The Association of Gut Microbiota 16s RNA Target Region with Obesity

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ABSTRACT
Background: Gut dysbiosis is strongly associated with obesity and T2DM pathogenesis.
Objective: To study the possible associations of gut microbiota 16s RNA target region with obesity. **Patients and Methods:** A case-control study that was done on 60 subjects (20 obese subjects without diabetes, 20 obese diabetic patients, and 20 control normal subjects). This study was based on gut microbiota 16s rRNA detection by DNA extraction from fresh frozen stool samples of study groups. Gene amplification was done by PCR. Using gene sequencing to identify the gut microbiota species.
Results: **2** cases were positive for 16s RNA gene in control group, the sequence of this gene was belonging to Succinivibrio dextrinosolvens. **3** cases were positive for 16s RNA gene in obese without diabetes group; all were belonging to Prevotella copri. **5** cases were positive for 16s RNA gene in obese with diabetes group; **3** cases were belonging to Prevotella copri and **2** cases were belonging to Succinivibrio dextrinosolvens. Statistically there was no significant difference among the three study groups regarding the 16s RNA gene, nor abundant organism. Although there was a significant difference between obese without DM group & lean group regarding type of organism as 100% of obese without DM group had Prevotella copri compared to 0% in group A **(P = 0.04)**. However, there was no statistical difference between lean group and obese with DM group as **(p = 0.20)**. In addition, there was no difference between obese group and obese with DM group **(P= 0.27)** regarding type of organisms.
Conclusion: Obesity is associated with microbial composition alteration using 16s rRNA sequences method.
Keywords: DNA extraction, 16S rRNA target region, Gut microbiota species.

INTRODUCTION
Obesity is becoming a serious health problem wide world [(1)]. Over the last decades the obesity prevalence and its associated complications has been continually increasing all over the world [(2)]. It is considered an intense risk factor for multiple complications of metabolism as type 2 diabetes and insulin resistance [(3)]. Many factors have an important role in these diseases development, as lifestyle, genetics and the gut microbiome [(4)]. Gut microbiota has an important role in the development of tissue homeostasis and immune system [(5)]. There are many functions of this microbiota, including digestion of indigestible nutrients leading to formation of short-chain fatty acids (SCFA), which are very important to the host. It also play an essential role in dehydroxylation of biliary acid and several vitamins synthesis [(6)].

Reduction of gut microbiota diversity has been associated with obesity [(7)]. Gut microbiota are trillions of microorganisms present in the human gut, their weigh about 1.5 kg. They are considered a microbial organ that has physical functions, and a bright participant to host health and disease [(8)]. Intestinal microbiota changes may affect insulin sensitivity, lipid & carbohydrate metabolism, and body weight. Gut dysbiosis leads to sensitization of insulin resistance, proinflammatory mechanisms, and metabolic toxicity [(9)].

The sequencing technology that based on advanced 16S rRNA has involved in establishing a bright role of the gut microbiota in human health [(10)]. Amplification of specific region in the 16S gene is done by using polymerase chain reaction (PCR); this product is subsequently sequenced [(11)]. Gene of 16s rRNA is an important component factor of the transcription process of all DNA-dependable live organisms, which helps in discrimination among specific different microorganisms as archaea, bacteria, and microbial eukarya [(12)].

The most abundant gut microbiota are Firmicutes and Bacteroidetes (Prevotellaceae) that represent about 90% of these microbiomes [(13)]. Gut microbiota differences may be affected by racial, socioeconomic, environmental, and dietary factors [(14)]. Therefore this work aimed to study the differences between gut microbiota profiles of obese subjects, obese with type 2 diabetes patients compared to lean subjects in Egyptian population.

SUBJECTS AND METHODS
This was a case-control study that was carried out at Outpatient Endocrinology Clinic of Internal Medicine Department, Menoufia University Hospitals through the period from December 2018 to December 2020.

Ethical considerations:
The study followed the ethical standards of the Faculty of Medicine, Menoufia University and was approved by the Institutional Review Board. A

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written informed consent was taken from each participant and biological sample was withdrawn only after his/her agreement.

60 subjects were divided into three groups according to **inclusion criteria:** group A "20 normal persons with body mass index (BMI) = 18.5-24.9 kg/m² as control". Group B "20 obese persons without diabetes with BMI ≥ 30 kg/m², fasting blood sugar < 100 mg/dl, 2 hours post prandial blood sugar < 140 mg/dl, and HbA1c < 5.7 %". Group C "20 diabetic obese patients with BMI ≥ 30 kg/m², fasting blood sugar ≥ 126 mg/dl, 2 hours post prandial blood sugar ≥ 200 mg/dl, and HbA1c ≥ 6.5%. Patients with chronic diseases as renal, hepatic, heart failure and other endocrinal causes of obesity were excluded.

Demographic and anthropometric measures:

All patients underwent full history taking including demographics, complete physical examination including anthropometric measures as body weight, height, waist circumference, hip circumference, and calculation of obesity indices. Waist to hip ratio and BMI = BW (kg)/height (m²). Visceral Adiposity Index (VAI) was measured as the following: For males = (WC/(39.68+(1.88*BMI)))*(TGS/1.03)*(1.31/ (HDL-C)) and for females = (WC/ (39.5+(1.89*BMI)))*(TGS/0.81)*(1.52/ (HDL-C)) [15]. Body Adiposity Index (BAI) = ((HC)/(Height (m)^1.5) - 18) [16]. Belarmino-Waitzberg (BeW) index: For females = −48.8+0.087×AC(cm)+1.147×HC(cm)−0.003×HC(cm)^2, For Males = −48.8+0.087×AC(cm)+ 1.147×HC(cm)−0.003×HC(cm)2-7.195 [17]. Conicity index (CI) = 0.109^{-1} × waist circumference (m) × (weight (kg)/height (m))$^{-1/2}$ [18]; A body shape index (ABSI) = 1,000× WC× Wt $^{-2/3}$×Ht$^{5/6}$ [19].

All subjects underwent abdominal ultrasound to detect the condition of the liver fibrosis & NAFLD score was calculated.

NAFLD Score = -1.675 + 0.037 × Age (years) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × Platelet (×10^9/L) – 0.66 × Albumin (g/dL).

• Laboratory investigations:

Complete Blood Count, Lipid profile (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C); high-density lipoprotein cholesterol (HDL-C), triglycerids (TG)), thyroid stimulating hormone (TSH), liver function tests and renal function tests.

Sample collection

1- Blood sample:

Five milliliters of venous blood were collected from each participant that was divided into two samples. The first (3 ml) were collected in a plain vacationer tube, centrifuged, and the resulting sera were used for biochemical investigations. The other (2 ml) were collected into a tube containing an anticoagulant; EDTA and centrifuged as soon as possible. Plasma was separated within 10 min of collection and used immediately for glycated hemoglobin (HbA1c) assay by Sysmex XT-1800i automated hematology analyzer (Sysmex, Japan).

2- Fecal sampling:

Fresh stool samples were collected in a container, which is sterile, then stored at -80 ℃ until DNA extraction for detection of gut microbiota 16s RNA by conventional PCR and detection of subspecies of positive cases by sequencer.

Methods:

A- DNA extraction:

Principle: QIAamp DNA Stool Mini Kit procedure comprises of four steps (lyse, bind, wash, elute) using QIAamp MinElute columns.

B- Measurement of samples' Extracted DNA Concentration and purity by nano drop:

Extracted DNA was evaluated for concentration, by using nano drop. Ratio of results at 260 nm & 280 nm (A260/280) represents an evaluation of DNA purity.

C- Detection of 16S ribosomal RNA gene microbiota by conventional PCR method:

PCR for the 16S ribosomal RNA gene of microbiota was carried out in a PCR tubes containing 2.5 ul of 10x Taq polymerase buffer, 1.5 ul 2 mM MgCl2 (Gene-craft, Germany), 0.25 ul, pTaq DNA polymerase (5 units/µl) (Gene-craft, Germany), 0.5 ul of dNTPS (10mM) (Strata-gene, USA), 1 ul of each primer (20µM) (Midland, Texas), 50 ng of extracted genomic DNA for each sample & amount of distal water to reach a total reaction volume of 25 ul. using the following primers, forward primer (5'-AGAGTTTGATCATGGCTCAG-3'), and reverser primer (GTATTACCGCGGCTGCTGG-3). Then PCR amplification of this gene was done in a programmable (Perkin Elmer) thermal cycler 2400 (USA), at 98ºC for 4 minutes followed by 25 denaturation cycles at (98°C) for 20 sec, then annealing at (65°C) for 20 sec, and extension at (72 °C) for 35 sec, followed by final extension at (72°C) for 10 min. Then the separation of amplificated products was done by using electrophoresis through 3% agarose which gel stained with ethidium bromide, observation of one band was occurred at 500 bp.

D- Purification of the PCR product:

PCR were purified before sequencing with the Invitrogen™ PureLink™ PCR Purification Kit - Fisher Scientific.

E- Cycle sequencing reaction:

Cycle sequencing reaction by the following component in a total reaction volume 20 ul, include, 4 ul of BigDye terminator, 4 ul of 5x sequencing buffer,

1 ul of Primer (3.2 pmol, DNAase-Free water and template PCR product. Thermal profile done in a Perkin Elmer thermal cycler 2400 (USA), which is a programmable application at 96º C for 1 minutes, followed by 25 cycles of denaturation at (96° C) for 10 sec, then annealing at (65° C) for 5 sec, and extension at (60° C) for 4 minutes. The reaction products were immediately cooled on ice after thermo cycling.

F- Centre-sep purification:

PRINCIPLE

The columns of CENTRI-SEP are used to remove the dye terminators prior to sequencing rapidly and influentially. The recommendations of CENTRI-SEP columns are by applied biosystems, Ink used for influential and trusted removal of excessive DyeDeoxy™ terminators from reactions of completed DNA sequences.

Procedure below is used in connection of the Taq DyeDeoxy™ and ABI Prism™ terminator cycle sequencing kits.

G- Suspending and Loading the Samples:

Resuspension of samples done by using the highly deionized (Hi-Di) formamid before electrokinetic injection in the systems of capillary electrophoresis. Then the samples were heated (at 95° C) for 2 minutes to denature. After that put on ice till ready to load.

H- Sequence analysis:

BigDye Terminator v3.1 cycle sequencing kit was used for sequencing, and an ABI PRISM 310 Genetic analyzer (Applied Biosystems, Foster City, CA, USA). The results of sequencing were electronically received as compressed sea files. Expansion of these files was done by program of the Aladdin expander, and visualized by using the Chromas program.

I- Identification of the organisms species:

- Sample 1, Prevotella copri, score: 830 bits (449), identities: 477/490(97%), gaps: 4/490(0%).
- Sample 2, Prevotella copri, score: 808 bits (437), identities: 470/486(97%), gaps: 1/486(0%).
- Sample 3, Prevotella copri, score: 826 bits (447), identities: 479/494(97%), gaps: 4/494(0%).
- Sample 4, Prevotella copri, score: 697 bits (377), identities: 440/471(93%), gaps: 2/471(0%).
- Sample 5, Succinivibrio dextrinosolvens strain, score: 614 bits (332), identities: 421/464(91%), gaps:6/464(1%)
- Sample 6, Succinivibrio dextrinosolvens strain, score: 375 bits (203), identities: 375/458(82%), gaps:12/458(2%)
- Sample 7, Prevotella copri, score: 808 bits (437), identities: 470/486 (97%), gaps:1/486(0%)
- Sample 8, Prevotella copri, score: 830 bits (449), identities: 477/490(97%), gaps: 4/490(0%)
- Sample 9, Succinivibrio dextrinosolvens, score: 614 bits (332), identities: 421/464(91%), gaps: 6/464(1%)

Statistical analysis

Data were collected and entered to the computer using SPSS (Statistical Package for Social Science; Inc., Chicago. IL) program for statistical analysis, version 13. Quantitative data were shown as mean, standard deviation (SD), and range. Qualitative data were expressed as frequency and percent. Chi-square test was used to measure association between qualitative variables. Fisher exact test was used for 2x2 qualitative variables when more than 25% of the cells have expected count less than 5. Kruskal-Wallis test was used for comparison between three or more groups having quantitative not normally distributed data. Sensitivity, specificity, +ve and –ve predictive values, and diagnostic accuracy were calculated. P-value is considered statistically significant when it is less than 0.05. P- Value < 0.01 is considered highly significant.

RESULTS

The three groups were comparable as regards age and gender. According to systolic blood pressure, there was a statistically significant difference among the three groups (P-value = 0.039). According to BMI, and WHR, there was a statistically significant difference among three groups (P-value = 0.001 and 0.003) respectively. Regarding visceral adiposity index (VAI), body volume index (BVI), conicity index (CI), A Body Shape Index (ABSI), Belarmino–Waitzberg (BeW) index there were statistically significant differences among the three study groups with (p-value = 0.001 for all) as shown in table 1.

Table (1): Demographic and clinical data of study groups:

Variables	Group A (no=10)	Group B (no=20)	Group C (no=20)	P-value	Post-hoc p value
Age/years					
• Mean ± SD	31.6 ± 12.8	34.0 ± 6.9	36.1 ± 14.8	0.464	
• Median	28.5	34.0	30.0		
• Range	19.0 – 63.0	20 – 47.0	19.0 – 67.0		
Gender					
• Male	220.0	15.0	525.0	0.210	
• Female	880.0	1995.0	1575.0		
Occupation					
• Nurse	00.0	1575.0	00.0	0.001*	
• Employee	220.0	15.0	420.0		
• Housewife	330.0	420.0	1365.0		
• Student	550.0	00.0	210.0		
• Teacher	00.0	00.0	15.0		
Systolic blood pressure(mmHg)					
• Mean ±SD	112.4±3.	119.8±3.08	128.0±5.9	0.039*	P1=0.867 P2=0.046* P3=0.011*
• Median	118.0	120.0	123.0		
BMI(Kg/m2)					
• Mean ±SD	22.5±0.69	38.6±5.1	39.5±3.4	0.001*	P1=0.001* P2=0.001* P3=0.51
• Median	22.4	36.1	40.3		
WHR					
• Mean ±SD	0.86±0.02	0.81±0.07	0.80±0.03	0.003*	P1=0.027* P2=0.014* P3=0.734
• Median	0.85	0.78	0.81		
VAI					
• Mean ±SD	2.6 ± 0.66	3.3 ± 0.76	4.5 ± 1.3	0.001*	P1=0.065* P2=0.001* P3=0.651
• Median	2.5	3.4	4.1		
BAI					
• Mean ±SD	13.1 ± 1.9	40.4 ± 3.2	40.8 ± 3.2	0.001*	P1=0.001* P2=0.001* P3=0.699
• Median	12.8	40.1	40.8		
CI					
• Mean ±SD	84.5 ± 5.8	112.0 ± 4.9	113.2 ± 6.6	0.001*	P1=0.001* P2=0.001* P3=0.521
• Median	84.1	112.0	113.0		
ABSI					
• Mean ±SD	0.27 ± 0.04	0.17 ± 0.04	0.145 ± 0.015	0.001*	P1=0.001* P2=0.001* P3=0.113
• Median	0.30	0.2	0.1		
BeW					
• Mean ±SD	16.1 ± 4.2	52.7 ± 4.6	53.3 ± 2.5	0.001*	P1=0.001* P2=0.001* P3=0.634
• Median	15.7	52.9	53.0		

K (Kruskal-Wallis test) -P1= Group A vs. Group B. -VAI=Viscera Adiposity Index. -P2= Group A vs. Group C. -BAI = Body Adiposity Index. -P3= Group B vs. Group C. -CI = Conicity Index. -ABSI =A Body Shape Index. - BeW= Belarmino–Waitzberg index. BMI=Body Mass Index WHR=waist to hip ratio *kg = Kilograms *m=meter
*cm = centimeter P-value was considered statistically significant when it is less than 0.05.
*(significant)

There were statistically significant differences among the three groups regarding T. Cholesterol, TG, LDL (P-value =0.001 for all). Concerning plasma glucose level there were statistically significant differences, for fasting plasma glucose level (P-value = 0.001), 2hrs post prandial plasma glucose level (P-value=0.001), and HBA1c (P-value = 0.001). According to NAFLD score, there were a statistically significant difference among groups (P-value = 0.001) (Table 2).

Table (2): Routine laboratory investigations:

Variables	Group A (no=20)	Group B (no=20)	Group C (no=20)	P-value	Post hoc p value
Cholesterol (mg/dl) • Mean ± SD • Median	140.7 ± 9.4 150.0	156.7 ± 14.7 160.5	221.9 ± 53.7 204.5	0.001*	P1=0.688 P2=0.001* P3=0.001*
TG (mg/dl) • Mean ± SD • Median	105.2±13.6 104.0	106.7±11.7 102.5	156.7±40.7 150.0	0.001*	P1=0.874 P2=0.001* P3=0.001*
LDL- C (mg/dl) • Mean ± SD • Median	10.7±0.75 10.7	10.9±1.7 10.7	15.7±3.9 14.9	0.001*	P1=0.840 P2=0.001* P3=0.001*
HDL(mg/dl) • Mean ± SD • Median	50.5±3.5 50.0	53.0±8.7 50.5	52.0±7.4 52.0	0.810	
FPG (md/dl) • Mean ±SD • Median	88.6±12.6 89.5	86.7±12.8 85.0	164.2±13.7 160.0	0.001*	P1=0.719 P2=0.001* P3=0.001
PPPG (mg/dl) • Mean ±SD • Median	124.6±8.7 112.0	131.3±11.5 129.0	283.4±39.5 290.0	0.001*	P1=0.10 P2=0.001* P3=0.001*
HBA1C % • Mean ±SD • Median	4.1±0.41 4.1	4.6±0.39 4.8	6.3±0.54 6.2	0.001*	P1=0.127 P2=0.001* P3=0.001*
NAFLD SCORE • Mean ±SD • Median	-4.5±0.91 -4.4	-3.4±0.4 -3.4	-1.7±0.03 -1.8	0.001	P1=0.025* P2=0.001* P3=0.001*

- K (Kruskal-Wallis test) - P1= Group A vs. Group B. - P2 = Group B vs. Group C. - P3 = Group A vs. Group C.
TG: Triglycerides - HDL: High Density lipoprotein - LDL: Low density lipoprotein. - FPG: Fasting Plasma Glucose.
-PPPG: Post Prandial Plasma Glucose. -HbA1C: Glycated Hemoglobin. NAFLD = Non Alcoholic Fatty Liver Diseases.
-P-value was considered statistically significant when it is less than 0.05. -*(significant)

For detection of 16s RNA gene, there was one positive case for 16s RNA gene (10%) in control (normal) group. The sequence of this gene belonged to Succinivibrio dextrinosolvens (100%). However, there were 3 cases (15%) that were positive for 16sRNA gene in obese without diabetes group (B) all belonged to Prevotella copri (100%). In addition, we found 5 (25%) cases that were positive for 16sRNA gene in obese with diabetes group; 3 belonged to Prevotella copri and 2 belonged to Succinivibrio dextrinosolvens. So, the difference among the three study groups regarding gut microbiota 16s RNA gene was statistically not significant with (p-value > 0.05) nor regarding the types of organisms. Although, the difference between group A and group B was statistically significant regarding type of organism as 100% of group B had Prevotella copri comparing to 0% in group A (P = 0.04). However, there was no marked difference between group A & group C (p-value = 0.20) and group B and group C (P-value = 0.27) regarding type of organisms (Table 3).

Table (3): Gut microbiota16sRNA gene detection among study groups:

Variables	Group A (no=20)	Group B (no=20)	Group C (no=20)	P-value
Gene • Negative (no=50) • Positive (no=10)	18.360 2.200	17.340 3.300	15.300 5.500	P1= 0.704 P2= 0.33 P3= 0.42
Organism • Prevotella Copri • Succinivibrio dextrinosolvens	0.00 2.1000	3.1000 00.0	3.600 2.400	P1=0.04* P2= 0.27 P3 = 0.20

-P1= group A vs. Group B -P2 = Group B vs. Group C -P3 = Group A vs. Group C
-P-value was considered statistically significant when it is less than 0.05. -* (significant).

Group C (-obese with DM) contained the majority of positive cases to target gene (16s rRNA) by percentage of 55.6%, followed by group B (-obese without DM) by 33.3% of positive cases, but group A (- control group), contained the lowest percentage (11.1%) of positive cases to the same gene (Figure 1).

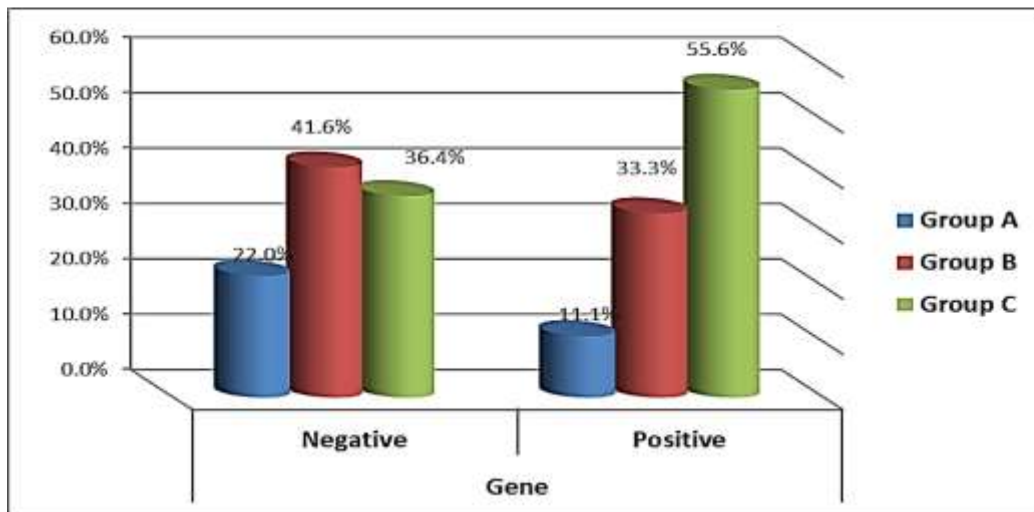

Figure (1): The positivity percentage of gene in each group.

Prevotella copri was more abundant in obese persons (100% of group B & 60% of group C), but not present in lean persons (group A). While, Succinivibrio was more abundant in lean persons (group A) by percentage of 100%, and also present in obese individuals with DM (group C) by percentage of 40%, but not present in obese subjects without diabetes (group B) (Figure 2).

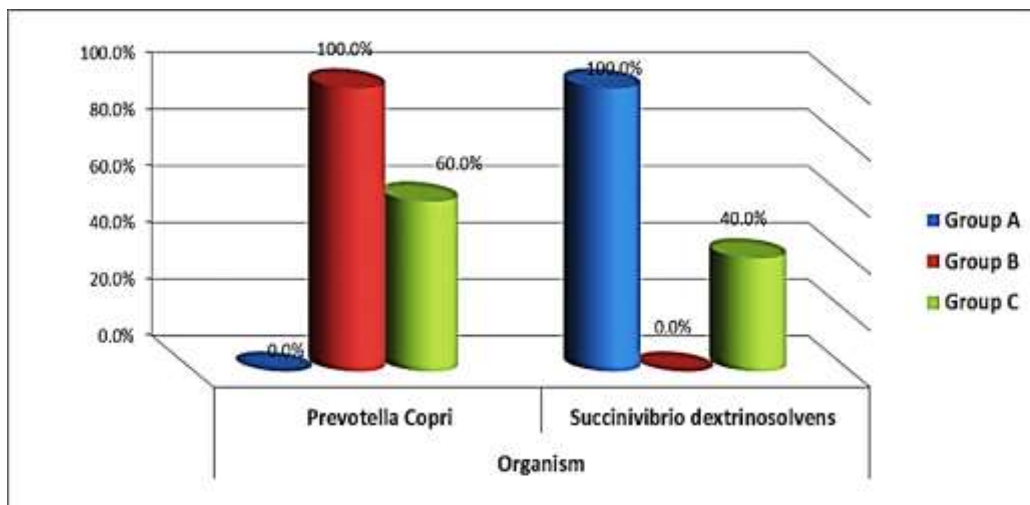

Figure (2): The organism abundance in study groups

DISCUSSION

In our study, we had three comparable groups with insignificant age and sex distribution. As consequence of using BMI as grouping factor in patient allocation in different study groups, the baseline clinical characteristics such as BMI, WC, WHR, ABSI, CI, VAI, BeW index, NAFLD score, fasting blood glucose, 2 hours postprandial blood sugar, HbA1C, total cholesterol, triglycerides, LDL, systolic blood pressure were significantly high among obese individuals of group B and with obese T2 DM patients of group C in comparison to control group; but no significant difference was found between group B & C, and no significant difference was found among three groups as regards HDL.

On analyzing the study results, there was no statistically significant difference among the three study groups regarding 16s rRNA positivity of gut microbiota; despite there were two positive cases (10%) for 16s RNA gene in group A, three cases (15%) were positive in group B and five (25%) cases were positive for 16s rRNA gene in group C.

Searching for the type of organism, positive samples for 16s RNA were selected and subjected to next generation sequencing for species level analysis. The found sequence was belonging to Succinivibrio dextrinosolvensi "Firmicutes" in the two positive cases (100%) in group A. All three cases (100%) of group B were belonging to Prevotella copri "Bacteroidetes". While the three cases (60%) of group C were belonging to Prevotella copri and two cases (40%) were belonging to Succinivibrio dextrinosolvens. This finding of Bacteroidetes predominance in obese groups (B and C) support the idea of dysbiosis associated with obesity despite that the statistically significant difference was found only between group A (Lean) and group B (Obese) and was not between group A (Lean) versus group C (Obese T2 DM).

The most two dominant bacterial phyla in human gut are Bacteroidetes Firmicutes. Bacteroidetes are mostly dominated by Bacteroides and Prevotella genera. Prevotella copri is associated with T2 DM, obesity, and hypertension [20].

The Dilemma of real association between microbiota and obesity is still ongoing without solid rules. Some studies showed that there was an association between obesity and changes in the Bacteroidetes/ Firmicutes ratio. Others found no correlation between obesity and this ratio [21]. Different researches investigated if there are other metabolic mechanisms to elucidate the effect of the gut microbiota on obesity and T2 DM. **Allin *et al.*** [22] reported that there are metabolic endotoxemia, bacterial translocation, decreased butyrate concentrations, and defective secretion of incretins.

Lipopolysaccharides (LPS) are endotoxins present in the gram-negative bacteria cell wall (as Prevotella) cause innate immune receptors activation leading to metabolic endotoxemia. This leading to release of inflammatory cytokines, as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF), and these mediators are primarily responsible for the endotoxemia observed in metabolic disorders [23]. In addition, **Everard and Cani** [24], in their study found that there was an association of increased C-reactive proteins, IL-6 plasma concentrations, and intestinal dysbiosis with obesity and T2 DM development.

Succinivibrio dextrinosolvens is a gram-negative anaerobic bacterium belongs to Proteobacteria phylam. It is considered as a succinate-producer microorganism [25]. By cross-feeding among different gut microbiomes, succinate which is a SCFA convert to propionate, so it is measured at a relatively low levels in the lumen of the gut [26]. There were several published studies that linked the succinate-enriched gut to pathological disorders, which are associated with dysbiosis as obesity and IBD. While other studies found that the healthy individuals gut microbiota showing a glucose metabolism improvement following barley kernel-based bread reduction, which was enriched for gut microbiota that produce succinate [27, 28]. Moreover, succinate is considered as an important bacterial product for the essential metabolic effects of dietary fiber consumption [29].

In agreement with our finding, the studies of **Radwan *et al.*** [30] **and Leite *et al.*** [31]**,** found that Prevotella species were present in obese-DM only and not present in lean persons, and may be considered a bright gut microbiota that is associated with development of T2DM. In meta-analysis done by **Stanislowski *et al.*** [32]**,** they found that in non-Westernized populations there was an enriched Prevotella diversity and prevalence, especially Prevotella copri, which is widespread in non-Westernized societies. Prevotella copri has four distinct clades that tend to be present together. Generally, these clades are absent in Westernized societies. Similar to our study, **Schwiertz *et al.*** [33] reported that the Firmicutes and Bacteroidetes were the most predominant bacterial phyla in feces of lean and obese subjects. The Firmicutes/Bacteroidetes ratio decreased in overweight and obese individuals.

Against the current study that showing abundance of Prevotella copri that considered Bacteroidetes, **Gallardo-Becerra *et al.*** [34], in their study that carried on 27 Mexican children divided into lean, obese, and obese with metabolic syndrome, they noted that there was a significant richness and diversity in obese children and children with MetS according to gut microbiome 16S rRNA profiling. In obese children, the 16S rRNA profiling reported an increased Firmicutes/Bacteroidetes ratio. This difference between the results of the current study and **Gallardo-Becerra *et al.*** [34] results may be due to that their study was carried out on children and in different geographic area. Lastly, **Zhang *et al.*** [35] study concluded that there was increase of Firmicutes/Bacteroidetes in the individuals with obesity and diabetes, which is opposit to our results in the present study of Prevotella abundance.

CONCLUSION

The dilemma is still going in type of phyla in obese and non-obese with studies like ours supporting the Bacterodietes predominance and others support Firmicutes predominance.

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