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A theoretical exploratory analysis on the risk of dysbiosis in individuals with inflammatory bowel diseases, using the DYS/FQM questionnaire

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Introduction

Thousands of bacterial species inhabit the human gastrointestinal tract. The number of microorganisms living in the intestine exceeds the number of cells in the human body by more than ten times. The human intestine is populated by several phyla of bacteria, including Bacteroidetes, Firmicutes (90% together), and Actinobacteria. The intestinal microbiota reveals the presence of 3.3million genes, compared to 23,000 genes present in the entire human body. Dysbiosis is an imbalance between the amount and type of microorganisms present in particular habitats of an individual, particularly the gut.

An individual's risk of dysbiosis has been investigated in human and animal models and is dependent on several factors. For example, there are differences in microbiota composition between young and old.[1–5] The mode of delivery is also decisive in defining the intestinal microbiota, and the microbiota of babies born by vaginal delivery differs from babies born by cesarean section.[6–8] Breastfeeding may also be essential in defining a different microbiota than formula-fed babies.[9–11] Other factors, such as diet composition[12–17] alcohol consumption[18,19] physical activity (20), and stress level[21–25] also play a role. In addition, there are reports of dysbiosis in patients with inflammatory bowel disease (IBD).[26–28] Italian researchers studied dysbiosis in patients with IBD, controlling for possible covariates present in these individuals, showing that there is a reduction in microbial diversity in patients with IBD, and identifying as covariate factors age, intake of yogurt and dairy products at least four times a week in ulcerative colitis. In contrast, age, sex, meat and bread intake at least four days a week are covariates related to Crohn's Disease.[29]

The DYS/FQM- Dysbiosis Frequent Questions Management questionnaire was developed, taking into account the risk factors associated with dysbiosis described in the literature and assigning an arbitrary score to each variable, according to its presence or absence or the frequency of exposure. The questionnaire is based on a theoretical presumption of the degree of risk. It was not validated through a direct comparison with the molecular determinants of the species present in the fecal samples of individuals carrying the variables related to dysbiosis.

The instrument is registered in Brazil at the National Institute of Industrial Property (INPI) for the Farmoquímica Laboratory, under number 914742353, and is available online.[30] The questionnaire classifies the individual as having a low, medium, high, or very high risk for dysbiosis. The higher the score obtained, the greater the risk.

Estimating the risk of dysbiosis for an individual with an inflammatory bowel disease must consider other variables besides the disease since several may be present in this individual.

A simple questionnaire could help identify those patients at higher risk of dysbiosis and more eligible for addressing factors that can be modified in clinical practice, such as diet, physical exercise, and weight loss. It could also serve as initial studies to indicate probiotics for these higher-risk patients.

Goals

The study's primary objective is to determine whether an inflammatory bowel disease constitutes an increased risk for dysbiosis compared to individuals without IBD, controlling for the other risk factors that comprise the DYS/FQM questionnaire.

Casuistics

Three hundred and twenty-five individuals from different regions of Brazil, with or without a diagnosis of IBD, took part in the study. The sample was taken from an IBD patients group on social media, ruled by one of the authors (WRC)

Methods

The Research Ethics Committee approved the study under number CAAE 52628021.3.0000.0087. Participants answered the DYS/FQM questionnaire adapted for sending over the internet to people with IBD. In addition, each patient with IBD was asked to send the questionnaire to another person without Crohn's disease or colitis, who constituted the control group. The results were collected and tabulated in an Excel spreadsheet.

In order to obtain the total score of each participant, we considered having IBD as an additional risk factor. So three more points were empirically added to the questionnaire score for individuals with IBD, and none for controls, based on the same theoretical knowledge of the literature that supports the attribution of points for the other variables.

From the global set of points of the 365 individuals, the 30% and 70% percentiles were obtained using the Graph Pad Prism software. According to their score, participants were allocated into three groups:

low risk of dysbiosis, intermediate risk, and high risk. For group allocation, those participants with a score less than or equal to the 30% percentile of the global score (i.e.,16 points or less) were allocated to the low-risk group. Participants at or above the 70% percentile of the global score (i.e., 22 points or more) were allocated to the high-risk group. Participants with scores between the 30th and 70th percentiles were classified as intermediate risk. The choice of cut-off levels was arbitrary to allow sufficient participants in each risk group.

Statistical methods

Graph Pad Prism software was used for all statistical analyses. The median scores between groups were compared using the Kruskal-Wallis test with Dunn's post-test for multiple comparisons.

Univariate analysis was used to assess the association of each questionnaire item individually with the classification of groups. Subsequently, the significant variables in the univariate analysis were studied together by multiple logistic regression. The value of p≤ 5% was used in all analyses.

Results

Among the 325 respondents to the questionnaire (257 females, 68 males), there were 126 controls, 134 with Crohn's disease, and 65 with ulcerative colitis. The mean age of the participants was 40years (range 18-73). The distribution of the global score among all participants shows that the minimum value of points obtained is 6, the maximum 33, the 30% percentile is 16, and the 70% is 22. In addition, the Kolmogorov-Smirnov test shows that the distributions of the groups are not parametric, indicating the use of non-parametric statistics.The score values in each group are shown in Table 1. The allocation of participants in groups is shown in Table 2.

Table 1 Minimum, maximum , median and percentile of points in each group

	Ccontrols	Crohn	Colitis
Number	126	134	65
Minimum	6	8	11
30% Percentile	14	19	17
Median	17	21	20
70% Percentile	20	24	23
Maximum	30	33	31

The scores in each group were compared using the Kruskal-Wallis test (p<0.0001). Dunn's post-test: controls x Crohn's (p<0.0001), controls x colitis (p=0.006), Crohn's x colitis (p>0.99)

Table 2 Number of participants in each risk group

Risk	Low	High
Controls	60 (57,6%)	32 (25,6%)
Crohn	25 (24,0%)	66 (52,8%)
Colitis	19 (18,2%)	27 (21,6%)
Total	104 (100%)	125 (100%)

For the logistic regression, the variables that were individually associated with high risk were the type of delivery, breastfeeding, frequency of intake of refined sugar, sweeteners, fruits, vegetables and greens, processed foods, frequency of exercise factors, the level of self-reported stress, smoking, recent use of antibiotics, proton pump inhibitors and probiotics, the number of patient comorbidities, the presence of diarrhea and the diagnosis of Crohn's disease or colitis.

The results of the multivariate analysis of the factors listed above by step-by-step multivariate analysis are presented in Table 3.

Table 3 Multivariate analysis of factors independently related to the risk of dysbiosis

Independent Variables	Risk	95% confidence interval	p
Cesarean delivery	6,8	2,8 a 14	0,0139
Fruits and vegetables less than once a day	19	8,5 a 42	0,0149
Fruits and vegetables once or twice a day	11	4,7 a 23	0,0109
Processed foods 2- 3 times a week	10	4,2 a 23	0,0209
Processed foods 4-5 times a week	20	9,3 a 45	0,0143
Processed foods more than 5 times a week	17	8,5 a 35	0,0059
Sedentary lifestyle	10	4,9 a 22	0,0089
Recent use of antibiotics	12	5,7 a 26	0,0107
Use of proton pump inhibitors	13	6,0 a 27	0,0099
Use of probiotics	-14	-29 a -6,4	0,0097
Diagnosis of Crohn´s disease	8,1	3,5 a 17	0,0099
Diagnosis of ulcerative colitis	13	4,5 a 29	0,0241

The adequacy of the model was verified using the log-likelihood ratio test, with p < 0.0001

Discussion

The study's objective was to define whether the diagnosis of Crohn's disease or ulcerative colitis constitutes a factor in classifying an individual as having a high risk of dysbiosis while adjusting for other risk factors. For example, many patients with IBD are also sedentary, smokers, have an excessive intake of sugars and processed foods, use antibiotics, and several other factors that increase the risk for dysbiosis. Among these factors, we studied those listed in the DYS/FQM questionnaire, treating each as a risk-related variable.

As we observe from the variation of scores in the control group, individuals who do not have inflammatory bowel diseases have a variable risk of dysbiosis, according to the presence or absence of risk factors analyzed in the questionnaire. Therefore, to assess whether Crohn's disease and ulcerative colitis diagnosis are risk factors for dysbiosis, it is also necessary to control for these other variables that determine risk and its presence in the groups with IBD.

The choice of nonparametric statistics is justified since the sample scores distribution is not normal. The median of the points in each group differs between them, being the median of the control group smaller than both groups with IBD, and these are similar to each other, as shown by the Dunn test (Table 1). Also, Table 2 shows that there are low-risk and high-risk individuals in the control group and IBD groups. In order to explore if the diagnosis of IBD is an independent factor related to the high risk, we performed the multivariate analysis controlling for the other variates related to the outcome.

The questionnaire proposes a classification for the risk of dysbiosis as low, medium, high, and very high, according to the cumulative score obtained by the individual. We did not adopted the four risk classifications proposed by the questionnaire. So, we established our cutoff values of scores setting the 30% percentile or less as the limit to define the low-risk and 70% or above for the high-risk and chose to compare these two groups, leaving aside the intermediate-risk group due to the need for a binary dependent variable for logistic regression.

The univariate analysis shows that those variables statistically associated with high risk were the type of delivery, breastfeeding, frequency of intake of refined sugar, sweeteners, fruits, vegetables, and greens, processed foods, frequency of physical exercise, level of self-reported stress, smoking, recent use of antibiotics, proton pump inhibitors and probiotics, number of patient comorbidities, presence

of diarrhea and diagnosis of Crohn's disease or colitis. Age and gender were not associated with the risk of dysbiosis.

The relative contribution of each variable, when analyzed together by step-by-step multiple logistic regression, shows that the diagnosis of Crohn's disease or ulcerative colitis is an independent factor for classifying the individual as having a high risk of dysbiosis when controlling for the other significant covariates. Table 3 shows the risk factor for each variable. Variables related to the risk in the univariate analysis were no more significant in the multiple regression step-by-step procedure, such as breastfeeding, self-reported stress, smoking, patient comorbidities, and the presence of diarrhea.

Table 3 displays the relative contribution of each significant variable to the risk classification. For example, cesarean delivery increases the risk by a factor of 6.8 compared to vaginal delivery. Infrequent consumption of fruits and vegetables increases the risk by 11 for eating once or twice a day and 19 for eating less than once a day, relative to eating five or more times a day. Using proton pump inhibitors increases the risk by a factor of 13 compared to those who do not use them, and so on for the other variables. However, using probiotics appears with an inverse relationship, with 14 times less risk in those who take probiotics.

Furthermore, the diagnosis of Crohn's disease has a risk factor of 8.1 and ulcerative colitis of 13 related to those who do not have these diseases.

Conclusion

This study is a theoretical exploratory analysis of the possible risk factors to define a high-risk individual with dysbiosis. Considering the chosen cutoffs to define risk, our results showed the diagnosis of IBD as an independent factor in placing the individual as high-risk of having dysbiosis. There are several pitfalls in this work. First, the questionnaire is empirical and was not validated by comparison with the genomic mapping of the microbiota in the feces of healthy and sick individuals. Attributing points to each variable relies on theoretical information, assuming that a greater frequency or intensity of exposure increases the risk of dysbiosis. The increase in score for each degree of exposure is entirely speculative. Some parameters are subjective, such as self-reporting the participant's degree of stress, without a validated measure. There may also be recall bias in certain variables, such as breastfeeding. We also do not know whether the IBD was in remission or active in the patients. Also, the diagnosis reported by the patients was made by other doctors external to the study and needed to be appropriately checked. Further work is needed after the validation of the questionnaire by mapping the fecal microbiota to check whether it will be helpful in clinical practice.

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Conflicts of Interest

Author declare there are no conflicts on interest towards the article.

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