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Irritable bowel syndrome and microbiota: preliminary study on correlations between gut bacteria, *Dientamoeba fragilis*, blastocystis, and eating habits

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Background: Irritable Bowel Syndrome (IBS) is a functional bowel disorder with no known organic etiology. Despite studies indicating a correlation between IBS and gut microbiota, the etiology is yet to be clarified. Here, we assessed the roles of gut microbiota (including *Dientamoeba fragilis* and *Blastocystis*) and eating habits in IBS patients and controls to identify an organic cause for IBS.

Material/methods: The Study Group (SG) consisted of 14 IBS patients diagnosed according to Rome III Criteria, and the control Group (CG) was selected from 14 age and sex-matched individuals who had undergone colonoscopy in Acibadem Hospital in Kocaeli-Turkey, due to suspected cancer and found cancer- (and IBS-) negative. All participants were from Kocaeli province, the southeastern neighbor of Istanbul. A questionnaire on eating habits was completed by all participants. DNA was isolated from stool samples obtained during colonoscopy. Microbiota profiles were determined by amplification of ribosomal genes (16S+18S), and PCR products were sequenced using Illumina technology. The prevalence of *D. fragilis* was determined by Real-Time PCR. Bioinformatic analyses were conducted by matching the raw data with the data of SG and CG, and a predictive model was developed using questionnaire data. Statistical significance was determined by chi square analyses.

Results: *Bacteriodes nordii*, *Phascolarctobacterium faecium*, *Escherichia/Shigella* spp., and Erysipelotrichaceae were significantly more common in IBS patients ($p < 0.05$). In addition, IBS patients having *Bacteriodes nordii* and *Escherichia/Shigella* spp. were also positive for

Blastocystis ($p>0.05$). Erysipelotrichaceae were common in individuals reporting high fat consumption, while a significant correlation was found between IBS, *Bacteriodes nordii* and consumption of simple carbohydrates ($p<0.05$). *D. fragilis* was identified equally in both groups and related to constipation.

Conclusions: The bacterial component of gut microbiota at species level as well as *Blastocystis* and *D. fragilis* were analyzed for the first time in a Turkish cohort in this pilot study, together with eating habits of participants. A relationship between *P. faecium*, *B. nordii* and IBS was identified, which may indicate an organic etiology of IBS. Current results for *Blastocystis* and *D. fragilis* warrant further investigation in microbiota studies with more samples in both groups. Despite the small sample size being a limiting factor of this preliminary study, the outcomes appear promising and may unveil some significant relationships between the eating habits of individuals, their gut bacteria and resident eukaryotes. The role of *D. fragilis* as well as *Blastocystis* in gut health or diseases deserves further studies as well. Addition of metabolomics may also present novel data about the complicated relationships within the gut. We plan to expand the number of participants and add metabolomics to the assessment methods to improve the outcomes of this pilot study.