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ARAŞTIRMA / RESEARCH

Factors affecting clinical phenotype in children with neurofibromatosis type 1

Nörofibromatozis tip 1 olan çocuklarda klinik fenotipi etkileyen faktörler

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Abstract

Purpose: In this study, it was aimed to evaluate the clinical, radiological and genetic features of children who were followed up with the diagnosis of Neurofibromatosis type 1 (NF-1).

Materials and Methods: Patients who were 0-18 years diagnosed with Neurofibromatosis according to National Institute of Health 1988 criteria between September 2012 and September 2019 were included in the study. Patient data were collected through patient files and hospital information system.

Results: A total of 50 patients were included in the study. The male/female ratio was 0.92. The median age at the time of diagnosis was 5.6 years (age range: 1-18 years). The most common finding was cafe-au-lait spots detected in all patients. Family history was found in 60% of the patients and consanguinity between parents in 14%. Neurofibroma was detected in 12%, Lisch nodule in 36% of the patients. Axillary freckling ratio was 82%, inguinal freckling ratio was 78%. Tumors were found in 22% of the patients, optic glioma in 12%, and plexiform neurofibroma in 6%. Focal areas of signal intensity (FASI) was found 56% in cranial magnetic resonance imaging (MRI).

Conclusion: The relationship between mutation type and clinical and radiological features in NF-1 was evaluated. There was no statistically significant difference in clinical and radiological findings between patients with or without mutation. Large-scale studies are needed to reveal the factors that determine the clinical phenotype in patients with NF-1 diagnosis.

Keywords:. Neurofibromatosis Type 1, phenotype, cafe-au-lait spots

Öz

Amaç: Bu çalışmada, Nörofibromatozis tip 1 (NF-1) tanısı ile izlenen çocukların klinik, radyolojik ve genetik özelliklerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Eylül 2012 ile Eylül 2019 tarihleri arasında Ulusal Sağlık Enstitüsü 1988 kriterlerine göre 0-18 yaş Nörofibramatozis tanısı almış hastalar çalışmaya dahil edildi. Hasta verileri, hasta dosyaları ve hastane bilgi sistemi üzerinden toplandı.

Bulgular: Toplam 50 hasta çalışmaya alındı. Erkek / kız oranı 0,92 idi. Tanı anındaki ortanca yaş 5,6 yıldı (yaş aralığı: 1-18 yıl). Tüm hastalarda saptanan en yaygın bulgu Cafe-au-lait lekeleriydi. Hastaların %60'ında aile öyküsü ve %14'ünde ebeveynler arasında akrabalık saptandı. Hastaların %12'sinde nörofibrom, %36'sında Lisch nodülü saptandı. Aksiler çillenme oranı %82, inguinal çillenme oranı %78 idi. Hastaların %22'sinde tümör, %12'sinde optik gliom ve %6'sında pleksiform nörofibrom saptandı. Kraniyal manyetik rezonans görüntülemede (MRG) %56 oranında fokal alanlarda sinyal yoğunluk artışı (FASI) saptandı.

Sonuç: NF-1'de mutasyon tipi ile klinik ve radyolojik özellikler arasındaki ilişki değerlendirildi. Mutasyonlu ve mutasyonsuz hastalar arasında klinik ve radyolojik bulgular açısından istatistiksel olarak anlamlı bir fark yoktu. NF-1 tanılı hastalarda klinik fenotipi belirleyen faktörleri ortaya çıkarmak için geniş ölçekli çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Nörofibromatozis Tip 1, fenotip, cafe-au-lait lekeleri

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INTRODUCTION

Neurofibromatosis is an autosomal dominant disease, causing complex multisystem involvement. The most common type is Neurofibramatosis type 1 (NF-1). The incidence of NF-1 is 1/3000[1]. Neurofibramatosis type 1 was first described by Friedrich Daniel von Recklinghausen in 1882[2]. The Neurofibramatosis type 1 (Nf1) gene, isolated in 1990, is a large gene containing 350kb of genomic DNA. Inactivation of the gene (by mutation or loss of allele) leads to loss of function and the subsequent development of many different tumor types seen in the disease[3]. 50% of neurofibromatosis type 1 cases are hereditary, the rest are due to de novo Nf1 mutation [4]. Patients with NF-1 have are at high risk for cancer, fractures, speech disorders, cardiovascular abnormalities, congenital anomalies and learning disabilities[5]. All ethnicities, races, and sexes are affected with equal frequency[6]. Approximately 46% of patients with sporadic NF-1 (ie, de novo mutations) do not meet criteria by age 1 year. If NF-1 is suspected, annual monitoring until late childhood is necessary because 97% of children with at least 1 feature of NF-1 eventually meet diagnostic criteria by age 8 years[7]. The Nf1 gene is so large, there are many mutations that have not been identified yet. Symptoms and signs in patients vary widely. It is one of the best known cancer predisposition syndrome. However, while some of the patients never have cancer in lifetime; some have more than one malignant tumor. It is thought that clinical phenotypic differences between patients may be related to genetic characteristics. In this study, it was aimed to evaluate the clinical, radiological and genetic features of children who were followed up with the diagnosis of NF-1.

Table 1. Clinical diagnostic criteria for neurofibromatosis type 1[8]

Criteria
1. ≥6 Cafe-au-lait macules (CALMs) >5 mm in diameter in prepubertal individuals and >15 mm in postpubertal individuals
2. ≥2 neurofibromas of any type or 1 plexiform neurofibroma
3. Freckling in the axillary or inguinal regions
4. Optic glioma
5. ≥2 Lisch nodules
6. A distinctive osseous lesion
7. First-degree relative with NF-1 based on above criteria
*At least 2 criteria must be met for diagnosis.

MATERIALS AND METHODS

This study was designed retrospectively. Patients who were 0-18 years diagnosed with NF according to National Institute of Health 1988 criteria and Nf-1 gene mutation analysis between September 2012 and September 2019 in Adana City Education and Research Hospital, Child Health and Diseases Clinic were included in the study. There were 124 patients who met the criteria for NF-1 and were followed-up at Adana City Education and Research Hospital, Pediatric Hematology/Oncology department. In 1988, the diagnostic criteria of Neurofibromatosis type 1 were defined by the National Institute of Health (NIH). The diagnosis is based on the presence of two or more of the criteria listed in Table 1[8]. Patients without genetic analysis were excluded from the study. Fifty patients who had previously undergone NF-1 gene analysis were included in the study.

Patient data were collected through standardized patient files designed specially for NF-1 patients. These files were completed for each patient with NF-1 by the same physician. This study was approved by the Adana City Education and Research Hospital Clinical Research Ethics Committee with the decision dated 23.10.2019 and numbered 580.

Gender, age, follow-up period, mother and father kinship, family history of Neurofibromatosis type 1, cafe-au-lait spots, neurofibroma, plexiform neurofibroma, Lisch nodule, axillary freckling, inguinal freckle, bone dysplasia, optic glioma, scoliosis , cranial and spinal magnetic resonance imaging (MRI), blood pressure, body weight and percentile, height and percentile, chemotherapy history, radiotherapy history, behavioral problems and learning disabilities based on parental statement and genetic test results were recorded and analyzed.

Whether patients had malnutrition was evaluated according to the Gomez classification. Accordingly, the patient's weight / weight of a healthy child of the same age x 100 is calculated. Found value; If> 90% is considered normal, 75-89% mild malnutrition, 60-74% moderate malnutrition, <60% severe malnutrition[9]. American Center for Disease Control (CDC) criteria were used to determine whether patients had obesity or not. According to this classification; between 85 and 95th percentile was classified as "overweight", 95th percentile and above were classified as "obese"[10].

Genetic analysis

The genetic analysis method used in all patients is DNA sequence analysis (next generation sequencing)[11]. In the method used, next generation DNA sequencing was performed using Ion S5TM in amplicons obtained from DNA material obtained from peripheral blood using primer pairs specific to Nf1 and Nf2 genes. Exons and exon-intron junctions have been studied. The detected variants were evaluated with the specified online databases and in-silico algorithms. Variants with an allele frequency <5% are reported. The variants detected were verified by Sanger sequencing. Pathogenicity classification of variants was made in accordance with the guideline published by The American College of Medical Genetics and Genomics (ACMG) in 2015[12].

Statistical analysis

The statistical analysis of the study was carried out with the program 'Statistical Package for Social Sciences' version 20 (IBM Corp., Armonk, NY, USA). Demographic data of the patients were given with descriptive statistics. Kolmogorov Smirnov test was used to determine normal distribution of numerical variables. In the comparison of categorical variables between groups, chi-square test and Fisher exact tests were used. The statistical significance level (p) was accepted as 0.05 and below in all analyzes.

RESULTS

A total of 50 patients were included in the study. The male / female ratio was 0.92. The median age at the time of diagnosis was 5.6 years (age range: 1-18 years), and the most common age group was 0-10 years. The mean follow-up time was 32.78 ± 26.46 months. Family history was found in 60% of the patients and kinship in parents were detected in 14%. Clinical fatures of NF-1 that found in patients were given in Table 2.

Table 2. Clinical fatures of neurofibromatosis type 1

Parameters		Number (n)	Percent (%)
Family history	Yes	30	60
	No	20	40
Cafe-au-lait	Yes	50	100
	No	0	0
Solitary neurofibroma	Yes	6	12
	No	44	88
Plexiform neurofibroma	Yes	3	6
	No	47	94
Lisch nodule	Yes	18	36
	No	32	64
Optic pathway glioma	Yes	6	12
	No	44	88
Axillary freckling	Yes	41	82
	No	9	18
Inguinal freckling	Yes	39	78
	No	11	22
Scoliosis	Yes	6	12
	No	44	88

An abnormality was found in cranial MRI in 42% of patients (n=21). Focal areas of signal intensity (FASI) was found in 56% (n=28). There was 1 patient with an abnormality detected in spinal MRI. Bulging was detected at the L3-S1 level in this patient. Scoliosis was detected in 12% of the patients Central nervous

system tumors were found in 11 of patients. Optic tract glioma was detected in 6 patients with tumor, pilocytic astrocytoma in 3 patients, medulloblastoma in 1 patient, and meningioma in 1 patient. The age range of the patients with tumors ranged from 2 years 6 months to 10 years 7 months, 8 of them were under 10 years old. Other lesions included periventricular gliosis, gliosis in the cerebellopontine region and mesencephalon, and hypointense lesions in the occipital region. Blood pressure was detected above 95th percentile according to height percentile in 2 patients. Short stature was detected in 6% of the patients. Moderate malnutrition was observed in 4% of patients and mild malnutrition in 30% of patients accoeding to Gomez classification.[9] Body mass index (BMI) values of children were converted to percentile values by using BMI percentile curves according to age. Eight of the patients (16%) were found to be "overweight" with a BMI of 85-95th percentile. 3 (6%) of the patients were found to be "obese" with BMI at the 95th percentile and above according to CDC criteria.[10]

Table 3. Pathogenicity distribution of variants of patients with NF-1

Parameters		Number (n)	Percent (%)
Pathogenicity	possibly benign	1	3
	unknown clinical significance	1	3
	possibly pathogenic	6	17
	pathogenic	27	77
Total		35	100

Table 4. Mutations detected regions of patients with neurofibromatosis type 1

Mutation Site	Number of patients	Percent (%)
Exon 1	1	2
Exon 14	1	2
Exon 16	1	2
Exon 17	1	2
Exon 18	2	4
Exon 20	1	2
Exon 21	4	8
Exon 23	1	2
Exon 24	2	4
Exon 25	1	2
Exon 26	1	2
Exon 28	2	4
Exon 3	1	2
Exon 30	1	2
Exon 30 and Exon 2	1	2
Exon 37	1	2
Exon 46	1	2
Exon 5	1	2
Exon 51	1	2
Exon 8	1	2
Exon 9	1	2
Exon27	1	2
Intron 11 (Detected in the NF2 gene.)	1	2
Intron 2	1	2
Intron 27	2	4
Intron 31 and Intron 21	1	2
Intron 7	1	2
NF1 and NF2 gene change not detected	15	30
Exon 6 (Detected in the NF2 gene.)	1	2
Total	50	100

Table 5. Mutated variants of patients with neurofibromatosis type 1

Variant	Number of patients	Percent (%)
c.1013A>G (p.Asp338Gly)	1	2
c.1123-6C>T	1	2
c.1541_1542delAG (p.Gln514Argfs*43)	1	2
c.1756_1759delACTA (p.Thr586Valfs*18)	1	2
c.1908delT (p.Ser637Valfs*51)	1	2
c.204+1G>A	1	2
c.2041C>T (p.Arg681*)	1	2
c.2072T>C (p.Leu691Pro)	1	2
c.233delA (p.Asn78Ilefs*7)	1	2
c.2407C>T (p.Gln803*)	1	2
c.2446C>T (p.Arg816*)	1	2
c.2533T>C (p.Cys845Arg)	1	2
c.2540T>C (p.Leu847Pro)	2	4
c.3005delT (p.Asn1004Ilefs*8)	1	2
c.3189delT (p.Thr1065Glnfs*12)	2	4
c.3301C>T (p.Gln1101*)	1	2
c.3462_3463delTG(p.Ala1155Glnfs*39)	1	2
c.3540delG (p.Glu118lysfs*3)	1	2
c.3709-2A>G	2	4
c.3826C>T (p.Arg1276*)	2	4
c.4084C>T (p.Arg1362*)	1	2
c.4084C>T (p.Arg1362*) and c.215T>C (p.Val72Ala)	1	2
c.4174-1G>A ve c.2851-16T>C	1	2
c.495_498delTGTT (p.Cys167Glnfs*10)	1	2
c.5110delA (p.Arg1705Glyfs*5) and c.5116C>G (p.Leu1706Val)	1	2
c.586C>T(p.Arg196*)	1	2
c.60+1delG	1	2
c.6852_6855delTTAC (p.Tyr2285Thrfs*5)	1	2
c.731-5T>G	1	2
c.7537C>T (p.Gln2513*)	1	2
c.784delC (p.Arg262Valfs*19)	1	2
NF1 and NF2 gene change not detected	15	30
Total	50	100

Table 6. Clinical fatures of NF-1 patients without mutations (n=15)

Parameters		Number (n)	Percent (%)
Family history	Yes	6	40
Cafe-au-lait	Yes	15	100
Solitary neurofibroma	Yes	2	13.3
Plexiform neurofibroma	Yes	1	6.6
Lisch nodule	Yes	4	26.6
Optic pathway glioma	Yes	1	6.6
Axillary freckling	Yes	11	73.4
Inguinal freckling	Yes	8	53.3
Scoliosis	Yes	3	20

Genetic abnormality frequency was 70%. Mutations were detected in 35 (70%) of patients and no mutation was detected in 15 (30%). Frameshift mutation was detected in 30% of the patients, nonsense mutation in 20%, missense mutation in 14%, and splice-site mutation in 14%. In addition, 4

patients had multiple mutations. The distribution of these mutations according to the pathogenicity classification is given in Table 3. Detailed information on the mutation sites and variants of mutations detected in NF-1 patients are shown in Tables 4 and 5. No novel mutations were detected in the patients. Genetic analysis was not performed in cases without mutation in terms of diseases associated with NF-1. The clinical characteristics of the patients with no mutations are given in Table 6. There was no statistically significant difference in terms of malignant tumor frequency between patients with mutation and without mutation.

DISCUSSION

Neurofibromatosis type 1 is a multisystemic, autosomal dominant inherited genetic disease that requires a multidisciplinary approach for management[1]. NF-1 patients have a high risk of cancer, skeletal fractures, speech disorders, cardiovascular abnormalities, congenital anomalies and learning difficulties[5]. The diagnosis is made according to the NIH 1988 clinical diagnostic criteria[8]. Clinical features of NF-1 detected in our patients were similar with many studies reported in literature previously[13,14].

Mutations were found in 35 (70%) of the patients in our study. Frameshift mutation was detected in 30% of the patients, nonsense mutation in 20%, missense mutation in 14%, splice-site mutation in 14%. Frameshift mutation was the most frequent mutation type detected in our patients. Interstingly, mutations in exon 6 and intron 11 were found on the Nf-2 gene in 2 patients. Among these patients who were diagnosed with NF-1 with clinical findings, it is seen that NF-2 disease does not cause a clinical picture because the intron 11 mutation in the Nf-2 gene is benign. Mutation in exon 6 is a pathogenic mutation. This patient, who has a mutation in the Nf-2 gene in molecular tests followed by the diagnosis of NF-1 with clinical findings, should be followed up closely in terms of clinical findings of NF-2 that can be overt in time. The genetic studies of Alkindy et al. revealed a new potential NF-1 genotype-phenotype correlation. NF-1 mutations are divided into five categories as frameshift mutations, splice-site mutations, nonsense mutations, missense mutations, and other mutations. It has been reported that NF-1 splice site mutations are associated with the tendency to develop neoplasms consisting mostly of central nervous system gliomas and malignant perypheral nerve sheath tumors[15]. In the study conducted by Xu et al., the frame shift mutation was detected in all three affected patients[16]. Corsello et al. reported the rate of nonsense mutations, splice mutations and missing mutations as 80% in their study[17].

In our study, the most common finding in patients without NF-1 mutation was cafe-au lait spots. However, a group of genetic disorders presenting with cafe-au-lait macules, which include Legius syndrome, Noonan syndrome with multiple lentigines or LEOPARD syndrome (Lentigines, Electrocardiographic conduction defects, Ocular hypertelorism, Pulmonary stenosis, Abnormalities of the genitalia, Retarded growth resulting in short stature, Deafness), and familial progressive hyperpigmentation are difficult to distinguish from NF-1 at early stages, using skin appearance alone[18].

Problems such as low body weight and short stature can be seen in NF-1[19]. In our study, the mild malnutrition rate was 30% and the moderate malnutrition rate was 4% according to the Gomez classification. Souza et al. found in their study that 6 (10%) of 60 NF-1 patients were low-weight, and 72% of patients had low calorie consumption[19]. In our study, the rate of short stature was 6%. Clementi et al. reported short stature rate of 15% in NF-1 patients in their study in 1999[20]. Szudek et al. reported the short stature rate of 13% in NF-1 patients in their study in 2000[21]. Souza et al. reported short stature at a rate of 28.3% in NF-1 patients in their study in 2016[22]. Obesity is one of the conditions that can be seen in NF type 1 patients. In our study, obesity was detected at a rate of 6%. Sani et al. reported the rate of obesity with insulin resistance as 11.1% in NF-1 patients in their study [23].

In our study, 11 of the 50 patients had malignant tumors (22%). Optic tract glioma was the most common tumor type detected in our patients. Malignant tumors associated with NF-1 include gliomas, malignant peripheral nerve sheath tumors, leukemias, pheochromocytomas, rhabdomyosarcoma, gastrointestinal stromal tumors, breast cancers, melanomas, non-Hodgkin lymphoma and carcinomas[24]. In the study of Lobbous et al. In 2020, NF-1 was found to be associated with a heterogeneous glioma pattern with different genetics from sporadic gliomas[25]. Taylor et al. reported that NF-1 commonly affects the optic nerves as well as many other sites in other regions[26]. In our study the frequency of tumor incidence in patients with NF-1 is similar to the literature. However, there are major

limitations of our study including short follow-up duration. In a longer follow-up period, tumor incidence might have been increased. Furthermore, our small study population might have not reflected the real association between clinical phenotype and Nf-1 mutation types.

In conclusion, NF-1 is the most common neurocutaneous disease in the population and has diverse clinical features. There is ongoing debate on which factors affect the clinical phenotype. While some of the patients present with only mild clinical features, a substantial number of patients present with full-blown disease with devastating manifestations such as malignant tumors, cognitive impairment and severe morphological and functional defects. Our study investigated factors affecting NF-1 clinical fenotype in a small number of patients. Large-scale multicentric studies are needed to reveal the relationship between Nf-1 mutation types and clinical phenotype in a large number of patients with NF-1.

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