# The Prevalence of Serum Anti Nuclear Antibodies in Children Treated With Anti-Epileptics

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**Abstract-** To evaluate the prevalence of positive serum antinuclear antibody (ANA) in children with epilepsy using three major antiepileptic drugs (phenytoin, carbamazepine and ethosuximide), 60 children under 18 years with epilepsy who were referred to pediatric neurology clinic or had admitted to neurology ward in Children Hospital in Tehran, Iran, were entered our study. They had been treated with one of the three antiepileptic drugs (carbamazepin, phenytoin, ethosuximide) with suitable dose for at least one month. The patients were divided into two groups according to the classification of the International League Against Epilepsy (ILAE): drug-resistant and drug-responsive. We studied the epidemiological and clinical characteristics and also serum ANA of the patients in both groups. In this research, we studied ANA in 60 epileptic children. 30 patients were diagnosed with drug resistant epilepsy and the other 30 were drug responsive. None of them showed the clinical manifestations of lupus erythematosus. As a whole, 7 patients (11.7%) were ANA-positive, 6.7% of drug resistant and 16.7% of drug responsive group showed this finding. There was no relationship between drug resistancy and ANA according to statistical studies ($P$=0.21). Although in our study, epidemiological and clinical data of the patients was reported in two separate groups of resistant or responsive to antiepileptic drugs, and no meaningful statistical difference was found between these two groups. Overally in our study, the prevalence of positive ANA in patients receiving antiepileptic drugs was less in comparison with previous studies and was more common in males. Finally, we suggest a more comprehensive and extensive study with more cases and further follow-up period in order to find the cause of immunological reactions to antiepileptic drugs in children with epilepsy.



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## Introduction

Many drugs have been reported to cause drug-induced lupus syndrome especially in children (1). This disorder is mild, compared to the spontaneous Systemic Lupus Erythematosus (SLE). Rashes and alopecia in such patients are less common than arthritis, pneumonitis or pericarditis. It is remarkable that hepatitis, which is rare in SLE, is more prevalent in drug-induced lupus (2,3). Unlike SLE, the prevalence of drug-induced lupus syndrome is equal in males and females. Antinuclear antibody (ANA) test is positive in 90 percent of children with such disorder and also anti-histone antibodies are positive in most of them (4).

It has been estimated that up to 10 percent of SLE cases are drug-inducedand is rare in children than adults

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(5). Procainamide, hydralazine, isoniazid, quinidine and anti TNFs are common drugs that cause drug-induced lupus. These drugs share a primary amine or hydrazine portion that is acetylated by the N-acetyl transferase system in the liver. Also, antiepilpetic drugs and phenothiazines have been reported in association with drug-induced lupus syndrome. Studies shown that phenytoin, ethosuximide, carbamazepine and trimethadione can cause drug-induced lupus syndrome in children (6,7).

Approximately 20 percent of children receiving antiepileptic drugs produce antinuclear antibodies who commonly have normal immunoglobulins and serum complement levels and remain symptom-free. The presence of antinuclear antibodies may persist for several years even after discontinuing antiepileptic drugs. It is suggested to check the serum antinuclear antibodies in two clinical conditions,: first, presenting recurrent seizures in patients with well controlled initial seizures, and second, when increasingly higher drug doses produce increased seizure activity (3).

The purpose of this study is to evaluate the prevalence of positive serum ANA in children with epilepsy using three common antiepileptic drugs (phenytoin, carbamazepine and ethosuximide).

As there are a few numbers of articles published about drug-induced Lupus in children with epilepsy and also, the importance of seizure control in patients suffer from drug-induced lupus, we decided to design this research in a specialized center for children's diseases.

## Materials and Methods

The number of 60 children under 18 years old with epilepsy who was referred to pediatric neurology clinic or had admitted to neurology ward in Children Hospital. they had been treated with one of the three antiepileptic drugs (carbamazepine, phenytoin, ethosuximide) with recommended dose for at least one month.

The exclusion criteria were Using other drugs that promote positive ANA other than antiepileptics and clinical presentations of other rheumatologic diseases.

The patients were divided into two groups according to the classification of the International League Against Epilepsy (ILAE): drug-resistant and drug-responsive. Drug resistant epilepsy is defined as failure of adequate trials of two tolerated and appropriately chosen and used anti-epileptic drugs (AED) schedules whether as monotherapies or in combination to achieve sustained seizure freedom, the patients not included in this group, comprise the drug-responsive group (8).

We studied the epidemiological and clinical characteristics and also serum ANA of the patients in both groups.

The research followed the tenets of the declaration of Helsinki. Informed consent was obtained from all parents of the children of this study. Furthermore, our study was approved by the local ethics committee of the Shahid Beheshti University of Medical Sciences.

Patients' data was recorded confidentially in pre-prepared questionnaires and then 1.5 cc venous blood sampling was prepared and then transferred to the hematology laboratory of Mofid Children Hospital Under standard conditions. Each blood sample was centrifuged and after separating the serum, was kept in -20º C temperature. At the beginning of the test, each collected blood sample was melted in room temperature and then was diluted to a ratio of one to forty. In the next step, 0.25 cc of a conjugated solution was added to 0.25 cc of the sample after incubating in 37º C temperature and drying. The last step was re-incubating in 37º C temperature and drying the samples to prepare them for studying under fluorescent microscope to recognize the ANA-positive cases and antibody titer.

We have used ANA-Hep-2 kit made by Generic Assays GMbH company of Germany.

All the samples were studied by a single laboratory technician unaware of the patients' data and their medications.

The data of the patients was classified in two groups according to drug resistancy. Also, patients' characteristics and their medication, duration of consumption and clinical presentations were recorded.

The data was analyzed by SPSS (V22.0) Statistical softwares. Mann-Whitney U test was used to compare between quantitative variables in different groups and Chi-Square test utilized for qualitative variables. If necessary, the Fisher Exact test was used. The quantitative variables were noted by mean (and standard deviation) and qualitative ones by numbers (and percent). *P* less than 0.05 was considered as statistically significant.

## Results

In this research, we studied ANA in 60 epileptic children. The number of 30 children had drug resistant epilepsy and 30 of them were drug responsive. None of them showed the clinical manifestations of lupus erythematosus like malar rash, discoid rash, photosensitivity, arthritis, serositis, psychosis, oral or nasal ulcer. The demographic and clinical characteristics

of the patients are noted in two groups according to drug resistance in table 1.

**Table 1. Comparison of the features of patients according to the drug resistance**

| Variables | | Drug resistance Yes (N=30) n (%) | Drug resistance No (N=30) n (%) | Total (N=60) n (%) | *P* |
|---|---|---|---|---|---|
| **Age (month) Mean(standard deviation)** | | 66.47(54) | 78.53(47.47) | 72.5(50.77) | 0.36 |
| **Sex (female/male)** | | 10/20 | 14/16 | 24/36 | 0.29 |
| **Drug type** | **Ethosuximide** | 7(23.3) | 2(6.7) | 9(15) | 0.19 |
| | **Carbamazepine** | 11(36.7) | 14(46.7) | 25(41.7) | |
| | **Phenytoin** | 12(40) | 14(46.7) | 26(43.3) | |
| **Epilepsy type** | **Idiopathic** | 6(20) | 12(40) | 18(30) | 0.08 |
| | **Cryptogenic** | 15(50) | 7(23.3) | 22(36.7) | |
| | **Symptomatic** | 9(30) | 11(36.7) | 20(33.3) | |
| **Duration of drug use Mean(standard deviation)** | **Ethosuximide** | 4(3.42) | 36(16.97) | 11.1(15.62) | 0.06 |
| | **Carbamazepine** | 17.64(15.5) | 16.14(21.18) | 16.8(18.54) | 0.37 |
| | **Phenytoin** | 2.83(2.48) | 8.21(7.3) | 5.73(6.16) | 0.04 |
| **positive- ANA** | | 2(6.7) | 5(16.7) | 7(11.7) | 0.21 |
| **Underlying disorder** | **Metabolic** | 1(11.1) | 1(9.1) | 2(10) | NA* |
| | **Perinatal** | 5(55.6) | 4(36.4) | 9(45) | |
| | **Neurocutaneous** | 2(22.2) | 1(9.1) | 3(15) | |
| | **Structural** | 1(11.1) | 1(9.1) | 2(10) | |
| | **Encephalitis** | 0(0) | 2(18.2) | 2(10) | |
| | **Cerebrovascular** | 0(0) | 2(18.2) | 2(10) | |

The mean age of the drug resistant cases is less than the drug responsive group. Also, the female/male ratio in drug resistant group is less than the drug responsives. But no meaningful difference is noted according to the statistical studies.

No relation was found between the type of the antiepileptic medication (ethosuximide, carbamazepine, phenytoin) and drug resistance(*P*=0.19).

The type of the seizure (idiopathic, cryptogenic or symptomatic) was also studied which had no meaningful statistical relation to drug resistance(*P*=0.08).

As a whole, 7 patients (11.7%) were ANA-positive, 6.7% of drug resistant and 16.7% of drug responsive group showed this finding. There was no relationship between drug resistancy and ANA according to statistical studies (*P*=0.21).

The duration of ethosuximide consumption in resistant group (4 months) was less than the responsive group (36 months). It is notable that the difference was not statistically meaningful (*P*=0.06).

The duration of treatment with carbamazepine in resistant group (17.64 months) was more than the responsive one (16.14 months) but was not statistically meaningful (*P*=0.37).

The duration of phenytoin consumption in the resistant group (2.83 months) was reported obviously less than the responsive group (8.21 months) and this difference was also confirmed by statistics (*P*=0.04).

## Discussion

Drug resistance in seizures is a big challenge with a poorly understood etiology, and investigating the side effects and effects of each drug helps in creating an efficient approach to control the recurrence of seizures. (9,10) this research is one of the few studies about the relationship between drug resistance and ANA in children with epilepsy.

The mean age of the patients of our study was 72.5 months and the male/female ratio was 1.5. Based on our physical examination, none of the patients had lupus erythematosus clinical manifestations (malar rash, discoid rash, photosensitivity, arthritis, serositis, psychosis, oral or nasal ulcer). Also, we did not find a statistically significant relationship between the type of seizure or the type of drug used with resistance to treatment, and like other studies, we can suggest the choice of drug based on clinical manifestations (11).

A prospective study after 10 months follows up of ethosuximide or diphenylhydantoin therapy showed that none of the asymptomatic children with positive ANA had lupus clinical manifestations. This finding is suggestive that discontinuing the antiepileptic medication or changing its type of drug is not necessary for the asymptomatic children with positive ANA. However follow up these patients in regular intervals seems to be reasonable (12).

In our study one patient treated with ethosuximide (11.1%), 4 patients treated with carbamazepine (16%) and 2 patients treated with phenytoin (7.6%) were ANA positive in which the most risk was related to carbamazepine. Previous studies also noted higher risk of carbamazepine than phenytoin and ethosuximide (13,14).

Asadi Pooya *et al*., (15) investigated the prevalence of ANA in a population close to our study who were under monotherapy with carbamazepine, but only one patient had a positive titer. In our study, more than half of the patients with our positive antibody were treated with carbamazepine and only one of them was under monotherapy with this drug, like the mentioned study of course, in our study, we divided the population of children into the group of responders and resistant to treatment, and at the same time, it was conducted in younger children, and there is a possibility of the effect of the age of the study group in the result. However, no significant relationship between age and prevalence was found in our study.

Among ANA positive patients in our research, 5 cases (13.8%) were male and 2 (8.3%) were female. Being higher ratio of male to female was in contrast to other studies like the last study (15). This difference may be attributable to the more number of antiepileptic drugs consumed by females and their synergic effects in creating ANA positivity.

In our research, overall 7 cases (11.7%) was ANA positive. This number is obviously less than previous studies in which the prevalence of ANA positive patients was reported between 17 to 19 percent (16-18). The above reported difference, may be caused by that previous studies are mostly conducted in adults rather than children, also our patients were few; On the other hand, in previous studies the medications (sodium valproate, phenobarbital and lamotrigine) were different from ours and finally, the racial differences between Iranians and European-Americans should not be forgotten as an effective underlying cause.

In our study 2 drug resistant patients (6.7%) and 5 drug responsive patients (16.7%) were ANA positive. However no meaningful statistical relationship was found ($P$=0.21). previous studies also showed no relationship between the drug resistance and ANA positivity in epileptic patients (17).

Of course, the report of common cases of healthy people with positive titers of antibodies such as ANA can partially diminish the clinical importance of the occurrence of positive titers following the use of anticonvulsants (19).

Although in our study, mentioned epidemiological and clinical data of the patients was reported in two separate groups of resistant or responsive to antiepileptic drugs, no meaningful statistical difference was found between two groups.

The study of drug lupus in the field of anti-epileptic drugs is not still enough. perhaps, anti-double-strand DNA titer measurement in future studies can better understand the possible connections between anticonvulsants and drug lupus and justify them (20). So extensive and multi-centered researches are recommended along with examining more characteristics of patients, especially growth indices and other blood factors like serologic antibodies in larger populations.

In our study the prevalence of positive ANA in patients receiving antiepileptic drugs was less in comparison with previous studies and also was more common in males and there was no correlation between the type of anticonvulsant drug used and the type of seizure in the occurrence of treatment-resistant epilepsy.

Finally, we suggest a more comprehensive and extensive study with more cases and further follow-up period in order to find the cause of immunological reactions to antiepileptic drugs in children with epilepsy.

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