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Prevalence of Gluten Sensitive Enteropathy antibodies in Restless Legs Syndrome

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Abstract

The prevalence of restless legs syndrome (RLS) is increased in gluten sensitive enteropathy (GSE); but prevalence of GSE is not known in RLS. 96 RLS patients and 97 healthy controls, both with or without iron deficiency were enrolled. All secondary RLS patients except iron deficiency were excluded. Subjects underwent a thorough biochemistry and routine blood analyses, and tissue transglutaminase antibodies (TTGA), endomysium antibodies (EMA) and gliadin antibodies (AGA) were also tested. In RLS patients positivity rates of all GSE antibodies were similar to those in controls. The rate of iron deficiency anaemia in RLS patients with at least one positive GSE antibody was significantly higher than that of RLS patients whose GSE antibodies were all negative. The prevalence of GSE antibodies in RLS patients is not increased. GSE might have a role in the aetiology of RLS in association with iron deficiency anaemia. Since the prevalence of GSE antibodies is not increased in RLS, it seems unlikely that GSE is involved in the aetiology of RLS through different mechanisms (e.g. immunological mechanisms) other than iron deficiency as proposed in some published papers.

Key words: Restless legs; gluten sensitive enteropathy; celiac disease; endomysium antibody; gliadin antibody; iron deficiency anaemia.

Introduction

RLS is a sensory-motor disorder characterized with intense, unpleasant, disagreeable sensations (paresthesia and dysesthesia) in the extremities (mostly in legs), which begin or worsen in the evening or at night during periods of rest and which are relieved by movement (1). It affects about 5-15% of general population (1). Diagnosis is based on clinic features (1, 2). The pathology may be primary or secondary. Iron deficiency is a well-documented cause for secondary RLS (1, 2).

Gluten sensitive enteropathy (GSE) is a chronic systemic immune-mediated gluten-dependent enteropathy, induced by ingestion of gluten-containing food and characterized by intestinal malabsorption and sub-total or total atrophy of intestinal villi, which improves after gluten-free diet (3). It may present with gastrointestinal symptoms (diarrhoea, abdominal pain, abdominal distension, weight loss, anorexia, lactose intolerance, irritability) and/or highly variable non-gastrointestinal findings (iron-deficiency anaemia, dermatitis herpetiformis, chronic fatigue, joint pain/inflammation, osteoporosis/osteopenia, infertility and/or recurrent foetal loss, vitamin deficiencies, short stature, failure to thrive, delayed puberty, dental enamel defects, and autoimmune or neurologic disorders) (4, 5). Classic GSE is characterized by mild to severe gastrointestinal symptoms and is less frequent than non-classic GSE, where gastrointestinal symptoms are absent (4, 5). GSE shows association with neurologic diseases such as peripheral neuropathy, cerebellar ataxia, autonomic dysfunction, myopathy, infantile hypotonia, growth retardation, learning disorder, attention deficiency-hyperactivity disorder, epilepsy and migraine (4, 6). Because evidence showing that a large percent is left undiagnosed is increasing more and more, the actual prevalence of GSE is not known. As with an iceberg, GSE patients with clinical findings represent the tip, while asymptomatic and latent patients make up the portion under water (7). GSE was reported to be present in approximately 1% of

general population (5). Because GSE accompanies many neurologic diseases and being a condition often leading to iron deficiency, a relation between RLS and GSE is possible (8).

Although RLS is reported to be more common among GSE patients, the prevalence of GSE in RLS has not been examined yet (3, 6). Through investigation of the prevalence of GSE antibodies in RLS patients, we aimed to demonstrate whether GSE has a place in the aetiology of RLS, and if there is a relation, whether there is any other cause than iron deficiency.

Methods and Material

Ninety six primary RLS patients or iron deficiency associated RLS patients among those who presented to or whom were referred to the outpatient clinics of Vakif Gureba Training and Research Hospital (VGH) were enrolled in the study. All other secondary RLS patients were excluded. Age, sex and BMI matching 97 healthy or iron deficient subjects were recruited to the control group. For both patient and control groups detailed history was taken, medication use was queried and physical examination was performed along with a thorough biochemistry study, routine blood analysis and urinalysis. Thyroid hormones, HBsAg, anti HCV, anti HIV 1-2, haemogram, sensitive CRP, erythrocyte sedimentation rate (ESR), faecal occult blood, iron and total iron binding capacity, ferritin, vitamin B12, folate, HbA1C, post-prandial glucose were also tested.

The four basic criteria developed by The International RLS Study Group (IRLSSG) in 1995 were used for the diagnosis of RLS (1).

As for anaemic patients in RLS and control groups, only those with iron deficiency probably of obscure origin were included. Thus menorrhagia and/or metrorrhagia, recurrent epistaxis, haematuria, melena, hematochezia, positivity of faecal occult blood test, vegetarian diet, gastrectomy, recent alteration in defecation habits and pronounced gastric complaints were also reasons for exclusion. Iron deficiency was defined as ferritin levels below 15 ng/mL and/or transferrin saturation below 15%. Iron deficiency accompanied by Hb levels below 12 g/dL was considered as iron deficiency anaemia. Additionally folic acid and vitamin B12 deficiency anaemia, thalassemia, haemolytic anaemia, aplastic anaemia, myelodysplastic syndrome and chronic disease anaemia were eliminated.

As GSE antibodies, antigliadin (AGA) IgA/IgG, antiendomysium (EMA) IgG/IgA, tissue transglutaminase (TTG) IgA and IgG were tested. In order to be considered as GSE antibody positive, at least one of the three types of antibodies was required to be positive as IgG or IgA. Intestinal biopsy was not performed in antibody positive patients. These patients were referred to gastroenterology outpatient clinics.

The study was approved by VGH ethics committee and patients gave their written informed consents according to Helsinki declaration of 2008.

Measurement of GSE antibodies

Anti-Gliadin Ig A, Ig G and Tissue transglutaminase Ig A Ig G were tested by ELISA method (kit brand: Generic Assay, Equipment: Biotek Inc. Brand, Elx 50 Washer and Elx 800 Reader). Anti-Endomysium Ig A and Ig G were tested by immune fluorescent antibody method (kit brand: Euroimmun, equipment: Eurostar, substrate: primate liver, 1/10 dilution).

Statistical analysis

Means and standard deviations of continuous variables are presented. Nominal variables are presented as proportions. Chi-square test and Compare of two proportion test, Student's t test, and Mann-Whitney u test were employed where needed. Two tailed p values lower than 0.05 were accepted significant.

Results

RLS and control groups were similar in terms of age, sex, and BMI (Table 1). RLS and control groups were similar in the numbers of subjects with iron deficiency and iron deficiency anaemia (Table 1).

Groups were similar in terms of haemoglobin (Hb), haematocrit (Htc), platelets (plt), sensitive C reactive protein (sCRP), ferritin, transferrin saturation rate (TSR) (Table 2).

RLS and control groups were similar in the positivity rates of IgG and IgA type GSE antibodies (Table 3).

46% of the GSE antibody positive RLS patients (13 subjects) had iron deficiency anaemia; whereas 13.3% of GSE antibody negative RLS patients (83 subjects) had iron deficiency anaemia. The frequency of iron deficiency anaemia was significantly higher in antibody positive RLS patients than antibody negative RLS patients ($p = 0.004$).

While 7.7% of the GSE antibody positive controls (13 subjects) had iron deficiency anaemia, 20.2% of antibody negative controls (84 subjects) had iron deficiency anaemia. Frequency of iron deficiency anaemia was similar in antibody negative and antibody positive controls ($p = 0.279$).

Table 1

Demographics, number of subjects with iron deficiency and iron deficiency anaemia in the RLS and control groups

Parameters	RLS Group		Control Group		P Value
Cases studied, n	96		97		
Female/male, n	70/26		68/29		0.665
Cases with iron deficiency, n	31		27		0.500
Cases with iron deficiency anaemia, n	17		18		0.878
	Mean	± sd	Mean	± sd	
Age, years	43.7	11.8	41.7	11.2	0.229
BMI, kg/m^2	27.4	4.3	27.8	4.3	0.492

Table 2

Selected blood analysis results in groups

Parameters	RLS Group		Control Group		Normal Range	P value
	Mean	± sd	Mean	± sd		
Hb, g/dL	13.2	1.3	13.2	1.5	12.7-18.1	0.745
Htc, %	38.9	3.9	39.5	3.3	37.7-53.7	0.290
Plt, number/mm^3 x10^3	271.9	57.3	269.5	62.2	142-424	0.777
Sensitive CRP, mg/dL	0.43	0.33	0.38	0.35	0-0.8	0.326
Transferrin saturation rate, %	23.2	11.9	25.2	10.5	20-40	0.227
Ferritin, ng/mL	57.1	65.4	54.7	67.7	30-400	0.797
Vitamin B12, pg/mL	380.4	250.8	343.5	250.8	191-663	0.309
Folic acid	9.1	3.1	8.8	2.5	4.5-32.2	0.470

Table 3

Positivity rates of GSE antibodies in the RLS and control groups

Groups	Number of cases	GSE antibody positivity						
		Tissue trans-glutaminase antibody IgA (+), *N* (%)	Tissue trans-glutaminase antibody IgG (+), *n* (%)	Antiendomysium antibody IgA (+), *n* (%)	Antiendomysium antibody IgG (+), *n* (%)	Antigliadin antibody IgA (+), *n* (%)	Antigliadin antibody IgG (+), *n* (%)	At least one antibody(+), *n* (%)
RLS	96	2 (2.1%)	5 (5.2 %)	3 (3.1 %)	6 (6.3 %)	5 (5.2 %)	11 (11.5%)	13 (13.5%)
CTRL	97	2 (2.1%)	4 (4.1%)	1 (1 %)	3 (3.1%)	6 (6.2%)	10 (10.4%)	13 (13.4%)
P value	–	0.992	0.721	0.307	0.298	0.770	0.798	0.977

In other words, in the RLS group, positivity rates of GSE antibodies were significantly higher in patients with iron deficiency anaemia than in patients without (Table 4). However, subjects with or without iron deficiency anaemia were similar in terms of positivity rates of GSE antibodies in the control group (Table 4).

Discussion

In the two original articles published recently, RLS was found to be more frequent in GSE patients than in controls. One of these studies found RLS prevalence 31% in GSE, and 4% in the control group ($p < 0.001$) (3). In the other study, RLS prevalence

Table 4

Positivity rates of GSE antibodies in subjects with and without iron deficiency anaemia among RLS and control groups

Groups		GSE antibody positivity						
		Tissue transglutaminase antibody IgA (+), *n* (%)	Tissue transglutaminase antibody IgG (+), *n* (%)	Antiendomysium antibody IgA (+), *n* (%)	Antiendomysium antibody IgG (+), *n* (%)	Antigliadin antibody IgA (+), *n* (%)	Antigliadin antibody IgG (+), *n* (%)	At least one antibody (+), *n* (%)
RLS	Iron def. anaemia (-) *n*: 79	0 (0%)	2 (2.5%)	0 (0%)	2 (2.5%)	1 (1.3%)	6 (7.6%)	7 (8.9%)
	Iron def. anaemia (+) *n*: 17	2 (11.8%)	3 (17.6%)	3 (17.6%)	4 (23.5%)	4 (23.5%)	5 (29.4%)	6 (35.3%)
P value		0.030	0.037	0.005	0.008	0.003	0.023	0.010
CTRL	Iron def. anaemia (-) *n*: 79	2 (2.5%)	4 (5.1%)	1 (1.3%)	3 (3.8%)	5 (6.3%)	10 (12.7%)	12 (15.2%)
	Iron def. anaemia (+) *n*: 18	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.6%)	0 (0%)	1 (5.6%)
P value		1	1	1	1	0.451	0.200	0.451

in GSE patients was found to be 25%, whereas it was found 10% in patients' spouses constituting the control group ($p < 0.02$) (6).

In our study, we could not detect a significant difference in GSE antibody positivity between the control and patient groups. Similar situations have been reported. For example, while prevalence of periodic limb movements of sleep (PLMS) in RLS was reported as 81%, prevalence of RLS in PLMS was 17% (2). Likewise, though RLS is seen frequently in subjects with rheumatoid arthritis, rheumatoid arthritis is rarely encountered in RLS (11).

Although a positive result on GSE disease-associated antibody testing is likely to be diagnostic of GSE disease, false-positive results occur. Conversely, normal GSE-associated antibody test results do not exclude the diagnosis of GSE (4). We did not take intestinal biopsies from GSE antibody positive subjects. However, since this is the case for both RLS and control groups, we consider that this would not impact the conclusion we draw from the findings. As we studied GSE antibodies at the same centre and with the same laboratory methods, and as sensitivity and specifity of these antibodies are already high (for EMA sensitivity 90-97%, specificity nearly 100%), we think the information we obtained about GSE prevalence are very close to the real facts (7).

The centre we recruited RLS patients acts as a secondary neurology referral centre. This in turn means that our patients were recruited without much selection, reflecting the RLS population in the community.

RLS is known to be closely associated with iron deficiency. An important part of the aetiology of iron deficiency of obscure origin is related to GSE (8). Therefore, we included only RLS patients and controls with iron deficiency anaemia of obscure origin in order to increase the number of subjects having GSE.

In our study, iron deficiency anaemia was significantly more common in GSE antibody positive patients than antibody negative ones in the RLS group.

The two previously published original papers confirm the association of GSE with iron deficiency or iron deficiency anaemia in RLS patients. In one of the articles, the frequency of iron deficiency was found significantly higher in GSE patients with RLS than GSE patients without RLS (6). The other article reported that iron deficiency anaemia was more common in GSE patients with RLS (3). In the published case reports, it was stated that GSE was detected in RLS patients during investigations performed to explain low ferritin levels (9, 10).

It was suggested that AGA IgG is the best marker of neurologic manifestations seen in GSE (12). AGA IgG was similarly the most frequent positive antibody of the RLS and control groups in our study. In our opinion, there is no reason to propose that GSE can lead to RLS through immune mechanisms which have not been clarified yet (3).

We do not think that GSE antibody testing is necessary for screening in primary RLS. The association between RLS and GSE might be related to iron deficiency anaemia. GSE antibody testing would be appropriate when an RLS patient presents with iron deficiency anaemia of obscure origin.

We declare there is no conflict of interest.

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