Correlation between Gut Microbiota, its Metabolic Products, and their Association with Liver Enzymes among Sample of Egyptian Females

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Abstract

BACKGROUND: Gut microbiota plays critical role in pathogenesis of obesity, liver metabolism and associated diseases.

AIM: The present study aimed to identify existing gut microbiota enterotypes and their metabolic products profiles, and investigate correlation between gut microbiota; body mass index (BMI); and liver enzymes among sample of normal weight and obese Egyptian females.

METHODS: A case-control cross-sectional study included 112 Egyptian females; 82 obese and 30 normal weights; aged 25–60 years. For each participant, anthropometric measurements (weight, height, and BMI), laboratory investigations (Aspartate amino transferase [AST], Alanine amino transferase [ALT], Short-chain fatty acids [SCFA], and C-reactive protein [CRP]) and microbiota analysis were done.

RESULTS: Obese females had higher significant values of CRP, AST, ALT, SCFA, log Lactobacillus, Firmicutes, Firmicutes/Bacteroidetes, Bifidobacteria, and Firmicutes/Bacteroidetes ratio had significant negative correlations with AST, ALT, and SCFA. Bifidobacteria had significant negative correlations with height and ALT. Bacteroidetes bacteria had significant positive correlations with SCFA, and significant negative correlations with age and height, Firmicutes bacteria had significant negative correlations with AST and ALT, and Firmicutes/Bacteroidetes ratio had significant negative correlations with AST, ALT, and SCFA. Among obese group, Lactobacillus, Bifidobacteria and Bacteroidetes bacteria had significant negative correlations with Firmicutes/Bacteroidetes ratio, and Firmicutes bacteria had significant negative correlations with ALT.

CONCLUSION: The beneficial Lactobacillus and Bifidobacteria have their good impact on improving obesity status and liver functions.

Introduction

Intestinal microbiota; the group of microorganisms inhabiting the gastrointestinal tract; plays a critical role in the host’s immune system regulation, natural defense against infection, and nutrient metabolism [1]. Its dysbiosis has been linked to pathophysiology of obesity and its related metabolic disorders. Gut microbial changes have been involved in chronic low-grade “metabolic” inflammation whose metabolic products could trigger the development of insulin resistance in obesity and its complications [2]. Therefore, alterations in gut microbiota composition were thought as a primary cause of inflammation observed in obesity and type 2- diabetes [3].

Short-chain fatty acids (SCFAs) represent products of fermentation of dietary fibers by the anaerobic intestinal microbiota that has shown to exert several beneficial effects on energy metabolism [4]. It became apparent that SCFAs can play a part in inhibiting inflammatory processes and might have a key role in the prevention and treatment of the metabolic complications [5].

The abundance of microbiota responsible for fermenting carbohydrates leads to increased biosynthesis of SCFA, providing an extra source of energy for the host that is later stored as lipids or glucose and so contributing to the pathogenesis of obesity and its related metabolic disorders [6].

Gut microbiota has also shown to impact liver diseases through the modulation of host immune response and the production of inflammatory metabolites [7]. Specific strains of gut microbiota...
were identified in liver diseases with different causes, extending from steatohepatitis, fibrosis, cirrhosis, and reaching to hepatocellular carcinoma [8]. The previous studies suggested that liver enzymes showed high sensitivity to metabolic disorders, and that liver enzymes may provide a diagnostic tool for the metabolic complications [9], [10].

C-reactive protein (CRP) is an acute-phase reactant and an inflammatory marker that can be regulated through the effects of inflammatory metabolic products of specific gut microbes in cardiovascular disease, type 2-diabetes and obesity [11]. Serum concentrations of CRP were found to be elevated in overweight and obese individuals, therefore, obesity especially visceral obesity is currently considered as a low-grade inflammatory disease [12].

Thus, understanding the relationship between the gut microbiota and inflammation is essential in clinical practice because anti-inflammatory microbiota can be engaged in probiotic supplements for treating various metabolic disorders.

The present study aimed to identify the existing enterotypes of gut microbiota and its metabolic products profiles, and to investigate the correlation between gut microbiota and its metabolic products; body mass index (BMI) and liver enzymes among a sample of normal weight and obese Egyptian females.

**Subjects and Methods**

This is a case-control cross-sectional study, included a total of 112 Egyptian females; 82 obese females with age ranges from 25 up to 60 years with mean age 41.62 ± 10.70 years, in addition to 30 normal weight females within the same age range.

The study was carried out at the Medical Research Excellence Center (MERC), National Research Centre (NRC) as part of a cross-sectional survey of a project funded by NRC Egypt, 2019–2022 entitled “Gut Microbiota in Obesity and Metabolic syndrome among obese women: Interactions of the Microbiome, Epigenetic, Nutrition and Probiotic Intervention.” (12th Research Plan of the NRC), which was approved from “Ethics Committee of NRC” (Approval no 19/236).

**Methods**

For each participated woman, anthropometric measurements, laboratory investigations, and microbiota analysis were done.

**Anthropometric measurements**

Body weight and height were measured, following the recommendations of the “International Biological Program” [13]. 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. BMI was calculated (BMI: Weight [in kilograms] divided by height [in meters squared]). The participant females were classified according to their BMI into two groups: 30 females normal BMI (BMI=18–<25 kg/m²) and 82 obese females (BMI ≥30 kg/m²).

**Blood sampling and laboratory investigations**

In the morning, venous blood samples were drawn from the participated females, using venipuncture. Biochemical parameters were performed on sera that were stored at –70°C until used for assessment of short chain fatty acids (SCFA), CRP and liver enzymes: Aspartate amino transferase (AST) and Alanine amino transferase (ALT). All were done in the laboratory of “Medical Excellence Research Center MERC” which is a part of “NRC,” Egypt.

Human short chain fatty acids (SCFA) were assessed in serum using Enzyme Linked Immunosorbent Assay (ELISA) kits; Catalog Number: MBS7269061 according to the method described by den Besten et al. [14].

The assay of CRP in serum was performed by ELISA kits, Cat No.: RAP002 [15]

Serum concentrations of AST and ALT were determined using the automated clinical chemistry analyzer Olympus AU 400 analyzer. [https://www.mybiosource.com](https://www.mybiosource.com).

**Microbiota analysis**

The proportion of *Lactobacillus* and *Bifidobacteria*; and *Firmicutes/Bacteroidetes* ratio strains were assessed in the stool of all participants using the real time polymerase chain reaction. 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 [16]. Reagents provided by kits: DNA extraction Kit. Assay procedure: DNA extraction: The QIAamp DNA Stool Minikit (Qiagen) was used to extract DNA from 1 g 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 the Social Sciences (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.

The 112 participated females were classified into two groups according to their BMI (30 normal weight and 82 obese). The parametric data were expressed as mean ± SD. The various parametric variables of the different groups were analyzed and compared using independent t test. Pearson’s correlation test was used to assess the relations between each enterotypes of microbiota and patient’s anthropometric and laboratory parameters among the two groups. P < 0.05 was regarded as statistically significant for all tests.

**Results**

Comparisons between the anthropometric measurements, laboratory and microbiota data in the two groups; normal weight and obese; are illustrated in Table 1. There were highly significant differences in body weight and BMI, where the obese females had the higher values. In respect to the laboratory investigations and microbiota, obese females had the higher highly significant values of CRP and higher significant values of AST, ALT, and SCFA. In addition, obese females had insignificant higher values of log bacteroidin, Firmicutes, Firmicutes/ Bacteroidetes ratio, and Lactobacillus, and insignificant lower values of log Bifidobacteria than normal weight females. Among obese females, the insignificant increase in log Firmicutes was more than the insignificant increase in log Bacteroides, this leads to insignificant minor increase in log Firmicutes/Bacteroides ratio.

Pearson’s correlations between each enterotypes of microbiota and their metabolic products among normal weight and obese females are presented in Tables 2–3. Lactobacillus had significant positive correlations with Bacteroides bacteria among the two groups (normal and obese), and significant negative correlations with Firmicutes/Bacteroides ratio among obese group. Among normal weight group (Table 2), Lactobacillus had significant positive correlations with SCFA, Bifidobacteria, and Firmicutes, and significant negative correlations with AST, ALT, and CRP.

Bifidobacteria had significant positive correlations with Lactobacillus among normal weight group, and had significant positive correlations with Bacteroides bacteria and significant negative correlations with Firmicutes/Bacteroides ratio among the obese group (Table 3). Among normal weight group, Bifidobacteria had significant negative correlations with Ht and ALT.

Bacteroides bacteria had significant positive correlations with Lactobacillus and significant negative correlations with Firmicutes/Bacteroides ratio among the two groups (normal and obese), and significant positive correlations with Bifidobacteria among the obese group only. Among normal weight group, Bacteroides bacteria had significant positive correlations with SCFA, and significant negative correlations with age and height.

Firmicutes had significant positive correlations with Firmicutes/Bacteroides ratio among the 2 groups (normal, obese). Among normal weight group, Firmicutes bacteria had significant negative correlations with ALT and AST. Among obese group, Firmicutes bacteria had significant negative correlations with ALT.

Firmicutes/Bacteroides ratio had significant positive correlation with Firmicutes and significant negative correlations with Bacteroides among the two groups (normal and obese). It had significant negative correlations with Lactobacillus and Bifidobacterium among obese group. Among normal weight group, Firmicutes/Bacteroides ratio had significant negative correlations with ALT, AST, and SCFA.

While among obese females, there were insignificant correlations between any type of studied microbiota and any of the anthropometric or laboratory parameters; except Firmicutes bacteria had significant negative correlations with ALT.

**Discussion**

The human gut harbors a large population of microorganisms, the gut microbiota, which exerts...
a notable influence on the host in modulating energy balance (host metabolism and energy uptake [17].

The gut microbiota appears to play a role in the pathogenesis of obesity and associated diseases [18]. This community can contribute to the development of obesity primarily by influencing dietary energy intake and intestinal absorption of nutrients [19], but it can also provide the human host with benefits besides energy extraction, including a reduction of low grade chronic inflammation associated with obesity and metabolic complications [1].

The present results showed that Bacteroides bacteria were the most prevalent type among the studied microbiota, followed by the gut microbes Firmicutes, followed by the two beneficial types the Lactobacillus and Bifidobacteria. Comparisons between the normal weight and obese groups revealed that obese females had insignificant higher values of log bacteroid, Firmicutes, Firmicutes/bacteroid ratio, than normal weight females. Similarly, studies in France [20] and in Brazil [21] showed that Bacteroides are one of the most abundant bacteria in the human gut and its dysbiosis was strongly linked to insulin resistance, altered metabolism, and obesity.

Among obese females, the insignificant increase in bacteroid was more than the insignificant increase in Firmicutes, this leads to insignificant difference in Firmicutes/bacteroid ratio between obese and control groups. The insignificant differences in statistical analysis of the microbiota might be related to the use of the log values.

Studies have reported controversial data about the Firmicutes/Bacteroides ratio and the proportion of Firmicutes and Bacteroides in obesity. The present data were in agreement with many other studies in China [22], United Kingdom [23], and Canada [24] where insignificant differences in Firmicutes to Bacteroides ratio was observed between lean and obese individuals, other studies found that the gut microbiota of obese people is characterized by an increase in the Firmicutes/Bacteroides ratio; as in Ukraine [25] and Italy [26]; while others even find an opposite relationship; as in China [27].

Difference in microbiota composition can be related to regional, environmental, or dietary causes. It could also be due to the insufficient number of subjects included in some studies, making their statistical power insufficient to detect small variations. Therefore, changes in microbiota composition from one population to another should not be generalized [28].

Obese females in the present study had insignificant higher values of log Lactobacillus, and insignificant lower values of Bifidobacteria; than normal weight ones. Similarly, a study showed that microbiota

### Table 2: Pearson’s correlations between each enterotypes of microbiota and its metabolic products among normal weight females

<table>
<thead>
<tr>
<th>Variables</th>
<th>Log Lactobacillus</th>
<th>Log Bifido</th>
<th>Log Bacteroid</th>
<th>Log Firmicutes</th>
<th>Log Firmicutes/Bacteroid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.243</td>
<td>0.195</td>
<td>-0.285</td>
<td>0.126</td>
<td>-0.338</td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.078</td>
<td>0.083</td>
<td>-0.044</td>
<td>0.817</td>
<td>-0.350</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.326</td>
<td>0.080</td>
<td>-0.400</td>
<td>0.029a</td>
<td>-0.575</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.167</td>
<td>0.379</td>
<td>0.341</td>
<td>0.065</td>
<td>-0.023</td>
</tr>
<tr>
<td>Lab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>-0.555</td>
<td>0.001**</td>
<td>-0.387</td>
<td>0.035*</td>
<td>0.297</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>-0.587</td>
<td>0.011**</td>
<td>-0.168</td>
<td>-0.376</td>
<td>0.328</td>
</tr>
<tr>
<td>SCFA (μmol/L)</td>
<td>0.406</td>
<td>0.023*</td>
<td>-0.231</td>
<td>0.220</td>
<td>0.460</td>
</tr>
<tr>
<td>CRP (mg/ml)</td>
<td>-0.417</td>
<td>0.022*</td>
<td>-0.074</td>
<td>0.699</td>
<td>-0.220</td>
</tr>
<tr>
<td>Microbiota</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log Lactobacillus</td>
<td>0.361</td>
<td>0.050*</td>
<td>0.394</td>
<td>0.031*</td>
<td>0.373</td>
</tr>
<tr>
<td>Log Bifido</td>
<td>0.394</td>
<td>0.031*</td>
<td>0.272</td>
<td>0.147</td>
<td>-0.320</td>
</tr>
<tr>
<td>Log Firmicutes</td>
<td>0.373</td>
<td>0.042*</td>
<td>0.120</td>
<td>0.529</td>
<td>-0.388</td>
</tr>
<tr>
<td>Log Firmicutes/Bacteroid ratio</td>
<td>0.106</td>
<td>0.576</td>
<td>-0.019</td>
<td>0.919</td>
<td>-0.694</td>
</tr>
</tbody>
</table>

R.B.: *p < 0.05=significantly different. **p < 0.01=highly significant differences. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CRP: C-reactive protein.

### Table 3: Pearson’s correlations between each enterotypes of microbiota and patient’s clinical and metabolic parameters among total obese females

<table>
<thead>
<tr>
<th>Variables</th>
<th>Log Lactobacillus</th>
<th>Log Bifido</th>
<th>Log Bacteroid</th>
<th>Log Firmicutes</th>
<th>Log Firmicutes/Bacteroid ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.066</td>
<td>0.622</td>
<td>-0.051</td>
<td>0.652</td>
<td>0.166</td>
</tr>
<tr>
<td>Anthropometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.120</td>
<td>0.284</td>
<td>-0.036</td>
<td>0.747</td>
<td>0.077</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>-0.043</td>
<td>0.705</td>
<td>-0.044</td>
<td>0.698</td>
<td>-0.050</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.160</td>
<td>0.153</td>
<td>-0.014</td>
<td>0.903</td>
<td>0.115</td>
</tr>
<tr>
<td>Lab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>-0.184</td>
<td>0.115</td>
<td>0.028</td>
<td>0.812</td>
<td>-0.107</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>-0.064</td>
<td>0.587</td>
<td>-0.082</td>
<td>0.484</td>
<td>0.045</td>
</tr>
<tr>
<td>SCFA (μmol/L)</td>
<td>0.156</td>
<td>0.176</td>
<td>0.098</td>
<td>0.402</td>
<td>-0.033</td>
</tr>
<tr>
<td>CRP (mg/ml)</td>
<td>-0.013</td>
<td>0.912</td>
<td>-0.068</td>
<td>0.562</td>
<td>-0.121</td>
</tr>
<tr>
<td>Microbiota</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log Lactobacillus</td>
<td>0.054</td>
<td>0.631</td>
<td>0.619</td>
<td>0.000**</td>
<td>0.038</td>
</tr>
<tr>
<td>Log Bifido</td>
<td>0.054</td>
<td>0.631</td>
<td>0.503</td>
<td>0.000**</td>
<td>0.020</td>
</tr>
<tr>
<td>Log Bacteroid</td>
<td>0.619</td>
<td>0.000**</td>
<td>0.503</td>
<td>0.000**</td>
<td>0.020</td>
</tr>
<tr>
<td>Log Firmicutes</td>
<td>0.038</td>
<td>0.733</td>
<td>0.020</td>
<td>0.859</td>
<td>0.129</td>
</tr>
<tr>
<td>Log Firmicutes/Bacteroid ratio</td>
<td>-0.304</td>
<td>0.006**</td>
<td>-0.249</td>
<td>0.025*</td>
<td>-0.432</td>
</tr>
</tbody>
</table>

R.B.: *p < 0.05=significantly different. **p < 0.01=highly significant differences. ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. CRP: C-reactive protein. 1800 https://oamjms.eu/index.php/mjms/index
composition of overweight and obese adults with dyslipideamia in China was characterized by over representation of *Lactobacillus* as a counter protective reaction but with reduction of *Bifidobacterium* [22]. It was also found that gut microflora in obese patients is characterized by greater numbers of *Lactobacillus* and lesser numbers of *Bifidobacterium* species [29].

Similarly, Long et al. found that *Bifidobacterium* was less abundant among obese African American individuals compared with normal-weight ones [30]. Moreover, Collado et al. [31] found a protective effect of high concentration of *Bifidobacterium* against obesity during infancy and adulthood. *Bifidobacterium* may decrease fat absorption through the deconjugation of bile acids [32], which are possibly related to its reduction in obese subjects. A study indicates that in mice on a high-fat diet, ingestion of sterilized *Bifidobacterium* suppressed fat accumulation, improved insulin resistance, and lowered blood glucose levels [33].

Among obese females, *Firmicutes/Bacteroidetes* ratio had significant negative correlation with *Lactobacillus* and *Bifidobacterium*. Confirming the present results, the genera *Lactobacillus* probiotic was found to have the potential to reduce the *Firmicutes/Bacteroidetes* ratio and obesity as the administration of *Lactobacillus* decreased the *Firmicutes/Bacteroidetes* ratio in obese mice and reduced fatty acid synthesis, in the liver [34]. In another study, *Lactobacillus* consumed with a high-fat diet prevented weight gain and decreased the *Firmicutes/Bacteroidetes* ratio [35].

The most abundant metabolic products of gut microbiota are short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, which are produced by anaerobic fermentation of undigested carbohydrates [36]. SCFAs play a crucial role in the development of obesity as they interact with adipose tissue promoting adipocytes formation and inhibiting lipolysis [37]. In addition, SCFAs decrease the synthesis of the hunger-suppressing hormones leptin, peptide YY, and glucagon-like peptide 1 [38].

In the present study, SCFAs had significant positive correlations with *Lactobacillus* and *Bacteroidetes*, in addition to significant negative correlation with *Firmicutes/Bacteroidetes* ratio among only normal weight group. These results could be related to the fact that acetate and propionate are mainly produced by the phylum *Bacteroidetes*, whereas butyrate is the predominant product of the phylum *Firmicutes* [14]. Studies also showed that acetate appears to be predominantly obesogenic, whereas butyrate and propionate are mainly anti-obesogenic [39].

These results confirm the beneficial effect of probiotic microorganisms as *Lactobacillus* on the balance of the intestinal microbiome and produced metabolites, including SCFAs as *Lactobacillus* showed the ability to increase in response to the concomitant rise in obesogenic acetate producing bacteria, that is, *Bacteroidetes*. Age had significant negative correlation with *Bacteroidetes* among normal group. Ageing has been suggested to cause changes in the intestinal microbial community. Similar to the present 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, and predicts decreased survival in a 4-year follow-up [40].

Concerning the laboratory investigations; among normal weight group; ALT and AST had significant negative correlation with *Lactobacillus*, *Firmicutes* and *Firmicutes/Bacteroidetes* ratio, while ALT only had significant negative correlation with *Bifidobacterium*. These correlations became insignificant among the obese group; except the significant negative correlation between ALT and *Firmicutes* which persist.

The gut microbiome appears to play a critical role in liver metabolism in healthy individuals as the present results showed that the decrease in the beneficial *Lactobacillus* and *Bifidobacterium* can be related to the increase in liver enzymes [41]. Many studies showed the efficacy of probiotic supplementation in liver diseases [42], [43]. The protective role of *Lactobacillus* and *Bifidobacterium* was insignificant among obese group in the present study, which could be due to intestinal dysbiosis.

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 metabolic syndrome [44].

In the present study, CRP had significant negative correlation with *Lactobacillus* among only normal weight group. Similarly, a study showed that high CRP levels were associated with decreased abundance of *Lactobacillus* and *Bifidobacterium*, and with increased abundance of *Bacteroides* [45]. Indeed a meta-analysis of 20 studies suggests that probiotic administration may significantly reduce serum CRP by reducing mucosal inflammation through modulation of cytokine levels and other inflammatory mediators [46].

Conclusion

Among obese group, *Lactobacillus* and *Bifidobacteria* had significant negative correlations with *Firmicutes/Bacteroidetes* ratio; however; these correlations were insignificant among normal weight group. Moreover, there were insignificant correlations between any type of studied microbiota and any of the anthropometric or laboratory parameters; except *Firmicutes* bacteria had significant negative correlations with ALT.
Among normal weight non-obese group, ALT had significant negative correlations with Lactobacillus, Bifidobacteria, Firmicutes and Firmicutes / Bacteroidetes Ratio; this correlation persists only with Firmicutes among obese group; while AST had significant negative correlations with Lactobacillus, Firmicutes and Firmicutes / Bacteroidetes Ratio among normal weight non-obese group only. SCFA had significant positive correlations with Lactobacillus and Bacteroidetes, and significant negative correlation with Firmicutes / Bacteroidetes Ratio, while CRP had significant negative correlations with Lactobacillus only.

The beneficial Lactobacillus and Bifidobacteria have their good impact on improving obesity status and liver functions.

Acknowledgments

We would like to acknowledge our institute “National Research Centre”; Egypt; without their support this study could not be done. Authors are also grateful to everybody participated in this study; the employers of our institute who were the participants of this study, the technicians who helped in the laboratory analysis and the doctors who participated in collection of the data. Without their help, this study could not have been completed.

Author Contribution

Nayera E. Hassan conceived and designed the study; she is the PI of the project from which this data were derived. Sahar A. El-Masy; statistical analysis and interpretation of the data, she is the Co-PI of the project from which this data were derived. Gamila SM El-Saeed; responsible about the laboratory investigations, Adel F. Hashish; responsible about the microbiota analysis, Ayat N. Kamal; wrote the draft of the article, Mohamed S. El Hussieny, Aya Khailil, and Manal Mouhamed Aly; supervision on collection of data and references. Mohamed Selim, Mahmoud A.S. Affy, and Ahmed Saied Ismaeil; 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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