Comparison of Immunoblotting and ELISA for Detection of Anti-Ganglioside Antibodies in Children with Guillain-Barre Syndrome

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ABSTRACT

Background: Anti-ganglioside antibody assays are widely used for diagnosis of autoimmune peripheral neuropathies. **Objective:** This study aimed to determine serum levels of anti-ganglioside antibodies in children with Guillain-Barre syndrome by immunoblotting technique and compare the results with those obtained by ELISA method. **Method:** In this investigation, 50 children with Guillain-Barre syndrome (GBS) who were admitted from July 2006 to July 2008, to Tabriz Children's hospital in the northwest of Iran were studied. 30 children admitted for various other reasons than GBS were randomly selected as a control group. The levels of anti-ganglioside antibodies in serum were measured by ELISA and immunoblotting methods using commercial kits. **Results:** Anti-ganglioside antibodies (IgG) were detected in 16 (32%) GBS patients and in 1 (3.3%) control using ELISA assay. However, by employing immunoblotting technique, antibodies against seven gangliosides were found positive in 28 (56%) GBS patients and none in the control group. The sensitivities of immunoblotting and ELISA methods were 56% and 32% and their specificities were 100% and 97%, respectively ($p<0.001$). **Conclusion:** According to the clinical criteria of GBS, the specificity and sensitivity of immunoblotting was better than those of ELISA. It is important to notice that the immunoblotting method is able to measure the seven types of antibodies (GM1, GM2, GM3, GD1a, GD1b, GT1b, and GQ1b) simultaneously and it is an easy, routine method with a lower cost.

Keywords: Antibody, ELISA, Ganglioside, Guillain-Barre, Immunoblotting

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INTRODUCTION

The rapidly progressive, paralytic syndrome associated with loss of tendon reflexes which was reported first by G. Guillain, JA. Barre, and A. Strohl in 1916 has been named Guillain–Barre´ Syndrome (GBS) (1). Guillain-Barre syndrome is an acute demyelinating paralytic disease of the peripheral nervous system (2) and is a heterogeneous disorder with different clinical, electrophysiological, and pathological subtypes including acute inflammatory demyelinating polyneuropathy (AIPD), acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAM), and Miller Fisher syndrome (MFS) (3,4). Gangliosides are components of the plasma membrane of Schwann cells, the myelin, and the axolemma. They represent membrane-sialylated glycolipids that bear autoantigenic epitopes confined to the carbohydrate moiety (5). Although carbohydrates are mainly associated with energy metabolism, evidence has accumulated since 1950 indicating distinct roles for such complex molecules. Defined sequences of carbohydrates have been correlated with a variety of cell processes. It is now generally accepted that carbohydrates, similar to proteins and nucleic acids, carry structural information and function as "address molecules" (6). They can bind to a protein moiety or to lipid moieties forming glycoproteins and glycolipids or lipopolysaccharides (7). Several studies have shown that infection with *Campylobacter jejuni* has been associated with AIDP and AMAN subtypes (8). Because anti-ganglioside antibodies are found in many GBS patients and the lipopolysaccharides of some *C. jejuni* strains isolated from GBS patients contain ganglioside-like epitopes, molecular mimicry between epitopes on the surface of *C. jejuni* and the neural targets has been proposed as a possible mechanism for *C. jejuni*-associated GBS (9,10). Increased anti-ganglioside antibodies have been found in some patients with GBS using ELISA. The frequency of anti-GM1 antibodies in different series of GBS patients varies from less than 2% to around 30% (11).

Because of limitations in preparing kits and their high cost, researchers often have analyzed a limited number of anti-ganglioside antibodies including anti-GM1, anti-GD1 and anti-GQ1. Recently, there is a tremendous increase in the use of anti-ganglioside antibody assays, as both diagnostic and research tools for studying autoimmune peripheral neuropathies have developed extensively (12). Most of the screening methods used in this context were ELISA (13). It seems that evaluating the anti-ganglioside antibodies for diagnosing above mentioned diseases is important and useful. In a study performed in the year 2000, these antibodies were tested using rapid latex agglutination method and were compared with ELISA (14). In 2007, measuring of eleven types of anti-ganglioside antibodies was done using immunoblotting method in adult autoimmune neuropathic patients (15). The rapid latex agglutination is simple but of low sensitivity. Thin layer chromatography with subsequent immunodetection is proposed to be the "golden standard".

The anti-ganglioside IgG type antibody panels are now commercially available and immunoblotting is employed by reference laboratories as a way for the clinicians to identify a possible autoimmune etiology in neuropathies otherwise considered idiopathic. Testing these panels by immunoblotting, which is simpler and less expensive than ELISA, one can assay several different autoantibodies to peripheral nerve antigens. Therefore, the aim of the present study was to measure the anti-ganglioside antibodies using commercially available immunoblotting and ELISA assay methods, and to evaluate the advantages and disadvantages of these methods for future screening.

MATERIALS AND METHODS

Sampling. In this study, 50 children with Guillain-Barre syndrome (GBS) who were successively admitted from July 2006 to July 2008 to Tabriz Children's Hospital in the northwest of Iran were studied. Furthermore, 30 children admitted for various reasons other than GBS disease were selected randomly as a control group. The average age of the patients was 5.3 ± 3.8 and of the control subjects was 5.4 ± 3.4. All subjects were examined by an expert child neurologist. The diagnosis of GBS was ascertained based on the criteria defined by Asbury and Cornblath (16). After obtaining an informed consent from the parents, the clinical data were collected. For each patient, data was collected with regard to the age, sex, date of onset of the disease, preceding illnesses, clinical features, results of cerebrospinal fluid analysis, electrophysiologic findings, course of the disease during hospitalization and the outcome. Serum samples were obtained 1-7 days after onset of GBS and before treatment, and then stored at -80°C until tested. This study was approved by the Ethics committee of Tabriz University of Medical Sciences.

ELISA Test. The quantity of antibodies (Anti GM1, GQ1, GD1) was measured by ELISA using a commercial kit (Buhlman, Switzerland) according to manufacturer's instructions. In this method, serum was diluted 1:50 with the sample buffer and measured using 800, 2400, 6000, 15000 BTU (Buhlman titer unit) standards. Cut off for Anti GM1 was considered ≥1200 BTU and for Anti GQ1 and GD1 equal to 2400 BTU, respectively, All the cut off values were greater than the mean +3SD for each measured antibody.

Immunoblotting Method. The profile of antibodies against GD1b, GT1b, GQ1b, GM1, GM2, GM3, and GD1a gangliosides were tested semiquantitatively using a commercial kit (Euroimmun, Germany). In this method, serum was diluted 1:50 with the sample buffer and tested according to manufacturer's instructions. The immunoblot strips staining were scanned after (Figure 1) and analyzed with a special Euroimmun software. Values ≥ +1 were considered positive.

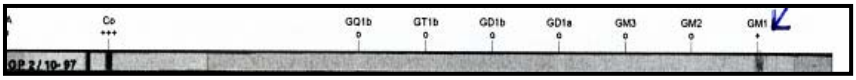
Statistical Analysis. Differences between proportions were statistically analysed by Chi-square and Fisher's exact test. All other numerical or quantitative comparisons were performed using Student's unpaired t-test or Mann-Whitney U test. All values were two tailed and were considered statistically significant at $p \leq 0.05$.

RESULTS

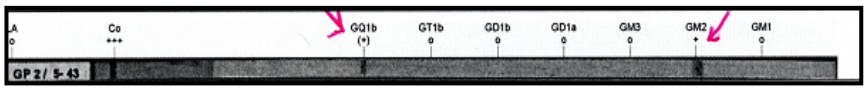
Using ELISA assay, anti-ganglioside antibody (IgG) levels were estimated in 16 (32%) patients with GBS and in 1 (33%) subject in the control group. Among 16 GBS patients, anti-ganglioside antibodies against GD1, GM1, and GQ1 were detected in 4 (8%), 11 (22%), and 6 (12%) patients, respectively (Table 1).

(a)

Antigen	Intensity	Class
GM1	20	+
GM2	1	o
GM3	1	o
GD1a	1	o
GD1b	1	o
GT1b	1	o
GQ1b	2	o
Control	152	+++



(b)



Antigen	Intensity	Class
GM1	3	o
GM2	14	+
GM3	4	o
GD1a	2	o
GD1b	2	o
GT1b	4	o
GQ1b	7	(+)
Control	125	+++

Figure 1. Representative figure illustrating the detection of (a) GM1, (b) GM2 and GQ1b by immunoblotting

Furthermore, immunoblotting assay was used for the detection of anti-ganglioside antibodies against GM1, GM2, GM3, GD1a, GD1b, GT1b, and GQ1b in order to compare with the ELISA findings. GD1a and GD1b were combined together as GD1a+b, and GM1, GM2 and GM3 were all combined and shown as GM1,2,3. According to immunoblotting results, among the 50 patients with GBS, anti-ganglioside antibodies were as follows: GD1a+b, 5 (10%); GM1, 2, 3, 23 (46%) and GQ1, 9 (18%).

Table 1. Anti-ganglioside IgG frequencies in GBS patients and controls.

	ELISA			Immunobloting			ELISA Total	Immunobloting Total
	GD1	GM_1	GQ_1	GD_{1a+b}	GQ_W	$GM_{1,2,3}$		
Patient	4(8%)*	11(22%)	6(12%)	5(10%)	23(46%)	9(18%)	16(32%)	28(56%)
Control	0	0	0	0	0	0	1(3.3%)	0

*Data shown as counts (percentages).

Using Immunoblotting technique, anti-ganglioside antibodies (at least one type) were found positive in 28 (56%) patients with GBS; while they were negative in the control group (Table 1). However, there was one False-positive result in the control subjects using ELISA method.

Specificity and Sensitivity. In the present study, the clinical and paraclinical criteria were considered as golden standard approach for diagnosis, and the specificity and sensitivity of the two methods were determined according those criteria. In this regard, the specificity was 100% in immunoblotting and 97% in the ELISA. Also, the sensitivity was 56% in Immunoblotting and 32% in ELISA ($p<0.001$). Detection of individual antibodies against only GD1, GM1 or GQ1 by ELISA and against GD1a+b and GQ1 by immunoblotting did not reveal any significant changes in the specificity and sensitivity. However, detection of antibodies against GM1,2,3 by immunoblotting showed a 46% specificity and a 97% sensitivity ($p<0.001$) (Table 2).

Table 2. Sensitivity and specificity of two assay methods (ELISA and Immunoblotting) in measuring IgG anti-ganglioside antibodies according to clinical criteria

	ELISA IgG	Immunoblot IgG	Immunoblot (IgG+IgM)
Sensitivity (%)	32	56	98
Specificity (%)	97	100	60

DISCUSSION

In this study, we measured the anti-ganglioside antibodies using two assay methods including immunoblotting and ELISA in 50 patients with acute and subacute GBS and 30 control subjects. Anti-ganglioside antibody positivity in GBS patients was 28 (56%) by immunoblotting and 16 (32%) by ELISA method. One positive subject was found among controls by ELISA. We also evaluated the specificity and the sensitivity of these

two methods according to clinical and paraclinical indices. Accordingly, it was demonstrated that the immunoblotting technique had a specificity of 100% and a sensitivity of 56%.

he key point in measuring anti-ganglioside antibodies using immunoblotting is that one can easily measure seven types of antibodies (GM1, GM2, GM3, GD1a, GD1b, GQ1b, and GT1b) simultaneously with a lesser cost. However, in ELISA method we can not measure all anti-ganglioside antibodies easily and simultaneously because of the use of specific kits for each individual antibody. Therefore it is more time consuming and impossible to do routine daily tests.

Lipopolysaccharides of *Campylobacter jejuni* (one of the causes of the disease) may lead to the production of IgG and IgM against gangliosides in rabbits (17). Several studies have confirmed that IgG and IgM antibodies are produced in GBS, AIDP, AMAN, MFS and AMSAM diseases in humans (2,3,4,18). Many investigations were also performed for assaying anti-ganglioside antibodies in GBS patients and other forms of neuropathies, and have reported IgG and IgM the frequencies from 2% to 30% (19,20,21). In a study performed on GBS patients, 18% of the patients showed IgG antibody, 9% IgM and 45% both IgG and IgM (22). In another study done in china (23), the existence of IgG anti-ganglioside antibody was reported in 54% of the patients in agreement with our immunoblot data. Antibody against GM1 is seen more than against other gangliosides in GBS patients. In a study using immunoblotting method for the multiparametric detection of anti-ganglioside autoantibodies, the degree of detection for at least one antibody (IgG or IgM) was 97.4% in patients with autoimmune peripheral neuropathies and 12.2% in control subjects (15). Using this method, a specificity of 93.3%-100% has been reported in their study. It is suggested that the immunoblotting technique is an easy, rapid and inexpensive method and of clinical importance in detecting the acute and chronic autoimmune peripheral neuropathies and GBS (24,25).

Detecting a certain type of antibody depends on the stage of the disease (acute, subacute, or chronic), because when the patients are in the acute or subacute stages, all of their antibodies will be of IgM type. Therefore, detecting only IgG antibodies by this assay method results in diminished sensitivity and specificity and perhaps, the result of our study in which the sensitivities of ELISA and immunoblotting were only 32% and 56% respectively, was influenced the stage of the disease. In our study, however, there was no false positive result in IgG type anti-ganglioside antibodies measured by immunoblotting, whereas a false positive case (3.3%) was found by ELISA method of in control subjects. Our findings demonstrated fairly good specificies and sensitivities for both methods.

As there is not enough research performed on this regard, it is suggested to evaluate both IgG and IgM anti-ganglioside antibodies in future studies. It seems that the immunobloting method which has the ability to identify both types of anti-ganglioside antibodies can be considered as a golden standard method.

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REFERENCES

1 Guillain G, Barre JA, Strohl A. Radiculoneuritis syndrome with hyperalbuminosis of cerebrospinal fluid without cellular reaction. Notes on clinical features and graphs of tendon reflexes.. Ann Med Interne. 1999; 150:24-32.

2 Arnason, BGW, Soliven, B. Acute inflammatory demyelinating polyradiculneuropathies. In: Dyck, P.J., Tohomas, P.K., Griffin, JW, Peripheral Neuropathy, 3rd ed. Philadelphia: W.B. Saunders Company Press; 1993; p. 1437-46.

3 Prineas JW. Pathology of the Guillain-Barre´ syndrome. Ann Neurol 1981; 9:6-19.

4 Walsh FS, Cronin M, Koblar S, Doherty P, Winer J, Leon A. Association between glycoconjugate antibodies and Campylobacter infection in patients with Guillain-Barre syndrome. J Neuroimmunol 1991; 34:43-51.

5 Sheikh, K.A., Deerinck T.J., Ellisman M.H. The distribution of ganglioside-like moieties in peripheral nerves. Brain 1999; 122:449-60.

6 Rademacher TW, Parekh RB, Dwek RA. Glycobiology. Ann. Rev. Biochem. 1988; 57:785-838.

7 Laine R.A. Invited commentary: a calculation of all possible oligosaccharide isomers both branched and linear yields 1.05 x 1012 structures for a reducing hexasaccharide: the Isomer Barrier to development of singlemethod saccharide sequencing or synthesis systems, Glycobiology 1994; 4:759-67

8 Ho TW, Mishu B, Li CY, Gao CY, Cornblath DR, Griffin JW, et al. Guillain-Barre´ syndrome in northern China: relationship to Campylobacter jejuni infection and anti-glycolipid antibodies. Brain 1995; 118:597-605.

9 Yuki, N, Taki, T, Takahashi M, Saito K, Yoshino H, Tai T, et al. Molecular mimicry between GQ1b ganglioside and lipopolysaccharides of Campylobacter jejuni isolated from patients with Fisher's syndrome. Ann. Neurol. 1994; 36,791-3.

10 Yuki N, Taki T, Inagaki F, Kasama T, Takahashi M, Saito K, et al. A bacterium lipopolysaccharide that elicits Guillain-Barre´ syndrome has a GM1 ganglioside-like structure. J Exp Med 1993;178:1771-5.

11 Walsh, FS, Cronin, M, Koblar, S, Doherty, P, Winer, J, Leon, A, et al. Association between glycoconjugate antibodies and *Campylobacter* infection in patients with Guillain–Barre´ syndrome. J. Neuroimmunol. 1991; 34,43-51.

12 Adams D, Kuntzer T ,Burger D ,Chofflon M, Magistris MR, Regli F, et al. Predictive value of anti- GM1 ganglioside antibodies in neuromuscular disease–astudy of 180 sera .J Neuroimmunol 1991; 32:223-30.

13 Ravindranath MH, Ravindranath RM, Morton DL, graves MC. Factors affecting the fine specifity and sensitivity of serum anti-ganglioside antibodies in ELISA. J Immunol Methods 1994; 169:257-72

14 Alaedini A, Latov N. Detection of anti-GMl ganglioside antibodies in patients with neuropathy by a novel latex agglutination assay. J Immunoassay 2000; 21:377-86.

15 Conrad K, Schneider H, Ziemssen T, Talaska T, Reinhold D, Humbel RL,. A new line immunoassay for the multiparametric detection of antiganglioside autoantibodies in patients with Autoimmune peripheral neuropathies. Ann. NY Acad. Sci. 2007; 1109:256-64.

16 Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barre syndrome. Ann Neurol. 1990; 27:21-4.

17 Ang CW, Endtz HP, Jacobs BC, Laman JD, de Klerk MA, van der Meche FG. Campylobacter Jejuni Lipopolysaccharides From Guillain-Barre Syndrome Patients Induce Igg Anti-Gm1 Antibodies In Rabbits**.** Journal of Neuroimmunology 2000 ;104: 133-8.

18 Jeremy H., Sara E, Norman A, Richard A. Campylobacter Jejuni Infection And Guillain–Barré Syndrome. The New England Journal Of Medicine. 1995; 333:1374-9.

19 Irie S, Saito T, Kanazawa N, Ogino M, Ogino Y, Sakai F. Detection of Serum Anti-ganglioside Antibodies by Latex Agglutination Assay in Guillain-Barre Syndrome: Comparison with ELISA Intern Med. 2003; 42:490-5.

20 Schessl J, Koga M, Funakoshi K, Kirschner J, Muellges W, Weishaupt A, et al. Prospective study on anti-ganglioside antibodies in childhood Guillain–Barre´ syndrome. Arch Dis Child 2007; 92:48-52.

21 Yuki N, Yoshino H, Sato S, Miyatake T. Acute axonal polyneuropathy associated with anti-GM1 antibodies following Campylobacter enteritis. Neurology. 1990; 40:1900-2.

22 Hao Q, Saida T, Kuroki S, Nishimura M, Nukina M, Obayashi H, et al. Antibodies to gangliosides and galactocerebroside in patients with Guillain–Barre syndrome with preceding Campylobacter jejuni and other identified infections. Journal of Neuroimmunology . 1998; 81:116-26.

23 Ho TW, Willison HJ, Nachamkin I, Li CY, Veitch J, Ung H, et al. Anti-GD1a antibody is associated with axonal but not demyelinating forms of Guillain-Barre´ syndrome. Ann Neurol. 1999; 45:168-73**.**

24 Caudie C, vial C, Petiot P, Bancel J, Later R, Gonnaud PM. Measurement of Antiganglioside Antibodies by immunodot-blot assay : clinical importance in peripheral neuropathies. Ann Biol Clin .1999; 57:579-88.

25 Caudie C, Vial C, Bancel J, Petiot P, Antoine JC, Gonnaud PM. Antiganglioside autoantibody profiles in Guillain-Barré syndrome. 1: Ann Biol Clin (Paris). 2002; 60:589-97.