 %) -Ve 20 (100 %)
ICA	+Ve 26(50.0%) -Ve (50.0 %)	+Ve 0 (0 %) -Ve 20 (100 %)

Distribution of Gut microbe in study groups

Seventy-two stool samples were collected. Group A consisted of 52 patients who were admitted to different Mansoura University children's hospitals. Group B consists of 20 health groups. All samples of stool were cultured on blood agar, MacConkey's agar and Colombia Blood agar.

Table (2): Result of Gut Microbe in stool culture in cases T1D and control.

Organisms	Group A (52) cases	Group B (20) control
E.Coli	27 (51.92 %)	13(65%)
Enterobacter ssp.	18(34.6)	0(0%)
Citrobaceria ssp.	3 (5.76%)	3 (15 %)
Sallmonella ssp.	3 (5.76%)	4 (20 %)
Proteus Vulgaris	1(1.92%)	0(0%)
Klebsiella ssp.	1(1.92%)	0(0%)

Autoantibodies and gut microbes

Antibodies play an essential role in the immune systems, as 27 patients had antibodies in the blood (ICA, ANA, and anti-dsDNA) and it was observed that 13 patients had antibodies with Escherichia coli, 6 with Enterobacter

cloacae and 2 Citrobaceria as described in table (3) and Figure (1).

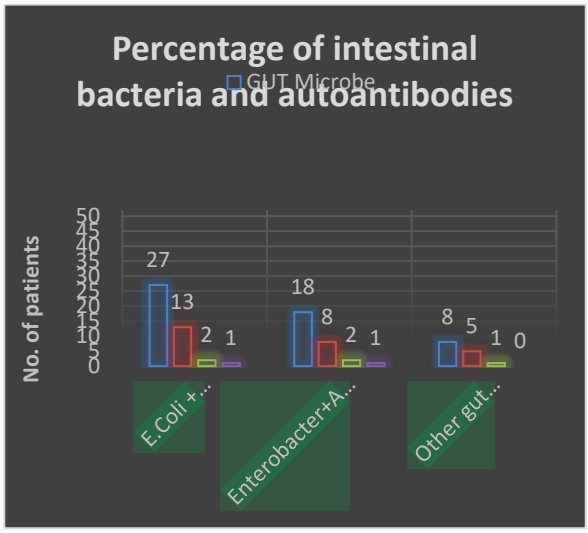

Figure (1): The percentage of intestinal bacteria and antibodies in diabetic patients

Table (3): Result of autoantibodies positive and the gut microbe in group A Type 1 diabetes cases

ANA	Anti-dsDNA	ICA	Organisms
7.3	13.4	47.7	E.coli
9.55	13.41	63.54	E.coli
28.4	15.9	53.7	E.coli
15.9	16.9	51.7	Citrobaceria
16.4	14.6	49.3	Sallmonella
18.9	20.5	48.2	E.coli
15.9	18.2	58.21	E.coli
12.3	11.52	43.75	E.coli
27.3	14.7	40.12	Enterobact cloacae
20.5	16.5	43.41	Enterobacter agglomerans group
13.4	11.4	51.49	Enterobact cloacae
27.1	12.1	43.6	Proteus vulgaris
32.5	27.4	17.2	Enterobacter
14.2	17.9	53.2	klebsiella
14.2	16.2	64.43	E.coli
17.1	20.3	47.2	E.coli
12.44	16.9	45.21	Enterobacter cloacae
29.4	29.4	60.0	E.coli
17.5	15.2	48.3	Enterobacter cloacae
9.8	13.9	46.6	Citrobaceria
13.2	15.4	45.2	Enterobacter cloacae
19.8	20.5	60.35	E.coli
18.9	21.3	41.2	Enterobacter
22.3	12.9	43.86	E.coli
13.4	15.6	43.1	E.coli
20.5	15.9	45.34	E.coli
13.4	19.6	48.0	Enterobact cloacae

N.V OF ANA (More than 25), N.V of ADNA (More than 25 and N.V of ICA (More than 40)

In group B there are no positive autoantibodies.

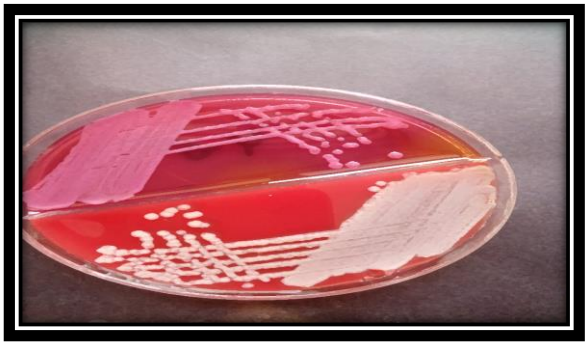

Photo (1): Growth of E.coli on Blood and MacconkeyvAgaar

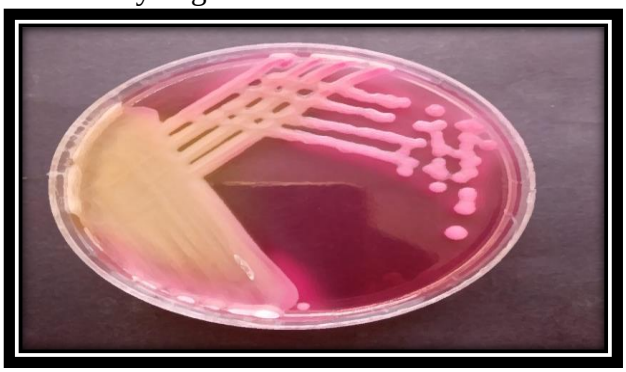

Photo (2): Growth of Enterobacter on Macconkey Agar.

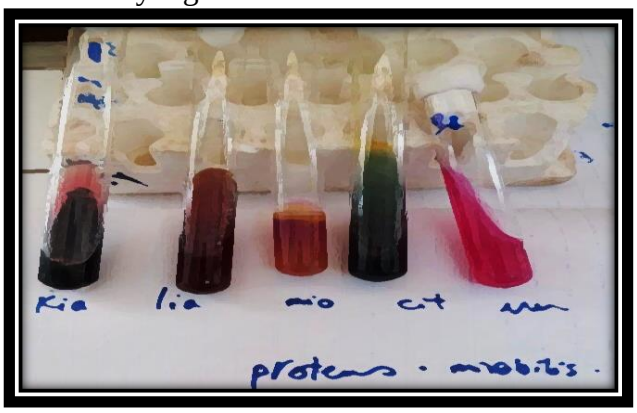

Photo (3): Biochemical reaction of Proteus vulgaris

Discussion

T1DM is caused by the loss of insulin-secreting beta cells in the pancreas' Langerhans islets. As a result, there is a total lack of insulin. Although evidence of autoimmune was not detected in a small percentage of individuals, autoimmune is thought to be the pathologic basis of T1DM. Clinical symptoms usually occur when beta-cell loss reaches 90% or more [11- 12]. The pathophysiology of T1DM is complicated by interactions between genetics, epigenetics, environmental variables, and immunologic factors. Gene damage has been linked to an increased risk of T1DM, and 40

gene loci linked to T1DM have been discovered [11–13].

Viral infection and nutrition are two environmental factors linked to T1DM. T1DM has been linked to congenital rubella syndrome and human enterovirus infection. T1DM is thought to be linked to cow milk consumption, early cereal eating, and low maternal vitamin D levels, while more research is needed [11].

In addition to the existence of antibodies in the affected person, Enterobacter and Escherichia coli are two of the most common causes of type 1 diabetes. The microbiological and immunological causes have a great impact on diabetes, and there have been many studies confirming these causes. It was found that out of 52 diabetic patients, 27 suffer from (E), 18 Enterobacter, and 7 with other intestinal microbes. And as for the antibodies, it was ICA the most prevalent (50.0%), ANA (9.62 %), Anti-dsDNA (3.84%) and normal (36.54%) in 52 diabetic patients, while in the healthy group there is no presence of any antibody.

It was found in this study that there is a microbiological and immunological association in TIDM patients, but larger studies are still needed to confirm this relationship.

Conclusion

T1DM is caused by a mixture of hereditary and environmental factors. It is caused by the killing of insulin-producing cells in the pancreas by the immune system. In genetically vulnerable people, Eisenbarth postulated that one or more environmental variables, such as enteroviruses, nutritional factors, or toxins, can induce the development of T-cell dependent autoimmunity [14].The conclusion of this study is that the intestinal microbes have a great influence on the incidence of type 1 diabetes, as they cause disorders and imbalance in the work of autoimmunity

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