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The Comparative Between Gut Microbiota in Type 2 Patients Diabetes and Health People

Hanan K Bokhamada, Intesar Ahmad Hasan Elmasli and Hind A Elzletni*

Biology Department, Faculty of Education, University of Benghazi, Benghazi, Libya

***Corresponding Author:** Hind A Elzletni, Biology Department, Faculty of Education, University of Benghazi, Benghazi, Libya.

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Abstract

The Diabetes it is a major contributor to the development of many pathological processes including hypertension, hyperlipidemia, and cardiovascular diseases. both animal and human studies indicates that gut microbial change is associated with diabetes, but such an association with T2DM in Libyan people is not known. Therefore, the aim of present study is to recognize if there is a difference in the bacterial composition between Libyan diabetic patients and a healthy control. Also, to find whether there is a relationship between bacterial composition and diverse factors such as FBS, HbA1c, and lipid profile and body composition. Two groups of participated in this study including 20 patients with type 2 diabetes and 28 healthy control subjects were involved. The fecal microbiota structure at level of species was investigated by using conventional culture method. There was significant difference in gut bacteria between diabetic patients and healthy control. The relative abundance of *B. vulgatus*, and *B. rodentium* were significantly declined in the diabetic group compared to non-diabetic group (P = 0.008, P = 0.018) but *B. vulgatus* negatively and significantly correlated to level of HDL-C (P = 0.015). Moreover, the relative abundance of *L. acidophilus* reduced significantly (P = 0.02) and correlated positively and significantly with Fasting blood sugar (P = 0.001) and HbA1c (P = 0.016) in diabetic patients compared to the healthy control group. Our results show that T2DM is associated with compositional alterations in gut microbiota. *B. vulgatus, B. rodentium* and *L. acidophilus B*. may be possible indicators of T2DM. The interaction of specific gut microbiota with FBG, HbA1c, and HDL-C should be considered as potential interest for future studies to develop better approaches for the prevention and treatment of T2DM by modulation of gut microbiota.

Keywords: Diabetes; Gut Microbiota; FSB; HbA1c; Lipid Profile

Introduction

Type 2 diabetes (T2DM) is the most top 10 cause of death worldwide. The incidence of diabetes has considerably increased in the recent years. According to the International Diabetes Federation (IDF) Atlas estimation (9th Edition), the number of diabetic patients is currently more than 400 million and it would be doubled by 2045 [1]. T2DM is a metabolic disease characterized by hyperglycemia, lipid profile dysfunction and is as a result of multiple factors. The main factors are genetic influences and environmental causes such as life style, diet, and gut macrobiota structure [2]. The gut microbiota alterations effect has been under spot light of recent studies. The first study connecting between the gut microbiota and alterations in glucose metabolism was published in 2004 by using germ-free mice [3]. Since that, several studies including animal and human samples have been prepared for studding this connection. However, the question is by which mechanism would gut micro-

biota affect T2DM and its disorders. A number of studies reported that the gut bacteria contribute to progression of T2DM and its compilations by mediating obesity-associated insulin resistance (IR) [2]. Accumulating evidence suggests that the gut microbiota has role in host metabolism by increasing immune system modulation, energy extraction, and altered lipid metabolism, all which have been demonstrated to contribute to development of T2DM [2-5]. This leads to ask about the role of this microbiota and the reason behind of its presence in the gut tract. The gut microbiota refers to all the parasitic microflora in gut tract, which include a variety of bacteria, fungi, and protozoa [6]. The human gut microbiota contains approximately 1014 bacteria [7]. The gut bacteria in healthy individuals with normal weight, is divided into five phyla: *Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria*, and *Verrucomicrobia* [8]. Both phyla Bacteroidetes and Firmicutes [9] are representative 90% of gut microbiota. Bacteroidetes phylum which is Gram-negative bacteria, have predominant genera such as Bacteroides and Prevotella. The *Firmicutes phylum* includes 274 genera of predominantly Gram-positive bacteria such as *Lactobacillus, Bacillus, Clostridium, Enterococcus,* and *Ruminicoccus. Actinobacteria phylum* is proportionally less abundant Gram-positive bacteria and contains Bifidobacteria [10].

The gut bacteria have a central role in the gut tract. These types of microbes are involved in vitamins produces, bile acids homeostasis, and amino acid supplements [6,7]. They also influence carbohydrate metabolism. While Bacteroidetes species degrades and ferments a great variety of polysaccharides and oligosaccharide side chains of mucins and glycosphingolipids, Firmicutes species convert them into short-chain fatty acids such as butyrate, propionate and acetate [6-8]. Therefore, any modification in gut microbiota will impact certainly on host health and disease through its role in regulation of energy metabolism and an essential material biosynthesis. The modification in gut microbiota, in usual, is as result of life style, food intake habits or some medication [10]. The link between gut bacteria alteration and development of T2DM and obesity was realized by previous studies. Difference in composition of fecal microbiota between diabetic patients and non-diabetic was also reported [11,12] and showing relationship with the level of glucose tolerance [13]. Study conducted by Cani., *et al*. [5] established that the gut bacteria influence the onset of IR and T2DM by triggering low-grade inflammation. IR is commonly associated with obesity [5]. The comparison between obese and lean in human [14] and animal [15] studies showed that there was difference in the rate of gut bacteria composition at level of phyla and class. Interestingly, weight loss improved this change demonstrating the relationship between gut bacteria balance and obesity [16].

In our knowledge, there have been limited researches in this field in Libya, in the despite of presence high incidence of T2DM cases. Specially, there is a high-energy food intake and low physical activity, all which lead to change in gut microbiota [17]. Thus, the aim of this study was, to find out whether there is a difference between diabetic patients and non-diabetic in the gut flora composition. Moreover, to find if there is relationship between this changes and factors such as age, BMI,WHR, lipid profile, FBS and HbAc1in Libyans people in Benghazi city.

Material and Methods

Study population

This study was conducted between March 2020 and January 2021on 20 diabetic patients (7 males, 13 females) and 28 healthy (14 males, 14females). People with T2DM were diagnosed as diabetic patients based on the WHO criteria. All the patients were selected randomly. By using standard procedures, height (in centimeters), weight (in kilograms), girth at hips and waist (in centimeters) were measured. Body mass index was calculated by the formula, BMI = weight (kg)/height $(m)^2$. Additionally, waist-to-hip circumference ratio (WHR) was calculated by dividing waist (WC in cm) by hip circumference (cm). Participants were asked about current use of medications, and these reports were checked by examining labels of drugs. Diabetes mellitus was defined as treatment with insulin or oral hypoglycemic agents. Of the eligible participants, 2 were excluded for lack of fecal sample or because of missing data, and 48 remained for analysis.

Laboratory methods

Blood samples were collected between 08:00 and 10:30 h. Lipid profile such as total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol, (HDL-C) and triglycerides (TG) were performed with a Cobas Integra analyzer (Roche Diagnostics, Indianapolis, Indiana). An enzymatic colorimetric assay was used for HDL-C, and triglycerides; LDL-C and VLDL-C were derived using the Friedewald calculation [18]. FBS was measured using enzyme method and the cutoff point of 126 mg/dl.2 was considered as diagnostic criterion for the diabetes whereas HbAc1was measured by using Cobas Integra Tina-quant Hemoglobin A1c Gen.2 kit (Roche Diagnostics, Germany).

All assays were performed according to the protocols recommended by the manufacturer. All stool samples were collected regularly from two groups in a standard specimen container without preservative and delivered to the laboratory for conventional culture method on the day of collection. The Human Research Ethics Committee of the University of Benghazi approved the study protocol. Both written and verbal consent was obtained from the subjects of the Study population.

Laboratory evaluation of bacterial activity

The blood agar method, the nutrient agar method and the bacterial agar diffusion method were used for the growth of microorganisms in the assay of samples of diabetic patients and non-diabetic. Inoculum suspension was applied to all surface isolated samples. The plates were left in a refrigerated incubator at 4 (± 2)°C for 1 hour and then incubated at 37 (± 2)°C for 24 hours for bacterial growth and Microbial colony, then identify the types of bacteria and the number of colonies were done.

Statistical analysis

Data were analyzed using SPSS version 20 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). The normal distribution of variables was assessed using the Kolmogorov-Smirnov test Results were presented as the mean +SD while categorical data were presented as frequency and percentage. The student *t* test was used to compare mean values of clinical data. The X^2 test was applied to determine difference between two samples groups in relative abundances of gut microbiota. Spearman correlation coefficient was performed to estimate relationship between bacteria construction and age, gender, BMI, WC, WHR, lipid profile, FBS and HbAc1 in diabetic group. All *P* values reported are two sided; a significance level of less than 0.05 was considered statistically significant.

Results

A total of 48 participants were included. 20 diabetic patients were with a mean of age (ranging from 39 to 70) and 28 healthy subjects were with a mean of age (ranging from 18 to 36). The characteristics and clinical data for both participants are summarized in (Table 1). The comparison between two groups in clinical data was conducted by *t* test sample. No significant difference was seen in age, WC, cholesterol and HDL-C between two groups. However, FBS, HbAc1 and BMI were significantly higher in diabetic patients than in healthy subjects (P, 0.0001, 0.0001, 0.004) respectively. The diabetic patients revealed also a significantly higher in W/HR (P, 0.047), TG (P, 0.021), and VLDL-C (0.019), while nearly significant in LDL-C (P, 0.06). To distinguish a difference in fecal microbial communities between control subject and diabetic patients, the X^2 test was uesd. The structure of gut bacteria in the diabetic patients sample showed in (Figure 1) and indicated that the *Prevotella copri* had highest density followed by *Escherichia coli* and *Bacillus subtilis* (96.19%, 90.64%, 76.13%) respectively. The *Bacteroides vulgatus* presented the lowest (42.85%) in additional to *Bacteroides rodentium* (47.61%). For non-diabetic group, the *Lactobacillus acidophilus* was the highest density (92.85%) while *E. coli* and *staphylococcus aureus* had a similar ratio (89.28%) and both are approximate to the *Prevotella copri* (82.14%). Furthermore, the percentage of *B. vulgatus* was (82.14%) and followed by *B. rodentium* (78.57%) whereas the *B. subtilis* was represented the lowest species (60. 71%). Significant difference in microbiota composition was found between two groups as showing in (Figure 1) Significant decline in the relative abundance of *B. vulgatus* 42.85% (P = 0.008) was seen in the diabetic groups comparing to control group 82.14 %. Similarly, the relative abundance of *B. rodentium* was significantly lower (P = 0.01) in the diabetic patients (47.61%) than in the non-diabetic group (78.57%). While, significant difference p = 0.002 was seen also in the relative abundance of lactobacillus in the diabetic group (52.38%) compared to control groups (92.85%), no significant differences was seen in *P. copri*, *E. coli* and *B. subtilis* between two groups. However, relative abundance of *S. aureus* was 71.42 % in cases and 89.28% in control and showed nearly significant difference P = 0.09. Determination of correlation between bacteria construction and clinical parameters in diabetic group using Spearman correlation coefficient was done and revealing in (Table 2). Positive and significant correlation was observed between *P. copri* and FBS (R = 0.492, P =0.02) and with HbcA1 (R = 0.449, P = 0.04). In the same way, the relative abundance of lactobacillus showed strong positive correlation with FBS (R = 0.680, P = 0.001) and, with HbcA1 and this correlation was significantly (R = 0.533, P = .01). In contrast, the relative abundance of *E. coli* correlated negatively and significantly with HbcA1 R = -0.48, P = 0.042) whereas nearly significantly with FBS (R = -.413, P = 0.071). For body composition, the ratios of *B. subtilis* just correlated positively and significantly with the BMI (R = 0.650, P =0.001), WC (R = 0.695, P = 002) and, with W/HR, even though not, significantly (R = 0.301, P = 0.162). Conversely, the ratios of *S. aureus* showed negative correlation with BMI and this was nearly significant (R = -0.397, P =

0.083). Related to lipid profile, there was no significant correlation between fecal bacteria and lipid profile except ratios of *B. vulgatus* which revealed negative and significant correlation with the values of HDL-C plasma (R = -0.533, P = .015) only.

Characteristic	Diabetic (n =20)	Non-diabetic (n =28)	p-value
Gender (male / female)	7/13	14/14	
Age (year)	55 ± 200 (39-70)	35 ± 2.15 (18-36)	0.287
BMI (kg/m^2)	32.19 ± 5.16 (24.4- 41.6)	30.3 ± 0.07 (19.3-47.5)	0.0001***
WC	109 ± 12.22 (90 -133)	96.46 ± 10.73 (70 -117)	0.651
W/HR	2.04 ± 0.15 (1.67-2.32)	1.81 ± 0.22 (1.37-2.33)	0.047*
FBS (mg/dl)	184.95 ± 56.92 (102-353)	98.89 ± 10.62 (74-126)	0.0001***
HbAc1	8.41 ± 1.77 (6.20-14.5)	5.61 ± 0.58 (4 -6.8)	0.004**
Cholesterol (mg/dl)	165.35 ± 29.51 (98-215)	162.07 ± 29.81 (114 -235)	0.861
TG (mg/dl)	149.50 ± 92.52 (83-422)	100.6 ± 46.84 (35 -213)	0.021*
LDL-C (mg/dl)	104.50 ± 80.27 (32-419)	97.50 ± 23.66 (57-152)	0.065
HDL-C (mg/dl)	45.35 ± 7.58 (29-61)	44.50 ± 5.99 (28-55)	0.408
VLDL-C (mg/dl)	29.99 ± 18.55 (17-84.8)	20.28 ± 5.2 (11-42.6)	0.019*

Table 1: Clinical characteristics of the subjects.

The data presented are the means and the SD (standarud diffusion) . *p < 0.05 vs control, **p < 0.01 vs control. ***p < 0.001 vs control M, male; F, female; FBS, fasting blood Sugar; HbA1c, hemoglobinA1c; TC, total cholesterol; HDL-C, high_density lipoprotein cholesterol; LDL-C, low_density lipoprotein cholesterol; TG, triglyceride; VLDL-C, very low density lipoprotein cholesterol; BMI, Body Mass Index; WC, waist circumference, W/HR; waist to hip to Ratio.

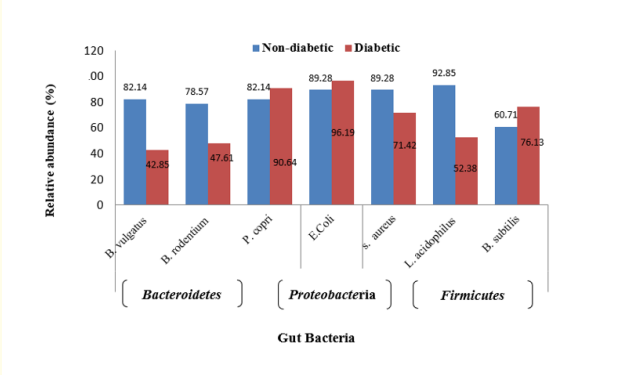

Figure 1: Relative abundance (%) of the gut bacteria in diabetic patient and healthy control at level of species. Bars represent the reads percentage found by conventional culture method using the X^2 test.

Phylum	Bacteria	Clinical parameters	R	p
Bacteroidetes	*B. vulgatus*	HDL	-0.533	0.01
	B. rodentium	Non	Non	Non
	P. copri	FBS	0.492	0.02
		HbAc1	0.449	0.04
Protobacteria	*E. coli*	FBS	-0.413	0.07
		HbAc1	0.432	0.05
Firmicutes	*S. aureus*	BMI	0.397	0.08
	B. subtilis	BMI	0.65	0.001
		WC	0.695	0.002
	L. acidophilus	FBS	0.68	0.001
		HbAc1	0 .533	0.01

Table 2: Correlations among the fecal bacteria and the clinical parameters in type 2 diabetic patients. Correlation was determined by using Spearman correlation coefficient.

The statistically significant items are only presented. FBS: fasting Blood Sugar; HbA1c: Hemoglobin A1c; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein; TG: Triglyceride; BMI: Body Mass Index; WC: Waist Circumference.

Discussion

Present study determined that there are significant difference in bacteria composition between type 2 diabetes patients and non-diabetic subjects. This difference was appeared at level of species level. The relative abundance *B. vulgatus*, and *B. rodentium* declined significantly in diabetic patients compared to non-diabetic subjects. This is in consistent with previous studies [19-21]. In the study conducted by Huang., *et al*. the decline in *B. vulgatus* was combined by blood sugar rose [22]. Similarly, Pakstnain study found that the relative abundance of Bacteroidetes was less in obese-T2DM compared to healthy people and correlated negatively with fasting glucose levels [23]. This correlation was not seen in the present study. Also, the same finding was observed by Zhang and coworkers [24] who assumed that there might other factor affected this reduction. Contrary to the present data, no significant change was seen in abundance of Bacteroidetes at phylum level in two previous diabetic studies [21,25] and an increase was seen in the study of Larsen., *et al*. [12], and Leite., *et al*. [20]. Larsen., *et al*. found that the abundance of Bacteroidetes at phylum level, was higher in diabetic patients compared to control group and the ratio of Bacteroidetes to Firmicutes was significantly and positively correlated with reduced glucose tolerance. According to studies using obese animal models [3,26,27] and humans [28], obesity changes gut bacteria composition and associated with declining in abundance of Bacteroidete*s* and increasing in Firmicutes proposing this change as biomarker of obesity. it has been suggested that the Firmicutes had high ability in harvest energy from the food than Bacteroidetes, hence stimulating a more efficient absorption of calories and the consequent weight gain [29]. However, this was opposite of Larsen., *et al*. result where the cases of that study were with average BMIs of 30. Furthermore, that study did not find relationship between this bacteria and BMI in diabetic patients, even though the obesity is identified as the main factor associated with T2DM [30]. Similar finding was stated by previous study that offered evidence on no association between the proportion of Bacteroidetes to Firmicutes and markers of human obesity after using weight loss diets [31]. This was match with our results where there was no correlation between the reduced of this bacterium with BMI or with WC of patient who were with average BMIs of 32. In further supportive to our data, Turnbaugh., *et al*. [28] and Furetetal. [32] found a lower representation of Bacteroidetes [Bacteroides/Prevotella] in obese individuals with no differences in Firmicutes phylum. Interestingly, we here found an inverse correlation between *B. vulgatus*, and HDL-C in diabetic patients who also had a slightly increase but not significantly in level of HDL-C plasma comparing to control group. The association between gut bacteria composition and lipid metabolism has been previously observed by several studies [33,34]. Flo., *et al*. found that variation in gut bacteria taxa was positively associated with HDL level and negatively with TG level [35]. These associations were at level of phyla, or genus and some of them were accompanied with BMI or alone with lipid. Also, recent study has demonstrated that Plasma lipoproteins can interact with bacterial toxins, such as endotoxin, (Enterohemolysin, Ehly) to reduce their toxicity *in vivo* [36] and *in vitro* [37]. In current study, this association was inversely between specific species and there was no affect for BMI. Furthermore, measuring level of gut bacteria endotoxin has not been involved in the present study. Consequently, it's difficult to explain this result. In addition, the diet nature is further and an important contributor in Bacteroidetes richness. For example, diets rich in saturated fat and animal protein lead to the increased of Bacteroidetes while diets rich in carbohydrates and simple sugars associated with the increased proliferation of Firmicutes [38]. Thus, it may be one of three above factors involved in an inverse correlation between *B. vulgatus* and HDl. It is notable that the recent appropriate modulation bacteria technique is either by using diet that promotes the growth of beneficial or desired bacteria, or by using methods to reduce the proportion of toxins secreted by bacteria affecting lipid profile [34]. Therefore, knowing the effect of these factors, it is undoubtedly will help in understanding this association and then reduced risk of T2DM and its dyslipidemia complication.

The next establish in present study is, the relative abundance of Lactobacillus. acidophilus decreased significantly in cases. This was in agreement with recent study [19] that reported that the amounts of Lactobacillus. acidophilus decline in patients with type 2 diabetes compared with healthy people. The same finding was seen in Chines [39] and Indian studies [40]. Our result was also in contact with Egyptian study [41]. In this study, there was low proportion of Stool Lactobacillus in type 2 diabetic patients without mentioning for correlation or causation of this relation. Also, the relative abundance of Lactobacillus acidophilus in the present study was positively correlated with FBS and HbAc1 level which it was not examined in the above three studies [19,39,41].

In this way, some studies found that reduction in glucose concentration and improved insulin homeostasis were observed after using some species from this type of bacteria as treatment [42-44]. To explain that, this type of bacteria is termed by probiotic bacteria and some of their strains were used to improve diabetes conditions because it acts to reduce FBS and HbA1c in patients with T2DM, meaning that it has an inverse relationship with the amount of glucose in the blood, and this is not in line with what we found in the current research. However, in diabetic mice treated with insulin, the relative abundance of Lactobacillales was decreased [45]. Other types of anti- diabetic medications (metformin) were reported in modulating of the genus Lactobacillus [46]. In the current study, the relation of treatment with this species did not determent. Therefore, it might that anti-diabetic medication was associated with this reduction of this species Further factor such markers of obesity might play effective role on level of this bacteria. For example, Egyptian study involving obese people found that *Lactobacillusa cidophilus* was also significantly lowered in the obese cases [47]. This result was likely consistent with our data because diabetic patients in the present study, were commonly obese and they had BMI and WHR significantly higher than non-diabetic patients. Furthermore, such assumption has been reached previously by Japanese study founding increase in this type of bacteria at level of order in non-obese individuals with type 2 diabetes. However, former researches conducted by Forslund., *et al*. [48] and Chinas [21] found an increase in frequency of this species in diabetic patients compared to control group. In addition, both studies of those Sedighi., *et al*. [49] and Larsen., *et al*. [12] found similarly increase but at level of genera and phylum respectively. It is possible that, the reason behind to inconstancy data might be related to geographic region and genetic background [41,48,50] or to small number of patients involving in our study and in above studies [12,49].

However, in our study, there are considerable limitations such as using conventional culture method comparing to other development technology aspects (metagenomic analysis method and the real-time PCR assays). Furthermore, some environmental factors were not included in the present study for investigation such as geographic regions, eating habits, anti-diabetic medication, dietary control, and genetic background.

Conclusion

Gut bacteria difference has been distinguished between diabetic patients and control healthy. Some species of bacteria including *B. vulgatus*, *B. rodentium* and. *L. acidophilus* decreased in diabetic patients compared to healthy subjects. Therefore, While, Latcobciulis could be consider as possible indicator of T2DM because its relative abundance significantly reduced and positively was linked with rising of FBS and HbAc1 in T2DM group, *B. vulgatus* specie might have role in lipid hemostasis as it had tendency to decrease with higher levels of HDL in diabetic patients. After this, further comprehensive studies are necessary to clarify the association between gut microbiota at level of species with T2DM development. These studies should be longitudinal research with high number size of individuals taking in consideration other important factors such as, anti-diabetic medications, diet habits, and genetic background. This will help to get better understanding for using gut microbiota composition as unique approach for treating T2DM or reduced its complication.

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