

## ORIGINAL ARTICLE

## Intestinal permeability is increased in bronchial asthma

Z Hijazi, A M Molla, H Al-Habashi, W M R A Muawad, A M Molla, P N Sharma

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See end of article for authors' affiliations

Correspondence to: Prof. Z Hijazi, Department of Paediatrics, Faculty of Medicine, Kuwait University, PO Box 24923, Kuwait 13110; zeinat@hsc.kuniv.edu.kw

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**Background:** Increased intestinal permeability has been reported in one study of adult asthmatics.**Aim:** To determine whether children with asthma have altered intestinal permeability.**Methods:** Thirty two asthmatic children, and 32 sex and age matched controls were recruited. The dual sugar (lactulose and mannitol) test was used to evaluate intestinal permeability, and the percentage of ingested lactulose (L) and mannitol (M) in the urine, and the L:M ratio were determined. All patients were skin prick tested for common aeroallergens, and specific IgE to some food items was determined.**Results:** The median value of L in asthmatic children (2.29, IQR 0.91–4.07) was significantly higher than that in controls (0.69, IQR 0.45–1.08), and that of M was almost similar. The ratio L:M was significantly higher in asthmatic children (0.20, IQR 0.11–0.40) than in controls (0.06, IQR 0.04–0.09). Intestinal permeability did not correlate with eczema, inhaled steroids, positive skin prick test to aeroallergens, or severity of asthma.**Conclusions:** Intestinal permeability is increased in children with asthma, suggesting that the whole mucosal system may be affected.

A possible relation between asthma and the gastrointestinal tract is an area of increasing interest. Food challenge can elicit wheezing in some children with asthma.<sup>1</sup> Gastrointestinal symptoms are common in children with asthma,<sup>2</sup> and duodenal histological changes and cytokine production mimicking those observed in bronchial asthma, have been shown.<sup>3</sup> Asthma is a T lymphocyte mediated inflammatory disease of the bronchus,<sup>4</sup> and it has been suggested that the whole mucosal immune system might be involved, with activated T lymphocytes migrating from one mucosal site to another.<sup>5</sup> Walleret and colleagues<sup>7</sup> have recently shown an airway-like inflammation of the minor salivary glands, and Benard and colleagues<sup>6</sup> showed increased intestinal permeability in adults with bronchial asthma. To our knowledge, this work has not been reproduced in adult asthmatics, and no similar study has so far been conducted in children with asthma.

We report the first study designed to evaluate intestinal permeability in asthmatic children, by using the dual sugar test, as an indirect measure of integrity of the intestinal mucosa.

## METHODS

## Patients

The study subjects were recruited from the Paediatric Asthma clinic at Mubarak Al-Kabeer hospital (the main teaching hospital in Kuwait), between January 2001 and June 2002. These patients fulfilled the International Consensus Criteria for the diagnosis of asthma,<sup>8</sup> and had the disease for no less than one year. Exclusion criteria included evidence of digestive disease, food allergy, or any other condition associated with increased intestinal permeability, such as cystic fibrosis, coeliac disease, rheumatological disease, or use of non-steroidal anti-inflammatory drugs, and those with positive skin test against common food allergens (see below). The control subjects were children attending a general paediatric clinic, their healthy siblings, and friends. The control subjects fulfilled the same exclusion criteria as the patients, and never had asthma or eczema.

Written informed consent was obtained from the parents of all subjects in the study. The Departmental Research

Committee approved the study in terms of ethics and scientific merits.

## Asthma severity

Asthma was divided into grades I–IV (I being the mildest and IV the most severe). Grading was based on events that took place over the past six months; including day and night symptoms, effect of asthma on daily activity, use of steroids, and peak expiratory flow rate in children  $\geq 6$  years of age, and as defined by the International Paediatric Consensus Group.<sup>9</sup>

## Skin testing

Sensitivity of patients to common aeroallergens (house dust mite, *Dermatophagoides pteronyssinus* and *D farinae* mites; grass, tree, and weed pollens; *Aspergillus fumigatus*; *Alternaria*, *Cladosporium* (Stallergenes, Paris)) was evaluated by skin prick tests (SPT) according to standardised protocol.<sup>10</sup>

## Allergen specific IgE

Allergen specific serum IgE for the same aeroallergens as well as food allergens (egg white, fish, peanuts, cows' milk) was determined using the Pharmania CAP-RAST test as described previously;<sup>11</sup> those with positive tests for food allergy were excluded from the study.

## Measurement of intestinal permeability

The dual sugar test was used in this study. After an overnight fast, children ingested 10 ml solution containing 1 gram lactulose (non-metabolised, high molecular weight sugar) and 1 gram mannitol (absorbable, low molecular weight sugar). Liberal intake of water was allowed after 30 minutes, and food after five hours. Urine was collected for a total of five hours, after ingestion of sugars, in a container with a few drops of 20% chlorohexidine as a preservative; urine volume was recorded. A 10 ml aliquot of urine was stored at  $-20^{\circ}\text{C}$  until analysis. Total urinary excretion of mannitol and lactulose was estimated according to the methods described previously,<sup>12,13</sup> but modified for the autoanalyser

**Abbreviations:** L, lactulose; M, mannitol; SPT, skin prick test

COBASMIRA<sup>14</sup>. The results were expressed as a percentage of the ingested dose (lactulose mg% (L) and mannitol mg% (M)). The ratio L:M was calculated.

### Statistical analysis

Results are presented as mean (SE) for age, weight, and height; the values were compared between patients and the control group using the *t* test for two independent samples. The values for different clinical variables are shown as median and interquartile range (25–75th centile) for each group as these data were not normally distributed. Hence, the groups were compared using the non-parametric Kolmogorov-Smirnov Z test and median test, in case of severity groups. The  $\chi^2$  test was applied to test the differences in proportions between patients and controls. A probability level of  $p < 0.05$  was considered significant. Statistical software (SPSS version 11.0) was used for analysis.

### RESULTS

Thirty two asthmatic children and 32 control subjects, aged 5–12 years, were included in the study. Table 1 shows sex, mean age, weight, height, and consanguinity. Mean age and gender in both groups were similar. However, weight and height were slightly higher in the control group. Consanguinity was high among parents of patients as well as controls.

Twelve patients had eczema (previous in nine and concurrent in three). The mean duration of symptoms was 4.1 (0.12) years (range 1.0–8.1 years). In seven patients asthma was of grade I severity, seven grade II, ten grade III, and eight grade IV. Twenty one patients were on inhaled steroids; none were receiving oral steroids or skin preparation containing steroids. Skin prick test was positive in 26 patients, of whom 19 were positive to more than one allergen; six patients were negative to all allergens used in the study.

The median value of the percentage of the ingested lactulose recovered from the urine of asthmatic children, was significantly higher than that for the controls ( $p < 0.002$ ), while that for mannitol was more or less similar (table 2). The ratio of L:M was significantly higher in children with asthma compared to controls ( $p < 0.001$ ) (table 2). The median values of L, M, and L:M of patients with or without inhaled steroids were similar (table 3). The median value of L:M for patients with eczema and those without were also not found significant (table 3). Patients with positive SPT had lower mean values than those who were negative, but the difference was not statistically significant. Intestinal permeability did not correlate with severity of asthma (table 4).

**Table 1** Characteristics of patients and controls

	Patients (n = 32)	Controls (n = 32)	p value
Males	21	18	NS
Females	11	14	NS
Age			
5–8 years	19	17	NS
9–12 years	13	15	NS
Mean (SE)	8.5 (0.18)	8.1 (0.21)	NS
Weight (kg), mean (SE)			
5–8 years	22.0 (0.21)	23.8 (0.37)	0.001
9–12 years	34.5 (0.19)	35.6 (0.21)	0.018
Height (cm), mean (SE)			
5–8 years	120.0 (0.39)	121.2 (0.21)	0.049
9–12 years	128.4 (0.05)	129.0 (0.04)	0.001
Consanguinity	13 (40.6%)	12 (37.5%)	NS

**Table 2** Percentage of lactulose (L) and mannitol (M) recovered from the urine and L:M ratio in asthmatic patients and control subjects

	Patients (n = 32)	Controls (n = 32)	p value
Volume of urine (l)	0.25 (0.11–0.45)	0.15 (0.08–0.27)	NS
L	2.29 (0.91–4.07)	0.69 (0.45–1.08)	0.002
M	11.82 (5.70–15.65)	11.28 (6.38–16.82)	NS
L:M	0.20 (0.11–0.40)	0.06 (0.04–0.09)	0.001

Results expressed as median (interquartile range).

### DISCUSSION

Histological examination of the small intestinal biopsy specimens remains the gold standard for the evaluation of the small intestinal mucosa. However, for various reasons most workers now prefer to use non-invasive techniques. Measurement of the differential absorption of monosaccharides of different molecular size, after an oral dose, has gained popularity in recent years.<sup>15</sup> In our study, two sugars, one with a smaller molecular size (mannitol) and another with a larger molecular size (lactulose) were chosen. The ratio of the urinary excretion of the lactulose over mannitol was determined in the urine collected for five hours after the oral dose. Determination of the ratio of the sugars makes the test more sensitive. It eliminates potential confounding factors such as defects in collection, gastric retention, transit time, and renal clearance,<sup>16</sup> as the physiological parameters affect the sugars equally but do not affect the ratio. The intestinal permeability of the larger molecules like lactulose is increased in case of damage of the intestinal mucosa, but for the smaller molecules like mannitol, remains unchanged or

**Table 3** Percentage of lactulose (L) and mannitol (M) recovered from the urine and L:M ratio in asthmatic patients on inhaled steroids, with eczema, positive SPT, and those without

	Category of patients	p value	
	<b>Steroids (n = 11)</b>	<b>No steroids (n = 21)</b>	
L	2.16 (0.91–4.08)	2.42 (0.86–4.13)	NS
M	11.74 (4.61–17.53)	12.20 (6.50–15.50)	NS
L:M	0.22 (0.11–0.43)	0.17 (0.07–0.32)	NS
	<b>With eczema (n = 12)</b>	<b>No eczema (n = 20)</b>	
L	2.36 (0.82–3.61)	2.22 (0.94–4.33)	NS
M	15.06 (8.14–17.54)	11.37 (5.13–14.58)	NS
L:M	0.16 (0.08–0.38)	0.22 (0.11–0.44)	NS
	<b>Positive SPT (n = 26)</b>	<b>Negative SPT (n = 6)</b>	
L	2.09 (0.89–4.16)	2.71 (0.95–6.78)	NS
M	11.72 (6.23–15.50)	14.85 (3.04–18.91)	NS
L:M	0.18 (0.10–0.38)	0.46 (0.19–0.99)	NS

Results expressed as median (interquartile range).

**Table 4** Percentage of lactulose (L) and mannitol (M) recovered from the urine of patients and L:M ratios according to severity of disease (n = 32 patients)

	Severity				p value
	I (n = 7)	II (n = 7)	III (n = 10)	IV (n = 8)	
L	1.02 (0.86–4.26)	2.67 (1.32–3.90)	2.22 (1.01–2.66)	3.73 (0.41–4.91)	NS
M	11.74 (3.79–23.00)	15.50 (3.30–17.55)	11.95 (9.94–15.27)	9.43 (2.92–14.96)	NS
L:M	0.15 (0.09–0.40)	0.24 (0.20–0.83)	0.14 (0.06–0.25)	0.29 (0.13–0.54)	NS

Results expressed as median (interquartile range).

decreased.<sup>12</sup> Therefore, slight changes in the excretion of both sugars result in significant changes in the ratio.

In this study, the dual sugar test has shown a significant increase of intestinal permeability in children with asthma compared with controls ( $p < 0.001$ ). Among the asthmatic children, increased intestinal permeability did not correlate with asthma severity, treatment with inhaled steroids, associated eczema, or allergy as indicated by positive SPT. Although many investigators have studied the permeability of nasal and respiratory mucosa in atopy,<sup>17</sup> reports of evaluation of the intestinal permeability in bronchial asthma are scarce. To our knowledge, the only study in the literature on intestinal permeability in asthma is that of Benard and colleagues<sup>6</sup> in France. They showed increased intestinal permeability in adult patients with bronchial asthma compared with patients with chronic obstructive pulmonary disease and healthy control subjects. In their study, they used radioactive material (CrEDTA) which was administered orally, and estimated its urinary recovery.

The mechanisms responsible for increased intestinal permeability in asthma remain unclear. However, gastrointestinal abnormalities have been reported in patients with asthma. Duodenal histological changes mimicking those observed in bronchial mucosa have been shown.<sup>3, 18</sup> Nevertheless, in asthma, it is not yet known whether increased intestinal permeability is correlated with gut inflammatory infiltrate.

Our study showed that increased intestinal permeability was not correlated with asthma severity and was not affected by inhaled steroids. Similar findings were reported by Benard and colleagues<sup>6</sup> who also found no correlation between serum IgE level, blood eosinophilia, and intestinal permeability.

Increased intestinal permeability has been shown in adult patients with eczema.<sup>19</sup> In addition, minor morphological abnormalities of the gastrointestinal tract have been shown in children with atopy.<sup>20</sup> In our study, increased intestinal permeability in the asthmatic children, was similar in those with and without eczema. It remains unclear whether histological and functional changes are associated primarily with asthma, eczema, or the atopic status in general. Alternatively, it may be that the difference in the phenotype of atopic patients may be related to differential expression of relevant cytokines and/or their receptors in different tissues. Our findings lend support to the view that gastrointestinal abnormalities are common in children with all atopic diseases.<sup>20</sup> Whether increased intestinal permeability is a primary inherited abnormality in patients with asthma or atopy remains to be elucidated, as well as the functional implications of such abnormalities.

In conclusion, this study has for the first time shown increased intestinal permeability in children with bronchial asthma. Intestinal permeability did not correlate with the

severity of asthma, treatment with steroids, or eczema. This lends further support to the view that the whole mucosal immune system may be involved in such patients. The functional implications of such findings need to be elucidated.

#### Authors' affiliations

**Z Hijazi, A M Molla, W M R A Muawad, P N Sharma**, Faculty of Medicine, Kuwait University, Kuwait

**H Al-Habashi**, Paediatric Department, Mubarak AlKabeer Hospital, Kuwait

**A M Molla**, Department of Biochemistry, Laboratory Medical Science, Faculty of Allied Health Science and Nursing, Kuwait University, Kuwait

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