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Neurol Neuroimmunol Neuroinflamm
2017;4:e344; doi: 10.1212/NXI.0000000000000344

ABSENCE OF NMDA RECEPTOR ANTIBODIES IN PATIENTS WITH OVARIAN TERATOMA WITHOUT ENCEPHALITIS

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Ovarian teratoma (OT) has been identified in 18%–50% of patients with anti–N-methyl-D-aspartate receptor (NMDAR) encephalitis, and an immunopathologic relationship between these conditions has been proposed.[1–3]

It is currently unclear whether a subset of patients with OT harbor NMDAR antibodies before developing anti–NMDAR encephalitis. Therefore, the usefulness of systematic NMDAR antibody screening to identify patients with OT who may develop encephalitic symptoms and/or benefit from additional therapy should be investigated. We thus conducted this study to determine the frequency and usefulness of serum NMDAR antibody detection in patients with OT compared with other benign ovarian cysts, to ascertain whether antibody testing should be routinely conducted in this patient group.

Methods. ***Patients.*** Female patients with OT (group 1) and other benign ovarian cysts (group 2) admitted to West China Second University Hospital from 2012 to 2013 were enrolled in this study. Diagnoses of OT were based on Doppler ultrasonography; all diagnoses were confirmed by postoperative histopathologic analysis. The study was approved by the local ethics committee, and informed consent was obtained from all participants.

Methods. Clinical data, including age, ethnicity, medical history, physical examination findings, and ovarian cyst features (diameter, unilateral or bilateral, and pathologic diagnosis), were collected for all participants. Serum samples were collected preoperatively and tested for the presence of the NMDAR antibody by examiners blinded to group allocation. NMDAR antibody detection was performed with NR1 heteromer–transfected HEK293 cells, according to clinical guidelines for the diagnosis of antineuronal antibodies. Serial diluted samples (starting with 1:10) of serum (30 μL) and fluorescein-labeled anti–human globulin (25 μL; Euroimmun, Lübeck, Germany) were reacted in separate reagent trays. BIOCHIP slides containing recombinant HEK293 cell substrates (Euroimmun) were incubated with serial diluted samples at room temperature for 30 minutes, rinsed, and then immersed in phosphate-buffered saline–Tween solution for 5 minutes. BIOCHIP slides were put into reagent trays containing fluorescein-labeled globulin to be incubated at room temperature for 30 minutes again. After being rinsed and immersed again, slides were embedded in glycerol, and fluorescence was observed under a microscope. The lowest dilution (1:10) showing a measurable degree of fluorescence was set as the threshold for positivity. Positive and negative controls were included. The sensitivity and specificity of this method in our center are 85.5% and 99.5%, respectively.

Statistical analysis. Given the nonnormal distribution of data, median values with 25th and 75th percentiles were calculated for continuous variables, and comparisons between groups were performed using Mann-Whitney *U* test. The Fisher exact test was used to compare categorical variables. Analyses were performed using SPSS software (version 17.0; SPSS Inc., Chicago, IL), with the level of significance set at $p < 0.05$.

Results. Ninety-six patients of Han ethnicity with OT diagnosed by Doppler ultrasonography were

Table Clinical features and NMDAR antibody test results of both groups

Characteristic	OT (n = 80)	Control (n = 95)
Age, y, median (IQR)[a]	29 (25–37)	29 (26–41)
Ethnicity		
Han	80 (100)	95 (100)
OT/cyst distribution		
Unilateral	71 (88.8)	70 (73.7)
Bilateral	9 (11.2)	25 (26.3)
NMDAR test result[b]		
Positive	0 (0)	0 (0)
Negative	80 (100)	95 (100)

Abbreviations: IQR = interquartile range; NMDAR = N-methyl-D-aspartate receptor; OT = ovarian teratoma.
Data are presented as n (%) unless otherwise indicated.
[a] $p = 0.231$, Wilcoxon 2-sample test.
[b] $p = 0.208$, Fisher exact test.

enrolled in group 1; diagnoses were confirmed pathologically in 80 cases. The group 2 comprised 95 patients with pathologically confirmed diagnoses of other benign ovarian cysts. The clinical characteristics of both groups are shown in the table. Serum NMDAR antibodies were not detected in any patient in both groups (table).

Discussion. As documented previously in smaller samples of patients with OT,[4,5] NMDAR antibodies were not detected in the serum of all patients with no encephalitis history or neurologic symptom in this study. These results might be explained by the absence of immunostimulatory events, such as infections, which could trigger antibody production.[6,7] The study results indicate that serum NMDAR antibody testing is unnecessary in patients with OT unless complicating encephalitis is suspected.

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Study funding: No targeted funding reported.

Disclosure: S. Gong, M. Zhou, and G. Shi report no disclosures. J. Guo received research support from National Natural Science Foundation of China. N. Chen and R. Yang report no disclosures. L. He received research support from National Natural Science Foundation of China. D. Zhou served on the editorial board for Neural Regeneration Research, Chinese Journal of Neurology, *and* Seizure: European Journal of Epilepsy*; and received research support from National Natural Science Foundation of China. Go to Neurology.org/nn for full disclosure forms. The Article Processing Charge was funded by the authors.*

Received September 29, 2016. Accepted in final form January 20, 2017.

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1. Dalmau J, Gleichman AJ, Hughes EG, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 2008;7:1091–1098.
2. Irani SR, Bera K, Waters P, et al. N-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. Brain 2010;133: 1655–1667.
3. Tuzun E, Zhou L, Baehring JM, Bannykh S, Rosenfeld MR, Dalmau J. Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. Acta Neuropathol 2009;118:737–743.
4. Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y, Sangha N, Martinez-Lage M, Dalmau J. Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. Neurology 2011;77:589–593.
5. Mangler M, Trebesch de Perez I, Teegen B, et al. Seroprevalence of anti-N-methyl-D-aspartate receptor antibodies in women with ovarian teratoma. J Neurol 2013; 260:2831–2835.
6. Tumbi A, Gilani A, Scarff JR, Kaur G, Lippmann S. Anti-N-methyl-D encephalitis. Innov Clin Neurosci 2011;8: 24–25.
7. Moscato EH, Jain A, Peng X, Hughes EG, Dalmau J, Balice-Gordon RJ. Mechanisms underlying autoimmune synaptic encephalitis leading to disorders of memory, behavior and cognition: insights from molecular, cellular and synaptic studies. Eur J Neurosci 2010;32: 298–309.