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Intestinal permeability and Ménière's disease

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ABSTRACT

Purpose: Ménière disease (MD) is a multifactorial chronic disabling condition characterized by episodic vertigo, ear fullness, and hearing loss. MD patients often complain of aspecific gastrointestinal symptoms associated with autonomic dysregulation, frequently outweighed by the otological manifestations. Dietary modifications have been reported to improve the typical MD symptoms in some cases. Our purpose was to test the urinary levels of lactulose and mannitol (double sugar test) and the fecal calprotectin, both markers of altered intestinal permeability, in subjects with definite MD in an active and inactive stage.
Materials and methods: Twenty-six with definite unilateral MD were studied: 14 patients were symptomatic for at least 3 months with moderate to severe vertigo spells and a functional level ≥4; 12 patients had been asymptomatic (no vertigo spells) for at least 3 months and had a functional level = 1 at the time of testing. Twenty healthy volunteers were recruited as "control group".
Results: Lactulose and mannitol absorption was significantly increased in the symptomatic M patients compared to the asymptomatic group ($p < 0.02$ and $p < 0.004$, respectively) and to the controls. FC were also higher than normal only in the symptomatic group. ($p < 0.01$).
Conclusions: An altered intestinal permeability, according to the two assays, was found only in symptomatic MD patients. The rationale for a possible relationship between MD and intestinal permeability is forwarded. The double-sugar test and FC quantification might be implemented in the MD diagnostic workup.

1. Introduction

Ménière disease (MD) is a multifactorial chronic disabling condition characterized by episodic vertigo, ear fullness, and hearing loss. The underlying pathologic condition has been identified in the endolymphatic hydrops, but its pathophysiology is not yet fully understood, as a causative role in the disease has been ascribed to many different etiological factors.

Several authors reported a significant improvement of the vestibular symptoms by generic or specific dietary modifications in MD patients [1,2,3]. A possible link between food allergies and MD symptoms has been firstly hypothesized in 1923; [4] after, that, since the 70's, targeted elimination diets for documented food allergies were proposed [5] after corroborating proof that avoidance might prevent an immune-mediated reaction to the inner ear [6]. However, double blinded food challenge tests [7] are very difficult to be implemented because of long follow-up required and the highly unpredictable time course of the symptoms in MD patients [8,9].

On the other hand, most MD patients complain of aspecific gastrointestinal symptoms, such as diarrhea, abdominal pain, dyspepsia, and weight fluctuation; [10] the association of MD with gastrointestinal lesions has also been described many years ago [11]. Given the enhancement of gastroenteric mucosal permeability during episodes of local inflammation, and the frequency of reported gastrointestinal symptoms in MD patients, we designed to evaluate the intestinal permeability by means of a couple of simple assays: a) the "*double sugar test*" is a well-known validated not-invasive method; it is easily applicable, independently from the etiology; [12] b) the dosage of *fecal calprotectin* (FC) in the stools has been recently proposed as a marker for differentiation between functional and inflammatory gastrointestinal disorders [13,14]. FC is a calcium binding protein of the S100 family, found mainly in neutrophils but also in other white blood cells [15,16].

The aim of this study was to evaluate "double sugar test" and FC in two groups of definite MD patients in the active versus quiescent phase of the disease and compare them to those obtained in normal controls.

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2. Material and methods

2.1. Patients

The study included 26 consecutive unilateral definite MD patients, recruited at the outpatient clinic of the Vestibular Disorders Unit in a tertiary referral university Hospital. They fulfilled all criteria for definite MD according to the consensus paper of the international societies revision [17] of the AAO-HNS guidelines; [18] all of them were in the clinical subgroup 1, stage 2 or 3 [19]. Patients were negative for retrocochlear lesion at magnetic resonance imaging. Those who had previously received systemic steroids or intratympanic injections (either gentamicin or steroids) were excluded from the study, as well as those following specific diets or being treated with steroids, gastrointestinal drugs and/or antihistamines. A positive history of gastrointestinal disease or familiarity for celiac disease or intestinal bowel disease, of abnormal thyroid hormones, of malignant tumours or autoimmune diseases was exclusion criteria. Hypertension was not an exclusion criterium, if well controlled by drugs.

Fourteen patients (6 females, 8 males, mean age: 45.3 ± 11.2 years) were defined as "*symptomatic*" if they had at least two major episodes of vertigo per month with sensorineural hearing loss and aural fullness during the 3 months prior to the admission and a functional level (FL) ≥ 4, according to the consensus paper guidelines [18]. Twelve patients (4 females, 8 males, mean age: 44.0 ± 14.4 years) were considered "*asymptomatic*" if they had been free from symptoms for at least 3 months at the enrolment time and a FL of 1.

Twenty healthy volunteers (10 females, 10 males, mean age: 39.0 ± 16.4 years) served as a "*control group*", in order to check the reference values. All healthy subjects were normally hearing, reported a negative familiar and personal history of vertigo or dizziness, and never suffered from otological diseases.

The participation in the study was voluntary and all the subjects were not paid for it. The work was carried out in accordance with the Helsinki Declaration of 1975, as revised in 2013, including, but not limited to, the absence of potential harm to participants, their guaranteed anonymity, and written informed consent.

2.1.1. Double sugar test

The intestinal permeability was assessed using a double sugar test. In principle, the test is based on the measurement of the urinary excretion of orally administered non-metabolized sugar probe molecules [20]. The urinary content of lactulose and mannitol can be considered an indirect index of the intestinal absorption [21]. When awakening in the morning, each patient was instructed to collect a pre-test urine sample. Then, they drank a solution containing 5 g lactulose plus 5 g mannitol dissolved in 100 ml water. Urines were collected during the next 5 h and kept refrigerated at 2–7 °C. Patients were instructed to avoid eating (not even a chewing-gum), drink or smoke during the test, but were allowed to drink a fixed dose of water (200 ml), only after 2 h. Total urine volume was recorded on completion of the test and a 10 ml fraction was stored at −20 °C until analysis.

Results were expressed as the percentage of recovery of the ingested dose of sugars.

2.1.2. Laboratory double sugar test analysis

Mannitol and lactulose concentrations were measured by gas chromatography mass spectrometry (GC-MS) [22]. A small aliquot (100 μl) of each urine sample was added with 200 μl of acetonitrile plus internal standards [methyl alfa-D-mannopyranoside 100 μl (0,5 μg/μl); D-(+)-turanose 50 μl (0,1 μg/μl)] in a glass tube. After evaporation under a gentle stream of nitrogen, the mixture was oximated by adding 100 μl of a 25 μg/μl solution of hydroxylamine in anhydrous pyridine and heated to 70 °C for 30 min. Sugar oximes were derivatizated with 100 μl of Chlorotrimethylsilane, N,O-*bis* (trimethylsilyl) trifluoroacetamide BSTFA with 1% Trimethylchlorosilane, Trimethylsilyl chloride TMCS at 70 °C for 30 min. Silylated derivatives, sealed in autosampler vials, were analyzed by a Clarus 600 GC-MS system equipped with an Elite column (30 m × 0.32 mm id × 0.25 mm film; Perkin Elmer, USA). Injection was performed with 1:20 split using helium (1 ml/min) as carrier gas. The temperature program was as follows: an initial temperature of 150 °C held for 2 min, followed by a linear ramp of 10 °C/min to 300 °C held for 5 min. The mass spectrometer operated in full mass scan. Peak integration was carried out manually and metabolites quantified against internal standards using calibration curves for the listed sugars. Based on preliminary studies, the assay was linear up to 500 μg for mannitol and 50 μg for lactulose. Intra-assay CV% was ~3% for the two biomarkers and recovery up to 99%.

2.1.3. Fecal calprotectin

In normal subjects, FC values <40 μg/g are considered as negative; values between 40 and 60 μg/g as borderline and values >60 μg/g as positive. A cut-off of 50 μg/g has a sensitivity and a specificity >80%, and a positive and negative predictive values of 70 and 90%, respectively [23]. Values between 40 and 60 μg/g suggest a possible intestinal inflammatory process; values ≥60 μg/g indicate a significant inflammatory process of the intestinal mucosa.

2.1.4. Laboratory FC analysis

Standardised laboratory protocol (ELISA) for FC analysis was used. A 100 mg quantity of stool was weighed and dispensed into an analysis pot using a 10 μl inoculation loop. As per local protocol, first morning sample was requested. The exact weight was recorded and 5 ml of extraction buffer was added. The samples were then vortexed for 30 min in order to ensure complete dissolution, and then centrifuged using an Eppendorf centrifuge. The supernatant was then removed for analysis using the IDK ® Calprotectin ELISA method (Immunodiagnostik, Bensheim, Germany).

2.1.5. Statistical analysis

Statistical analysis was performed using the SPSS statistical package version 24.00 (SPSS Inc., Chicago, Illinois). The significance of difference between the two groups compared with each other and with controls was evaluated by student *t*-test for independent samples. Results are expressed as means and standard deviations (SD), median and first and third quartiles. A $p < 0.05$ was considered statistically significant. Spearman correlation test was performed to investigate the association between the variables.

3. Results

The two MD groups resulted homogeneous for age, sex, stage, onset time of MD, ear side, degree of hearing loss, absence of comorbidities; they differed only for the number of vertigo episodes during the last 3 months and the functional level, accordingly to the inclusion/exclusion criteria.

A significant increase of both sugars was found in symptomatic MD compared to the asymptomatic MD patients and to the controls, with a most consistent rise observed for mannitol in the symptomatic MD group. ($p = 0.004$) (Fig. 1).

Similarly, the levels of FC were significantly higher in the symptomatic MD group resulting in a range between 46 and 380 μg/g (median: 120.4 μg/g) compared to a range of 2–80 μg/g (median: 28.5 μg/g) in the asymptomatic MD subjects ($p = 0.01$); all subjects in the control group showed normal values (<40 μg/g). (Fig. 2) A positive correlation was observed between FC levels and mannitol absorption values. ($r = 0.768$).

Table 1 summarizes the results of the double sugar test and FC. The overall outcomes were comparable between asymptomatic MD and the control groups, except for the amount of urinary lactulose that resulted slightly but significantly higher in the former than in controls (median: 1.2% [range, 0.0–1.8] vs 0.5% [0.13–1.1]). ($p = 0.02$).

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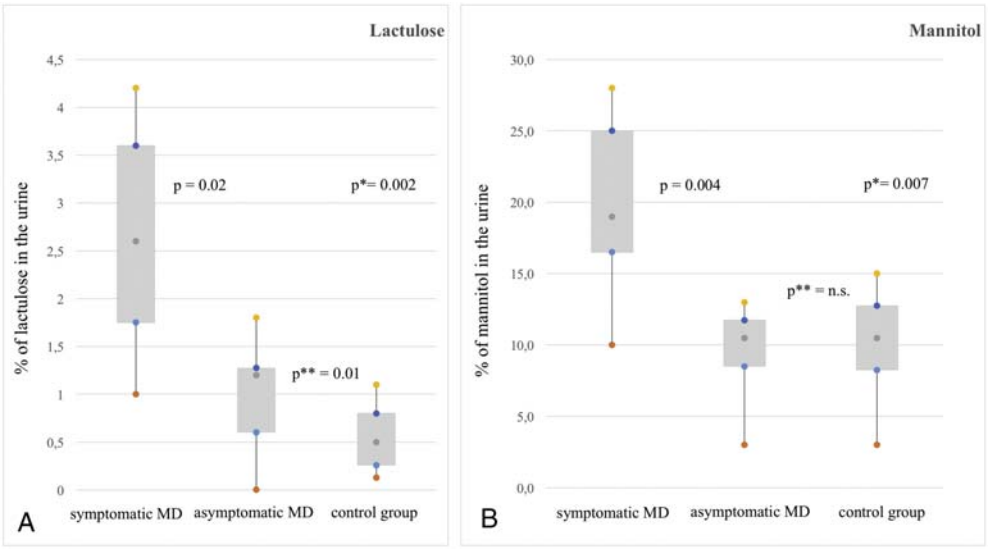

Fig. 1. The boxplots represent the urinary levels of lactulose and mannitol in the three groups.

4. Discussion

Several conditions often act as comorbidities of MD, including arthritis, psoriasis, gastroesophageal reflux disease, irritable bowel syndrome, and migraine [17]. On the other hand, scanty and heterogenous reports have included dizziness, [24] numbness [25,26] and/or sensorineural hearing loss [27,28,29] among the various comorbid symptoms in irritable bowel syndrome and/or intestinal bowel disease [30]. Since 1997, Derebery [31] supposed that there was considerable clinical and immunological evidence in favour of a probable role of allergies in MD symptoms: inhalant and food allergies have both been linked with the symptoms. In a previous work, we found an increased rate of atopic subjects among the MD patients (82.7%): food most frequently inducing hypersensitivity in MD were wheat, cow's milk protein and tomato [32].

Dietary modifications have sometimes proven effective in improving the typical MD symptoms [1]. More recently, Heather stated that allergy avoidance and immunotherapy should be considered as part of the treatment plan to help controlling MD symptoms [33].

As food intolerance is frequently expressed in atopic patients, our hypothesis in the present study was that the acute MD crises might be associated with conditions of acutely altered intestinal permeability [2,5]. However, a proof of this correlation is difficult to assess, because MD patients often show hypersensitivity to many foods, and a combination of dietary deprivation and challenge tests require very long observation periods and repeated trials.

Our preliminary data seem to support the hypothesis that an impaired intestinal barrier function may be pathologically related with the active/inactive stage of MD symptoms.

In healthy subjects, <1% of lactulose passes the intestinal mucosal barrier, only through the intercellular junctions (a para-cellular pathway); practically, all lactulose remains unabsorbed and can be found in the stools. Conversely, under normal conditions, about 14% of mannitol is absorbed through the hydrophilic pores of the enterocytes. Diseases causing a definite damage to the villi (e.g. celiac sprue, giardiasis, etc.) determine a reduced absorption of mannitol whereas other chronic diseases of the bowel (e.g. Crohn's disease, ulcerative colitis) show an increased permeability to lactulose [12].

In both our symptomatic and asymptomatic MD patients, lactulose absorption was significantly increased compared to the control group. Interestingly, instead of a reduction, an increase of mannitol intake was observed in the symptomatic MD group. Similarly, also FC was higher in the symptomatic MD group. FC is a calcium-binding protein, classified as damage-associated molecular pattern protein that has antimicrobial properties [34]. It is released mainly from neutrophilic leukocytes and secreted into the whole enteric tract during inflammation of the intestinal mucosa [15,16]. Therefore, it is a marker of inflammation of the intestinal tract. It is an expression of the activation of the mucosal innate defense system against a pro-inflammatory challenge, such as in irritable bowel syndrome patients. Interestingly, it can be detected also in the absence of macroscopic signs of inflammation [35].

The increased absorption of lactulose and mannitol, together with higher values of FC, clearly indicates an altered intestinal permeability in the active phases of MD. We might speculate that the impaired intestinal barrier function in symptomatic MD, could be possibly linked to a decreased tightness of the intercellular junctions caused by the inflammation, rather than by a direct damage to the intestinal cells. However, to confirm this hypothesis a further study with a longer follow-up in the same patients has been planned, in order to crosscheck the two groups.

A limit of this study is the small size of the sample group. Nevertheless, we are not aware of other studies addressing the possible link

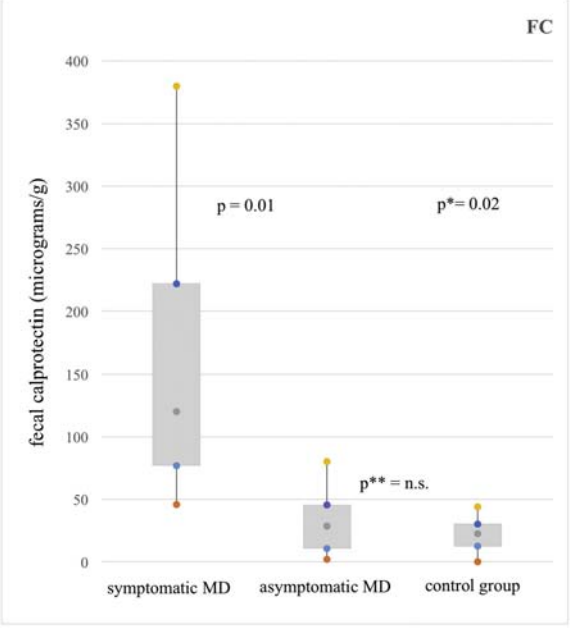

Fig. 2. The boxplots show the values of FC.

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Table 1
Recovery ratio of the administered sugars (percentage of the ingested dose of sugars) and the amount of fecal calprotectin (FC).

Patients	Lactulose % (n.v. <1)	Mannitol % (n.v. <14)	FC μg/g (n.v. <40)
Symptomatic MD group	2.64 ± 1.3	20 ± 6.4	163.4 ± 119.4
Asymptomatic MD group	1.22 ± 0.34	9.5 ± 3.6	32.5 ± 27
Non-MD control group	0.56 ± 0.39	10 ± 4.3	21.8 ± 15.6

Mean ± standard deviation; normal reference values = n.v.

between altered intestinal permeability and symptomatic MD and we will proceed with the recruitment of new patients in order to increase the statistical power of the research. These interesting results might also allow to think that testing these parameters after specific therapies or in the intercritical phase of MD in the same subjects may provide interesting insights in the follow-up. We also advocate further trials by other centres in order to assess if the investigation of intestinal permeability and FC can be added to the current clinical practice, aimed at identifying those MD patients who would benefit from deprivation diets.

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