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Original Article

Biofilm Forming Intestinal *Escherichia coli* as a Risk Factor for Increasing BMI in Type 2 Diabetic Patients

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Abstract

Background: The term diabetes mellitus (DM) describes a group of chronic diseases and metabolic disorders that accompany hyperglycemia (high blood glucose) which may be the result of an abnormality in secretion or insulin action. Diabetes type 2 is the most common type of diabetes, it usually occurs in adults but is increasingly with overweight. On the other hand, gut bacteria have a key role in the regulation of different metabolic pathways that are important in glucose homeostasis and diabetes type 2 pathogenesis. **Objectives:** This study aimed to analyze the effect certain gut and biofilm formation on the glucose homeostasis and BMI in diabetic females. **Materials and Methods:** This study included the collection of 180 samples from diabetic and non-diabetic participants (20–50 years) during the period from December 2022 to March 2023. BMI was calculated for study participants, and *Escherichia coli* was microbiologically and biochemically identified. **Results:** The results demonstrated a substantial ($P < 0.05$) increase of 80% in biofilm formation *E. coli* in diabetic patients with greater BMI than those without diabetes with lower BMI. **Conclusion:** This study showed a significant relationship between intestinal bacteria (specially *E. coli*) and diabetes causing increased BMI, leading to probable useful tools for developing strategies to prevent obesity.

Keywords: Biofilm, BMI, diabetes, *Escherichia coli*, *Kirkuk*

Introduction

Diabetes is a chronic, metabolic disease characterized by increased levels of blood glucose.[1] As estimated by the World Health Organization statistics, the predicted number of diabetic patients will reach 366 million in 2030.[2] In addition, there is a continuous increase in the incidence of this disease, in Iraq where the statistic for the year 2019 indicated that more than 13.9% of adults suffering from diabetes,[3,4] and those statistics were elevated in 2022 recording more than 19.7% of the adults where diabetes type 2 was the most common type of diabetes and most patients with this form of diabetes were obese.[5,6]

Moreover, diabetes type 2 was found to have a wide relationship with obesity, in such away the likelihood and severity of diabetes type 2 were closely linked with body mass index (BMI).[7] It was found that, there was a seven times greater risk of diabetes in obese people compared to those of healthy weight, with a threefold increase in risk for overweight people.[8] Whilst, it is known that body fat distribution is an important determinant of increased risk of diabetes where gut bacteria was found to play a great role in it.[9,10]

Furthermore, *Escherichia coli* is considered as one of the most common intestinal bacteria that grows as a normal flora in the intestines of humans.[11] It is gram-negative bacteria and has the ability to ferment sugars, and have the ability to interact and bind with Toll-like receptor 4 (TLR4) through lipopolysaccharide from its capsule which increases inflammation and in turn reduces insulin sensitivity.[12,13] Additionally, the pilli of *E. coli* are the most

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important factors that help the bacteria stick to tissues, giving them the ability to form a biofilm in its pathogenic state and thus increasing their ability to acquire antibiotic resistance.[14]

Therefore, this study implicated to determine the effect of intestinal *E. coli* as a potential risk factor increasing BMI in diabetes type 2 patients.

Materials and Methods

Study group

The total of 180 participated in this study at the age range of 20–50 years for the period from December 2022 to March 2023 after signing a consent to participate. They were divided into two groups according to their BMI as in Table 1. The BMI was calculated depending on the law cited in following weight equation:

$$BMI = \text{Weight (kg)}/ \text{Height}^2 (m^2)$$

Sampling collection and processing

Stool samples collection and analyses

The stool sample was collected in a clean container according to the method by Doerr *et al.*[15] Once the sample was collected, it was transferred to the laboratory directly for stool examinations; including culture.

Blood samples collection and analyses

Whilst, then, 5 mL of blood was drawn by venipuncture from study participants and placed in gel tube for the purpose of separating the serum and process biochemical analyzes and left it for 15 min for clotting. The serum was separated by centrifugation at 3000 rpm for 15 min, and used to measurement fasting blood glucose (FBG) by device Cobas Integra Plus 400 (Roche, Vaud, Switzerland) as in Mohammed *et al.*[16]

Identification of *E. coli* in stool samples

Total of 180 stool samples were directly inoculated onto (Blood agar, MacConkey agar, Eosin methylene blue(Himedia, Mumbai, India) and incubated at 37°C for 24 h for Macroscopic, biochemical, and antibiotic susceptibility tests according to the procedure mentioned in Hadeel *et al.*[17] Complete diagnosis of *E. coli* was performed by using API 20E system for Enterobacteriaceae (BioMerieux, Craponne, France). Stock cultures were inoculated into (heart and brain infusion broth medium/ Himedia/India) and kept in the temperature at −20°C for about 6 to 8 months as mentioned in Tawfeeq and Taher.[18]

Biofilm formation of *E. coli*

Bacterial isolates were inoculated in test tubes containing brain heart infusion broth, then incubated at a temperature of 37°C for 48 h, then the supernatant was poured out and crystal violet at a concentration of (0.1%) was added to the tubes, then the tubes were washed with distilled water (three times), and left to dry in the air, and the appearance of a layer of colored materials attached to the wall of the inner tube was evidence of a positive test.[19]

Antibiotic sensitivity test of *E. coli*

E. coli was confirmed and susceptibility to different antibiotics was determined using the Vitek-2 compact system.

Statistical analysis

Finally, to analyze and perform statistical tests, SPSS 20 software is used at a significance level of 0.05.

Ethical approval

Before each woman signed up for the study, her husband or other family members also received informed consent. Each person was extensively briefed on the procedure prior to the blood and stool samples being obtained to ensure they understood every step. This has been acknowledged by Kirkuk's health department.

Results

The results of general stool examination showed signs of gut inflammation in the diabetes females (specially in obese). Stool samples were black and diarrhea in 68.89%, 68.89% of the diabetes group compared to 7.78%, 4.44% of the non-diabetes group. Higher number of pus and RBC were detected in the stool samples of diabetes group compared to non-diabetes group, 72.22% of stool samples have 10–19 pus cells/HPF in the diabetes group compared to 7.78% in the non-diabetes group, 61.11% of stool samples have 10–19 RBC/HPF in the diabetes group compared to 7.78% in the non-diabetes group. 77.78% of stool samples in the diabetes group have *monilia* compared to 33.33% in the non-diabetes

Table 1: Classification of study group according to body mass index (BMI)

Classification	Diabetic patient ($N = 90$)			Non-diabetic patient ($N = 90$)		
BMI	Obese (BMI >30 kg/m²)	Thin (BMI 16–18.5 kg/m²)	Normal (BMI 18.5–25 kg/m²)	Obese (BMI >30 kg/m²)	Thin (BMI 16–18.5 kg/m²)	Normal (BMI 18.5–25 kg/m²)
Total	62	15	13	13	7	70

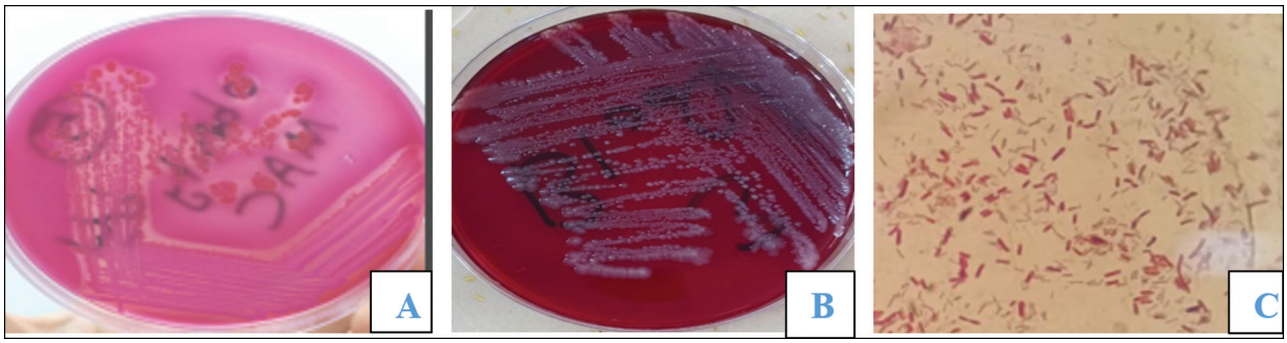

Figure 1: Photo of *E. coli* bacteria cultivated on selective media (A—MacConkey ager, B—blood agar, C—gram's stain of *E. coli*) for 24 h at 37°C isolated from the stool samples of the study groups

Figure 2: API test result of *E. coli*

Table 2: Characteristic of general stool examination of the study groups

Characters	Diabetic patient	Non-diabetic patient
Consistency		
Semisolid	15	83
Solid	15	3
Diarrhea	60	4
Color		
Brown	28	83
Black	62	7
Pus cells (cells/HPF)		
<10	25	83
10–19	65	7
RBC (cells/HPF)		
<10	35	83
10–19	55	7
Monilia		
Negative	20	60
Positive	70	30
pH		
Normal range (6.6)	3.5–6.2	5.9–7.2

group[Figures 1 and 2]. Stool samples recorded pH value of about 3.5–6.2 of the diabetes females samples compared to pH value of about 5.9–7.2 of the non-diabetes females as in Table 2.

The results revealed that the age group 40–50 years had the highest mean fasting glucose. Figure 3 shows that the percentages in non-diabetic subjects were within the usual range across all age categories. Following that, diabetic study participants were sub-classified based on their BMI, as illustrated in Figure 4. As a result, bacteria isolated from feces samples were identified further in order to validate these assertions, as shown in Figure 5. The results showed that *E. coli* levels were substantially ($P < 0.05$) higher in diabetes type 2 individuals (about 80%) and non-diabetic participants (about 40%) in obese participants than in thin and normal participants [Figure 5]. Thus, isolated intestinal *E. coli* was subjected to biofilm formation, and the results revealed that the *E. coli* bacteria has a 70% ability to develop a biofilm by attaching to the tube, as evidenced by a colored coating of material adhering to the tube wall, as shown in Figure 6. Furthermore, as shown in Figure 7, Vitek test results showed an absolute sensitivity of 100% to Nitrofurantoin, 83% to Meropenem, 66% to Nalidixic acid, and 50% to Amikacin, while Aztreonam and Imipenem were very low, 0%.

Discussion

Since gut microbiota is a dynamic community of microorganisms where bacteria make up their majority.[20,21] Besides, a number of researches had claimed an important role played by those bacteria in the immunity and disease state especially obesity.[10,20,22] Therefore, this study implicated to determine the effect of intestinal bacteria as a potential target to increase the waist circumference in obese diabetic patients in relation with some health index. In accordance, a number of obese, diabetic and non-diabetic participants in Kirkuk community were subjected to the questionnaire of the study and the total of 180 participants with age range of 20 to 50 years met the criteria of the survey were selected. Obtained results became in consistent with a study by Jouda *et al.*[23], where, general stool examination of diabetes group results, showed the appearance of solid consistency and black color of stool. They pointed out to the elevation of RBC in the

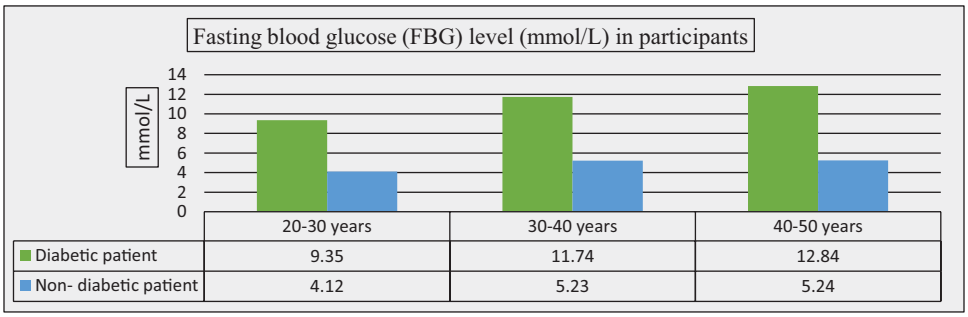

Figure 3: Fasting blood glucose level in participants according to age group

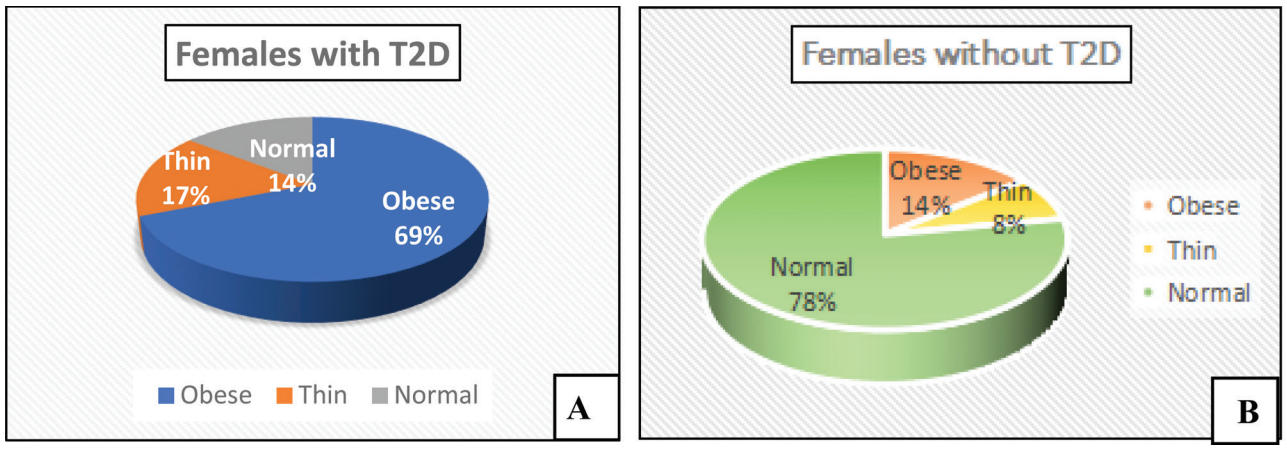

Figure 4: The percentage of diabetes type 2 recorded in the study classified according to BMI prime

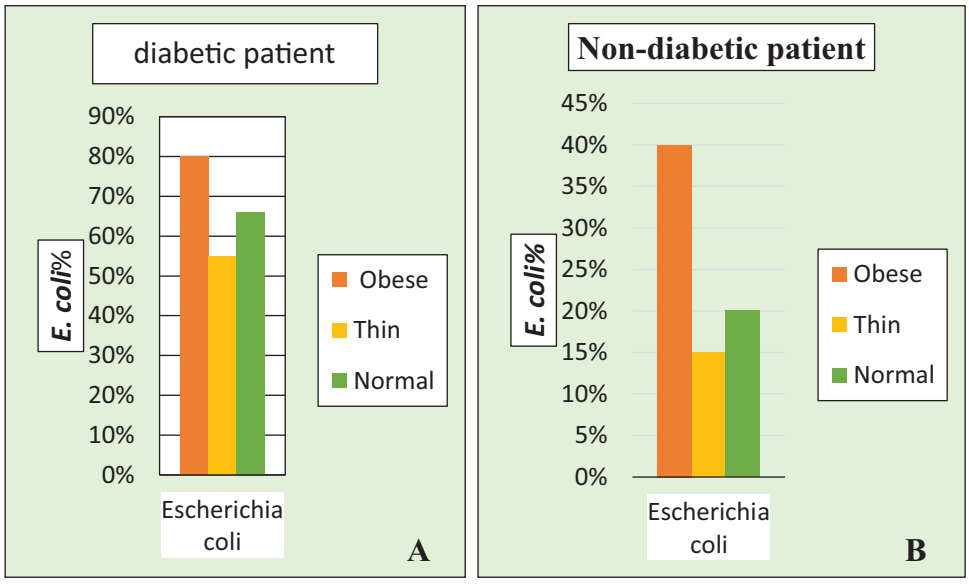

Figure 5: Distribution of identified *E. coli* bacteria isolated from stool samples according to the diabetic situation of the participants

stool, might give the black color to the stool of diabetes group instead of the normal brown color which come from the bilirubin excreted from liver. Normally, stool contains 74.6% water which gives it soft consistency but the decrease in the bowel movement due to the fat diet could cause the solid consistency of stool as the current study showed and agreed by Sommers *et al.*[24] and Seid *et al.*[25] Diabetic diarrhea was diagnosed in 68.89%. Patients with diabetic diarrhea have abnormal motility of the small bowel during fasting and postprandially; migrating motor complexes develop prematurely during the postprandial period, was found previously as it.[26]

In addition, Acidosis detected in the pH values of the diabetic patients probably was due to decreased bicarbonate secretion of the colon which may decrease the pH of the patient as[23,24] declared. Moreover, acidosis could kill the normal flora in intestine, replacing them with monilia.[23] This explains the high number of monilia detected in the samples of the diabetic patients of the study.

These result, however, agreed with the results of[27-29] which showed a high level of blood glucose increase with age. This increase in the blood sugar level changes with age as a result of poor glucose tolerance, which decreases with age, with poor control of blood sugar level and deterioration in glucose tolerance result from aging.[30,31]

Current results however, agreed with those results of Khaled and Husam,[10] where they explained that, the gut microbiome plays the main role in regulating glucose and energy balance, it also plays a main role in obesity and controlling blood sugar, type 2 diabetes mellitus, it has been hypothesized that people with type 2 diabetes have a different gut microbiota composition than healthy people, and then compared with the control group.[10,19] In addition, the current results confirmed that gram-negative bacteria, especially *E. coli*, prefer high blood sugar because they have the ability to ferment, so their numbers increase and the pH in the stool decreases to 3.5 as a result of the presence of alcohol resulting from the growth of the increased numbers of *E. coli.*

As indicated by the results agreed with those results of Kamelija, and her colleagues in 2018, which showed administration of hormone insulin in concentration of 2.5 U/mL resulted in significant increase in proliferation of all tested *E. coli* strains under *in vitro* conditions at 37°C.[23] Some previous studies already established that human insulin stimulates *E. coli* growth and its ability to form a biofilm.[13,27,32] Moreover, several studies have shown that, biofilm *E. coli* could initiate a state of obesity and insulin resistance as a metabolic syndrome following the activity of lipopolysaccharides (LPS; a component of the cell wall of gram-negative bacteria), which could play a role in the pathogenesis of *E. coli* which increases inflammation and in turn reduces insulin sensitivity increasing BMI the participants.[33-35] Thus, isolated intestinal *E. coli* was objected biofilm formation and results showed that the *E. coli* bacterium has the ability to produce a biofilm by sticking to the tube by 70%, as it showed a colored layer of material sticking to the wall of the tube. In addition, Vitek test results showed an absolute sensitivity of 100% to Nitrofurantoin, while the sensitivity rate was 83% to Meropenem, 66% to Nalidixic acid, and Amikacin 50%, while Aztreonam and Imipenem were very low, 0%, Similar to the results mentioned previously.[22]

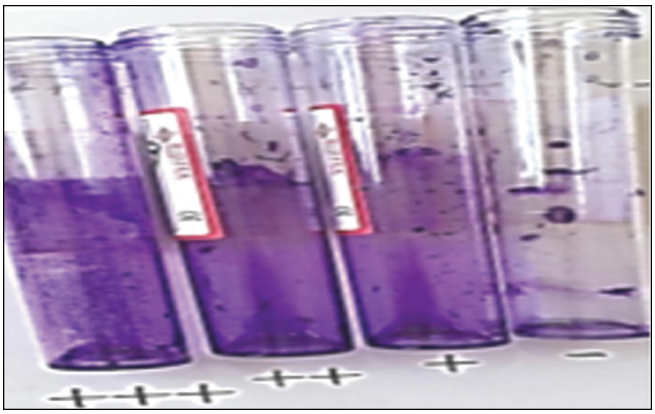

Figure 6: Biofilm forming intestinal isolated bacteria *E. coli* strain

Conclusion

This study showed a significant relationship between *E. coli* and diabetes with increased BMI, therefore, these findings could be useful for developing strategies to prevent obesity in addition to some metabolic changes which might modify some intestinal flora to be used as a therapeutic tool in the management of the body weight.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

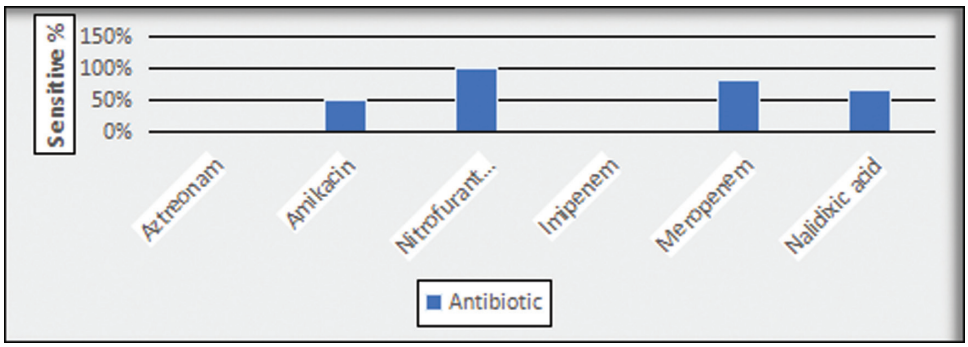

Figure 7: Antibiotic susceptibility assay of intestinal *E. coli* isolated from stool sample

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