A Study of Fecal Microbiota in Newly Diagnosed Egyptian patients with Type 1 Diabetes Mellitus

Thesis

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Youssef Heshmat William

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List of Abbreviations

AUC : Area under the curve

BMI : Body mass index

CDC : centers for disease control

CS : Caesarian section

ELISA : Enzyme-linked immune sorbent assay

GAD : Glutamic acid decarboxylase

HBA1C : Hemoglobin A1C

HLA : Human leukocyte antigen

IAA : Anti insulin antibody

ICA : Islet cell antibody

PCR : Polymerase chain reaction

ROC : Receiver operating characteristics

SD : Standard deviation

T1DM : Type one diabetes mellitus

T2DM : Type 2 diabetes mellitus

WHO : World health organization

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Abstract

Background: Type 1 diabetes mellitus (T1DM), characterized by Beta-cells destruction and insulin deficiency, is one of the most common autoimmune diseases in childhood that leads to significant burden and mental problems. Gut microbiota, habitating in human intestinal tract, comprises a total genome that is near 150 times more than the human genome**..** Recently, it has been proven that gut microbiota plays an important role in regulating metabolic functions and is associated with many diseases such as obesity, insulin resistance, autoimmune diseases, and tumor.

Objectives: is to evaluate the alteration of gut microbiota between children with newly diagnosed T1DM and healthy controls and to determine if gut microbiota could partly explain the etiology of this disease.

Methods: This case control study was carried out on 50 children, 30 newly diagnosed TIDM patients (less than 6 months) recruited from Diabetes clinic, children hospital, Ain Shams University. In addition 20 age, gender, race, mode of delivery, and duration of breastfeeding matched controls were recruited in the study. All participants were subjected to full history, clinical examination, and laboratory measurement of HbA1C, fasting C peptide (in patients), anti insulin and anti islet cell antibody titre and fecal microbiota assessment in stool.

Results: In this study the total number of microbiota is significally less in patients with diabetes mellitus in contrast with healthy controls, also total number of microbiota was significally decreased in antibody positive than antibody negative patients. The proportion of Dysgonomonas, Parabacteroides, Clostridium, Ruminococcus, Bilophila and Trabulsiella were significantly increased in healthy controls in comparison diabetic patients. While proportion of prevotella, Faecalibacterium, Veillonella and Dialister were significally decreased in healthy controls in comparison to diabetic patients.

Conclusion: The gut microbiota was associated with the development of T1DM by affecting the autoimmunity, and the result suggested a potential therapy for T1DM via modulating the gut microbiota.

Keywords: Type one diabetes Mellitus, anti insulin antibody, anti islet cell antibody, fecal microbiota.

Introduction

Type 1 diabetes mellitus (T1DM), characterized by Beta-cells destruction and insulin deficiency, is one of the most common autoimmune diseases in childhood that leads to significant burden and mental problems **(ISPAD, 2014)**. In addition, the worldwide prevalence of T1DM keeps increasing in recent years. The overall increase rate in Europe is about 3%-4% per annum, and the incidence is anticipated to double by 2020 **(Patterson, 2012).**

However, the pathogenesis of T1DM has not been clearly understood yet. Nowadays, it has been acknowledged that T1DM is not only caused by genetic susceptibilities, such as human leukocyte antigen *DRB1* and *DQB1* alleles, **(Keskin, 2012)** but also more importantly by environmental factors, because the increasing incidence might not be explained only by host genetic factors. Reports have shown that lack of breastfeeding and viral infections would contribute to the development of T1DM, **(Patelarou, 2012).** The intestinal microbiota, as another environmental factor currently under study, might also play a role in T1DM, **(Rodriguez-Calvo, 2015).**

Gut microbiota, habitating in human intestinal tract, comprises a total genome that is near 150 times more than the human genome **(Lozupone, 2012).** Recently, it has been proven that gut microbiota plays an important role in regulating metabolic functions and is associated with many diseases such as obesity, insulin resistance, autoimmune diseases, and tumor **(De Vos, 2012).**

The role of the intestinal microbiota as an integral determinant of human health has become increasingly evident over the past decade **(Borody, 2012, de Vos, 2012, Lozupone, 2012).** The human host and its intestinal microbes have co-evolved, resulting in the development of complex immune mechanisms that confine the commensal bacteria to the intestinal lumen and protect the host from invading pathogens. The intestinal microbiota thrives mainly on diet-derived nutrients, while the host benefits from metabolites (e.g. butyrate) from the microbial fermentation of carbohydrates that are otherwise poorly digested **(Neish, 2009)**.

The intestinal microbiota is one of the environmental factors currently under study, partly as a result of observations in both non-obese diabetic (NOD) mice and BioBreeding diabetes-prone rats, where the use of

antibiotics was shown to prevent the onset of diabetes **(Brugman, 2006 and Schwartz, 2006).** Moreover, a recent study using mice suggested that the development of type 1 diabetes can be prevented through modulation of the intestinal microbiota **(King, 2005).**

Clinical studies found that the gut microbiota composition was different between T1DM children and healthy controls, in terms of decreased ratio of phylum *Firmicutes* to *Bacteroides* **(Soyucen, 2015 and Murri, 2013).** However, there are no such data in Egyptian children with T1DM.

Aim of the work

Is to evaluate the alteration of gut microbiota between children with newly diagnosed T1DM and healthy controls and to determine if gut microbiota could partly explain the pathogenesis of this disease.

Chapter 1
Type One Diabetes Mellitus

Definition

Type 1 diabetes Mellitus is a chronic illness characterized by inability of the human body to produce insulin due to the autoimmune destruction of the beta cells in the pancreas **(Aathira, 2014).**

It is the leading cause of blindness, amputations, and end-stage renal disease, and contributes to premature death. The most frequent age of its onset is 12-13 years, but it may occur at any age, in all racial groups, with equal prevalence (about 1/300) in males and females. The incidence of type 1 diabetes has been increasing in many countries **(Gottlieb, 2002).**

Etiology

Type 1 DM results from autoimmune destruction of the pancreatic beta cells and involves both genetic predisposition and an environmental component. Although the genetic aspect of type 1 diabetes mellitus is complex, with multiple genes involved, there is a high sibling relative risk **(Redondo, 2001)** . Whereas dizygotic twins have a 5-

6% concordance rate for type 1 DM **(Steck, 2005)**, monozygotic twins will share the diagnosis more than 50% of the time by the age of 40 years **(Redondo, 2008).**

The genetic contribution to type 1 diabetes mellitus is also reflected in the significant variance in the frequency of the disease among different ethnic populations. Type 1 diabetes mellitus is most prevalent in European populations, with people from northern Europe more often affected than those from Mediterranean regions **(Andrea, 2010).**

Extragenetic factors also may contribute. Potential triggers for the immunologically mediated destruction of the beta cells include viruses (eg: enterovirus **(Yeung, 2011),** mumps, rubella, and coxsackievirus B4), toxic chemicals, exposure to cow's milk in infancy **(Paronen, 2000)** and cytotoxins. Combinations of factors may be involved. **Lempainen et al**. found that signs of an enterovirus infection by 12 months of age were associated with the appearance of type 1 DM-related autoimmunity among children who were exposed to cow's milk before three months of age. These results suggest an interaction between the two factors and provide a possible explanation