ORIGINAL ARTICLE

Effect of Predominant Methanogenic Flora on Outcome of Lactose Hydrogen Breath Test in Controls and Irritable Bowel Syndrome Patients of North India

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Abstract The relationship between methanogenic flora and hydrogen (H_2) production is considered to be a possible confounding factor in the interpretation of hydrogen breath tests (H_2BT). Therefore, the present study was conducted prospectively and included 154 IBS patients (fulfilling Rome II criteria) and 286 age-and-sex-matched apparently healthy controls. Each subject underwent H_2BT after overnight fasting using 25 g lactose. Methane and H_2 were measured using an SC Microlyser from Quintron, USA, at baseline and every 30 min for a total of 4 h. Subjects with fasting methane concentration <10 ppm were labeled as low methane producers (LMP) and >10 ppm as predominant methane producers (PMP). A rise >20 ppm over base line in hydrogen concentration was taken as +ve hydrogen breath test. IBS and control groups included 66.78% and 67.53% males, respectively. Mean age in the two groups were 48.52 ± 30.54 years (range 15–68 years) and 45.67 ± 30.54 years (range 15–78 years), respectively. Hydrogen breath test was +ve in 77/154 (50%) IBS patients and in 142/286 (49.65%) in controls ($P > 0.05$). It was also observed that the hydrogen breath test was −ve due to PMP in 5/77 (6.49%) of IBS patients and in 29/154 (20.14%) in controls. PMP affected lactose hydrogen breath tests in 6.49–20.14% subjects. This effect is more apparent in apparently healthy subjects as compared to patients with IBS.

Introduction

Breath methane measurements reflect in situ activity of methanogenic colonic flora, but it remains to be determined if methanogens actively influence human physiology or are simply a marker of colonic function [1]. In clinical and field conditions, breath gas analysis has been widely used in evaluating carbohydrate digestion [2]. The relationship between hydrogen (H_2) and methane (CH_4) production is a possible confounding factor in the interpretation of H_2 breath tests (HBT), but is usually disregarded for the interpretation of HBT. Nonetheless, hydrogen breath tests are commonly used in clinical practice for the diagnosis of carbohydrate malabsorption, as the concentration of breath hydrogen parallels the intestinal production from fermented carbohydrates [3]. No relevant clinical use has so far been identified for the measurement of CH_4 [4], another important end product of the metabolism of the strictly anaerobic Archeobacteria. In contrast to hydrogen, methane concentration remains relatively constant during the day, and does not depend directly upon the availability of fermentable substrate [5]. Methane production consumes 4 moles of hydrogen to reduce 1 mole of CO_2 to methane, a process greatly reducing the volume of gas in the colon. Some authors concluded that methane production can be disregarded in the interpretation of hydrogen breath test, as in children a positive correlation is found between the magnitude of the rise in methane and hydrogen concentrations [6]. However, it has been reported that evaluation of breath methane might enhance the accuracy of HBT [7] and that a decreased prevalence of methane production is

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observed in lactose malabsorbers [8]. In most instances, only H_2 excretion is measured. The assumption that H_2 excretion is an effective means of quantifying the amount of malabsorbed carbohydrates is questionable in PMP. Methane-producing patients likely have a higher "false-negative" rate as compared to LMP after an oral load of lactose. Nonetheless, as symptoms are related to the amount of gas produced in the colon, HBT identifies patients with "lactose intolerance," irrespective of the presence of lactose malabsorption, and helps in predicting the effect of a lactose-restricted diet [9]. Kajs et al. [10] investigated the possibility that a variant of the normal colonic flora, a high concentration of methanogens, influences the host's response to ingestion of non-absorbable, fermentable materials. The study of fecal samples from four subjects with low breath H_2 excretion after lactulose showed that absolute H_2 production was normal, and the low H_2 excretion apparently reflected increased consumption due to rapid methanogenesis (two subjects) and decreased luminal stirring (two subjects) [11]. The presence of a methanogenic flora is associated with a reduced symptomatic response to ingestion of non-absorbable, fermentable material in healthy subjects. Manipulation of the normal flora could be of therapeutic value in non-methanogenic patients with irritable bowel syndrome [10]. The effect of predominant methanogenic flora on the outcome of lactose breath tests in irritable bowel syndrome north Indian patients has not yet been studied, therefore, this study was planned.

Materials and Methods

For this study, 345 adult patients from GE Clinic between 15 and 60 years old with a diagnosis of IBS based on the Rome II criteria were referred to our laboratory to evaluate lactose tolerance by means of a HBT. Age and sex matched, 254 apparently healthy relatives of the patients were also enrolled for this study. Institute ethical clearance was taken before starting this study and informed consent was also given by each subject. Predominant methane producers (PMP) ($CH_4 > H_2$) were the patients with a concentration of methane exceeding 10 parts per million (ppm). Data of these patients was compared with those of age-and-sex-matched IBS patients from the same patient series with fasting methane <10 ppm and predominant ($H_2 > CH_4$) fasting hydrogen excretion, low methane producers (LMP). Effect of predominant methanogenic flora on the outcome of lactose hydrogen breath test in controls and IBS patients from north India was also calculated. After 72 h on a low-fiber diet and 12 h of fasting, HBT [12] was performed following an oral load of 25 g lactose. End-alveolar air samples were collected prior to the administration of lactose

and after every 30 min up to 4 h of administration of lactose. Hydrogen and methane concentration were measured in ppm by using a Quintron SC Microlyser (Quintron Instrumentals, Milwaukee, WI, USA). The test was defined as positive when an H_2/methane peak exceeding 20 ppm over the baseline value was observed in two or more samples. Tests not fulfilling the above-mentioned criteria were defined as negative HBT. The patients testing negative for hydrogen breath test who did not excrete an increased amount of hydrogen or methane after oral administration of 10 g lactulose in a subsequent HBT were defined as non-hydrogen producers. Symptoms and bowel movements before the test and occurring during the test were also recorded.

Statistics

Data was analyzed using the Chi-square test.

Results

In this study, a 25-g lactose hydrogen breath test (LHBT) was done in 345 IBS patients from GE Clinic of PGI, Chandigarh. The age range of these patients was 15–60 years and 217 out of 345 (62.9%) were males and 128 out of 345 (37.1%) females in IBS patients. In the control group, 165 out of 254 (64.9%) were males and 89 out of 254 (35.1%) females.

We performed lactose hydrogen breath tests in our lab in both IBS patients and controls with methanogenic flora to determine the effect of methanogenic flora on lactose hydrogen breath test in these subjects. The results in Table 1 show that hydrogen breath test was positive in only six out of 17 (35.3%) of IBS patients with PMP, while it was found to be higher, i.e., in 23 out of 33 (69.7%) in IBS patients with LMP. This difference was statistically significant ($P < 0.04$). A similar type of effect was observed in the control group. The hydrogen breath test was positive in 16 out of 52 (30.8%) with PMP as compared to 20 out of 36 (55.5%) with LMP in the control group (Table 1). This difference was also statistically significant ($P < 0.035$).

Table 1 Effect of methanogenic flora on lactose H_2BT in IBS patients and controls

		H_2BT +ve	H_2BT −ve
IBS pts ($n = 50$)	PMP ($n = 17$)	6/17 (35.3%)[a]	11/17 (64.7%)
	LMP ($n = 33$)	23/33 (69.7%)	10/33 (30.3%)
Controls ($n = 88$)	PMP ($n = 52$)	16/52 (30.8%)[b]	36/52 (69.2%)
	LMP ($n = 36$)	20/36 (55.5%)	16/36 (44.5%)

[a] $P < 0.04$, HBT positive in PMP and LMP of IBS patients

[b] $P < 0.035$, HBT positive in PMP and LMP of controls

Table 2 Mean ± SD of fasting and peak hydrogen concentration (ppm) in controls and IBS patients with methanogenic flora

Hydrogen conc. (ppm)	Controls (n = 88)				IBS patients (n = 50)			
	PMP (n = 52)		LMP (n = 36)		PMP (n = 17)		LMP (n = 33)	
	H_2BT +ve (n = 16)	H_2BT −ve (n = 36)	H_2BT +ve (n = 20)	H_2BT −ve (n = 16)	H_2BT +ve (n = 6)	H_2BT –ve (n = 11)	H_2BT +ve (n = 23)	H_2BT −ve (n = 10)
Fasting	10.7 ± 1.8	8.6 ± 2.5	12.7 ± 1.9	10.3 ± 1.4	11.5 ± 1.7	12.3 ± 2.4	11.4 ± 2.1	10.8 ± 1.6
Peak	43.5 ± 5.7[b]	13.8 ± 2.3	85.6 ± 10.4	12.9 ± 1.8	72.4 ± 9.5[a]	15.7 ± 2.3	121.6 ± 15.6	16.3 ± 2.4
Rise	*32.8*[b]	5.2	*72.9*	2.6	*60.9*[a]	3.4	*110.2*	5.5

[a] $P < 0.05$ between LMP and PMP in IBS

[b] $P < 0.01$ between LMP and PMP in controls

Italicized values denote the difference of the mean of peak and fasting hydrogen concentration

Peak Hydrogen Concentration

Mean ± SD of peak breath hydrogen concentration in IBS patients with PMP was less (72.4 ± 9.5 ppm) as compared to those with LMP where it was 121.6 ± 15.6 ppm. The difference was statistically significant ($P < 0.05$). A similar trend was observed in the control group, where the peak hydrogen concentration was less (43.5 ± 5.7 ppm) in controls with PMP compared to 85.6 ± 10.4 ppm in controls with LMP (Table 2). This difference was statistically significant ($P < 0.01$).

Symptoms During Lactose Hydrogen Breath Tests

During 4 h of lactose hydrogen breath test, symptoms consisting of diarrhea or flatulence were noted. The results of the data obtained during this period have shown less flatulence in PMP, i.e., in five out of 52 (9.6%) as compared to LMP, i.e., in ten out of 36 (27.8%) in controls. This difference was statistically significant ($P < 0.05$). Similarly, less flatulence was observed in IBS patients with PMP, i.e., in four out of 17 (23.5%) as compared to LMP, i.e., in 19 out of 33 (57.6%), as is shown in (Table 3). This difference was statistically significant ($P < 0.05$) and it may be due to the reason that production of methane consumes hydrogen, thereby reducing hydrogen gas in the intestines. Symptom of diarrhea in controls with PMP was also less, i.e., in three out of 52 (5.8%) as compared to LMP, i.e., in five out of 36 (13.9%). Similarly, diarrhea in

fewer patients was observed in IBS patients with PMP, i.e., in two out of 17 (11.8%) as compared to IBS patients with LMP, i.e., in 12 out of 33 (36.4%), (Table 3).

Methane Production

During LHBT, fasting methane (24.6 ± 8.5 ppm) as well as rise (23.9 ± 8.9 ppm) in methane excretion was more in IBS patients with predominant methanogenic flora as compared to fasting methane (3.4 ± 1.0 ppm) and rise (2.4 ± 1.3 ppm) in methane in patients with low methanogenic flora (Table 4). Similarly, fasting methane (27.5 ± 10.6 ppm) as well as rise (23.1 ± 9.5 ppm) in methane excretion was more in controls with predominant methanogenic flora compared to fasting methane (3.6 ± 1.2 ppm) as well as rise in methane (1.9 ± 1.1 ppm) in low methanogenic flora (Table 4).

Ten-gram lactulose hydrogen breath test was also done in 145 out of 345 (42%) IBS patients and 135 out of 254 (53%) controls in whom lactose hydrogen breath test was normal. Methane production was not observed at any time up to 4 h during the test in any of these subjects of both the groups. But hydrogen production was there in all subjects of both the groups. This indicates that there was not a single subject of non-hydrogen producer in the present study (neither in IBS patients nor in apparently healthy north Indians).

A less number of IBS patients with methanogenic flora gave history of flatulence, i.e., 23 out of 50 (46%) as

Table 3 Symptoms during test in controls and IBS patients with methanogenic flora

Symptoms	Controls (n = 88)		IBS patients (n = 50)	
	PMP (n = 52)	LMP (n = 36)	PMP (n = 17)	LMP (n = 33)
Flatulence	5/52 (9.6%)[a]	10/36 (27.8%)	4/17 (23.5%)[b]	19/33 (57.6%)
Diarrhea	3/52 (5.8%)	5/36 (13.9%)	2/17 (11.8%)	12/33 (36.4%)

[a] $P < 0.0417$ flatulence in PMP vs. LMP in controls

[b] $P < 0.0467$ flatulence in PMP vs. LMP in IBS patients

Table 4 Mean ± SD of fasting, peak and rise in methane concentration (ppm) in controls and IBS patients with methanogenic flora

CH_4 conc. (ppm)	Controls (n = 88)		IBS patients (n = 50)	
	PMP (n = 52)	LMP (n = 36)	PMP (n = 17)	LMP (n = 33)
Fasting	27.5 ± 10.6	3.6 ± 1.2	24.6 ± 8.5	3.4 ± 1.0
Peak	50.6 ± 16.3	5.5 ± 1.4	48.5 ± 15.6	5.8 ± 1.2
Rise	23.1 ± 9.5	1.9 ± 1.1	23.9 ± 8.9	2.4 ± 1.3

Table 5 History of symptoms in IBS patients with methanogenic and non-methanogenic flora

Symptoms	Methanogenic flora (n = 50)	Non-methanogenic flora (n = 295)
Flatulence	23/50 (46%)[a]	240/295 (81.4%)
Diarrhea	14/50 (28%)[a]	197/295 (66.8%)

[a] $P < 0.001$ for diarrhea and flatulence in patients with methanogenic and non-methanogenic flora

Table 6 History of symptoms in IBS patients with predominant and low methanogenic flora

Symptoms	Predominant methanogenic flora (n = 17)	Low methanogenic flora (n = 33)
Flatulence	4/17 (23.5%)[a]	27/33 (81.8%)
Diarrhea	3/17 (17.6%)[b]	24/33 (72.7%)

[a] $P < 0.002$ flatulence between PMP vs. LMP

[b] $P < 0.007$ diarrhea between PMP vs. LMP

compared to IBS patients with non-methanogenic flora, i.e., 240 out of 295 (81.4%). The difference was statistically significant $P < 0.001$. Similarly, history of diarrhea in IBS patients with methanogenic flora was in less number of patients, i.e., in 14 out of 50 (28%) as compared to IBS patients with non-methanogenic flora, i.e., 197 out of 295 (66.8%), Table 5. This difference was also statistically significant $P < 0.001$.

History of flatulence in IBS patients with PMP was in less number of patients, i.e., four out of 17 (23.5%) as compared to IBS patients with low methanogenic flora, i.e., in 27 out of 33 (81.8%). Similarly, history of diarrhea was also in less number of patients, i.e., three out of 17 (17.6%) as compared to IBS patients with low methanogenic flora, i.e., in 24 out of 33 (72.7%), Table 6. This difference was also statistically significant $P < 0.01$.

Discussion

Methane is produced extensively by strict anaerobic bacterial fermentation in the gut and generally has not been found to have a physiologic role in humans [13]. Since approximately 20% of colonic methane is excreted via the breath, breath methane analysis has been used as an indirect assessment of intracolonic bacterial metabolism [13]. The predominant methanogenic bacteria found in humans is *Methanobrevibacter smithii* [14, 15], which preferentially colonizes the left colon [15, 16].

The prevalence of a positive lactose breath test was surprisingly low (35.3%) in PMP of IBS patients compared to LMP (69.7%). In the first group, we would have expected similar overall figures, or the excretion of increased amounts of CH_4, as the availability of H_2 is the main limiting step for CH_4 production [17]. Actually, the concentration of methane excretion was more (24.6 ± 8.5) PPM fasting and (23.9 ± 8.9) PPM rise over the fasting values in PMP patients of IBS and (27.5 ± 10.6) PPM fasting and (23.1 ± 9.5) PPM rise over the fasting value in controls. Moreover, splitting these data, we observed that CH_4 excretion was more in patients with negative HBT and less in positive HBT. This is exactly what would be expected considering that CH_4 production is associated with H_2 consumption. Moreover, a marked increase in CH_4 excretion was hardly ever observed after the rise in H_2. Conversely, the increase in H_2 concentrations in expired air was associated with the reduction or suppression of CH_4 excretion. Similar results were observed by Vernia et al. [9], in an Italian population. As expected, subjects with predominant CH_4-producing flora excrete lower amounts of H_2 as compared to low CH_4 producers. Indeed, in our series, the peak values of H_2 excretion in PMP showed lower values than in the LMP group.

On the other hand, the patients with a positive HBT and increased CH_4 excretion showed higher H_2 excretion rates than those in whom CH_4 excretion showed no increase during the test. This is in contrast to what might have been expected, and suggests that the mutual interference of differing bacterial species, and their metabolism, is far more complex than anticipated by in vitro fermentation studies.

These data also indicate that PMP patients excrete less H2 than LMP patients and that an HBT is burdened by a high false-negative rate in those tests aimed at identifying "lactose malabsorbers," in epidemiologic studies. The assumption that H_2 excretion is an effective indirect means for quantifying the amount of malabsorbed carbohydrates is questionable, at least in PMP. These data also suggest that the use of gas chromatography measuring H_2 excretion only is, in this respect, less than ideal. Conversely, if the

test is aimed at identifying those patients with "lactose intolerance," in which symptoms are, to some extent, related to the amount of gas produced in the colon, the measurement of H_2 seems to be adequate. The lower prevalence of severe lactose intolerance in PMP is, indeed, related to lower and slower H_2 excretion, as well as to a lower incidence of symptoms characteristic of lactose intolerance such as abdominal pain, gaseousness, bloating, and diarrhea.

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