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Antiphospholipid Antibody in Serum of Guillain-Barre Syndrome Patients

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Abstract

Background Studies have provided convincing evidence that Guillain-Barre syndrome [GBS] is caused by an infection-induced aberrant immune response that damages peripheral nerves. Despite intensive research over the past two decades, the immune target is still unknown in patients with acute inflammatory demyelinating polyradiculoneuropathy [AIDP], the most frequent variant of GBS.

Objective Measuring of immunoglobulin G [IgG] and immunoglobulin M [IgM] antiphospholipid antibodies [aPL] of incidental untreated GBS patients and comparing them with that of normal population.

Methods This is an age and gender matched paired case-control study at Al-Kadhimiya Teaching Hospital between 1-Dec-2008 and 31-Jan-2010. Each patient was paired with the age and sex matched control which was useful in controlling the confounding effect of age and gender on possible case-control differences. The aPL were measured by Immunometric Enzyme Immunoassay.

Results Eleven patients with GBS (cases) and eleven age and gender matched controls included in current study. The GBS cases have higher IgM and IgGaPL titers than healthy controls [P=0.026, P=0.13 respectively]. The GBS cases IgMaPL titers have negative correlation with duration of illness [r=-0.494, P=0.12], while the cases IgGaPL titer have positive correlation with duration of illness [r=0.243, P=0.47]. The GBS cases that need mechanical ventilation have lower IgM and IgGaPL titers than cases that do not need mechanical ventilation [P=0.1, P=0.06 respectively].

Conclusion GBS cases have statistically significant higher IgMaPL titers during the first week [p=0.028] and the first two weeks [p=0.026]of illness than healthy controls, and aPL may have a protective effect in GBS.

Keywords Guillain-Barre syndrome, Demyelination, Antiphospholipid antibodies and autoantibodies.

Introduction

Until 2010, GBS has remained a descriptive diagnosis of a disorder for which there are no specific diagnostic tests. The combination of rapidly progressive symmetrical weakness in the arms and legs with or without sensory disturbances, hyporeflexia or areflexia, in the absence of a CSF cellular reaction, remains the hallmark for the clinical diagnosis of GBS [(1)].

Based on well-controlled population-based studies the incidence of GBS in Europe is 1.2-1.9 cases per 100 000, while worldwide, the incidence is 0.6-4 cases per 100 000 [(2,3)]. Despite the effect of intravenous immunoglobulin [IV Ig] or plasma exchange [PE] treatment, 10% to 20% of patients are left with disabling motor deficits and 4% to 15% of patients die by 1 year after onset [(4)]. Up to one-third of GBS patients need to make substantial changes in their job, hobbies or social activities due to the residual functional deficit [(5)]. Even 3-6 years after onset, GBS has a large impact on social life and the ability to perform activities

[6,7,8]. GBS often remains a severe disease for which better treatment is required, at least in some patients.

About two-thirds of GBS cases have an antecedent infection within 6 weeks prior to symptom onset, generally an upper respiratory tract infection or gastroenteritis [9]. Autoimmune diseases such as graft-vs.-host disease, systemic lupus erythematosus, scleroderma, and sicca syndrome are sometimes associated with GBS [10]. Some of these are also characterized by the presence of aPL. This relationship between the above autoimmune diseases and GBS raises the question of whether aPL are encountered in patients with this syndrome or not.

Nearly any neurological manifestation may occur in patients have aPL[11]. Non-thrombotic manifestations were described in relation to the presence of aPL like epilepsy, chorea, transverse myelitis, multiple sclerosis, GBS, dementia, and psychiatric disease [12]. Also subtle abnormalities in neurocognitive function may be found in patients have a PL, such as memory loss or behavioral disturbances [13].

The aim of study is to reveal if IgG and IgM aPL associated with GBS and if there a significant deference in the titers of IgG and IgM aPL between incidentals untreated GBS patients without known autoimmune disorders and normal population.

Methods

Study design: Age and gender matched paired case-control study. Patients with GBS [cases] were recruited from patients admitted to Al-Kadhimiya Teaching Hospital between 1-Dec-2008 and 31-Jan-2010 [14 months] who fulfilled the following eligibility criteria:-

1. Patient is examined and diagnosed as GBS according to Asbury and Cornblath criteria [1].
2. Patient with no signs of improvement.
3. The period from appearance of first symptom of GBS till time of blood sample aspiration is less than two weeks.
4. The patient did not receive steroidal therapy since the appearance of first GBS symptoms.
5. The patient did not receive intravenous immunoglobulin since the appearance of first GBS symptoms.
6. The patient did not start plasmapheresis sessions since the appearance of first GBS symptoms.
7. Patient is not a known case of autoimmune disorder.
8. Patient is not diabetic

The controls were recruited from Al-Kadhimiya Teaching Hospital medical staff and patient's relatives who were matched with cases according to age and sex on an individual bases.

The serum have been separated from each blood sample and stored in the refrigerator of Hospital's Blood Bank at temperature range from -35°C to -40°C till time of analysis.

The patients that fulfill the inclusion criteria during the period of this study were only eleven patients, (Table 1), with an age and gender matched healthy control was paired with each case, (Table 2). The selected controls showed evidence of effective case-control age matching, (Table 3).

The presence of aPL antibodies was investigated using the ORGENTEC Anti-Phospholipid Screen IgG/IgM kit [ORG 529] for Immunometric Enzyme Immunoassay (EIA) for the quantitative determination of the sum of autoantibodies against Cardiolipin, Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and ß2-Glycoprotein I (IgG and/or IgM class).

Statistical analysis of data was done using SPSS version 13 computer software (Statistical Package for Social Sciences). Frequency distributions for selected variables were done first. The primary outcome for the present study was the IgG and IgM aPL titers, which were non-normally distributed continuous quantitative variables. Such variables are conveniently described by median and inter-quartile range. The non-parametric tests of significance, which do not require the

assumption of normal distribution, are applicable here. The statistical significance of paired case-control differences in median was assessed by Wilcoxon Signed Ranks test. The statistical significance of difference in median between two groups (like males and females) was assessed by Mann-Whitney test. The paired t-test was used to assess the statistical significance of paired case-control difference in mean age (a normally distributed variable). P value less than the 0.05 level of significance was considered statistically significant. The statistical significance, strength and direction of linear correlation between two quantitative variables (one of which being non-normally distributed) were assessed by Spearman's rank linear correlation.

Table 1. Demographic features of the studied cases

No.	Sex	Date of 1st symptom [Day zero]	Day of blood aspiration [D]	Significant history	Need mechanical ventilation	Age (years)	aPL titer	
							IgG U/ml	IgM U/ml
1	Male	05-12-2008	D11	NS	No	55	0.489	0.154
2	Female	11-12-2008	D10	Resp. inf.	No	3.5	16.363	6.311
3	Male	17-12-2008	D6	GIT inf.	No	5.5	11.331	7.03
4	Female	13-12-2008	D10	Resp. inf.	No	60	2.543	2.127
5	Male	28-12-2008	D2	Resp. inf.	No	8	0.498	12.295
6	Male	27-12-2008	D8	Resp. inf.	No	6	6.071	0.829
7	Male	05-09-2009	D5	NS	No	33	5.094	7.009
8	Male	05-11-2009	D4	Resp. inf.	Yes	30	0.093	0.119
9	Female	18-11-2009	D6	Resp. inf.	No	14	0.098	3.254
10	Female	31-12-2009	D4	Resp. inf.	No	6	0.799	9.049
11	Male	27-12-2009	D9	Resp. inf.	Yes	30	0.109	1.15

Table 2. Demographic features of the controls

No.	Sex	Date of Blood aspiration	Note	Age years	Control aPL titer	
					IgG U/ml	IgM U/ml
1	Male	22-12-2008	Hospital analyzer	54	0.042	0.004
2	Female	10-01-2008	Miner head trauma	3	0.97	9.058
3	Male	28-12-2008	Child of a patient	5.5	0.074	0.028
4	Female	03-01-2008	Relative of a patient	60	0.744	0.014
5	Male	22-01-2008	Miner head trauma	8	0.017	0.017
6	Male	24-01-2008	Relative of a patient	6	0.01	0.01
7	Male	12-09-2009	Medical staff	33	0.305	4.69
8	Male	10-11-2009	Doctor	30	0.069	0.041
9	Female	27-11-2009	Relative of a patient	16	8.505	1.363
10	Female	14-01-2010	Candidate of elective tonsillectomy	6	0.075	0.057
11	Male	07-01-2010	Doctor	30	4.909	0.033

Table 3. Case-control age matching

Age in years	Cases	Control	Case-control age difference	P (paired t-test)
Range	3.5-60	3-60	-2 to 1	
Mean	22.82	22.86	-0.05	
SD	20.4	20.21	0.72	0.84[NS]
SE	6.15	6.095	0.218	
N	11	11	11	

Results

The cases that needed mechanical ventilation showed IgG and IgM aPL medians less than that of cases that did not need mechanical ventilation, but the difference between medians failed short of statistical significance, (Table 4).

The cases were also classified according to time between onset of symptoms [Day zero] and blood aspiration into two groups, first week and second week group. By comparing these two groups, the IgG aPL median of second week cases was higher than that of first week cases but the difference between medians was not significant. While the IgM aPL median of first week cases was higher than that of second week cases but the difference between medians failed short of statistical significance, (Table 5).

Table 4. The aPL titer medians difference between cases who need and cases who don't need mechanical ventilation

Cases		Need for mechanical ventilation		P (Mann-Whitney)
		Negative	Positive	
Serum IgG aPL titer	Range Median Inter-quartile range Number of cases	0.098-16.363 2.543 0.494-8.701 9	0.093-0.109 0.101 0.093-** 2	0.06[NS]
Serum IgM aPL titer	Range Median Inter-quartile range Number of cases	0.154-12.295 6.311 1.478-8.04 9	0.1-1.15 0.635 0.119-** 2	0.1[NS]

"**" refers to "cannot be calculated"

Table 5. The aPL titer medians difference between 1st week and 2nd week cases

Cases		1st week cases	2nd week cases	P (Mann-Whitney)
Serum IgG aPL titer	Range Median Inter-quartile range Number of cases	0.093-11.331 0.649 0.097-6.653 6	0.109-16.363 2.543 0.299-11.217 5	0.47[NS]
Serum IgM aPL titer	Range Median Inter-quartile range Number of cases	0.119-12.295 7.02 2.47-9.861 6	0.154-6.311 1.15 0.492-4.219 5	0.1[NS]

The cases IgG aPL median was higher than that of controls in both time strata, but the median case-control difference was not significant, (Table 6). The cases IgM aPL median was also higher than that of controls in both time strata, but the median case-control difference in the total and first week groups was statistically significant, while in the second week group was not significant, (Table 7).

The cases positive correlation with duration of symptoms, while the cases IgM aPL showed a statistically insignificant moderately strong negative correlation with duration of symptoms, (Table 8).

By comparing IgG aPL with IgM aPl, the controls IgG aPL showed statistically significant strong positive correlation with their IgM aPL, while the cases IgG aPL showed a statistically

insignificant moderately strong positive correlation with their IgM aPL, (Table 8).

Table 6. The IgGaPL titer difference between cases and controls

Case-control group		Serum IgG aPL titer			P (Wilcoxon Signed Ranks Test)
		Cases	Controls	case-control difference	
1st week	Range Median Inter-quartile range Number	0.093-11.33 0.649 0.097-6.65 6	0.017-8.50 0.075 0.056-2.35 6	-8.40-11.25 0.603 -2.08-6.40 6	0.25[NS]
2nd weeks	Range Median Inter-quartile range Number	0.10-16.36 2.543 0.29-11.21 5	0.01-4.90 0.744 0.026-2.94 5	-4.8-15.39 1.799 -2.17-10.72 5	0.23[NS]
Total	Range Median Inter-quartile range Number	0.093-16.36 0.799 0.10-6.07 11	0.01-8.50 0.075 0.042-0.97 11	-8.40-15.39 0.724 0.024-6.06 11	0.13[NS]

Table 7. The IgM aPL titer difference between cases and controls

Case-control group		Serum IgG aPL titer			P (Wilcoxon Signed Ranks Test)
		Cases	Controls	case-control difference	
1st week	Range Median Inter-quartile range Number	0.11-12.29 7.02 2.47-9.86 6	0.017-4.69 0.049 0.023-2.19 6	0.078-12.27 4.661 1.43-9.81 6	0.028
2nd weeks	Range Median Inter-quartile range Number	0.154-6.31 1.15 0.492-4.21 5	0.004-9.05 0.014 0.007-4.54 5	-2.74-2.11 0.819 -1.29-1.61 5	0.5[NS]
Overall	Range Median Inter-quartile range Number	0.119-12.295 3.254 0.829-7.03 11	0.004-9.05 0.033 0.014-1.36 11	-2.74-12.27 1.891 0.15-7.002 11	0.026

Table 8. The linear correlation coefficient between aPL titer, age, and duration of symptoms

Group		Serum IgG aPL titer	Serum IgM aPL titer
Control	Serum IgM aPL titer Age (years)	r= 0.636 P = 0.035 r= 0.087 P = 0.8[NS]	r=-0.324 P = 0.33[NS]
Cases	Serum IgM aPL titer Age (years) Duration of symptoms (days)	r= 0.409 P = 0.21[NS] r=-0.516 P = 0.1[NS] r= 0.243 P = 0.47[NS]	r=-0.457 P = 0.16[NS] r=-0.494 P = 0.12[NS]

Note: r = 0–0.2: very weak. r = 0.2–0.4: weak. r = 0.4–0.6: moderately strong. r = 0.6–0.8: strong. r = 0.8–1.0: very strong

To determine the best cut-point values by which the aPL test shows the highest accuracy, we use the ROC (Receiver Operator Characteristic) Curve analysis, which determined the best cut-off value for IgG aPL as 0.084U/ml with 77.3% accuracy and for IgM aPL as 0.088 U/ml with 86.4% accuracy, (Table 9 and Figure 1).

Table 9. Validity parameters of IgG and IgM aPL test at selected cut-off values when used to diagnose GB syndrome differentiating it from healthy controls.

Positive if ≥ cut-off value	Sensitivity	Specificity	Accuracy	PPV at pretest probability =50%	PPV at pretest probability =90%	NPV at pretest probability =10%
Serum IgG aPL titer						
0.084	*100.0*	*54.5*	*77.3*	*68.7*	*95.2*	*100.0*
0.096	90.9	54.5	72.7	66.6	94.7	98.2
0.104	81.8	54.5	68.2	64.3	94.2	96.4
0.207	72.7	54.5	63.6	61.5	93.5	94.7
0.397	72.7	63.6	68.2	66.6	94.7	95.4
0.494	63.6	63.6	63.6	63.6	94.0	94.0
0.621	54.5	63.6	59.1	60.0	93.1	92.6
0.772	54.5	72.7	63.6	66.6	94.7	93.5
0.885	45.5	72.7	59.1	62.5	93.8	92.3
1.757	45.5	81.8	63.7	71.4	95.7	93.1
3.726	36.4	81.8	59.1	66.7	94.7	92.0
5.002	36.4	90.9	63.7	80.0	97.3	92.8
5.583	27.3	90.9	59.1	75.0	96.4	91.8
7.288	18.2	90.9	54.6	66.7	94.7	90.9
9.918	18.2	100.0	59.1	100.0	100.0	91.7
Serum IgM aPL titer						
0.088	*100.0*	*72.7*	*86.4*	*78.6*	*97.1*	*100.0*
0.137	90.9	72.7	81.8	76.9	96.8	98.6
0.492	81.8	72.7	77.3	75.0	96.4	97.3
0.990	72.7	72.7	72.7	72.7	96.0	96.0
1.257	63.6	72.7	68.2	70.0	95.4	94.7
1.745	63.6	81.8	72.7	77.8	96.9	95.3
2.691	54.5	81.8	68.2	75.0	96.4	94.2
3.972	45.5	81.8	63.7	71.4	95.7	93.1
5.501	45.5	90.9	68.2	83.3	97.8	93.8
6.660	36.4	90.9	63.7	80.0	97.3	92.8
7.020	27.3	90.9	59.1	75.0	96.4	91.8
8.040	18.2	90.9	54.6	66.7	94.7	90.9
9.054	9.1	90.9	50.0	50.0	90.0	90.0
10.677	9.1	100.0	54.6	100.0	100.0	90.8

When aPL test used to differentiate GBS cases from healthy controls, the ROC area analysis showed that IgG aPL test showed a statistically significant moderately strong ROC area, while the IgM aPL test showed statistically significant test of high validity, (Figure 1).

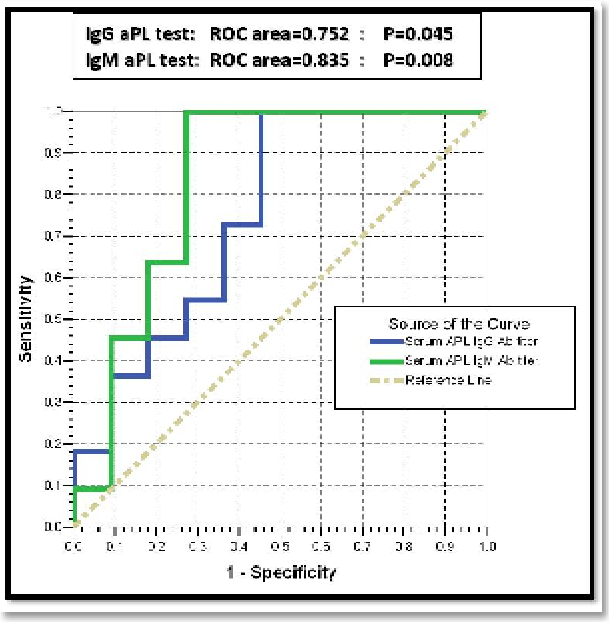

Figure 1. ROC curves for IgG and IgM aPL tests differentiating GBS cases from healthy controls

Using aPL test to differentiate cases with <8 days symptoms from cases with longer duration, the IgG aPL test was not significant, while the IgM aPL test showed high validity, but failed short of statistical significance, (Table 10).

Table 10. The aPL test ROC area and P value for different positive finding

	Test				
	Symptoms duration < 8 days differentiating them from longer duration		Males differentiating them from females		Need for mechanical ventilation Vs not needing
	Serum IgG aPL	Serum IgM aPL	Serum IgG aPL	Serum IgM aPL	
ROC area	0.633	0.8	0.571	0.643	
P value	0.47[NS]	0.1[NS]	0.71[NS]	0.45[NS]	

Note: ROC area = 0.5–0.7: weak. ROC area = 0.7–0.8: moderately strong. ROC area = 0.8–0.9: strong. ROC area = 0.9–1: very strong. ROC area = 1: perfect

Discussion

The current study findings about the elevated IgM aPL titers in cases and it's negative correlation with duration of illness have been reported by Nakos *et al* 2005 [xiv]. The important difference between results of our research and that of Nakos *et al*is that, the later investigates GBS cases treated with IV Ig, and explain the negative correlation of IgM aPL with time from onset of GBS as a result to the blocking effect of IV Ig that have been given to the cases which resulted in the decrement of IgM aPL titers with time. While current study showed this negative correlation as part of the natural history of the GBS without the effect of any given drug.

Our observations can be explained by the history of recent common infections [Respiratory infection, GIT Infection] that are usually reported within two weeks before the onset of GBS. A recent study identified aPL as a common phenomenon in patients with

common infections, independent from the type of infection. These aPL have pathogenic abilities that can be assumed by the presence of prolonged activated Partial Prothrombin Time [aPTT] in the infected patients and these aPL may be part of the immunoreactions against the inoculated pathogen [(xv)].

It is crucial to investigate if there is a direct relation between aPL and specific antigens in Schwann cell membrane or not. Keeping in mind that there is no known antiganglioside antibodies associated specifically with AIDP subtype of GBS [which is the most common subtype] till 2005 [(16-19)].

The findings of current study about IgG and IgM aPL titers difference between cases who need mechanical ventilation and who do not need, may point out to a protective effect for aPL in GBS. This can be explained by two ways. First the ventilated cases immune system; for unknown host factor; cannot synthesize the reactionary aPL in response to the common pathogens which other cases immune system can synthesize aPL in response to them. Second, the ventilated cases have been infected with different pathogen which is not known to be associated with production of reactionary aPL.

The IgM aPL test showed statistically significant very good sensitivity and acceptable specificity when used to differentiate between GBS cases and healthy controls at the cut-off value suggested by ROC curve analysis. These test performance characteristics suggest its use as screening test for GBS among subjects with unclear neurologic symptoms and as a very early additional marker of diagnostic significance for GBS cases in the first two weeks of illness, given the fact that both electromyographic techniques and cerebrospinal fluid biochemical findings lag for days or even weeks in the early diagnosis of the syndrome.

Both IgG and IgM aPL tests have very good overall test accuracy reflected by the tests ROC areas which failed short of statistical significance, when used to differentiate between ventilated and not ventilated GBS cases. Although the possibility of chance effect cannot be excluded as an alternative explanation for any statistically insignificant finding due to the very small number of cases of our research, but these results is still very important. If these results are confirmed by other larger studies, the aPL test can be used as early test to detect cases that will need mechanical ventilation.

Conclusion

From this study it was concluded that:-

1. GBS cases have statistically significant higher IgM aPL titers during the first week and the first two weeks of illness than healthy controls. This reflects a more extensive immune reaction beyond the well known antiganglioside production, which has been related to the demyelination of the peripheral nerves.
2. There is a statistically not significant negative time trend for IgM aPL titer and duration of illness in GBS.
3. The aPL may have a protective effect in GBS.

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