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Serum nerve growth factor levels in autistic children in Turkish population: A preliminary study

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***Background & objectives*: It has been hypothesized that abnormal levels of serum nerve growth factor (NGF) may represent a serological marker for autistic children who may develop cognitive impairment, regression and finally epilepsy. The objective of this preliminary study was to measure serum NGF concentrations of autistic children and compare these levels with those of healthy children.**

***Methods*: Consecutive children who were referred to the Paediatric Neurology and Child Psychiatry Policlinics of Dr. Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital, Turkey between February and September 2008 were included in the study. Serum samples were analyzed for NGF levels using ChemiKine NGF Sandwich ELISA Kit. Comparisons between the study and the control groups were made using student's t test and Chi-square test.**

***Results*: Forty-nine autistic children and an equal number of healthy children (control group) were included in the study. No significant difference was found between the study and the control groups in terms of children's age, while number of boys was significantly higher ($P<0.05$) in the study group. Average serum NGF concentrations were 46.94 ± 51.40 and 32.94 ± 12.48 pg/ml in the study and control group, respectively. Serum NGF concentrations were significantly higher ($P<0.05$) in the study group compared with the control group.**

***Interpretation & conclusions*: Our preliminary findings show that enhanced serum NGF concentration may be used as a potential diagnostic tool in autism, however, further studies including a large number of patients are required to confirm the findings.**

Key words Autism - child - epilepsy - nerve growth factor - neurodevelopment

Autism is a neurodevelopmental disorder of unknown origin characterized by both verbal and nonverbal abnormalities in social interaction and communication, repetitive (stereotypic) behaviours and restricted interests. Autism starts at early years of life and has lifelong effects on the patient. Although the first studies suggested a biological tendency, psychological causes have been considered the major problem in the aetiology of autism for decades. Genetic, environmental and biochemical factors have also been reported to play a role in autism aetiology[1,2].

The nerve growth factor (NGF), a member of neurotrophin family, was first characterized by Levi Montalcini and colleagues in 1951[3,4]. NGF is involved in growth, differentiation, survival and regeneration of nerve cells by stimulating Trk A (a transmembrane tysosine kinase) and p75 receptors[5,6]. NGF is mainly present in highly functional brain regions containing major cholinergic pathways such as hippocampus and cerebral cortex. It is primarily expressed in neurons under control of neural activity[7]. Glutamate and acetylcholine increase NGF expression while gamma amino butyric acid decreases its expression. Enhancement in NGF levels after brain injury is a part of neuronal recovery process[8]. NGF concentrations in serum and cerebrospinal fluid (CSF) may be enhanced in various cerebral pathologies such as hypoxia, ischaemia, trauma, age-dependent atrophy, hydrocephalus, seizures, neuroimmunological diseases and intracranial hypertension syndrome[4,8,9]. It has been hypothesized that high levels of serum NGF may represent a serological marker for autistic children who will later develop cognitive impairment, regression and finally epilepsy[10].

The aim of the present study was to detect serum NGF levels in children with autism and compare with that in healthy children is as to suggest if serum NFG levels can be considered as a serological marker for early diagnosis.

Material & Methods

In the present study, children who were referred to Dr. Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital's Paediatric Neurology and Child Psychiatry policlinics at Izmir, Turkey, between February and September 2008 were consecutively included prospectively. The study protocol was approved by the institutional ethical committee and written informed consent was obtained from parents of children. Forty nine children who were diagnosed with autism according to DSM-IV criteria[11] were included in the study group while the control group consisted of 49 healthy children with no known acute or chronic disease. Healthy children were included from children coming to healthy children polyclinics for periodic vaccination. Data regarding socio-demographic characteristics and pre-, and postnatal risk factors as well as EEG and brain magnetic resonance imaging (MRI) findings were recorded.

Blood samples (2 ml) were obtained from children in both the study and control groups and the serum samples were kept under -80ºC in polystyrene tubes and were thawed in room temperature one day prior to analysis. All samples were analyzed for NGF using ChemiKine NGF Sandwich ELISA Kit (Chemicon, Temechula, California, USA) the same day under the same laboratory conditions following manufacturer's instructions.

Results were analyzed using SPSS (version 12.0 for Windows Chicago, IL) software. Data were presented as mean ± SD, average (minimum-maximum), number of patients and per cent (%). Differences between two groups were analyzed using student's t test and Chi-square test, and $P<0.05$ was considered significant.

Results

Socio-demographic characteristics of subjects in autism (study) and healthy (control) groups are listed in Table I. The study group contained 39 boys and 10 girls while the control group contained 27 boys and 22 girls and number of boys was significantly higher ($P<0.05$) in the study group compared to the control group. No significant difference was found between the two groups in terms of mean age and educational status of their parents. While 43 (87.7%) mothers in the study group had a healthy pregnancy, six (12.2%) mothers had health problems such as repetetive urinary tract infection (4 mothers), hypertension (1 mother) and upper respiratory tract infection (1 mother). On the other hand, 44 (89.7%) mothers in the control group had a healthy pregnancy while five (10.2%) had various health problems. No significant difference was found between the two groups in terms of progress of

Table I. Socio-demographic characteristics of patients and healthy controls

Characteristics	Autism group (No. 49)	Control group (No. 49)
Age (yr)	8.38 ± 2.92	7.34 ± 3.48
Sex ratio (M : F)	39 : 10*	27 : 22
Age at motherhood (yr)	27.8 ± 6.25	28.6 ± 6.71
Age at fatherhood (yr)	31.6 ± 5.71	32.4 ± 6.61
Educational level of mother No. (%) Low High	46 (93.8) 3 (6.1)	45 (91.8) 4 (8.1)
Educational level of father No. (%) Low High	40 (81.6) 9 (18.3)	37 (75.5) 12 (24.4)

*$P<0.05$ compared to controls

pregnancy. Similarly, duration of pregnancy in the two groups did not reveal a significant difference. Although history of birth complication such as anus presentation, cord entanglement, and prolonged delivery was present in three (6.12%) children and history and findings of asphyxia at birth were present in eight (16.3%) children in the study group, no significant difference was found between the two groups in terms of birth complications. The history of a chronic psychiatric disease (depression, mental retardation, minimal brain dysfunction) was significantly higher ($P<0.05$) in the families of children in the study group compared with that in the control group (Table II). Mean nursing duration in the study group (11.5 ± 7.62 months) did not statistically differ from that in the control group. Similarly, rate of sibling death did not statistically differ between the two groups.

Mean age of diagnosis was 3.61±1.60 (range: 1.5-8) years. Sixteen patients (32.6%) had retardation in motor functions such as crawling and walking. Vaccinations were completed in 45 (91.8%) patients. While none of the patients had a history of epilepsy, pathologic EEG was observed in nine children. Pathological findings were also detected in brain MRI of four patients. Average serum NGF concentrations in the study and control groups were 46.94 ± 51.40 and 32.94 ± 12.48 pg/ml, respectively. Serum NGF concentrations were significantly higher ($P<0.05$) in the study group compared with those in the control group.

Table II. Clinical characteristics of patients and controls

Characteristics	Autism group n=49 (%)	Control group n=49 (%)
Health problem during pregnancy	6 (12.2)	5 (10.2)
Preterm birth	10 (20.4)	12 (24.4)
History of asphyxia	8 (16.3)	10 (20.4)
Birth complications	3 (6.1)	6 (12.2)
Consanguineous marriage	11 (22.4)	9 (18.3)
Chronic and/or psychiatric disease in family	13 (26.5)*	4 (8.1)
Duration of nursing (months) (mean ± SD)	11.5 ± 7.62	11.5 ± 7.11
History of sibling death	3 (6.1)	2 (4.0)

*$P<0.05$ compared to controls

Discussion

The main objective of our study was to investigate serum NGF concentrations in autistic children and compare with those in healthy children. Concentrations of NGF in serum has been shown to be increased in various neurological diseases[10,12]. Therefore, it is reasonable to assume that NGF may be utilized as a serological marker in autism, a disease with cognitive dysfunction which originates from an organic impairment. Serum NGF concentrations were found to be significantly higher in autistic children compared to the healthy children in our study. Our results corroborated with those of Nelson *et al*[13] who detected high serum concentrations of various neuropeptides (BDNF, NT3, NT4/5) including NGF and neurotrophins in blood samples obtained in the first days of life from children with autism and mental retardation. Similar results have been reported by others also[12,14,15].

In conclusion, we found that the serum NGF concentrations of autistic children were significantly higher compared with those of healthy controls and this could be used as a potential serological marker for early diagnosis. Limited data from limited number of subjects are available on serum NGF concentrations in autistic children. These findings need to be investigated thoroughly by future studies with large sample.

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