RELATION OF LACTOBACILLI ACIDOPHILUS TO OBESITY IN EGYPTIAN POPULATION

By
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Current considerations are existed about the sharing role of gut microbiota in the enhancement of obesity and allied comorbidities. This observational case-control study assessed the possible relation of Lactobacilli acidophilus to obesity in a sample of Egyptian population by real-time PCR in stool. The study enrolled 20 healthy slim subjects and 40 subjects who had BMI >25 kg/m². Routine laboratory analysis and identification of stool L. acidophilus by quantitative real time PCR technique was performed for all enrolled subjects. Lactobacillus acidophilus was expressed in 21/40 (52.5%) fecal samples of obese cases and 16/20 (80%) of fecal samples of non-obese ones. In rest of samples in both groups, the expression was below the detection limit. The results showed that the mean lactobacilli CT was expressed in obese cases (38.89±2.57) compared to (36.08±4.63) in non-obese cases, with high significant difference (P =0.04). Lactobacillus acidophilus was significantly lowered in obese Egyptian patients. The argument about significance correlation between imbalance in gut microbiota and obesity is one of the hot topics in medicine.

Key words: Obesity, Gut Dysbiosis, Lactobacilli acidophilus.

Introduction

Globally, obesity prevalence has been surged in the last 50 years, touching a pandemic state (Blüher, 2019). Surplus body weight ranked as the 6th most crucial risk factor added to entire burden of diseases worldwide with about 1.1 billion adults and 10% of children are nowadays categorized either overweight or obese (D’Ugo et al, 2019).

In Egypt, around 36% of the adult population was considered obese (Salman et al, 2018) and increasing number of diabetic patients (El-Tawdy et al, 2017).

Obesity heralds copious of morbidities and is also allied to higher mortality. For instance, it's is a risk factor for type 2 diabetes mellitus, elevated blood pressure, dyslipidemia, cardiovascular diseases (CVD), respiratory ailments, joint complaints, psychosocial conditions, and multitude of cancers including colon, esophagus, pancreas, breast, and prostate (Cornejo-Pareja et al, 2019).

Considerations are existed about the sharing role of gut microbiota in the obesity enhancement and its allied comorbidities (Al-Assal et al, 2018). Dysbiosis, imbalance in the microbial classes' abundance, was frequently related to gut barrier malfunction and inflammatory cell instigation (Bäckhed et al, 2012).

The failure to amply adjust the microbial diversity configuration occurs at the onset and chronicity of several illnesses, involving inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), diabetes, obesity and cancer (Zuo and Ng, 2018; Hills et al, 2019). In the early 21st century, conclusions were built on divergent gut microbiota constitution amongst lean and obese mice, which were subsequently validated in humans proposing that this configuration could influence obesity. Ever since, many studies declared an intimate relation between microbiota and obesity, postulating that gut microbiota may be fundamental to energy homeostasis regulation (Cuevas-Sierra et al, 2019).

Obese patients have a minor diversity and richness in bacterial component of gut microbiota than eutrophic subjects. Obese mice presented proliferated Firmicutes and declined Bacteroidetes in faeces, irrespective of diet intake. Thus, obese humans displayed an amplified Firmicutes/Bacteroidetes ratio in faecal microbiota. But, a meta-analysis did
not authorize the lower Bacteroidetes proportion in overweight persons, suggested that disparities in the gut microbiota makeup by weight or by body mass index may not be true (Al-Assal et al, 2018).

Furthermore, probiotics which are health beneficial microbes predominantly categorized underneath genus Lactobacillus as L. acidophilus and Bifidobacterium, which when administered in suitable quantities confer health profits to the host, and have been concerned with innumerable physiological tasks including energy homeostasis and an up-and-coming zone of research (Arora et al, 2012).

This study aimed to assess the possible relation of lactobacilli acidophilus to obesity in a sample of Egyptian population by real-time PCR of lactobacilli acidophilus in stool.

**Subjects and Methods**

The present study enrolled 60 outpatient clinics at Kafr El-sheikh General Hospital and Ain Shams University during the period from May 2017 to June 2018. They were divided accord-ing to body mass index (BMI) into two groups G1:20 healthy slim cases and G2:40 obese cases with BMI >25 kg/m².

Exclusion criteria: Patients with hypothyroidism, Cushing syndrome, inflammatory bowel diseases, as well as pregnant women and patients with ascites were excluded.

The selected patients were subjected to history taking and complete general and abdomi-nal examination. BMI was calculated by using formula: BMI= weight (kg)/height (cm). Waist to hip ratio was calculated by dividing waist circumference (WC) by hip circumference (HC).

Sample collection: Morning blood samples were drawn after a 12hr fast, a part was collect-ed on EDTA tube for determination of glycated hemoglobin, extraction of RNA, and routine CBC by Sysmex the automated hematology analyzer SF-300 (Corporation, Japan). Second part was left to clot at room temperature, and serum was separated by centrifuging for 10 minutes at 3000r.p.m, for other biochemical investigations. Fresh stool samples were put in labeled clean and sterile containers. The samples collected at home or at the hospital were kept in a refrigerator at 4°C for not more than 2hr before stored in deep-froze at -70°C until use.

Laboratory measurements: Glycosylated Haemoglobin-A1c (HbA1c) was determined by cation exchange resin method using commercial kits (Intermedical, Italy). Serum cholesterol concentration, triglycerides, HDL-cholesterol concentration were determined by using Colorimetric enzymatic method using commercial assay kit (Stanbio Laboratory, USA) and the LDL concentration. ALT & AST were determined by the enzymatic colorimetric method using a Kit (Abnova). Assessment of thyroid function was done using reagents (IBL International GmbH, Germany) by solid phase sandwich-ELISA.

Identification of stool L acidophilus by quantitative real time PCR: DNA isolation: DNA from bacterial colony grown in Diamond Rogosa Sharp agar (MRS) broth was extracted using proteinase K protocol after centrifugation of the broth for 2min. Briefly, bacterial cells were re-suspended in 467 µl of freshly prepared (50mMTris-HCl, 10Mm EDTA, pH 7.5) 30UL of 10% SDS & 3µl of 20mg/ml proteinase-k (Sigma, Aldrich). After incubation at 65°C for 45min with shaking, DNA was obtained by phenol/chloroform/isoamyl alcohol precipitated with isopropanol washed with 70% ethanol, dried by vacuum. DNA pellet was re-suspended in 25 µl sterile distilled water and stored at 20°C.

PCR primer: DNA pellet was confirmed as bacterial origin using the universal 16-S primer. F (5-AGA GTT TCC TGG CTC AG-3) and R (5-ACG ACC TTG TTA GCT CTA-3) amplified a 1500bp fragment. Genus specific primers of Lactobacilli identification were designed on 16SrRNA. Genus specific primers, (Sigma, USA) for Lactobacilli amplified a 123bp fragment. LbLMA1-rev (5-CTC AAA ACT AAA CA AG-3) and R16-1 (50-CTT GTA CA C A CC GCC CGT CA-3).
PCR conditions and procedure: PCR reaction mixture (25 μl) composed of 25 pmol of each primer, 0.2 mM of each dNTP, PCR buffer 50 mM Tris-HCl, PH value was carried out in a Touchdown Thermal Cycler (Hybaid Middlesex, UK).

Amplification program was as follows: Initial denaturation at 95°C for 5 min, followed by 30 cycles consisted of denaturation at 95°C for 30s, annealing at 55°C for 30s, extension at 72°C for 30s, & 7 min final extension step at 72°C. Amplification products were subjected to electrophoresis in 1% agar gels (Electrophoresis grade, Invitrogen) in TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.2) followed by ethidium bromide staining under UV light.

Statistical analysis: Data were analyzed by using SPSS version 23. Shapiro-Wilks test evaluated variables distribution. Data were expressed as M±SD or median and range. Categorical data were given in percentages. Significance for difference between groups was determined by using two-tailed Student’s t-test. Qualitative variables were assessed by chi-squared test. Correlations between different parameters were done using Spearman’s and Pearson’s correlation coefficient. Probability (P) values of <0.05 were considered statistically significant indicated, but P > 0.05 was considered not significant.

Results

The results were given in tables (1 & 2) and figures (1 & 2).

![Table 1: Comparison of demographic and clinical characteristic of both groups at study beginning.](image-url)
cent (35%) of control subject are suffering from mal-digestion. Obese cases showed a significant increase in percentage of patient who had male digestion compared to control subjects (P=0.01). Quantification of \textit{L. acidophilus} in faeces by real-time PCR: Lactobacilli were expressed in 21/40 (52.5%) of obese cases and 16/20 (80%) of control cases, with expression was below the detection limit in both. The mean CT at which lactobacilli expressed in the obese cases was (38.89±2.57) compared to (36.08±4.63) in control cases with higher significant difference (P =0.04). Fold change in lactobacilli expression was 0.53 in obese cases compared to control ones (Tab. 2, Figs. 1-2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fold changes in expression of Lactobacilli</th>
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</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>0.038</td>
</tr>
<tr>
<td>CT</td>
<td>-0.744**</td>
</tr>
<tr>
<td>BMI (Kg/m$^2$)</td>
<td>-0.268*</td>
</tr>
<tr>
<td>WHR</td>
<td>-0.264*</td>
</tr>
<tr>
<td>Medical history of diabetes</td>
<td>-0.052</td>
</tr>
<tr>
<td>Stool analysis</td>
<td>-0.080</td>
</tr>
<tr>
<td>HbA1c (per gm %)</td>
<td>-0.052</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>-0.093</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>0.254*</td>
</tr>
</tbody>
</table>

Note: BMI: Body-mass index

Pearson's correlation test showed significant negative correlation between fold change of Lactobacilli expression and CT, BMI, & WHR (r=-0.744, P<0.001, r=-0.268, P=-0.038, & r=-0.264, P=0.041, respectively). Also, a significant positive correlation between expression of lactobacilli and triglyceride level was observed (r=0.254, P=0.05).

In contrast, there was no significant correlation between Lactobacilli expression and age (r=0.038, P=0.774), HbA1c (r=0.052, P=0.691) as well as cholesterol levels (r=-0.093, P=0.478). Spearman correlation did not show significant correlation between Lactobacilli expression and medical history of diabetes & stool analysis (r=-0.052, P=0.693 & r=-0.080, P=0.542; respectively).

**Discussion**

The gut dysbiosis, namely dysregulation of intestinal microbiota, and increased gut per-meability led to enhanced inflammation and are commonly seen in chronic conditions such as obesity and aging (Ouyang et al., 2020). Besides, Obesity and diabetes were concomitantly increased in Firmicutes and lower proportion of Bacteroidetes and Proteobacteria (Greathouse et al., 2019).

In the present study, \textit{Lactobacilli acidophilus} was expressed in 21/40 (52.5%) obese cases faecal samples and 16/20 (80%) in control ones. The mean cycle threshold (CT) which Lactobacilli expressed in obese cases was (38.89±2.57) compared to (36.08±4.63) in control cases. The expression of lactobacilli was statistically higher in control cases than obese cases (P =0.04). The fold change in the expression of lactobacilli was 0.53 in obese cases compared to control subjects. Pearson's correlation test did not show significant correlation between Lactobacilli expression and BMI, WHR and positive correlation with triglyceride level. Quantitative PCR was more sensitive beyond culture to detect \textit{Lactobacillus} species (Herbel et al., 2013).

Lactobacilli generally comprised less than 1% of faecal bacterial media, yet the composition of the distal parts of the gastrointestinal tract was still not clear. Lactobacilli were 90% of faecal specimens by the culture and 100% by RT-PCR (Štšepetova et al., 2011). Also, Million et al. (2012a) reported that different Lactobacillus species were associated different effects on weight change that were host-specific, and must clarify the role of Lactobacillus species in the human energy harvest and weight regulation, with attention to the potential effects of commonly
marketed lactobacillus-containing probiotics on weight gain Million et al. (2012b) found that the *Lactobacillus & Bifidobacterium* genus representatives have a critical role in weight regulation as an anti-obesity effect in experimental models and humans, or as a growth-promoter effect in agriculture depended on the strains. Wu et al. (2015) found that supplementation with K21 *L. plantarum* restricted weight gain in DIO mice. Halawa et al. (2019) by PCR found significantly lower stool *L. acidophilus* count among obese diabetic patients compared to healthy controls. Thus, lactobacilli played a main role in dairy industry as utilized in cheese and yogurts manufacture. Karami et al, (2017) stated that probiotics such as lactobacilli prevent the development of a wide range of human and animal's pathogens. They concluded that these bacteria can be raised for the production of various kinds of food, pharmaceutical products, and functional foods. Yogurt inclosing Lactobacillus strains provoke resistance to body weight induced by diet- and increased plasma cholesterol and triglyceride levels. Although several potential mechanisms have been proposed, how lactobacilli reduce serum cholesterol levels has not been well established. One of the postulated mechanisms is the assimilation of cholesterol by *L. acidophilus* as some lactobacilli, as *Lactobacillus* species, *Bifidobacterium longum*, *Clostridium perfringens*, and Bacteroides fragilis fragilis, stimulated bile salt hydrolase; in turn facilitated the de-conjugation of bile acid salts, catalyzed hydrolysis of glycine- or taurine-conjugated bile salts into Amino acid residue and free bile salts and hindered entero-hepatic circulation of bile salts. This decreased serum cholesterol concentrations as cholesterol is the precursor for the de novo production of fresh bile acids. Added mechanism by which cholesterol is decreased is the production of short-chain FA (SCF) by probiotics upon fermentation, which inhibited hepatic cholesterol synthesis (Song et al, 2015).

Štšepetova et al. (2011) found great proportions of intestinal lactobacilli in elderly persons, influenced by diet type were in those (70-73%) who consumed western diet, while in a vegetarian-type diet, an upper lactobacilli prevalence was measured.

 Lê et al. (2013) assessed the relationship between lactobacilli and gut inflammation allied to obesity found that *Lactobacillus* levels did not correlate to plasma C-reactive proteins concentrations. They hypothesized that such organisms in situ provoke inflammation or other cytokines that they did not estimate, such as TNF-α or IL-6.

Armougom *et al.* (2009) by real-time PCR found that the expression profiles Firmicutes concentration in obese and controls were the same. They concluded that an opposite firmicutes/bacteroidetes ratio only reduced bacteroid levels in obese.

However, Duncan et al. (2008) found no significant relationship between BMI or absolute weight loss and the relative bulky population of the top most groups of human colonic bacteria, comprising bacteroidetes in obese and non-obese persons. The Bacteroidetes and Clostridia cluster XIV (Firmicutes) quantities analysed by qPCR and FISH modalities, did not show correlation between altered microbiota structure & BMI, and thus people ingesting high-fat diet had lesser Clostridia, while on consuming a fiber-rich food there was an upsurge of lactic acid bacteria levels (Abenavoli et al, 2019).

Patil et al. (2012), by comparative analysis and quantification of dominant gut microbiota among lean, normal, obese and surgically-treated obese individuals of Indian origin by 16S rRNA sequencing, found no evident in distribution of the predominant bacterial phyla (Bacteroidetes & Firmicutes) with an even of Bacteroides species at genus level in obese cases with positive RT-PCR correlation between Bacteroides and BMI. The unique populations with dissimilar particular habits of life, or genetic and socioeconomic status can clarify these contradictory results.
Conclusion

The argument about the gut microbiota alterations in obesity is still on-going and it may be affected by diet and many other factors. Lactobacilli are good candidate for further investigation with in vivo studies to determine its potential health profits.

*Lactobacillus acidophilus* was significantly lowered in the obese cases. Modulation of gut microbiota with probiotics, prebiotics, antibiotics, or other therapeutic interventions may be beneficial in obesity control.

References


Explanation of figures

Fig.1: Frequencies and percentages of fecal samples showed positive expression of Lactobacilli among controls and obese cases.

Fig.2: Comparison between relative Lactobacilli expression levels in groups by RT-PCR. Lactobacilli expression levels were significantly down-regulated in obese cases compared to slim controls.