International Clinical and Medical Case Reports Journal
Research Article (ISSN: 2832-5788)

Anti-Gliadin and Anti-Tissue Transglutaminase Antibodies in the Serum of Patients with Cerebellar Ataxia

Kumari A[1,6#], Singh I[2#], Verma A[1,3], Singh I[1], Hooda U[1], Faruq M[4], Swarup V[2], Garg A[5], Ahuja V[1], Makharia G[1*] and Srivastava A[2*]

[1]Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, India

[2]Neuroscience Centre, All India Institute of Medical Sciences, India

[3]Department of Pediatrics, Celiac Disease Research Laboratory, Università Politecnica delle Marche, Italy

[4]CSIR-Institutes of Genomics and Integrative Biology, India

[5]Department of Neuroradiology, All India Institute of Medical Sciences, India

[6]Department of Biochemistry, All India Institute of Medical Sciences, India

[#]Authors have equal contribution

Citation: *Kumari A, Singh I, Verma A, Singh I, Hooda U, Makharia G, et al . Anti-Gliadin and Anti-Tissue Transglutaminase Antibodies in the Serum of Patients with Cerebellar Ataxia. Int Clinc Med Case Rep Jour. 2025;4(5):1-12.*

Received Date: 01 May, 2025; **Accepted Date:** 07 May, 2025; **Published Date:** 10 May, 2025

[*]**Corresponding author:** Govind K Makharia, Department of Gastroenterology and Human Nutrition, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India; Achal Kumar Srivastava, Department of Neurology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India

1. ABSTRACT

1.1. Background: Cerebellar ataxia is a heterogeneous group of disorders which can be familial or sporadic. Sensitivity to gluten has been implicated in the pathogenesis of sporadic cerebellar ataxia. Since there is a paucity of data on prevalence of gluten sensitivity in ataxia patients from Asian countries, we screened well-characterized progressive cerebellar ataxia patients for the presence of gluten sensitivity.

1.2. Materials and Methods: A cohort of 192 familial and sporadic patients with progressive cerebellar ataxia, were screened for the presence of gluten sensitivity using anti-gliadin Ab IgA and IgG, Anti-Transglutaminase 2 Ab (TG2) and IgA Anti-Transglutaminase 6 Ab (TG6) ELISA kits. The data on their genetic testing for Spinocerebellar Ataxia (SCA) 1, 2, 3, 12, Friedreich's Ataxia (FRDA), and brain imaging were reviewed.

1.3. Results: Out of 192 patients, 110 and 82 had sporadic and familial cerebellar ataxia, respectively. Genetic mutation for SCA 1, 2, 3, 12 and FRDA was confirmed in 76 (40%) patients. Forty-Two (21.8%) patients had either one or more serological test positive for gluten sensitivity; IgA-AGA (20/192; 10.4%), IgG-AGA (2/87; 2.3%), Anti-TG2 Ab (1/141; 0.71%) and IgA Anti-TG6 Ab (23/186; 12.3%). 20 of 32 (63%) seropositive patients had cerebellar atrophy on brain imaging. None of 10 patients, who agreed to undergo duodenal biopsy examination, had evidence of celiac disease.

1.4. Conclusion: In conclusion, our study suggests that an ataxia syndrome related to gluten sensitivity may occur in a subgroup of patients and those antigliadin antibodies and anti-TG6 autoantibodies may be a marker for identifying gluten sensitive and diagnosing sporadic idiopathic ataxia.

2. INTRODUCTION

Gluten Related Disorders (GRDs) are autoimmune harbouring spectrum of clinical manifestations triggered by the immunological response to the dietary gluten in genetically predisposed individuals [1]. The Gastrointestinal Symptoms (GIS) of Gluten Sensitivity (GS) are most popularly recognized, but GS is implicated significantly in the pathogenesis of neurological manifestations [2]. Coeliac Disease (CeD) is the most after sought disease among GRDs spectrum affecting 1.0% of the population. It defines a patient with mostly small bowel enteropathy after exposure to the gliadin protein [3,4]. Non-Coeliac Gluten Sensitivity (NCGS) refers to patients with mainly GIS after ingestion of wheat, barley and rye, but do not have enteropathy and symptomatically benefited from a Gluten-Free Diet (GFD) [5]. Interestingly, the patients with neurological symptoms either do not harbour GIS or they are rare. The patients with neurological disorders are referred as GS after showing presence of circulating anti-gliadin antibodies (AGA IgA and AGA IgG) and Transglutaminases Antibodies (TG2 and TG6) in the serum, however the exact diagnostic criteria remain uncertain for patients with suspected GS [6]. Among the most frequent neurological deficit in gluten hypersensitivity is ataxia that exhibits heterogeneous phenotypes including variability in the age at disease onset and disease severity. Genetic basis has been established in 60% to 75% of cerebellar ataxia patients, rest remains genetically undiagnosed [7,8]. While the most common mutation known in them is trinucleotide repeat (TNR-e.g., CAG and GAA) expansions, others include point mutations, duplications, and deletions [9]. Many environmental factors have also been proposed that could lead to cerebellar ataxia, one of them being sensitive to gluten [10].

A subgroup of ataxia patients those are described sensitive to gluten are termed as Gluten Ataxia (GA) [11]. GA is an autoimmune disorder characterized by the presence of a cerebellar injury, affecting mainly Purkinje cells. Post-mortem brain histopathology of patients with gluten ataxia has shown loss of Purkinje cells throughout the cerebellar cortex. There is cross-reactivity between antigenic epitopes located at the level of Purkinje cells and gluten-related antibodies [12]. Several studies such as impaired intestinal absorption leading to impaired vitamin E deficiency, malabsorption damaging serotonin containing neurons in cerebellum and brainstem have suggested possible mechanisms for the development of GA in CeD [13,14]. It is estimated that GA accounts for approximately 15% of all ataxias and 40% of all idiopathic sporadic cerebellar ataxias in males and females of over the age of 50 years [15] and in general population it affects only 5% to 12% [16]. Besides the evidence of circulating anti-TG6 autoantibody, missense mutations pathogenic for the protein stability and catalytic activity of TG6 have also been reported in TGM6 gene of Han-Chinese patients with SCA-35, supporting the role of TG6 in cerebellar functioning [17]. Cerebellar IgA deposits with TG6 have also been traced from the tissues of patients with GA [18].

Traditionally, it was thought that GRDs do not occur in Asia. However, there has been an emergence of CeD in multiple Asian countries including India (0.6%) and China [9,19]. Consumption of dietary grain is different in northern and southern regions of India. Northern India predominantly consumes wheat, whereas people in southern Indian states predominantly consume rice. A gradient is observed in the frequency of CeD among

healthy adults in India with highest being in North India, followed by North East and least in South India [20]. Chinese provinces also have different ratio for consumption of rice and wheat, overall wheat is the second staple food of the Chinese, after rice. Most of the studies on the association of GS and cerebellar ataxia have originated from USA and UK, so far there are no studies to find whether GA is prevalent in India among patients with cerebellar ataxia. Hence, the objectives of the study were to estimate the frequency of angliadin antibody and transglutaminase of GS in Indian ataxia cohort for the presence of GA. Probably, this is the first detail and largest study from Asia which tried to link a possible association between cerebellar ataxia and GS.

3. PATIENTS AND METHODS

This study is a prospective cross-sectional, conducted in a well-established Ataxia Clinic, Department of Neurology in collaboration with Department of Gastroenterology and Human Nutrition of All India Institute of Medical Sciences (AIIMS), New Delhi, India. We recruited 192 patients with progressive cerebellar ataxia. They were tested using standard laboratories protocols for the ataxia-causing known genes in India SCA-1, 2, 3, 12 and Friedreich's Ataxia (FRDA). Patients were divided into two groups. The Familial Group (Grp1) consisted of patients with a family history of ataxia (autosomal recessive or dominant) and/or established genetic testing for one of the SCAs and Friedreich's Ataxia (FRDA). The Sporadic Idiopathic (Grp2) consisted of sporadic idiopathic patients with history of ataxia, absence of family history and unconfirmed genetic diagnosis of SCAs and FRDA. This group also includes the two probable Multiple System Atrophy-Cerebellar (MSA-C) patients. Patients having cerebellar ataxia due to alcohol abuse, prolonged use of the anticonvulsant medication(s), multiple sclerosis, stroke, vitamin E deficiency and paraneoplastic cerebellar degeneration were excluded from the study.

A questionnaire-based evaluation was done for gastrointestinal symptoms. CT/MRI brain images were reviewed for evidence of cerebellar atrophy. The study was approved by the Institutional Ethics committee and written informed consent was documented from each participant.

3.1. Screening of patients for gluten sensitivity

Four millilitres of blood were drawn from each participant for serological assays. For measuring concentration following commercially available ELISA kits were used: IgA anti tTG ab by QUANTA Lite® h-tTG IgA kits. The AGA IgA and AGA IgG by QUANTA Lite®Gliadin IgA II and QUANTA Lite® Gliadin IgG II kits respectively and TG6 by kits procured from ZEDIRA GmbH, Darmstadt, Germany. The ELISA was done in duplicate in all the sera samples as per manufacturer's instructions. Positive and negative controls were used. For the study serum titre of >4 U/mL, >20 U/mL and >41 U/mL were considered positives for IgA anti-TG2 Ab, AGA (IgA and IgG) and anti-TG6 Ab, respectively.

3.2. Assessment of intestinal mucosal biopsies

Individuals screened positive for either anti-tTG-Ab and/or AGA were invited for upper gastrointestinal endoscopic examination and duodenal biopsies. The examination was done using an endoscope and four pieces of mucosal biopsies were collected from the second/third part of the duodenum. Each duodenal biopsy specimen was examined at least 20 steps-sections and was evaluated in terms of architectural changes, crypt depth, villous height, Intra-Epithelial Lymphocytes (IELs), lamina propria inflammation and presence or absence of parasites. Modified Marsh grading system of Oberhuber was used for grading of the mucosal changes [21]. Diagnosis of

CD was made on the basis of a combination of a positive anti-tTG Ab and biopsies showing villous abnormalities of Marsh grade 2 or more.

3.3. Statistical analysis

Statistical analysis was performed using Software Program Stata System (SPSS), version 17.0 (IL, Chicago). Frequencies between two variables were compared by Fisher's exact test and mean and standard deviations were compared by Student's T-test. Correlation among variables such as age at disease onset and pathogenic repeats of CAG were estimated using regression analysis. The level of statistical significance was set at p values < 0.05.

4. RESULTS

4.1. Demographic characteristics of patients

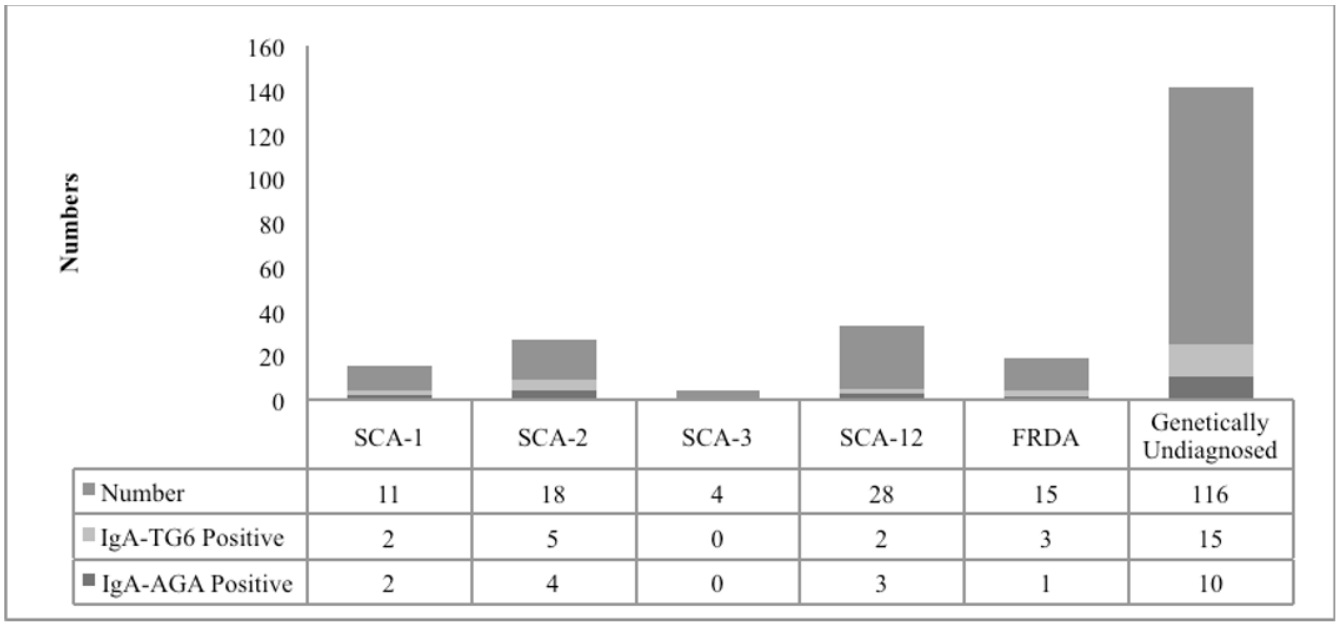
Clinical demography of all the cases recruited is shown in Table 1. In the total 192 (146 male and 46 female) patients with progressive cerebellar ataxia; the mean age was 42.4 ± 16.4 years and the age of onset of symptoms in them was 36.8 ± 15.9 years. Grp1 had 110 (57.2%) patients, rest 82 (42.7%) were in Grp2. Presenting symptoms were gait ataxia, hand tremor and/or the slurring of speech. Gait ataxia was evident in all the patients recruited. Cerebellar atrophy was evident in 53% patients from whom brain imaging could be reviewed. Two patients had hot cross bun sign and qualified for probable MSA-C. Four subjects showed cerebral atrophy and three patients had cerebral atrophy with ischemic changes.

Table 1: General demography of the patients.

Total no of cases:	192
Male/Female:	146/46
Age (mean ± SD); range (yrs):	42.44 ± 16.24; 9-87
Age at onset (mean ± SD); range (yrs):	36.88 ±15.93; 3-67
Disease Duration (mean ± SD):	6.05 ± 6.21
Familial cases/sporadic idiopathic cases:	110/82
Genetically Diagnosed/ Genetically Undiagnosed:	76/116

4.2. Frequency estimates of SCAs and FRDA

Confirmed genetic mutation diagnosis of SCA-1, 2, 3, 12 and FRDA was established in 76 (40%) cases, whereas 116 (60%) cases remained genetically undiagnosed. The percentage of sporadic ataxia with positive genetic test was 21.9% (23/105). The frequency of confirmed genetic mutation in our cohort showed that SCA-12 was the most common (28; 14.6%) type of SCAs followed by SCA-2 (18; 9.4%), FRDA (15; 7.8%), SCA-1 (11; 5.3%) and SCA-3 (4; 2.1%) [Figure1]. A significant inverse correlation between disease-causing CAG repeats and age at onset was observed for SCA-1 (P=0.033, r2=0.719), SCA-2 (P=0.038, r2=0.273) and SCA-12 (P=0.000, r2=0.488). This correlation could not be measured for SCA-3 due to the low number of SCA-3 positives in our cohort.



	SCA-1	SCA-2	SCA-3	SCA-12	FRDA	Genetically Undiagnosed
Number	11	18	4	28	15	116
IgA-TG6 Positive	2	5	0	2	3	15
IgA-AGA Positive	2	4	0	3	1	10

SCA- Spinocerebellar Ataxia; FRDA- Friedreich's Ataxia; 76 patients were genetically diagnosed as (SCA-1, SCA- 2, SCA-3, SCA12 and FRDA); 116 patients remained genetically undiagnosed.

4.3. Results of screening of patients for GS

Out of 192 subjects included, the results of serological tests were concluded as follow; AGA IgA in 192, antigliadin AGA IgG in 87, anti-TG2 in 141 and anti-TG6 in 186. In few of the cases some serological tests were compromised due to difficulty in drawing blood sample from the patients and limited availability of the assay for these antibodies at the start of this study. The prevalence of GA after testing positive for selected antibodies was 21.8% (42/192) among all ataxia patients. 21.8% (24/110) in Grp1 and 21.9% (18/82) in Grp2, 25% (19/76) in genetically established cases and 20.6% (23/116) were in genetically unexplained cases. The frequency of a positive antibodies was highest in SCA 2 patients; 33.3% (6/18).

4.4. Anti-Gliadin Antibodies (AGA)

The total number of patients with circulating IgA AGA was in 10.4% cases (20/192), IgG AGA was in only 2.2% cases (2/87). These two patients with positive family history also had higher serum level of IgA AGA. In Grp1 frequency of both circulating antibodies was 11.8% (13/110) and in Grp2 it was 8.5% (7/82). The mean concentration of IgA AGA was 28.9 U/L ± 18.7 U/L, but there was no significant difference in the serum concentration levels between sporadic and familial cases.

4.5. Anti-TG2 autoantibodies

Only one out of 141 patients (0.7%), in whom anti-TG2 could be done, tested positive for anti-TG2. This index patient with positive family history harbours SCA-2 genetic mutation and was also positive for circulating both AGA (IgG and IgA). While the patient also had diarrhoea, abdominal pain, weight loss, and high likelihood of having CD, he however refused further testing including endoscopic examination for the presence of enteropathy [Table 2]. He also refused empirical trial of a gluten-free diet.

Table 2: Description of patients with cerebellar ataxia having evidence of gluten sensitivity.

ID	G	anti-TG2	IgA-AGA	IgG-AGA	IgA-TG6	FH	GD (CAG repeats-NA/EA)	AOS	DD	CA	Presenting symptom
AG6	M	-	-	-	+	-	UNK	30	10	+	GA, DYS, HT
AG7	F	-	+	-	-	+	SCA-2 (22/41)	40	1		GA, HT
AG15	M	-	-	-	+	-	FRDA			-	DYS, HT
AG16	F	-	-	-	+	+	SCA-12 (15/52)	48	12		GA, DYS
AG18	F	-	-	-	+	+	UNK	28	17		DYS, HT
AG19	F	-	-	-	+	+	UNK	20	14	-	GA, HT
AG26	M	-	-	-	+	-	FRDA	17	9	+	GA, DYS
AG35	M	-	-	-	+	+	SCA-2 (22/49)	35	5	+	GA, HT
AG36	F	-	+	-	-	-	SCA-2 (22/44)	24	4	+	GA
AG38	M	-	-	-	+	-	FRDA	19	5	-	GA
AG41	M	-	+	-	-	-	SCA2 (24/40)	39	6	+	HT
AG47	M	-	+	-	-	+	UNK	48	2	+	GA
AG48	M	-	+	-	-	+	FRDA			-	HT
AG51	M	-	-	-	+	-	UNK	52	4	+	GA, DYS, HT
AG54	M	+	+	+	-	+	SCA-2 (22/45)				BD
AG63	M	-	+	-	-	-	UNK	48	7	-	HT
AG69	M	-	+	-	-	+	SCA-12 (24/63)	41	2	+	GA
AG71	M	-	+	-	-	+	SCA-12 (12/59)	49	5	+	GA, HD
AG85	M	-	+	-	-	-	UNK	13	8	+	HT
AG86	M	-	+	-	-	-	UNK				DYS
AG89	F	-	+	-	-	+	SCA-12 (10/53)	49	5		GA, DYS, HT
AG91	M	-	-		+	-	UNK	62	11	-	HT, GA
AG96	M	-	-		+	-	UNK	15	3	+	GA

AG97	M	-	-		+	+	SCA-1 (26/ 54)	28	6	+	DYS, GA
AG104	M	-	-		+	+	SCA12 (15/ 59)	29	18		HT, GA, DYS
AG111	M	-	-		+	-	UNK			+	GA
AG113	F	-	-		+	-	UNK	52	3	+	HT
AG114	F	-	+	-	-	+	SCA-1 (29/ 50)			+	HT, DYS
AG117	M	-	-		+	-	UNK	58	2	-	GA
AG121	M	-	-		+	-	UNK	25	12	-	GA
AG127	M	-	+	-	-	-	UNK	46	2	+	GA, HT
AG148	M	-	+	-	-	-	UNK	58	4	+	GA
AG152	F		+	-	-	+	SCA-1 (29/ 44)	56	5		HT, GA, DYS
AG153	F		+	+	-	+	UNK	54	8		GA, DYS, HT
AG163	F		-		+	+	SCA-2 (21/ 37)	48	7	-	HT, DYS
AG165	M		-		+	-	UNK	63	2	-	GA, DYS, HT
AG173	M		-		+	-	UNK	54	11		HT
AG175	M		+		+	-	UNK	65	22	-	GA
AG183	M		-		+	-	UNK	52	20	-	GA, DYS
AG192	M		-		+	+	SCA-2	30	15	+	HT, GA
AG142	F	-	+		-	+	UNK	35	10	+	HT
AG200	M		+			-	UNK	47	1	+	HD

GA- Gait Ataxia; DYS- Dysarthria; HT- Hand Tremor; BD- Body Tremor; HD- Head Tremor; G-Gender; M-Male; F-Female; FH-Family History; GD-Genetic Diagnosis; NA-Normal allele; EA-Expanded allele; UNK-Unknown; AOS- Age of Onset; DD- Disease Duration; CA-Cerebellar Atrophy

4.6. Anti-TG6 autoantibodies

The availability of TG6 antibody testing was limited as TG6 autoantibodies were not discovered until 2006, and their importance in neurological manifestations of GRD was not established until 2008 (Ref). TG6 was found circulating in 12.3% (23/186) patients. Anti-TG6 was found circulating in 10% (11/110) of Grp1 cases and 14.6% (12/82) of Grp2 cases. The titre of IgA anti-TG6 Ab was 56.4 U/L ± 19.4 U/L but there was no significant difference in the serum concentration levels between sporadic and familial cases.

Description of all serological assays (frequency, mean concentration, age at onset, disease duration, frequencies among sporadic, familial cases, genetically diagnosed and genetically undiagnosed) is shown in Table 2. While

complete demographic features, disease characteristics and test results of all 42 serologically positive patients are shown in Table 3.

Table 3: Description of patients with cerebellar ataxia having IgA AGA and IgA TG6 positivity.

	IgA-AGA	IgA-TG6
Total number (%)	20/192 (10.4)	23/186 (12.3)
Titer of antibody; Mean ± SD	28.9 ± 18.7	56.4 ± 19.4
Age at onset; Mean ± SD	42.5 ± 10.2	31.7 ± 11.6
Disease Duration; Mean ± SD	4.0 ± 1.8	9.6 ± 4.8
Sporadic cerebellar ataxia; N (%)	7/82 (8.0)	12/82 (13.8)
Familial cerebellar ataxia; N (%)	13/110 (12.3)	11/110 (10.4)
Patients with genetic mutation; N (%)	10/76 (13.1)	9/76 (13.1)
Patients having no genetic mutation; N (%)	10/116 (8.6)	14/116 (12.1)

Due to lack of sufficient number of patients having a positive anti-TG2 and IgG antigliadin, we did not perform the statistical analysis; N-Numbers; SD- Standard Deviation, Cut off values of AGA <20; anti TG 6: <41

Table 4a: Prevalence and comparison of IgA antigliadin in patients with sporadic ataxia and familial ataxia in different ethnicity and studies.

Group; Population	Sporadic cases N (%)	Familial cases N (%)	Overall N (%)
Numbers from the Sheield ataxia clinic (MH)	101/215 (47)	15/116 (13)	116/331 (35)
Hadjivassiliou et al, 2003 [21]; British	59/143 (41)	8/59 (14)	67/202 (33)
Pellecchia et al 1999; Italy	3/24 (13)	0/23 (0)	03/65 (6.5)
Bürk et al, 2001; Germany	12/104 (12)	-------	12/104 (11.5)
Bushara et al 2001; USA	7/26 (27)	9/24 (38)	16/40 (40)
Abele et al 2002; Germany	13/98 (13)	1/15 (6)	14/113 (12)
Luostarinen et al 2001; Finland	4/24 (17)	-------	04/24 (16.6)
Abele et al, 2003; Germany	6/32 (19)	-------	6/32 (18.7)
Ihara et al, 2005 [22]; Japanese	5/14 (36)	1/27 (4)	06/41 (14.6)
Anheim et al; 2006; French	12/33 (36)	-------	12/33 (36)
Guan W J et al; 2013 [21] Chinese	22/100 (22)	3/25 (12)	25/125 (20)
Present study; Indian	7/82 (8.0)	13/110 (12.3)	20/192

Table 4b: Prevalence and comparison of Transgluatminase (IgA-TG6) in patients of different origin with sporadic ataxia and familial ataxia cases.

Group; Population	Sporadic cases N (%)	Familial cases N (%)	Overall N (%)
Hadjivassiliou et al, 2013;			
British	21/65 (32)	8/59 (14)	29/124 (23)
Guan W J et al; 2013 Chinese	35/100 (35)	6/25 (24)	41/125 (33)
Present study; Indian	12/82 (13.8)	11/110 (10.4)	23/186 (12.3)

Due to infrequency of anti-TG2 in our study, statistical analysis was not performed

4.7. Mucosal biopsies for villous abnormalities

While we invited all the 42 patients detected positive for anti-tTG Ab, IgG-AGA or IgA AGA, only 10 agreed to undergo endoscopic examination and duodenal biopsies. None of these 10 biopsies showed any evidence of enteropathy.

5. DISCUSSION AND CONCLUSION

The principal findings of our study are:

- Mutations in SCAs and FRDA accounts for 39.5% among all ataxia patients included and SCA type 12 being the most common.
- 21.9% are genetically positive from sporadic cases.
- Antigliadin antibodies and transglutaminase autoantibodies are found in the serum of both groups of patients in the same frequency.
- No evidence of enteropathy in GA patients.

GA is more common in the USA and Europe than in Asia, affecting males and females aged over 50 years [15]. The data available from China and Japan has limited sample size and have concluded GA and CeD uncommon in their ethnicities. While the prevalence of GA estimated through circulating IgA AGA in patients with cerebellar ataxia, has been reported to vary from 6.5% to 40% in all forms of ataxia, 12% to 47% in sporadic idiopathic ataxia, 4% to 38% in familial group of patients and 2% to 12% among healthy controls Table 4a [6, 21], but in the present study, it was 10.4% in all ataxia cohort, 8.5% in sporadic idiopathic and 11.8% in the familial group. We found only two patients of antigliadin IgG AGA, this infrequency in our study is in concordance with other studies suggesting lower specificity and less useful of this marker for diagnosis of CeD or gluten ataxia [22]. Although the exact role of TG6 in GA remains unclear, growing evidence indicates that antibodies against TG6 could serve as a biomarker of GS in patients with neurological involvement in addition to conventional antigliadin IgA/IgG and anti-TG2 Ab [23]. It is primarily expressed in brain and its prevalence

is estimated 62% in ataxia cases. The TG6 antibodies are sensitive and specific in GA, but it may be a secondary effect of neuronal damage rather than being pathogenic directly [23]. It is also postulated that anti-TG6 IgA antibodies, seems to more useful for diagnosing gluten ataxia not anti-TG6 IgG antibodies [20]. Not many studies are available estimating the prevalence of anti-TG6 IgA antibodies in GA patients. Data from two different cohort and ethnic background from UK and China respectively estimated prevalence of TG6 32% and 35% for sporadic cases, 14% and 24% for familial cases and 4% and 12% for healthy controls Table 4b [21,23]. In present study it was 14.6% for sporadic idiopathic and 10% for familial group Table 4b. The frequencies of antigliadin IgA, IgG and anti-TG6 in ataxia patients from diverse studies were much higher than what we observed in present study. The data on the prevalence of these antibodies in the healthy volunteers is not present from India. Since the epidemiological data of GS from general Indian population is still evolving, we were not able to compare GS among ataxia cases and in general Indian population. Approximately half of the patients with cerebellar ataxia having SCA-2 mutation had one or more serological test positive for GS suggesting a probable role of GS as a consequence or as disease modifier in SCA-2 patients.

Proportions of cerebellar ataxia patients with evidence of GS have been described to have enteropathy and thus qualify for the diagnosis of the CeD. Hadjivassiliou et al. [16] in 2003 reported that 24% of their ataxia patients with GS had evidence of enteropathy [16]. Several studies have also revealed the presence of CeD among ataxia patients at varying frequencies [6]. While our study had only 10 patients with evidence of GS who underwent duodenal biopsies and none were found to have enteropathy. More efforts are needed to link CeD and ataxia not just by using an invasive procedure of the endoscopic examination but also by identifying various clinical relevance biomarkers using modern day tools including transcriptomics, proteomics, and metabolomics [24]. We could not see the effect of gluten-free diet on the cerebellar symptoms and the titre of the gluten sensitive antibodies including SCA2 patient who has elevated level of anti TG2 and both AGA assays. This remained one of the limitations of the study. Most of the sero-positives in our study did not have GIS, which was in agreement from previous work suggesting that most patients who present with neurological manifestations of GS do not have gastrointestinal symptoms [22]. The high frequency variations of serological markers for GS and CeD with cerebellar ataxia amongst different populations could be due to the geographical differences in the prevalence of CeD, disproportionate ingestion of the gluten in their diet, presence of mutations in linkage disequilibrium with the causative mutations, referral bias, variability in the assays used, selection of patients, variable study size, and absence of controls. These results suggest an unknown correlation between GS and cerebellar ataxia.

The present study is the first study from India to describe an association between cerebellar ataxia and gluten sensitivity. The strength of the present study includes involvement of well-characterized patients from a well-established Indian Ataxia Clinic. There are certain limitations too. First and foremost, evaluation for enteropathy could be done only a few subjects showing evidence of gluten sensitivity. Second, we did not see the effect of gluten-free diet on the cerebellar symptoms and the titre of the gluten sensitive antibodies.

Observations from this study and frequency variations among different ethnicities raise the question whether circulating antigliadin and transglutaminase in serum are the consequence or the pathogenic cause of the cerebellar degeneration in the brain. In conclusion, an ataxia syndrome related to GS may occur in a subgroup of patients and those antigliadin antibodies and anti-TG6 autoantibodies may be a marker for identifying gluten sensitive and diagnosing sporadic idiopathic ataxia. However, to access the reliability and clinical relevance of these markers there is a need of a biomarker replication of our findings, more evaluation of enteropathy, and

strict monitoring of the patients those are on a gluten-free diet. In the future studies we expect more advance technologies with more reliable biomarkers to relate them to ataxia for the confirm diagnosis, better treatment and monitoring.

6. ACKNOWLEDGMENT

We acknowledge the support of patients for their kind participation in the study. Also, we acknowledge the support of blood collection and laboratory staff of Department of Gastroenterology and Human Nutrition and Department of Neurology, All India Institute of Medical Sciences, New Delhi, India.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

8. REFERENCES

1. Pennisi M, Bramanti A, Cantone M, Pennisi G, Bella R, Lanza G. Neurophysiology of the "Celiac Brain": Disentangling Gut-Brain Connections. Front Neurosci. 2017;11:498.
2. Julian T, Hadjivassiliou M, Zis P. Gluten sensitivity and epilepsy: a systematic review. J Neurol. 2019;266(7):1557-65.
3. Mustalahti K, Catassi C, Reunanen A, Fabiani E, Heier M, McMillan S, et al. The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. Ann Med. 2010;42(8):587-95.
4. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterology. 2001;120(3):636-51.
5. Sapone A, Bai JC, Ciacci C, Dolinsek J, Green PH, Hadjivassiliou M, et al. Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Med. 2012;10:13.
6. Hadjivassiliou M, Sanders DS, Grünewald RA, Woodroofe N, Boscolo S, Aeschlimann D. Gluten sensitivity: from gut to brain. Lancet Neurol. 2010;9(3):318-30.
7. Fleckenstein B, Qiao SW, Larsen MR, Jung G, Roepstorff P, Sollid LM. Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. J Biol Chem. 2004;279(17):17607-16.
8. Guo YC, Lin JJ, Liao YC, Tsai PC, Lee YC, Soong BW. Spinocerebellar ataxia 35: novel mutations in TGM6 with clinical and genetic characterization. Neurology. 2014;83(17):1554-61.
9. Yuan J, Zhou C, Gao J, Li J, Yu F, Lu J, et al. Prevalence of Celiac Disease Autoimmunity Among Adolescents and Young Adults in China. Clin Gastroenterol Hepatol. 2017;15(10):1572-9.e1.
10. Hadjivassiliou M, Rao DG, Grìnewald RA, Aeschlimann DP, Sarrigiannis PG, Hoggard N, et al. Neurological Dysfunction in Coeliac Disease and Non-Coeliac Gluten Sensitivity. Am J Gastroenterol. 2016;111(4):561-7.
11. Hadjivassiliou M, Sanders DD, Aeschlimann DP. Gluten-related disorders: gluten ataxia. Dig Dis. 2015;33(2):264-8.

12. Ghezzi A, Filippi M, Falini A, Zaffaroni M. Cerebral involvement in celiac disease: a serial MRI study in a patient with brainstem and cerebellar symptoms. Neurology. 1997;49(5):1447-50.
13. Gordon N. Vitamin E deficiency and illness in childhood. Dev Med Child Neurol. 1987;29(4):546-9.
14. Finelli PF, McEntee WJ, Ambler M, Kestenbaum D. Adult celiac disease presenting as cerebellar syndrome. Neurology. 1980;30(3):245-9.
15. Ford RPK. The gluten syndrome: a neurological disease. Med Hypotheses. 2009;73(3):438-40.
16. Hadjivassiliou M, Grünewald R, Sharrack B, Sanders D, Lobo A, Williamson C, et al. Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. Brain. 2003;126(Pt 3):685-91.
17. Guo YC, Lin JJ, Liao YC, Tsai PC, Lee YC, Soong BW. Spinocerebellar ataxia 35: novel mutations in TGM6 with clinical and genetic characterization. Neurology. 2014;83(17):1554-61.
18. Hadjivassiliou M, Aeschlimann P, Strigun A, Sanders DS, Woodroofe N, Aeschlimann D. Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase. Ann Neurol. 2008;64(3):332-43.
19. Ramakrishna BS, Makharia GK, Chetri K, Dutta S, Mathur P, Ahuja V, et al. Prevalence of Adult Celiac Disease in India: Regional Variations and Associations. Am J Gastroenterol. 2016;111(1):115-23.
20. Oberhuber G. Histopathology of celiac disease. Biomed Pharmacother. 2000;54(7):368-72.
21. Guan WJ, Liu XJ, Tang BS, Liu YT, Zhou Y, Jiang H, et al. Gluten ataxia of sporadic and hereditary cerebellar ataxia in patients from mainland China. Neurol India. 2013;61(3):226-30.
22. Ihara M, Makino F, Sawada H, Mezaki T, Mizutani K, Nakase H, et al. Gluten sensitivity in Japanese patients with adult-onset cerebellar ataxia. Intern Med. 2006;45(3):135-40.
23. Gadoth A, Nefussy B, Bleiberg M, Klein T, Artman I, Drory VE. Transglutaminase 6 Antibodies in the Serum of Patients With Amyotrophic Lateral Sclerosis. JAMA Neurol. 2015;72(6):676-81.
24. Singh A, Pramanik A, Acharya P, Makharia GK. Non-Invasive Biomarkers for Celiac Disease. J Clin Med. 2019;8(6):885.