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OTİZM SPEKTRUM BOZUKLUĞU OLAN HASTALARDA RİSK FAKTÖRLERİ ve DİSMORFOLOJİNİN İNCELENMESİ

Investigation of Risk Factors and Dismorphology in Patients with Autism Spectrum Disorders

Dilşad Yıldız Miniksar (0000-0002-6389-4377)

1 Yozgat Bozok Üniversitesi Tıp Fakültesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Ana Bilim Dalı, Yozgat

Sorumlu yazar yazışma adresi:

Dilşad Yıldız MİNİKSAR: Yozgat Bozok Üniversitesi Tıp Fakültesi, Çocuk ve Ergen Ruh Sağlığı ve Hastalıkları Ana Bilim Dalı, Yozgat

E-mail:
dr_dilsad1984@hotmail.com

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ÖZ

GİRİŞ ve AMAÇ: Çalışmamızda Otizm Spektrum Bozukluğu (OSB) tanısına sahip çocuk ve ergenlerin sosyodemografik özellikleri, prenatal-postnatal öyküleri,dismorfik bulguları ve olguların annelerinin gebelik öykülerinin araştırılması amaçlanmıştır.

YÖNTEM ve GEREÇLER: Bu prospektif tanımlayıcı çalışmaya OSB tanısı alan yaşları 5-17 arasında olan 74 çocuk ve ergen dahil edilmiştir. Bu çocukların zeka düzeyini belirlemek için Wechsler Çocuklar İçin Zeka Ölçeği (WISC –R) kullanılmıştır. Otistik semptomların şiddetini değerlendirmek için Çocukluk Otizm Derecelendirme Ölçeği (CARS) kullanılmıştır. Olguların annelerinin gebelik öyküsü, olguların prenatal ve postnatal öyküleri, varsa o zamanın görüntüleme raporları ile birlikte aile anamnezi ve epikriz kayıtları değerlendirilmiştir. Olguların fizik muayene ile baş çevresi ölçülmüş ve dismorfik bulguları Minör Konjenital Anomaliler için Yüzey Değerlendirme ölçeği kullanılarak belirlenmiştir

BULGULAR: Olguların annelerinin gebelik öyküleri, olguların prenatal-postnatal risk faktörleri ve dismorfoloji ASD etyolojisinde etkili bulunmuştur

TARTIŞMA ve SONUÇ: OSB etyolojisi karışık ve tam olarak aydınlatılamamış olmakla birlikte, çalışmamızdaki verilerle bu spektrumun risk faktörleri değerlendirilmiş, etyopatogenezinin aydınlatılmasına katkı sunulmuş ve OSB'nin önlenebilir risk faktörleri belirlenmiştir.

Anahtar Kelimeler: OSB, risk faktörleri, dismorfi

ABSTRACT

INTRODUCTION: The aim of the current study was to investigate the sociodemographic characteristics, prenatal and postnatal history, and dysmorphic findings of children and adolescents diagnosed with Autism spectrum disorder (ASD) and the pregnancy history of the mothers of these cases.

MATERIALS and METHODS: The prospective descriptive study included 74 children and adolescents aged 5-17 years, diagnosed with ASD. The Wechsler Intelligence Scale for Children-Revised (WISC)-R was used to determine intelligence level of these children. Childhood Autism Rating Scale (CARS) was used to evaluate the severity of autistic symptoms. The cases were evaluated in respect of the pregnancy history of the mothers, the prenatal and postnatal histories of the cases, family anamnesis with any imaging reports of that time if available, and epicrisis records. In the physical examination of the cases, head circumference was measured, and dysmorphic findings were determined according to the Surface Evaluation for Minor Congenital Anomalies scale.

RESULTS: Prenatal, postnatal risk factors, dysmorphology and pregnancy history of mothers were found to be effective in the etiology of ASD in children with ASD.

CONCLUSIONS: Although the etiology of ASD is complex and has not been fully elucidated, the data obtained in this study from the evaluation of the risk factors of this spectrum have contributed to the clarification of the etiopathogenesis, and identified the preventable risk factors of ASD.

Key words: ASD, risk factors, dysmorphia

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by deficiencies in social interaction, delayed communication skills, and repeated behaviours ignoring the social norms (1). Prevalence has been determined to be approximately 16.8/1000, and it has been reported to be seen 3-4-fold more in males than females (2). Using the criteria of DSM-5, Brown et al. (3) reported IQ (intelligence quotient) prevalence and indicated that about 40% of the ASD population are likely to present ID. Although the etiology of ASD has not been fully elucidated, it is thought to be a multifactorial hereditary disease. A recently suggested theory of ASD etiology is the older age of the parents at the time of pregnancy (4). McGrath et al also found that the children of older fathers were at a higher risk of mental disability, schizophrenia and ASD (5). Apart from the older age of the parent, a greater age difference between the mother and father has also been reported to be a high risk factor for ASD (6). In terms of psychiatric diseases, ASD is one of the most important diseases with genetic transmission, and the hereditary rate has been reported to be 90% in data obtained from family, children and twin studies (7). Concordance has been reported as 36%-96% in monozygotic twins, 0-27% in dizygotic twins, and 16% in non-twin siblings (8). Clinical studies have revealed a higher rate of psychiatric disorders in the relatives of children with ASD (9).

Several medical disorders may accompany ASD, the most common of which is epilepsy, seen in individuals with ASD at a rate 10-30-fold higher than that of the general population (10). Although the gene-environment interaction is accepted as important in the etiology of ASD, the increasing frequency of the disease demands a more detailed examination of the environmental factors. Environmental toxins, maternal diseases, pregnancy-related factors, prenatal and postnatal factors explain the heterogenous clinical table in the etiology of ASD (11,12).

The neurodevelopmental model of ASD accepts that the etiological roots of the disease can be seen up to prenatal events. As both the face and the brain develop from the neuroectoderm, which is the same embryological layer, this warrants close examination of the craniofacial anomalies which can be seen in neurodevelopmental disorders (13).

In this study , by identifying the risk factors of the disease, it was aimed to contribute to the clarification of ASD etiology, emphasize the importance of early diagnosis, and increase the possibility of preventing the disease.

MATERIALS and METHODS

Subjects: Patients were selected from those who presented at the Child and Adolescent Psychiatry Department of Bozok University Hospital between October 2019 and November 2019, and had not started treatment. The study included 74 children and adolescents aged 5-17 years, diagnosed with ASD according to DSM-5 diagnostic criteria. Patients were excluded from the study if they had severe intellectual disability (ID). Informed consent was obtained from parents and children and adolescents to participate.

Instruments for Assessment of Children and Adolescents

Structured interview form, was prepared by the researchers and from the data collected, cases were evaluated in respect of sociodemographic characteristics, family data, comorbidities, pregnancy-related factors of the mother, prenatal and postnatal history of the child, and whether or not there were any dysmorphic characteristics.

The Childhood Autism Rating Scale (CARS) consists of 15 items and all the items contribute equally to one total score. Each item is rated by half scoring between 1 and 4 (14). 15–29 points represent the child does not have autism, 30–36.5 points represent mild-moderate autism, and 37–60 points represent severe autism. For the Turkish version of the scale, item analysis shows that all items (except item 14) differentiate children with mild to severe autism (15). According to CARS total score, children with ASD were divided into two groups: children with mild-moderate ASD and children with severe ASD.

Wechsler Intelligence Scale for Children-Revised, which is the revised form of WISC developed by Wechsler in 1949, was re-edited in 1974 (16). Turkish standardization and validity and reliability studies were performed (17). The severity of intellectual disability was defined according to the international criteria proposed by the American Association on Intellectual and Developmental Disabilities: mild intellectual disability (IQ 50–69), moderate intellectual disability (IQ 35–49), severe intellectual disability (IQ 20–34) (18).

Surface Evaluation for Minor Congenital Anomalies Scale was developed by Samuel Zinner in 2000 to evaluate dysmorphic findings of children with birth defects or genetic problems. In the 6th edition of Smith's Recognizable Patterns of Human Malformation, it was accepted that there are normal standards of body measurements, and a deviation of <4% (not causing a functional or cosmetic problem) is accepted as a minor anomaly, >4% not causing functional or cosmetic problems as a variant of normal, and >4% causing significant functional or cosmetic problems as a major anomaly (19).

Procedure: CARS was administered to 74 of the children and adolescents diagnosed with ASD. WISC-R was used to assess the intellectual disability in patients with ASD. The patients were assessed using the family anamnesis, imaging reports if available, and epicrisis records from that time. In the physical examination of the cases, head circumference was measured, and dysmorphic findings were determined according to the Surface Evaluation for Minor Congenital Anomalies scale.

Statistical Analysis: The results are stated in the tables as number and percentage, mean and standard deviation values. The Independent Samples t-test was used to assess the difference in the mean age of the mothers and fathers. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

The study included 74 children and adolescents diagnosed with ASD, comprising 57 (77%) males and 17 females (23%), with a mean age of 8.89±3.43 years (range, 5-17 years). The sociodemographic characteristics of the cases are shown in Table 1.

Table 1. The findings related to the sociodemographic characteristics of the children and adolescents diagnosed with ASD (n:74)

Demographics	**n**	**%**
Gender		
Female	17	23.0
Male	57	77.0
***Mean age* (mean±SD)**	8.89±3.43	
Maternal age		
≤35 years	36	48.6
≥35 years	38	51.4
***Maternal mean age* (mean±SD)**	36.21±6.44	
Paternal age		
≤35 years	12	16.2
≥35 years	62	83.8
***Paternal mean age (mean*±SD)**	41.54±7.90	
***Mean number of siblings* (mean±SD)**	2.25±0.95	
Birth order status		

1st child	30	40.5
2nd child	27	36.5
3rd child	12	16.2
4th child	3	4.1
5th child	1	1.4
6th child	1	1.4
Parental consanguinity		
Present	5	6.8
Absent	69	93.2
Diseases concomitant to ASD		
Present	19	25.7
Absent	55	74.3
Diseases concomitant to ASD (n:19)		
Epilepsy	13	17.6
Other Diseases	6	8.1
History of Medication Use		
Used	7	9.5
Not used	67	90.5
Familial History of Psychiatric Disease		
Present	20	27.0
Absent	54	73.0
Psychiatric Diseases seen in the Family (n:20)		
ASD	7	9.5
Epilepsy	1	1.4
Panic attack	1	1.4
Cerebral Palsy	1	1.4
Mental retardation	5	6.8
Depression	2	2.7
Schizophrenia	3	4.1

*1. Findings related to the sociodemographic data:*Age >35 years was determined in 51.4% of the mothers and 83.8% of the fathers of the children and adolescents with ASD. The mean age of the fathers was determined to be statistically significantly higher than that of the mothers ($p<0.001$). The mean difference between the ages of the parents was 5.32±4.22 years (Table 2). The mean number of siblings was 2.25±0.95 and 40.5% of the cases were the first-born child.

In 25.7% of the children, a concomitant organic disease was determined and epilepsy constituted 17.6% and 8.1% the other diseases (lactose intolerance 1.4%, hearing loss 1.4%, hydronephrosis 1.4%, obesity 1.4%, congenital hypothyroidism 1.4%, subarachnoid cyst 1.4%) of this rate. In 7 (9.5%) cases, there was a history of medication; 2, taking risperidon, and 5, anti-epileptic drugs (carbamazepin, fenobarbital, topiramat, levetiracetam, valproic acid). There was determined to be a psychiatric disease in a first-degree relative of 27% of the children, with ASD in a sibling of 9.5%, followed by mental retardation in a sibling at the rate of 6.8%.

Table 2. The ages of the mothers and fathers of the children and adolescents diagnosed with ASD (n:74)

Item	**mean±SD**	**Min-Max**	**Test**
Maternal age	36.21± 6.44	26.00-57.00	*p=0.000* *t*:-4.492*
Paternal age	41.54±7.90	29.00-66.00	
Age Difference between the mother and father	5.32±4.22		

**Independent Samples t test*

2. Findings related to the İntelligence Quotient (IQ) score and The Childhood Autism Rating Scale (CARS) score; All the children with ASD had the moderate form (IQ 35–49), The mean number of IQ score was 44.6± 3.5. Symptoms of children with ASDs were evaluated with CARS. Mean number of CARS in this group was 44.6± 3.5. According to the cars total score, all children in this study had severe autism (Table 3).

Table 3. Characteristics Of The Children with ASD Included In The Study (mean ± SD)

	ASD (n=74)	
Item	**mean±SD**	**Min-Max**
Total CARS score	44.4 ±5.5	37.00-55.00
Total IQ Score	44.6± 3.5	35.00-49.00

3. Pregnancy-related findings of the mothers of the children and adolescents diagnosed with ASD; There were found to be negative factors related to the pregnancy of 33.8% of the mothers, with gestational diabetes in 6.8%, followed by pre-eclampsia in 5.4% and gestational hyperthyroidism in 5.4%. Psychiatric medical treatment had been taken during pregnancy by 2 (2.7%) mothers. The other negative factors include; radiation exposure 1.4%, hyperemesis gravidarum 1.4%, factor 5 leiden mutation 1.4%, non-steroid anti-inflammatory drug use 1.4%, traffic accident 1.4%, heart failure 1.4%, myoma uteri 1.4%, assistive reproductive techniques treatment 1.4%, active measles infection in pregnancy 1.4%, abnormal vaginal bleeding in pregnancy 1.4%. Of the total 74 mothers, 62.2% gave birth by caesarean section, and 37.8% by the normal spontaneous vaginal route (Table 4).

4. Findings related to the prenatal and postnatal risk factors of the children and adolescents diagnosed with ASD; Prenatal risk factors were found in 9.5% of the children with ASD, and 4.1% of these were determined to have a history of large for gestational age (LGA), 5.6% the other prenatal risk factors (abortus risk 1.4%, oligohydroamnios 1.4%, cord prolapse 1.4%, retarded intrauterine development 1.4%). Postnatal risk factors were determined in 31.1%, with neonatal jaundice in 12.2%, followed by hypoxia at 9.5%. The other postnatal risk factors include; premature birth 2.7%, macrosomic infant 2.7%, low birth weight 1.4%, meconium aspiration syndrome 1.4%, neonatal hypoglycaemia 1.4%. There was a history of postnatal hospitalisation in 22 (29.7%) of the 74 cases with ASD (Table 4).

Table 4. Findings related to the prenatal and postnatal periods of the children and adolescents diagnosed with ASD

Item	**n**	**%**
Negative factors in pregnancy		
Yes	25	33.8
No	49	66.2
Negative factors experienced (n:25)		
Gestational diabetes	5	6.8
Gestational hyperthyroidism	4	5.4
Pre-eclampsia	4	5.4
Use of Psychiatric Medication in Pregnancy	2	2.7
Other negative factors	10	14
Manner of Birth		
Caesarean section	46	62.2
Normal Spontaneous vaginal birth	28	37.8
Prenatal Risk Factors		
Present	7	9.5
Absent	67	90.5
Prenatal Risk Factors (n:7)		
Large for Gestational Age (LGA)	3	4.1
Other prenatal risk factors	4	5.6
Postnatal Risk Factors		
Present	23	31.1
Absent	51	68.9
Postnatal Risk Factors (n:23)		
Neonatal jaundice	9	12.2
Neonatal Hypoxia	7	9.5
Other postnatal risk factors	7	9.5
Postnatal Hospitalisation		
Yes	22	29.7
No	52	70.3

5. Findings related to the dysmorphic characteristics of the children and adolescents diagnosed with ASD; Dysmorphic findings were determined in 25 (33.8%) of the 74 children. Macrocephaly (head circumference >97th percentile) was seen in 13.5% of all cases, and ear anomalies in 12.2% of all cases (low placed ear, preauricular tag, asymmetric ears, large ears, small ears) (Table 5).

Table 5. The dysmorphic findings of the children and adolescents diagnosed with ASD (n:74)

Item	n	%
Dysmorphic findings		
Present	25	33.8
Absent	49	66.2
Dysmorphic findings (n:25)		
Macrocephaly	10	13.5
Short palpable fissure	1	1.4
Ear anomaly	9	12.2
Umblical hernia	1	1.4
Microcephaly	1	1.4
Epicantal fold	2	2.7
Ptosis	1	1.4

DISCUSSION

It has been reported in literature that ASD is seen 3-4-fold more in males than females (2). Similarly in the current study, the children and adolescents diagnosed with ASD comprised 77% males and 23% females. The effect of gender difference on the disease may be related to the developing fetal brain being exposed to a high level of testosterone. A low 2nd finger to 4th finger ratio has been found to be related to high fetal testosterone levels compared to oestrogen levels (20). There are also studies in literature that have used the 2nd finger to 4th finger ratio as a biomarker in ASD (21). In the current study, 51.4% of the mothers and 83.8% of the fathers were aged over 35 years, and in all the studies related to ASD, “advanced parental age” is accepted as the most consistent data (22). In addition to the older age of the parents, the greater age difference between the parents was an interesting finding, with the

mean age difference between the mother and father determined to be 5.32±4.22 ($p<0.001$). The effect of the older age of the father is responsible for increased de novo mutations in gametogenesis and for hereditary characteristics (7). It was determined that 40.5% of the cases were the firstborn child, but no data could be found in literature related to this. However, in a study by Lyall et al, the risk of ASD was seen to be reduced by preconceptional folic acid supplementation (23). This could be associated with the mother having less experience in the first pregnancy and neglecting vitamin support. In 25.7% of the current study ASD cases, a history of another medical disease was determined, and epilepsy comprised 17.6% of these. Although epilepsy was seen to be the most common medical condition accompanying ASD, in literature the rate of epilepsy has been reported as 20% in ASD cases with mental disability and 8% in ASD cases with normal mental status 10 . The findings of the current study were seen to be consistent with the literature. Like ASD, epilepsy is a brain disease, the etiopathogenesis of which is multifactorial, including genetic and environmental factors, just as in ASD (24).There was seen to be a psychiatric disease in a first-degree relative of 27% of the children in the current study, of which 9.5% comprised ASD in a sibling. This rate of presence of ASD in a sibling was lower than rates reported in literature. This can be explained by the small sample size of our study. The second most common psychiatric disease in the family was mental retardation. 60% of individuals diagnosed with ASD are under 50 and 50 in IQ tests (25), 20% between 50 and 70 (26) , 20% above 70 and 70 score points (27). All patients in our study had moderate mental retardation. ASD and severe ID share diagnostic features, such as deficits in social behavior, and problems with communication and stereotypies (28). Therefore, children and adolescents with severe ID were excluded from the study to avoid diagnosis confusion. All children and adolescents in our study according to CARS scores have severe ASD. ASD diagnoses sometimes change due to misdiagnosis, maturation, or treatment. In longitudinal studies of small clinic-based samples of children diagnosed with ASD at two years of age, 18% (29) and 37% (30) of the children did not show signs of autism two years later. Early and intensive therapy is one mechanism by which a child may be able to achieve optimal outcome and lose his or her ASD diagnosis (31). The fact that all cases in our study have severe ASD may be related to the age range being over 5 years old. Probably those who lost their diagnosis until the age of 5 years were eliminated and were not included in our study group. When the pregnancies of the mothers of the children and adolescents with ASD in this study were studied, pregnancy-related negative factors were determined in 33.8%, and this included

gestational diabetes at the rate of 6.8%. The results obtained from previous studies which have examined the frequency of gestational diabetes in the mothers of children with ASD have been inconsistent, but a recent meta-analysis showed that gestational diabetes is an additional risk factor for ASD (32). Maternal hyperglycemia has been associated with increased free radical production, and oxidative stress in the cord blood and placental tissue results in an impaired anti-oxidant defence system (33). In the current study, gestational hyperthyroidism and pre-eclampsia were seen to be the second most commonly experienced negative events in pregnancy (5.4% for both). There is a need for normal thyroid hormone production for there to be brain development in the fetus. In the early and mid gestation periods, the fetus is completely dependent on the mother for thyroid hormone. Low thyroid-stimulating hormone (TSH) measured in mid pregnancy and low neonatal TSH have been associated with an increased risk of ASD (34). In the current study, pre-eclampsia was seen in 5.4% of the mothers of children with ASD. In a study of a Swedish population of 1216 ASD cases born between 1987 and 2002 and a healthy control group of 6080 subjects, pre-eclampsia was determined to have increased the risk of ASD by 50% (35). As oxygenation of the placenta is disrupted in pre-eclampsia, retarded intrauterine development, fetal death, fetal thrombocytopenia, bronchopulmonary dysplasia, neurodevelopmental defects and low birth weight may be seen (36). Severe pre-eclampsia can cause the development of primary hyperthyroidism (37). Similarly, there has been reported to be a higher risk of pre-eclampsia in pregnancies with gestational diabetes compared to normal pregnancies (38). This could be the case in the current study as hyperthyroidism was found to be both associated with pregnancy and was found at a higher rate than normal in those with gestational diabetes.

The infant was born by caesarean section to 62.2% of the mothers in the current study and by normal spontaneous vaginal route in 37.8%. Previous studies have associated an impairment in cognitive functions and an increase in the frequency of ASD with caesarean section delivery (39). On the other hand, just as the increase in cesarean deliveries can be explained by medical reasons, it can also be because of a lack of communication between the doctor and the patient, the fears of women, and the influence of social and cultural beliefs. When the findings related to the prenatal and postnatal risk factors of the children and adolescents with ASD in this study were examined, there were found to be prenatal risk factors in 9.5% of the cases, and 4.1% of this rate was determined to be LGA infants. Previous studies have found a relationship between maternal hyperglycemia and several negative complications such as

LGA infant, primary caesarean rate, neonatal hypoglycemia, pre-eclampsia and birth trauma (40).

Postnatal risk factors were determined in 31.1% of the cases, of which 12.2% were seen to have neonatal jaundice. The most important neuropathological finding of neurotoxicity caused by bilirubin is cerebellar damage progressing with loss of purkinje cells (41). Similarly, one of the most repeated findings in postmortem studies in autism is the reduction of purkinje cells in the cerebellum (42). Neonatal jaundice is seen as a postnatal risk factor in ASD cases. The second most common postnatal risk factor was determined to be a history of hypoxia in 9.5% of the 74 cases. Although neonatal hypoxia has the potential to damage the central nervous system, as hypoxia also increases dopaminergic activity, it has been shown that there could be a link between this condition and the excessive dopamine activation determined in ASD (43). In 22 of the 74 cases with ASD in the current study, there was a history of postnatal hospitalisation in 22 (29.7%). Of these, 9 were determined with neonatal jaundice, and phototherapy was applied to all the infants with neonatal jaundice. Seven cases were hospitalised because of neonatal hypoxia.

When the relationship with dysmorphic findings was examined in children and adolescents diagnosed with ASD, there were determined to be dysmorphic findings in 25 (33.8%). Of these cases, macrocephaly (head circumference >97th percentile) was determined in 13.5% of all cases. In children with ASD with a normal or small head circumference measurement at birth, there is thought to be normal growth in the brain or it occurs within the first 2 years after birth (44). Especially between the ages of 2 and 4 years in ASD, there is excessive growth of the frontal lobe, temporal lobe, and amygdala, but this is not seen in the occipital lobe. The pathological growth of areas of the brain that play a role in cognitive, social and emotional functions and language development may contribute to the understanding of the functional deficits clinically observed in ASD (45-47) . An ear anomaly (low placed ears, pre-auricular tag, asymmetric ears, large ears, small ears) was determined in 12.2% of all cases of the cases with dysmorphia (33.8%). Development of the craniofacial area and the central structures of the brain responsible for the etiopathogenesis of ASD, occurs simultaneously in early fetal morphogenesis (48). When the embryonal development of the face is examined, the outline of the auricle starts to develop at the end of the 5th week, which is in the organogenesis period, 4-16 weeks after implantation (49). Exposure to teratogens in this period of the pregnancy or more different risk factors, can affect the pregnancy and increase the risk of ASD.

In conclusion, while the familial characteristics determined in these cases can be seen to contribute to the genetic aspect of ASD, the prenatal and postnatal factors, and the pregnancies of the mothers contribute to the environmental aspect.

Limitations of this study can be said to be the low number of the sample, and the prenatal and postnatal risk factors were defined from the family anamnesis, imaging reports if available, and epicrisis records from that time. Further studies of more extensive groups would be useful to strengthen the results obtained in this study.

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Ethical Standarts: The author asserts that all procedures contributing to this work comply with the ethical standards of the relevant national guidelines on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and has been approved by the institutional committees. Ethics approval for the study was obtained from the Bozok University Ethics Committee (Ref. No. 2017-KAEK-189_2019.11.13_18).

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