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Linking Gut Microbiota, Metabolic Syndrome and Metabolic Health among a Sample of Obese Egyptian Females

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Abstract

BACKGROUND: Studies of the gut microbiota have revealed a great link to obesity and metabolic syndrome (MetS).

AIM: The aim of this study was to review the dysbiosis of gut microbiota in terms of the components of MetS among a sample of obese Egyptian female patients and to assess current potential gut microbiota targeted therapies for the treatment of MetS.

SUBJECTS AND METHODS: This study is a cross-sectional study included 82 obese Egyptian women. All participants were subjected to anthropometric assessment; and laboratory evaluation of fasting blood sugar (FBS), insulin, C-reactive protein (CRP), lipid profile, and insulin resistance homeostasis model assessment (HOMA), in addition to fecal microbiota analysis for *Lactobacillus*, *Bifidobacteria*, *Firmicutes* and *Bacteroid*.

RESULTS: Among obese group with MetS, *Firmicutes*/*Bacteroidetes* (F/B) ratio was negatively associated with HOMA and positively associated with serum cholesterol and low-density lipoprotein (LDL), while *Lactobacillus* was negatively associated with serum cholesterol. Among obese group without MetS, (F/B) ratio is negatively associated with Waist circumference (central obesity marker) and positively associated with CRP (inflammatory marker), while *Lactobacillus* was positively correlated with FBS and HOMA, and *Bifidobacteria* was negatively associated with serum cholesterol and LDL.

CONCLUSION: The two beneficial types the *Lactobacillus* and *Bifidobacteria* supplementation in the form of probiotic with therapeutic treatment and decreasing of WC have their important role in controlling and treating hypertension, serum cholesterol and LDL levels, among obese females even with MetS.

Introduction

Metabolic syndrome (MetS) is rapidly growing worldwide health concern with estimation that over one billion people globally [1]. It is a cluster of co-occurring pathological conditions, including insulin resistance (IR), abdominal obesity, hypertension and dyslipidemia [2].

The criteria for diagnosing MetS are designated by values for obesity (e.g. waist circumference [WC] or body mass index [BMI]), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and hypertension [3]. Obesity and metabolic disease are complex multi-factorial diseases that result from interaction of genetic and environmental factors [4]. Several studies demonstrated that the human gut microbiota, the complex microbial community living inside the human gastrointestinal tract, plays a significant role in the pathogenesis of MetS [5], [6], [7]. They postulated that the composition of microbiota found in the gut of obese individuals showed dissimilar diversity in comparison with that of the microbiota of lean individuals [8], [9].

Alterations in the gut microbiota composition or diversity are known as dysbiosis [10], [11]. Human Gut dysbiosis is linked to many pathologic conditions disturbing the energy metabolism; such as obesity, type 2-diabetes, and atherosclerosis [12]. The microbiota in the human gut is mostly composed of bacterial phyla: *Firmicutes* and *Bacteroidetes* (F/B); in addition to *Bifidobacteria* and *Lactobacillus* [13]. The association between the two dominant phyla, expressed as the (F/B) ratio, has been associated with several pathological disorders [14].

Dietary choices influence human health through modification of the gastrointestinal microbiota. Regarding the impact of dietary protein on gut microbial composition, a study showed that protein consumption positively correlated with overall microbial diversity, and that consumption of pea and whey protein extract has been found to increase gut Bifidobacterium

and *Lactobacillus* [15]. On the contrary, bile-tolerant anaerobes such as Bacteroides were found to increase with animal-based protein consumption [16]. A study of Fava *et al*. [17], showed that a high-fat diet increased total counts of Bacteroides, while consumption of a low fat diet led to increased fecal abundance of Bifidobacterium with concomitant reductions in fasting glucose and total cholesterol (TC). Carbohydrates ability to modify the gut microbiota was also studied, Eid *et al*. [18], found that human subjects fed high levels of glucose, fructose, and sucrose in the form of date fruits had increased relative abundance of Bifidobacteria, with reduced Bacteroides. On the other hand, Carvalho-Wells *et al*. [19], reported that. non-digestible carbohydrates rich in wheat bran and whole grain were linked to the increase in gut Bifidobacteria and Lactobacilli.

Thus, describing the microbiota involved in the dysbiosis is essential as they are emerging as a hopeful target for the nutritional or therapeutic strategies for management of MetS. For example, the overgrowth of potentially pathogenic species could be treated through targeted antimicrobial agents while the disappearance of beneficial microbiota could be treated by the administration of specific probiotics such as *Lactobacillus rhamnosus* and *Bifidobacterium lactis* [20].

Hence, the purpose of this research is to review the dysbiosis of gut microbiota in terms of the components of MetS among a sample of obese Egyptian female patients; recognize the relationship between microbiota status and laboratory markers of MetS and to assess current potential gut microbiota targeted therapies for the treatment of MetS among a sample of obese Egyptian females.

Subjects and Methods

This study was cross-sectional study, included 82 obese Egyptian women. Their ages were ranged between 25 and 60 years with mean age 41.62 ± 10.70 years. They were recruited and randomly chosen, from all employees and workers; of all categories; of the "National Research Centre (NRC)," Egypt. A written informed consent was obtained from all participants after being informed about the purpose of the study. This research paper was derived from a cross-sectional survey of a project funded by NRC Egypt, 2019–2022 entitled "Gut Microbiota in Obesity and MetS among obese women: Interactions of the Microbiome, Epigenetic, Nutrition and Probiotic Intervention." (12th Research Plan of the NRC), with an approval obtained from Ethics Committee of NRC (Registration Number is19/236).

Methods

For each participated woman, blood pressure (BP), anthropometric measurements, laboratory investigations, and microbiota analysis were done.

BP

BP was measured using the standardized mercury sphygmomanometer with a suitable cuff size. It was measured on the left arm while the participated women were sitting relaxed for 5 min. Two readings were obtained, and the average was recorded. Systolic BP (SBP); determined by the onset of the "tapping" Korotkoff sounds (K1), while the fifth Korotkoff sound (K5), or the disappearance of Korotkoff sounds, as the definition of diastolic BP (DBP) were recorded.

Anthropometric measurements

Body weight, height and WC were measured, following the recommendations of the "International Biological Program" [21]. Body weight (Wt) was determined to the nearest 0.01 kg using a Seca Scale Balance, with the woman wearing minimal clothes and with no shoes. Body height (Ht) was measured to the nearest 0.1 cm using a Holtain portable anthropometer. WC was measured using non-stretchable plastic tape; approximated to the nearest 0.1 cm. WC was measured atthe midpoint between the lower curvature of the last fixed rib and the superior curvature of the iliac crest, with the woman in an upright standing position and their arms alongside the body, feet together, and abdomen relaxed. BMI was calculated (BMI: weight [in kilograms] divided by height [in meters squared]). The participated women were all chosen as obese; as their BMI ≥30 kg/m^2.

Blood sampling and laboratory investigations

In the morning, venous blood samples (after 12-h fasting) were drawn from the participated women, using venipuncture. Biochemical parameters were performed on fasting sera that were stored at –70°C until used for assessment of fasting blood sugar (FBS), insulin, C-reactive protein (CRP), and lipid profile. All were done in the laboratory of "Mediucal Excellence Research Center MERC" which is a part of "NRC," Egypt.

FBS level was measured using the automated clinical chemistry analyzer Olympus AU 400 analyzer. Serum insulin was assessed using Enzyme Immunoassay Test Kit Catalog No. E29-072 (Immunospec Corporation). Then IR was calculated according to Matthews *et al*. [22] using the following equation: IR = fasting glucose (mg/dl) × fasting insulin (μIU/ml)/405.

The assay of the serum CRP was performed by Enzyme Linked Immunosorbent Assay (ELISA) kits, Cat No.: RAP002 [23], (https://www.mybiosource.com.)

Estimation of lipid profile: Serum levels of TC, TG, and HDL-C were measured by standardized enzymatic procedures; using kits supplied by Roche Diagnostics (Mannheim, Germany) on the Olympus AU 400 automated clinical chemistry analyzer. Low-density lipoprotein cholesterol (LDL-C) was calculated according to formula of Friedewald *et al*. [24] as follows: LDL-C = TC–TG/5 + HDL-C (This formula is valid when TG must be <400 mg/dl).

Clinically, a patient is considered to have MetS when three or more of the following five conditions exist, which are (i) WC ≥88 cm in women, (ii) BP ≥135/85 mmHg, (iii) TG ≥150 mg/dl, (iv) HDL-C <50 mg/dl in women, and (v) fasting glucose ≥100 mg/dl [25].

Microbiota analysis

The proportion of *Lactobacillus* and *Bifidobacteria*; and (F/B) ratio strains were assessed in the stool of all participants using the real time polymerase chain reaction (PCR). Specimen collection and preparation: Stool was collected by defecation in a plain sterilized container allowed to be frozen. Specimen Storage and Preparation: stool was frozen on at –20°C. The primers and probes were used to detect *Bifidobacterium* spp. and *Lactobacillus* spp; and *Firmicutes* spp. and *Bacteroidetes* spp., where based on 16S rRNA gene sequences retrieved from the National Center for Biotechnology Information databases by means of the Entrez program [26].

Reagents provided by kits: DNA extraction Kit. Assay procedure: DNA extraction: The QIAamp DNA Stool Minikit (Qiagen) was used to extract DNA from one gram of fresh or frozen stool sample according to the manufacturer's instructions. Bacterial quantification by real-time PCR was done.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) (SPSS/Windows Version 18, SPSS Inc., Chicago, IL, USA). Normality of data was tested using the Kolmogorov-Smirnov test. The data were normally distributed. Hence, the parametric tests were used. All participated women were obese; with BMI >30 kg/m^2. They were classified according to the presence of MetS criteria into two subgroups: 59 obese without MetS (have no or <2 criteria of MetS), and 23 obese with MetS (have 3 or more criteria of MetS).

The parametric data were expressed as mean ± SD, The various parametric variables of the two groups were analyzed and compared using independent t-test. Pearsons correlation test was used to assess the relations between each enterotypes of microbiota and patient's clinical and metabolic parameters among the two groups. $p < 0.05$ was regarded as statistically significant for all tests.

Results

Table 1 showed means ± SD and the range of the BP, characteristic anthropometric parameters, and laboratory and microbiota investigations of the obese women with and without multiple sclerosis (MS). The obese women with MetS were significantly older, and had highly significant higher values of BP (both systolic and diastolic), BMI, WC, homeostasis model assessment (HOMA) and TG, and significant higher value of serum insulin; than those without MetS.

Table 1: Mean ± SD and range of the Characteristic anthropometric parameters and BP of the obese non Met S women and Met S patients (n = 82)

Parameters	Without Met S (n: 59)	With Met S (n: 23)	p
	Mean ± SEM and range		
Age (year)	43.61 ± 9.89 (25–60)	48.52 ± 9.95 (29–60)	0.037*
BP (mm Hg)			
Systolic	115.38 ± 10.38 (90.0–150.0)	137.50 ± 22.35 (100.0–190.0)	0.000**
Diastolic	72.92 ± 6.95 (60.0–90.0)	84.32 ± 14.50 (60.0–110.0)	0.000**
Anthropometry			
Weight (kg)	91.82 ± 16.29 (54.0–134.10)	100.10 ± 20.40 (73.8–136.20)	0.058
Height (cm)	159.12 ± 5.85 (146.0–171.0)	157.59 ± 7.28 (146.0–172.0)	0.324
BMI (kg/m^2)	36.19 ± 5.64 (24.99–49.42)	40.01 ± 5.89 (29.41–51.43)	0.008**
WC (cm)	102.66 ± 15.42 (70.0–133.0)	113.96 ± 12.85 (93.138.0)	0.003**
Lab			
FBS (mg/dl)	112.77 ± 42.57 (70.0–191.0)	130.13 ± 46.37 (90.0–285.0)	0.116
Serum Insulin (µIU/ml)	12.22 ± 6.76 (1.6–28.00)	16.73 ± 7.40 (2.0–31.5)	0.011*
HOMA	3.47+2.19 (0.21–9.48)	5.27+2.46 (0.56–9.67)	0.002**
CRP (ng/ml)	7243.15+2981.27 (1900–12000)	6933.48+1795.72 (4200–8900)	0.655
Lipid profile			
Cholesterol (mg/dl)	195.57 ± 5.80 (99.0–277.0)	208.52 ± 36.86 (134.0–281.0)	0.169
HDL (mg/dl)	57.00 ± 11.52 (39.0–74.0)	54.35 ± 10.23 (40.0–75.0)	0.344
LDL (mg/dl)	117.55 ± 33.45 (68.00–188.0)	128.25 ± 5.04 (73.0–190.0)	0.477
TG (mg/dl)	98.51 ± 34.33 (50.00–172.0)	154.39 ± 79.17 (69.0–254.0)	0.000**
Microbiota			
Log *Lactobacillus*	6.11 ± 0.13 (4.67–7.91)	5.85 ± 0.15 (4.61–7.83)	0.167
Log bifido	6.14 ± 0.11 (4.61–7.79)	6.12 ± 0.11 (4.85–7.74))	0.926
Log *Bacteroid*	13.24 ± 1.51 (10.57–16.68)	13.05 ± 0.26 (10.67–14.68)	0.576
Log *Firmicutes*	9.45 ± 1.49 (4.52–11.88)	8.91 ± 1.43 (6.79–11.69)	0.141
Log *Firmicutes/Bacteroid* ratio	0.73 ± 0.13 (0.43–1.10)	0.69 ± 0.14 (0.46–1.07)	0.350

BMI: Body mass index, BMR: Basal metabolic rate, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBS: Fasting blood sugar, TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein–cholesterol, LDL-C: Low density lipoprotein cholesterol, SGPT: Alanine aminotransferase, SGOT: Aspartate aminotransferase, Met S: Metabolic Syndrome, $p < 0.05$: Significant difference, $p < 0.01$: Highly significant difference, BP: Blood pressure.

Reviewing Table 1, it was also, found that *Bacteroidetes* bacteria were the most prevalent type

Table 2: Pearson's correlations between each enterotypes of microbiota and patient's clinical and metabolic parameters among obese females without MetS

Variables	Log *Lactobacillus*		Log bifido		Log *Bacteroid*		Log *Firmicutes*		Log *Firmicutes/Bacteroid* ratio	
	r	p	r	p	r	p	r	p	r	p
Age (years)	0.172	0.192	0.025	0.853	0.282	0.031*	–0.060	0.651	–0.234	0.075
BP (mm Hg)										
SBP	0.016	0.908	–0.113	0.423	–0.085	0.549	–0.199	0.157	–0.151	0.284
DBP	–0.107	0.452	–0.124	0.383	–0.252	0.072	–0.236	0.092	–0.079	0.579
Anthropometry										
Weight (kg)	0.171	0.196	–0.036	0.788	0.089	0.505	0.043	0.748	–0.018	0.895
Height (cm)	–0.139	0.294	–0.066	0.619	–0.147	0.267	–0.120	0.367	–0.029	0.827
BMI (kg/m^2)	0.256	0.051	–0.073	0.584	0.165	0.210	0.111	0.402	0.002	0.990
WC (cm)	0.040	0.763	0.230	0.080	0.106	0.423	–0.214	0.103	–0.272	0.037*
Lab										
FBS (mg/dl)	0.296	0.031*	0.059	0.676	0.312	0.023*	0.030	0.832	–0.170	0.223
Serum Insulin (µIU/ml)	0.176	0.208	–0.088	0.529	0.207	0.136	0.147	0.293	–0.019	0.892
HOMA	0.301	0.029*	–0.012	0.930	0.342	0.012*	0.197	0.158	–0.064	0.648
CRP (ng/ml)	–0.108	0.443	–0.101	0.471	–0.194	0.163	0.210	0.131	0.273	0.048*
Lipid profile										
Cholesterol (mg/dl)	0.242	0.080	–0.295	0.032*	0.040	0.774	–0.072	0.608	–0.071	0.614
HDL (mg/dl)	0.256	0.065	–0.074	0.600	0.197	0.156	–0.156	0.265	–0.233	0.093
LDL (mg/dl)	0.236	0.089	–0.320	0.019*	–0.004	0.980	–0.076	0.589	–0.048	0.734
TG (mg/dl)	–0.061	0.665	–0.009	0.947	0.043	0.762	0.063	0.653	0.007	0.963
Microbiota										
Log *Lactobacillus*			0.087	0.512	0.645	0.000**	0.131	0.321	–0.281	0.031*
Log bifido	0.087	0.512			0.547	0.000**	0.079	0.554	–0.261	0.046*
Log *Bacteroid*	0.645	0.000**	0.547	0.000**			0.220	0.094	–0.415	0.001**
Log *Firmicutes*	0.131	0.321	0.079	0.554	0.220	0.094			0.788	0.000**
Log *Firmicutes/Bacteroid* ratio	–0.281	0.031*	–0.261	0.046*	–0.415	0.001**	0.788	0.000**		

p < 0.05: Significant difference, p < 0.01: Highly significant difference, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein–cholesterol, LDL-C: Low density lipoprotein cholesterol, Met S: Metabolic syndrome, FBS: Fasting blood sugar.

among the studied microbiota, and followed by the gut microbes *Firmicutes*, followed by the two beneficial types the *Lactobacillus* and *Bifidobacteria*. The insignificant decrease in bacteroid was less than the insignificant decrease in *Firmicutes*, this lead to insignificant minor decrease in F/B ratio.

Pearson 's correlations between each enterotypes of microbiota and patient's clinical and metabolic parameters among obese women in the two groups are presented in Tables 2 and 3. *Lactobacillus* had significant positive correlations with *Bacteroidetes* bacteria, and significant negative correlations with (F/B) Ratio among both obese with and without MS groups. Among obese without MS group, *Lactobacillus* had significant positive correlations with FBS and HOMA. While among obese with MS group, *Lactobacillus* had significant negative correlations with serum cholesterol.

Bifidobacteria had significant positive correlations with *Bacteroidetes* bacteria among both groups. Among obese without MS group, *Bifidobacteria* had significant negative correlation with serum cholesterol, LDL and (F/B) Ratio.

Bacteroidetes bacteria had significant positive correlations with *Lactobacillus* and *Bifidobacteria*, and significant negative correlations with (F/B) ratio among the two groups. Among obese without MS group, *Bacteroidetes* bacteria had significant positive correlations with age, FBS and HOMA. Among obese with MS group, *Bacteroidetes* bacteria had significant positive correlations with weight and significant negative correlations with serum cholesterol.

Table 3: Pearson's correlations between each enterotypes of microbiota and patient's clinical and metabolic parameters among obese females with MetS

Variables	Log *Lactobacillus*		Log bifido		Log *Bacteroid*		Log *Firmicutes*		Log *Firmicutes/Bacteroid* ratio	
	r	p	r	p	r	p	r	p	r	p
Age (years)	0.035	0.872	–0.059	0.790	0.006	0.977	0.111	0.614	0.082	0.710
BP (mm Hg)										
SBP	0.337	0.125	0.422	0.050	0.409	0.059	–0.336	0.16	–0.391	0.072
DBP	0.286	0.196	0.048	0.832	0.125	0.578	0.084	0.712	–0.103	0.647
Anthropometry										
Weight (kg)	0.271	0.212	0.044	0.844	0.414	0.050*	–0.101	0.648	–0.251	0.248
Height (cm)	0.157	0.475	0.124	0.574	0.372	0.080	–0.055	0.805	–0.203	0.352
BMI (kg/m^2)	0.265	0.222	–0.003	0.991	0.344	0.108	–0.114	0.604	–0.231	0.299
WC (cm)	0.180	0.412	–0.027	0.904	0.330	0.125	0.010	0.965	–0.145	0.510
Lab										
FBS (mg/dl)	–0.114	0.606	–0.054	0.807	–0.068	0.758	–0.147	0.502	–0.097	0.658
Serum Insulin (µIU/ml)	–0.092	0.677	0.412	0.051	0.224	0.305	–0.385	0.070	–0.374	0.079
HOMA	–0.153	0.485	0.294	0.174	0.134	0.543	–0.441	0.035*	–0.392	0.064
CRP (ng/ml)	0.218	0.318	0.004	0.987	0.191	0.384	–0.323	0.132	–0.301	0.163
Lipid profile										
Cholesterol (mg/dl)	–0.443	0.034*	–0.260	0.231	–0.451	0.031*	0.454	0.029*	0.562	0.005**
HDL (mg/dl)	–0.048	0.827	–0.020	0.928	–0.095	0.666	0.181	0.409	0.222	0.308
LDL (mg/dl)	–0.391	0.065	–0.229	0.295	–0.403	0.056	0.368	0.084	0.485	0.019*
TG (mg/dl)	–0.296	0.170	–0.184	0.402	–0.265	0.222	0.288	0.183	0.300	0.164
Microbiota										
Log *Lactobacillus*			0.166	0.449	0.659	0.001**	–0.248	0.253	–0.468	0.024*
Log bifido	0.166	0.449			0.526	0.010*	–0.138	0.530	–0.300	0.156
Log *Bacteroid*	0.659	0.001**	0.526	0.010*			–0.261	0.230	–0.631	0.001**
Log *Firmicutes*	–0.248	0.253	–0.138	0.530	–0.261	0.230			0.907	0.000**
Log *Firmicutes/Bacteroid* ratio	–0.468	0.024*	–0.300	0.156	–0.631	0.001**	0.907	0.000**		

p < 0.05: Significant difference, p < 0.01: Highly significant difference, WC: Waist circumference, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBS: Fasting blood sugar, TC: Total cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein–cholesterol, LDL-C: Low density lipoprotein cholesterol, Met S: Metabolic syndrome, BMI: Body mass index.

Firmicutes had significant positive correlations with (F/B) ratio among the two groups. Among obese with MS group, *Firmicutes* had significant positive correlation with serum cholesterol and significant negative correlations with HOMA.

(F/B) Ratio had significant positive correlation with *Firmicutes* and significant negative correlations with *Lactobacillus* and *Bacteroidetes* among the two groups. It had significant negative correlations with *Bifidobacteria* among obese without MS group. Among obese without MS group, (F/B) ratio had significant positive correlation with CRP, and significant negative correlation with WC. Among obese with MS group, (F/B) ratio had significant positive correlations with serum cholesterol and LDL, and significant negative correlations with HOMA.

Discussion

For years, a great deal of research was undertaken for better understanding of the factors leading to MetS. Several studies discussed the significant role played by the human gut microbiota; the complex microbial community living inside the human gastrointestinal tract, in the pathogenesis of MetS [6]. The interaction between gut microbiota and host metabolism was found to either threaten or protect the host from metabolic diseases [27].

Some studies showed that this alterations in the gut microbiota composition; intestinal dysbiosis; causes low-grade inflammation, obesity and consequently MetS [28], while others revealed that dysbiosis could be the result of low-grade inflammation during obesity and MetS [29].

The present study aimed to characterize the gut microbiota of obese women with and without MetS and identify relationship between their microbiota status and metabolic parameters among a sample of obese Egyptian females. Present results revealed that, in spite of the insignificant differences between the 2 studied groups concerning microbiota; obese women without MetS had insignificant higher values of all types of the studied microbiota than those with MetS. While *Firmicutes*/*Bacteroid* ratio became insignificant lower among obese with MetS than among those without MetS. This means that development of MetS led to decrease the amount of microbiota and particularly decrease the *Firmicutes*/*Bacteroid* ratio; which may be one of the causes of MetS. The insignificant differences in statistical analysis of the microbiota might be related to the use of the log values.

Among obese females with and without MS, F/B ratio had significant negative correlation with *Lactobacillus*. Confirming our results, the genera *Lactobacillus* Probiotics were found to have the potential to reduce the F/B ratio and obesity as the administration of *Lactobacillus* decreased the F/B ratio in obese mice and reduced fatty acid synthesis, in the liver [30]. In another study, *Lactobacillus* consumed with a high-fat diet prevented weight gain and decreased the F/B ratio [31].

In the current study, ageing has been suggested to cause changes in the intestinal microbial community. Age had significant positive correlation with *Bacteroidetes* only among obese females without MS and insignificant in obese females with MS. Similar to current results, the identified microbiome pattern of healthy ageing is characterized by depletion of Bacteroides, while retaining a high Bacteroides dominance into older age due to dysbiosis of obesity, predicts decreased survival in a 4-year follow-up [32].

In the present study, BP had insignificant correlations with *Lactobacillus*, *Firmicutes* and F/B ratio among the 2 obese groups. In contrast, some studies have suggested an association between intestinal dysbiosis and hypertension. For example, treating mice with *Lactobacillus* prevented salt sensitive hypertension [33]. In another experimental study by Adnan *et al*. [34], gavage feeding the normotensive rats with microbiota from hypertensive rats, led to increases in the F/B ratio and systolic BP.

Concerning the anthropometry, among obese females with MetS, body weight had significant positive correlations with *Bacteroidetes.* Similarly, Schwiertz *et al*. [35]; in Germany; studied the fecal microbiota of lean and obese volunteers of both sexes and noted that the proportions of Bacteroides were greater in overweight volunteers than lean ones. On the other side, Crovesy *et al*. [36] revealed that *Bacteroidetes* have been associated with normal body weight but the *Firmicutes* with obesity. The possible cause may be due to dysbiosis in MetS or could be diet-induced effect. In fact, *Bacteroidetes* encode a greater number of carbohydrate-degrading enzymes than *Firmicutes* [37].

In the current study, WC had significant negative correlation with F/B Ratio among obese females without MS; confirming dysbiosis. However, Davis, 2016 revealed that the increase in *Firmicutes* can be associated with augmented uptake of fatty acids, storage of TG in adipocytes and increased hepatic lipogenesis [38]. On the other hand, *Bifidobacterium* could have the ability to help Bacteroides degrade polysaccharides and inhibit exogenous cholesterol absorption from the small intestine [39], therefore the decrease in *Bifidobacterium* will increase body adiposity and WC [40].

There was a significant negative correlation between HOMA only with *Firmicutes* and F/B ratio among obese group with MS. While among obese group without MS, FBS, and HOMA had significant positive correlations with *Lactobacillus* (which may be

a protective effect against the development of MS) and *Bacteroidetes*.

Gut microbiota may affect our body's response to insulin, for this reason, many researchers are interested in targeting the gut microbiota to improve obesity-associated IR and hyperglycemia [41]. Glycemic improvement by probiotics supplements in pre-diabetic individuals has been supported by an Iranian study [42]. Serum CRP is a marker of systemic inflammation, which is elevated in the presence of chronic conditions including obesity, type 2 diabetes, and several components of the MetS [43].

In current study, CRP had significant positive correlations with F/B Ratio among obese group without MetS. Studies had revealed that inflammation in MetS may be triggered by intestinal dysbiosis and involves many chronic multisystem conditions including obesity, atherosclerosis, type 2 diabetes mellitus, and inflammatory bowel disease [44]. In current study, among obese group with MS serum cholesterol and LDL had significant positive correlations with F/B ratio which may be due to a protective increase in *Firmicutes* to lower hypercholesterolemia and decrease high LDL associated with MS. Furthermore, both serum cholesterol and LDL had significant negative correlation with *Bifidobacteria* only among obese group without MS, while serum cholesterol only showed significant negative correlation with *Lactobacillus* and Bacteroides among obese group with MS.

Similarly, meta-analysis studies showed reduction in the level of TC and LDL cholesterol after the use of probiotics, including *Lactobacillus* and *Bifidobacterium* [45], [46]. A study in Brazil also showed potential effects of *Bifidobacterium* in reducing obesity, blood lipids, and some inflammatory markers, which may reduce cardiovascular risk in obese patients [47]. A meta-analysis established that consumption of *Lactobacillus* has beneficial effects on serum TC and LDL-C levels, while no noticeable changes in serum HDL-C and TG levels [48].

Firmicutes participate in the metabolic process of phenolic compounds, which act as ant diabetic and anti-obesity agents [49]. Thus, this bacterial phylum could have a potential role in the maintenance of normal blood lipids. A study identified that higher abundances of *Firmicutes* and lower abundances of *Bacteroidetes* were associated with an optimal therapeutic effect of the lipid lowering agent Rosuvastatin [50].

Conclusion

Among obese women, gut microbiota had insignificant correlation with either BP or anthropometry, except WC which was negatively associated with F/B Ratio among obese group without MS. Among obese group with MS, F/B Ratio is negatively associated with HOMA and positively associated with serum cholesterol and LDL, while *Lactobacillus* and *Bacteroidetes* are negatively associated with serum cholesterol.

Among obese group without MS, F/B Ratio is negatively associated with WC (obesity marker) and positively associated with CRP (inflammatory marker), while *Lactobacillus* and *Bacteroidetes* are positively associated with FBS and HOMA, and *Bifidobacteria* is negatively associated with serum cholesterol and LDL.

Current findings indicate that gut microbiota may play a crucial role in dyslipidemia. As well, gut micro flora can be implicated in the regulation of lipid, glucose, and energy metabolism.

Limitation of this Study

The fund of the research was limited, so we did not be able to increase the sample number of obese women regarding laboratory and microbiota analysis. COVID-19 pandemic also was one of the most difficult challenges we met which was a barrier for collection of the study sample.

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Author Contribution

Nayera E. Hassan conceived and designed the study; she is the PI of the project from which this data was derived. Sahar A. El-Masry; statistical analysis and interpretation of the data, she is the Co-PI of the project from which these data were derived. Enas Abdel Rasheed and Mai Magdy Abdel Wahed; responsible about the laboratory investigations. Ayat N. Kamal and Mohamed S. El Hussieny; wrote the draft of the article, Aya Khalil and Manal Mouhamed Aly; supervision on collection of data and references. Mohamed Selim,

Khadija M Alian and Darine Amin; collected the data. All authors contributed to the collection of references, drafting of the article and final approval of the version to be submitted. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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