



## **Exploring the Relationship between Bowel Microbiota and Impaired Glucose Tolerance**

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### **Authors' contributions**

Conceptualization, A.E.; validation, A.E; G.E, and A.A.A; formal analysis, A.A.A; investigation, A.E; data curation, G.E; writing—original draft preparation, A.E.; writing—review and editing, A.E, and A.A.A. All authors have read and agreed to the published version of the manuscript.

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## **ABSTRACT**

**Background and Aim:** Because of the large number of genes found in the gut microbiome, it has recently been determined that numerous human microorganisms have significant implications for human health.

**Aim:** The significance of gut bacteria in the development of T2DM was investigated in this study.

**Materials and Methods:** Microbial species were extracted from fecal materials; they were identified and quantified using genomic spectrophotometric equipment, and certain biochemical parameters for Diabetes were quantified.

**Result:** We observed a concentration of firmicutes, Bacteroides, and proteobacteria, with the *Escherichia coli* population predominating. Biochemical parameters reveal a several-fold raised value for some biomarkers in T2DM. In a paired sample test results gave significant differences for all tested pairs.

**Conclusion:** Microbiomes can affect the gut environment and trigger alterations that embolden the development of T2DM, according to study findings.

**Keywords:** *Microbiota; type 2 DM; impaired glucose tolerance.*

## 1. INTRODUCTION

In both industrialized and emerging countries, type 2 diabetes mellitus (T2DM) has become one of the most rapidly expanding public health issues [1]. Understanding the mechanisms responsible for the development of T2DM and thus ways to prevent it requires a more comprehensive understanding of the dynamic development processes of glucose intolerance [2]. Emerging evidence of the function of the gut microbiota as a potential novel component of this epidemic exemplifies the growing evidence that numerous environmental factors contribute to their genesis [3]. The human microbiome comprises 3.3 million non-redundant genes, with over 99 % of them being of bacterial origin, according to metagenomics sequencing [4].

Recently, it was discovered that some human bacteria have significant implications for human health, owing to the large number of diseases that can be related to the gut microbiome due to the large number of genes it contains [5]. There is now a spark of hope that identifying the hub microbiota may help researchers target species responsible for microbe interactions and improve target therapies. T2DM has been increasingly common throughout the years, resulting in a widespread metabolic condition. Our microbiota has now been connected to obesity-related T2DM [6]. The process of energy harvesting, which accounts for the development of obesity and host immune development, is known to involve intestinal flora. The involvement of the microbiota in the progression of prediabetes, which includes insulin resistance, is particularly important [7].

Bacteroidetes and *Escherichia coli* are known to impact the intestinal mucus and glycocalyx layer, reducing permeability [8]. Furthermore, alterations in the gut-junction protein, endocannabinoid system, and intestinal alkaline phosphatase are caused by changes in microbiota-dependent bacteria. Some probiotics have been shown to reduce the onset of diabetes by acting as interleukin (IL), IFN, or even influencing anti-inflammatory IL-10 production, according to research [9]. Other research has found that high fructose-fed rats have lower levels of serum glucose, insulin, leptin, c-peptide, glycated hemoglobin, GLP, inflammatory IL-6, and TNF in adipose tissue, and PPAR- and Glut4 gene expression [10].

Furthermore, testing of lipid profiles after probiotic usage revealed a decrease in serum total cholesterol and low-density lipoprotein, both of which are implicated with T2DM [11]. Microbiota and T2DM have been extensively investigated in diabetes retinopathy, atherosclerosis, cystic fibrosis, diabetic foot ulcer, and Alzheimer's disease. The gut microbiota contributes considerably to unique biological activities such as energy metabolism, gut integrity management, and metabolic signaling, according to accumulating evidence from earlier investigations [12].

The generation of secondary bile acids is a notable example of an endogenous metabolite that has been transformed into a beneficial secondary metabolite by the microbiome. They are very hydrophobic and have the potential to induce colitis when produced in the liver; nevertheless, intestinal bacteria have been shown to convert them to more hydrophilic, less toxic secondary bile acids that block nuclear receptors with important physiologic functions [13]. Therefore, the significance of gut microbiota in the development of T2DM was investigated in this study.

## 2. MATERIALS AND METHODS

After obtaining consent from subjects that had glucose concentrations >190 mg/dl, eighty patients (n=80) fecal and fasting blood samples were taken from known T2DM patients aged 39 to 55 years at the Benha General Hospital, Benha University, Benha, Egypt. Another group of samples was taken from thirty non-diabetic participants (n = 30) with fasting blood glucose levels ranging from 88 to 105 mg/dl.

### 2.1 Determination of Biochemical Tests

The glucose test (Spinreact, Spain) was used to assess glucose concentration, and glycated hemoglobin (HbA1C) was quantified using a chemistry analyzer (Biomed, Egypt). For the automated analysis, an Automated instrument (Beckman) was utilized with the Beckman Assay reagent to measure the levels of insulin.

The Westergreen technique, Naubear counting chamber, and ELISA were used to measure inflammatory features such as erythrocyte sedimentation rate (ESR), white blood count (WBC), and C - reactive protein (CRP).

## 2.2 Determination of Faecal Bacterial DNA

Approximately 3 ml of fecal sample was used to extract bacterial DNA using the standard methodology for ZR fecal DNA miniprep™ D6010 (Halt Research, USA). The concentration of extracted DNA was measured using a UV-Visible spectrophotometer (Nano-Drop, model 13,300, Thermo Fisher Scientific, USA). Samples having a DNA content of >300 g/L, or the equivalent of 60 - 100 ng, were deemed eligible for further analysis and were probed with bacterial 16s primers and cloned plasmid as a reference [14]. To compare quantitative data, the Pearson chi-square test

was employed, and a two-way analysis of variance was utilized to find changes in bacterial abundance with each group utilizing bacterial species and concentration count as factors.

## 3. RESULTS

This study characterized the gut microbiota of 110 people who satisfied the study's inclusion criteria. The taxonomic diversity of fecal microbiomes was studied at the phylum, class, order, genus, and species levels. Firmicutes, Bacteroides, and proteobacteria were found. Table (1) and Table (2) demonstrate our findings in the various subgroups.

**Table 1. The study's species variables and concentrations of chosen microbiota**

Types	Control group	Diabetic group
<i>Staphylococcus</i>	2.6	3.2
<i>Enterococcus</i>	2.4	3.9
<i>Streptococcus</i>	1.9	2.6
<i>Bacteroides</i>	1.6	2.2
<i>Prevotella</i>	1.1	1.5
<i>Helicobacter</i>	1.9	2.1
<i>Enterobacter</i>	1.7	1.9
<i>Klebsiella</i>	2.1	3.3

*10<sup>9</sup>/ml is the unit of measurement*

**Table 2. Link between the optical density of standards and numerical equivalents of some of the major microorganisms**

Types	Number	Median+ SD
<i>Staphylococcus aureus</i>	10	2.1± 0.42
<i>Streptococcus pyogenes</i>	10	1.4± 0.35
<i>Enterococcus</i>	10	1.3± 0.39
<i>E. Coli</i>	10	1.5± 0.29
<i>Klebsiella</i>	10	1.8± 0.98
<i>H.pylori</i>	10	1.4± 0.78
Optical density	10	3.5± 2.23

**Table 3. Diabetics and non-diabetics were tested on a variety of glycaemic and inflammatory markers**

Parameters	Control group	Diabetic group	P-value
FBS (mg/dl)	89.5± 5.42	202.5± 6.59	0.001
Insulin Level (uU/ml)	5.5± 1.35	1.6± 0.89	0.001
HbA1C	5.6± 1.41	10.2± 2.31	0.001
TG (mg/dl)	102.5± 4.56	158.5± 8.56	0.001
TC (mg/dl)	118.5± 6.53	189.5± 7.56	0.001
LDL (mg/dl)	82.5± 3.89	135.5± 4.56	0.001
CRP (mg/l)	2.5± 0.89	6.9± 2.21	0.001
ESR (mm/hr)	5.5± 2.56	22.5± 3.68	0.001
WBC (×10 <sup>3</sup> cells)	4.9± 1.39	11.2± 2.34	0.001

*Data are expressed by mean±SEM; Abbreviations: FBS: Fasting blood sugar; HbA1C: Glycated hemoglobin; TG: Triglycerides; TC: Total cholesterol; LDL: Low-density lipoproteins; CRP: C-reactive proteins; ESR: Erythrocyte sedimentation rates; WBC: White blood cells*

Characteristics thought to be indicators of inflammation and diabetes development, such as glucose concentration, insulin level, glycosylated hemoglobin HbA1C, ESR, C - reactive protein, and white blood count, were used to compare biochemical parameters of T2DM with non-diabetes as revealed in Table (3).

The following are the findings of a statistical comparison of biochemical parameters between diabetics and control groups. Mean and standard error of the mean for glucose  $202.5 \pm 6.59$ , insulin  $1.6 \pm 0.89$ , HbA1C  $10.2 \pm 2.31$ , CRP  $6.9 \pm 2.21$ , ESR  $22.5 \pm 3.68$ , and WBC  $11.2 \pm 2.34$  were all significant at  $P < 0.05$ . The concentrations of parameters in the patients and controls were found to be significantly different between all parameters.

#### 4. DISCUSSION

The recognition that microbiomes and the microbiota population have a role in T2DM is a new and developing finding [15]. Fasting, random, 2 hours post-prandial glucose levels, glycosylated hemoglobin, insulin sensitivity test, and clinical findings of polydipsia, polyphagia, and polyuria have all been used to diagnose T2DM [16]. The complication of diabetes, which has concurrent effects on key organs of the body such as the kidney, nephron, sex organs, and neurons, as well as a high mortality and morbidity rate and related co-morbidities, necessitates a growing body of investigations [17].

Diabetes prevention strategies are still a mystery, with a variety of management alternatives available to alleviate the deteriorating condition [18]. The goal of this work was to identify the pivot microbiota to highlight target intervention as a potential research area, especially because in situ microbiome engineering is becoming a crucial topic.

Changes in the microbiome, microbial metabolome, and their interaction with the biological system have now been linked to several diseases [19]. We found bacteria in the Firmicutes, Bacteroides, and proteobacteria phyla. Modified diets are known to play a role in microbiome diversity. We isolated the bacteria using culture procedures and used spectrophotometric methods to assess their concentration by measuring their turbidity coefficients. Fiber-degrading bacteria responsible for the production of short-chain fatty acids

(SCFA) and other metabolites favorable to the human body are known to be abundant in vegetable-rich foods [20]. Imported or westernized diets, on the other hand, are high in animal protein, high fats, and a plethora of harmful microbial metabolites such as indole derivatives, phenolic acid, and Trimethylamine N-oxide (TMAO), all of which have the potential to alter the gut's environment and have been linked to an increase in inflammatory bowel disease (IBD), particularly ulcerative colitis and Crohn's disease [21].

The target of RNA III activating protein (TRAP), Nuclear Kappa Beta (NF- $\kappa$ B), and Extracellular Signal Regulated Kinase are some of the signaling pathways discovered in *Staphylococcus aureus* (ERK). Another cohort study has discovered that *Staphylococcus* enhances the synthesis of ATP and has been implicated in a variety of superficial and deep skin infections, including cellulitis, erysipelas, and impetigo, all of which are aided by the N.F. KB65 signaling pathway [22].

Increased plasma Lipopolysaccharides, triglyceride buildup in the liver, and inflammatory response are all linked to the gut microbiota's involvement in regulating metabolic processes [23]. This study's increased triglyceride level is consistent with prior findings. Furthermore, the features of the measures assessed for T2DM and controls differed significantly. When compared to normal persons, clinical research has revealed that obese people with insulin resistance have a different gut microbiota composition, with a higher Firmicutes/Bacteroides ratio. Obesity-related changes in the microbiota have been linked to increased metabolic endotoxin production, which increases insulin resistance and leads to T2DM [24].

On the intestinal mucus and glycocalyx layer, *Escherichia Coli* and Bacteroidetes have been found to behave differently, which can alter permeability [25]. Some probiotic strains have an antioxidative action that helps to reduce pancreatic oxidative stress, which can lead to inflammation and B-cell death [26].

#### 5. CONCLUSION

Microbiomes can alter the gut environment and cause changes that encourage the development of T2DM. Understanding how the microbiome influences disease states, utilizing therapeutic

bacterial microbiomes, and developing biomarkers for illness diagnosis, patient stratification, and individualized intervention were the driving forces behind this study. Individual bacterial strains that are aligned with a certain functional activity, illness route, or mechanism will improve therapy outcomes.

## CONSENT

As per international standard or university standard, Participants' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

The Ethics Committee of Benha General Hospital in Egypt (CASE #2021-2020-185 IRB/AAA) gave its approval to the research.

## DATA AVAILABILITY STATEMENT

All data described in this study will be provided on reasonable request by the authors.

## COMPETING INTERESTS

There are no competing interests between the authors and the product makers when it comes to releasing this work. All ethical standards were strictly followed by the authors, including plagiarism, misconduct, data fabrication and falsification, multiple publications and/or submission, and redundancy.

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