Blood Brain Barrier: The Role of GAD Antibodies in Psychiatry

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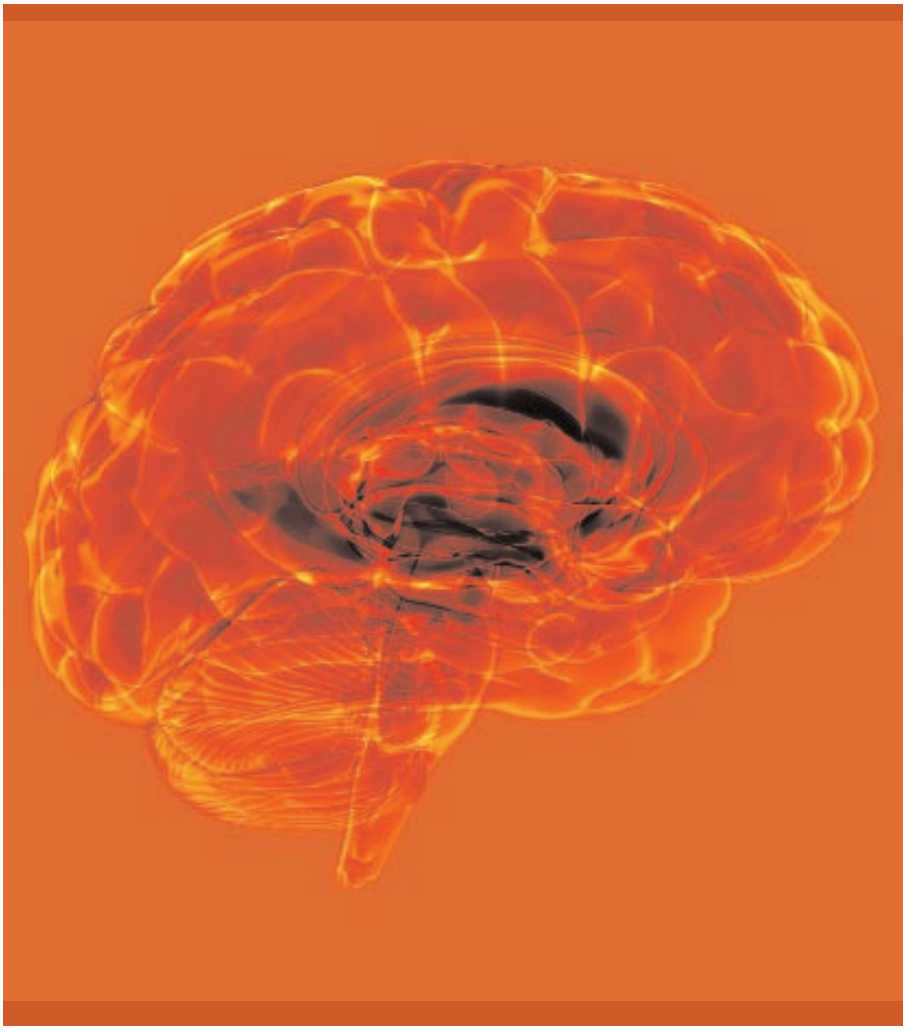
ABSTRACT

Objective: Goal of our case control study was to establish the presence of antibodies to glutamic acid decarboxylase (GAD) in patients with chronic psychotic disorders.

Methods: Serum levels of GAD antibodies in 12 patients with chronic psychotic disorders (schizophrenia and schizoaffective disorders) and 10 age-matched healthy control subjects were evaluated utilizing enzyme linked immunosorbitent assay (ELISA).

Results: Antibodies to GAD in patients with chronic psychotic disorders have a higher mean than nonpatient control individuals.

Conclusion: Our findings provide the first *in-vivo* evidence of positive GAD antibodies in chronic psychotic disorders and potentially may be used as a screening for these disorders.



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KEY WORDS: blood brain barrier, glutamic acid decarboxylase (GAD), GAD antibodies, schizophrenia, diabetes mellitus (DM), screening tests

INTRODUCTION

More complicated than other organs, the central nervous system (CNS) is a closed, highly selective system with complicated neurophysiological mechanisms. The brain differs significantly from other organs in its unique capability for processing and storing information. There are no well established biomarkers that can be used to diagnose or monitor the development

and progression of psychiatric disorders. Reliable treatment options for many psychiatric disorders, as a result, have been and remain a challenge. Some clinical data supported by recent advances in technology have opened doors to possible associations between the brain and thought. Functional assessment of the working brain may provide some insight into these complicated, yet related, processes. However, bridging the gap between structural and functional abnormalities in the CNS by identifying a potential marker peripherally linking these two processes still remains a challenge.

The role of GABAergic neurotransmission in epilepsy, anxiety disorders, schizophrenia, and premenstrual dysphoric disorder has been a subject of some recent investigations.[1] Absence of structural abnormalities in the brains of most patients with chronic psychotic disorders has always raised suspicion for an alternative pathogenesis and a possible functional disturbance at the neuronal/cellular level. Glutamic acid decarboxylase (GAD), a rate limiting and a pyridoxal phosphate-dependant enzyme, is involved in the formation of gamma aminobutyric acid (GABA) a central inhibitory neurotransmitter of the nervous system. Antibodies to GAD may impair GABA formation or inhibitory function. Antibodies to GAD are primarily cytoplasmic and previously have been reported in a neurological entity called Stiff Person Syndrome (SPS)[2] and in type I diabetes.[3] Recent evidence points to their role in pathogenesis via two mechanisms: 1) blocking the function of intact cells without structurally changing the neurons and 2) expulsion of certain peptide fragments of GAD via exocytosis of GABA consequently presenting to the T-cell receptors. In the context of diabetes, GAD65 antibodies have been associated with an infectious etiology, especially entero and coxsackie viruses.[4,5] However, GAD65 antibodies interestingly bear a striking similarity with P2-C protein of coxsackie virus suggesting a cross-reactivity and interference with neuronal development.

We hypothesized that GAD antibodies are increased in patients with chronic psychotic disorders. The aim of this pilot study was to compare the level of GAD antibodies in patients with chronic psychotic disorders with normal controls.

METHODS

This study was approved by the University of Virginia Human Investigation Committee. Twelve patients with chronic psychotic disorders (schizophrenia and schizoaffective disorder) comprised the patient group. Exclusion criteria included presence of diabetes mellitus (type 1 or 2) and SPS. An informed consent was obtained from each participant in the study. Ten age-matched healthy controls were recruited from the medical professionals at the facility. Assessment of GAD antibodies was performed on a 3mL serum sample, collected and frozen immediately utilizing ELISA kit from ICN pharmaceuticals (now Valeant pharmaceuticals International). Antibodies to GAD, specifically to GAD65, were measured on duplicate samples from each patient and control. Data were calculated manually by using a dose response curve (DRC) on linear graph paper, plotting each calibrator value (as indicated on the calibrator vial label) on the X-axis and corresponding absorbance value on the Y-axis. A straight line was drawn between three points. Values of GAD antibodies were determined from each patient's serum using its absorbance value and extrapolating from the DRC on the X-axis. A mean value of GAD antibodies was calculated for each participant based on the two samples. The assay was run in strict conformity with the guidelines and instructions provided by the manufacturer. Results were considered negative if antibodies to GAD65 were <1.00, indeterminate (borderline) if between 1.00 to 1.05, and positive with GAD65 antibodies >1.05.

RESULTS

GAD65 antibodies levels in patients and control group are listed in Table 1. Negative results of GAD65 antibodies in nine out of 10 healthy controls were present with a mean of 0.62 and a standard deviation of 0.49. Strongly positive levels of antibodies to GAD65 were found in five out of 12 patients with a mean of 1.19 and a standard deviation of 1.08.

GAD65 antibodies values in a group of 12 patients with chronic psychotic disorders have a higher mean and show greater variability than GAD65 antibodies values in a group of 10 healthy controls. Although the difference in mean values did not achieve the statistical significance criteria of 0.05 (t=-1.53, df+20, p=0.14), this small sample study has minimal power. To achieve a large effect size (d=0.80), more subjects would be needed; this sample of 10 analyzable subjects in the control group and 12 patients with chronic psychotic disorders (N=22) provided 56-percent power to detect a difference at the 0.05 significance level (critical t (20)=1.72).

...FROM A PRACTICING PSYCHIATRIST'S point of view, measurements of antibodies to GAD65 could potentially be used to screen for chronic psychotic disorders and for diabetes mellitus very early on in the disease process.

TABLE 1. Antibodies to glutamic acid decarboxylase (GAD)

Controls	0.5	0.4	0.8	1.9	0.6	0.3	0.2	0.4	0.3	0.8	X	X
Patients	0.6	1.3	0.4	2.8	0.7	0.7	3	3.6	0.3	0.8	1.7	0.5

DISCUSSION

The recent work of Kalkman and Loetscher,[6] showed a decreased expression of 67 kDa isoenzyme to glutamic acid decarboxylase(GAD67) in patients with bipolar disorder and schizophrenia. In another study, Hashimoto, et al., [7] demonstrated reduced expression of GAD67 mRNA in 15 postmortem brains of schizophrenic patients. However, this reduced expression was in combination with a calcium binding protein, parvalbumin (PV) mRNA expression in the prefrontal cortex, leading to increased levels of free calcium as hypothesized previously.[8] These studies did not evaluate for the presence of antibodies to GAD, which, although through a different mechanism, would have a similar effect, resulting in decreased concentrations of neurotransmittor GABA and lack of inhibition. Association of GABA with various neuropsychiatric entities is expanding.[1] Considering that GABA is a central inhibitory neurotransmitter, its fluctuations are critical, with a potential to result in seizures.[9]

Taking into consideration the small sample size, our preliminary results suggest that a reasonably sized effect exists between antibodies to GAD65 and chronic psychotic disorders. However, the study was underpowered. To increase statistical power in future research, a larger sample size is required. For group comparisons using t-tests for independent samples and to achieve a large effect size (d=0.80), a sample of 21 analyzable subjects in the control and 21 patients with chronic psychotic disorders (total N=42) would provide 80-percent power to detect such a difference at the 0.05 significance level (critical t (40)=1.68).

CONCLUSION

Antibodies to GAD65 are peripherally present in patients with chronic psychotic disorders (schizophrenia/schizoaffective disorders) and may potentially be used as a tool to screen for these disorders. The presence of such antibodies also suggests a possible role for autoimmune mechanism in the pathogenesis of these disorders. In summary, from a practicing psychiatrist's point of view, measurements of antibodies to GAD65 could potentially be used to screen for chronic psychotic disorders and for diabetes mellitus very early on in the disease process.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Acharya Ganesh of the Tromso University, Norway, for his expert editorial coments.

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