Article presented at 2nd International Nineveh Conference on Medical Sciences (2nd INCMS 2023)

Original Article

Isolation and Identification of Individual Intestinal Bacteria Associated with Waist Circumference Increase

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Abstract

Background: The bacterial flora of the gastrointestinal tract (GIT) of humans, there are differences in the composition of the flora which are influenced by age, diet, cultural conditions, and the use of antibiotics. Since the origin of obesity is multifactorial and complex, resulting from the interaction between genetic/epigenetic, environmental, and socio-psychological determinants, in addition to the bacterial composition of the intestinal flora could be a third element involved in the development of excess weight. Therefore intestinal flora plays an intermediary role in the metabolism and inflammation and is involved in the pathogenesis of diabetes type 2 and obesity. **Objectives:** This study aimed to draw the relationship between identified intestinal bacterial types on increasing the waist circumference among study participants. **Materials and Methods:** This study included the collection of 180 samples from participants (20–60 years) during the period from December 2022 to June 2023. Body mass index and waist circumference were calculated for study participants and intestinal bacteria was microbiologically and biochemically identified. **Results:** Results obtained showed that the intestinal flora of obese participants is significantly ($P < 0.001$) different from that of thin and normal participants. This flora was characterized by the presence of high levels of Gram's negative at the percentage (80.6% in obese), and Gram's positive at the percentage (19.4% in obese), with higher body mass index and waist circumference than those participants, with lower body mass index and waist circumference. **Conclusion:** This study showed a significant relationship between intestinal bacteria and diabetic patients with increased body mass index and waist circumference, leading to probable useful tools for developing strategies to prevent obesity.

Keywords: BMI, diabetes, intestinal bacteria, obesity, waist circumference

Introduction

The bacterial flora of the gastrointestinal tract (GIT) of humans, there are differences in the composition of the flora which are influenced by age, diet, cultural conditions, and the use of antibiotics.[1] In addition, the flora of the large intestine (colon) is qualitatively similar to that found in feces.[2] Populations of bacteria in the colon reach levels of (1.5 kg) from feces.[3] Coliforms become more prominent, and *enterococci*, *clostridia,* and *lactobacilli* can be regularly found, but the predominant species are anaerobic *Bacteroides* and anaerobic lactic acid bacteria in the genus *Bifidobacterium* (*Bifidobacterium bifidum*).[4] These organisms may outnumber *Escherichia coli* by 1000:1 to 10,000:1. It is now known that significant numbers of anaerobic methanogenic bacteria (up to 10^{10}/gm) also reside in the colon of humans.[2]

During the past decade, several studies have reported a causal relation between gut bacteria and development of metabolic diseases such as diabetes and obesity.[5–7] A new paradigm suggesting that bacteria may contribute to the regulation of energy homeostasis emerges.[8]

In addition, it is hypothesized that interrelation of environmental circumstances with gut bacteria may

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Quick Response Code:

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DOI: 10.4103/MJBL.MJBL_958_23

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Submission: 10-Jul-2023 **Accepted:** 16-Dec-2023 **Published:** 21-Nov-2024

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How to cite this article: Hussein SS, Tawfeeq, AA. Isolation and identification of individual intestinal bacteria associated with waist circumference increase. Med J Babylon 2024;21:S217-23.

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cause an energy imbalance leading to metabolic changes that would promote development of obesity.[7] However, the increase in Firmicutes seen in humans, and also in obese subjects, could be associated with an increased capacity for digesting some indigestible polysaccharides to monosaccharides and short-chain fatty acids that may be absorbed by the host, thus obtaining more energy from substances which in thin subjects would be excreted in feces unabsorbed.[6,9–11] An additional important finding in humans was that switching from a fat-rich, low-fiber diet to a low-fat, fiber-rich diet induced significant changes in gut bacteria intestinal in only 24 h, moreover, multiple evidence suggests that, in humans, an increase in dietary fat decreases the number of Lactobacillus organisms and causes an increase in gram-negative bacteria.[10] Specifically, a higher level of *Lactobacillus reuteri* and lower levels of *Lactobacillus casei/paracasei* and *Lactobacillus plantarum* were associated with obesity.[10] On the other hand, gut bacteria may decrease production of fasting-induced adipocyte factor by intestinal cells, thus inhibiting activity of the enzyme lipoprotein lipase, this enzyme promotes release of non-esterified fatty acids to tissues such as the liver and adipose cells.[10,12] Several studies have investigated the association of given species to human obesity, and greater numbers of *Staphylococcus*, Enterobacteriaceae, and *E. coli* have been reported in overweight subjects as compared to normal weight.[6,10]

Therefore, this study aimed to determine the effect of intestinal bacteria as a potential risk factor in increasing body mass index (BMI) and waist circumference.

Materials and Methods

Study group

The total of 180 females in this study at the age range of 20–60 years for the period from December 2022 to June 2023 after signing a consent to participants. They were divided according to their BMI as in Table 1.

Sample collection and processing

Stool sample collection and analyses

The stool sample was collected in a clean container according to the method by Doerr *et al.*[13] Once the sample was collected, it was transferred to the laboratory directly for culture.

Blood sample collection and analyses

Whilst, then, 5 mL of blood was drawn by venipuncture from study participants and placed in an EDTA tube for HbA1c and gel tube for vitamin D3 for the purpose of biochemical analyzes and used to measurement HbA1c by device Cobas Integra Plus 400 (Roche/Switzerland) and vitamin D3 levels by device Cobas e 411 as in Mohammed *et al.*[14]

Identification of Intestinal bacteria in stool samples

A total of 180 stool samples were directly inoculated onto (Blood agar, MacConkey agar, Eosin methylene blue, and MRS agar/Himedia/India) and incubated at 37°C for 24–48 h for macroscopic and biochemical tests according to the procedure mentioned in the study of Zaky *et al.*[15] Complete diagnosis of isolated bacteria was performed by using API 20E system for Enterobacteriaceae, API 50CHL for *Lactobacillus*, API test for *Staphylococcus* and *streptococcus* (BioMerieux, France). Stock cultures were inoculated into (heart and brain infusion broth medium/Himedia/India) and kept at the temperature of −20°C for about 6–8 months as mentioned in Tawfeeq and Taher.[16]

Statistical analysis

The Chi-square test was used with conventional equations to compare the results and reported with P value < 0.001 as the accepted level of significance accordingly.

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

Results obtained showed that the intestinal flora of obese females (diabetic type 2 and nondiabetic) are significantly different from that of thin and normal females. This flora was characterized by the presence of high levels of Gram's negative at the percentage (80.6% in obese T2D and 61.5% in obese without diabetes), Gram's positive at the percentage (19.4% in obese T2D and 38.5% in obese without diabetes) [Table 2].

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

Classification	Diabetic patient ($N = 90$)			Nondiabetic 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

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Accordingly, to give a better interpretation of the results, the previously identified Gram's positive bacteria (*Lactobacillus* spp., *Staphylococcus aureus,* and *Streptococcus* spp.) were subsequently classified according to the major study groups to analyze the results more deeply as displayed by Figures 1 and 2.

Results obtained from Figures 1 and 2 showed that the main *Lactobacillus* spp. isolated from the obese diabetes type 2 females and nondiabetes were *L. acidophilus, L. salivarius, L. fermentum, L. ingluviei, L. reuteri,* whilst, *Lactobacillus* spp. isolated from the thin diabetes and nondiabetes females were *L. plantarum, L. fermentum, L. gasseri, L. pentosus, L. rhamnosus, L. lactis* and in normal *L. paracasei, L. pentosus, L. delbrueckii, L. rhamnosus.*

Results obtained showed that *E. coli* were slightly elevated in diabetics about 80% compared to the nondiabetic obese females 60% than thin and normal participants as shown in Figures 3 and 4.

Moreover, the current results showed that the highest mean of HbA1c was in the age group of 40–60 years in diabetic females. Whereas, the percentages were normal in nondiabetic females as shown in Figure 5 at all age groups.

On the other hand, the role of vitamin D3 deficiency was also evaluated and Table 3 shows the results.

Discussion

Since gut microbiota is adynamic community of microorganisms where bacteria make up their majority.[15,17] Besides, a number of researches had claimed an important role played by those bacteria in the immunity and disease state, especially obesity.[2,5,15] Therefore, this study aimed to determine the effect of intestinal bacteria as a potential target to increase the waist circumference in obese diabetic patients in relation to some health index such as vitamin D3 level. Thus, a number of obese, diabetic, and

Table 2: Distribution of intestinal bacteria according to their isolation group

Classification						
BMI subgroups	**Females with type 2 diabetes (T2D) *N* = 90**			**Females without type 2 diabetes (T2D) *N* = 90**		
Bacterial isolated	**Obese**	**Thin**	**Normal**	**Obese**	**Thin**	**Normal**
Gram's negative	50	7	6	8	2	30
Gram's positive	12	8	7	5	5	40
Total	62	15	13	13	7	70

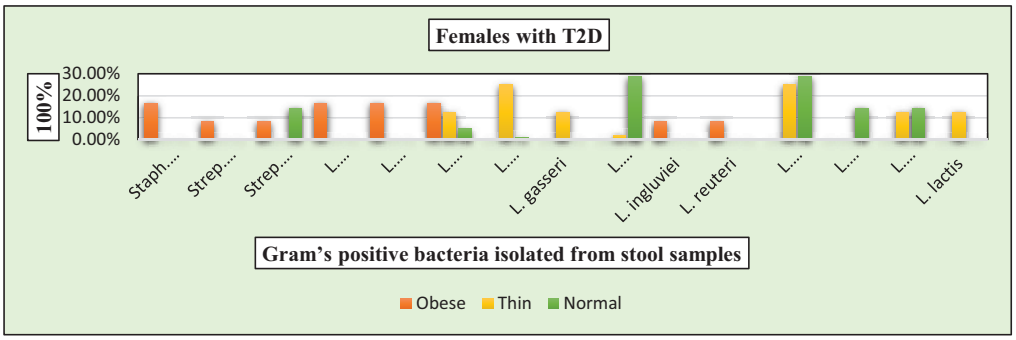

Figure 1: Distribution of identified Gram's positive bacteria isolated from stool samples according to the body mass indexed diabetic patients of the study

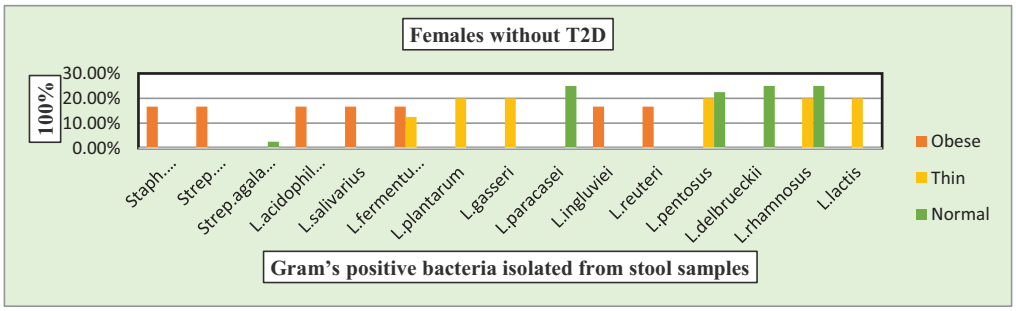

Figure 2: Distribution of identified Gram's positive bacteria isolated from stool samples according to the body mass indexed study participants without diabetes

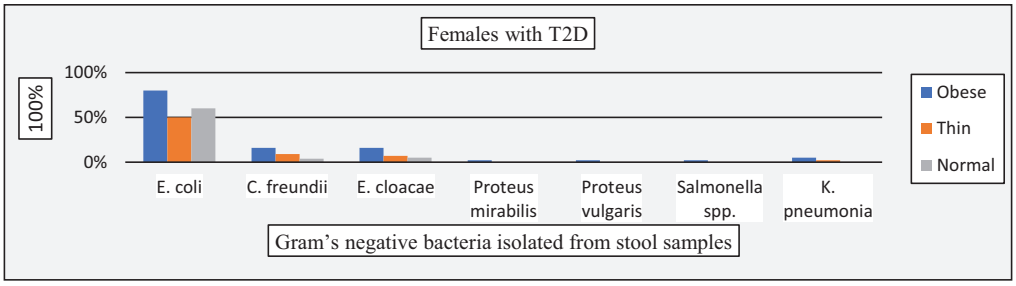

Figure 3: Distribution of identified Gram's negative bacteria isolated from stool samples according to the body mass indexed diabetic patients of the study

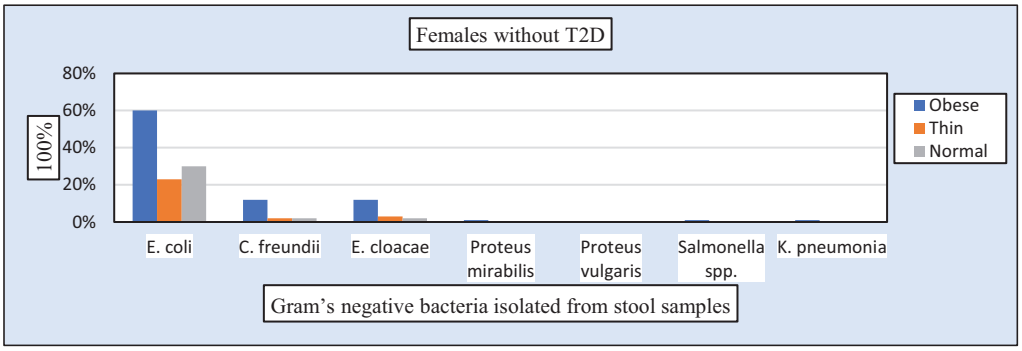

Figure 4: Distribution of identified Gram's negative bacteria isolated from stool samples according to the body mass indexed study participants without diabetes

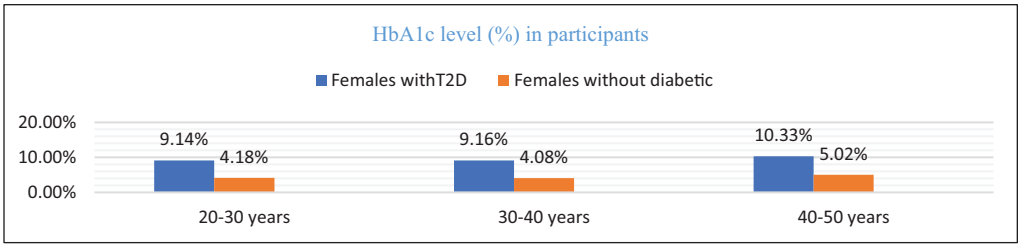

Figure 5: HbA1c level in participants according to age group

Table 3: Serum vitamin D3 levels in the study group according to body mass index (BMI)

Classification	(Females with T2D) $N = 90$			(Females without T2D) $N = 90$		
BMI subgroups	**Obese**	**Thin**	**Normal**	**Obese**	**Thin**	**Normal**
Total	62	15	13	13	7	70
Vitamin D3 Normal range (10–30 ng/mL)	7.33 ng/mL	10.21 ng/mL	12.04 ng/mL	8.23 ng/mL	11.03 ng/mL	18.37 ng/mL

nondiabetic females in Kirkuk community were subjected to the questionnaire of the study and the total of 180 participants with an age range of 20–60 years met the criteria of the survey were selected.

Figures 6–10 illustrate the results of cultural charecteristics and API system for all gram-positive and gram-negative bacterial isolates recovered in the present study. Additionally, waist circumference is used to assess the patients abdominal fats and consequently, the increased likelihood of developing diabetes type 2.[18,19] Thus, all the obese participants were subjected to waist circumference measurement where they showed a circumference of 80–88 cm at all the age groups. The result obtained was in consistent with the results of Jabbar, in 2018 where a proportional increase in the cases of diabetes type 2 as the waist circumference increased among the population of Kirkuk city.[20] Whilst it was well known that, body fat disruption is an important determinant of increased risk of diabetes.[1] In the same context, a number of studies have suggested a specific bacterial role in obesity and subsequently in developing diabetes.[2,21,22]

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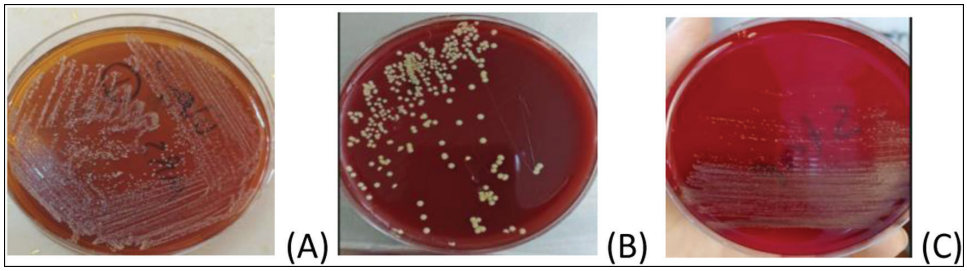

Figure 6: Photo of Gram's positive bacteria cultivated on selective media for 24–48 h at 37°C isolated from the stool samples of the study groups showing: (a) *Lactobacillus acidophilus* on MRS agar, (b) *Staphylococcus aureus* on blood agar, and (c) *Streptococcus pyogenes* on blood agar

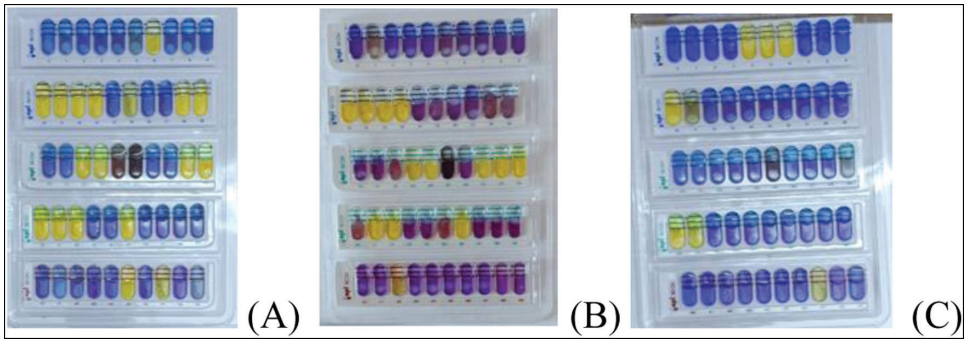

Figure 7: API 50CHL test results for *Lactobacillus* bacteria isolated from stool samples: (a) *L. salivarius*, (b) *L. acidophilus*, and (c) *L. Fermentum*

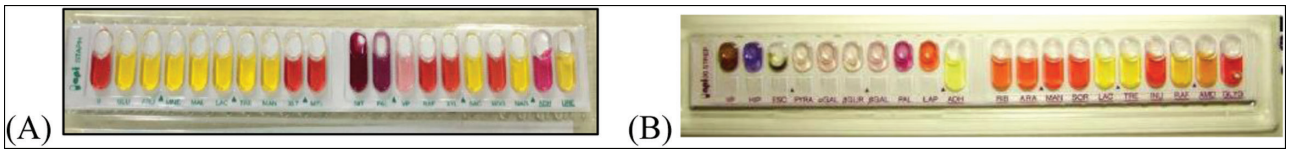

Figure 8: API test results: (a) *Staphylococcus aureus, and* (b) *Streptococcus pyogens*

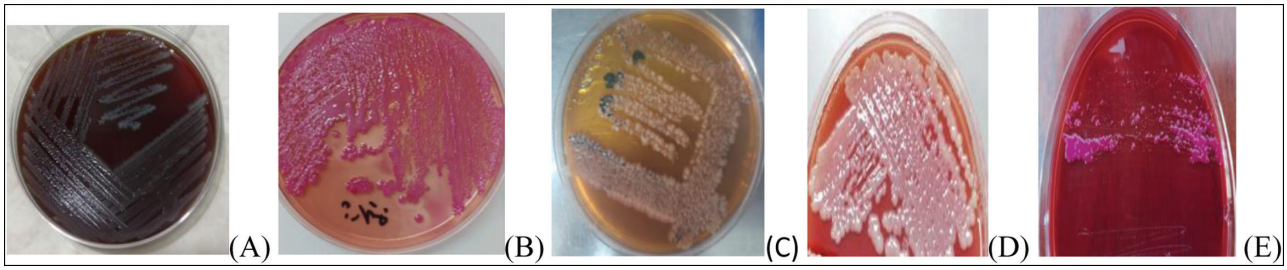

Figure 9: Photo of Gram's negative bacteria cultivated on selective media for 24 h at 37°C isolated from the stool samples of the study groups showing

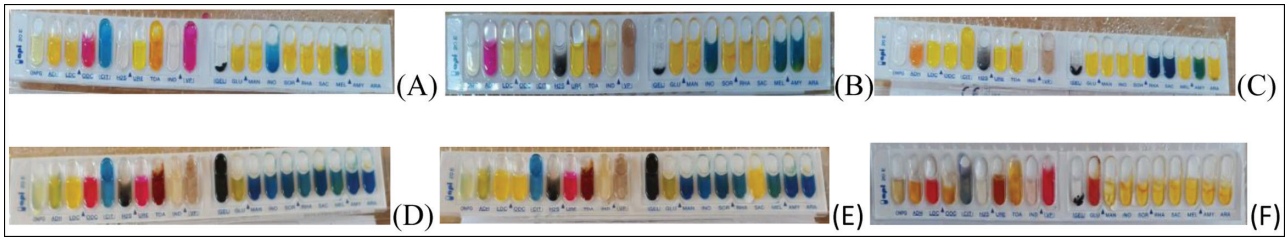

Figure 10: API test result: (a) *Enterobacter cloacae,* (b) *Citrobacter freundii,* (c) *Sallmonella* spp.*,* (d) *Proteus mirabilis,* (e) *Proteus vulgaris, and* (f) *Klebsiella pneumoniae*

Current results, however, were in consistent with the results of Khaled and Husam[2] who 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, and type 2 diabetes mellitus. It has been hypothesized that people with type 2 diabetes have a

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different gut microbiota composition than healthy people and then compared with the control group. Proteobacteria bacteria were significantly higher in the pre-diabetic mellitus group.[2,23] The results of the current study were compatible with Dawood and Thalij[24] who found that the types of lactic acid bacteria (*L. acidophilus, L. fermentum, L. reuteri*) may cause weight gain, and that the reason for weight gain is that lactic acid bacteria stimulate appetite, fermented sugar, produce digestive enzymes, improve the microbial balance in the intestine, and their ability to reduce the amount of the lipids available in the intestine are triglycerides or cholesterol, which have the largest role in the occurrence of weight gain, through their association with other compounds of steroids that are able to fully exploit and metabolize the lipids present in food, turning them into fat layers in the body, which causes an increase in body weight.[24-26]

In addition, Zaky *et al.*[15] demonstrated in 2021 that *Staphylococcus aureus* was increased in intestinal flora from patients with T2D compared to control and that BMI and women weight correlated with concentration of *Staphylococcus*, therefor, these results agreed with the current study.[15,27] However, 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*, and this was confirmed by the results in the stool examination. These results, however, were compatible with the results of Khaled and Husam[2] who showed that *Proteobacteria* were in a higher percentage in DM2 patients than in healthy people.

These results, however, agreed with the results of Mahmmod and Hussein,[28] and Liu *et al.*[29] which showed a high level of glycated hemoglobin 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] Therefore, microbiological examination showed the isolation of many different bacterial isolates with increased *E. coli* percentage in diabetic patients than nondiabetic ones. In addition to isolated other types of intestinal bacteria (*Lactobacillus* spp*., Staphylococcus aureus, Streptococcus* spp., *Citrobacter freundii, Enterobacter cloacae, Proteus mirabilis, P. vulgaris, Salmonella* spp., and *Klebsiella pneumonia*) in obese participants with diabetic.

When comparing average BMI values between the approved vitamin D3 levels, it was found that there was a difference in the BMI values according to the three vitamin D3 levels. Where the results of the current study showed that the increase in the BMI value is associated with a decrease in the concentration of vitamin D3, as shown in Table 3.[3,26] The conclusion of this study was consistent with other previous studies in Iraq 2022 that revealed that patients with insufficiency vitamin D3 patients are more likely to be overweight or obese than patients with sufficient vitamin D3 levels.[31,32] Moreover, the results of this research agreed with the other two studies showing the lowest tertile of vitamin D3 intake correlated with an increase in LPS level likely due to an increase in Gram-negative bacteria (*Proteobacteria*) which have LPS in their outer membrane.[33–35]

Conclusion

This study showed a significant relationship between intestinal bacteria and diabetic patients with increased BMI and waist circumference, leading to probable useful tools for developing strategies to prevent obesity.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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