Extended Abstract
Volume 5:4, 2021

Hepatology and Pancreatic Science

Open Access

Study of the gut enterotypes in some Egyptian patients with Type 1 diabetes mellitus

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Abstract

Introduction: Gut microbiota cluster into three enterotypes named the Bacteroides, Prevotella and Ruminococcus. While each person???s microbial ???fingerprint??? is unique, there are specific patterns seen in those that are healthy and those that have specific illnesses.

Objective: The objective of the present study was to identify the enterotypes that are possibly associated with Type I Diabetes Mellitus (T1DM) Egyptian patients as well as their possible role in the course of the disease.

Subjects & Methods: The study included 40 T1DM patients as well as control group of 20 healthy subjects of matched age and sex. Stool specimens were taken from each. Quantitative SYBR Green Real-Time PCR was done for the identification and quantitation of Bacteroides, Prevotella and Ruminococcus which constitute the core of the three major enterotypes.

Results: Enterotype 1 was the most common enterotype detected in T1DM and control cases (75% versus 65% respectively) with no significant differences between the two groups (P=0.418). Regarding enterotype 2 no significant differences was noted between T1DM patients and control group (25% vs. 35% respectively P=0.324). For enterotype 3, it was detected neither in patients with T1DM nor in control cases.

Conclusion: There was no significant difference in the distribution of enterotypes in all study groups. Therefore, collapsing the whole microbiome variations into dominant enterotypes was not appropriate to identify disease association or to be used as a disease biomarker.

Diabetes mellitus (DM) is a diverse metabolic disorder characterized by elevated blood sugar levels as a result of deficiency of insulin secretion, defective insulin action or both . DM can cause complications if uncontrolled, including, stroke, cardiovascular disease and kidney failure . Globally, DM is the ninth major cause of death; one in eleven adults worldwide have DM.The International Diabetes Federation (IDF) ranked Egypt ninth worldwide in number of diabetes cases, with prevalence of 15.56% among adults.

Diabetes cases can be categorized into 3 classes. Type I diabetes (TID) is characterized by the autoimmune destruction of pancreatic B cells. Over the past 50 years there was an increase in incidence of TID that may be attributed to genetic predisposition and several environmental factors including stress and viral infections. Type II diabetes (TIID) represents about 90% of all diabetes cases worldwide. It is associated with an unhealthy lifestyle and diet, obesity, lack of exercise and physical activity, in addition to other poor dietary habits [9]. The third class is known as MODY is a rare but increasingly recognized cause of diabetes in young people. MODY is commonly misdiagnosed as type 1 or type 2 diabetes and, as a result, patients are often inappropriately managed with insulin when they can be more effectively managed with oral sulfonylureas.

Recent research, driven by advances in high throughput 16S rRNA amplicon sequencing and shotgun metagenomics, has established that the gut microbiome includes100-fold or more genes than the human genome. These microbial genes are considered key to metabolic processes with impact to the host, including catabolism of dietary fibers to short-chain fatty acids, amino acid and vitamin biosynthesis, as well as aiding the production of neurotransmitters and hormones. The previous decade witnessed many studies that aimed to explain the role of the gut microbiota in TIID and glycemic control. Recent studies have proposed that disturbance of gut microbiota could influence TIID development]. Significance of diversity of the microbiota in controlling metabolic processes was revealed by Le Chatelier et al. and Cotillard et al. [16] who reported association between low diversity of the gut microbiome with obesity, non-alcoholic fatty liver disease and a higher prevalence of insulin resistance.

Type I diabetes is associated with a well-known genetic mutation in the human leukocyte antigen genes. High incidence of this disease can also be attributed to environmental factors . A study of eight children, four with newly developed TID and four matched controls, found differences in the composition of the gut metagenome between the two groups and reduced diversity in the TID-associated microbiomes. Another study in non-obese diabetic (NOD) mice have demonstrated that germ-free NOD mice are more likely to have diabetes, suggesting a role for the gut microbiota in the development of

autoimmune diabetes.To better understand the features of gut microbiota in TID and TIID, here we investigated the gut microbiome of 47 Egyptian citizens (7 healthy controls, 22 TID and 18 TIID), using the conserved V4 region of the bacterial 16S ribosomal DNA. We compared the composition, diversity and richness (number of species) of the fecal microbial ecosystem of healthy, TID and TIID patients. To our knowledge, this is the first metagenomic study comparing the gut microbiome among TID and TIID patients in Egypt.

Isolated microbial DNA from each sample was used for amplification of the hypervariable region 4 (V4) of the 16S rRNA gene, as described previously . Briefly, the universal 515F/806R primer set was used in a single-step 30-cycle PCR reaction to generate multiplexeddual-indexed library molecules.

Controls included the ZymoBIOMICSTM D6311 Microbial DNA Community Standard II (mock community) and a no-template negative control, which was included in each PCR plate. An additional PCR reaction was conducted using P5 and P7 library amplification primers with 15 cycles to help further amplify these library molecules.

To confirm amplification, libraries were visualized on an agarose gel. Library molecules were purified using Sera-Mag beads (GE Life Sciences) to select for DNA fragments larger than 150bp. Purified library molecules were quantified using Quant-iT Broad-Range dsDNA kit (Invitrogen) according to manufacturer's protocols. An equimolar concentration of each library was combined, and the final pool was brought to a concentration of 4 nM.

This work is partly presented at 14th Euro-Global Gastroenterology Conference