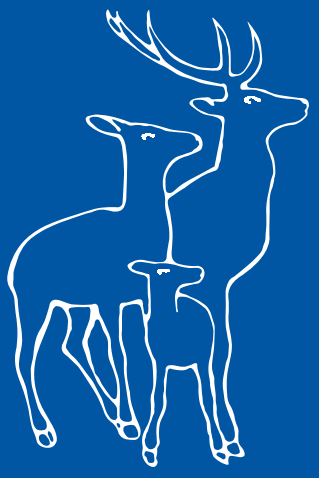

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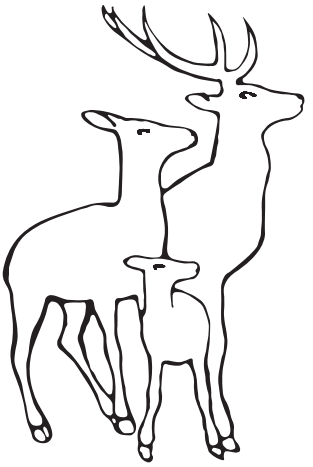

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Review

Neurofibromatosis type 1-associated tumors in children

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ABSTRACT

Neurofibromatosis type 1 (NF1) is characterized by the involvement of multiple systems, including dermatological, neurological, skeletal, and cognitive manifestations. NF1 exhibits almost complete penetrance, with a wide range of symptoms that usually develop over the course of a person's lifetime. The most obvious signs are café-au-lait macules, neurofibromas and axillary or inguinal freckling.Patients with NF1 are predisposed to developing benign and malignant tumors. Some of these tumors are exhibited during childhood. The rate of cancer development over a person's lifetime is higher for patients with NF1 than for the general population. Malignancies associated with NF1 include low grade gliomas, malignant peripheral nerve sheath tumors, juvenile myelomonocytic leukemias, pheochromocytomas, gastrointestinal stromal tumors, rhabdomyosarcomas, breast cancers, malignant melanomas, acute lymphoblastic leukemias, non-Hodgkin lymphomas, carcinoid tumors, and Wilms tumors. The identification of patients with NF1 and their interittent follow-up are important for the early detection of potential complications, especially tumorigenesis. This review aimed to summarize NF1-associated tumors in pediatric patients and recently developed targeted therapies for treating these tumors.

Key words: child, neurofibromatosis type 1, neoplasms, *NF1* gene.

Neurofibromatosis type 1 (NF1) is an autosomal dominant neurocutaneous syndrome resulting from mutations in the NF1 gene found on chromosome 17q11.2. The involvement of multiple systems is a characteristic feature, with manifestations occurring in the dermatological, neurological, skeletal and cognitive functions. . Approximately one in 3,000 people across the globe are affected by NF1, with no difference in terms of ethnicity or sex.[1] De novo mutations account for around 50% of cases, while the remaining 50% are inherited in an autosomal dominant manner.[2] NF1 exhibits almost complete penetrance, with a wide range of symptoms that usually develop over the course of a person's lifetime.[2] The most obvious signs are café-au-lait macules, neurofibromas and axillary or inguinal freckling.[3] Although café-au-lait macules are the most common symptom of NF1, the presence of café-au-lait macules alone may also be seen in other genetic disorders such as constitutional mismatch repair deficiency syndrome, McCune-Albright Syndrome, Legius syndrome, multiple familial café-au-lait, Cowden syndrome, and Leopard/multiple lentigenes syndrome.[4] A number of other systemic complications have been identified, including optic pathway gliomas and skeletal abnormalities. There is also an increased risk of malignancy. The variability in phenotype emphasises the importance of a personalized approach to diagnosis and treatment. This is crucial for ensuring effective treatment and improving patients' quality of life. The revised 2021 guidelines state that a diagnosis of NF1 can be made if an individual exhibits two or more of the following manifestations:

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a. Six or more café-au-lait macules (greater than 5mm in pre-pubertal children or greater than 15 mm in post-pubertal individuals).

b. Freckling in the axilla (armpit) or groin.

c. Two or more neurofibromas of any type, or one plexiform neurofibroma.

d. Two or more Lisch nodules or two or more choroidal abnormalities.

e. Optic pathway glioma.

f. A distinctive osseous lesion such as sphenoid dysplasia; anterolateral bowing of tibia (tibial dysplasia) or pseudarthrosis of a long bone.

g. A pathogenic *NF1* gene variant, or a first-degree relative meeting diagnostic criteria.[1]

Neurofibromin, which is primarily found in neurons, Schwann cells and glial cells, is a large, multifunctional protein that is encoded by the tumor suppressor gene *NF1*.[3] Neurofibromin is involved in several cell signaling pathways, including the Ras/MAPK, Akt/mTOR, and cAMP/PKA pathways, and regulates many cellular processes. The loss of neurofibromin results in uncontrolled cell proliferation, leading to tumor development associated with NF1.[3] Patients with NF1 are predisposed to developing benign and malignant tumors. The lifetime cancer development rate in patients with NF1 is increased compared to the normal population. Table I shows the cancers associated with NF1. The identification of patients with NF1 and their intermittent follow-up are important for the early detection of potential complications, especially tumorigenesis. This review aimed to summarize NF1-associated tumors in pediatric patients and recently developed targeted therapies for treating these tumors.

Cutaneous Neurofibroma and Plexiform Neurofibroma (PN)

The presence of cutaneous neurofibromas on the face and limbs can cause distress and social anxiety. Their raised appearance can cause itching or pain, and friction or pressure from clothing when moving around.It is estimated that 20–50% of patients with NF1 will develop PN, which may appear at birth or during the first few years of life, localizing to the craniofacial, paraspinal, mediastinal, extremities, and retroperitoneal regions, leading to significant complications. Congenital ones, in particular, gradually enlarge and feel worm-like when palpated. Additionally, PN exhibits progression along the nerve trunk. Among patients with PN, 8%–12% develop malignant peripheral nerve sheath tumors (MPNSTs).[5] A careful examination is needed to check for PN in all individuals with NF1. This should be followed by monitoring to detect any growth of PN. . A standard evaluation includes a medical history, physical and neurological examination.[5] The symptoms of PNs include pain, facial disfigurement, neurological deficits, deformities, orthopedic problems, and airway obstruction. Although PNs are benign tumors, treatment may be necessary due to their location and the resulting morbidity and functional

Table I. NF1-related malignancies

Strongly associated malignancies	Possibly related malignancies
Low grade gliomas	Breast cancer
Malignant peripheral nerve sheath tumor	Malignant melanoma
Juvenile myelomonocytic leukemia	Acute lymphoblastic leukemia
Pheochromocytoma	Non-Hodgkin lymphoma
Gastrointestinal stromal tumor	Carcinoid tumor
Rhabdomyosarcoma	Wilms tumor

NF1: neurofibromatosis type 1

impairment.[6] The goal of treatment is usually to improve or prevent PN-associated morbidity. The presence of morbidity, especially when it does not respond to symptomatic treatment, is of paramount importance. The preferred therapeutic strategy for PNs is total surgical excision if the surgery can be performed without causing morbidity.[7] Most superficial PNs can be surgically excised, alleviating the symptoms. However, most patients are not eligible for surgery as the tumor is located deeply along the nerve tract. Most patients undergoing subtotal excision exhibit PN progression. The discovery of the molecular pathogenesis and the biological basis of this disorder has enabled the development of targeted therapies. In the last two decades, clinical trials have evaluated the therapeutic efficacy of imatinib, sirolimus, tipifarnib, pirfenidone, peginterferon, trametinib, cabozantinib, and selumetinib in NF1-associated PN.[8-16] Selumetinib treatment resulted in a 70% reduction in pain and a reduction in tumor size of between 30% and 50%.[15] However, Food and Drug Administration (FDA)-approved drugs were not available for PNs until recently. MAPK (mitogen-activated protein kinase) kinase (MEK) inhibition is an effective treatment strategy for PN. In April 2020, the FDA approved selumetinib, an oral MEK-1/2 inhibitor, for treating symptomatic and inoperable PN in pediatric patients with NF1 aged ≥ 2 years. Additionally, the MEK inhibitor mirdametinib has been reported to exert therapeutic effects on PN[17] and was approved by the FDA in February 2025 for the treatment of pediatric patients (aged ≥ 2 years) with symptomatic and inoperable PN. The results of clinical trials demonstrated that mirdametinib effectively reduced the size of PNs by 41–52% in both adult and pediatric patients.[17] Studies evaluating the efficacy of MEK inhibitors in PN are summarized in Table II.

Malignant Peripheral Nerve Sheath Tumor (MPNST)

MPNST is an aggressive spindle cell sarcoma that arises from peripheral nerve sheath cells. It is one of the most common non-rhabdomyosarcoma soft tissue sarcomas in children. The incidence rate of MPNST is rare, but MPNST has been diagnosed in 20% to 50% of patients with NF1.[18] In around half of the cases, MPNST develops on the basis of a pre-existing PN. Compared to other non-rhabdomyosarcoma soft tissue sarcomas, MPNST has particular characteristics. For example, it often arises at axial sites, such as the trunk and head–neck region, while most other non-rhabdomyosarcoma soft tissue sarcomas generally develop in the extremities. MPNST also shows marked local invasiveness. In recent years, the main international pediatric sarcoma cooperative groups have published two prospective protocols specifically designed for non-rhabdomyosarcoma soft tissue sarcomas[19,20], and have defined the current risk-adapted multimodal standards of care for non-rhabdomyosarcoma soft tissue sarcomas.

Table II. Efficacy of MEK inhibitors in children with NF1 and PN

MEK inhibitor	NCT Number	Phase	>20% decrease from baseline PN volume
Selumetinib	NCT01362803	1	17/24 (71%)
	NCT01362803	2	34/50 (68%)
Mirdametinib	NCT02096471	2	8/19 (42%)
	NCT03962543	2	7/20 (35%)
Cabozantinib	NCT02101736	2	8/19 (42%)
Trametinib	NCT02124772	1/2	12/26 (46%)
Binimetinib	NCT03231306	2	13/20 (65%)

Adapted from Armstrong et al.[6]
MEK: mitogen-activated protein kinase kinase, NCT: national clinical trial, NF1: neurofibromatosis type 1, PN: plexiform neurofibroma.

The presentations of MPNST are pain, bleeding and rapid growth by 20% of a previously known PN. The magnetic resonance imaging (MRI) and positron emission tomography – computerized tomography (PET-CT) results support the suspicion of conversion to MPNST. MPNST is an aggressive, fatal disease with an overall survival rate of 20%–40%.[21] Total surgical excision is the preferred treatment for MPNST as the tumor is chemoresistant. R0 resection is important for improving survival rates. However, adequate surgery is often not possible for deep tumors that extend to adjacent structures. The involvement of major nerves, which is typical of MPNST, often makes the tumor unresectable. The lack of local control is generally reported as the main cause of treatment failure, which can have a considerable effect on patient outcomes. The role of radiotherapy in MPNST is controversial, especially for pediatric patients. Potential side effects must be considered before using radiotherapy. Although radiotherapy is used to provide local control in MPNST, its effect on overall survival has not been demonstrated. If radiotherapy is unavoidable, it is essential to limit the total dose and field size. For NF1 patients who require radiation therapy and are not limited by financial constraints, proton beam therapy is a sensible option.[22] Proton therapy reduces the dose to organs at risk, making a lower integral dose achievable.[23] Given the critical locations of MPNSTs and the young age of patients, proton therapy seems an appropriate treatment strategy in order to ensure local control for this group. A recent series by Ferrari et al. suggests that a combined local treatment that included both surgical resection and radiotherapy could improve local control.[24] Systemic chemotherapy could be considered as the primary medical treatment. In most cases, systemic chemotherapy formed part of the treatment scheme. Patients with high-grade tumors larger than 5 cm generally received adjuvant chemotherapy after initial R0/R1 resection.[24] Chemotherapy protocols, including ifosfamide and adriamycin, are preferred in non-metastatic and metastatic cases.[25]

The potential therapeutic targets for MPNST include receptor kinases, the MAPK pathway, and the phosphoinositide 3-kinase – protein kinase B – mammalian target of rapamycin (PI3K-AKT-mTOR) pathway. These targets are present at all three levels of physiological signal transduction. Similar to PN, MAPK pathway inhibition with MEK inhibitors is a therapeutic strategy for NF1-associated MPNST. The FDA has not approved selumetinib for the treatment of MPNST. However, studies are ongoing to evaluate the efficacy of MEK inhibitors in PN and MPNST (NCT03433183 and NCT02124772). The use of MEK inhibitors is not recommended as a standalone treatment. The SARC031 study (NCT03433183) examined the efficacy of the combination of selumetinib and the mTOR inhibitor sirolimus in patients with nonresectable or metastatic MPNST. Positron emission tomography-computed tomography scans revealed that this combination achieved partial metabolic responses but did not translate into treatment success.[26]

Targeting tyrosine kinase receptors alone or in combination with chemotherapy may inactivate the MAPK or mTOR pathways. In a randomized phase 2 trial, the event-free survival rate in patients treated with doxorubicin and olaratumab (an anti-platelet-derived growth factor receptor alpha antibody) combination therapy was higher than that in patients treated with doxorubicin monotherapy.[27] Receptor tyrosine kinase inhibitors, such as erlotinib, sunitinib, sorafenib, cediranib, and dasatinib, can exert growth-inhibitory effects on NF1-related tumors. However, several phase I trials have reported that receptor tyrosine kinase inhibitors are ineffective. Limited studies have evaluated the efficacy of receptor tyrosine kinase inhibitors for NF1-related tumors.[28-33] Additionally, receptor tyrosine kinase inhibitors are associated with several side effects. In a study on 25 adult patients with MPNST, only three patients achieved stable disease with the combination therapy of everolimus and bevacizumab.[34] Anti-programmed cell death-1 ligand 1 (PD[L]-1) inhibitors, which are a type

of immunotherapy, are effective in treating various cancers, including melanoma, non-small cell lung cancer, and mesothelioma. One study investigating the effect of pembrolizumab on MPNSTs is currently ongoing (NCT02691026). Oncolytic viruses are reported to be effective in vivo. One study is investigating the effects of the oncolytic measles virus on patients with MPNST (NCT02700230).

Optic Pathway Glioma and Low Grade Glioma

Optic pathway glioma (OPG)

OPGs, which are the predominant pilocytic astrocytomas, are typically diagnosed within the first decade of life. OPGs affect the axons of the visual pathway and may affect the optic nerve, optic chiasm, optic tracts, optic radiation and hypothalamus individually or in combination. Children without a known OPG should undergo annual vision screening until the age of eight, and then every two years until the age of 18, since vision loss is less common in older age groups.[35,36] Routine MRI follow-up is not recommended if there are no visual symptoms.[36] The standard imaging modality for OPGs is an MRI scan of the brain and orbit.[37,38] Children with NF1 who experience unexplained vision loss or new-onset optic nerve pallor should undergo an MRI scan to evaluate their visual pathways. Although OPGs are asymptomatic, they may present with visual complaints or endocrinological aberrations.[39] It can be difficult for parents to recognise deterioration of vision in a young child, and it can often go unnoticed. The symptoms vary depending on where the tumor is located. Those confined to the optic nerve usually present with decreased visual acuity. Other symptoms include loss of colour vision, loss of visual field, nystagmus, proptosis and strabismus. In patients with NF1-associated OPGs, treatment is initiated when there is evidence of progressive visual loss.[35] Impaired visual field and visual acuity require treatment. Visual acuity is the most important factor in deciding whether to treat NF1-related OPGs or not.[40] Visual acuity is measured using the Snellen chart. The compliance of pediatric patients in visual examinations is challenging. These children may experience difficulties such as young age, developmental delays, attention problems and adaptation issues. In addition, as visual maturation is not complete in children under six years of age, normal visual acuity thresholds vary according to age. Visual field evaluation is very important in OPG; however, computerized and kinetic visual field tests may be difficult to perform in young children due to compliance problems. Thus, ocular coherence tomography can be used as an objective measure of visual acuity in pediatric patients.[41] The unpredictableinimize of OPGs has led to much controversy surrounding follow-up and treatment decisions. Once detected on an MRI scan, they may remain the same size, grow or spontaneously shrink during the follow-up period.[38] Most clinicians accept visual examination as the follow-up criterion, as changes in vision influence decisions regarding follow-up and treatment. However, when visual acuity is unreliable due to problems with children's compliance, MRI results can inform follow-up and treatment. The primary objective of therapy is to minimize the risk of long-term, substantial visual impairment. Decreased visual acuity and radiographic tumor progression are the most common primary indications.[42] In the event of clinical progression, the main treatment option is chemotherapy. Surgery and radiotherapy are not preferred treatment options for NF1-related OPGs, despite being commonly used for other brain tumors. The first-line systemic chemotherapy for patients with OPGs is carboplatin and vincristine.[42] Report of good outcome has been documented concerning the use of first-line cisplatin and etoposide in combination.[43] For patients who do not respond to these drugs, vinblastine or a combination of irinotecan and bevacizumab may be preferred.[42] Radiotherapy is not preferred owing to the risk of developing secondary malignant tumors.[44] It is not possible to perform a total surgery due to the location of the tumor.[35]

Low grade glioma (LGG)

The incidence rate of brainstem gliomas among patients with NF1 is 10%, with most being low-grade tumors.[45] Brainstem gliomas are asymptomatic and rarely obstructive. Close follow-up without treatment is preferred. However, hydrocephalus can develop due to aqueductal stenosis, which can cause headaches and vomiting.[39] The initial treatment approach is conservative. Chemotherapy and surgery are preferred in case of disease progression.[39,46] Most other NF1-related glial tumors are asymptomatic and of low grade, with the most common occurrence site being the temporal lobe, cerebellum, thalamus, basal ganglia, or spinal cord.[46] The treatment choice is dependent on the location and symptoms and includes surgical resection, chemotherapy, and/or conservative approaches.[47]

Targeted therapy for OPG and LGG

Molecular profiling studies have revealed that the pathogenesis of pediatric OPGs and LGGs is driven by aberrations in the Ras-Raf-MEK-ERK (MAPK) pathway, which can serve as a therapeutic target. Selumetinib is reported to exert antitumor effects in pediatric patients with NF1-associated recurrent or refractory LGG. Selumetinib is a potential alternative to chemotherapy in LGG and OPGs.[48,49] Currently, studies are investigating the efficacy and dosage of the MEK inhibitor binimetinib (NCT02285439), as well as the efficacy of the pan-RAF inhibitor tovorafenib (NCT05566795) in NF1-related LGG. For pediatric patients with newly diagnosed *BRAF* V600E mutant LGG, the response to the combination of dabrafenib and trametinib is higher than that to chemotherapy.[50] Additionally, treatment with the mTOR inhibitor everolimus resulted in tumor shrinkage and the stabilization of visual acuity in patients with NF1-associated recurrent or progressive LGG. [51,52] The combination of rapamycin and erlotinib (an EGFR inhibitor) stabilized the disease in some pediatric patients with recurrent LGG.[53]

High Grade Glioma (HGG) and Other Brain Tumors

HGG is rare in children and is usually observed in early adulthood.[54] The most common occurrence sites for HGG are the cerebral hemispheres. The prognosis of HGG is poor. The therapeutic strategies for HGG are surgical resection, radiotherapy, and various chemotherapy agents.[55] An ongoing trial is investigating the efficacy of dabrafenib, trametinib, and hydroxychloroquine in recurrent LGG or HGG with a *BRAF* mutation (NCT04201457). In a series by Rosenfeld et al., five out of 145 patients with NF1 and central nervous system tumors were found to have high-grade tumors, including one case of medulloblastoma and four cases of high-grade glial tumors.[55] Two patients with high-grade gliomas had previously undergone radiotherapy for OPGs. Given the tendency for secondary malignancies to develop, it is important to use radiation therapy in NF1 patients carefully. The correlation between NF1 and medulloblastoma is unclear, although some case reports have been published. Information regarding the medulloblastoma histology of the reported patients is limited, and only one case has a known molecular subgroup.[56] Further studies are needed to elucidate the underlying molecular mechanisms.[56,57] Although most central nervous system tumors in patients with NF1 are low-grade gliomas, clinicians should be highly suspicious of malignancy in patients whose tumors are in an unusual location or behave in an aggressive manner.

Leukemia and Lymphoma

The risk of developing myeloid disorders, particularly juvenile myelomonocytic leukemia (JMML), is increased by up to 500-fold in children with NF1. NF1 is correlated with JMML, and 15% of JMML cases are associated with NF1.[58] Patients with JMML, a rapidly progressing condition, typically present with symptoms, such as hepatomegaly, fever, pallor, rash, and lymphadenopathy.[59] T lymphoblastic

lymphoma is the most common lymphoma in patients with NF1 and is associated with low survival rates.[60] Case reports have described an association between NF1 and Hodgkin's lymphoma, although this is considered coincidental.[57] The potential therapeutic effect of MEK inhibitors on JMML can be attributed to the activation of RAS signaling. An ongoing study is evaluating the efficacy of the MEK inhibitor trametinib in patients with relapsed or refractory JMML (NCT03190915).

Rhabdomyosarcoma (RMS)

RMS is the most common type of soft tissue cancer in children, accounting for around 40–50% of cases.[61] The incidence of RMS in patients with NF1 is higher than that in the general population. Compared to sporadic RMS, tumors are almost exclusively the embryonal subtype.[62] The most common site for RMS occurrence is the urogenital system.[62] The age of RMS occurrence in patients with NF1 is lower than that in patients with sporadic NF1.[62,63] There is no data to suggest that the outcomes of RMS in the NF1 population differ from those of the general population. The treatment options and survival rates are similar for patients with and without NF1.[64] In a series by Crucis et al. they reported 16 RMS cases with NF1.[62] The long-term sequelae related to chemotherapy are not obviously different from those of non-NF1 patients. The 5-year event-free survival and overall survival were 67% and 87%, respectively.

Malignant Melanoma

Although the development of malignant melanoma in patients with NF1 has been documented in various clinical reports, very little is known about the characteristics of melanomas that occur in this patient group.[65] Some case reports have suggested a correlation between malignant melanoma and NF1. However, large population studies have not provided clear evidence.[66] Due to these scarce results, surveillance for malignant melanoma has typically not been recommended.[67,68] The MEK inhibitors cobimetinib and trametinib have been used to treat malignant melanoma in adult patients with NF1.[69,70]

Other Tumors

The incidence rates of breast cancer, gastrointestinal stromal tumors, carcinoid tumors, and pheochromocytoma in adult patients with NF1 are higher than those in pediatric patients with NF1.[71-73] The prognosis and treatment are similar to those of patients without NF1, except for breast cancer.[74] The increased breast cancer risk among younger women with NF1 means they should be offered more screening than females in the general population. Annual mammography should be recommended in patients with NF1 in national high-risk screening programs. The National Comprehensive Cancer Network recommends annual mammography beginning at age 30, as well as consideration of breast MRI between ages 30 and 50.[75] The aggressiveness of breast cancer with NF1 is higher than that of sporadic breast cancer. Patients with NF1-associated breast cancer exhibit poor prognostic features, such as a high frequency of high-grade tumors, hormone receptor negativity, and HER2 overexpression.[76] The incidence of pheochromocytoma associated with NF1 has been reported as ranging from 0.1% to 5.7%. They cause hypertension in most individuals who experience symptoms. Histopathology is more often benign than malignant.[77]

Conclusion

NF1 is an autosomal dominant neurocutaneous disease that significantly increases the risk of developing cancer. Patients with NF1 are also predisposed to developing benign tumors. Although there is no cure for NF1, identifying patients with NF1 and conducting intermittent follow-ups are important for early detection of potential complications, particularly tumorigenesis. The treatment options for NF1-

related cancers, which include chemotherapy, radiotherapy, and surgery, are planned according to the type of cancer.

PNs are usually benign, but as they grow, they can cause serious health problems, including pain and damage to the surrounding tissues. Treatment may be necessary due to their location and the resulting morbidity and functional impairment. Most patients are not eligible for surgery as the tumor is located deeply along the nerve tract. The discovery of the molecular pathogenesis and the biological basis of this disorder has enabled the development of targeted therapies.

Treatment is required for patients with OPG, another type of benign tumor, if there is impairment to their visual field or acuity. In the event of clinical progression, the main treatment option is chemotherapy. Surgery and radiotherapy are not preferred treatment options for NF1-related OPGs, despite being commonly used for other brain tumors. Targeted therapy is a potential alternative to chemotherapy in OPGs.

New insights into the pathogenesis of the disease now offer hope for the development of specific, less toxic, and more precise molecularly targeted treatment methods.

Author contribution

The author confirm contribution to the paper as follows: Review conception and design: HSŞ; literature review: HSŞ; draft manuscript preparation: HSŞ. The author reviewed the results and approved the final version of the manuscript.

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Original Article

Effectiveness of an autism spectrum disorder screening and follow-up training program for primary health care professionals in Türkiye

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ABSTRACT

Background. Autism spectrum disorder (ASD) screening and follow-up programs are implemented in all provinces in Türkiye as part of the National Action Plan for Individuals with ASD. Primary health care professionals are trained regarding ASD by child and adolescent psychiatrists, aiming to ensure that risky children are diagnosed and referred earlier and diagnosed in early childhood. The aim of this study is to objectively evaluate the effectiveness of an ASD training program provided to primary healthcare professionals.

Methods. Three hundred and three individuals consisting of family physicians and family healthcare workers (FHW) who participated in the ASD training program were recruited in the study in the Muğla province of Türkiye. The Knowledge About Childhood Autism Among Health Workers Questionnaire (KCAHW) was completed by all participants before and after the training.

Results. The mean total KCAHW scores pre- and post-training were 13.12±3.14 and 16.48±2.02, respectively. There was a statistically significant difference in Domains 1, 2, 3, and 4 and the total scores pre- and post-training ($p<0.001$). The effect sizes of the differences in KCAHW domains 1, 2, 3, 4, and the total score for family physicians and FHWs' pre- and post-test means were 0.24, 0.01, 0.08, 0.14, and 0.22, respectively.

Conclusions. Family physicians and the FHWs benefited from the intervention in all domains of the KCAHW. The training program provided within the scope of the ASD screening and follow-up program significantly increased knowledge and awareness of ASD in primary healthcare providers.

Key words: autism spectrum disorder, general practitioners, primary care physicians, screening, training.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that emerges in early childhood. In DSM-5, ASD is categorized into two main domains: deficits in social communication and interaction and restricted, repetitive patterns of behavior, interests, or activities.[1] Recent studies have focused on predictors of loss of autism diagnosis and have demonstrated the impact of early diagnosis and intervention.[2-5] The most important step towards diagnosis is recognizing the early signs of ASD and referring the child to a child and adolescent psychiatrist for further evaluation. However, the age of diagnosis remains suboptimal. According to the results of a meta-analysis study, the mean age of ASD diagnosis was determined as 60.48 months.[6]

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In a recent review examining the impact of implemented policies on early diagnosis of autism, it was found that both screening models and training programs increased early diagnosis.[7] The American Academy of Pediatrics recommends that all children aged 18-24 months be screened for ASD symptoms.[8] Today, some countries have implemented nationwide screening programs to facilitate the early detection of ASD.[9-12] In addition to increasing ASD awareness among healthcare professionals, studies are recommended to raise awareness of risk symptoms of autism in children in all environments where they come into contact with children, such as schools[13] and among parents.[14,15] A study conducted in the United States emphasizes that early autism diagnosis is shaped by diverse state-level screening practices, early intervention efforts, and existing disparities, suggesting that refining national and state policies is essential to support timely identification and access to services.[16] Countries are trying to produce and implement health policies for the early diagnosis of autism. In Türkiye, the Autism Spectrum Disorder Screening and Follow-up Program is conducted by the Ministry of Health under the National Action Plan for Individuals with Autism Spectrum Disorder, ensuring that all children aged 18-36 months are screened for ASD symptoms.[17,18] As part of this program, family physicians are required to evaluate children aged 18-36 months at least once for ASD risk. In the literature, various studies have assessed the knowledge of physicians and other healthcare professionals about ASD. However, a significant portion of these studies indicate that levels of knowledge are either insufficient or lower than expected.[19-21] Research suggests that postgraduate training on ASD significantly enhances awareness and knowledge.[22-25] This is particularly important for primary healthcare professionals and pediatric clinic staff, as they have a greater chance of encountering children aged 18-36 months and hence identifying ASD risk at an early stage.

The aim of this study is to evaluate the effectiveness of ASD training provided to primary healthcare workers on their knowledge on ASD.

Materials and Methods

Ethics and consent

The study was conducted in compliance with the principles of the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Participants. Approval was obtained from the Muğla Provincial Directorate of Health and the Muğla Sıtkı Koçman University Human Research Ethics Committee (180171/150). All participants who volunteered to the study were provided with detailed verbal information regarding the study and the procedures involved, and written informed consent was obtained.

Study sample

A total of 303 family physicians and family healthcare workers (FHWs) employed in family health centers and social health centers who had received ASD training and participated in the ASD Screening and Follow-up Program organized by the Ministry of Health in Muğla city and its districts between November 1, 2018, and February 1, 2019, were included in the study.

Family physicians and FHWs who had previously participated in the Turkish validity and reliability study of the "Knowledge About Childhood Autism Among Health Workers Questionnaire" (KCAHW) in Muğla were excluded from the study. The KCAHW was administered to participants before and after the training to assess changes in their knowledge.

Training content

Each training session consisted of a maximum of 20 participants and lasted approximately

2 hours. The sessions were held in a meeting room supplied with a computer and projector. Pen and paper questionnaires were given to participants before the training and given again after the training ended. The training sessions were conducted interactively by the same child and adolescent psychiatrist and included:

- A brief slide presentation prepared by the Ministry of Health, covering theoretical information on ASD, including its definition, diagnostic criteria, etiopathogenesis, frequency, social-emotional and language development stages in typically developing children, autism symptoms, prognosis, course and treatment.
- A video presentation illustrating case examples of ASD. These were videos of children from different age groups, shot in the presence of a child psychiatrist and parents in an examination environment, showing the symptoms of normal development and autism (such as stereotypy, speech delay) and the findings detected during the examination (such as calling out to one's name, eye contact, joint attention, imitation games).[18]

Data collection tools

Sociodemographic data form: Participants completed a questionnaire designed by the researchers to assess their sociodemographic and occupational characteristics.

The Knowledge About Childhood Autism Among Health Workers Questionnaire (KCAHW): KCAHW, developed by Bakare et al., consists of 19 items.[26] This questionnaire is used to measure health workers' knowledge of ASD and evaluates four domains related to autism:

1. Social Interaction Deficits (8 items) – Evaluates impairments in social interaction observed in children with ASD.
2. Communication and Language Development (1 item) – Assesses symptoms related to communication and language skills.
3. Repetitive and Stereotypical Behaviors (4 items) – Examines obsessive-compulsive tendencies, repetitive actions, and stereotypical behavioral characteristics of ASD.
4. General Knowledge About Autism (6 items) – Covers autism as a disorder, its possible comorbid conditions, and the typical age of onset.

The total score from the questionnaire ranges between 0 and 19. Response options were categorized as "I don't know," "Yes," and "No," with correct answers scoring 1 point and incorrect responses receiving 0 points. In Domain 4, three items were reverse-scored. The total score of the scale consists of the sum of the four domain scores. Higher scores indicate greater knowledge of ASD.

The mean scale score in the studied population reflects the overall knowledge level of childhood ASD within that group. The Turkish validity and reliability of the KCAHW was established by Gürbüz Özgür et al.[27]

Data analysis

Descriptive statistics are presented as mean, median, standard deviation, number, and percentage. The normality of the distribution was assessed using the Shapiro-Wilk test. Pre- and post-training scale domain scores were compared using the Wilcoxon signed- ranks test due to failure to provide conditions of normal distribution conditions, and Cohen's effect size (ES) statistics was also used in the comparisons. In brief, ES was calculated with the "(Group 1 Mean - Group 2 Mean)/ Pooled Standard Deviation" formula.[28]

Confounding and effect-modifier variables were determined with the stratified analysis method by using Wilcoxon signed- ranks tests. Since neither the dependent nor independent variables were dichotomous, certain conditions were used instead of using the Mantel-Haenszel analysis to determine confounding or effect-modifier variables.

Criteria for identifying confounding and effect-modifying variables

Condition 1: If a significant result is achieved in the $p<0.05$ level in the hypothesis test during the before-after comparison which is done before the related independent variable is categorized and if the before-after analyses done (stratified) for all the sub-categories of the same variable are significant at the $p<0.05$ level, then this variable is neither confounding, nor is an effect modifier.

Condition 2: For a variable to be accepted as a "confounding variable," the before-after comparison done before the independent variable is categorized needs to be significant at the $p<0.05$ level; a "non-significant" result should be achieved at the $p<0.05$ level in all the strata of the related variable in the stratified analyses (or in the before-after analyses for all the sub-categories of the same variable).

Condition 3: For a variable to be accepted as an "effect modifier variable," the before-after comparison done before the independent variable is categorized needs to significant at the $p<0.05$ level; "significant" result should be achieved in the $p<0.05$ level in one of strata of the related variable and "non-significant" in another stratum in the stratified analyses (or in the before-after analyses for all the sub-categories of the same variable).

The data were analyzed using SPSS Version 17.00 for Windows (Chicago: SPSS Inc., 2008). For statistical significance, a type 1 error (p-value) threshold of 0.05 was applied.

Results

A total of 303 participants were included in the study, of whom 197 were female and 106 were male. Among them, 48.2% (n=146) were family physicians, while 51.8% were FHWs, including midwives, nurses, and health officers.

The mean age of the participants was 43.2 ± 8.49 years, with an average number of children of 1.4 ± 0.82. The mean duration of work experience in the field was 20.18 ± 8.66 years (ranging from 0 to 46 years). The sociodemographic characteristics of the health workers included in the study are presented in Table I. Among the family physicians, 7% had completed a child psychiatry internship during their medical training.

A statistically significant increase was observed in all domains and total scores of the KCAHW questionnaire administered before and after the training sessions (Table II). Additionally, family physicians and FHWs were compared in terms of pre- and post-training score differences across each domain (Table II). In this comparison, Cohen's effect size was used. A significant difference between family physicians and FHWs was observed only in the first domain, where the effect size indicated a weak effect. In other words, physicians showed greater improvement in the first domain compared to FHWs, but no significant differences were found in the other domains.

As a result of the stratified analysis, none of the independent variables were found to be confounding factors. However, for the 2nd domain, age, gender, marital status, income-expense level, years of experience in the field, and ASD knowledge perception level were identified as effect modifier variables (Table III). Participants' correct response rates to the KCAHW scale items before and after the training are presented in Table IV.

Table I. The sociodemographic characteristics of the participants

		n (%)
Gender	Female	197 (65)
	Male	106 (35)
Occupation	Family physician	146 (48.2)
	Family healthcare worker	157 (51.8)
Physician's specialty	Practitioner	135 (92.5)
	Family physician specialist	10 (6.8)
	Unknown	1 (0.7)
Educational status	High-school	15 (5)
	Undergraduate	58 (19.1)
	Graduate	84 (27.7)
	Post-graduate	135 (44.6)
	Doctorate/practice in medicine	11 (3.6)
Marital status	Married	247 (81.5)
	Single/divorced	56 (18.5)
Income-expense level	Low	99 (32.7)
	Medium	106 (35)
	High	89 (29.4)
	Unknown	9 (3)
Work history at a mental health center	Yes	21 (6.9)
	No	276 (91.1)
	Unknown	6 (2)
Child psychiatry internship	Yes	36 (11.9)
	No	252 (83.2)
	Unknown	15 (5)
Having child diagnosed with ASD	Yes	5 (1.7)
	No	298 (98.3)
Having child with a chronic disease / disability	Yes	25 (8.3)
	No	278 (91.8)
Child with ASD in one's environment	Yes	93 (30.7)
	No	210 (69.3)
Completed the follow-up of a child with ASD	Yes	57 (18.8)
	No	246 (81.2)
Previous ASD training	Yes	50 (16.5)
	No	244 (80.5)
	Unknown	9 (3)
Perceived knowledge level	Insufficient	138 (45.5)
	Medium	102 (33.7)
	Sufficient	54 (17.8)
	Unknown	9 (3)

ASD: autism spectrum disorder.

Table II. The Knowledge About Childhood Autism Among Health Workers Questionnaire domains and total score means of the participants and their comparison before and after training

	Training status	Family physician (Mean±SD)	p*	Family healthcare worker (Mean±SD)	p*	The effect size of the differences** (p)***	Whole group (Mean±SD)	p*
Domain 1	Before	7.03±1.30	<0.001	6.51±1.65	<0.001	0.24 (0.035)	6.76±1.51	<0.001
	After	7.78±0.76		7.65±0.97			7.71±0.87	
Domain 2	Before	0.88±0.32	0.01	0.88±0.31	0.02	0.01 (0.90)	0.88±0.32	0.001
	After	0.95±0.19		0.95±0.20			0.95±0.20	
Domain 3	Before	2.67±1.15	<0.001	2.49±1.09	<0.001	0.08 (0.45)	2.58±1.12	<0.001
	After	3.43±0.82		3.35±0.83			3.39±0.83	
Domain 4	Before	3.23±1.24	<0.001	2.57±1.27	<0.001	0.14 (0.22)	2.89±1.30	<0.001
	After	4.63±1.18		4.20±1.31			4.40±1.27	
Total score	Before	13.82±2.87	<0.001	12.47±3.24	<0.001	0.22 (0.067)	13.12±3.14	<0.001
	After	16.81±2.05		16.17±2.30			16.48±2.20	

* Wilcoxon signed ranks test; ** Cohen's effect size (ES): (Group 1 Mean - Group 2 Mean) / Pooled Standard Deviation; *** Mann-Whitney U test

Table III. Results of stratified analysis for the Knowledge About Childhood Autism Among Health Workers Questionnaire domains (pre-test and post-test)

Possible confounding effect / effect modifier variable	Variable categories	Median scale scores (before / after training)				
		Domain 1	Domain 2	Domain 3	Domain 4	Total scale
Age group (years)	<39 (n=95)	7/8*	1/1, p=0.40	2/3*	3/4*	13/16*
	40-45 (n=88)	7/8*	1/1*	3/4*	3/4*	14/17*
	46+ (n=120)	8/8*	1/1**	3/4*	3/5*	14/17*
Gender	Female (n=197)	7/8*	1/1, p=0.09	3/4*	3/4*	13/17*
	Male (n=106)	8/8*	1/1*	3/4*	3/4*	14/17*
Marital status	Married (n=247)	7/8*	1/1*	3/4*	3/4*	14/17*
	Single/divorced (n=56)	7/8*	1/1, p=0.09	3/3.5*	3/4*	13/16.5*
Income-expense level	Low (n=99)	7/8*	1/1, p=0.17	2/4*	3/4*	13/17*
	Medium (n=106)	7/8*	1/1**	3/4*	3/4*	13.5/17*
	High (n=89)	7/8*	1/1**	3/4*	3/5*	14/17*
Years working in the field (years)	0-14 (n=79)	7/8*	1/1, p=0.16	2/3*	3/4*	13/16.5*
	15-24 (n=107)	7/8*	1/1, p=0.08	3/4*	3/4*	14/17*
	25+ (n=117)	7/8*	1/1*	3/4*	3/5*	14/17*
Perceived knowledge level	Insufficient (n=138)	7/8*	1/1*	2/4*	3/4*	13/17*
	Medium (n=102)	78*	1/1, p=0.36	3/4*	3/4*	14/17*
	Sufficient (n=54)	8/8*	1/1, p=0.20	3/4*	3/4*	15/17*
Occupation	Family physician (n=157)	7/8*	1/1*	3/4*	3/4*	13/17*
	Family healthcare worker (n=146)	7/8*	1/1**	3/4*	3/5*	14/17*
	Whole group (n=303)	7/8*	1/1*	3/4*	3/4*	14/17*

*p ≤ 0.001; **p between 0.001 and 0.05.

Table IV. Correct response rates of family physicians and healthcare workers to the Knowledge About Childhood Autism Among Health Workers Questionnaire items before and after training

	Family physician (n=157), n (%)		Family healthcare worker (n=146), n (%)	
	Before training	After training	Before training	After training
Domain 1				
Item 1	147 (93.6)	156 (99.4)	137 (93.8)	145 (199.3)
Item 2	140 (89.2)	150 (95.5)	140 (95.9)	144 (98.6)
Item 3	115 (73.2)	150 (95.5)	133 (91.1)	145 (99.3)
Item 4	126 (80.3)	151 (96.2)	124 (84.9)	142 (97.3)
Item 5	149 (94.9)	155 (98.7)	130 (89)	140 (95.9)
Item 6	118 (75.2)	145 (92.4)	120 (82.2)	139 (95.2)
Item 7	138 (87.9)	152 (96.8)	138 (94.5)	144 (98.6)
Item 8	90 (57.3)	143 (91.1)	105 (71.9)	138 (94.5)
Domain 2				
Item 9	139 (88.5)	150 (95.5)	129 (88.4)	140 (95.9)
Domain 3				
Item 10	138 (87.9)	156 (99.4)	125 (85.6)	143 (97.9)
Item 11	67 (42.7)	110 (70.1)	72 (49.3)	105 (71.9)
Item 12	128 (81.5)	147 (93.6)	126 (86.3)	141 (96.6)
Item 13	58 (36.9)	114 (72.6)	68 (46.6)	113 (77.4)
Domain 4				
Item 14	70 (44.6)	119 (75.8)	102 (69.9)	119 (81.5)
Item 15	57 (36.3)	92 (58.6)	96 (65.8)	119 (81.5)
Item 16	119 (75.8)	150 (95.5)	116 (79.5)	142 (97.3)
Item 17	42 (26.8)	111 (70.7)	54 (37)	109 (74.7)
Item 18	25 (15.9)	101 (64.3)	44 (30.1)	94 (64.4)
Item 19	92 (58.6)	87 (55.4)	60 (41.1)	93 (63.7)

Discussion

Primary healthcare workers interact with infants and children regularly during vaccination appointments and developmental follow-ups. During these visits, family physicians play a crucial role in identifying children at risk for ASD at an early stage through screening tests. As a result, various countries have implemented training programs for healthcare workers, including practicing physicians, within the framework of ASD action plans.[10,11] In the literature, numerous studies using different assessment tools have reported that healthcare professionals' knowledge of ASD is often insufficient.[20] In our study, we evaluated ASD knowledge using KCAHW, and our findings are discussed below in relation to existing literature.

When analyzing intervention studies aimed at increasing knowledge levels on ASD, various training programs can be identified, including face-to-face education, computer-assisted distance learning, case-based education, intensive one-day training, and weekly or monthly repetitive sessions.[20] In our study, a 2-hour theoretical training combined with video-based case presentations was implemented, as planned by the Ministry of Health. The findings revealed a significant increase in ASD knowledge levels among both

family physicians and other primary healthcare workers following the training. Similarly, another training study conducted in Türkiye reported that family physicians' knowledge levels improved after a 2-hour ASD training program.[22] Additionally, Carbone et al. found that after training, the ASD screening rates of 26 primary physicians increased during their 18- to 24-month age group medical examinations.[23] Studies evaluating the effectiveness of ASD training programs generally rely on researcher-developed questionnaires or assess differences in participants' subjective perceptions. In contrast, we used the KCAHW measurement tool, which is internationally recognized for assessing training effectiveness. Therefore, our findings will allow for comparisons with other studies that have used the same measurement tool, contributing to a more standardized evaluation of ASD training programs. The knowledge level measured in the study measures the gains immediately after the training. The level of knowledge of health workers cannot be determined in the long term with this study alone. However, it is possible to see that this training is effective in our other study where we examined the number of children screened and referred by the same sample in the same province after the training and the rate of children diagnosed with autism.[29]

Eseigbe et al. used the KCAHW in their study to assess the ASD knowledge levels of physicians from different specialties and reported that 34% of 76 family physicians had a KCAHW mean total score above 15.[30] Similarly, in Salama's study, the KCAHW was administered to 70 family physicians, revealing a mean score of 11.2 ± 3.5 In a study by Eray and Murat, it was found that 65.3% of family physicians lacked sufficient knowledge about ASD.[22] In contrast, in our study, only 17.8% of family physicians reported that they did not have a sufficient level of knowledge. Additionally, in a study by Gürbüz Özgür et al. on primary healthcare workers, 21.3% of participants perceived their ASD knowledge as sufficient or highly sufficient.[27] In our study, 16.5% of participants had received prior training on ASD. Similarly, a study conducted in the United Kingdom reported that approximately two-thirds of practitioners had not received any ASD training during their medical school training or family physician specialization training.[31] In our study, it was found that only 7% of physicians had completed an internship in child psychiatry during their medical training, while 11.9% of all participants (nurses, midwives, and other health workers as well as physicians) had undergone internship training in child psychiatry.

Since theoretical and practical training related to ASD diagnosis is typically provided during child psychiatry internships, we believe that incorporating ASD-focused lectures into the education curriculum of all healthcare-related disciplines could be an effective strategy for enhancing knowledge levels after graduation. In another study conducted among senior students of medical, nursing, and psychology faculties, the mean KCAHW score was 10.67 ± 3.73 overall, with faculty-specific scores as follows: 12.24 ± 3.24 for medical students, 10.76 ± 3.5 for nursing students, 9.01 ± 3.76 for psychology students.[32] This study found that KCAHW scores had a positive relationship with both the number of weeks spent in psychiatry and pediatrics rotations and the number of psychiatry/abnormal psychology course hours.[32] These findings emphasize the importance of integrating ASD-related training into both pre- and post-graduate curricula to enhance knowledge and awareness. The study also evaluated the impact of the training program on primary healthcare workers, who were the main target audience. The results showed that while both family physicians and FHWs benefited equally across all domains, including total scores, family physicians demonstrated a significantly greater improvement in Domain 1 when effect size was analyzed. One possible explanation for this finding is that Domain 1 contains the highest number of items and

is primarily based on clinical observation. Additionally, physicians frequently encounter differential diagnosis scenarios, which may have led them to reframe their past knowledge and clinical observations in light of the new training. Since the scale applied in the study was not developed to directly evaluate the effectiveness of the training, the lack of a high increase in the response rates (in Domain 4 items) to some information not included in the content of the training (e.g. comorbidity, detailed etiology) suggests that this training corresponds to greater gains, especially in domains 1, 2 and 3 of KCAHW (Table IV). This information may provide practical implications for future program development and policy planning.

This study found that none of the analyzed independent variables were confounding factors for KCAHW scores. However, in Domain 2, age, gender, marital status, income-expense level, years of work experience, and ASD knowledge perception level were identified as effect modifier (moderator) variables. As is well known, the absence of confounding variables suggests that the obtained results are robust and reliable without requiring additional multiple analyses. However, effect modifier variables cannot be controlled through statistical adjustments; they can only be reported, as was done in this study. Given that multiple variables influenced Domain 2, we recommend interpreting the results with caution in this domain. Domain 2 of the scale consists of a single item. This item asks whether the respondents know that patients with ASD have a speech delay problem. We see that a significant majority of the respondents (both family physicians and FHW) knew this before the educational intervention. The vast majority of health workers knew this before the intervention, and very few did not. In the subcategories of the independent variables, the number of those who did not know decreased further. In the research sample, this homogeneity (the overwhelming majority of respondents knowing the subject before the educational intervention) is also due to the fact that the 2nd Domain in the scale was questioned with only one question. There is no such homogeneity in any of the other dimensions because the other dimensions were questioned with at least 4 items.

Among the strong points of our study, we can highlight the following: Objective measurement of training effectiveness using a validated and reliable questionnaire in Turkish; a large sample size, enhanced statistical power due to minimized variability, as the same trainer conducted all sessions, reducing potential bias.

Limitations

The study also has some limitations: Knowledge assessment was conducted immediately after the training, without a follow-up evaluation to measure long-term knowledge retention. Although our findings demonstrate strong internal validity, a regionally confined sample may limit the generalizability of results to the broader population of trained professionals across the country.

In developing countries, educational initiatives on ASD remain insufficient compared to Western countries.[33] Determining which educational interventions are most effective is challenging. Factors such as the use of different training groups, cultural variations, and the lack of standardized measurement tools make direct comparisons difficult.

Conclusion

The ASD Screening and Follow-up Program is currently being implemented nationwide in Türkiye. Although our study showed an increase in level of knowledge immediately after the training, studies are needed to evaluate the knowledge levels in the long term. Examining the long-term effects of increase in knowledge levels of family physicians and other primary healthcare workers with data sources, such as referral statistics, diagnostic outcomes, or follow-up assessments over time, will yield

objective results. We believe that this study will contribute to future research evaluating the effectiveness of ASD training programs, both in Türkiye and internationally.

Ethical approval

The study was approved by Muğla Provincial Directorate of Health and the Muğla Sıtkı Koçman University Human Research Ethics Committee (180171/150).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BGÖ, HA, EE; data collection: BGÖ; analysis and interpretation of results: EE, BGÖ; draft manuscript preparation: BGÖ, HA, EE. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

The role of non-coding RNAs miR-98, miR-19a and lncRNA MALAT1 and oxidative stress in the pathogenesis of food allergy

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ABSTRACT

Background. Food allergy is a public health concern affecting quality of life and increasing in prevalence. Numerous studies suggest that the rapid increase in the prevalence of allergic diseases may be linked to epigenetic mechanisms, particularly microRNA (miRNA), long non-coding RNA (lncRNA). The aim of this study was to investigate the effects of oxidative stress and selected non-coding RNAs on the development and pathogenesis of food allergy.

Methods. A total of 26 children with food allergy and 30 healthy children were enrolled in this study. Real-time polymerase chain reaction (RT-PCR) was performed to detect the expressions of serum miR-19a, miR-98 and lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) in all the participants. Serum levels of interleukin-4 (IL-4), IL-10, IL-13 and transforming growth factor beta (TGF-β), along with levels of oxidative stress markers 8-isoprostane and cysteinyl leukotrienes, were measured by enzyme-linked immunosorbent assay.

Results. Our study found that the expression of miR-98 was significantly lower in children with food allergies compared to healthy controls ($p < 0.05$), whereas there was no significant difference in the expression levels of miR-19a between the two groups ($p > 0.05$). There was no difference in gene expression levels ($p > 0.05$) of lncRNA MALAT1 between children with food allergies and healthy children. TGF-β levels of healthy children were found to be significantly higher than those of children with food allergies ($p < 0.05$). There was no statistical difference in cysteinyl leukotriene levels between patients and controls ($p = 0.804$). However, 8-isoprostane levels were significantly lower in patients (6.68 pg/mL; interquartile range [IQR]: 1.57-26.55) compared to controls (37.20 pg/mL, IQR: 18.55-167.58) ($p < 0.001$).

Conclusions. Considering our findings in conjunction with existing literature, miR-98 appears to be a promising candidate biomarker for food allergy.

Key words: epigenetics, food allergy, lncRNA MALAT1, miR-98, miR-19a, oxidative stress.

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Food allergy is an inappropriate immune response that occurs after food intake or exposure.[1] The prevalence of food allergies is increasing all over the world.[2] The clinical findings of food allergies can range from mild itching to life-threatening anaphylaxis.[3] Along with reducing the quality of life, food allergy consumes public health resources significantly.[4,5] Depending on the type of immune response, food allergies are classified into three groups: immunoglobulin E-mediated (IgE-mediated), non-IgE-mediated food allergy, and mixed-type food allergy.[6] IgE-mediated food allergies are the most common type and show immediate symptoms.[7] During the sensitization phase of IgE-mediated food allergy, ingestion of the allergenic food protein triggers the production of food-specific IgE antibodies, which subsequently bind to tissue basophils and mast cells. When food crosses the disrupted barrier, dendritic cells are activated via danger signals and release inflammatory cytokines. These activated dendritic cells present the antigen to naïve T cells, the T cells differentiate into a T helper cell 2 (Th2) phenotype, which in turn promotes inflammatory signals that induce food antigen-specific B cells to class switch and produce food antigen-specific IgE. In the effector phase of IgE-mediated allergic reactions, re-exposure to the sensitized food allergen leads to mast cell degranulation and mediator release, triggered by cross-linking of the allergen with allergen-specific IgE bound to Fc epsilon receptor 1 (FcεRI) on mast cells. Therefore, mast cells release mediators such as histamine and leukotrienes and allergic reactions to food occur.[8]

Epigenetic changes are mechanisms that regulate genome activity that do not involve altering the DNA sequence.[9] The main epigenetic mechanisms are divided into three as DNA methylation, histone modifications and non-coding RNAs.[10] MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are important regulators of gene expression, belonging to the class of non-coding RNAs.[11]

MiRNAs are untranslated transcripts found in clusters within the introns of other genes.[12] miRNAs are about 18-26 nucleotides long and are responsible for the translational repression of messenger RNA (mRNA). They cause translational repression by partial binding to the non-protein-translated region (3′-UTR) located at the end of the target mRNA via RNA-induced silencing complex (RISC), or mRNA degradation by full conjugation.[13] MiRNAs play a role in biological processes such as apoptosis, cancer, cell differentiation, and inflammation. MiRNAs are also involved in the regulation of the immune response. MiRNAs affect the development of diseases or the strength of inflammation in the affected tissues.[14]

LncRNAs are non-coding RNAs longer than 200 nucleotides. It is estimated that there are approximately 16,000 lncRNAs in the human genome.[15] LncRNAs are structurally similar to mRNAs, but lncRNAs do not have an open reading frame (ORF). Therefore, they cannot code for proteins.[16] LncRNAs are found in the nucleus and cytoplasm and are involved in the regulation of almost every step of gene expression by various mechanisms. As epigenetic modulators, lncRNAs bind to chromatin-modifying enzymes and direct their activity to specific regions of the genome. In these regions, lncRNAs direct chromatin modification by changing the pattern of gene expression. LncRNAs act on transcription factors and cause suppression or activation of the target gene. LncRNAs regulate alternative splicing by acting on splice factors. In addition, lncRNAs prevent the miRNA from binding to the target mRNA by acting as a sponge between the target mRNA and the miRNA.[17] Emerging evidence suggests that lncRNAs are involved in the transcriptional or epigenetic regulation of gene expression, various biological processes such as cell differentiation, embryonic development, cancer metabolism, and inflammation.[18]

The expression pattern of miRNAs and lncRNAs may vary according to different cell types and disease conditions. miRNAs and lncRNAs are

stable and detectable in different body fluids such as serum, urine and saliva. Furthermore, it has been shown that miRNAs and lncRNAs may play a role in the prognosis of the disease, in predicting the response to the treatment, and following the disease process. Therefore, it is thought that miRNAs and lncRNA can be used as biomarkers in airway and allergic diseases.[14,19]

In our study, we aimed to identify candidate biomarkers that could be used to distinguish children with food allergy from healthy controls and to determine the effects of selected non-coding RNAs on inflammation. Additionally, by identifying a potential diagnostic biomarker for food allergy, we sought to reduce the reliance on food challenge tests, which are both challenging and carry inherent risks. We also aimed to explore the relationship between food allergy and oxidative stress.

Materials and Methods

Patients

Twenty-six children with IgE-mediated food allergy from the Division of Allergy, and 30 healthy children from the Department of Child Health and Diseases General Outpatient Clinic who agreed to participate in the study were enrolled between June 2020 and July 2021, at İhsan Doğramacı Children's Hospital of Hacettepe University, Ankara, Türkiye. All patients and parents gave their informed written consent to participate in the study. The criteria for inclusion in the food allergy group were as follows: food-specific IgE ≥0.35 IU/L, a swelling of 3 mm or more compared to the negative control in the epidermal prick test and the presence of clinical symptoms. In the healthy control group, the criteria for inclusion in the study were the absence of food allergy and atopy. Blood samples were collected from all study participants for miRNA and mRNA isolation from serum. The study was approved by Hacettepe University Non-interventional Clinical Research Ethics Committee (approval no. GO 20/343).

The demographic characteristics of the study population are given in Table I.

MiRNA and lncRNA isolation and quantification

The serum samples were separated from the blood by centrifugation and stored at -80°C until use for miRNA isolation by miRNeasy Serum/Plasma Kit (QIAGEN, Germany).

MiRNAs were reversely transcripted to complementary DNA (cDNA) according to the

Table I. The demographic and clinical characteristics of the study population.

	Food allergy (n=26)	Controls (n=30)	p value
Age (year), mean±SD	4.54 ± 2.77	4.53 ± 2.25	p > 0.05
Female sex, n (%)	5 (19)	6 (20)	p > 0.05
Total IgE (IU/mL), median (Q1-Q3)	222 (115-597)	n/a	NA
Eosinophil count (x10^3/μL), median (Q1-Q3)	400 (200-500)	n/a	NA
Eosinophils (%), median (Q1-Q3)	4.8 (2.8-6.6)	n/a	NA
Food allergy pattern, n (%)			NA
Milk	7 (27)		
Milk and egg white	4 (15)		
Nuts	12 (46)		
Milk and nuts	3 (12)		

SD: standard deviation

instructions of the miScript II RT Kit (QIAGEN, Germany) by using HiFlex Buffer, which allows reverse transcription of both mRNA and miRNA into cDNA. The expression levels of miR-19a and miR-98 were derermined by using the miScript SYBR Green Kit (QIAGEN, Germany). The primer assays for miR-98, miR-19a, and miR-16 were purchased from QIAGEN. Maxima SYBR Green/ROX qPCR Master Mix" kit (ThermoFisher Scientific, USA) was used to determine expression levels of lncRNA MALAT1 and elongation factor 1-alpha (EF1-α), housekeeping gene. Primers for MALAT1 and EF1-α were purchased from Integrated DNA Technologies (Iowa, USA). The expression analyzes were performed by using Applied Biosystems Fast 7500 Real Time PCR System device. The relative expression levels of each miRNA were normalized by endogenous miR-16, and EF1-α was used as a housekeeping gene for lncRNA MALAT1. Differentiation in expression levels between samples was revealed by using the $2^{-\Delta\Delta Ct}$ method.

Enzyme-linked immunosorbent assay (ELISA)

The serum levels of interleukin IL-4, IL-10, transforming growth factor beta (TGF-β) and IL-13, 8-isoprostane and cysteinyl leukotrienes were measured by ELISA by using commercial kits, according to the manufacturer's instructions. Briefly, standards and samples were added to the wells of the capture antibody-coated plates and incubated at room temperature for the specified time. Unbound molecules were then washed away, and the enzyme-conjugated secondary antibody was added and incubated at room temperature for the specified time. After the washing process, the enzyme-specific substrate was added and incubated at room temperature for the specified time. After the reaction was stopped, measurements were made at the appropriate wavelengths. The concentrations of the samples were determined by using the standard graphs obtained.

Statistical analysis

The SPSS 22 for Windows program was used for statistical analysis. The comparison of numerical variables was performed using parametric or non-parametric tests depending on whether they were normally distributed. Categorical variables were evaluated using the chi-square test or Fisher's exact test. Expressions of target miRNAs and lncRNA were normalized according to the expression of control miRNA and EF-1α, respectively. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method. The results are given as fold change. The Mann-Whitney U test was used to analyze expression levels. In all analyses, $p < 0.05$ was considered statistically significant.

Results

Expression levels of miRNAs

The expression levels of selected miRNAs miR-19a, and miR-98 were compared between children with food allergies and healthy controls. The mean of the ΔCt values of the control group was calculated. The miRNA expression level of the individual whose ΔCt value was closest to the mean ΔCt values of the control group was accepted as $2^{-\Delta\Delta Ct} = 1$, and changes in expression levels of other individuals in the control group and food allergy patients were compared with the expression level of this reference individual.

There was no difference in the level of miR-19a expression between children with food allergies and healthy children ($p=0.85$, Fig. 1A), while the expression levels of miR-98 were significantly downregulated in children with food allergy compared with healthy children. ($p<0.001$, Fig. 1B).

Expression levels of lncRNA MALAT1

Although MALAT1 expression levels seem to be higher in patients, no significant difference was observed between the patient and control groups due to high inter-sample variability ($p = 0.993$, Fig. 2).

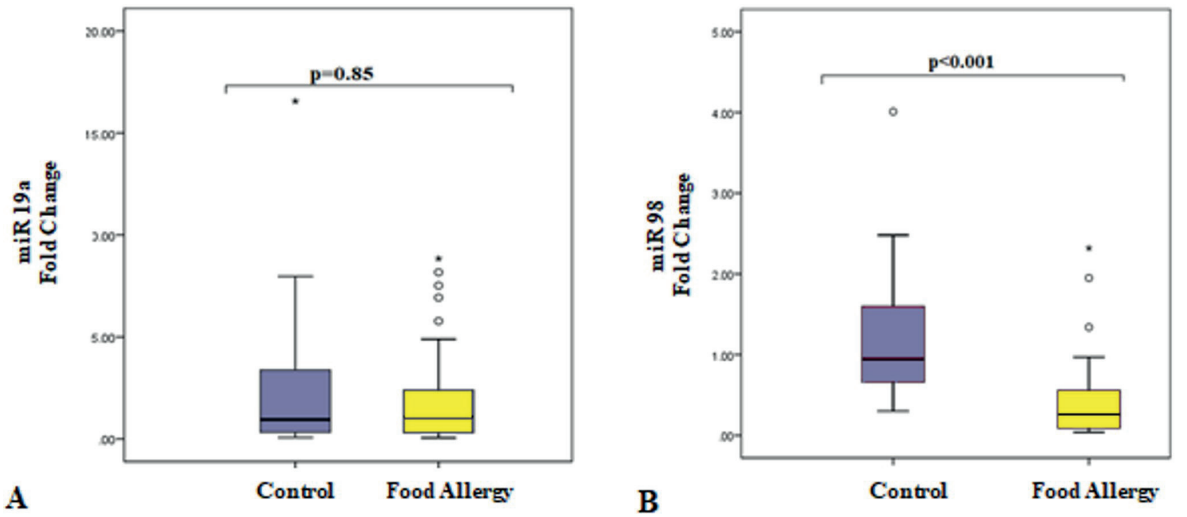

Fig. 1. A) Comparison of miR-19a expressions of food allergy patients (median: 1.08, Q1-Q3: 0.28-3.02) and control group (median: 0.95, Q1-Q3: 0.31-3.46). **B)** Comparison of miR-98 expressions of food allergy patients (median: 0.26, Q1-Q3: 0.09-0.57) and control group (median: 0.95, Q1-Q3: 0.65-1.60).

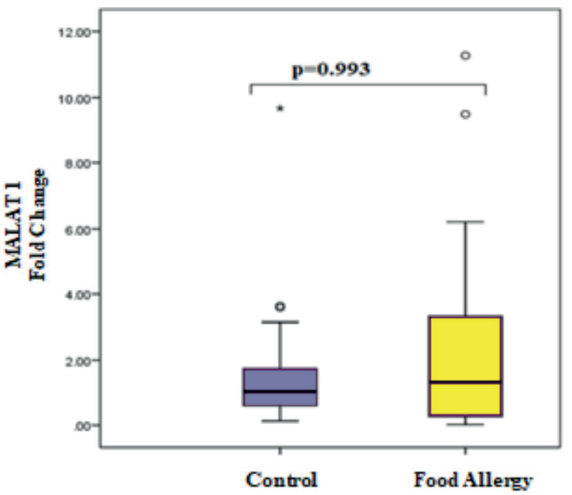

Fig. 2. Comparison of MALAT1 expressions of food allergy patients (median: 1.31, Q1-Q3: 0.25-3.36) and control group (median: 1.03, Q1-Q3: 0.57-1.78).

MALAT1: metastasis-associated lung adenocarcinoma transcript 1

Cytokine levels

The serum level of TGF-β was found to be significantly lower in the patient group compared to the control group (p=0.027, Fig. 3A). In contrast, the IL-13 protein levels in the serum samples of the patient group were not different from the control group (p=0.85, Fig. 3B). IL-4 and IL-10 proteins were not detectable at measurable levels in the serum samples of either group.

Oxidative stress markers

There was no statistical difference in cysteinyl leukotriene levels (p=0.804) between controls and patients (Fig. 4A). However, 8-isoprostane levels were significantly lower in patients (6.68 pg/mL; interquartile range [IQR]: 1.57-26.55) compared to controls (37.20 pg/mL; IQR: 18.55-167.58) (p< 0.001, Fig. 4B).

Discussion

Food allergy, defined as an inappropriate immune response to a harmless food antigen, is an important disease that can recur when exposed to the same food and can be life-threatening.[20] The prevalence of food allergy and other allergic diseases is increasing all over the world.[2] A growing body of evidence suggests that this increase may be associated with epigenetic mechanisms, particularly involving miRNA and lncRNA.

MiR-19a, a member of the miR-17~92 cluster, promotes Th2 cytokine production by simultaneously targeting inhibitors of the

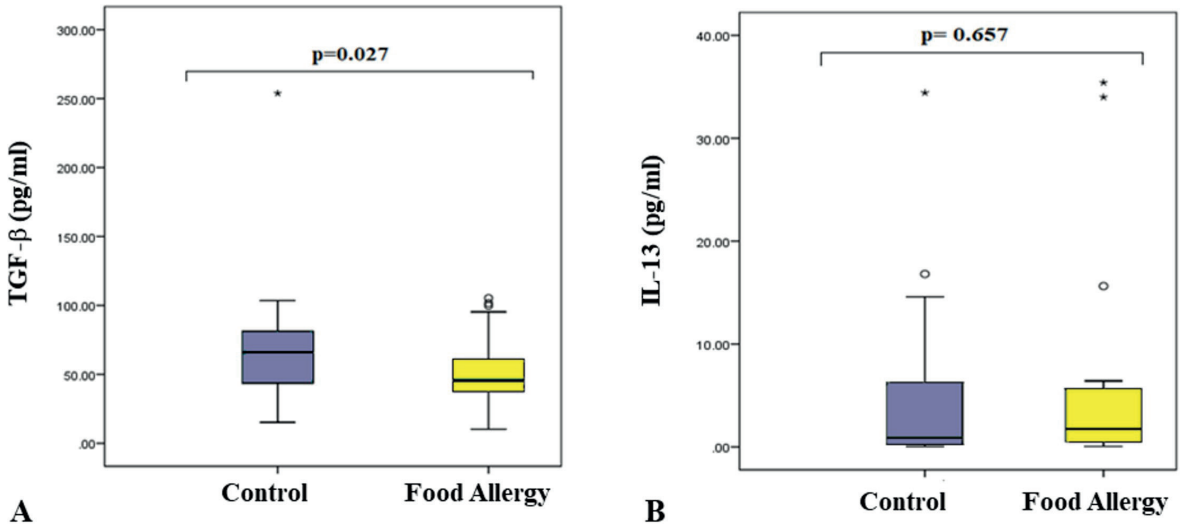

Fig. 3. A) Comparison of TGF-β protein levels in serum samples obtained from food allergy group (median: 45.39, Q1-Q3: 35.7-61.32) and healthy controls (median: 65.98, Q1-Q3: 43.07-81.59) **B)** Comparison of IL-13 protein levels in serum samples obtained from food allergy group (median: 1.76, Q1-Q3: 0.41-6.15) and healthy controls (median: 0.85, Q1-Q3: 0.26-7.29).

IL-13: interleukin-13; TGF-β: transforming growth factor-beta

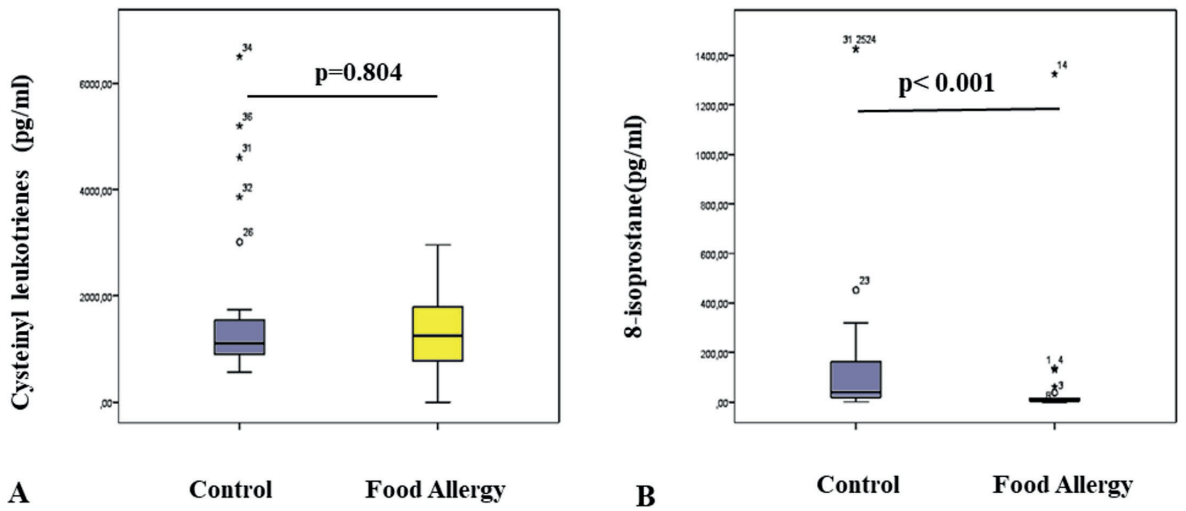

Fig. 4. A) Comparison of cysteinyl leukotriene levels in serum samples obtained from food allergy group (median: 1292.35, Q1-Q3: 842.46-1858.87 pg/mL) and healthy controls (median: 1102.62, Q1-Q3: 894.91-1557.52 pg/mL) **B)** Comparison of 8-isoprostane levels in serum samples obtained from food allergy group (median: 6.68, Q1-Q3: 1.57-26.55 pg/mL) and healthy controls (median: 37.20, Q1-Q3: 18.55-167.58 pg/mL).

NF-KB, JAK-STAT and PI3K pathways. It has also been observed that mir-19a is increased in allergic inflammation and supports the production of IL-5 and IL-13.[21] Another study revealed that the levels of thrombospondin-1 (TSP1), molecule involved in the maintenance of immune tolerance, were significantly decreased, while the levels of miR-19a were significantly increased in intestinal CD35+ B cells of mice sensitized to ovalbumin (OVA) as compared to naïve controls. They concluded that IL-4 suppresses the expression of TSP1 in the intestinal CD35+ B cells by up regulating miR-19a.[22]

In a study that aimed to investigate the role of miR-17-92 cluster in the induction of food allergen-related inflammation in the intestine, the authors found that the levels of miR-19a were significantly higher in the B cells of the intestine of food allergic mice than those in naïve control mice. They also showed that exposure of B cells, which were isolated from the mouse spleen, to IL-4 in the culture led to increased expression of miR-19a and suppressed expression of IL-10 in these cells.[23]

As a result of our study, when miR-19a gene expression levels were compared between the patient and control groups, there was no significant difference ($p > 0.05$). However, a negative correlation was found between miR-19a levels and patient eosinophil count ($p=0.009$, $r= -0.505$).

MiR-98, another miRNA selected in our study, is an important member of the Let-7 family. MiR-98 may serve as a regulator of the T cell mediated immune response.[24] Xie and Xu[25] found that patients with systemic lupus erythematosus had lower expression of miR-98 and higher Fas mRNA and protein levels in CD4+ T cells compared to healthy donors. In the study by Xie et al.[26] CD4+ cells were isolated from the lamina propria mononuclear cells of mice exposed to peanut extract and the expression level of miR-98 was examined. As a result of the study, an increase in miR-98 expression level was found.

Luo et al.[27] showed that the levels of IL-10 in peripheral B cells were significantly lower in patients with airway allergy as compared with healthy subjects. High levels of miR-98 were detected in peripheral B cells of patients when the B cells were stimulated with IL-4 to mimic allergy status. In this study, it was shown that miR-98 mediated IL-4 inhibited IL-10 expression in B cells.

In a study performed by Chen et al.[28] the levels of miR-98 were found to be higher, but the levels of TSP1 were lower, in B cells isolated from the peripheral blood in patients with asthma. A negative correlation was identified between the levels of miR-98 and TSP1 in B cells.

In our study, when miR-98 gene expression levels were compared between children with food allergy and healthy controls, statistically significant differences were found between the two groups ($p<0.05$). The expression results of miR-98 we obtained are inconsistent with the results obtained in other studies in the literature. But we also found a positive correlation ($p= 0.032$, $r= 0.494$) between miR-98 levels and IL-13 levels in patients with food allergy. When we evaluated both groups together, no correlation was found between miR-98 levels and IL-13 levels ($p= 0.273$, $r= 0.175$).

MALAT1 is a prominent intergenic lncRNA known to be associated with metastasis in non-small cell lung cancer.[29] Through its essential role in T helper cell differentiation and function, MALAT1 has important roles in immune response.[30] MALAT1 regulates the innate immune response.[31] In lung tissue biopsy samples taken from patients with chronic obstructive pulmonary disease (COPD) and a control group, MALAT1 expression levels of patients with COPD were found to be significantly higher than those of the control group.[32] In the study by Qiu et al.[33] it was observed that MALAT1 was expressed more in CD4+ cells from asthmatic patients compared to healthy patients. A recent study used bioinformatics to uncover the lncRNA-miRNA-mRNA regulatory network of bronchial epithelial cells in severe asthma. Five mRNA datasets from bronchial brushing samples from severe asthmatic patients and healthy controls were downloaded from the Gene Expression Omnibus (GEO) database, and MALAT1 was identified as one of the top 10 competing endogenous RNAs (ceRNAs) upon analysis.[34]

A study conducted by Yu et al. investigated *Morinda officinalis* extract (MOE) and its interaction with the long non-coding RNA MALAT1 in the treatment of atopic dermatitis. They showed that MOE inhibited MALAT1 expression in atopic dermatitis, leading to

reduced expression of C-C chemokine receptor type 7 (CCR7), which is regulated through a ceRNA mechanism involving MALAT1 acting as a sponge for miR-590-5p. This, in turn, suppressed tumor necrosis factor alpha (TNF-α) / interferon gamma (IFN-γ)-induced cellular proliferation and inflammation.[35] In another study, the authors demonstrated that MALAT1 increased NLRP3 expression by targeting miR-124-3p in a mouse model of atopic dermatitis. They reported that suppression of MALAT1 suppressed NLRP3 inflammasome activation and attenuated the Th1/Th2 imbalance in Th2-conditioned CD4+ T cells.[36] Feng and colleagues showed that MALAT1 was highly expressed in mice with food allergy, and its silencing relieved allergic reactions with reduction in intestinal inflammatory cells and mast cells in food allergy mice by using BALB/c mice that were sensitized to ovalbumin in accordance with a model of food allergy protocol. They also found that MALAT1 aditionally promotes IL-6 secretion by dendritic cells and their maturation. As a result of their findings the authors suggested that therapeutically blocking MALAT1 in food allergy could reduce the severity of food allergy by decreasing the secretion of IL-6 by dendritic cells and suppressing the immunomodulation of T regulatory cells.[37]

In our study, there was no difference between the two groups in the expression levels of MALAT1 ($p>0.05$). When we examine the existing literature, most studies have utilized tissue samples or inflammatory cells. In contrast, our study used serum samples, which may explain why we did not observe any significant differences between the groups.

We determined that the 3′-UTRs of the IL-6, IL6R, IL-8, IL-10, IL-13, TGFBR1, TGFBR3 and IL22RA1 genes contain putative binding sites of miR-98 using target estimation programs TargetScan (http://www.targetscan.org/), miRDB (http://mirdb.org/), and miRBase (https://www.mirbase.org/). Among these target genes we selected IL-10, IL-13, TGF-β, which are effective in allergic diseases and the pathogenesis of food allergy, and IL-4, which has been shown to be associated with miR-98 in the literature, to investigate the role of miRNAs in disease pathogenesis. Protein levels were measured by the ELISA method. When the TGF-β protein levels were examined, there was a statistically significant difference between the patient and control groups ($p<0.05$).

TGF-β, the main regulator of the immune response, has important anti-inflammatory and immunosuppressive functions. TGF-β, which has a chemoattractant effect, leads to rapid accumulation of macrophages, granulocytes, and other cells at the site of inflammation. TGF-β stimulates the secretion of other inflammatory cytokines, and in the meantime recruits granulocytes to the site, which strengthens the immune response. TGF-β1 inhibits immune cell differentiation (Th1 and Th2 cells and B cells) and cytokine production (IFN-γ and IL-2) and is also involved in the development and differentiation of T regulatory cells.[38] In a study that included 37 patients with allergic rhinitis and 30 healthy people, TGF-β protein levels were found to be significantly lower in patients with allergic rhinitis than in healthy controls.[39] The concentration of TGF-β-1 was found to be significantly higher in patients with rhinosinusitis compared to the control group. In our study, TGF-β level was found to be significantly higher in the control group.[40] This finding is in line with the data in the literature due to the anti-inflammatory properties of TGF-β and its effects on the differentiation and development of Treg cells.

In our study, IL-4 and IL-10 proteins were not found in the samples at a measurable level, however, no significant difference was observed between food allergy patients and the control group in terms of IL-13 protein levels ($p>0.05$).

Oxidative stress is often defined as an imbalance of pro-oxidants and antioxidants, which causes damage to cells or tissues. 8-isoprostane, a marker of oxidative stress, is a non-enzymatic peroxidation product of arachidonic acid. Levels of 8-isoprostane were found to be higher than normal in the exhaled breath condensate (EBC)

of asthmatic adults or children.[41] It has also been shown that elevated levels of 8-isoprostane is associated with exercise-induced bronchoconstriction in asthmatic children and adolescents.[42] In our study, contrary to the literature, 8-isoprostane levels were found to be significantly lower in patients than in controls. The available data in the literature primarily stem from studies on other allergic conditions, such as asthma and atopic dermatitis, and to the best of our knowledge, no study has specifically investigated isoprostane levels in food allergy. Due to the small sample size in our study and the lack of comparable data in the literature, the relationship between oxidative stress and food allergy remains unclear. Further comprehensive studies are needed to elucidate this connection.

Our study has several limitations. The most significant is the small sample size in the study groups. Additionally, the lack of correlation analysis between miRNA expression levels and inflammatory markers, such as cytokines and oxidative stress indicators, represents a notable gap in our investigation. One of the aims of the study was to find a biomarker that could distinguish those with food allergies from those who are healthy, thereby reducing the need for risky and laborious food provocation tests. However, the data obtained at the end of the study, although weak, indicated that miR-98 alone could be a candidate for this purpose. Another weakness of our study is the lack of a comparison group for allergic diseases such as asthma, in addition to a healthy control group. Including another allergic disease group would have allowed us to determine whether miR-98, which was prominent in our study, is a food allergy-specific biomarker.

Another limitation of our study is that blood samples were collected outside the context of active food allergic reactions. This may partly explain the discrepancies between our findings and those reported in the literature. We believe that conducting future studies using samples collected both before and after a food challenge test with the suspected allergen would provide a clearer understanding of the relationship between food allergies and non-coding RNAs.

One of the strengths of our study is the limited number of clinical studies demonstrating the relationship between food allergies and non-coding RNAs. Most of these studies are related to milk and peanut allergies. The miRNAs we selected, miR19a and miR-98, have been previously shown to be associated with food allergy in animal models and have not previously been included in clinical studies. In this respect, despite the small sample size, they contribute new data to the literature. Similarly, as mentioned above, to the best of our knowledge, our study is the first to date to explore the relationship between food allergy and oxidative stress.

In conclusion, when considered alongside the existing literature, miR-98 may serve as a potential biomarker for food allergy. There is a need to replicate these findings in a larger patient population and to further investigate the relationship between miR-98 and inflammatory markers.

Ethical approval

The study was approved by Hacettepe University Non-interventional Clinical Research Ethics Committee (date: 17.04.2020, number: GO 20/343). Informed consent was obtained from all individual participants before included in the study.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EB, HE, ÜMŞ; data collection: HE, HÜ, ÖUS, BES; analysis and interpretation of results: HE, EB, ÜMŞ; draft manuscript preparation: EB, HE. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Flexible bronchoscopy in children: complications and predictive factors

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ABSTRACT

Background. Although flexible bronchoscopy (FB) is frequently performed in children, there is limited information on the potential complications and risk factors. In this study we aimed to evaluate the complications associated with pediatric FB and identify predictors of these complications.

Methods. Patients aged 0-18 years who underwent FB at the Akdeniz University Pediatric Pulmonology Department between February 1, 2015 and June 30, 2023 were included in the study. We retrospectively recorded the patients' demographic data, known diseases, pulmonary function test results, chest computed tomography findings, bronchoscopy time/indication/route/findings, vital signs, minor and major complications associated with the FB procedure, post-procedure intensive care unit admission, procedure and sedation durations, and American Society of Anesthesiologists physical status (ASA-PS) classification, Mallampati score and anticipated need for post-procedural intensive care as evaluated in the pre-procedure anesthesiology consultation.

Results. The study included a total of 292 patients; 157 (53.8%) girls and 135 boys, with a mean age of 9.9±4.8 years. There were a total of 55 FB-related complications (18.8%), 19 major (6.5%) and 36 minor (12.3%), and 10 patients (3.4%) required intensive care unit admission due to the procedure. The most common complication was hypoxia (11.3%). Patient age, height, anticipated need for intensive care, and baseline oxygen saturation values were significant predictors of the development of bronchoscopy-related complications, while patient age, baseline diastolic blood pressure, anticipated need for intensive care, and route of insertion were predictors of major complications after bronchoscopy. ASA-PS score, pulmonary function test values, and procedure/sedation durations had no effect on the development of complications.

Conclusion. Although FB is a fairly safe diagnostic method in children, extra caution regarding possible complications is warranted in young children, when using the nasal route of insertion, or if the patient is evaluated as high-risk in the pre-procedure assessment performed by the anesthesiologist.

Key words: flexible bronchoscopy, complication, laryngeal mask, pediatrics, anesthesia.

Flexible bronchoscopy (FB) is an interventional procedure commonly used by pediatric pulmonologists. FB serves multiple purposes in the diagnosis and treatment of pediatric respiratory diseases. It is generally used diagnostically for anatomical imaging of the respiratory tract in conditions such as stridor, persistent/recurrent wheezing, chronic cough, recurrent pneumonia, suspected foreign body aspiration, hemoptysis, pulmonary hemorrhage, and radiological abnormalities (atelectasis, recurrent/permanent consolidations), but can

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also be used for therapeutic purposes, such as restoring airway patency in patients with mucus plugs, treating alveolar filling disorders (alveolar proteinosis), controlling bleeding, and guiding difficult intubations.[1,2]

Although FB is frequently performed in children, there is limited information about its complications and potential risk factors. Many studies have focused on the clinical importance and areas of application of FB, while few have evaluated the reliability and complications of the procedure.[3] FB is generally considered safe. Nevertheless, it is an invasive intervention that requires anesthesia, and various studies have documented the occurrence of mild or severe complications such as desaturation, airway trauma, and laryngeal spasm. These complications may be related to numerous patient or procedure-related variables. The risk of complications depends on various factors, including the patient's pre-existing disease, their condition during the procedure, and the way the procedure is performed.[1,3]

Our aim in this study was to evaluate the complications associated with the pediatric FB procedure in children and to identify predictors of these complications.

Materials and Methods

Patients and study setting

Patients aged 0-18 years who underwent FB in the Akdeniz University Pediatric Pulmonology Department between February 1, 2015 and June 30, 2023 were included in this retrospective single-centre study. Those who underwent bronchoscopy while hospitalized in the intensive care unit (ICU) were excluded from the study due to the likelihood of comorbidities. We retrospectively examined patient records, bronchoscopy documents, anesthesia consultation notes, anesthesia follow-up charts, laboratory test results, and radiology reports. We evaluated the patients' demographic data, pulmonary function test results, chest computed tomography (CT) findings, indication, duration and findings of bronchoscopy, route of insertion of the bronchoscope, vital signs, bronchoscopy-related complications, need for post-procedure intensive care, duration of procedure and sedation, and anesthetic agents used during the procedure. Body mass index (BMI) percentiles and thresholds for tachycardia, tachypnea, hypotension, and hypertension were determined according to the Centers for Disease Control (CDC) and Pediatric Advanced Life Support (PALS) data.[4,5]

Consultation from the Department of Anesthesiology was requested before bronchoscopy, and the procedure was performed in our Pediatric Chest Diseases Bronchoscopy Unit with a bed ready in the ICU, if deemed necessary by the anesthesiologist, based on their assessment. The patients' American Society of Anesthesiologists physical status classification (ASA-PS) (I: A normal healthy patient; II: A patient with mild systemic disease, III: A patient with severe systemic disease that limits activity but not incapacitating, IV: A patient with incapacitating disease that is a constant threat to life, and V: A moribund patient not expected to survive 24 h with or without surgical operation)[6] and Mallampati score (Class 1: Faucial/tonsillar pillars, uvula and soft palate are all visible; Class 2: Partial visibility of the faucial/tonsillar pillars, uvula and soft palate; Class 3: Base of the uvula, soft and hard palate visible; and Class 4: Only hard palate is visible)[7] were evaluated by the anesthesiologist before the procedure and recorded in the consultation notes, as well as whether they anticipated the need for intensive care were recorded retrospectively.

Anesthesia

Informed consent was obtained from each patient's parents after a detailed explanation of the bronchoscopy procedure in detail. Pediatric Chest Diseases Bronchoscopy Unit was equipped with all necessary resuscitation materials, with continuous monitoring consisting of pulse oximetry, capnography, non-invasive electrocardiogram, and blood pressure

measurement throughout the procedure. The EB530S and EB530P (Fujinon Fujifilm Europe GmbH, Düsseldorf, Germany) devices were used with a bronchoscope tip suitable for the age and weight of the patient (3.8/4.9 mm).

FB under general anesthesia with the use of a laryngeal mask airway (LMA) was predominantly utilized, as it ensured patient comfort, maintained the stability of the upper airway, and provided a less contaminated pathway for the introduction of the bronchoscope into the lower airway. In cases requiring dynamic airway assessment or when suspicion of concomitant upper respiratory tract pathologies existed, the nasal route was preferred. Sedoanalgesia was achieved with sevoflurane, propofol, fentanyl, and rocuronium in cases where an LMA was selected as the route of insertion, or with midazolam, propofol, and ketamine when using the nasal route.

Procedure duration was calculated as the time from the insertion of the bronchoscope tip into the nose or laryngeal mask to its removal from the insertion site after completing the procedure. Total sedation duration was calculated as the time from the administration of the first anesthetic agent to the patient's return to baseline sedation level.[8]

Definition and classification of complications

Minor complication: 1) Mild desaturation during the procedure ($80\% \leq SpO_2 < 90\%$); 2) Laryngospasm or bronchospasm without $SpO_2 < 90\%$; 3) Mild systemic allergic reaction without hypoxia or hypotension; 4) Temporary need for oxygen support after bronchoscopy; 5) Transient cough, stridor, or dyspnea after the procedure; 6) Fever > 38.5 °C after bronchoscopy; 7) Mild bleeding during the procedure.

Major complication: 1) Sustained bradycardia associated with severe desaturation ($SpO_2 < 80\%$) during bronchoscopy; 2) Laryngospasm or bronchospasm with desaturation ($SpO_2 < 90\%$); 3) Severe allergic reaction accompanied by hypoxia or hypotension; 4) Pulmonary or endobronchial bleeding requiring procedure interruption; 5) Need for mechanical ventilation after bronchoscopy; 6) Need for unplanned ICU observation after bronchoscopy; 7) Arterial hypotension requiring intravascular volume expansion or inotropic support; 8) Cardiorespiratory arrest or need for cardiopulmonary resuscitation.[3]

Statistical analysis

Statistical analyses were performed using SPSS version 20 statistical software. Distribution of the variables was assessed using both visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). The chi-square test was used to compare categorical data, and the Mann-Whitney U test was used to compare continuous data. Variables analyzed with Mann-Whitney U test were summarized using median (interquartile range, Q1-Q3) values, and number and percentage values were given for categorical variables. Logistic regression analysis was performed to determine the association between patient- and procedure-related factors and the occurrence of complications. Variables found to be statistically significant in the univariate logistic regression analysis for the development of complications — namely age, nasal route of bronchoscopy, anticipated ICU need, and initial oxygen saturation — were subsequently included in a multivariate logistic regression model. Due to missing data in some predictor variables, the final multivariate analysis included 278 patients. Among these, 52 patients had complications. Collinearity diagnostics indicated that all parameters had a variance inflation factor (VIF) of 1. The model demonstrated a sensitivity of 13.5% and a specificity of 98.2%. Similarly, for the analysis of major complication development age, nasal route of bronchoscopy, and anticipated ICU need, identified as significant in the univariate logistic regression, were entered into a multivariate logistic regression model. This analysis included 284 patients, of whom 17 had major complications. In this model, all

variables had VIF values of 1, indicating no multicollinearity. The model's sensitivity and specificity were determined to be 5.8% and 99.3%, respectively.

Ethical approval

This study was conducted in line with the principles of the Declaration of Helsinki. Approval was granted by the Akdeniz University Ethics Committee (Approval no KAEK-545; date 19/7/2023).

Results

The study included a total of 292 patients; 157 girls (53.8%) and 135 boys, with a mean age of 9.9±4.8 (range: 0.1-18) years. The patients had a mean BMI of 17.3±3.9, with 63.1% being of normal weight, 23.7% underweight, 8.1% overweight, and 5.1% obese. A total of 172 patients (58.9%) had a known disease, including asthma (n=61, 20.9%), cystic fibrosis (n=24, 8.2%), primary ciliary dyskinesia (n=18, 6.2%), immunodeficiency (n=14, 4.8%), bone marrow transplant (n=13, 4.5%), malignancy (n=13, 4.5%), renal transplant (n=7, 2.4%), operated esophageal atresia (n=6, 2.1%), congenital heart disease (n=6, 2.1%), neuromuscular disease (n=5, 1.7%), interstitial lung disease (n=4, 1.4%), and achondroplasia (n=1, 0.3%). The most common finding on chest CT before bronchoscopy was atelectasis (36%). This was followed by nodular lesion (32.5%) and peribronchial thickening (29.1%). The most common indication for bronchoscopy was chronic cough (33.6%), followed by bronchoalveolar lavage sampling (23.6%) and right middle lobe syndrome/atelectasis (19.2%). Other indications included recurrent pneumonia (11%), hemoptysis (10.3%) and suspected endobronchial lesion (2.3%).

Pre-bronchoscopic data

The potential need for ICU admission after bronchoscopy was noted in the anesthesia department consultation for 55 patients (19.4%). ASA-PS classification was I in 66 patients (29.1%), II in 127 patients (55.9%), III in 33 patients (14.5%), and IV in 1 patient (0.4%). Mallampati score was 1 for 160 patients (76.6%), 2 for 47 patients (22.5%), and 3 for 2 patients (1%). In the 131 patients who were able to perform the pulmonary function test, the mean percent predicted forced expiratory volume in the first second (FEV_1%) was 77.6±24.6, percent predicted forced vital capacity (FVC%) was 71.1±22.7, and the percent predicted maximal mid-expiratory flow rate (MEF25-75%) was 89±34.2. The mean preoperative SpO_2 was 98.1%±2.0%, heart rate was 111±21.5 beats/min, systolic blood pressure was 113.9±13.7 mmHg, diastolic blood pressure was 68.5±11.9 mmHg, and respiratory rate was 23.9±5.3 breaths/min. A total of 116 patients (40.3%) had tachycardia, 49 (17.2%) had tachypnea, 42 (14.8%) had hypertension, and 14 (4.9%) had hypotension.

Bronchoscopy data

The mean duration of bronchoscopy was 19.2±7.8 minutes (range: 6-50) and the duration of sedation was 32.9±10.9 minutes (range: 13-65). The route of insertion used was a laryngeal mask in 266 (91.1%) and the nasal route in 26 patients (8.9%). A total of 108 patients (41.4%) required an additional dose of anesthetic agent during the procedure. Bronchoscopy findings were evaluated as normal in 96 patients. Among the other patients, the most common abnormal bronchoscopy finding was mucus plugs (n=114, 39%). Complications associated with the FB procedure occurred in 55 patients (18.8%). Of these, 19 (6.5%) had major and 36 (12.3%) had minor complications. The most common complication was hypoxia (11.3%). In addition, 10 patients (3.4%) required ICU admission due to the procedure (6 patients had persistent severe desaturation, 2 had bronchospasm, 1 had pulmonary edema, and 1 had respiratory distress due to increased secretions; Table I).

Variables associated with bronchoscopy complications

The demographic and clinical data of patients with and without bronchoscopy-related complications are compared in Table II.

Table I. Bronchoscopy-related data (N=292)

Bronchoscopy findings	n (%)
Mucus plug	114 (39)
Normal	96 (32.9)
Chronic inflammatory changes*	26 (8.9)
Bronchomalacia	21 (7.2)
Bronchial stenosis	5 (1.7)
Endobronchial lesion	5 (1.7)
Tracheal bronchus	4 (1.4)
Tracheomalacia	4 (1.4)
Endobronchial white plaque	3 (1)
Polypoid lesion	3 (1)
Bleeding	3 (1)
Bronchoesophageal fistula	2 (0.7)
Tracheoesophageal fistula	2 (0.7)
Blind ending fistula orifice	2 (0.7)
Foreign body	1 (0.3)
Vocal cord edema	1 (0.3)
Bronchoscopy-related complications	
None	237 (81.2)
Major complications	19 (6.5)
Sustained or bradycardia associated severe desaturation (SpO2 < 80%) during bronchoscopy	12 (4.1)
Need for unplanned intensive care observation after bronchoscopy	10 (3.4)
Bronchospasm with desaturation <90%	4 (1.3)
Bleeding requiring interruption of the procedure	1 (0.3)
Minor complications	36 (12.3)
Mild desaturation (80% ≤ SaO2 < 90%) during bronchoscopy	21 (7.2)
Laryngospasm without desaturation < 90%	7 (2.4)
Bronchospasm without desaturation < 90%	3 (1)
Fever > 38.5 °C after bronchoscopy	2 (0.7)
Mild systemic allergic reaction without hypoxia or arterial hypotension	1 (0.3)
Agitation after anesthesia probably related to medication	1 (0.3)
Mild bleeding during the procedure	1 (0.3)

*Chronic inflammatory changes: erythema, edema, and friability of the bronchial mucosa.

Characteristics associated with the development of any bronchoscopy complication included anticipated post-procedure intensive care need as noted in the anesthesiologist's pre-procedure consultation (p=0.021), younger age (p=0.002), shorter height (p=0.002), lower baseline diastolic blood pressure (p=0.026), nasal route of bronchoscope insertion (p=0.001), and lower baseline oxygen saturation (p=0.012).

When the patients' demographic and clinical data were compared based on the development of major complications, patients with major complications were found to have a significantly lower age (p=0.035) and baseline diastolic blood pressure (p=0.018). A higher Mallampati score (p=0.042), anticipated ICU need (p<0.001), and nasal route of bronchoscopy (p<0.001) were also significantly associated with major complications (Table III).

Table II. Relationship between presence of complications and other parameters (N=292)

	Complication present	Complication absent	p
	n (%)	n (%)	
Sex			0.898
Female	30 (19.1)	127 (80.9)	
Male	25 (18.5)	110 (81.5)	
BMI percentile			0.477
Normal	28 (18.8)	121 (81.2)	
Underweight	9 (16.1)	47 (83.9)	
Overweight	5 (26.3)	14 (73.7)	
Obese	4 (33.3)	8 (66.7)	
Comorbidity			0.301
Yes	29 (16.9)	143 (83.1)	
No	26 (21.7)	94 (78.3)	
ASA-PS classification			0.120
1	9 (13.6)	57 (86.4)	
2	28 (22)	99 (78)	
3	3 (8.8)	31 (93.9)	
Mallampati score			0.180
1	23 (14.4)	137 (85.6)	
2	11 (22.4)	38 (77.6)	
Anticipated ICU need			0.021
Yes	16 (29.1)	39 (70.9)	
No	36 (15.7)	193 (84.3)	
Bronchoscopy route			0.001
Laryngeal mask	44 (16.5)	222 (83.5)	
Nasal	11 (42.3)	15 (57.7)	
Additional dose of anesthetic agent requirement during procedure			0.277
Yes	21 (19.4)	87 (80.6)	
No	22 (14.4)	131 (85.6)	
	Median (Q1-Q3)	Median (Q1-Q3)	
Age (years)	6 (4-13)	10 (7-14)	0.002
Height (cm)	125 (106-155)	143 (124-160)	0.002
Weight (kg)	21 (17-41)	30 (20- 46.5)	0.357
BMI (kg/m^2)	16 (14.4-18.4)	16.8 (14.6-19.5)	0.364
Baseline heart rate (bpm)	113 (102-129)	110 (97-125)	0.143
Baseline systolic BP (mmHg)	112 (100-120)	115 (105-123)	0.234
Baseline diastolic BP (mmHg)	63 (57-73)	69 (60-77)	0.026
Baseline respiratory rate (/min)	24 (20-29)	24 (20-28)	0.269
Baseline oxygen saturation (%)	98 (97-99)	99 (97-100)	0.012
FEV1%	79 (68-92)	77 (56-93)	0.853
FVC%	67 (61-84)	68 (54-86)	0.730
MEF25-75%	93 (72-115)	91 (61-112)	0.595
Procedure duration (min)	20 (15-24)	19 (15-20)	0.216
Sedation duration (min)	30 (25-43)	30 (25-40)	0.324

ASA-PS: American Society of Anesthesiologists physical status, BMI: body mass index, BP: blood pressure, FEV1: forced expiratory volume in the first second, FVC: forced vital capacity, MEF25-75%: maximal mid expiratory flow rate

Table III. Relationship between presence of major complications and other parameters

	Major complication present	Major complication absent	p
	n (%)	n (%)	
Sex			0.709
Female	11 (7.0)	146 (93.0)	
Male	8 (5.9)	127 (94.1)	
BMI percentile			0.584
Normal	7 (4.7)	142 (95.3)	
Underweight	5 (8.9)	51 (91.1)	
Overweight	2 (10.5)	17 (89.5)	
Obese	1 (8.3)	11 (91.7)	
Comorbidity			0.565
Yes	10 (5.8)	162 (92.4)	
No	9 (7.5)	111 (92.5)	
ASA classification			0.083
1	0 (0.0)	66 (100)	
2	7 (5.5)	120 (94.5)	
3	3 (8.8)	31 (91.2)	
Mallampati score			0.042
1	5 (3.1)	155 (96.9)	
2	5 (10.2)	44 (89.8)	
Anticipated ICU need			<0.001
Yes	9 (16.4)	46 (83.6)	
No	8 (3.5)	221 (96.5)	
Bronchoscopy route			<0.001
Laryngeal mask	13 (4.9)	253 (95.1)	
Nasal	6 (23.1)	20 (76.9)	
Additional dose of anesthetic agent requirement during procedure			0.349
Yes	7 (6.5)	101 (93.5)	
No	6 (3.9)	147 (96.1)	
	Median (Q1-Q3)	Median (Q1-Q3)	
Age (years)	7 (2-13)	10 (6-14)	0.035
Height (cm)	126 (103-158)	143 (122-160)	0.080
Weight (kg)	18 (10-45)	30 (20-46)	0.070
BMI (kg/m^2)	15.22 (13.31-18.99)	16.73 (14.65-19.47)	0.321
Baseline heart rate (bpm)	116 (100-138)	110 (98-126)	0.219
Baseline systolic BP (mmHg)	109 (100-118)	115 (105-123)	0.095
Baseline diastolic BP (mmHg)	59 (51-71)	69 (60-77)	0.018
Baseline respiratory rate (/min)	24 (20-25)	24 (20-28)	0.768
Baseline oxygen saturation (%)	98 (97-99)	99 (97-100)	0.346
FEV1%	80 (49-98)	78 (58-93)	0.959
FVC%	76 (61-90)	67 (56-84)	0.720
MEF25-75%	112 (41-131)	92 (65-112)	0.814
Procedure duration (min)	20 (15-30)	19 (15-20)	0.066
Sedation duration (min)	40 (21-45)	30 (25-40)	0.360

ASA-PS: American Society of Anesthesiologists physical status, BMI: body mass index, BP: blood pressure, FEV1: forced expiratory volume in the first second, FVC: forced vital capacity, MEF25-75: maximal mid expiratory flow rate

In the univariate logistic regression analysis for development of complications, age (odds ratio [OR = 0.902, 95% confidence interval [CI]: 0.846–0.962, p = 0.002), height (OR = 0.979, 95% CI: 0.967–0.991, p = 0.001), anticipated ICU need (OR = 2.199, 95% CI: 1.112–4.350, p = 0.024), nasal route of bronchoscopy (OR = 3.700, 95% CI: 1.593–8.592, p = 0.002), and initial oxygen saturation (OR = 0.866, 95% CI: 0.754–0.994, p = 0.040) were significant predictors.

In the univariate logistic regression analysis for major complication development, age (OR = 0.889, 95% CI: 0.801–0.986, p = 0.026), initial diastolic blood pressure (OR = 0.939, 95% CI: 0.896–0.984, p = 0.009), anticipated ICU need (OR = 5.405, 95% CI: 1.981–14.750, p = 0.001), and nasal route of bronchoscopy (OR = 5.838, 95% CI: 2.004–17.006, p = 0.001) were also significant predictors. The results of the multivariate logistic regression analysis performed to identify independent predictors of bronchoscopy-related complications and major complications are presented in Table IV.

The development of major complications and need for intensive care were statistically significantly more common among patients who

Table IV. Multivariate logistic regression analysis of parameters associated with complications and major complications after bronchoscopy

	p	OR	95% CI
Parameters associated with complications			
Age	0.004	0.905	0.846-0.968
Anticipated ICU need	0.047	2.109	1.010-4.406
Nasal route of bronchoscopy	0.005	4.360	1.569-12.114
Initial oxygen saturation	0.049	0.864	0.745-1.003
Parameters associated with major complications			
Age	0.052	0.896	0.803-1.001
Anticipated ICU need	0.002	5.387	1.891-15.345
Nasal route of bronchoscopy	0.029	4.414	1.160-16.787

CI: confidence interval, ICU: intensive care unit, OR: odds ratio.

Table V. Relationship between anticipated intensive care need and ASA-PS classification and complications after bronchoscopy

	Anticipated ICU need in pre-procedure anesthesia consultation, n (%)		p
	Yes	No	
Need for intensive care			<0.001
Yes	8 (80)	2 (20)	
No	47 (17.2)	227 (82.8)	
Major complication			<0.001
Yes	9 (52.9)	8 (47.1)	
No	46 (17.2)	221 (82.8)	
	ASA-PS classification, n (%)		
	I-II	III-IV	
Complication			0.144
Yes	37 (92.5)	3 (7.5)	
No	156 (83.4)	31 (16.6)	
Major complication			0.173
Yes	7 (70)	3 (30)	
No	186 (85.7)	31 (14.3)	

ASA-PS: American Society of Anesthesiologists physical status, ICU: Intensive care unit.

were recommended for intensive care in the pre-procedure anesthesia consultation notes. There was no significant difference between the ASA-PS I-II and ASA-PS III-IV groups in terms of the presence of complications and the development of major complications (Table V).

Discussion

In our study, we found that FB was generally well tolerated, with the frequency of FB-related complications, major complications, and intensive care needs being 18.8%, 6.5%, and 3.4%, respectively. As in previous studies, there was no bronchoscopy-related mortality.[9] Patient age, height, anticipated ICU need noted in the pre-procedure anesthesia consultation, and pre-procedure oxygen saturation values were predictors for the development of bronchoscopy-related complications. Furthermore, patient age, baseline diastolic blood pressure, anticipated ICU need and bronchoscopy insertion route were predictors of the development of major bronchoscopy-related complications. We observed no association between complications and ASA-PS score, pulmonary function test values, or procedure and sedation duration. In the literature, the reported incidence and severity of FB-related complications are quite inconsistent, varying between 5% and 30%.[1,3,10,11] Carlens et al.[3] stated that this inconsistency may be due to differences in patient selection, complication definitions, and the procedural techniques performed. In their study, they reported intraprocedural minor complications in 7.2%, postprocedural minor complications in 25.8%, major complications in 5.2%, and unplanned intensive care need in 3.1% of their patients. In our study, we used the complications definitions of Carlens et al.[3] and found similar rates of major complications and ICU need.

There are few studies in the literature examining risk factors for bronchoscopy-related complications. These studies indicated that young age was a risk factor with younger age groups more susceptible to desaturation.[12,13] While Schnapf[12] stated in his study that infants aged 6-12 months were more susceptible to desaturation, Carlens et al.[3] found that the major complications were significantly more frequent in patients younger than 2 years compared to those aged 2 years and older (9.2% vs. 3.3%, p=0.009). In addition, they found that additional diagnostic or therapeutic interventional procedures during FB were associated with longer anesthesia duration and an increased risk of serious complications. Similarly, DeBoer et al.[9] found that complications occurred more frequently in patients undergoing multiple procedures on the same day. However, as they did not evaluate anesthesia duration in their study, they could not determine whether this was a contributing risk factor. Consistent with the literature, our findings showed that younger age was associated with bronchoscopy-related complications, while procedure duration and sedation duration were not predictors of complication development. This may be due to the fact that we did not perform many additional interventional procedures during FB. These findings suggest that the risk of complications may depend more on the complexity of the procedure than on its duration or the duration of sedation.

To our knowledge, no previous study has evaluated the relationship between the post-procedure bronchoscopy complications and the pre-procedure anesthesiologist assessment. In our study, we found that the physician's opinion was an important predictor of complications, major complications, and need for intensive care. The physician's anticipation of a potential need for ICU prior to the procedure was associated with a 2.10-fold higher risk of complications and a 5.38-fold higher risk of major complications. We consider this important because it provides a valuable guide for anticipating complications that may develop before FB procedures, particularly in centers that have a limited number of pediatric ICU beds, such as our center.

The use of ASA-PS score as a pre-procedure risk assessment tool in the pediatric population

is controversial because of its inconsistent results.[3,14,15] There are two studies in the literature evaluating ASA-PS scores in pediatric FB. Carlens et al.[3] reported a statistically nonsignificant relationship between high ASA scores and the development of serious complications. In contrast, DeBoer et al.[9] observed fewer unexpected events in the ASA-PS I-II group compared to the ASA-PS III group (18% vs. 55.6%). In our study, ASA-PS score was not a significant predictor of complications or major complications. However, a high Mallampati score was associated with the occurrence of major complications. In a study of obese patients, desaturation occurred more frequently during bronchoscopy in patients with higher Mallampati scores, although this had no effect on bronchoscopy duration or the successful completion of the procedure.[16] To our knowledge, no previous study has evaluated the Mallampati score in relation to bronchoscopy complications in children, and we believe further studies are needed to clarify the role of ASA-PS and Mallampati scoring in risk assessment before pediatric bronchoscopy.

In our study, the most common complication associated with bronchoscopy was hypoxia (11.3%). De Blic et al.[11] in their study on FB complications, reported that complications were detected in 6.9% of patients and the most common complication was hypoxemia, at a rate of 2.7%. Carlens et al.[3] detected hypoxemia in 4.8% of patients during FB. Hypoxemia may occur as a result of depressed respiratory effort due to sedation or from partial or complete airway obstruction by the bronchoscope. It may also occur due to FB-related laryngospasm, bronchospasm, or excessive cough. The condition is usually temporary and reversible.[17] In a study evaluating systemic and cerebral oxygen saturations during FB, systemic desaturation was detected in 18.5% of patients. Male sex, smoking, baseline oxygen saturation, and FEV_1% were identified as the most important factors contributing to FB-related systemic desaturation.[18] In our study, we observed that patients with low pre-procedure saturation values were at higher risk of developing complications, whereas no relationship with sex or pulmonary function parameters were found.

The most common route of bronchoscope insertion in our study was laryngeal mask (91.1%). We excluded patients hospitalized in the ICU because they were more likely to have comorbidities that could impact the rate of bronchoscopy-related complications. Therefore, FB was not performed via an intubation tube and tracheostomy cannula in any patients. There are a limited number of studies in the literature evaluating the effect of the bronchoscopic route on the development of complications. Carlens et al.[3] stated that endotracheal intubation is associated with serious complications. Similarly, Naguib et al.[19] reported a higher rate of hypoxia in cases intubated for FB compared to those using a laryngeal mask. To our knowledge, the literature includes no study comparing the nasal and laryngeal bronchoscopy routes in children. However, Alon et al.[20] reported in a study conducted with adults that desaturation rates were significantly lower in the LMA group compared to the nasal mask group (37% vs 63.4%, p=0.008) and that the use of LMA provided better respiratory support and more stable oxygen saturation. Similarly, in our study, bronchoscopy performed via the nasal route was associated with a 4.36-fold higher risk of complications and a 4.41-fold higher risk of major complications compared to the laryngeal mask approach. Based on this, we believe that the preferential use of an LMA for FB procedure is appropriate in children who do not require dynamic airway evaluation.

This study has several limitations. First, its retrospective design and single-center setting may limit the generalizability of the findings. Additionally, due to the retrospective nature of the study, we were unable to obtain ASA-PS and Mallampati scores for all patients, nor could we evaluate the anesthesiologists' level of experience or the factors influencing their clinical judgment. Furthermore, the relatively low sensitivity values observed in both logistic regression models indicate a limited ability

to correctly identify patients who developed complications or major complications. This is likely attributable to the class imbalance in the outcome variables, as well as the reduction in sample size caused by missing data in some predictor variables. These factors may have affected the overall performance of the models. Future prospective, multicenter studies with more comprehensive data collection and balanced group distributions are needed to validate and improve upon these findings.

Conclusion

Although FB is a fairly safe diagnostic method in pediatric patients, additional caution in terms of possible complications is warranted in young children, when using the nasal route of insertion, or if the patient is evaluated as high-risk in the pre-procedure risk assessment performed by the anesthesiologist. Further studies are needed to evaluate the effect of procedure and sedation times, ASA-PS, and Mallampati score on the development of FB-related complications in children.

Ethical approval

The study was approved by the Ethics Committee of Akdeniz University (Approval no KAEK-545; date 19/7/2023).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AB, AEB, AB², İÖA, BBP; data collection: AB, BBP, AEB; analysis and interpretation of results: all authors; draft manuscript preparation: AB, AEB, AB², İÖA; All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Dietary adequacies and anthropometric measurements in children with poor appetite according to their mothers

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ABSTRACT

Background. This cross-sectional study aimed to evaluate the dietary adequacy and growth patterns of children considered to have a poor appetite by their mothers and to compare these findings with established reference values.

Methods. A total of 200 volunteer mothers who applied because they thought their children (2-9 years) had poor appetites participated in the study. Maternal reports were obtained through a comprehensive questionnaire, encompassing socio-demographic characteristics, dietary habits, the Children's Eating Behavior Questionnaire (CEBQ), anthropometric measurements, and three-day food consumption records. Children's anthropometric measurements were evaluated according to z-scores based on the World Health Organization standards, and daily energy and nutrient intake amounts were determined from food consumption records. Dietary adequacy was calculated according to dietary reference intakes (DRI).

Results. It was found that 90.5% of the children had normal height, and 6.0% were stunted/severely stunted. According to body mass index (BMI)-for-age z-scores (BAZ), 92.5% of the children had normal weight. All mothers perceived that their children had poor appetite, and 55% also believed their children to be underweight, whereas objective measurements indicated that 90% of these children had normal weight. The scores of sub-dimensions of "Food Responsiveness" and "Emotional Overeating" of CEBQ for girls (12.6±2.7 and 9.6±2.3, respectively) were significantly higher than those for boys (11.2±2.8 and 8.7±2.4, respectively) ($p<0.05$). Regarding dietary adequacy, both boys and girls met their daily energy (91.4±8.5% and 88.3±7.8%, respectively) and protein requirements (196.4±47.7% and 210,3±41,8%, respectively). However, fiber, folate, and potassium intakes were relatively low in both sexes. The mean adequacy ratio (MAR) was significantly higher in boys (145.0±16.0) than girls (140.2±15.7). Nonetheless, the MAR values for both sexes were notably high, suggesting an adequate nutrient intake overall. There was a significant weak positive correlation between children's daily protein intake and z-scores of weight, height and BMI for age. Similarly, a significant weak positive correlation was observed between calcium and iron intake and BAZ.

Conclusions. This study highlighted divergence between perceived and objective nutritional assessments in this population. Comprehensive evaluation, including anthropometric and dietary data, is needed to accurately characterize children's status.

Key words: eating behaviors, body mass index, appetite, dietary adequacy.

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Nutrition plays a fundamental role in the growth and development of individuals, commencing from the earliest stages of life and persisting throughout the lifespan. The consequences of inadequate, excessive, or erroneous energy and nutrient intake manifest as malnutrition, creating a dual burden that significantly impacts the health, physical well-being, and cognitive development of children.[1] The family/home environment is an essential determinant of children's dietary intake and its impact on body weight. Parents have the opportunity to positively influence their children's body weight and food intake by providing healthy foods at home, giving information about healthy nutrition to their children, and being role models in healthy food choices.[2] In numerous countries, the responsibility for ensuring children's nutritional needs primarily falls upon parents and caregivers, with mothers often bearing the primary responsibility. Mothers' beliefs and behaviors regarding their children's dietary habits are shaped by perceptions of their children's nutritional status. Mothers' awareness of changes in their child's nutritional status, which may lead to health concerns, initiates the first step in seeking medical assistance and health care.[3]

Growth during the toddler (ages 1-3 years) and preschool (ages 3-5) years is slower than in infancy but is steady. This decrease in growth velocity is reflected in reduced appetite. However, young children still require an adequate amount of energy and nutrients to meet their nutritional needs. The eating and health habits established during these early years may influence dietary habits and subsequent health in adulthood. The energy needs of toddlers and preschool-age children reflect their slower growth rate compared to earlier stages.[4]

The World Health Organization (WHO) defines poor appetite as an eating difficulty, rejecting eating, or excessive selectivity in the presence of appropriate and sufficient nutritional sources, in the presence of an individual providing care for children who do not have an underlying organic pathology.[5] Remarkably, a substantial proportion of healthy children, estimated at 20-35%, are brought to healthcare facilities due to concerns related to poor appetite and eating difficulties.[6] In some societies, being overweight or having a large body is important, and parents evaluate their children's growth based on this. Although children receive adequate amounts of energy and nutrients, this may not meet the expectations of their parents.[3] Studies show that parents generally adopt controlling feeding practices (i.e., food restriction and eating pressure) in response to concerns regarding their children's appetite and body weight.[2,7] It is reported that more than half of parents misjudge their child's weight status, which is called 'parental misperception'.[8] Unrealistic parental expectations can give rise to unwarranted feelings of anxiety, while inappropriate threats or punishments may intensify a child's resistance to eating.[9] When children with poor appetite seek medical attention in outpatient settings, evaluating their body weight gain, growth trajectory, and developmental progress is imperative.[10] The most crucial indicator of a child's adequate and balanced nutrition is their growth and development. The adequacy of growth is understood by determining the body weight and height children should have according to their age and gender. It is critical to determine whether the preschool and school age child is adequately nourished, determine at an early stage any deviations from normal, and take necessary precautions.[11] This study aimed to assess the dietary adequacy and growth patterns of children considered to have a poor appetite by their mothers and to compare these findings with established reference values.

Materials and Methods

Study design, setting, and participants

The study was conducted between June 2022 and March 2023 with 200 mother-child dyads aged 2-9 years who applied to the Social Pediatrics Polyclinic because they thought their children had poor appetite. The power analysis,

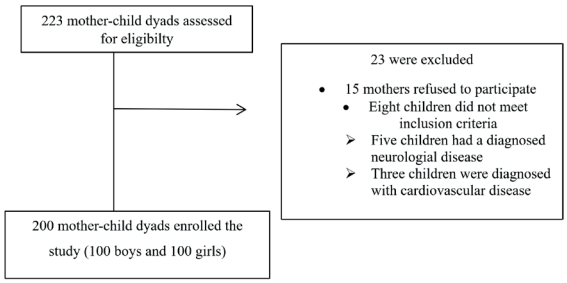

Fig. 1. Study flow diagram

conducted using G*Power version 3.0.10, indicated that a total sample size of at least 199 was necessary to achieve 80% power, with a 5% margin of error, and effect size of d=0.20 with 95% confidence. The study included mothers who thought their child had poor appetite, volunteered to participate, had a child aged between 2 and 9 years, and did not present with communication problems. Encompassed children with chronic, metabolic, or neurological conditions, those accompanied by individuals other than their mothers during clinic visits, those exhibiting abnormal laboratory findings, and those seeking acute care for conditions such as fever, diarrhea, infection, or respiratory distress which are thought to be confounding factors, were determined as exclusion criteria. The flow chart of participants' enrollment is given in Fig. 1. Informed written consent was obtained from the mothers following the principles outlined in the Declaration of Helsinki, and the study protocol was approved by the Gazi University Ethics Commission (approval number: 2022-537, 22.04.2022).

Data collection and evaluation

Following the completion of their medical examination, the children who had applied to the Social Pediatrics Outpatient Clinic were referred to the dietitian for the study. Data were collected through face-to-face interviews utilizing a comprehensive questionnaire by the same dietitian. The questionnaire, which was developed in line with the literature, comprised sections on children's socio-demographic characteristics, dietary habits, a 35-item Children's Eating Behavior Questionnaire (CEBQ), anthropometric measurements, and a three-day food record.[3,5]

Dietary assessment

Mothers were asked to keep a three-day food record, consisting of one weekend day and two weekdays. The average energy and nutrient values of consumed foods were determined using the Nutrition Information System (BeBiS).[12] These values were then evaluated in accordance with the dietary reference intake (DRI) guidelines.[13] To evaluate the adequacy of nutrient intake, nutrient adequacy ratios (NARs) were calculated for eleven essential nutrients (protein, fiber, vitamin B_6, vitamin B_{12}, vitamin C, folate, calcium, phosphorus, magnesium, iron, and zinc) using the DRI recommendations (%). The mean adequacy ratio (MAR) was obtained by dividing the NARs by the number of nutrients considered.

Children's Eating Behavior Questionnaire (CEBQ)

The Children's Eating Behaviour Questionnaire (CEBQ) was utilized to determine the eating behaviors of children. This questionnaire consists of 35 items and employs a 5-point Likert-type scale.[14] Eating style is assessed on eight scales: food responsiveness (4 items), enjoyment of food (4 items), emotional overeating (4 items), desire to drink (3 items), satiety responsiveness (5 items), slowness in eating (4 items), and emotional undereating (4 items), and fussiness (7 items). Participants rate the frequency of their child's behaviors and experiences on a 5-point scale: 1 = never, 2 = rarely, 3 = sometimes, 4 = often, and 5 = always. The questionnaire evaluates both food-approach behaviours and food-avoidant behaviours through these sub-dimensions. Food-approach behaviours include food responsiveness, enjoyment of food, emotional overeating, and desire to drink. Food-avoidant behaviours comprise satiety responsiveness, slowness in eating, emotional undereating, and food fussiness. The internal consistency of the CEBQ was assessed using Cronbach's alpha

coefficients in the original study, ranging from 0.74 to 0.91.[14] The CEBQ was adapted for the Turkish population by Yılmaz et al.[15] in 2011 with 2–9-year-old children and their parents.

Anthropometric measurements

The body weight and height were obtained by the dietitians and assessed by established reference values. Height was measured (cm) with feet close together and the head in Frankfort plane with a 0.1 cm portable stadiometer. Weight was recorded to the nearest 0.1 kg, using an electronic weighing scale (Seca 285).

The body mass index (BMI, kg/m^2), a measure calculated using the body weight and height measurements, was used to evaluate the children's nutritional status. To assess the growth status, height-for-age Z-score (HAZ), weight-for-age Z-score (WAZ), and BMI-for-age Z-score (BAZ) values were calculated using the World Health Organization (WHO) Anthro/AnthroPlus software.[16] The BAZ and HAZ were evaluated based on the WHO Child Growth Standards cut-off values. These Z-scores enable standardized comparison of individual children's measurements with a reference population. The HAZ value was classified as ''severely stunted'' (< -3 SD), ''stunted'' ([-2]–[-3] SD) ''normal'' ([-2]–[+2] SD), and ''tall'' (> +2 SD). The BAZ value was classified as "normal" ([-2]-[+2] SD), "underweight" ([-2]-[-3] SD), "severely underweight" (< -3 SD) for children aged 2-5 years[17] and "normal" ([-2]-[+1] SD), "underweight" ([-2]-[-3] SD), and"severely underweight" (< -3 SD) for children aged 5-9 years.[18]

Statistical analysis

Statistical analyses of the study data were performed using SPSS 24.0 (Statistical Package for the Social Sciences, Inc.; Chicago, Illinois, United States). The dependent variables of the study were HAZ and BAZ. Numerical variables were expressed as mean and standard deviation (±SD), and qualitative variables were expressed as number (n) and percentage (%). The Kolmogorov–Smirnov test was conducted to ascertain the normality of the distribution of variables. As the numerical variables between sexes were normally distributed, independent t-test was used to evaluate the difference. Relationships between numerical variables with normal distribution were evaluated with the Pearson correlation coefficient. Statistical significance was evaluated at a $p<0.05$ level.

Results

Two hundred mother-child dyads participated in the study. The mean age for boys was 4,9±1,9 years, and for girls, it was 5.6±2.0 years. The mean age of the mothers was 32.0±6.3 years (min-max: 18-47 years). Table I shows the distribution of children based on the general characteristics of their families. Of the children, 56.0% were the firstborn in their families. More than half of the mothers (59.0%) and fathers (54.5%) had a high school education or lower. Separation of parents was reported in 16.0% of the families, and 30.0% of the families indicated that their expenses exceeded their income. All mothers perceived that their children had poor appetites, while 55% also perceived that they were underweight. Notably, among the children whose mothers perceived them as underweight, 90% had normal body weight, 8.2% were underweight, and 1.8% were severely underweight. Conversely, only 4.4% of mothers who perceived their children as having a normal body weight had underweight children (Table II).

When the anthropometric measurements were evaluated for the children, it was revealed that 90.5% had a height within the normal range, while 6% were classified as stunted. Based on BMI z scores, 92.5% of the children had a normal body weight (Table II).

Table I. Distribution of children according to the general characteristics of their families and daily activities

	Boys (n=100)		Girls (n=100)		Total (N=200)		p
	n	%	n	%	n	%	
Birth order							
First child	63	63.0	49	49.0	112	56.0	0.087
2-4 child	37	37.0	51	51.0	88	44.0	
Mother's educational status							
High school and below	56	56.0	62	62.0	118	59.0	0.388
University and above	44	44.0	38	38.0	82	41.0	
Father's educational status							
High school and below	59	59.0	50	50.0	109	54.5	0.201
University and above	41	41.0	50	50.0	91	45.5	
Mother's working status							
Not working	34	34.0	31	31.0	65	32.5	0.651
Working	66	66.0	69	69.0	135	67.5	
Father's occupation							
Civil Servant/Worker	52	52.0	59	59.0	111	55.5	0.319
Self-employment	48	48.0	41	41.0	89	44.5	
Family type							
Nuclear	59	59.0	59	59.0	118	59.0	0.903
Extended	24	24.0	26	26.0	50	25.0	
Broken	17	17.0	15	15.0	32	16.0	
Family income status							
Income is less than expenses	35	35.0	25	25.0	60	30.0	0.281
Income is more than expenses	29	29.0	39	39.0	68	34.0	
Income is equal to expenses	36	36.0	36	36.0	72	36.0	
Parental assessment of child's body weight							
Underweight	52	52.0	58	58.0	110	55.0	0.394
Normal	48	48.0	42	42.0	90	45.0	
Free time activities							
Outdoor activity	25	25.0	23	23.0	48	24.0	0.741
Sedentary activity	75	7.0	77	77.0	152	76.0	
Outdoor play time							
Less than 1 hour	37	37.0	73	73.0	110	55.0	<0.001*
1 hour or more	63	63.0	27	27.0	90	45.0	
Average TV viewing time per day							
Less than 2 hours	77	77.0	67	67.0	144	72.0	0.063
More than 2 hours	23	23.0	33	33.0	56	28.0	
Average daily computer/tablet usage time							
Less than 2 hours	94	94.0	97	97.0	191	95.5	0.306
More than 2 hours	6	6.0	3	3.0	9	4.5	
Regular sports activity							
Yes	26	26.0	29	29.0	55	27,5	0.635
No	74	74.0	71	71.0	145	72,5	
Average daily sleep time							
Less than 9 hours	25	25.0	51	5.0	76	38,0	<0.001*
More than 9 hours	75	75.0	49	49.0	124	62,0	
Number of meals per day							
1-2 meals	18	18.0	33	33.0	51	25,5	<0.001*
3-4 meals	48	48.0	56	56.0	104	52,0	
5-6 meals	34	34.0	11	11.0	45	22,5	

Chi-square test. *p<0,05

Table II. Evaluation of anthropometric measurements of children

	Boys (n=100)		Girls (n=100)		Total (n=200)		p
	Mean ± SD	Min-Max	Mean ± SD	Min-Max	Mean ± SD	Min-Max	
Height (cm)	106.8±12.98	75-130.1	112.4±13.65	81.1-138.5	-	-	-
Body weight (kg)	16.5±3.89	8.5-25.9	19.0±5.17	9.9-31.6	-	-	-
BMI (kg/m^2)	14.3±1.02	11.2-16.4	14.8±1.33	11.7-17.3	14.6±1.10	11.2-17.3	0.633
WAZ	-0.73±1.05	(-3.57)-(1.68)	-0.25±0.74	(-2.87)-(0.90)	(-0.49)±0.94	(-3.57)-(1.68)	0.001*
HAZ	-0.22±1.46	(-3.22)-(2.96)	0.12±0.72	(-2.80)-(1.73)	(-0.47)±1.16	(-3.22)-(2.96)	<0.001*
BAZ	-0.96±0.81	(-3.07)-(0.75)	-0.48±0.78	(-3.10)-(0.85)	-0.72±0.82	(-3.10)-(0.85)	0.292
	n	%	n	%	n	%	
HAZ classification**							
Tall	7	7.0	-	-	7	3.5	
Normal	82	82.0	99	99.0	181	90.5	
Stunted	8	8.0	1	1.0	9	4.5	
Severely stunted	3	3.0	-	-	3	1.5	
BAZ classification***							
Normal	89	89.0	96	96.0	185	92.5	
Underweight	10	10.0	3	3.0	13	6.5	
Severely underweight	1	1.0	1	1.0	2	1.0	

Actual weight of child	Maternal assessment of child's body weight			
	Underweight (n=110)		Normal weight (n=90)	
	n	%	n	%
Normal weight	99	90	86	95.6
Underweight	9	8.2	4	4.4
Severely underweight	2	1.8	-	-

*p<0.05, independent groups t-test, **Tall: > +2 SD, normal: (-2)-(+2) SD, stunted: (-2)-(-3) SD, severely stunted: < -3 SD, ***For 2-5 years: normal: (-2)-(+2) SD, underweight: (-2)-(-3) SD, severely underweight: < -3 SD; for 5-9 years: normal: (-2)-(+1) SD, underweight: (-2)-(-3) SD, severely underweight: < -3 SD.
BAZ: BMI for age z-score, BMI: body mass index, HAZ: height for age z-score, SD: standard deviation, WAZ: weight for age z-score.

Table III. Evaluation of scores obtained from the sub-dimensions of the CEBQ according to sex

	Boys (n=100)		Girls (n=100)		Total (n=200)		
	Mean ± SD	Min-max	Mean ± SD	Min-max	Mean ± SD	Min-max	P
Food responsiveness	11.2±2.85	5-20	12.6±2.74	6-20	11.9±2.88	5-20	0.001*
Emotional overeating	8.7±2.44	4-15	9.6±2.35	5-16	9.2±2.43	4-16	0.013*
Enjoyment of food	12.5±3.58	6-20	12.6±3.64	5-20	12.6±3.60	5-20	0.830
Desire to drink	7.9±2.58	4-14	7.9±2.98	3-15	7.9±2.78	3-15	0.939
Satiety responsiveness	21.6±2.76	16-27	21.2±3.03	14-29	21.4±3.00	14-29	0.342
Slowness in eating	11.1±2.83	4-20	11.4±2.36	6-17	11.3±2.61	4-20	0.402
Emotional undereating	12.1±2.84	6-20	12.5±2.79	6-20	12.3±2.81	6-20	0.422
Food fussiness	7.8±2.68	3-14	8.1±2.50	3-14	7.9±2.59	3-14	0.496

*p<0.05, independent groups t-test.
CEBQ: Children's Eating Behaviour Questionnaire, SD: standard deviation.

Table III presents the evaluation of CEBQ sub-dimension scores according to sex. The scores for girls (12.6±2.7 and 9.6±2.3, respectively) were significantly higher than those for boys (11.2±2.8 and 8.7±2.4, respectively) in the sub-dimensions of "Food Responsiveness" and "Emotional Overeating" (p=0.001 and p=0.013, respectively). No significant sex differences were found in the scores of other sub-dimensions (p>0.05).

Table IV provides information on the mean daily energy and nutrient intakes NAR and MAR of children by sex. Both boys (91.4±8.5%) and girls (88.3±7.8%) met the energy requirements specified in the DRI. Additionally, they consumed approximately double the recommended protein intake (196.4±47.7% in boys and 210.3±41.8% in girls). However, both sexes exhibited relatively low intakes of fiber

Table IV. Evaluation of children's mean daily energy and nutrient intakes, NAR, and MAR by sex

	Boys (n=100)			Girls (n=100)			
	Daily intake amounts		NAR	Daily intake amounts		NAR	
	Mean ± SD	Min-max	Mean ± SD	Mean ± SD	Min-max	Mean ± SD	p*
Energy (kcal)	1366.8±248.93	761.0-2017.1	91.4±8.50	1476.6±242.49	988.5-2183.2	88.3±7.87	0.008**
Carbohydrate (g)	165.6±35.25	81.1-269.8	-	180.9±35.68	109.1-281.6	-	-
Carbohydrate (%)	48.3±4.16	40.6-56.4	-	48.3±4.16	40.8-56.4	-	-
Protein (g)	34.0±8.46	12.3-60,.	196.4±47.72	39.4±9.20	23.1-66.0	210.3±41.88	0.030**
Protein (%)	13.1±1.93	9.8-17.6	-	13.0±1.88	9.3-16.7	-	-
Protein (g/kg)	2.6±0.57	1.5-3.9	-	2.5±0.57	1.2-4.2	-	-
Fat (g)	58.4±12.07	35.7-90.1	-	62.3±12.62	35.9-97.8	-	-
Fat (%)	38.5±4.45	28.8-47.7	-	38.0±4.10	27.5-46.5	-	-
Saturated fatty acids (%)	13.4±1.85	9.2-18.2	-	13.6±1.78	9.7-17.1	-	-
MUFA (%)	13.6±2.17	9.3-18.7	-	13.0±1.77	9.5-17.6	-	-
PUFA (%)	10.0±1.64	6.9-13.4	-	9.9±1.52	7.0-13.4	-	-
Cholesterol (mg)	282.7±64.81	167.0-480.0	-	288.5±78.72	146.0-451.0	-	-
Fiber (g)	14.6±2.72	8.0-21.0	63.6±15.67	14.7±2.53	9.0-22.0	62.0±13.32	0.434
Vitamin A (mcg)	245.1±56.33	159.0-409.0	66.5±17.63	327.0±90.40	166.0-602.0	84.9±29.53	<0.001**
Thiamine (mg)	0.60±0.16	0.28-0.94	105.7±31.88	0.65±0.14	0.39-0.92	109.2±27.17	0.409
Riboflavin (mg)	1.15±0.35	0.54-1.96	200.9±62.44	0.77±0.30	0.43-1.78	129.7±54.92	<0.001**
Niacin (mg)	14.1±2.57	8.9-20.6	191.5±44.40	15.4±3.25	8.7-22.3	199.4±58.01	0.284
Vitamin B_6 (mg)	0.93±0.34	0.35-1.77	162.0±60.37	0.79±0.26	0.42-1.85	131.4±46.67	<0.001**
Folate (mcg)	140.7±31.05	84.0-214.0	75.7±16.70	155.0±3.38	92.0-222.0	78.9±15.33	0.163
Vitamin B_{12} (mcg)	3.34±1.09	1.50-7.30	278.5±90.51	3.17±0.80	1.70-6.10	263.8±66.61	0.194
Vitamin C (mg)	44.4±10.16	24.0-78.4	177.7±40.65	44.7±11.23	21.3-69.8	178.7±44.92	0.874
Calcium (mg)	605.8±198.96	317. -1152.0	88.5±36.39	740.2±231.48	315.0-1210.0	100.7±45.50	0.036**
Potassium (mg)	1245.2±394.33	691.0-2156.0	55.8±15.94	1382.7±762.74	688.0-769.0	61.2±32.57	0.139
Magnesium (mg)	223.5±60.33	105.0-341.0	195.0±53.17	217.1±55.75	122.0-375.0	173.7±45.12	0.003**
Phosphorus (mg)	507.6±186.23	193.0-971.0	101.1±36.91	534.9±155.48	219.0-963.0	102.7±35.90	0.753
Iron (mg)	8.4±2.32	4.18-17.17	93.5±24.78	8.3±1.95	5.09-13.25	91.1±21.88	0.453
Zinc (mg)	5.4±1.65	3.19-9.70	122.5±27.95	6.0±1.70	2.35-9.32	125.1±26.88	0.505
MAR		145.0±16.03			140.2±15.77		0.033***

*Difference between NAR values, **p<0.05, independent groups t-test, ***Difference between MAR values, independent groups t-test.
MAR: mean adequacy ratio, MUFA: monounsaturated fatty acids, NAR: nutrient adequacy ratio, PUFA: polyunsaturated fatty acids, SD: standard deviation.

(63.6±15.6% in boys and 62.0±13.3% in girls), folate (75.7±16.7% in boys, 78.9±15.3% in girls), and potassium (55.8±15.9% in boys, 61.2±32.5% in girls). The MAR value, although significantly higher in boys, was found to be relatively high in both sexes (145.0±16.0% in boys and 140.2±15.7% in girls).

Results revealed a significant but weak positive correlation between children's daily protein intake and WAZ, HAZ, and BAZ. Similarly, a significant but weak positive correlation was observed between calcium and iron intake and BAZ. Among the sub-dimensions of the CEBQ, the "Food Responsiveness" sub-dimension exhibited an association with WAZ, HAZ, and BAZ. In addition, a significant weak positive relationship was found between enjoyment of food and emotional undereating and BAZ and between satiety responsiveness and HAZ ($p<0.05$) (Table V).

Table V. Evaluation of the relationship between WAZ, HAZ, and BAZ scores, adequacy ratio of nutrient requirements, and CEBQ subscale scores of children

	WAZ		HAZ		BAZ	
	r	p	r	p	r	p
NAR						
Energy	0.088	0.217	0.105	0.138	-0.003	0.968
Protein	0.316	<0.001*	0.300	<0.001*	0.152	0.032*
Fiber	0.106	0.134	0.079	0.268	0.072	0.313
Vitamin B_6	-0.011	0.877	0.016	0.819	-0.037	0.602
Folate	0.029	0.681	0.012	0.863	0.039	0.580
Vitamin B_{12}	0.093	0.189	0.099	0.163	0.021	0.763
Vitamin C	-0.050	0.478	-0.040	0.575	-0.003	0.962
Calcium	0.068	0.336	-0.001	0.991	0.144	0.041*
Potassium	-0.154	0.030*	-0.100	0.160	-0.135	0.057
Magnesium	-0.087	0.221	-.0.076	0.285	-0.047	0.509
Phosphorus	0.114	0.109	0.091	0.199	0.027	0.699
Iron	0.050	0.483	-0.055	0.441	0.156	0.028*
Zinc	-0.057	0.419	-.0.132	0.062	0.003	0.963
MAR	0.139	0.050	0.074	0.299	0.147	0.037*
CEBQ subscale scores						
Food responsiveness	0.383	<0.001*	0.359	<0.001*	0.237	0.001*
Emotional overeating	0.097	0.172	0.086	0.228	0.051	0.472
Enjoyment of food	0.120	0.089	0.035	0.621	0.148	0.037*
Desire to drink	-0.046	0.520	-0.003	0.971	-0.051	0.474
Satiety responsiveness	-0.120	0.090	-0.151	0.032*	-0.015	0.833
Slowness in eating	-0.060	0.395	-0.048	0.499	-0.023	0.745
Emotional undereating	0.079	0.265	-0.002	0.977	-0.156	0.028*
Food fussiness	-0.021	0.768	-0.014	0.841	0.041	0.564

* $p<0.05$, Pearson correlation test.
BAZ: body mass index for age z-score, CEBQ: Children's Eating Behavior Questionnaire, HAZ: height for age z-score, MAR: mean adequacy ratio, NAR: nutrient adequacy ratio, WAZ: weight for age z-score.

Discussion

This study highlighted a significant divergence between parental perceptions and objective measurements of the nutritional status of children with poor appetite. The findings also underscored the importance of accurately assessing children's nutritional status through comprehensive methods, including objective measurements and dietary assessments.

Although poor appetite may serve as an indicator of an underlying organic condition resulting in lower than expected body weight or impaired weight gain, the majority of cases involving poor appetite still demonstrate normal growth. In our study, the findings revealed that the majority of children exhibited normal height (90.5%) and BMI (92.5%) for their age. Also, it was determined that the majority of the children were able to meet their energy needs and nearly double their protein requirements. Nutrition is the most important factor affecting growth during childhood.[1] Since we excluded confounding factors such as certain diseases (such as endocrine, gastrointestinal, and anemia) that affect growth and development, the effect of appetite on growth was observed.

However, parental expectations regarding their children's nutrition may not always align with their actual nutrition and growth status, leading to concerns about body weight, appearance, or appetite.[19] In this study, it was observed that more than half of the mothers who brought their children to the outpatient clinic due to poor appetite believed their children were underweight, while almost all of these children were found to have normal body weight. Another study focusing on 1-4-year-old children and their caregivers revealed that 4.5% of the children were inaccurately perceived as having a "poor appetite" by their caregivers despite demonstrating a good appetite.[20] Yılmaz et al. reported a high prevalence of parental misperceptions regarding their child's growth, with only 16.7% of mothers and fathers accurately estimating their child's growth.[21] Similarly, Pinasco et al. found that 45.8% of the mothers incorrectly estimated their children's growth.[22] These perceptions of underweight or overweight can be influenced by the social environment, socioeconomic status, and cultural traditions.[23] It has been reported that many parents do not consider the body weight of their children to be adequate because they believe that a child with a good appetite and a higher body weight is healthier.[3] In this study, we found that more than half of the mothers had high school education or below, and more than half of the children were the firstborn. Baughcum et al. showed that, higher maternal educational levels were associated with more accurate predictions of their children's body weight status.[24] Another study reported that a significant proportion of children with poor appetite (32%) were the eldest child in the family, possibly due to increased attention from parents and the experience gained as parents have more children.[3]

Each child has their own growth rate, and children may not show the same growth rate in each period. Depending on individual differences in growth rate, appetite may decrease or increase in children.[25] Poor appetite is a prevalent symptom in childhood, particularly among children aged 1 to 5 years. It affects approximately 30% of typically developing children and up to 80% of children with growth and developmental issues.[26] Children with poor appetites often have limited food intake, leading to deficiencies in essential nutrients such as energy, protein, vitamins, and minerals crucial for their growth and development.[5] Despite maternal concerns about their children's nutritional status, the analysis of children's food intake in relation to reference values revealed that daily energy and nutrient intakes were generally sufficient, with the exception of fiber, folate, potassium (in both sexes), and vitamin A (in boys). A previous study reported similar daily intakes of energy, macronutrients, and micronutrients among children aged 6 to 60 months, regardless of their mothers' evaluations of their appetite. However, energy, folate, and calcium intakes were below reference values,

while protein, vitamin A, zinc, and magnesium intakes exceeded reference values in children aged 12 to 36 and 37 to 60 months.[5] Interestingly, we observed that children consumed nearly all of their recommended daily energy intake requirement and twice the recommended protein intake. Despite maternal perceptions of insufficient energy intake, children can obtain high-energy foods in small portions, and some can meet their energy requirements even with one or two meals per day.[3] It is important to note that children possess the capacity to self-regulate their energy intake. While meal intakes may fluctuate throughout the day, the total daily energy intake generally remains consistent among both young children and school-age children.[3] Notably, meals with a high protein content have been associated with increased satiety and reduced hunger. Proteins are known to induce satiety, stimulate the secretion of gastrointestinal hormones, and enhance diet-induced thermogenesis.[27]

In terms of nutritional status, the majority of the children in this study exhibited normal HAZ and had normal BAZ. Consistent with previous research, Kaymaz et al. reported that 65% of school-age children (aged 6-15 years) who were brought to the outpatient clinic due to perceived poor appetite and low weight-for-height, without any underlying organic causes, fell within the normal range based on BMI percentile values.[10] Another study demonstrated no significant difference in WAZ and HAZ between children whose appetite was considered good or poor by their mothers, with a predominance of low scores in both groups.[5] In our study, we observed that children displaying food cravings, which indicate a healthy appetite, had higher WAZ, HAZ, and BAZ. Conversely, children exhibiting emotional undereating, a sign of reduced appetite, had lower BAZ values.

Furthermore, this study observed significant correlations between WAZ, HAZ, and BAZ, and increased protein adequacy ratio, calcium adequacy ratio, and iron adequacy ratio. Adequate intake of macronutrients and micronutrients during infancy and childhood plays a critical role in promoting growth and preventing failure to thrive.[28] Energy requirements per kilogram of body weight are lower for school-age children (5-10 years) compared to toddlers and preschoolers.[29] Insufficient energy and protein intake, in particular, can lead to growth delay and loss of muscle and fat mass.[28] In this study, the protein adequacy ratio, calcium adequacy ratio, and iron adequacy ratio values of the children were found to be high, and the fact that the majority of them had normal BAZ scores is thought to be a good indicator of this.

Evaluation of dietary adequacies by obtaining 3-day food records from children, evaluation of nutritional behaviors with a validated scale, and the large number of samples despite being a single center experience are the strengths of the study. However, this study also has some limitations. First, only mothers participated, thus there might be less generalization of the findings in terms of parent gender. Second, we did not evaluate maternal nutrition-related knowledge, attitudes, and practices, which could have offered a more comprehensive understanding of maternal perception. In addition, the fact that the anxiety and depression status of the mothers was not evaluated is another limitation. Third, the fact that non-nutritional confounding factors such as genetic predisposition, sleep patterns, and physical activity levels that affect growth and development were not evaluated may also be a limitation.

Conclusion

This study revealed a discordance between maternal perceptions of poor appetite and objective assessments of nutritional status in children. While all mothers reported reduced appetite, quantitative analysis showed adequate daily energy and nutrient intake as well as normal growth parameters in the majority of children. This divergence between subjective parental impression and standardized outcome metrics suggests qualitative evaluations may be influenced foremost by mothers sentiment. It

is crucial to provide comprehensive training to individuals involved in childcare, particularly mothers, through a multidisciplinary team comprising a pediatrician, dietitian, psychologist, and child development specialist, to enhance their awareness of normal child growth and development. A detailed discussion with the mother regarding the type and quantity of food provided to the child is essential.

Ethical approval

The study was approved by Gazi University Ethics Committee (date: 22.04.2022, number: 2022-537).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ADÇ, RB, BÇ; data collection: RB, ADÇ, BÇ; analysis and interpretation of results: EY, RB; draft manuscript preparation: RB, EY, ADÇ, BÇ. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Abdominal massage as an adjunctive therapy for pediatric functional constipation: a randomized controlled trial

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ABSTRACT

Background. Chronic functional constipation affects approximately 3% of children globally, leading to painful defecation, fecal incontinence, and abdominal discomfort. Abdominal massage may improve gastrointestinal motility by stimulating vagal activity and reducing abdominal muscle tension. This study aimed to evaluate the effectiveness of abdominal massage therapy as an adjunct treatment for chronic functional constipation in children.

Methods. This randomized controlled trial included 61 children aged 4–10 years (mean age 6.36 ± 1.77) diagnosed with functional constipation. Participants were randomly assigned to two groups: the control group, receiving standard drug therapy, and the intervention group, receiving 12 sessions of Swedish abdominal massage involving effleurage and gentle pressing, vibration of the small and large intestines, kneading of the abdomen, and clockwise circular movements in addition to drug treatment. Outcomes assessed included stool consistency (using the Bristol Stool Scale), constipation severity (measured by the Constipation Assessment Scale), and associated symptoms.

Results. Both groups showed improvements in stool consistency; however, no statistically significant difference was found between them. The intervention group demonstrated a significantly greater reduction in constipation symptoms (Constipation Assessment Scale scores decreased from 14.70 ± 1.29 to 10.21 ± 1.45, $P < 0.001$.) and fewer episodes of fecal incontinence (from 3.82 ± 1.33 to 2.70 ± 1.33 days/week, $P < 0.001$) compared to the control group.

Conclusion. A 12-session abdominal massage therapy program appears to be an effective adjunct treatment to standard pharmacological therapy for alleviating constipation-related symptoms in children. Larger, multicenter trials are needed to confirm these findings.

Key words: Swedish abdominal massage, children, chronic constipation, functional constipation, Bristol Stool Scale, Constipation Assessment Scale.

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Chronic functional constipation is one of the most common gastrointestinal disorders in children, with a global prevalence ranging from 7% to 30%. It often presents with symptoms such as painful defecation, abdominal discomfort, fecal incontinence, bloating, and reduced appetite. These symptoms can significantly impair a child's physical, emotional, and social well-being, interfering with daily activities, school performance, and peer relationships. Constipation accounts for approximately 3–5% of general pediatric consultations and 10–25% of referrals to pediatric gastroenterologists.[1-5]

The management of chronic constipation typically involves pharmacological and non-pharmacological approaches. Pharmacological interventions include the use of laxatives such as stimulant laxatives, stool softeners, and fiber supplements, though long-term use may result in side effects and dependency.[6,7] Non-pharmacological approaches aim to modify diet and behavior and include increased fiber and water intake, behavioral therapy, and complementary methods such as abdominal massage.[8-12] Abdominal massage has been proposed as a supportive therapy that enhances colonic motility and facilitates bowel movements. Its physiological mechanism is thought to involve stimulation of the parasympathetic nervous system and vagal activity, leading to improved colonic transit and reduced fecal retention.[13-15] Furthermore, it may help reduce dependence on laxatives and improve comfort during defecation.[16-19] Despite emerging evidence, abdominal massage therapy remains underexplored as a treatment for chronic functional constipation in children. High-quality randomized controlled trials are needed to determine its efficacy as a supportive therapy in children with functional constipation.

This study aims to assess the effectiveness of abdominal massage therapy as an adjunct pharmacological treatment for chronic functional constipation in children, based on a randomized controlled trial conducted at Bahrami Children's Hospital.

Materials and Methods

Study design and participants

This randomized controlled trial was conducted to assess the effects of abdominal massage therapy on children aged 4 to 10 years diagnosed with chronic functional constipation. Participants were recruited from the pediatric gastroenterology clinic at Bahrami Children's Hospital between 2022 and 2023. Diagnosis was confirmed according to the Rome IV criteria for functional constipation.[5,19] Written informed consent was obtained from the parent or legal guardian of each participant.

Inclusion and exclusion criteria

Children were eligible if they experienced fewer than three spontaneous bowel movements per week, exhibited symptoms such as painful defecation, large-diameter stools, fecal incontinence, and difficulty during bowel movements. Exclusion criteria included metabolic, endocrine, or anatomical causes of constipation, neurological disorders, Hirschsprung's disease, severe systemic diseases (liver, kidney, heart), and current use of medications that may interfere with bowel function.

Sample size calculation

Sample size was calculated based on a power analysis from a previous study by Van et al.[20,21] considering a power of 80%, a confidence interval of 95%, and an improvement rate of 63% in the control group and 93% in the intervention group. The calculated sample size was 26 per group, resulting in a total of 28 participants in the control group and 33 participants in the intervention group.

Intervention protocol

Eligible participants were randomly assigned to either the control or intervention group using a Google Random Generator software to ensure equal allocation. The randomization process was not blinded, and both the participants and

researchers were aware of group assignments. To minimize potential bias resulting from the lack of blinding, outcome assessors and data analysts were blinded to group allocation. All statistical analyses were conducted using de-identified and coded data. The control group received standard medical care, which included laxatives (polyethylene glycol) at a dose of 0.5 g/kg, adjusted based on individual needs. The intervention group received 12 sessions of Swedish abdominal massage therapy, in addition to the same drug treatment. The massage sessions were conducted twice a week for six weeks by a trained therapist with over four years of pediatric massage experience. This therapy involved Swedish abdominal massage, following a protocol described by Sinclair.[22] Daily dietary intake of all participants was documented using food diaries throughout the study. Although no formal statistical comparison of dietary fiber or fluid intake was conducted between the groups, all participants and their families received standardized dietary advice. This included recommendations for adequate intake of fruits, vegetables, and fluids, and the avoidance of low-fiber foods and unhealthy snacks. Families were instructed not to make major dietary changes during the study or use dietary supplements unless approved by the study clinicians. We monitored the diet by reviewing the food diaries weekly. Laxative was administered based on each child's clinical requirement, with the frequency monitored through follow-up consultations. Adverse events were monitored throughout the study using standardized forms completed by caregivers and verified by study clinicians. Events were classified as mild, moderate or severe based on predefined criteria.

Outcome measures

Primary outcomes included stool consistency, assessed using the Bristol Stool Scale (BSS)[23], which categorizes stool types from 1 (hard lumps, severe constipation) to 7 (watery, severe diarrhea)[24]; and the severity of constipation symptoms, assessed using the Constipation Assessment Scale (CAS).[25] CAS is a validated tool consisting of 8 items, each scored from 0 to 2. The total score ranges from 0 (no symptoms) to 16 (most severe symptoms). Higher scores indicate greater severity of constipation-related complaints, such as painful or infrequent defecation, bloating, and straining. The scale was completed at baseline and post-intervention to evaluate changes in symptom severity.[25] Secondary outcomes included the frequency of fecal incontinence and the time until initiation of bowel movements. Symptoms were also monitored by evaluating straining, painful defecation, and withholding behavior.

Statistical analysis

All data were analyzed using SPSS version 22. Descriptive statistics, including means (± standard deviation) for continuous variables and frequencies (percentages) for categorical variables, were computed. The chi-square test was used for categorical data, and repeated measures ANOVA was performed to analyze differences in primary outcomes between groups over time. Non-parametric tests were applied for non-normal distributions. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted by analysts blinded to group allocation.

Ethical considerations

The study was approved by the Research Council of the Faculty of Medicine at Tehran University of Medical Sciences, and the ethical code (IR.TUMS.MEDICINE.REC.1401.268) was obtained. The study was also registered in the Iranian Registry of Clinical Trials (IRCT20230929059549N1). Informed consent was obtained from the parents or legal guardians, and confidentiality of patient information was maintained throughout the study. The research adhered to the ethical guidelines outlined in the Declaration of Helsinki.

Results

A total of 66 participants were enrolled in the study with parental consent, with 5 children discontinuing participation (Fig. 1). The baseline demographic and clinical characteristics, including age, gender, height, and weight, showed no significant differences between the control and intervention groups ($P > 0.05$), indicating balanced allocation (Table I). Among the 61 participants, 41% were female and 59% were male, with an average age of 6.36 ± 1.77 years.

At baseline, no significant differences were observed between the groups in terms of stool consistency, constipation severity, frequency of straining, or frequency of retentive fecal incontinence ($P > 0.05$) (Table II). The frequency of laxative use was also similar between the groups ($P > 0.05$).

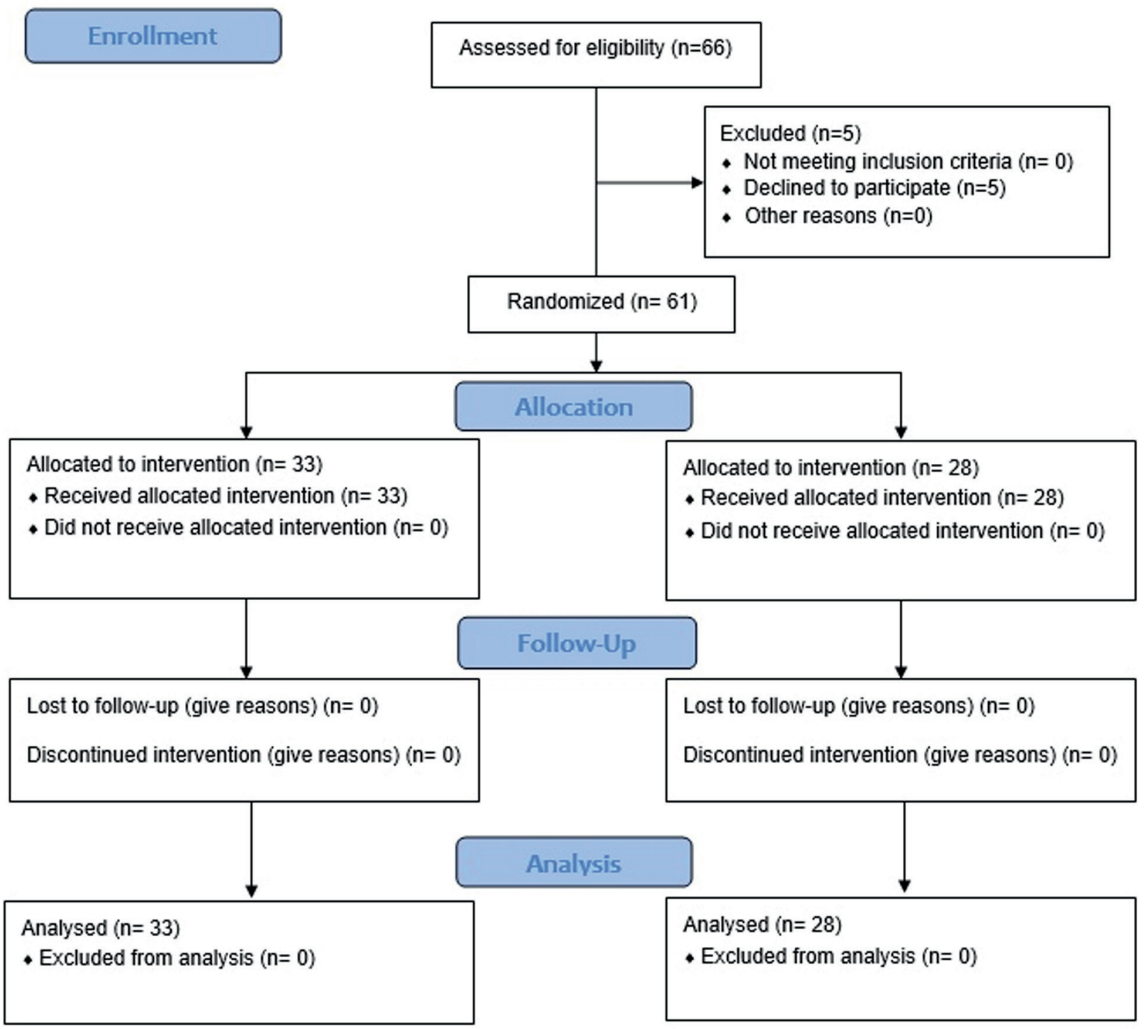

Fig. 1. Study population (CONSORT Flow Diagram).

Table I. Demographic characteristics of participants

Characteristic	All (n=61)	Control group (n=28)	Intervention (massage) group (n=33)	P-value
Age (years)	6.36 ± 1.77	6.36 ± 1.75	6.43 ± 1.81	0.897
Female gender	25 (41%)	11 (39.3%)	14 (42.4%)	0.506
Height (cm)	118.49 ± 19.482	117.93 ± 20.80	118.97 ± 18.60	0.839
Weight (kg)	20.29 ± 5.44	20.34 ± 5.28	20.24 ± 5.65	0.94

Table II. Analysis of stool variables

Variable	All (n=61)	Control group (n=28)	Intervention (massage) group (n=33)	P-value between groups
Bristol Stool Scale				
Before treatment				0.589
Type 1	26 (42.6%)	12 (42.9%)	14 (42.4%)	
Type 2	35 (57.4%)	16 (57.1%)	19 (57.6%)	
After treatment				0.138
Type 3	38 (62.3%)	20 (71.4%)	18 (54.5%)	
Type 4	23 (37.7%)	8 (28.6%)	15 (45.5%)	
P-value within group		<0.001	<0.001	
Constipation Assessment Scale				
Before treatment	14.62±1.42	14.54±1.57	14.70±1.29	0.661
After treatment	12.03±2.49	14.18±1.56	10.21±1.453	<0.001
P-value within group		0.398	<0.001	
Straining				
Before treatment	50 (82)	23 (82.1%)	27 (81.8%)	0.620
After treatment	24 (39.3)	11 (39.3%)	13 (39.4%)	0.601
P-value within group		0.001	<0.001	
Painful defecation				
Before treatment	44 (72.1)	20 (71.4%)	24 (72.7%)	0.567
After treatment	16 (26.2)	7 (25%)	9 (27.3%)	0.538
P-value within group		0.001	<0.001	
Retentive fecal incontinence				
Before treatment	3.80±1.44	3.79±1.59	3.82±1.33	0.932
After treatment	3.10±1.33	3.57±1.20	2.70±1.33	0.009
P-value within group		0.573	0.001	
Withholding behavior				
Before Treatment	40 (65.6%)	18 (64.3%)	22 (66.7%)	0.529
After Treatment	19 (31.1%)	9 (32.1%)	10 (30.3%)	0.548
P-value within group		0.016	0.003	

Control group only received drug treatment. Intervention group additionally received 12 sessions of Swedish abdominal massage.

Stool consistency

Stool consistency was assessed using BSS. At baseline, stool consistency was predominantly classified as type 1 or type 2, indicative of constipation. After treatment, both groups showed improvement in stool consistency, with a shift towards types 3 (sausage-shaped, with cracks) and 4 (smooth and soft, resembling a sausage). However, there was no significant difference in stool consistency between the two groups (P > 0.05) (Table II).

Constipation severity

Constipation severity was assessed using CAS, where higher scores indicate more severe symptoms. In the control group, there was no significant reduction in constipation severity, with average CAS scores decreasing slightly from 14.54 ± 1.57 to 14.18 ± 1.56 (P = 0.398). Conversely, the intervention group showed a significant decrease in constipation symptoms, with the average CAS score reducing from 14.70 ± 1.29 to 10.21 ± 1.45 (P < 0.001) (Table II).

Straining and painful defecation

The frequency of straining decreased in both groups, from 82.1% to 39.3% in the control group and from 72.7% to 39.3% in the intervention group; however, the difference between groups was not statistically significant (P = 0.601). The frequency of painful defecation decreased significantly in both groups. In the control group, the percentage decreased from 71.4% to 25% (P < 0.001), while in the intervention group, it decreased from 72.7% to 27.3% (P < 0.001). No significant difference in the frequency of painful defecation was found between the two groups (P = 0.538) (Table II).

Retentive fecal incontinence and withholding behavior

The frequency of retentive fecal incontinence decreased significantly in the intervention group, from 3.82 ± 1.33 days per week at baseline to 2.70 ± 1.33 days per week post-treatment (P < 0.001). In the control group, this change was not significant, decreasing from 3.79 ± 1.59 to 3.57 ± 1.20 days per week (P = 0.372). Withholding behavior also decreased in both groups: from 64.3% to 32.1% in the control group (P = 0.016) and from 66.7% to 30.3% in the intervention group (P = 0.003), but there was no significant difference between the groups (P = 0.548) (Table II).

Adverse events

In the intervention group, 5 out of 33 participants (15.2%) reported mild abdominal discomfort during massage sessions, which resolved without intervention. No serious adverse events were reported in either group. In the control group, 2 out of 28 participants (7.1%) experienced mild diarrhea, likely related to laxative use.

Discussion

The findings demonstrated that abdominal massage significantly improved CAS and reduced fecal incontinence compared to pharmacological treatment alone. However, no significant difference was observed in stool consistency between the groups (P > 0.05), indicating that the effect of abdominal massage on stool consistency remains inconclusive based on our results. Therefore, claims regarding improvements in stool consistency should be made cautiously and require further investigation.

The therapeutic efficacy of abdominal massage may be attributed to its potential to enhance gastrointestinal motility and stimulate bowel movements, although the exact physiological mechanisms in children with chronic functional constipation require further research. Previous studies have reported that abdominal massage can improve colonic transit time and reduce constipation symptoms, supporting our findings.[15,26]

The abdominal massage intervention was well-tolerated, with no serious adverse effects reported. Mild abdominal discomfort

observed in a few children was transient and did not necessitate discontinuation of therapy. These results align with prior studies suggesting that abdominal massage is a safe complementary therapy for children with functional constipation. A 2019 systematic review confirmed the effectiveness and safety of several complementary interventions, including massage, without any reported adverse effects.[27]

Our results are consistent with a randomized controlled trial comparing drug therapy with manual physiotherapy—including abdominal massage—in children with functional constipation, which demonstrated symptom relief and improved stool consistency, although no statistically significant difference between treatment groups was found.[28] Moreover, systematic reviews have highlighted abdominal massage as a useful intervention to alleviate constipation severity and associated symptoms such as bloating and pain.[28] Participants in our study showed improvement in stool form, transitioning from severe constipation types (1 and 2) to more normal stool types (3 and 4) after treatment, indicating clinical benefit. This is aligned with findings from other pediatric studies, including research on infants with early-onset constipation who experienced symptom relief following abdominal massage.[29] However, our study focused on older children with more established behavioral and dietary patterns, suggesting that the benefits of massage extend across a wider pediatric age range.

A randomized controlled trial comparing drug therapy to manual physiotherapy in children with functional constipation found both methods effective in symptom relief and stool consistency improvement, although no statistically significant superiority was observed.[30] Although quality of life (QoL) and psychosocial factors were not formally assessed in our study, these are important dimensions to consider in future research. Future research should incorporate validated QoL assessments to examine this potential benefit more rigorously.

Functional constipation is influenced by complex interactions among physiological, psychological, social, and cultural factors. A systematic review and meta-analysis revealed that children with functional constipation report a lower quality of life compared to their healthy peers.[31] Previous literature highlights that parental education, psychological health, and caregiving strategies significantly affect constipation management outcomes.[32,33] In our study, parents were provided with information about the pathophysiology of constipation and the mechanisms through which massage could help, which likely enhanced their engagement. Although no formal satisfaction assessment was conducted, the observed clinical improvements and regular follow-up sessions may have contributed to maternal satisfaction. Furthermore, a recent systematic review underscored the association between pediatric constipation and exposure to stress, suggesting that life stressors—both at home and in school—can act as contributing factors.[34] In our context, it is reasonable to assume that symptom relief positively affected maternal emotional well-being, as improvements in the child's condition may reduce caregiver stress.

Limitations

Several limitations should be noted. First, the absence of a massage-only group limits the ability to isolate the specific effects of abdominal massage apart from pharmacological treatment. Second, the lack of a sham massage (placebo control) group reduces the ability to distinguish true therapeutic effects from placebo responses. Third, QoL and caregiver satisfaction were not evaluated. Lastly, the relatively small sample size and single-center design may limit generalizability. Larger, multicenter studies with longer follow-up and more comprehensive outcome measures are needed to validate and expand upon these findings.

Conclusion

In conclusion, this study provides evidence that abdominal massage combined with

conventional drug therapy is an effective safe complementary intervention for children with chronic functional constipation. The underlying physiological mechanisms—such as parasympathetic stimulation and enhanced gastrointestinal motility—support its therapeutic potential. While further research is needed to address study limitations, our findings support abdominal massage as a safe, cost-effective, and non-invasive complementary strategy. Future investigations should evaluate long-term efficacy, impacts on quality of life, and caregiver-administered protocols to improve adherence and accessibility.

Ethical approval

The study was approved by the Research Council of the Faculty of Medicine at Tehran University of Medical Sciences, and the ethical code (IR.TUMS.MEDICINE.REC.1401.268) was obtained. The study was also registered in the Iranian Registry of Clinical Trials (IRCT20230929059549N1). Informed consent was obtained from the parents or legal guardians, and confidentiality of patient information was maintained throughout the study. The research adhered to the ethical guidelines outlined in the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AM, MM, HSM, MTOY, KE; data collection:AM, MA, MM, HSM, KE; analysis and interpretation of results: AM, MA, MM, MTOY, KE; draft manuscript preparation: AM, MA, MM, HSM, MTOY, KE. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Changes in developmental-behavioral pediatric referral trends from a non-western country during the COVID-19 pandemic

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ABSTRACT

Background. The global spread of coronavirus disease 2019 (COVID-19) and associated policies have caused negative consequences at the level of children, families, and services, resulting in physical, mental, and developmental issues in children, as well as limited access to healthcare. We evaluated the referral numbers, sources, and trends of a developmental-behavioral pediatrics (DBP) department in Türkiye as a Eurasian country, as well as the effects of the COVID-19 pandemic on referral variables.

Methods. This retrospective cohort study examined patient referral data to the Division of Developmental Behavioral Pediatrics, Department of Pediatrics, Hacettepe University between the years 2014 and 2021. We analyzed the changes in the number of referrals over time in 3-month intervals using polynomial regression models. The impact of the COVID-19 pandemic on referral reasons was evaluated.

Results. Polynomial regression analyses demonstrated significant nonlinear trends in consultation volumes across all categories. During the pre-pandemic period, referrals showed a marked increase, reaching a peak around 2018 before declining. In the post-pandemic period, an initial surge in consultations was followed by a notable decline after 2021. Notably, referral numbers had dropped to their lowest levels during the pandemic. Similarly, referrals to neonatal and pediatric clinics increased sharply until 2020, after which a plateau or slight decrease was observed, indicating a deceleration in growth over time. Referrals for perinatal-neonatal risks were 1.359 (95% confidence interval: 1.269-1.456) times higher than in the pre-pandemic period, and those for suspected autism were 1.209 (95% confidence interval: 0.987-1.478) times higher.

Conclusions. Although it is encouraging that our referral trends have improved in the 1.5 years since the COVID-19 pandemic, it is thought that health service constraints caused a considerable increase in prenatal risk and suspicion of autism referrals following the pandemic. Improvement and innovation in healthcare systems to prevent the long-term detrimental impacts of periodic interruptions in healthcare on children's development and behavior is needed.

Key words: coronavirus disease 2019, referral trends, developmental and behavioral pediatrics, pandemic.

Early childhood is a sensitive developmental period, and it's known that all interventions that will reduce developmental risks and increase resilience in this period have positive effects on health, academic skills, and economic productivity in adulthood.[1] Currently, the

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effectiveness of family-centered strategies during developmental follow-ups is known and primary healthcare providers have a key role in this regard. Primary care providers frequently do have not the competence to promote early childhood development due to the deficiency of educational curricula.[2,3] However, in a study conducted in Türkiye, most parents stated that developmental issues were more frequently addressed in primary healthcare services.[4] Time constraints, inadequate reimbursements, staffing shortages, and a lack of knowledge concerning referral alternatives for positive screening results are the most critical problems that pediatricians experience when completing developmental screening and building a referral chain for early intervention.[5,6] All of these situations demonstrate the need for regulations in health systems, and it is emphasized that cooperation between all sectors, not just health and education, is required to improve early childhood development globally.[3]

Developmental behavioral problems affect approximately 15% of children in high-income countries (HICs), and this rate is expected to be higher in low-middle-income countries (LMICs).[7] Pediatricians are crucial in preventing, identifying, and properly referring these conditions. Friedman, who used the definition "behavioral pediatrics" for the first time, asserted in 1975 that this field encompassed prevention, advocacy, delivering integrative healthcare, and clinical administration in addition to dealing with psychological, social, and learning problems. The need for a subspecialty with a central role in these fields was emphasized among pediatric departments.[8,9] The American Board of Medical Specialties accepted the developmental-behavioral pediatrics (DBP) sub-specialty in 1999, despite long-standing conflict concerning department task definitions, particularly between representatives of psychiatry, neurology, and developmental and behavioral pediatric divisions.[9] Developmental-behavioral pediatrics has been accepted as a subspecialty in Türkiye over the last decade and efforts to improve the maturation of the department are currently ongoing. There are comprehensive studies evaluating the workforce and referral volumes of the department, as well as revealing its competition with other pediatric subspecialties in HIC where developmental pediatrics has existed for years.[7,10,11] In countries where DBP has newly been established, comparable research is currently limited.[12] In Türkiye, as in the rest of the world, almost all developmental pediatricians work in academic medical centers, which are tertiary-level health institutions.[11] These institutions are unique facilities for training and supporting pediatric residents in all patient visits, not just throughout their rotation, and for establishing collaborations with other disciplines.[13]

Since 2020, the COVID-19 pandemic has affected the entire world, and data on the long-term effects of such outbreak periods, particularly on the growth and development of children, are limited.[14] In addition, the stress caused by the isolation; school closures; disrupted social lives; decreased physical activity; changes in daily routines, sleeping habits, and diet; exposure to home discord; and longer screen use, all had an impact on children's and adolescents' physical and mental health.[15] It has been demonstrated that changes in the economic, psychosocial, and educational environments since the pandemic has caused declines in children's cognitive functions and performance, as well as negative effects on mental health.[16,17] COVID-19, which is predicted to have devastating effects on early childhood development, caught countries off guard, disrupting their healthcare infrastructure. The public's access to health services has been restricted as a result of both government's closure measures and policies aimed at reducing population movement, as well as the resulting economic challenges. Concerns about the potential of COVID-19 infection have also exacerbated the problem.[18] Reports of a decline in childhood vaccines led us to believe that access to primary healthcare has diminished, interrupting the follow-up of healthy children and pregnant women.[19-21]

Access to health and support services, including early intervention, has grown more limited for at-risk children who are socioeconomically, culturally, and geographically disadvantaged and have developmental issues.[22,23] High-Risk Infant Follow-Up (HRIF) programs in the United States of America (USA) highlight the importance of monitoring the effectiveness of clinical services on neonatal outcomes that were interrupted during the pandemic.[24] Pediatric emergency applications decreased by about half during the pandemic, whereas child mental health applications increased.[25,26] It is critical to investigate changes in clinical service capacity, patient volume, and reasons for hospital admissions during the pandemic, a period marked by uncertainty. Examining referrals to child health professionals for developmental and behavioral issues allows policymakers to develop long-term strategies through a better understanding of the consequences of this process.

This study aimed to investigate the referral volume, sources, and trends of a DBP department, as well as the factors influencing these trends, in one of the largest academic centers in Türkiye, a country where DBP has been established. Additionally, we hypothesized that the COVID-19 pandemic's devastating effect on early childhood development would significantly increase department referral trends.

Materials and Methods

The Turkish Ministry of Health formally established DBP as a subspecialty of pediatrics in 2011. The Division of DBP was established in 2013 at Hacettepe University. The department provides services to families and children based on family-centered strategies. The primary patient group is children aged between 0 and 6 years who have developmental risks and delays. Patients who present directly or through referrals from family physicians and pediatric departments are evaluated on an average of 2 months. Since its establishment, the division has had a 1-month rotation in DBP for pediatric residents, and a 3-year fellowship training in the subspecialty.

Referral data for the Division of DBP, Department of Pediatrics, Hacettepe University between May 1st, 2014, and October 21st, 2021, were used in this retrospective cohort study. Approximately 8 years of patient referral data, including the pandemic period, were analyzed. Referral resources were classified as general pediatrics, pediatric subspecialties, and non-pediatric departments. Self-referrals were not included in the study, that is, these numbers only represent referrals from other specialists. Pediatric departmental referrals with fewer than 20 total referrals were classified as 'other'. Due to the low number of referrals, pediatric surgery, orthopedics, neurosurgery, cardiovascular surgery, and urology departments were combined under 'pediatric surgery branches'. The main reasons for referrals were categorized collaboratively by the researchers after reviewing the content and considering the potential referral reasons identified by the American Academy of Pediatrics (AAP) for the DBP; incomplete or inappropriate referrals were not considered.[27] Changes in referral sources and reasons were initially investigated on an annual basis, followed by a review of the trend of change over time since 2019, both before and after the COVID-19 pandemic. Informed consent from the families was not obtained since this study was retrospective in design. The Ethics Committee of Hacettepe University approved this study (GO 21/1261).

Statistical analyses

The age and sex of children referred to the Division of Developmental Behavioral Pediatrics, Department of Pediatrics, Hacettepe University, as well as the quantity and distribution of referrals, were analyzed using descriptive statistics. To model the temporal trend in the number of consultations, a second-degree (quadratic) polynomial regression model was applied, as the dependent variable was continuous. Both the linear and quadratic

terms of the year variable were included as independent predictors. The overall significance of the model was tested using the F-test, and the statistical significance of individual coefficients was assessed based on p-values. To evaluate model fit, performance metrics such as Mean Absolute Error (MAE), Root Mean Square Error (RMSE), Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC) were calculated. In addition, graphical comparisons between observed and predicted values were conducted to assess the model's predictive accuracy.

We analyzed the changes in the number of referrals over time for the general pediatric outpatient clinic, neonatology, pediatric genetics, and pediatric metabolism departments that request the most referrals, in 3-month periods between 2014 and 2021. The effect of COVID-19 on perinatal risk and autism spectrum disorder (ASD) risk was calculated with 95% confidence relative risk. Polynomial regression analysis was conducted using the lm function from the base stats package in R, with both linear and quadratic terms of the year variable included in the model.[28,29] The "ggplot2" package were used to plot polynomial regression curves.[30] A p-value of less than 0.05 was considered significant.

Table I. Numbers of referrals to the department of developmental and behavioural pediatrics from other departments between 2014 and 2021 (N=8412).

	n (%)
General pediatric outpatient clinic	2608 (31.0)
Neonatology	2373 (28.2)
Pediatric metabolism	827 (9.8)
Pediatric genetics	824 (9.8)
Child and adolescent psychiatry	329 (3.9)
Otorhinolaryngology	256 (3.0)
Pediatric neurology	190 (2.3)
Pediatric allergy	168 (2.0)
Pediatric gastroenterology	149 (1.8)
Pediatric endocrinology	100 (1.2)
Pediatric pulmonology	98 (1.2)
Pediatric cardiology	82 (1.0)
Pediatric hematology and oncology	69 (0.8)
Pediatric immunology	66 (0.8)
Plastic and reconstructive surgery	60 (0.7)
Pediatric infectious diseases	57 (0.7)
Other surgery clinics*	54 (0.6)
Pediatric emergency	40 (0.5)
Other non-surgical clinics**	35 (0.4)
Ophthalmology	27 (0.3)
Total	8412 (100.0)

*Pediatric surgery, orthopedics, neurosurgery, cardiovascular surgery, and urology
**Pediatric nephrology, rheumatology, intensive care, physical medicine and rehabilitation

Results

After excluding self-referrals, over approximately 8 years, 8412 children were referred to the Division of DBP, Department of Pediatrics, Hacettepe University. 59.5% were males and 40.5% were females. The median age was 18 months.

The departments that requested the most referrals were the general pediatric outpatient clinic (31.0%), neonatology (28.2%), pediatric metabolism (9.8%), and pediatric genetics (9.8%), respectively (Table I). Child and adolescent psychiatry (3.9%) and otolaryngology (3.0%) had the most referrals among non-pediatric departments. Other pediatric subspecialties, pediatric surgical branches, and non-pediatric departments all had referral rates of less than 3%. Patients were referred to the Division of DBP, Department of Pediatrics, Hacettepe University due to perinatal and neonatal risks (33.5%), speech delay (15.7%), a likelihood of developmental delays (14.1%), and developmental evaluation of patients with metabolic disorders (7.0%), respectively. The distribution of reasons for the children's referrals during the study period is shown in Table II.

Between 2014 and 2021, the number of referrals grew each year, the number of referrals increased from 302 in 2014 to 1394 in 2021. Even

though the referrals from the last two months were not considered, the highest number of referrals was reached in 2021. The number of referrals reduced after the COVID-19 pandemic began in 2020. Comparing the number of referrals over the last 3 years, Fig. 1 shows that there was a considerable drop in referrals during the 3-month lockdown period following March 2020, the start of the outbreak and then began to rise.

Table II. Referral reasons (2014 to 2021).

Reasons	n (%)
Infants and children at risk due to perinatal and neonatal history	2820 (33.5)
Speech delay	1319 (15.7)
Children at risk of developmental delays	1182 (14.1)
Developmental evaluation of patients with metabolic disease	589 (7.0)
Feeding difficulties	542 (6.4)
Developmental evaluation of patients with a genetic syndrome	535 (6.4)
Global developmental delay	445 (5.3)
Autism diagnosis or suspicion	425 (5.1)
Behavioral problems	240 (2.9)
Motor development delays	181 (2.2)
Sleep problems	75 (0.9)
Problems with toilet habits	46 (0.5)
Children considered as being gifted	13 (0.2)
Total	8412 (100.0)

In the pre-pandemic period, the polynomial regression model revealed that both the linear (β = 37.03, $p < 0.001$) and quadratic (β = –0.95, $p < 0.001$) year terms were statistically significant. These results indicate a strong initial increase in consultation numbers, followed by a deceleration in growth and a shift toward decline around 2018. The model demonstrated excellent explanatory power, accounting for 81.0% of the variance (R^2 = 0.810; Adjusted R^2 = 0.792) with strong overall significance (F = 46.8, $p < 0.001$). Additional performance metrics included MAE = 40.77, RMSE = 48.12, AIC = 272.63, and BIC = 277.51 (Supplementary Table S1). As visualized in Fig. 2a, the number of referrals showed a consistent increase from 2014 to around 2018–2019, followed by a slight decline toward 2020. This quadratic pattern suggests a possible plateau or saturation effect in referral numbers shortly before the onset of the pandemic.

In the post-pandemic period, the polynomial regression model revealed that both the linear (β = 199.29, p = 0.02) and quadratic (β = –18.59,

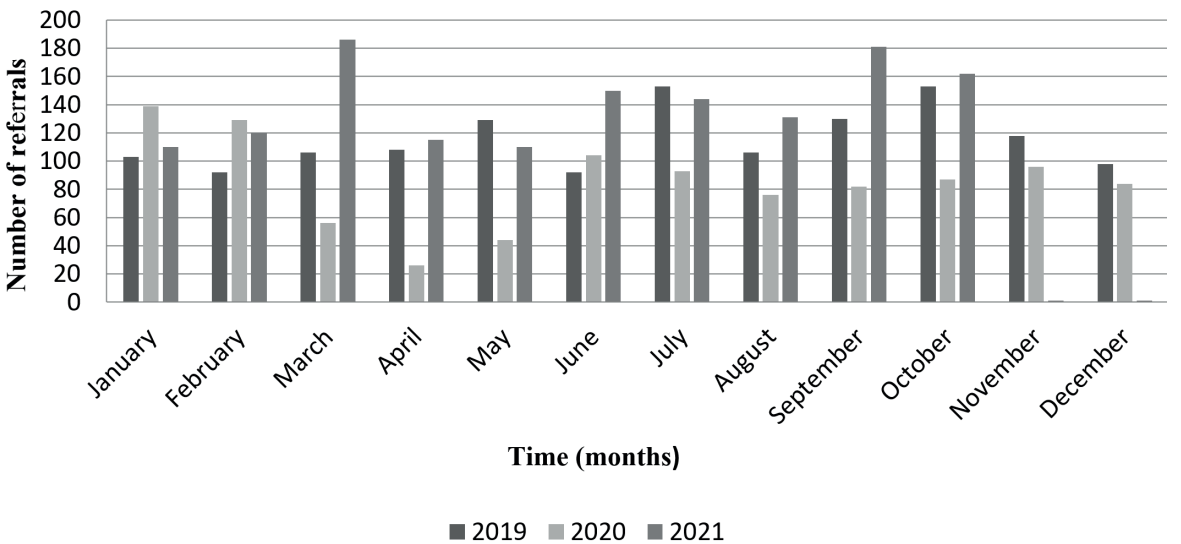

Fig. 1. Monthly Distribution of children referred between the years 2019-2021 (before, during and after the COVID-19 pandemic).

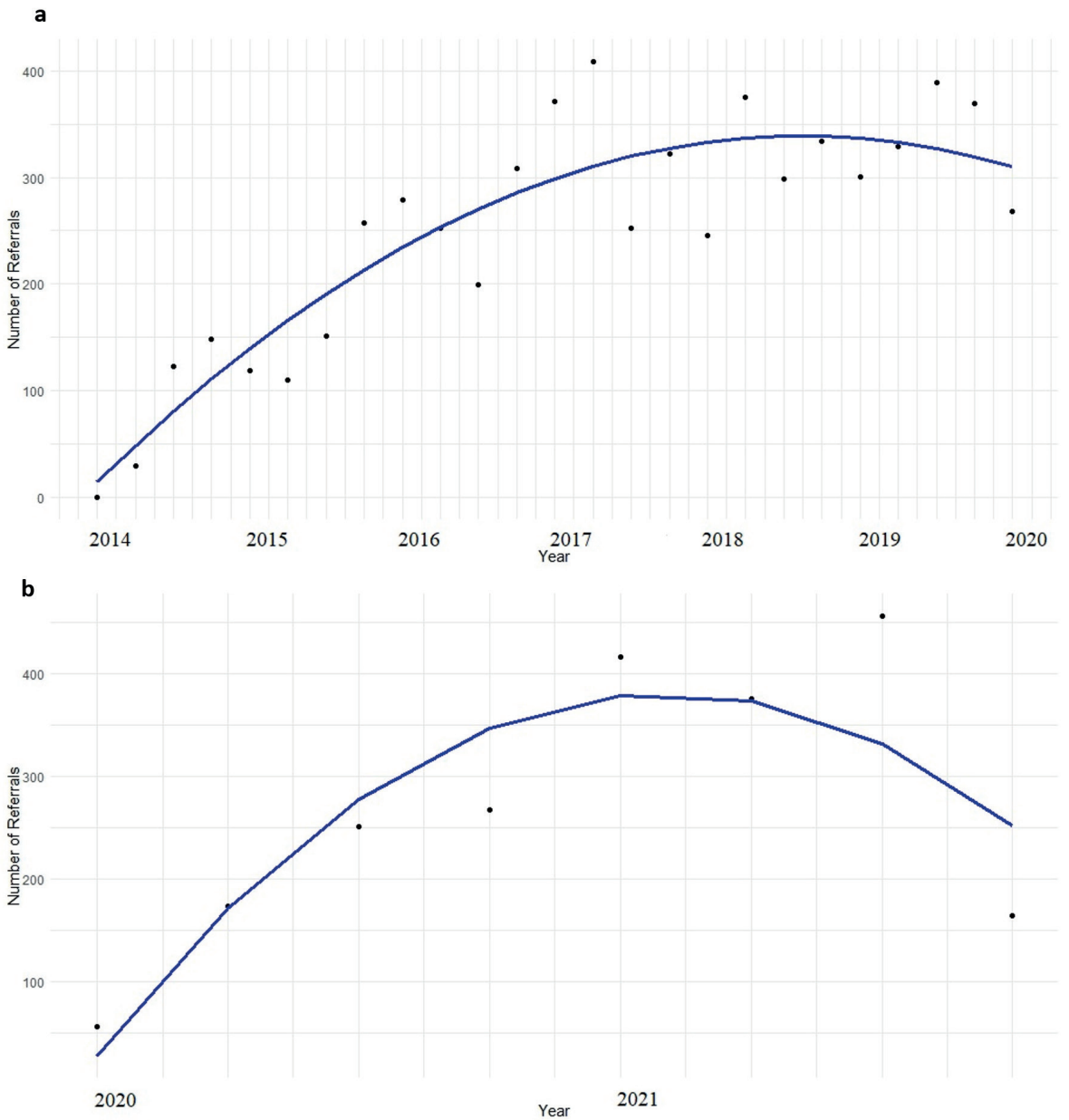

Fig. 2. Quadratic polynomial regression models for total referral trends to the Hacettepe University Division of Developmental Behavioral Pediatrics over three-month intervals, **a)** before the pandemic (2014-2020) and **b)** after the pandemic (2020-2021).

p = 0.03) year terms were statistically significant. These results indicate a sharp initial increase in consultation numbers, followed by a loss of momentum and a downturn beginning after 2021. The model demonstrated good explanatory power, accounting for 75.7% of the variance (R^2 = 0.757; Adjusted R^2 = 0.659) with overall significance (F = 7.77, p = 0.029). Model performance metrics included MAE = 48.55, RMSE = 63.76, AIC = 97.19, and BIC = 97.50 (Supplementary Table S2). As illustrated in Fig. 2b, the number of referrals increased sharply from early 2020 through 2021, reaching a peak in mid-2021. However, a noticeable decline followed, suggesting a non-linear recovery pattern. This may reflect healthcare system

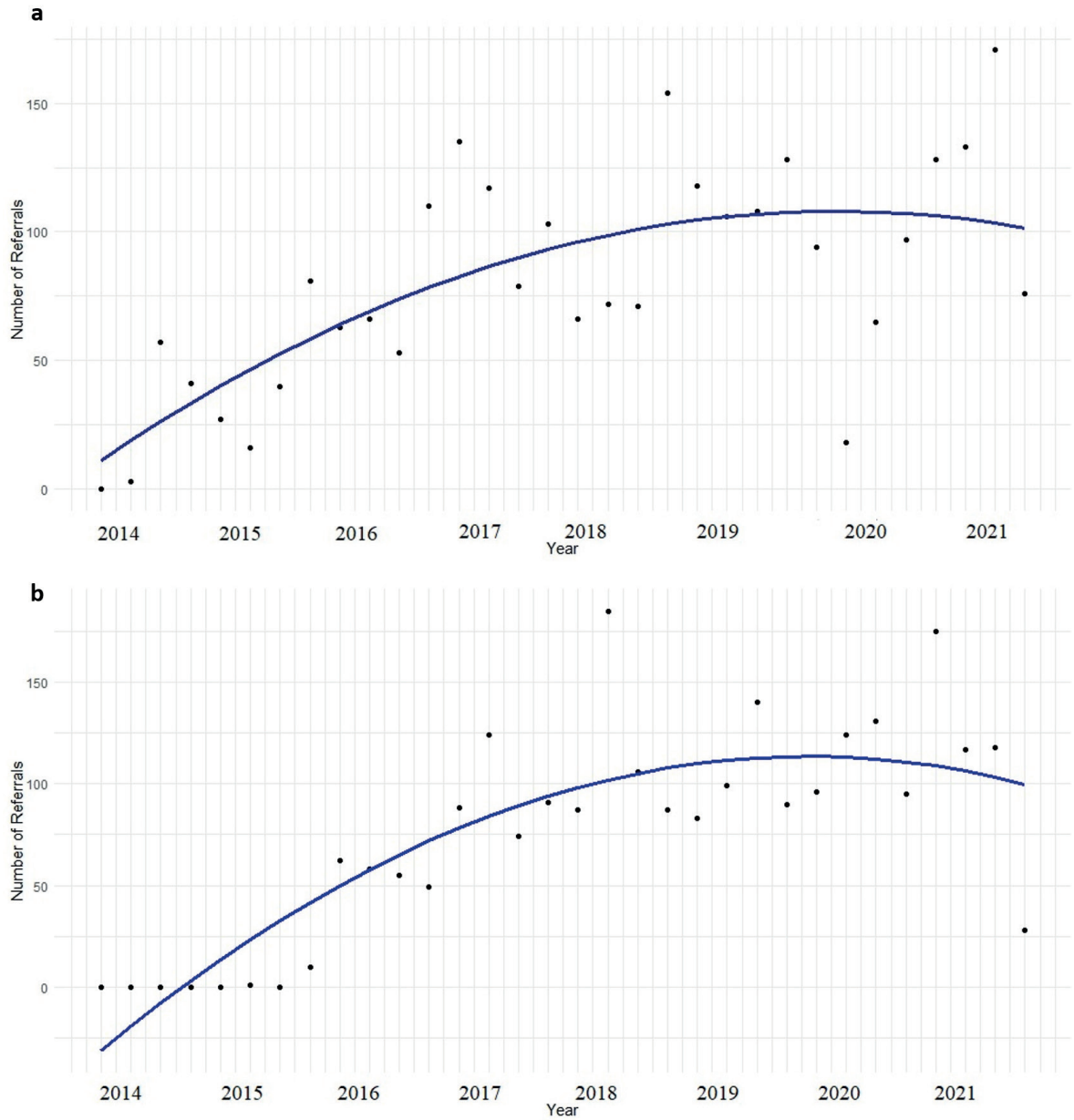

Fig. 3. Quadratic polynomial regression models for referrals to the Hacettepe University Division of Developmental Behavioral Pediatrics over three-month intervals between 2014 and 2021, **a)** from the pediatric outpatient clinic, and **b)** from the neonatology clinic.

adaptations and fluctuations in patient behavior during the later phases of the pandemic.

The polynomial regression model for pediatric outpatient clinic referrals indicated that both the linear (β = 8.18, p = 0.004) and quadratic (β = –0.16, p = 0.044) year terms were statistically significant. These results suggest a curvilinear trend characterized by an initial increase in consultation numbers that gradually slowed and leveled off over time. The model explained 47.5% of the variance (R^2= 0.475; Adjusted R^2 = 0.439) and was overall statistically significant (F = 13.1, p < 0.001). Model performance metrics

were MAE = 24.28, RMSE = 31.09, AIC = 318.77, and BIC = 324.63 (Supplementary Table S3). As illustrated in Fig. 3a, the number of pediatric referrals increased steadily between 2014 and approximately 2020, reaching a plateau, followed by a slight decline towards 2021. This non-linear trend may reflect the combined influence of service expansion during the earlier years and pandemic-related disruptions in later years.

The polynomial regression model for neonatology outpatient clinic referrals demonstrated that all year-related terms were statistically significant: the intercept ($\beta = -43.65$, $p = 0.018$), linear term ($\beta = 12.71$, $p < 0.001$), and quadratic term ($\beta = -0.26$, $p = 0.001$). These findings indicate a clear initial increase in referrals, followed by a gradual deceleration and eventual decline, suggesting a non-linear trajectory. The model explained 69.0% of the variance ($R^2 = 0.690$; Adjusted $R^2 = 0.669$) and was overall statistically significant ($F = 32.26$, $p < 0.001$). Model performance indices supported this fit, with MAE = 22.10, RMSE = 29.74, AIC = 314.92, and BIC = 320.79 (Supplementary Table S4). As visualized in Fig. 3b, referral rates exhibited a steady upward trajectory from 2014 through 2020, followed by a modest decline in 2021. The significant quadratic effect suggests a nonlinear shift in referral patterns, potentially attributable to systemic changes in neonatal care provision or the indirect effects of the COVID-19 pandemic.

The relative risk was analyzed to evaluate the change in referral reasons from before the COVID-19 outbreak. Following the pandemic, the number of referrals for infants and children at risk due to perinatal and neonatal history was 1.359 (95% confidence interval: 1.269-1.456) times higher than before the pandemic. Furthermore, the number of children referred with a diagnosis or suspicion of autism was 1.209 (95% confidence interval: 0.987-1.478) times higher after the pandemic.

Discussion

This study reveals the referral trends of the DBP sub-specialty, which provides training, research, and service within one of the largest and best-equipped academic institutions in Türkiye. Furthermore, the study had to include the COVID-19 pandemic periods to evaluate the possible effects of the pandemic on developmental and behavioral issues, particularly in developing countries.

DBP training and services persisted in a few centers, one of which was an academic facility, throughout the period when referrals to the DBP, Department of Pediatrics, Hacettepe University were examined between 2014 and 2021. In Türkiye, a similar two-center study reported an annual 1.18-fold increase (95% confidence interval: 1.09-1.28) in DBP referrals between 2010 and 2017.[12] The number of referrals increased rapidly in the pre-pandemic period, peaked in 2018, and declined toward 2020, reaching a low point during the pandemic. Following an initial post-pandemic surge, a marked decline was observed after mid-2021, indicating an early recovery that later lost momentum. These trends likely reflect the expansion and eventual saturation of the division's services and workforce over time. Additionally, given that the number of referrals in longitudinal studies evaluating the DBP workforce trend in HICs has mostly remained constant in recent years, it is clear that the demand for DBP training programs, research, and services will continue to grow in countries where DBP's have recently been established.[10,11]

A significant upward trend was observed in pediatric outpatient clinic referrals from 2014 to 2020, followed by a slight decline in 2021. This pattern suggests that the rapid increase in referrals may have reached a plateau. In a comprehensive study conducted in the USA, pediatric generalists were responsible for the majority of referrals to the DBP for both time points.[10] This improvement in awareness is quite

encouraging, given the critical requirement of a developmental perspective in pediatrics and the lack of knowledge of pediatric residents on psychological and developmental issues.[5,27,31] We believe that time and rotation training was the most effective factors in this trend in referrals. As a result, the establishment of DBPs in developing countries, the increase in the number of training and service institutions, and subsequently, the support and collaboration of DBP specialists with young pediatricians throughout their residency training in chronic/inpatient follow-ups, as well as DBP rotations, allows them to improve their necessary knowledge and skills about developmental issues before serving in primary care.[13]

The model for neonatal outpatient referrals demonstrated a sharp upward trend beginning in 2016, which plateaued and showed a slight decline after 2020, indicating a deceleration and eventual reversal in referral growth. Likewise, referrals from the neonatology department and perinatal risk-related referrals ranked first in a multicenter study analyzing referrals to DBP outpatient clinics in Türkiye.[32] In HICs, the number of neonatal follow-up referrals is relatively low.[5,10] This condition in developing countries is likely to be attributed to an increase in high- risk babies, caused by the inability to entirely remove maternal social, environmental, and biological risk factors, as well as improved neonatal survival and limited health services in rural regions.[33,34] There is a definite need for policies that will improve mother and newborn health as well as health services. Furthermore, the high and growing awareness of the neonatal department, which serves a growing population of high-risk infant patients, is of utmost importance. The most common reasons for referral, after perinatal issues, were children with speech delays and developmental delay risk. A longitudinal study of pediatricians' developmental screening and referral trends in the USA reported that most referrals to a developmental or medical specialist were due to developmental delays in milestones and global developmental delays.[5]

DBP is a subspecialty that addresses the possible causes of complicated developmental and behavioral issues among children and uses system-based practices and neurodevelopmental approaches to achieve optimal developmental outcomes. Neurologists, child psychiatrists, DBP professionals, and physical medicine and rehabilitation specialists frequently collaborate in the care of children with behavioral, developmental, and learning difficulties, which have become much more common worldwide.[27] The multidisciplinary nature of DBP education and practice is essential. Unfortunately, since the establishment of DBP, specialists working in this discipline have remarked that they face competition with other pediatric subspecialists and practice constraints due to a lack of clinical support from other professionals.[7,10,11] According to research, pediatric subspecialists screen and refer children and their families for psychological concerns at an extremely low rate.[31] However, it is well-known that the majority of children followed in these subspecialties have chronic medical conditions and are at high risk for developmental and behavioral problems, and family-level psychosocial stressors.[35] Most pediatric subspecialties, as well as child and adolescent psychiatry, had referral rates of less than 5% in this study. It is well-known that collaboration practices between various disciplines and professions are linked with improved health outcomes, accordingly, we need appropriate strategies to reduce potential competition, recognize DBP's education, service, advocacy, and research roles in pediatrics, and ensure effective collaborations.[36,37] Given that the majority of DBP specialists in countries where the division was newly established are university-based, improved awareness and collaboration of other pediatric subspecialties in these institutions will eventually have a positive influence on primary care services.

The policies associated with the global COVID-19 pandemic caused negative implications at the level of children, families, and services, leading to physical, mental, and developmental issues in children, increased

parental anxieties and household stress, and restricted access to healthcare.[22] The pandemic has resulted in a decline in vaccine orders and vaccination admissions, according to reports from the Centers for Disease Control and Prevention (CDC).[19] Diminished vaccine administrations and, as a consequence, interruptions in well-child follow-up could preclude the diagnosis of developmental delays and referral to early intervention programs for children, who are the most vulnerable to the pandemic's devastating effects.[38] These potential morbidities in children are likely to occur more frequently in developing countries, where healthcare restrictions and economic challenges are more severe. In this study, the number of referrals steadily increased after 2014, reaching its peak in 2018, followed by a plateau and subsequent decline. Following a sharp drop to its lowest levels during the pandemic, referral numbers began to rise again in the post-pandemic period. This demonstrates that the pandemic's detrimental effects on access to healthcare are being mitigated. Furthermore, as compared with the pre-pandemic period, the increase in referrals due to perinatal risk was a remarkable finding in our research. As shown by studies, pregnant women are minimizing their pregnancy follow-up checkups because of concerns about the danger of COVID-19 infection[39], and they are experiencing significant mental health issues, particularly depression and anxiety symptoms, as a result of pandemic-related conditions.[40] Additionally, access to healthcare may have negatively affected maternal, fetal, and neonatal health[22,41], increasing the number of high-risk babies and the requirement for follow-up. According to comprehensive studies, there would be a significant increase in maternal and child deaths in LMICs if the deterioration in healthcare during the pandemic is not resolved and the health system's sustainability is not preserved.[18]

ASD is a biologically based neurodevelopmental disorder with an increasing frequency, characterized by persistent deficits in social communication and social interaction. In the USA, ASD was diagnosed in one in every 59 children in 2018.[42] Identification of this increasingly prevalent disorder, as well as enrolment of children in early intervention services, improves outcomes and reduces long-term costs for families and governments.[43,44] Even though the necessity of face-to-face field evaluations in the autism diagnostic process is well known, the barriers and solutions in autism examinations during the outbreak are being challenged all over the world.[45,46] Telehealth has recently been adopted for DBP monitoring, mainly in HICs.[47,48] Clinic access for children with suspected or diagnosed autism may be further prolonged in Türkiye where healthcare interruptions and social inequalities are more severe and telehealth services cannot be structured. The accumulation of children who cannot receive health services due to these disadvantages may be the cause of the 1.209-fold increase in referrals requested from our department with the suspicion of autism after the pandemic. Children lost opportunities for social interaction with their peers as a result of social distancing measures within their daily life during the pandemic period, and their social skills regressed. Parent-child interactions were also damaged by the chaotic home environment caused by school closures, parental jobs lost, economic challenges, or the requirement of working from home.[38,49,50] Furthermore, during the pandemic, children's screen time increased.[51,52] Significant longitudinal research has indicated that screen time in 1-year-olds is associated with ASD and autism-like symptoms.[53,54] All of these potential consequences could have resulted in an increase in DBP referrals for suspected autism. It will take time to determine the potential impact of the COVID-19 outbreak on the prevalence of autism and our study's findings draw attention to this emerging topic.

One of the study's strengths is that our center is one of the three major academic institutions providing residency training and family-centered services in the field of DBP in Türkiye

and it analyses patient referrals over an 8-year period. The changes in the numbers and reasons for referrals, as well as the departments that request them, over time, indicate that developing strategies and collaborations for both our department and countries where DBP will be re-established is recommended. Another strength of our study is that it is the first to evaluate DBP referrals during the COVID-19 pandemic, demonstrating the pandemic's potential deleterious impacts on early childhood, including at-risk children. The fact that it was a single-center study can be considered a limitation, despite the fact that it was conducted in one of the rare and comprehensive academic centers where a DBP clinic is located. One further limitation of the study is the lack of data on the crucial final diagnosis. Additional research on diagnostic processes or delays in diagnosis in DBP clinics could contribute to the body of literature.

We believe that time and assistant rotations were the most critical factors in the significant increase in referrals following the establishment of the DBP department. It is possible that the services and training we provide are in response to a lack of knowledge and support in pediatric practice regarding developmental difficulties. This should be encouraging to countries where DBPs will be established. To effectively meet awareness and referral increases, interdisciplinary collaborations should be established and practices that will improve productivity should be planned, given the limited DBP workforce in countries where the division has newly been established. Although it is encouraging that our referral trends have improved in the 1.5 years since the COVID-19 pandemic, health service constraints may have caused a considerable increase in prenatal risk and suspicion of autism referrals to our department following the pandemic. Identifying the pandemic's indirect effects is critical for policymakers. Governments in developing countries should improve their healthcare systems to prevent the long-term detrimental impacts of periodic interruptions in healthcare on children's development and behavior.

Supplementary Materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.4560

Ethical approval

The study was approved by Ethics Committee of Hacettepe University Faculty of Medicine (date: 07.12.2021, number: GO21/1261).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EÖ, AMY, HA, ENÖ; data collection: EÖ, AMY, HA; analysis and interpretation of results: EÖ, AMY, HA; draft manuscript preparation: EÖ, ENÖ. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Assessment of hormone measurement methods in girls with premature adrenarche, polycystic ovary syndrome, and non-classical congenital adrenal hyperplasia

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ABSTRACT

Introduction. Hyperandrogenism is a clinical condition in girls, resulting from excessive androgen production originating from the adrenal glands or ovaries. The measurement of androgen hormones plays an essential role in supporting the clinical diagnosis. These hormone levels can be assessed using immunoassay methods or liquid chromatography – tandem mass spectrometry (LC-MS/MS). Our study aimed to assess the efficacy of hormone measurement with both methods in girls clinically diagnosed with hyperandrogenism.

Methods. Girls presenting with hyperandrogenism were included in this cross-sectional retrospective study. The exclusion criteria included a diagnosis of precocious puberty, classical congenital adrenal hyperplasia (CAH), adrenocortical tumors, and the use of medications known to affect androgen levels. Hormones measured simultaneously by both methods were compared. Regression analysis was performed to adjust hormone levels for age and pubertal stage. Receiver operating characteristic (ROC) analysis was performed based on diagnosis, and androgen hormones with the highest specificity and sensitivity for diagnosis were identified.

Results. A total of 96 girls with hyperandrogenism were included in the study. 60 (62.5%) were diagnosed with premature adrenarche (PA), 31 (32.3%) with polycystic ovary syndrome (PCOS), and 5 (5.2%) with non-classical congenital adrenal hyperplasia (NCCAH). Dehydroepiandrosterone sulfate (DHEAS) measured by LC-MS/MS was significantly lower ($p<0.001$) but less concordant with clinical diagnosis than electrochemiluminescence immunoassay in PA cases. The androgen hormone with the highest area under the curve (AUC) value was androstenedione for PCOS (AUC: 0.949), and 17-hydroxyprogesterone (AUC: 0.994) using LC-MS/MS for NCCAH.

Conclusions. The measurement of DHEAS levels by both methods has low specificity. Androstenedione and total testosterone measured by LC-MS/MS had the highest sensitivity and specificity in PCOS.

Key words: hyperandrogenism, liquid chromatography – tandem mass spectrometry, premature adrenarche, polycystic ovary syndrome.

Hyperandrogenism is a clinical condition caused by excessively high levels of androgen hormones, which result from increased ovarian, adrenal, or peripheral androgen production.[1] The etiology of hyperandrogenism varies between the prepubertal and pubertal periods.

The primary cause of prepubertal hyperandrogenism is premature adrenarche

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(PA). In girls, PA typically presents with clinical signs before the age of 8 years, including early axillary and pubic hair growth, apocrine body odor, greasy hair, acne, and transient growth acceleration. Adrenarche is characterized by increased adrenal androgen hormone precursors, primarily dehydroepiandrosterone (DHEA) and its sulfate form (DHEAS). A serum DHEAS level exceeding 40 μg/dL is accepted as a biochemical marker of adrenarche. It is essential to exclude other pathological causes of androgen excess, such as non-classical congenital adrenal hyperplasia (NCCAH), precocious puberty, and androgen-producing tumors, and exogenous androgen exposure.[2,3]

After the onset of puberty, polycystic ovary syndrome (PCOS) emerges as the most common cause of hyperandrogenism. The primary symptoms of PCOS include menstrual irregularities and hyperandrogenism symptoms such as treatment-resistant acne and hirsutism.[4] The international consensus on diagnostic criteria for PCOS involves identifying evidence of ovulatory dysfunction through abnormal menstrual patterns, along with clinical and biochemical hyperandrogenism. Clinicians should first evaluate total or free testosterone levels to assess biochemical hyperandrogenism. In cases with normal testosterone levels, it is recommended that DHEA and androstenedione (AS) be measured secondarily.[5] However, it should be noted that biochemical hyperandrogenism is not essential for the diagnosis of PCOS mainly as there is no consistent correlation between clinical and biochemical hyperandrogenism.

NCCAH should be considered in the differential diagnosis of PA and PCOS. Heterogeneous *CYP21A2* variants primarily cause a mild deficiency of the 21-hydroxylase enzyme.[6] Clinicians may perform an adrenocorticotropic hormone (ACTH) stimulation test to rule out NCCAH. However, some clinicians suggest that basal serum 17-hydroxyprogesterone (17OHP) levels can be helpful for exclusion. Genetic analysis should be conducted for a conclusive diagnosis if an elevated 17OHP level is detected at baseline or after ACTH stimulation.[7,8]

Accurate measurement of androgen hormones and precursors is essential for differential diagnosis and clinical evaluation. Immunoassay methods, including enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescence immunoassay (ECLIA) are commonly used for measuring steroid hormones due to their simplicity, cost-effectiveness, and sensitivity. However, because of the matrix effect and low specificity, immunoassay methods can be prone to inaccuracies when measuring steroid hormones.[9,10] The liquid chromatography – tandem mass spectrometry (LC-MS/MS) method offers several advantages, such as effectively measuring low concentrations of steroid hormones, reducing interference from analytes, and simultaneously measuring multiple hormone levels.[11]

This study aimed to assess the efficacy of androgen hormone levels measured by both methods in girls presenting with clinical hyperandrogenism.

Materials and Methods

Study population

Prepubertal and pubertal girls from the pediatric population presenting with symptoms of hyperandrogenism at a single tertiary center between January 2017 and December 2018 were included in the study. Cases diagnosed with precocious puberty, classical CAH, or adrenocortical tumors, along with those undergoing medical therapy that could interfere with androgen hormone measurement, were excluded from the study. Demographic data, auxologic measurements, bone age assessments, laboratory results, and molecular variant analysis of the cases were obtained retrospectively from the clinical records. The diagnoses of the cases were determined by experienced pediatric endocrinologists based on the latest guidelines.[12] Cases younger than

8 years with adrenarche symptoms onset, after other causes of hyperandrogenism were excluded, were diagnosed with premature pubarche. PCOS was diagnosed in patients whose menstrual irregularities persisted for two years after menarche and who had clinical hyperandrogenism. A standard dose ACTH test was performed on patients with baseline 17OHP levels >2 ng/mL. Genetic variant analysis was performed in patients with stimulated 17OHP levels >10 ng/mL, and cases with a detected biallelic variant were accepted as NCCAH.

This study was approved by Ege University Medical Research Ethics Committee (Approval No: 18.11T/27), and the principles of the Declaration of Helsinki were followed during this study.

Hormone measurements

The hormones simultaneously measured by immunoassay (IA) and LC-MS/MS methods were 17OHP, DHEAS, and total testosterone (TT). Hormone levels were assessed on the third day of menstruation in PCOS patients. Serum TT and DHEAS concentration were analyzed using the electrochemiluminescence (ECLIA) method (Cobas e-801, Roche Diagnostics GmbH, Germany), serum 17OHP levels were analyzed using the enzyme-linked immunosorbent assay (ELISA) method (Diamtera, SNL, Italy). Plasma concentrations of 17OHP, DHEA, DHEAS, TT, dihydrotestosterone (DHT), and AS were measured using LC-MS/MS (Eureka Lab Division, Code LC72310; 6460 Triple Quadrupole LC-MS/MS, Agilent Technologies, USA). The analytical procedure included protein precipitation using a reagent containing internal standards, followed by centrifugation, dilution, and direct injection into the LC-MS/MS system.

The instrument was operated in multiple reaction monitoring (MRM) mode under positive and negative atmospheric pressure chemical ionization (APCI). The analytical column used was an RRHD Eclipse Plus C18 column with dimensions 50 × 2.1 mm and a particle size of 1.8 μm. Fragmentation parameters for each analyte included the Q1 and Q3 mass-to-charge (m/z) ratios and compound-specific collision energies. The transitions monitored were m/z 331 to 109 for 17-OHP, 271.2 to 231.1 for DHEA, 289.1 to 97 for TT, and 273.1 to 255 for both DHT and AS, with collision energies ranging from 20 to 30 eV.

The analytical performance characteristics were as follows: lower limits of quantification (LLOQ) were 0.015 ng/mL for 17OHP, 0.1 ng/mL for DHEA, 0.6 ng/mL for DHEAS, 0.003 ng/mL for TT, 0.02 ng/mL for DHT, and 0.003 ng/mL for AS. The method also demonstrated lower limits of detection (LLOD) ranging from 0.001 to 0.2 ng/mL, depending on the analyte.

Precision of the assay was evaluated at low, medium, and high concentrations. Intra-day coefficients of variation (%CV) values ranged from 2.0% to 15.2%, and inter-day %CV values ranged from 2.2% to 15.9%, with the highest variability observed at the lowest concentrations of DHEA and TT. The recovery rate for all analytes was reported to be approximately 100% based on the manufacturer's validation data.

Genetic variant analysis

Genetic analysis of the *CYP21A2* gene, which encodes the 21-hydroxylase enzyme, was conducted on all cases showing an increase in the standard dose ACTH stimulation test. Variant analysis of the *CYP21A2* gene was performed using a reverse dot blot (RDB) assay. Using the RDB assay, an assessment was conducted on the eleven regions of the *CYP21A2* gene where variants are most commonly detected. The results were confirmed by performing complete gene sequencing on cases where the RDB assay was positive and those with clinical suspicion despite negative results.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 25.0 (SPSS Inc, Chicago, IL, USA). A p-value less than 0.05 was used to indicate

statistical significance. The Kolmogorov-Smirnov test was used to assess normality. Descriptive statistics for parametric data were expressed as mean and standard deviation values. Non-parametric data of hormone levels were expressed as median and interquartile range. The comparison of androgen hormones TT, DHEAS, and 17OHP, measured simultaneously by LC-MS/MS and IA methods, was performed using the Wilcoxon signed-rank test. Given the effect of age and Tanner stage on androgen hormone levels, regression analysis was performed to obtain adjusted hormone levels. Receiver operating characteristic (ROC) curve analysis was performed in clinically diagnosed cases, and the assay with the highest area under the curve (AUC) was identified. The optimal cut-off value was determined using the Youden index by identifying the point with the highest sensitivity and specificity.

Results

This study included 96 girls with hyperandrogenism. The diagnostic distribution was as follows: 60 (62.5%) had PA, 31 (32.3%) had PCOS, and 5 (5.2%) had NCCAH. One NCCAH case was pubertal, while the others were prepubertal. The hormone levels obtained using the LC-MS/MS and immunoassay methods during the diagnostic evaluation are shown in Table I.

The mean age of the PA cases was 6.7±0.9 years, their body weight standard deviation score (SDS) was 1.10±1.04, height SDS was 0.92±1.2 and BMI SDS was 0.93±0.91. The bone and chronological age difference in PA cases was 1.4±1.29 years. The comparison of hormone measurements by the two methods revealed that hormone levels of DHEAS measured by the ECLIA method were significantly higher ($p<0.001$). The scatter dot plot showed that 11% of cases had DHEAS levels below the biochemical adrenarche threshold value by both methods (Fig. 1). It was also noted that 39% of the cases had DHEAS levels above the threshold according to the ECLIA but below the threshold when measured by LC-MS/MS. However, no cases were found where DHEAS levels were measured above the threshold by LC-MS/MS but below the threshold by ECLIA. ROC analysis of adjusted hormone levels showed that the highest AUC value was observed for DHEAS measured by the ECLIA method (AUC: 0.728). A cut-off value was 44 μg/dL with a sensitivity of 84% and specificity of 62% (Fig. 2A).

Table I. Comparison of androgen hormone levels measured simultaneously by immunoassay and LC-MS/MS according to diagnostic subgroups.

Analyte	Method	PA (n=60)	PCOS (n=31)	NCCAH (n=5)
17OHP (ng/dL)	LC-MS/MS	28.2 (14.3-54.2)	59.2 (33.6-85.8)	601.4 (223.5-1915)
	ELISA	114 (2-164)	261 (157.5-319)	210 (229.9-967.5)
	p value	0.002	0.002	0.144
DHEAS (μg/dL)	LC-MS/MS	44.3 (20.6-84.8)	143.1 (78.3-266.4)	51.1 (22.7-98.5)
	ECLIA	111.7 (62.2-148.2)	312 (160.9-481.5)	67.8 (59.9-153.7)
	p value	0.001	0.001	0.225
TT (ng/dL)	LC-MS/MS	9.2 (5.72-15.2)	31.1 (21.4-55.7)	10.2 (8-30.3)
	ECLIA	6 (3-12)	37 (24.2-53.2)	13.4 (3.5-24.3)
	p value	0.124	0.677	0.07

Data presented as median (Q1-Q3).
17OHP: 17-hydroxyprogesterone, DHEAS: Dehydroepiandrosterone sulfate, ECLIA: Electrochemiluminescence, ELISA: Enzyme-linked immunosorbent assay, LC-MS/MS: Liquid chromatography – tandem mass spectrometry, NCCAH: Non-classical congenital adrenal hyperplasia, PA: Premature adrenarche, PCOS: Polycystic ovary syndrome, TT: Total testosterone.

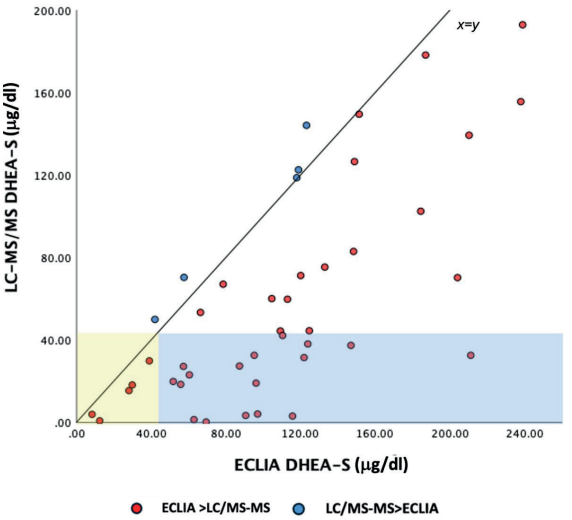

Fig. 1. Scatter dot plot of simultaneous measurement of DHEAS with ECLIA and LC-MS/MS. The yellow box represents the cases of premature adrenarche with normal DHEAS levels. The blue box indicates cases measured below the threshold value by the LC-MS/MS method and those above the ECLIA method's threshold value.

DHEAS: Dehydroepiandrosterone sulfate ECLIA: Electrochemiluminescence immunoassay, LC-MS/MS: Liquid chromatography – tandem mass spectrometry.

The mean age of PCOS cases was 15.3±1.2 years, and their body weight SDS was 0.95±1.55, height SDS was -0.1±1.2 and BMI SDS was 1.03±1.30. No significant difference was found in the comparison of TT levels performed simultaneously by the ECLIA and LC-MS/MS methods (p=0.677). ROC analysis showed that TT and AS levels measured with the LC-MS/MS method had the highest AUC values in diagnosis of PCOS (AUC: 0.792). AS level measured by LC-MS/MS with cut-off values of 49 µg/dL yielded a sensitivity of 79% and specificity of 82%, while TT cut-off of 36 ng/dL resulted in a sensitivity of 83% and specificity 68% (Fig. 2B).

Genetic variant analysis was performed on 41 cases. Variants were detected in 5 cases, while variant analysis was negative in 36 cases. Among these variant negative cases, 18 (50%) of them were diagnosed with PA, and 18 (50%) of them had PCOS. The V281L variant of *CYP21A2* was detected in 4 cases, and the P453S variant in 1 case. The mean age of NCCAH

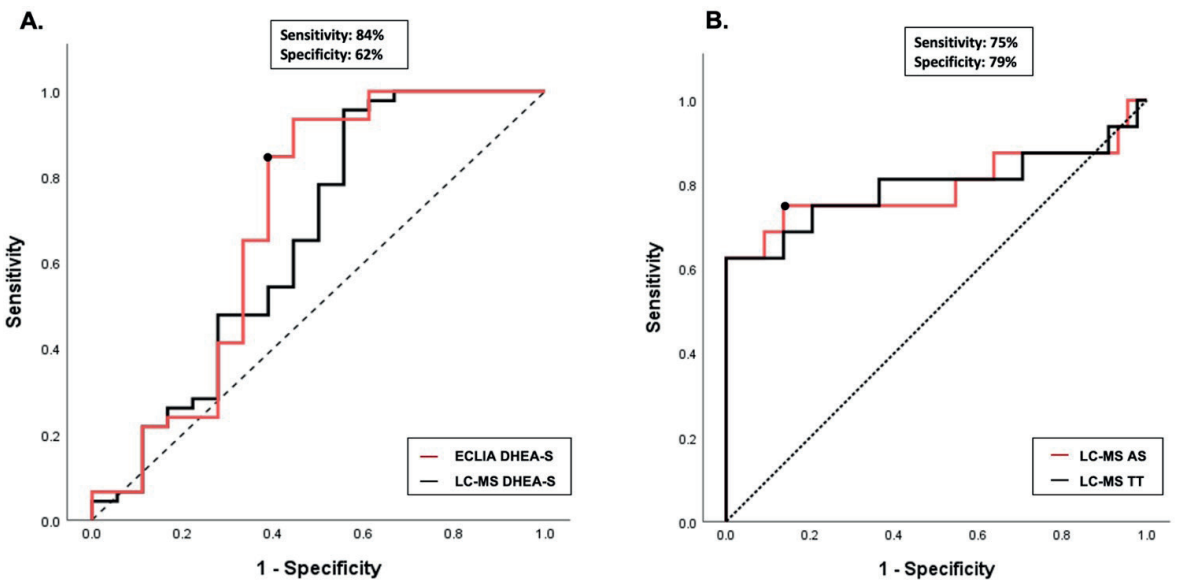

Fig. 2. Receiver operator characteristic (ROC) curve of androgen hormones by diagnostic groups. The two assays with the highest area under curve values are illustrated. **A.** DHEAS measured by ECLIA is superior to LC-MS/MS in diagnosing PA (sensitivity: 84%, specificity: 62%) **B.** In PCOS, AS and TT show comparable results, with AS having slightly higher specificity at similar sensitivity levels (sensitivity: 75%, specificity: 79%).

AS: Androstenedione, DHEAS: Dehydroepiandrosterone sulfate, ECLIA: Electrochemiluminescence immunoassay, LC-MS/MS: Liquid chromatography – tandem mass spectrometry, PA: Premature adrenarche, PCOS: Polycystic ovary syndrome, TT: Total testosterone.

cases was 8.2±2.78 years, their height SDS was 0.72±1.5, their body weight SDS was 1.76±1.1, and their BMI SDS was 1.65±0.84. Among the five cases, four presented with premature adrenarche and were at Tanner stage 2, while one case was diagnosed during the evaluation of menstrual irregularities with Tanner stage 5. No statistically significant difference was found between the 17OHP levels measured by LC-MS/MS and ELISA methods (p=0.144).

Discussion

The auxiliary data and hormonal evaluation of girls diagnosed with PA, PCOS, and NCCAH are presented here. The mean height, weight, and BMI SDS were above the average for healthy children of the same age in all diagnostic subgroups but still within normal limits. Current studies show that PA, PCOS, and NCCAH cases tend to have higher height, weight, and BMI SDS.[13-15]

In the present study, DHEAS levels, the biochemical indicator of adrenarche, measured by the LC-MS/MS method, were significantly lower than those of ECLIA.[10] The percentage of cases with DHEAS levels below the threshold by both methods was lower than that reported in the literature.[15]

Biochemical adrenarche is defined as DHEAS levels exceeding 40 μg/dl. However, a definitive threshold has not been established for PA. Measurement of DHEAS by LC-MS/MS is more sensitive compared to IA methods. In our study, although the two methods demonstrated comparable diagnostic performance, DHEAS measured by the ECLIA method exhibited higher sensitivity and specificity. Nonetheless, despite relatively high sensitivity, specificity was low. This is thought to be primarily due to the fact that DHEAS is not a bioactive androgen and is not strongly correlated with clinical findings.[16] Recent studies have shown that 11-ketotestosterone (11-KT), a potent androgen receptor agonist, may serve as a more effective marker in identifying premature adrenarche.[17] To the best of our knowledge, this is the first study in the literature to evaluate the efficacy of DHEAS in clinically diagnosed premature adrenarche cases.[18] The International Evidence-based Guideline for Assessment and Management of Polycystic Ovary Syndrome recommends that total or free testosterone measurements be evaluated initially for biochemical hyperandrogenism in the diagnosis of PCOS. DHEAS and AS measurements can be analyzed secondarily in cases with normal testosterone levels.[5] No significant difference was observed between TT measurements conducted by different methods. In the literature, the LC-MS/MS method is superior in evaluating low total testosterone levels. However, similar superiority has not been demonstrated in patients with hyperandrogenism.[19]

In our study, AS and TT levels measured by LC-MS/MS were found to have equal AUC values. However, AS demonstrated slightly higher specificity compared to TT at a similar level of sensitivity. The AUC values of AS and TT in our study are consistent with those reported in a recently published meta-analysis. However, in contrast to the meta-analysis, our findings demonstrated that AS exhibited similar sensitivity compared to TT.[20]

Due to the small number of cases, the evaluation of NCCAH cases is limited. However, similar to the literature, the effectiveness of 17OHP measurement with LC-MS/MS in diagnosing NCCAH cases with detected genetic variants has been demonstrated. In the present study, the most common *CYP21A2* variant was V281L, which is consistent with the literature.[6]

Study limitations

The study's main limitations are its retrospective design and the lack of a control group. Another area for improvement is the need for hormone values from alternative steroidogenesis pathways. Additionally, due to the small number of detected genetic variants in NCCAH cases, the evaluation should be considered suboptimal. Prospective studies are needed

to evaluate the clinical efficacy of hormone measurement using the LC-MS/MS method.

Conclusion

The specificity of DHEAS measured by the ECLIA and LC-MS/MS is low in PA cases. Recent studies have reported 11-KT levels measured by LC-MS/MS as more useful in the diagnosis of PA. The sensitivity and specificity of AS and TT levels measured by LC-MS/MS in PCOS were similar to those in the literature. In conclusion, a detailed evaluation of the main and backdoor pathways of steroidogenesis in patients presenting with androgen excess is important for differential diagnosis.

Ethical approval

The study was approved by Ege University Medical Research Ethical Committee (date: November 16th, 2018, number: 18.11T/27). Before the study, an informed written consent form was obtained from the legal caregivers of all participants, and the principles of the Declaration of Helsinki performed this study.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MU, SÖ, AA; data collection: MU, AAanalysis and interpretation of results: AA, BB, ZP, GA, SH, SÖ, DG, ŞD; draft manuscript preparation: MU, AA, SÖ, DG, ŞD. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Trends and clinical features of childhood diabetes subgroups: 28 years of single center experience

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ABSTRACT

Objective. This study aimed to explore the distribution, trends, and clinical characteristics of various types of childhood diabetes, including type 1 diabetes (T1DM), type 2 diabetes (T2DM), and maturity-onset diabetes of the young (MODY) in a tertiary health center.

Methods. We conducted a comprehensive review of medical records of individuals aged 0–18 years who were diagnosed with diabetes between January 1996 and December 2023. Clinical and laboratory characteristics at the time of diagnosis, along with the specific diabetes type, were meticulously documented.

Results. A total of 1219 patients were included in the study, of whom 48.4% were female, with a mean age at diagnosis of 9.1 ± 4.3 years. T1DM was diagnosed in 85.8% of patients, T2DM in 6.3%, clinical MODY in 5.2%, and rare forms of diabetes in 2.6%. An increasing trend in T2DM and MODY cases has been observed since 2007. Diabetic ketoacidosis (DKA) was most prevalent in T1DM (47.1%), followed by T2DM (5.2%) and MODY (1.6%). Mean C-peptide levels at diagnosis were 0.57 ± 0.5 ng/mL in T1DM, 3.2 ± 1.3 ng/mL in T2DM, and 1.4 ± 0.9 ng/mL in MODY. Antibody positivity was observed in 78.8% of T1DM, 6.5% of T2DM, and 15.9% of MODY cases. Among the MODY group, genetic analysis was performed in 48 (75%) patients, with *GCK* gene mutations identified as the most common genetic abnormality in 27 (56.2%) of these patients.

Conclusion. This study demonstrates that T1DM is still the most commonly diagnosed type of diabetes in childhood, while T2DM and MODY are less frequent. However, a temporal increase in the incidence of MODY and T2DM subtypes was observed. The incidence of DKA at diagnosis was significantly higher in T1DM patients compared with those diagnosed with MODY or T2DM.

Key words: type 1 diabetes, type 2 diabetes, monogenic diabetes, matury-onset diabetes of the young (MODY), childhood.

Recent trends have shown that the incidence of diabetes is increasing rapidly worldwide[1], and there has also been a dramatic increase in its incidence in Turkish children.[2,3] Type 1 diabetes mellitus (T1DM) is the most common type of diabetes in children and continues to increase in different parts of the world.[1,4-6] Childhood obesity, which is increasing worldwide depending on ethnicity and country of residence, has been associated with a variable increase in the prevalence of type 2 diabetes (T2DM).[7] The prevalence of T2DM in children has been reported to be 11% in the USA[8], compared to 1.3% in Europe.[9] In six

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regions of the USA, the prevalence of diabetes in children and adolescents has been reported to be increasing significantly for both type 1 and type 2 diabetes.[10]

Childhood T2DM can be confused with maturity-onset diabetes of the young (MODY) due to family history, presentation and a possible confounding factor of obesity/overweight.[11] MODY can also be misclassified as T1DM, particularly due to *HNF1A* mutations.[12] Determining the type of diabetes is important for therapeutic evaluation and genetic counselling.[13] The most common type of monogenic diabetes is MODY, a clinically and genetically heterogeneous group of endocrine disorders affecting 1-5% of patients with diabetes.[14] The increasing availability of molecular diagnostics has made it possible to identify other types of monogenic diabetes in addition to type 1 and type 2 diabetes with polygenic inheritance. In this classification, the concept of monogenic diabetes includes MODY, mitochondrial diabetes, Wolfram syndrome, neonatal diabetes and specific syndromes due to genetic defects causing insulin resistance.[15]

Until about 15 years ago, the distinction between T1DM, T2DM and MODY was somewhat simpler. However, with the increase in genetic studies and the definition of associations, it has become clear that these distinctions are not clear-cut. It has shown us that there are intertwined forms of diabetes. In daily practice, the differential diagnosis includes clinical findings, laboratory tests and diabetes-associated autoantibodies. This study will discuss these parameters in the differential diagnosis of diabetes.

The aims of this study were: (1) to evaluate the aetiological distribution and temporal changes of childhood diabetes, (2) to compare the clinical and laboratory characteristics of different types of diabetes seen in the pediatric diabetes centre of a tertiary care hospital during the last 28 years. The aim was also to contribute to the differential diagnosis of childhood diabetes subgroups.

Materials and Methods

The study was approved by the Ethics Committee of İnönü University (approval no: 2024/5706). Data of children and adolescents aged 0-18 years who were diagnosed with diabetes mellitus at İnönü University Turgut Özal Medical Center Training and Research Hospital in Malatya, Türkiye during the 28-year period between January 1996 and December 2023 were analyzed. Patients with a follow-up period of less than one year and those with undetermined diabetes type due to insufficient data were excluded from the study. In addition, because we could not clearly determine the number of patients who developed drug-associated diabetes, these patients were also excluded from the study. The observational and retrospective study included 1219 patients.

Sex, age at diagnosis, height, weight, body mass index (BMI), glucose, insulin, HbA1c, C-peptide levels at diagnosis, presence of symptoms, presence of diabetes-associated autoantibodies (islet cell antibodies, glutamic acid decarboxylase antibodies and insulin autoantibodies), presence of ketone bodies, pH at diagnosis and type of diabetes were recorded. The islet cell antibody (ICA), insulin autoantibody (IAA), and glutamic acid decarboxylase (GAD) levels were measured based on antigen-antibody detection using enzyme-linked immunosorbent assays with the Isletest kit in the Seac Brio 410499 model instrument. Patients were classified according to the ISPAD Consensus 2022 (Table I).[16] Clinical findings were queried as polyuria, polydipsia, polyphagia and weight loss, and patients with one of these four clinical findings were considered symptomatic. Standard deviation scores (SDS) for height, weight, and BMI were calculated based on reference data from healthy Turkish boys and girls.[17]

T1DM was diagnosed based on the presence of severe insulin deficiency, diabetes-associated autoantibodies positivity, and the absence of any clinical signs suggestive of alternative causes of diabetes. The diagnostic criteria for

T2DM included overweight or obesity, clinical features of insulin resistance (such as acanthosis nigricans, hypertension, and dyslipidemia), a family history of T2DM, and good metabolic control achieved with metformin alone, or with metformin combined with a low dose of long-acting insulin (<0.5 U/kg/day).[7,15,16] Patients with a family history of diabetes for at least two generations on one side of the family, negative diabetes-associated autoantibodies, no evidence of insulin resistance and good metabolic control with diet, sulfonylurea or low-dose insulin were clinically classified as MODY. *HNF1A, HNF4A, GCK* and other genes have been analysed in clinically suspected cases of MODY, and a MODY panel has been studied in recent years. To differentiate between mutations and polymorphisms among the detected variants, segregation analyses were conducted within families. In silico analysis tools, including Provean, SIFT (Sorting Intolerant From Tolerant), PolyPhen, Franklin, and MutationTaster, were employed to evaluate the potential pathogenicity of the variants. In accordance with the 2015 ACMG guidelines[18], variants were classified as "pathogenic", "likely pathogenic", "variant of uncertain significance (VUS)", "likely benign" or "benign". Individuals carrying variants classified as "pathogenic" or "likely pathogenic" based on in silico analyses were considered to have monogenic diabetes. Children with onset of diabetes before the age of six months were diagnosed with neonatal diabetes mellitus (NDM) and appropriate genetic testing was performed.

Statistical analysis

All statistical data were analysed using SPSS statistical software for Windows, version 21.0 (SPSS, Chicago, IL, USA). Variables were calculated using descriptive statistics. Data were presented as mean ± standard deviation (SD) or median (interquartile range, IQR, Q1-Q3). Normality was assessed using the Kolmogorov-Smirnov test. Parametric and non-parametric tests were used for comparisons between groups. The chi-square test was used for categorical variables. Student's t-test was used for continuous variables in independent groups. Mann-Whitney U test was used for continuous variables that were not normally distributed. In statistical analyses, $p < 0.05$ is the accepted threshold for significance.

Results

The mean age at diagnosis of 1219 patients (48.4% female) was 9.1±4.3 (range 0.0-18.0) years. 23.1% (n=242) of patients with T1DM were younger than 5 years. 1046 patients were diagnosed with T1DM (85.81%), 77 patients with T2DM (6.31%), 64 patients with MODY (5.25%) and 32 patients with other types of diabetes (2.62%) (Table I).

Table I. Distribution of diabetic patients according to subgroups

	n	%*
Type 1 diabetes mellitus	1046	85.81
Type 2 diabetes mellitus	77	6.31
MODY	64	5.25
Neonatal DM	7	0.57
Wolfram syndrome	5	0.41
Diseases of the exocrine pancreas	4	0.33
Alström syndrome	3	0.24
Mitochondrial DM	3	0.24
Genetic defect in insulin action	2	0.16
TRMA	2	0.16
Subtotal pancreatectomy**	2	0.16
IPEX syndrome	1	0.08
Woodhouse-Sakati syndrome	1	0.08
Prader-Willi syndrome	1	0.08
Generalized lipodystrophy	1	0.08
Total	1219	100

*Column percentages.
**The development of diabetes following surgery for hyperinsulinemic hypoglycemia.
DM: diabetes mellitus; IPEX: immune dysregulation, polyendocrinopathy, enteropathy, X-linked; MODY: maturity onset diabetes of the young, TRMA: thiamine-responsive megaloblastic anemia.

Seven of the cases were definitively diagnosed with NDM. During follow-up, four cases were classified as transient NDM, while three cases were confirmed as permanent NDM. The clinical characteristics at diagnosis of T1DM, T2DM and MODY are compared in Table II. The median age at diagnosis was 8.6 years (IQR: 5.1-12.0) for T1DM, 15 years (12.6-16.2) for T2DM, and 10.9 years (7.2-13.0) for MODY. A single case of T2DM diagnosed before the age of 10 years was identified. This patient, a 9.8-year-old girl at the time of diagnosis, also presented with precocious puberty. Positivity for at least one diabetes-associated autoantibody was 78.8% in T1DM, 6.5% in T2DM and 15.9% in MODY. IAA positivity before the age of 5 years was significantly higher in T1DM cases than > 5 years (41.1% vs. 32.6%, p=0.045).

T2DM and MODY cases started to be seen in the cohort from 2007 onwards, this increasing trend over time is shown in Fig. 1. T1DM, on the other hand, seemed to increase over time, but the number of type 1 diabetes cases

Table II. A comparative analysis of metabolic and clinical characteristics of patients diagnosed with type 1 diabetes, type 2 diabetes, and maturity-onset diabetes of the young at time of diagnosis.

	n*	T1DM	T2DM	MODY	T1DM vs T2DM, p	T1DM vs MODY, p	T2DM vs MODY, p
Sex (F/M), n	1187	504/542	46/31	25/39	0.050[a]	0.156[a]	0.014[a]
Age at diagnosis (year)	1187	8.6 (5.1-12.0)	15 (12.6-16.2)	10.9 (7.2-13.0)	<0.001[c]	0.003[c]	<0.001[c]
Height SDS	336**	0.3 (-0.4-1.2)	0.2 (0.0-0.7)	-0.2 (-0.7-0.6)	0.890[c]	0.001[c]	0.001[c]
Weight SDS	336**	-0.3 (-1.1-0.5)	2.1 (1.4-2.5)	-0.2 (-1.0-1.0)	<0.001[c]	0.280[c]	<0.001[c]
BMI SDS	336**	-0.5 (-1.5-0.1)	2.0 (1.6-2.3)	-0.2 (-1.2-1.1)	<0.001[c]	0.013[c]	<0.001[c]
BMI SDS ≥2	336**	4 (2.0%)	43 (58.1%)	5 (8.1%)	<0.001[a]	0.023[a]	<0.001[a]
BMI SDS <2	336**	27 (13.6%)	0	6 (9.7%)	0.001[a]	0.414[a]	<0.001[a]
Presence of symptoms	898	718 (93.7%)	26 (37.1%)	22 (35.4%)	<0.001[a]	<0.001[a]	0.811[a]
Parental consanguinity	1040	413 (39.4%)	17 (23.9%)	25 (39.1%)	<0.001[a]	0.289[a]	<0.001[a]
Age <5 years	1187	242 (23.1%)	0	7 (10.9%)	<0.001[a]	0.073[a]	0.001[a]
Fasting glucose (mg/dL)	1040	479±182	184±92	185±172	<0.001[b]	<0.001[b]	0.992[b]
Insulin (μU/mL)	1040	1.8 (1.1-3.4)	20.4 (12.1-31.0)	5.2 (2.8-9.3)	<0.001[c]	<0.001[c]	<0.001[c]
C-peptide (ng/mL)	1040	0.57±0.5	3.2±1.3	1.4±0.9	<0.001[b]	<0.001[b]	<0.001[b]
HbA1c (%)	1040	12.2±2.6	8.1±2.3	7.6±2.7	<0.001[b]	<0.001[b]	0.258[b]
pH	891	7.24±0.15	7.37+0.04	7.38±0.25	<0.001[b]	<0.001[b]	0.684[b]
DKA	891	354 (47.1%)	4 (5.2%)	1 (1.6%)	<0.001[a]	<0.001[a]	0.663[a]
Ketosis	891	290 (38.6%)	10 (13.1%)	8 (12.5%)	<0.001[a]	<0.001[a]	0.876[a]
Hyperglycemia	891	107 (14.2%)	62 (81.5%)	55 (85.9%)	<0.001[a]	<0.001[a]	0.506[a]
Antibody positivity	831	546 (78.8%)	5 (6.5%)	10 (15.9%)	<0.001[a]	<0.001[a]	0.066[a]
Anti-GAD positivity	831	419 (60.4%)	5 (6.7%)	10 (15.9%)	<0.001[a]	<0.001[a]	0.073[a]
ICA positivity	831	309 (44.5%)	0	3 (4.8%)	<0.001[a]	<0.001[a]	0.093[a]
IAA positivity	831	240 (34.6%)	0	1 (1.6%)	<0.001[a]	<0.001[a]	0.457[a]

Data were presented as n (%), mean±standard deviation, or median (Q1-Q3).
*The n values represent the number of patients for whom data was available.
**T1DM: 198, T2DM: 74, MODY: 62.
[a]Chi-square test. [b]Independent samples t test. [c]Mann-Whitney U test. In statistical analyses, p<0.05 is the accepted threshold for significance.
BMI: body mass index, DKA: diabetic ketoacidosis, F: female, GAD: glutamic acid decarboxylase, HbA1c: hemoglobin A1c, IAA: insulin autoantibody, ICA: islet cell antibody, M: male, MODY: maturity onset diabetes of the young, SDS: standard deviation score, T1DM: type 1 diabetes mellitus, T2DM: type 2 diabetes mellitus.

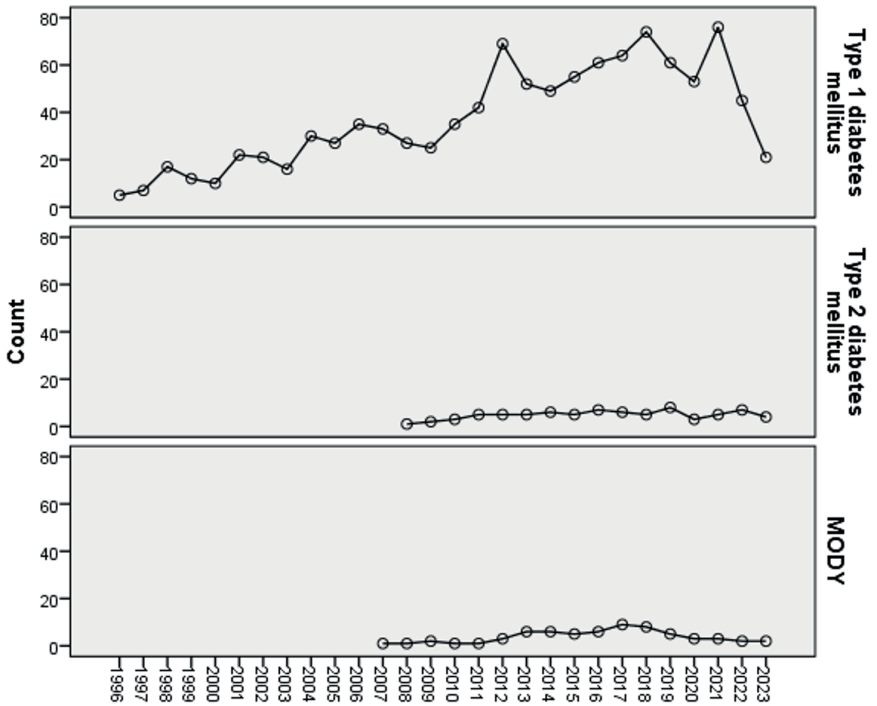

Fig. 1. Changes in the number of newly diagnosed patients with diabetes subgroups over the years. The steep decline in the number of cases diagnosed with type 1 diabetes mellitus in 2023 may be attributable to the migration of some of the population out of Malatya after the devastating twin Kahramanmaraş eartquakes in February 2023.

MODY: maturity onset diabetes of the young.

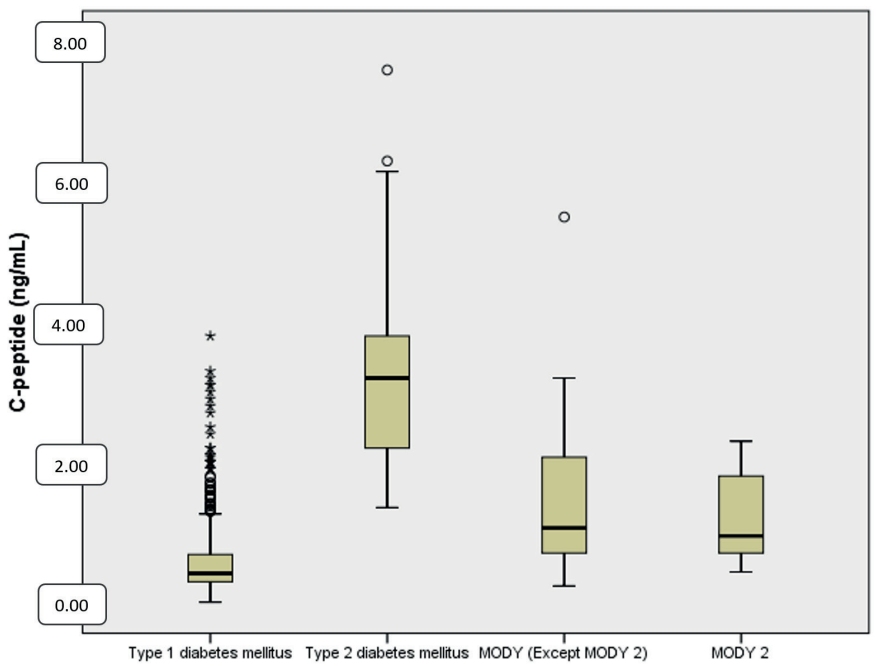

Fig. 2. C-peptide values of patients with diabetes at diagnosis.

MODY: maturity onset diabetes of the young.

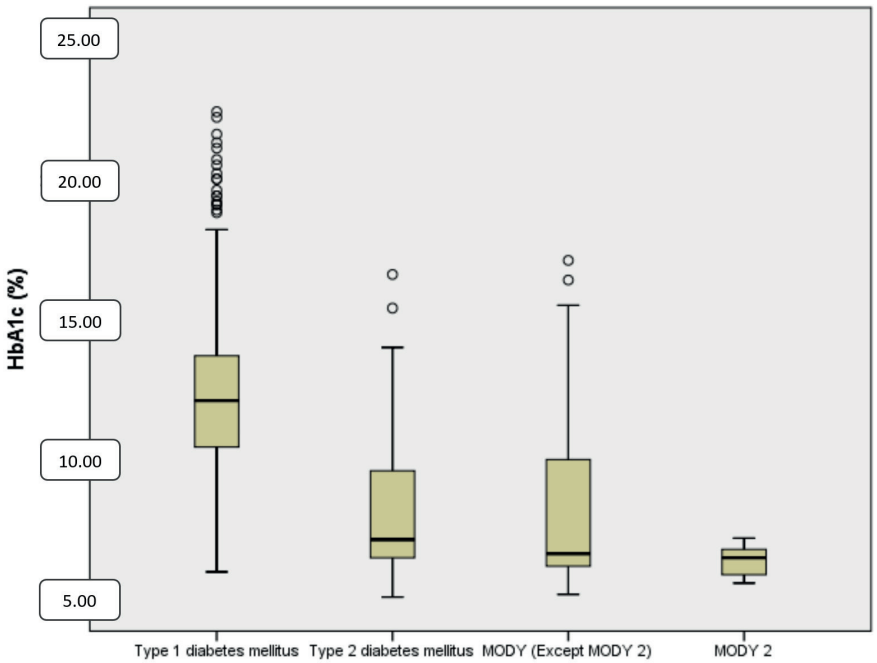

Fig. 3. HbA1c values of patients with diabetes at diagnosis.
MODY: maturity onset diabetes of the young.

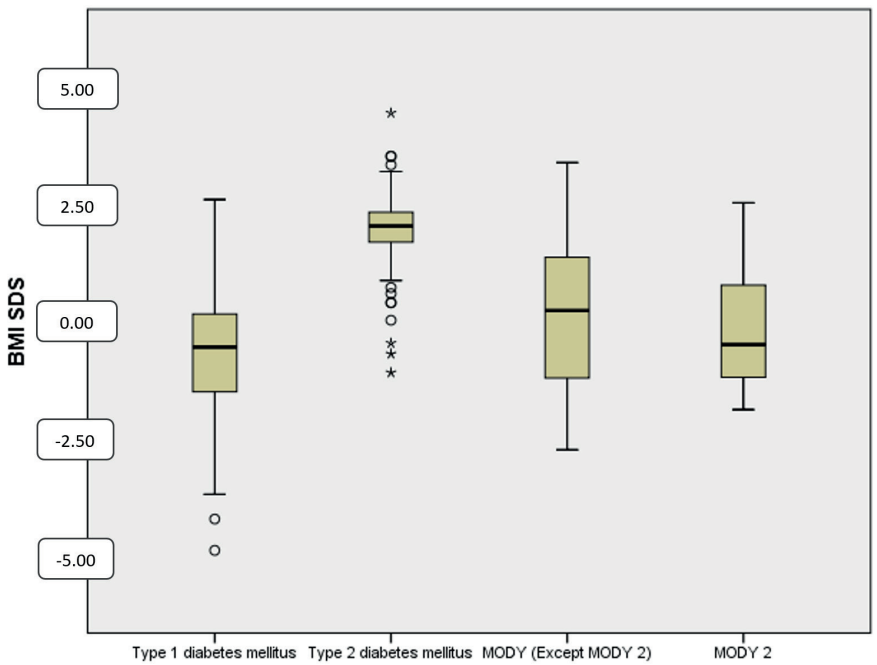

Fig. 4. BMI SDS values of patients with diabetes at diagnosis.
BMI: body mass index, MODY: maturity onset diabetes of the young, SDS: standard deviation score.

decreased in 2023. The prevalence of diabetic ketoacidosis (DKA) in T1DM at diagnosis was 47.1%, 47.8% in patients <5 years and 44.8% in patients >5 years (p=0.486). The prevalence of DKA decreased to 61.5% between 1996-2002, 51.0% between 2003-2009 and 32.9% between 2010-2016. However, it increased again between 2017 and 2023, reaching 41.2%. At the time of diagnosis, 62 (81.6%) patients with T2DM had hyperglycemia, ten (13.2%) had ketosis and only four (5.2%) had DKA (Table I).

Mean C-peptide levels at diagnosis were 0.57±0.5 ng/mL in T1DM, 3.2±1.3 ng/mL in T2DM and 1.4±0.9 ng/mL in MODY patients (p<0.001) (Table II and Fig. 2). The mean HbA1c level at diagnosis was 12.2% ± 2.6% in T1DM, 8.1% ± 2.3% in T2DM and 7.6% ± 2.7% in MODY patients. HbA1c levels were significantly higher in T1DM (p<0.001) (Table II and Fig. 3).

The median BMI-SDS at diagnosis was –0.5 (–1.5-0.1) in T1DM, 2.0 (1.6-2.3) in T2DM, and –0.2 (–1.2 -1.1) in MODY patients. It was found to be significantly higher in T2DM (p<0.001). Obesity was present 2% of T1DM, 10.9% of MODY and 93.7% of T2DM patients. Malnutrition was found in 13.6% of T1DM patients, 9.7% of MODY patients and was absent in T2DM patients (Table II and Fig. 4). Genetic analysis was available in 48 (75%) of 64 clinical MODY patients, and *GCK* mutations were detected in 27 (56.2%) patients.

Discussion

In the present study, we analysed the prevalence of diabetes subgroups in our population and these data allowed us to make comparisons with the literature. The overall prevalence of T1DM, T2DM, MODY and other types of diabetes was 85.81%, 6.31%, 5.25% and 2.62%, respectively. T1DM is still the most common cause of diabetes in children and its prevalence varies from 83-95% in different parts of the World.[5,6,8,9,12,19] This difference is due to the number of children with T2DM and MODY. In the SEARCH study (USA, multicenter), the prevalences of T1DM, T2DM and MODY were 85.6%, 10.8% and 1.2%, respectively, whereas in the SWEET study (48 participating centers, 37 from Europe) these rates were 95.5%, 1.3% and 1.5%, respectively.[9,20] In the SEARCH studies, the burden of T1DM was highest among non-Hispanic White youth, whereas the burden of T2DM was greatest among minority youth, particularly among American Indian and Alaska Native populations.[8,20] The variation in frequencies may be explained by the accessibility of genetic testing and also by the prevalence of obesity in this region. In this study, the diagnoses of T2DM (6.3%) and MODY (5.2%) were more common than in SWEET. As this was not a national multicentre study, it is thought that this difference may be explained by the referral of rare types of diabetes to our tertiary care centre and easier access to genetic testing.

The country with the highest incidence of T1DM in childhood is Finland, followed by Sweden. Recent studies show an increasing trend in the Arabian Gulf countries and Türkiye.[1-6] Previously, we found an annual increase of 8.3% in the incidence of T1DM in our centre.[2] The incidence of T2DM, thought to be rare in children, is gradually increasing, and in parallel, the incidence of obesity in children is increasing, making it difficult to clinically differentiate between different types of diabetes.[5,6] In studies of MODY in children, misdiagnosis of type 1 or type 2 diabetes has been observed.[5] Recently, the number of cases diagnosed with MODY has been increasing with increasing awareness and molecular diagnostic capabilities.

The most important and accessible data in the differential diagnosis of diabetes subgroups are clinical findings (polyuria, polydipsia, polyphagia, and weight loss), glucose, insulin, C-peptide, HbA1c and the presence of diabetes-associated autoantibodies. Polyuria, polydipsia, polyphagia and weight loss are more suggestive of T1DM, but can also be seen in MODY.[21] Clinical findings indicate the severity of insulin deficiency.[21] In our study, the clinical findings were 93.7% in T1DM, 37.1% in T2DM and 35.4%

in MODY. In T1DM, clinical findings are the most important.

One of the most confusing clinical findings in diabetes classification is obesity. At the time of diagnosis, BMI is considered to be a less determinant feature in classification. This is because the increase in obesity has led to the emergence of obese children with T1DM and MODY.[22] Although different studies have used different criteria, the prevalence of obesity at the time of diagnosis in T1DM patients varies from 3.1% to 9%.[11,12] In this study, obesity was found in 2.0% of patients with T1DM. This may be due to the lower rates of obesity in our pediatric population compared to North America and Western Europe.[23] Consistent with previous reports, T2DM is more common in girls and in adolescents.[14,15,23] In this study, the youngest documented case of T2DM was that of a 9.8-year-old girl who presented with precocious puberty. No additional cases of T2DM were identified in children under the age of 10 years, the mean age at diagnosis was 14.4 years, and almost all cases were in the adolescent age group. MODY can occur at any age.

While glucose and HbA1c are very effective in diagnosis, they are not very helpful in differential diagnosis. While HbA1c is generally below 7.5% in MODY 2 and 8-9% in other MODYs, this rate is above 10% in type 1 diabetes.[21] Similar results were found in our study; in general, HbA1c is higher in T1DM. In diagnosis, C-peptide level is more valuable than insulin level in demonstrating reserve, and a C-peptide level >1.2 ng/mL in diagnosis suggests T2DM or MODY.[24] In our study, mean C-peptide level was 0.57 ng/mL and significantly low in T1DM, high in T2DM and moderate in MODY. In this study, there was little overlap in C-peptide levels at the time of diagnosis, especially in T1DM and T2DM, which helped us to differentiate these two types of diabetes. It also led us to distinguish T2DM from MODY, albeit weakly.

Antibody positivity in T2DM has been reported up to 15% and these antibody positive patients tend to be younger, less overweight/obese and have higher HbA1c levels.[25]Therefore, different terms such as type 1.5 diabetes, double diabetes and occult autoimmune juvenile diabetes have been used. In the present study, antibody positivity in T2DM was found to be 6.5%. In T2DM patients aged 10 years and older, antibody positivity was reported in 9.8% in the TODAY study[26] and 21.2% in the SEARCH study.[27] The diagnosis of this group of seropositive patients is controversial. Studies have shown antibody positivity in 1% of people with MODY.[20] In the present study, 15.9% of our MODY patients had at least one antibody positivity. We were unable to explain the high antibody positivity observed in MODY patients. Therefore, in this study, antibody positivity was not used as an exclusion criterion for MODY.

In studies conducted in developed countries, the prevalence of DKA at the time of diagnosis of type 1 diabetes is reported to be 20-43%, whereas in the Arabian Peninsula these rates can be as high as 80%.[28,29] In different studies conducted in Türkiye, the prevalence of DKA in T1DM at diagnosis varies between 44% and 65%.[6,30-33] In our study, the rate of DKA was 47.1% and the current rate is still very high. The prevalence of DKA in pediatric T2DM at the time of diagnosis is variable, ranging from 4% to 40%.[6,25,34] This rate was not high in our study (5.2%). The rate of DKA was lower in our MODY patients (1.6%). Ketosis without acidosis was found in 13% of our T2DM patients and 12.5% of our MODY patients. More than 80% of patients with T2DM and MODY were diagnosed after detection of hyperglycemia without ketosis or diabetic ketoacidosis. The most likely reason for this is that our centre is a tertiary care centre and we perform oral glucose tolerance test in all obese children with risk factors.

The frequency of MODY in children with diabetes varies from 1% to 6% in different studies.[6,9,12,21] In studies reporting a higher frequency of MODY, *GCK* mutation is the most common cause.[6,12,21] Similarly, we found a *GCK* mutation in 56.2% of genetically tested clinical MODY patients, which may be explained

by the widespread use of random glucose measurement in general pediatric clinics in Türkiye. On the other hand, since genetic analysis was not available before 2010 and not all MODY genes could be analysed, the rate of genetic analysis in clinical MODY patients in this study was relatively low (75%). A mutation in one of the known MODY genes was found in 75% of these patients. This rate varies between 27-89% in different studies.[6,21,35] The reasons why not all patients in our study received a genetic diagnosis may be due to the inability to detect mutations, inability to perform a MODY gene panel in all patients, inclusion criteria for genetic testing, mutation in a gene not yet identified, or diagnostic overlap of different types of diabetes.

Study limitations

This study has some limitations; (1) data from a tertiary care centre, (2) specifically, prior to 2010, the diagnosis of MODY was only made clinically, (3) not all patients with MODY after 2010 could be interviewed, (4) the frequency of some specific types of diabetes such as cystic fibrosis-related diabetes (CFRD) may not be the actual frequency, (5) cases with drug-induced diabetes could not be included in the study.

Conclusion

This study examines trends in pediatric diabetes over the past 28 years in a large patient population from a large city with access to a tertiary care diabetes centre. It aimed to identify the distinguishing characteristics of different types of diabetes in children. While the prevalence of T2DM is increasing among the paediatric population in Türkiye, it remains lower than reported rates in North America and Western Europe. In recent years, the recognition of MODY has increased, largely due to the wider availability of autoantibody testing and, more importantly, genetic testing. Although overlapping clinical features—such as obesity, ketosis, and autoantibody positivity—can complicate the differential diagnosis, demographic (e.g., age, sex, pubertal status, consanguinity) and laboratory parameters (e.g., autoantibody measurements, C-peptide levels, HbA1c) are valuable tools for accurately classifying diabetes types in the paediatric population.

Ethical approval

The study was approved by the local Ethical Committee of İnönü University (approval no: 2024/5706).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: İD, AA; data collection: İD, EK, ZYY; analysis and interpretation of results: İD, EÇ; draft manuscript preparation: İD, AA, EÇ, EK, ZYY. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Heart rate variability as a marker of autonomic dysfunction in children with primary Raynaud's phenomenon

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ABSTRACT

Background. Primary Raynaud's phenomenon (RP) is a functional vasospastic disorder triggered by cold or emotional stress, often occurring without an underlying systemic disease. As autonomic dysfunction is thought to contribute to RP pathogenesis, heart rate variability (HRV) analysis may provide insights into underlying mechanisms. This study aimed to assess autonomic nervous system activity in children with primary RP using HRV parameters.

Methods. The study included 36 primary RP patients (0–18 years) and age- and gender-matched 30 healthy controls with normal 24-hour Holter electrocardiograms (ECG). Data on demographics, laboratory results, 24-hour Holter ECG, capillaroscopy, and treatment were collected. HRV was analyzed in both the time and frequency domains.

Results. In the patient group, 11 (30.4%) were male, and 25 (69.6%) were female, with a median age of 15 (8–18) years. Symptom onset occurred at a median age of 14. The attack patterns were biphasic in 36.1% of patients, triphasic in 30.6%, and monophasic in 33.3%. Capillaroscopy was normal in 16 (44.4%) patients, with minor changes in 20 (55.6%). Six (16.6%) patients had positive antinuclear antibody (ANA) with no autoimmune disease diagnoses. Holter ECG monitoring results were compared with those of healthy controls (median age 15 years), showing significant differences in standard deviation of all normal-to-normal intervals (SDNN) and standard deviation of successive differences between adjacent RR intervals (SDSD) between primary RP patients and controls, but no differences in root mean square of successive differences (RMSSD) or HRV index values.

Conclusion. Pediatric patients with primary RP showed significant autonomic changes compared to controls, though it remains unclear if these changes favor sympathetic or parasympathetic pathways. Further multicenter, prospective studies are needed to clarify these findings.

Key words: Raynaud's phenomenon, heart rate variability, autonomic nervous system activity, antinuclear antibody, capillaroscopy.

Raynaud's phenomenon (RP) is characterized by transient vasospastic episodes in peripheral blood vessels, leading to a characteristic three-phase color change—pallor, cyanosis, and hyperemia—typically affecting the extremities.[1] In some cases, the tip of the nose and the ears can also be involved.[1] While data on the prevalence of RP in childhood is scarce, a study by Jones

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et al.[2] found a prevalence of 18% in girls and 12% in boys aged 12–15 years. Because RP episodes are frequently triggered by cold and emotional stress—both of which involve autonomic nervous system activity—it has been suggested that dysregulation of autonomic function may play a role in the pathophysiology of RP.[1,2]

Heart rate variability (HRV) reflects fluctuations in the time intervals between consecutive heartbeats, and can be measured over short-term (e.g., 5 minutes) or long-term (e.g., 24 hours) electrocardiogram (ECG) recordings. HRV analysis provides a non-invasive window into autonomic nervous system function, and is typically expressed using time-domain or frequency-domain indices.[3,4] For example, time-domain indices such as standard deviation of all normal-to-normal intervals (SDNN) or root mean square of successive differences (RMSSD) reflect beat-to-beat variability, while frequency-domain indices capture oscillatory patterns associated with sympathetic and parasympathetic activity.[5] Specifically, the high-frequency (HF) component is linked to parasympathetic activity, while the low-frequency (LF) component reflects a mix of sympathetic and parasympathetic influences.[4,6] Given this background on HRV as a marker of autonomic regulation, it is relevant to explore how these indices behave in patients with RP. Studies in adults with primary RP have demonstrated signs of autonomic imbalance, including increased sympathetic activity, reduced parasympathetic tone, and altered HRV parameters compared to healthy controls.[7,8] These findings suggest that autonomic dysfunction may contribute to the pathogenesis of RP. However, studies on this topic in childhood are rare. A pediatric study evaluating primary RF by Oflaz et al.[9] demonstrated abnormalities in time-domain HRV parameters, including SDNN, SDNN index (mean of the SDNN for all 5-min segments; SDNNi), standard deviation of the average NN intervals (SDANN), RMSSD, percentage of NN50 (pNN50), and the triangular index. Exploring HRV changes in children with primary RP could help clarify whether similar autonomic disturbances occur early in the disease course. Elucidating autonomic nervous system involvement in primary RP during childhood could improve our understanding of the disease's pathophysiology and potentially guide management, since autonomic markers like HRV might eventually serve as early indicators of dysfunction. Given the role of the autonomic nervous system in mediating vascular responses to stress, we hypothesized that children with primary RP may exhibit measurable autonomic dysfunction. By analyzing HRV, which is a non-invasive marker of autonomic activity, this study seeks to fill a knowledge gap in pediatric RP.

This study aimed to assess heart rate variability in children with primary RP compared to age-matched healthy peers to determine whether RP in childhood is associated with autonomic nervous system dysfunction by evaluating the time—and frequency-dependent HRV in children diagnosed with primary RP.

Materials and Methods

Patients aged 0–18 years, diagnosed with RP and followed up at the Pediatric Rheumatology Outpatient Clinic, were enrolled in the study. Initially, patients with primary RP were documented. The diagnosis of primary RP was based on clinical assessment and fulfilled the International Consensus Criteria for Raynaud's Phenomenon, which include episodic, symmetrical, and reversible color changes of the extremities in response to cold exposure or emotional stress, in the absence of an underlying systemic disease.[10] In all cases, a comprehensive work-up including medical history, physical examination, immunologic screening, and echocardiography was performed to exclude secondary RP causes. Subsequently, the medical records of these patients were reviewed to collect data on demographic characteristics, the time of diagnosis, symptoms, comorbidities, treatments, clinical course, laboratory findings, and capillaroscopic evaluations. Capillaroscopic patterns were classified according to the criteria

proposed by Ingegnoli et al.[11], including normal, minor abnormalities, major abnormalities, and scleroderma pattern. Minor abnormalities were defined as a capillary density of 6–8/mm with <10% elongated loops, <50% tortuous loops, all arranged in parallel rows, and no hemorrhages. Evaluations were performed by an experienced pediatric rheumatologist using standard magnification videocapillaroscopy. Finally, heart rhythm analysis was performed on data from 24-hour three-channel Holter ECG monitoring before initiation of medical therapy for RP. All Holter recordings were obtained during asymptomatic periods, as confirmed by patient reports and absence of active color changes during monitoring. This approach was chosen to evaluate baseline autonomic function independent of acute vasoactive fluctuations during RP episodes.

Exclusion criteria included the presence of connective tissue diseases, conditions like skin ulceration, telangiectasia, muscle weakness, scleroderma, and factors affecting heart rate such as hypothermia, hyperthermia, chronic diseases (e.g., diabetes, hypertension), structural heart disease, or the use of medications affecting heart rhythm.

Patients were advised not to engage in strenuous exercise during the 24-hour Holter ECG monitoring. For the HRV analysis, all patients' Holter recordings were manually assessed to exclude artifacts and ectopic beats. Only recordings with at least 85% of data composed of normal R-wave morphology were included. HRV parameters were automatically extracted using dedicated Holter data processing software. Time-domain measurements included SDNN, standard deviation of successive differences between adjacent RR intervals (SDSD), SDANN and RMSSD. Frequency-domain measurements were presented, including HF band, LF band, and LF/HF ratio.[4] Time-domain parameters quantify the variability in successive RR intervals over time. SDNN reflects the overall variability and is influenced by both sympathetic and parasympathetic activity. SDSD and RMSSD primarily reflect parasympathetic (vagal) activity. RMSSD is calculated by determining successive RR interval differences, squaring each, averaging the squared values, and taking the square root of this average.[4,5]

Frequency-domain analysis involves spectral decomposition of RR interval time series into frequency bands. The high-frequency (HF: 0.15–0.40 Hz) band reflects parasympathetic activity, especially associated with respiratory sinus arrhythmia. The low-frequency (LF: 0.04–0.15 Hz) band reflects a combination of sympathetic and parasympathetic modulation. The LF/HF ratio is used as an indicator of sympathovagal balance. Very low frequency (VLF) and ultra-low frequency (ULF) bands are also detectable but were not analyzed in this study due to methodological limitations.[4,6,12] The LF band (0.04–0.15 Hz) is comprised of rhythms with periods between 7 and 25 s. The HF or respiratory band (0.15–0.40 Hz) is comprised of rhythms with periods between 7 and 2.5 s. HRV parameters were extracted using the Spacelabs Healthcare Pathfinder SL software (Spacelabs Healthcare Inc., Snoqualmie, WA, USA).

The control group consisted of age- and gender-matched healthy children with normal 24-hour Holter ECG recordings.

The KocaeliUniversity Ethics Committee received approval under the approval number on December 13th, 2022. (GOKAEK-2022/20.23).

Statistical analysis

The study employed both parametric and non-parametric statistical analyses, utilizing IBM SPSS 20.0 for data processing. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Variables following a normal distribution were expressed as mean ± standard deviation, while those not conforming to normal distribution were reported as median values with minimum and maximum. Categorical data were summarized as frequencies and percentages. Group comparisons of continuous variables were conducted using the Student T-test for normally

distributed data and the Mann-Whitney U test for non-normally distributed data. A two-tailed p-value of <0.05 was considered statistically significant for all analyses, with a 5% threshold set for significance testing.

Results

Study group

A total of 36 patients were evaluated of whom 11 (30.4%) were male and 25 (69.6%) were female. The median age of symptom onset was 14 (5–16) years, the median age at diagnosis was 15 (7–17) years, and the median age at study enrollment was 15 (8–18) years. Three patients (8.3%) had a family history of RP, and the rate of consanguineous marriage among the parents was 13.9% (n=5).

The control group consisted of 30 healthy volunteers including, 20 females (66.7%) and 10 males (33.3%). No significant difference in gender distribution was observed when compared to the patient group (p=0.894). The median age of the control group was 15 (9–18) years with no significant age difference compared to the patient group (p=0.546). Body mass index (BMI) was not different between groups (The patient group: 19.9±4 kg/m^2, The control group: 20.1±3.2, p=0.88).

Clinical and laboratory characteristics of patients

Regarding clinical findings, 13 (36.1%) patients exhibited biphasic symptoms, 11 (30.6%) patients presented with triphasic symptoms, and 12 (33.3%) patients had monophasic symptoms. Discoloration patterns were observed as follows: cyanosis in 33 (91.7%) patients, pallor in 22 (61.1%) patients, and redness in 21 (58.3%) patients. None of the patients developed digital ulcers, though 7 patients (19.4%) reported experiencing sweating. RP was noted in the upper extremities of 34 (94.4%) patients and in the lower extremities of 19 (52.8%) patients. Seventeen (47.2%) patients had symptoms affecting both upper and lower extremities, while 2 (5.6%) patients had signs restricted to the lower extremities and 17 (47.2%) patients had symptoms confined to the upper extremities. Additionally, RP symptoms were reported in the ears of 3 (8.3%) patients and in the nose of 2 (5.6%) patients.

Seasonal triggers revealed that symptoms first appeared in 16 (44.4%) patients during the winter, 15 (41.7%) in the autumn, 2 (5.6%) in the spring, and 3 (8.3%) in the summer. Symptom exacerbation was noted in 31 (86.1%) patients during the winter, while 5 (13.9%) patients experienced symptoms consistently throughout all seasons. Stress was identified as an aggravating factor in 16 (44.4%) patients.

Laboratory investigations revealed a median white blood cell count of 7245/mm^3 (4150–11060), hemoglobin level of 13 g/dL (10.7–16.7), and platelet count of 265,500/mm^3 (177,000–460,000). C-reactive protein (CRP) levels were within normal limits for all patients, although 4 patients had elevated erythrocyte sedimentation rate (ESR) levels. The median was 5.5 mm/h (2–52). Complement levels were within normal ranges for all patients, with a median complement (C) 3 level of 1.1 (0.9–2) and a median C4 level of 0.2 (0.1–0.5). Six (16.6%) patients tested positive for antinuclear antibodies (ANA), though all were negative for anti–double-stranded DNA antibody (anti-dsDNA). No significant antibody positivity was observed in the extractable nuclear antigen (ENA) panel or antiphospholipid antibody tests. None of the patients fulfilled the diagnostic criteria for autoimmune diseases.

Capillaroscopic examination revealed normal findings in 16 (44.4%) patients, whereas 20 (55.6%) exhibited minor abnormalities, such as tortuous capillaries.

In terms of treatment, all patients were initially advised on preventive measures. Medical treatment was administered to 15 patients, with 12 receiving acetylsalicylic acid, 5 of whom were also prescribed pentoxifylline concurrently, and 3 patients were started on nifedipine.

Evaluation of heart rate variability

Holter ECG analysis of the patients with primary RP revealed statistically significant differences in SDNN and SDSD when compared to healthy controls. However, no significant differences were found in RMSSD and HRV index values between the groups (Table I). Minimum-maximum heart rate and average heart rate were not statistically different between the groups. Although HF and LF band powers were lower and LF/HF was higher in patients with RP than in the control group, there was no statistically significant difference between the groups. In the patient group, when comparing 24-hour Holter monitoring parameters based on the presence of capillaroscopy findings, there were no significant differences between patients with capillaroscopic abnormalities and those with normal capillaroscopy findings.

Discussion

This study found that children with primary RP exhibited significantly lower SDNN and SDSD values than healthy controls, suggesting altered autonomic nervous system function. These findings highlight a potential role for HRV analysis in identifying early autonomic dysregulation in pediatric RP.

Raynaud's phenomenon typically manifests with a triphasic pattern of pallor, cyanosis, and redness. However, some patients may present with monophasic or biphasic patterns. Maricq et al.[13] reported that only 1% of patients exhibited a triphasic pattern. Furthermore, Nigrovic et al.[14] found that only 24% of patients with primary RP and 19% with secondary RP displayed the classic triphasic pattern, with nearly half of the cases showing a monophasic pattern. In our study, 13 patients (36.1%) exhibited biphasic symptoms, 11 patients (30.6%) presented with triphasic symptoms, and 12 patients (33.3%) had monophasic symptoms.

Raynaud's phenomenon predominantly affects the distal extremities. In a multicenter cohort study by Falcini et al.[15], 99% of RP patients had upper extremities involvement with 37.2% also displaying symptoms in the lower extremities and face. In our study, RP was observed in the upper extremities of 34 patients (94.4%) and in the lower extremities of 19 patients (52.8%).

Table I. Comparison of 24-hour Holter electrocardiogram monitoring analyses between the patient and control groups

	Patient Group (n=36)	Control Group (n=30)	p value
Heart rate			
Average of heart rate, bpm	78 (68-100)	80 (60-105)	0.914
Minimum heart rate, bpm	57 (50-85)	58 (45-71)	0.239
Maximum heart rate, bpm	139 (96-171)	140 (122-166)	0.870
Time-domain HRV parameters			
SDNN, ms	132.3 (64.3-201.6)	145.3 (99-226.9)	0.04
SDSD, ms	34.9 (14.5-70.5)	41.7 (20.4-75.8)	0.04
RMSSD, ms	49.6 (20.2-90.5)	56.9 (27.3-88.6)	0.09
HRV index	39.9 (15.4-59)	38.4 (25.2-86.2)	0.50
Frequency-domain HRV parameters			
HF band, ms^2	697 (24-39542)	991 (76-5406)	0.488
LF band, ms^2	1134 (57-50954)	1636 (152-8694)	0.475
LF/HF ratio	1.75 (0.58-6.25)	1.42 (0.60-3.02)	0.425

HF: high frequency, HRV: heart rate variability, LF: low frequency, SDNN: standard deviation of all normal-to-normal intervals, SDSD: standard deviation of successive differences between adjacent RR intervals, RMSSD: root mean square of successive differences.

Seventeen patients (47.2%) exhibited symptoms in both the upper and lower extremities. Additionally, RP symptoms were reported in the ears of 3 patients (8.3%) and in the nose of 2 patients (5.6%).

In terms of capillaroscopic findings, limited research exists on pediatric patients with primary RP. Pavlov-Dolijanovic et al.[16] reported that 80% (173 of 191) of patients with primary RP had normal capillaroscopic findings, while 20% showed nonspecific changes. Importantly, no reduction in capillary density or enlargement in capillary size was observed in any of the primary RP cases. In our study, capillaroscopic examination showed normal findings in 16 patients (44.4%), while 20 patients (55.6%) displayed minor abnormalities, including tortuous capillaries. In the patient group, 24-hour Holter ECG monitoring parameters showed no significant differences between those with capillaroscopic abnormalities and those with normal findings. This is expected, as all patients had primary RP, a functional vasospastic condition without structural microvascular damage. Unlike secondary RP, minor capillaroscopic changes in primary RP are unlikely to affect autonomic function.

Previous studies considered normal ESR and ANA levels essential for a primary RF diagnosis. However, in the newly established consensus criteria, the requirement for negative ESR has been fully eliminated, and the negative ANA criterion has been relaxed to allow for either negative or low-titer ANA (e.g., 1:40 by indirect immunofluorescence).[10] In the present study, six patients (16.6%) were positive for low-titer ANA, but none of them had other evidence of systemic connective tissue disease.

Heart rate variability parameters are significantly influenced by average heart rate. Therefore, in the interpretation of HRV in different groups, the average heart rate must be considered.[17] Both groups in our study had similar average heart rate (p=0.914). Although there is still controversy over how sympathetic and vagal stimulus affect the LF band formation, the LF band likely represents the balance between the sympathetic and vagal stimuli. With predominant sympathetic excitation, a decrease in the LF component is observed. It has been shown that during exercise LF band is markedly reduced. The HF or respiratory band is influenced by breathing from 9 to 24 bpm.[4] An overactive local vasoconstrictive response in the digital cutaneous vessels is the fundamental starting point of the primary RP. Defective sympathetic and parasympathetic responses localized in the digital cutaneous vessels might be implicated in the pathogenesis of primary RF. Some adult studies investigating HRV parameters in primary RP found a slight sympathetic predominance with decreased LF band power.[7,18] Although it did not reach statistical significance, our study showed a slight decrease in LF band power in the patient group.

Both sympathetic and parasympathetic activity contribute to SDNN, and it is highly correlated with VLF and LF band power. The SDNN is more accurate when calculated over 24 h than during the shorter periods (5 min). The SDNN is a significant predictor of cardiac risk in adults with cardiac disease when recorded over a 24 h period. SDNN values predict both morbidity and mortality.[3] Based on 24 h monitoring, patients with SDNN values below 50 ms are classified as unhealthy, 50-100 ms have compromised health, and above 100 ms are healthy.[3] Both groups in our study had SDNN values above 100 ms, but patients with primary RP had lower SDNN value than the control group (p=0.04). Low SDNN values in patients with primary RP means that the ability of the sympathetic and parasympathetic stimuli to produce large fluctuations in heart rate (i.e. RR interval) is diminished in this group of patients. Low SDNN values have consistently been demonstrated in adult patients with primary and secondary RP.[19] Furthermore, the SDSD is an index of successive beat-to-beat variability determined largely by autonomic influences on the heart. Therefore, decreased SDSD can be used as a sign of impaired and deranged

sympathetic and parasympathetic activation. Oflaz et al.[9] evaluated autonomic nervous system function in 32 children with primary RP using time-domain HRV analysis, including parameters such as SDNN, SDNNi, SDANN, RMSSD, pNN50, and the triangular index. These results were compared with those of 30 healthy controls. The study found that children with RP exhibited significantly higher average heart rates and significantly lower values in several HRV measures (SDNN, SDNNi, SDANN, and triangular index) compared to the control group. The authors concluded that these findings indicate a shift toward sympathetic dominance and reduced autonomic modulation in children with PRP. Both our study and the study by Oflaz et al.[9] were conducted in pediatric populations; however, there are notable differences in the reported HRV parameters. While we observed significant alterations in SDNN and SDSD in primary RP patients compared to controls, we did not find significant differences in RMSSD or triangular index. Although the age and gender distribution and the measurement methodology were similar between these two studies, differences in disease duration or severity may account for the discrepancy in findings. While the study by Oflaz et al.[9] does not provide detailed information on these parameters, our patient group had a relatively short disease duration and exhibited mild clinical symptoms. This may explain the more limited alterations observed in HRV parameters in our study.

The lack of significant changes in RMSSD or frequency-domain parameters may suggest that mild autonomic dysfunction in pediatric RP may selectively affect short-term time-domain parameters. Given the predominant parasympathetic mediation of SDSD and RMSSD, the selective decrease in SDSD might reflect subtle alterations in vagal tone, which is not sufficient to affect all indices. These findings may indicate early or partial autonomic dysregulation in pediatric RP, in contrast to more pronounced findings reported in adult populations.

A recent study exploring autonomic-targeted interventions, such as neurofascial vascular training, demonstrated symptomatic improvement in patients with primary RP through autonomic nervous system stimulation.[20] A detailed evaluation of HRV in children with primary RP may assist in identifying early functional alterations in the autonomic nervous system. This could be essential for developing non-pharmacological approaches that target autonomic modulation in this population.

The main limitation of our study is its single-center design, which limits the generalizability of the findings to a specific population rather than the broader population. Due to the rarity of primary RP in childhood, no formal power analysis was performed; however, including all eligible patients provides valuable preliminary data in this underexplored area. The relatively small sample size may reduce the ability to detect more subtle differences in HRV parameters and should be considered when interpreting borderline or non-significant findings. Additionally, the cross-sectional design prevents longitudinal follow-up and further evaluation of patient outcomes over time. However, our study has some strengths that should be emphasized. Notably, it included frequency-domain HRV analysis and nailfold capillaroscopy, which allowed for a more comprehensive evaluation of both autonomic function and microvascular involvement. Although the differences between primary RP patients and healthy controls were not statistically significant in these parameters, the use of these advanced methodologies enhances the depth and quality of the assessment.

In conclusion, pediatric patients with primary RP exhibited significant alterations in autonomic nervous system activity compared to healthy controls, particularly reflected by reduced SDNN and SDSD values. These changes suggest subclinical autonomic dysregulation, which may have implications for early identification

or monitoring of affected children. Although the specific balance between sympathetic and parasympathetic influence remains unclear, identifying the predominant autonomic pathway could guide future diagnostic or therapeutic strategies. The lack of significant changes in RMSSD or frequency-domain parameters may indicate that dysregulation is mild or selectively affects short-term time-domain indices. Given the rarity of pediatric RP, our findings provide valuable preliminary insight into autonomic function in this population. Future multicenter, longitudinal studies with larger sample sizes and standardized autonomic assessments are essential to confirm these results, explore their clinical relevance, and determine whether HRV markers could support risk stratification or intervention planning.

Ethical approval

The study was approved by Kocaeli University Ethics Committee (date: December 13th, 2022, number: E-80418770-020-334984).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SU, GA, HES; data collection: SU, YEB, GA, EZB, NŞ, HES; analysis and interpretation of results: SU, YEB, GA, EZB, NŞ, HES; draft manuscript preparation: SU, YEB, GA, EZB, NŞ, HES. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

The danger of magnet attraction: an 11-year cohort of pediatric intestinal complications due to magnet ingestion

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ABSTRACT

Introduction. In recent years, there has been a significant rise in the number of pediatric cases involving multiple magnet ingestion, resulting in increased incidence and morbidity of injuries. When a metal object and magnet are ingested, either single or multiple, they can cause serious complications such as intestinal obstruction, ischemia, necrosis, fistula, perforation, and even death. This study aims to detail the complications and treatment approaches associated with magnet ingestion in children.

Materials and Methods. In our study, we conducted a retrospective analysis of all cases involving the ingestion of a magnet along with a second metal object at two training and research hospitals in our province, which admit pediatric patients, between the years of 2013 and 2023.

Results. A total of 42 patients had a history of magnet ingestion, with the number of ingested magnets ranging from 1 to 41. The median magnet size was 11 mm (range: 5.5-17.5 mm) and the median time to presentation was 24 hours (range: 3-48 hours). Thirteen patients (30.9%) required either endoscopic or surgical intervention to extract the magnets or address complications. Endoscopy was performed on eight patients, while surgical intervention was required for five patients. Among those who underwent surgery, four experienced complications, including intestinal perforation, ileoileal fistula, and internal herniation. Notably, no fatalities occurred following intervention. There was no statistically significant difference in age or magnet size between the interventional and non-interventional groups. However, the length of hospital stay was significantly longer in the interventional group compared to the non-interventional group ($P<0.05$).

Conclusions. The ingestion of magnets by children can result in serious complications, such as intestinal fistula, perforation, and volvulus. These conditions pose significant health risks and may require endoscopic or surgical intervention.

Key words: magnet, foreign body, children, endoscopy, surgery.

Foreign body ingestion poses a significant health concern due to its prevalence, particularly among pediatric patients. Annually, approximately 100,000 incidents of foreign body ingestion are documented in the United States, with 80% of these cases involving children aged between 6 months to 3 years.[1-3] While many cases of foreign body ingestion result in uncomplicated transit through the gastrointestinal tract, the ingestion of button batteries and multiple magnets has been associated with complications, including potentially life-threatening conditions.[4,5] In fact, fatalities have been reported following the ingestion of multiple magnets.[6] The tendency

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of toddlers to explore their environment orally leads to with increased instances of ingestion, while adolescents often ingest foreign bodies as a result of improper tongue and lip piercings. Less than 1% of foreign bodies in the stomach require surgery or cause complications, whereas 10–20% require endoscopic intervention.[7,8]

The notable rise in pediatric injuries related to magnets over the past two decades is a significant concern.[4,9-12] This increase coincides with the growing sales of small, potent neodymium-iron-boron magnets, which are frequently sold as part of toy sets. This study aims to increase awareness of the potentially life-threatening consequences associated with the ingestion of multiple magnets by children, which may include obstruction, fistula formation, and perforation. This article serves as a reminder of the dangers associated with magnet ingestion and aims to highlight this issue.

Materials and Methods

This study was a retrospective, two-center analysis. The study was approved by the Kocaeli University Faculty of Medicine Clinical Research Ethics Committee (reference number GOKAEK-2024/01.13). A comprehensive collection and analysis of all cases of magnet ingestion admitted to Kocaeli University Hospital and Kocaeli City Hospital from January 2013 to December 2023 was conducted. The collected data encompassed demographic information, including age and sex, as well as medical history, symptoms, physical examination findings, diagnostic methods, treatments, post-treatment follow-up, and details regarding the ingestion of magnets, such as their quantity, size, localization, and the endoscopic and surgical interventions performed. Morbidity was defined as any injury directly attributable to magnets, encompassing perforation, fistula formation, obstruction, bleeding, infection, volvulus, and/or intestinal herniation. Patients who ingested magnets were categorized as follows: those who passed them spontaneously through the gastrointestinal tract were classified as mild; those requiring intervention were classified as moderate; and those presenting with complications were classified as severe. Patients were categorized into groups based on the ingestion of a single magnet or multiple magnets. Because patients who ingested multiple magnets exhibited similar clinical behavior to those who ingested a magnet alongside a metallic object, the latter were included in the multiple magnet ingestion group. Furthermore, distinct groups were established for patients who required medical intervention and those who did not. Statistical analysis was conducted using the chi-squared test to compare these groups. Categorical variables are presented as frequencies (percentages), whereas numerical variables are reported as the median with the interquartile range (25th-75th percentile, Q1-Q3). Statistical significance was determined by a p-value of less than 0.05, consistent with established statistical conventions.

Results

During the period spanning 2013 to 2023, the total number of emergency department visits for pediatric patients was 1,110,812. Of these, 2,078 patients (0.18%) had swallowed foreign bodies, and 42 patients (2.02%) had ingested magnets. The study sample comprised 42.8% male and 57.1% female patients, with 47.6% of the participants being under the age of four years. Statistical analysis revealed no significant difference between the groups concerning sex ($P>0.05$).

The median age, sex, magnet size and number, localization, duration of presentation, duration of hospital stay, and comparisons between patients who underwent intervention and those who did not, as well as between those who ingested a single magnet and those who ingested multiple magnets, are presented in Table I. The study revealed that severe consequences resulted in 9.5% of the cases. The outcomes for the 13 patients who underwent the intervention are outlined in Table II.

Table I. Comparison of patients undergoing and not undergoing intervention, and single and multiple magnet ingestion groups

	Total	Patients undergoing intervention	Patients not undergoing intervention	P	Single magnet	Multiple magnets, or magnet with metals	P
Number, n (%)	42	13 (30.9%)	29 (69.1%)		22 (52.3%)	20 (47.7%)	
Sex (M/F), n	42	4/9	14/15	0.72	11/11	7/13	0.70
Age, yr, median (Q1-Q3)	3.5 (2-6)	2.0 (1-4.5)	4.0 (2-7)	0.67	3.5 (2-6)	4.0 (2-5.7)	0.71
Time to application, hr, median (Q1-Q3)	24 (3 - 48)	48 (13.5-96)	24 (3-24)	0.01	24 (3.7-30)	24 (3–48)	0.47
Magnet size, mm, median (Q1-Q3)	10 (5.5-15.2)	5.5 (5.5-10.2)	10.5 (5.5-15.5)	0.049	10.5 (7.7 – 20.7)	5.5 (5.5–11.7)	0.02
Number of magnets, median (Q1-Q3)	1 (1-2)	2 (2-14)	1 (1-2)	0.002	1 (1-1)	2 (2-6)	<0.001
Hospitalization period, hours, median (Q1-Q3)	12 (0-48)	72 (24-132)	0 (0-24)	<0.001	0 (0-0)	36 (24-90)	<0.001
Location, n (%)							
Upper GI tract	10 (23.8 %)	7 (70.0 %)	3 (30.0 %)		1 (10 %)	9 (90 %)	
Esophagus	1 (2.3 %)	1 (100 %)	0 (0 %)		1 (100 %)	0 (0 %)	
Stomach / duodenum	9 (21.5 %)	6 (66.6 %)	3 (33.3 %)		0 (0 %)	9 (100 %)	
Lower GI tract	32 (76.2 %)	6 (18.7 %)	26 (81.3 %)		21 (65.6 %)	11 (34.4 %)	
Ileum	18 (42.6 %)	3 (16.6 %)	15 (83.3 %)		13 (72.2 %)	5 (27.8 %)	
Cecum	7 (16.7 %)	0 (0 %)	7 (100 %)		2 (28.6 %)	5 (71.4 %)	
Colon	7 (16.7 %)	3 (42.8 %)	4 (57.2 %)		6 (85.7 %)	1 (14.3 %)	

GI: gastrointestinal

Table II. Characteristics of patients undergoing intervention.

Patient no.	Age	Sex	Number of magnets	Magnet size, mm	Ingestion time, hour	Location	Symptom	Damage	Intervention
1	12 mo	M	7	5.5	96	Duodenum and colon	Abdominal pain and diarrhea	Perforation and fistula	Primary repair + appendectomy
2	24 mo	F	27	5.5	48	Stomach and jejunum	Abdominal pain	Perforation, fistula and peritonitis	Resection anastomosis
3	16 mo	F	1	3.6	336	Colon	-	-	Appendectomy
4	3 yr	M	2	30	120	Ileum	Abdominal pain and fever	Perforation and fistula	Resection anastomosis
5	10 yr	M	1*	5.5	240	Ileum	Abdominal pain and fever	Perforation, fistula and internal herniation	Resection anastomosis
6	4 yr	F	1	19.5	1	Esophagus	-	-	Endoscopy
7	20 mo	M	2	7	96	Stomach and jejunum	-	-	Endoscopy
8	18 mo	F	41	5.5	48	Stomach and jejunum	Abdominal pain	-	Endoscopy
9	5 yr	F	2	8.5	72	Cecum	-	-	Colonoscopy
10	12 mo	F	2	12	4	Stomach	-		Endoscopy
11	5 yr	F	2	5.5	2	Stomach	-	-	Endoscopy
12	3 yr	F	2	5.5	3	Stomach	-	-	Endoscopy
13	13 mo	F	3	5.5	2	Stomach	-	-	Endoscopy

* 1 magnet + 28 staple wires

Among the children who ingested magnets, 22 (52.3%) ingested a solitary magnet, while 20 (47.7%) ingested multiple magnets. The number of magnets swallowed ranged from 1 to 41.

Median magnet size was 11 mm (Q1-Q3: 5.5-17.5 mm). A statistically significant difference was observed between the single and multiple magnet groups, as well as between the interventional group and that did not ($P < 0.05$). Median hospital stay was 24 hours (Q1-Q3: 3-48 hr). A statistically significant difference was identified between the single- and multiple-magnet groups, as well as between the intervention and non-intervention groups ($P < 0.05$) (Table I).

Most patients were asymptomatic (88.2%), while one patient experienced diarrhea (2.3%), and four patients had abdominal pain, restlessness, and fever (9.5%). The ingestion of magnets in asymptomatic patients was confirmed through the patient's statement, family observation, and incidental plain radiography. Indications for intervention and medical follow-up in the interventional and non- interventional groups are given in Fig. 1.

Among the patients who underwent endoscopic procedures, seven received esophagogastric interventions, while one underwent a colonoscopy. In one case, a single magnet located in the initial esophageal stricture was extracted via esophagoscopy. In six patients, magnets ranging from 1 to 41 were identified in the stomach, all of which were successfully removed through esophagogastroscopy (Fig. 2A). In one patient, a magnet was located in the stomach, with another found in the jejunum, adhering to the stomach magnet (Fig. 2B). The portion of the magnet attached to the jejunum, which was removed from the stomach by gastroscopy, passed through the digestive tract spontaneously the following day.

Five patients required surgery. Each patient underwent laparotomy; with the exception of one patient whose surgery was incidental. The indications for surgery included peritoneal signs, intestinal perforation, or intestinal obstruction. A 12-month-old male patient who ingested seven magnets presented with two perforations in her duodenum (Fig. 2C). A two-year-old female patient who ingested twenty-seven magnets presented with two

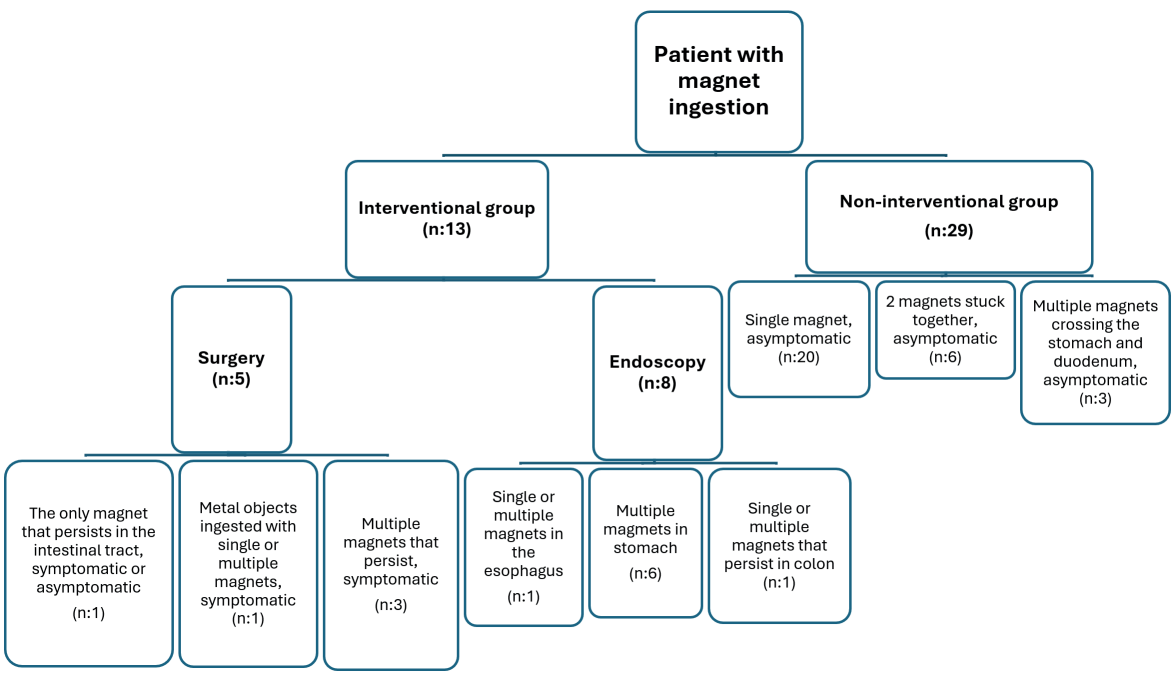

Fig. 1. Treatment algorithm in the groups with and without interventional procedures.

perforations in the stomach and jejunum, which were surgically repaired using primary closure (Fig. 2D). A ten-year-old male patient who ingested 28 staples after swallowing a magnet developed an ileoileal fistula and internal herniation (Fig. 3A1-A3). A three-year-old male patient who ingested two magnets presented with an ileoileal fistula and a perforation (Fig. 3B1-B2). Primary repair was performed in three patients and resection in one patient. In one patient, a magnet found incidentally in the cecum was removed from the appendix and an appendectomy was performed. There were no postoperative or postprocedural complications, and all patients were discharged after recovery. No deaths occurred during the study period.

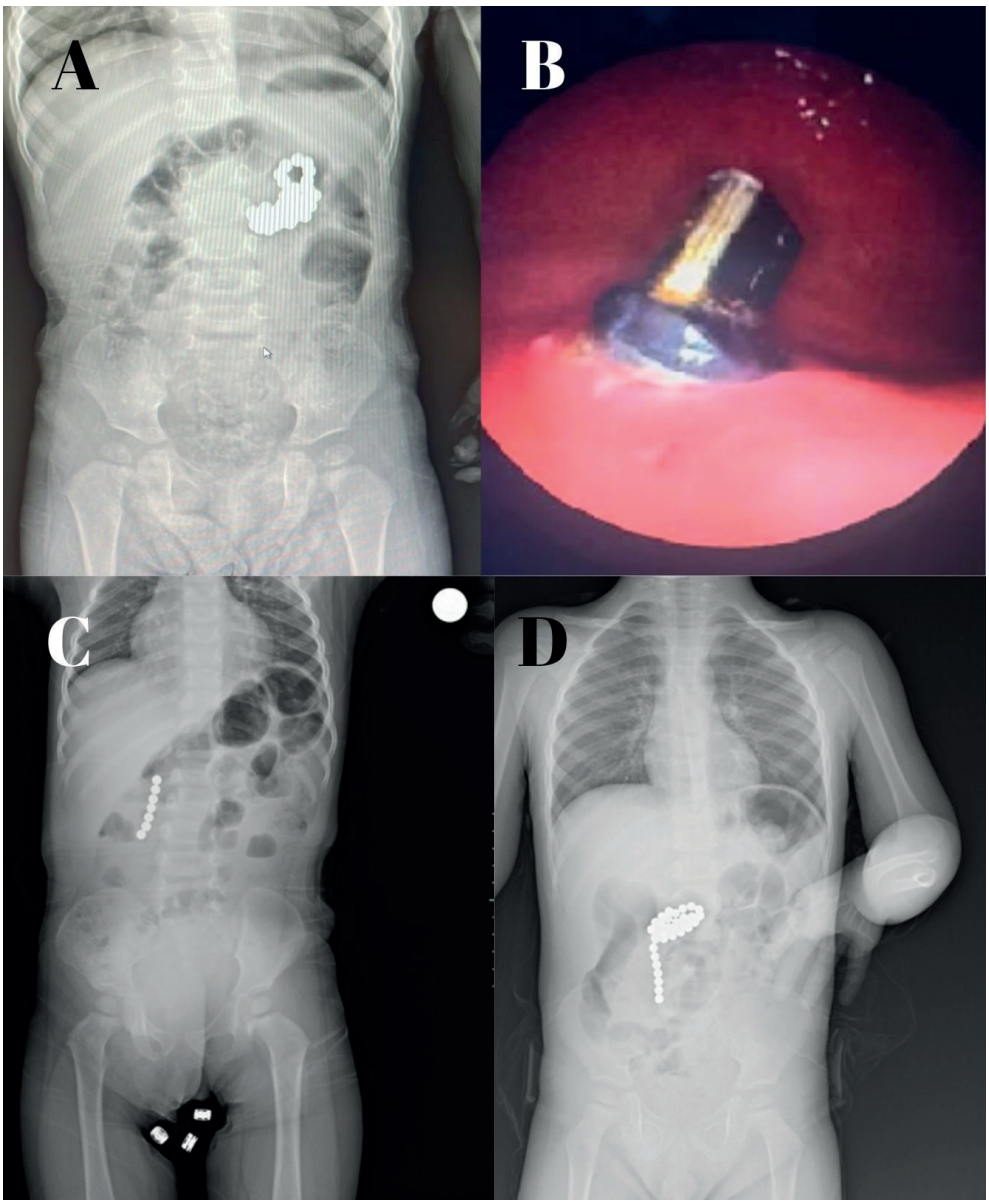

Fig. 2. A) Radiographic image of magnets in the stomach of an 18-month-old female, who ingested 41 magnets. **B)** Endoscopic image of the stomach in a 20-month-old male. **C**) Radiographic image of magnets in the duodenum and colon (hepatic flexure) of a 12-month-old male, who ingested seven magnets. **D)** Radiographic image of magnets in the stomach and jejunum of a 24-month-old female, who ingested 27 magnets.

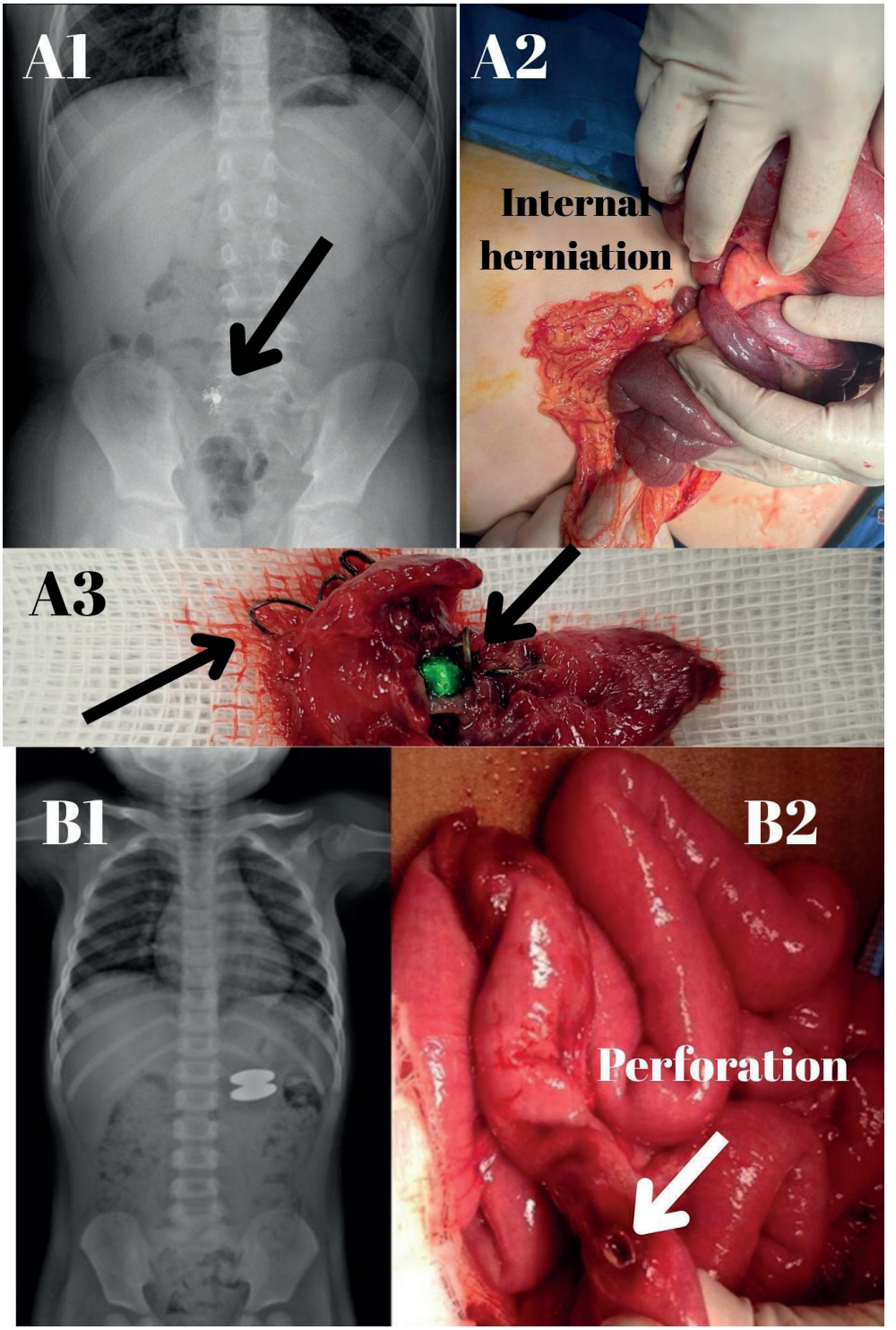

Fig. 3. A) Depictions of a 10-year-old male, who ingested a single magnet along with multiple metallic objects. **A1**: Radiographic image illustrating numerous metal staples adhering to the magnet. **A2:** Intraoperative photograph depicting an ileoileal fistula and internal herniation induced by the magnet. **A3:** Magnet and metallic objects affixed to the resected intestinal segment. **B)** Images of a 3-year-old male, who ingested two magnets. **B1:** Radiographic image of the magnets causing ileal fistula and perforation. **B2:** Intraoperative photograph of ileal fistula and perforation in the ileum.

Discussion

The ingestion of foreign bodies represents a significant concern across all age groups, with evidence indicating that 75% of such cases involve patients under the age of four.[13,14] In our study, we observed that only 20 (47.6%) patients were under the age of 4 years, and therefore, pediatric patients of any age should be considered in the differential diagnosis.

Rare earth metals, such as neodymium-iron-boron, are extensively employed in industry due to their exceptional power-to-size ratio, rendering them ideal for use in high-power magnets. These magnets exhibit a confining force that is 5-30 times stronger than that of conventional magnets.[4,15,16] Specifically, the magnetic attraction force between 5 mm balls is approximately half a kilogram. This strong magnetic attraction between the intestines results in compression at pressure points, which cuts off blood flow and causes rapid damage to the wall of the intestine. This damage can cause ischemia, necrosis, and perforation.

Since the groundbreaking report by McCormick et al on magnet ingestion, there has been a proliferation of published material detailing the clinical manifestations, complications, and management of this phenomenon.[4,11,12,17] In a single-center study conducted in China, the first instance of magnet ingestion was registered in 2015. By 2019, magnets accounted for a staggering 80% of all young children's foreign body ingestion cases, and an astonishing 76.8% of these cases required surgical intervention.[18] The literature is replete with evidence suggesting that the ingestion of multiple magnets significantly heightens the likelihood of hospitalization and surgical intervention, as well as the risk of developing complications.[14,16,19] There have been reports of a high incidence of magnet ingestion in older children who use magnets to mimic facial piercings such as those in the lips, tongue, and nostrils.[9] One such adolescent patient ingested two magnets while attempting to pierce his lip.

In the initial stages of magnet ingestion, children typically exhibit no symptoms. While the majority of children who ingested a single magnet remained asymptomatic, those who ingested multiple magnets exhibited symptoms of gastrointestinal distress, including fever, vomiting, diarrhea, and abdominal pain. Nausea/vomiting and abdominal pain are the most common non-specific symptoms, with their intensity and duration influenced by the quantity, strength, and location of the ingested magnets, as well as the time elapsed between ingestion and symptom manifestation.[20] Following the confirmation of magnet ingestion through radiographic examination, the subsequent step involved assessing whether a single magnet, multiple magnets, or other metallic objects had been ingested. The expulsion of a foreign body, such as a coin or a small round object, can occur naturally and can be managed as an outpatient with daily X-ray examinations. In our study, laxatives were employed as a conservative treatment for 69% of the patients, and this method generally proved to be effective in most cases.

The likelihood of sustaining significant gastrointestinal injuries, such as perforation and obstruction, increases with the ingestion of an increased number of magnets. In instances where multiple magnets or a single magnet is ingested with additional metallic objects, it is advisable to conduct serial radiographic examinations at 4 to 6 hour intervals. In this study, all patients underwent plain radiographs (anteroposterior and lateral views) of the neck, chest, and abdomen, as plain X-rays have a high diagnostic yield. Computerized tomography scans were performed in more complex cases to detect inflammation and small perforations. If it is not possible to distinguish between single and multiple magnet ingestions with certainty, inpatient treatment following the multiple-magnet regimen should be initiated. When there are several magnets, one magnet, or a secondary metallic foreign body in the stomach, endoscopic intervention is necessary. Multiple magnets or a single magnet with a secondary

metallic foreign body in the stomach should be surgically removed, especially if there are symptoms or obstructive findings visible on abdominal radiographs. Nonsurgical inpatient therapy, including a bowel regimen and serial radiographic surveillance, is recommended for asymptomatic patients with several magnets or one magnet plus another metallic foreign body in the stomach. Endoscopic or surgical intervention may be considered if there is no progression of the magnet after 48 hours. Modifications to the algorithm proposed by Sola et al. in 2018 were made by Mostafa et al. in 2021.[11,21]

It is imperative to acknowledge that the ingestion of metal objects, aside from a solitary magnet, can pose substantial risks comparable to those associated with the ingestion of multiple magnets. One of our cases involved a patient who ingested 28 staples along with a magnet, resulting in a fistula and perforation in the intestines, a scenario similar to the ingestion of multiple magnets (Fig. 3A1-A3). The management of magnet ingestion in pediatric patients should be tailored to the specific characteristics of the magnet, the duration since ingestion, and the patient's clinical condition. Prompt endoscopic or surgical intervention can prevent severe complications. The diagnostic clues and treatment algorithms for clinicians dealing with pediatric patients are presented in Fig. 4.

Patients who ingest multiple magnets should be closely monitored in a controlled clinical setting due to their potential for complications, even if they do not exhibit clinical symptoms.

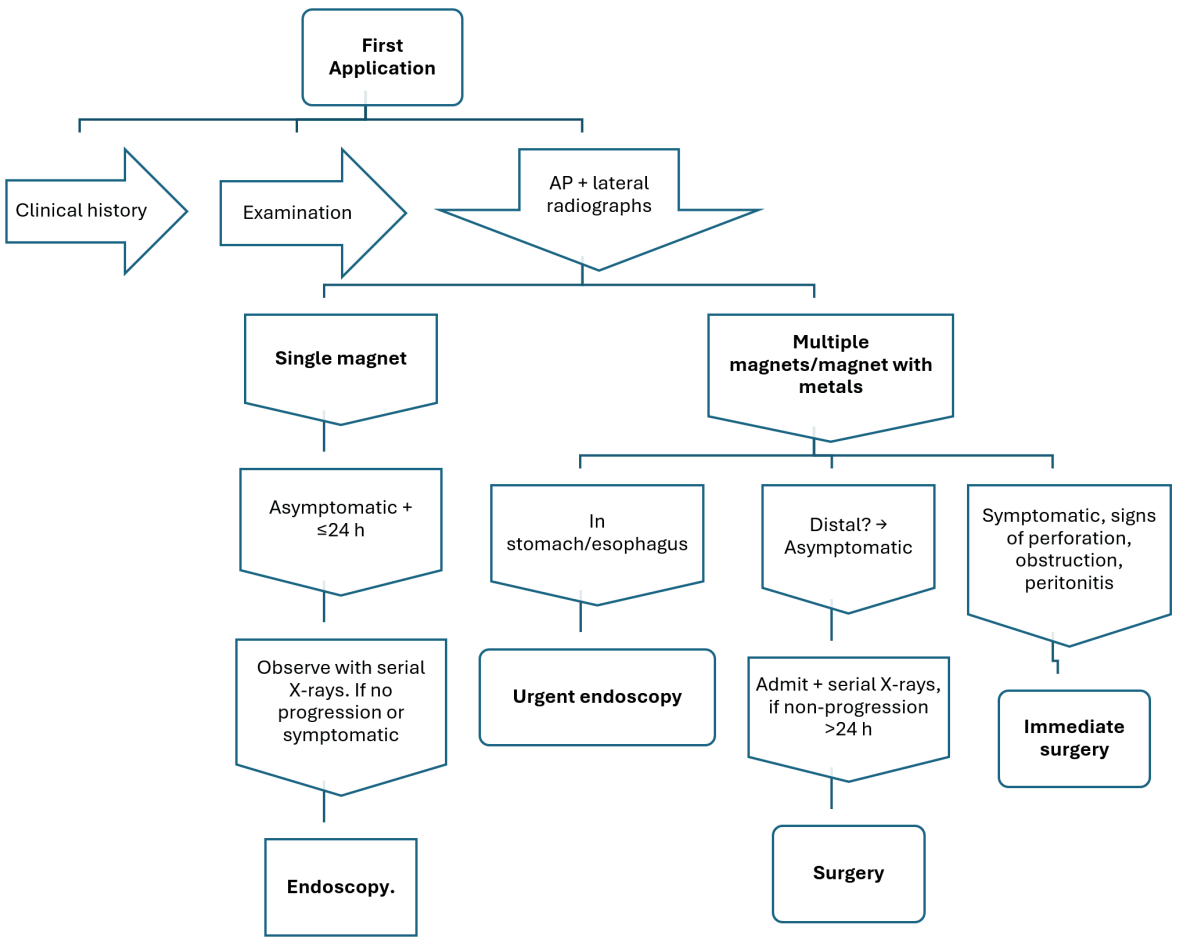

Fig. 4. Diagnostic clues and treatment algorithms for single and multiple magnets.

Magnets with smaller diameters pose a greater risk of complications and should be approached with caution. When more than one magnet is ingested, complications can arise in 11-50% of cases, including intestinal perforation, which may require surgical intervention.[22,23] Of the 13 patients who underwent surgery in our series, 30.7% had bowel perforation at baseline, and the most common site of perforation was the small intestine.[19] In our study, the intervention rate in the multiple magnet group also showed a significant difference (P<0.05). Our hospital admission rate was 36.1%, aligning with the reported range of 27-68%.[24,25] Notably, in our study, the duration of hospitalization for patients who ingested multiple magnets showed a statistically significant difference between those who required intervention and those who did not (P<0.05).

If magnet fragments are in the esophagus, stomach, duodenum, or colon, prompt removal by endoscopy within the first 24 hours is crucial. Delaying medical intervention allows magnetic beads to travel from the stomach and eventually reach the intestine, thereby increasing the risk of complications. The success rate of endoscopic removal of magnets is reported to be between 66% and 89% in most cases.[11,21] In our series, 62.5% of the patients who underwent endoscopy has magnets successfully removed within the first 24 hours, while the others were removed after 24 hours.[20,26] The efficacy of endoscopic removal is significantly influenced by the positioning of the magnets, the duration before the endoscopic procedure, and the expertise of the medical professionals involved. Magnets that have penetrated the mucosa are particularly difficult to remove. It is important to note that magnets may adhere to the device or be displaced during the endoscopy process. In one of our cases, we were unable to detect the magnets in the cecum during colonoscopy, but we were able to locate them after they had adhered to the device and were identified after removing it from the rectum. In instances where endoscopic removal is unfeasible or complications arise during the procedure, surgical intervention may become necessary. Depending on the available resources and the expertise of the medical team, surgical removal can be performed using either laparotomy or laparoscopy. Nonetheless, the laparoscopic extraction of magnets presents challenges due to their tendency to adhere to surgical instruments.

Due to the significant health hazards associated with strong magnets, it is imperative to prevent young children from ingesting these objects. The small and shiny nature of magnets can attract the curiosity of children. In order to reduce these risks, parents and caregivers must make sure that magnetic toys adhere to strict safety regulations and keep all magnets, especially those made of rare earth elements like neodymium, in places that are out of children's reach. Public awareness initiatives, child-resistant packaging, and clear warning labels on products that contain magnets are essential. Ultimately, prevention can be achieved through responsible product design and vigilant adult supervision.

One possible limitation of this study is that the data were collected retrospectively, which inherently carries the risk of bias or missing information. Data may not have been fully transferred due to changes in the hospital database system. Thus, we may have missed cases of ingested foreign bodies and not reached the true incidence rates. However, we would like to highlight the strengths of our study. This study presents comprehensive information about why magnetic attraction continues to be a danger to children and how it poses risks. In addition, it offers physicians a detailed algorithm for both diagnosis and treatment.

Conclusions

The incidence of severe injuries resulting from magnet ingestion in children is on the rise. In instances where a child presents to the pediatric emergency department with suspected magnet ingestion, it is crucial to achieve a prompt and accurate diagnosis to prevent serious complications. Early detection is essential, as

delays may lead to severe outcomes, including gastrointestinal perforation, volvulus, and fistula formation, particularly when multiple magnets are involved. These high-risk scenarios can often be mitigated through timely imaging and intervention. Therefore, it is imperative that magnet ingestion is neither overlooked nor misdiagnosed, as early recognition is vital for preventing life-threatening sequelae and ensuring optimal patient outcomes.

Ethical approval

The study was approved by Kocaeli University Faculty of Medicine (date: January 18, 2024, number: GOKAEK-2024/01.13).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: OV, MAA; data collection: NGS, SM; analysis and interpretation of results: OV, MAA, NGS, SM; draft manuscript preparation: GEY. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Immunodeficiency and hemolytic uremic syndrome: a case report

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ABSTRACT

Background. Ataxia-telangiectasia (A-T) is a rare, autosomal recessive disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, and immunodeficiency, predisposing affected individuals to recurrent and severe infections. This case report presents a rare example of Shiga toxin-producing *Escherichia coli* (STEC)-associated hemolytic uremic syndrome (HUS) in a 12-year-old boy with a known diagnosis of A-T. To our knowledge, this is the first reported case of STEC-HUS in a patient with A-T.

Case Presentation. The patient presented with vomiting and bloody diarhea Investigations revealed hemolytic anemia, thrombocytopenia, and acute kidney injury. The patient received intravenous immunoglobulin, albumin, and continuous renal replacement therapy and recovered.

Conclusion. This case highlights the increased susceptibility of individuals with A-T to infections and the potential for life-threatening complications, such as HUS. The coexistence of A-T and STEC-HUS presentes unique challenges in diagnosis and management. Early recognition and targeted treatment led to a successful recovery and underscored the importance of close follow-up in immunodeficient patients.

Key words: ataxia telangiectasia, hemolytic uremic syndrome, Shiga toxin, immunodeficiency.

Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy characterized by microangiopathic hemolytic anemia, thrombocytopenia, and renal failure. While it is often caused by Shiga toxin-producing *Escherichia coli* (STEC), it can also arise from inherited mutations in complement-regulating proteins and endothelial damage disorders. The activation of the complement system leads to vascular wall thickening and the formation of fibrin- and thrombocyte-rich thrombi in the microcirculation, resulting in impaired end-organ function, primarily affecting the kidneys or brain. Schistocyte formation and hemolysis are triggered by increased shear stress in partially obstructed vessels, while thrombocytopenia results from platelet consumption and immune-mediated destruction.[1]

Ataxia telangiectasia (A-T) is an autosomal recessive disorder marked by cerebellar degeneration, telangiectasia, immunodeficiency, cancer susceptibility, and radiation sensitivity.[2] A-T is considered a genome instability syndrome, with a global prevalence of approximately 1 in 40,000 to 1 in 100,000 live births.[3] Patients with A-T commonly present with immunological abnormalities, including deficiencies in immunoglobulins and antibodies, as well as lymphopenia. These immunodeficiencies lead to an increased risk of infections and malignancies, particularly of lymphoid origin, alongside frequent issues

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related to pulmonary function, feeding, swallowing, and nutrition.[4]

Approximately two-thirds of patients with A-T exhibit significant immune system abnormalities, including low levels of immunoglobulins (IgG, IgA, IgM, or IgG subclasses), impaired antibody responses to infections and vaccinations, and T-lymphocyte lymphopenia. All these factors predispose individuals to severe infections and complications.[4,5]

This case presentation aims to highlight the clinical features and complications of HUS associated with STEC infection in a patient with underlying A-T, emphasizing the importance of recognizing and managing infections in this disorder.

Case Report

A 12-year-old male, weighing 28 kg, with a known diagnosis of A-T, presented to an tertiary care center with complaints of vomiting and bloody diarrhea for 3-day history. The patient was receiving monthly intravenous immunoglobulin (IVIG) therapy at a dose of 0.4 g/kg due to IgA and IgG deficiency. Prior to the onset of HUS, the patient's baseline serum creatinine level was 0.5 mg/dL, which was within normal limits.

The patient's vital signs at presentation were as follows: SpO_2 93% on room air (98% with 6 L/min oxygen via mask), heart rate 140 bpm, and blood pressure 140/90 mmHg (95th percentile: 120/80 mmHg). Physical examination revealed crepitant rales, a tense abdomen, pretibial edema, and ascites.

Laboratory tests revealed elevated uric acid and lactate dehydrogenase (LDH) levels and decreased complement 3 (C3; 0.81 g/L) and C4 (0.06 g/L) levels. Haptoglobin was notably low at 0.02 g/L, and peripheral blood smear confirmed microangiopathic hemolytic anemia with the presence of schistocytes. Additionally, the patient exhibited significant proteinuria (3+) with a urinary protein-to-creatinine ratio of 3 mg/mg (normal range: <0.2 mg/mg). Renal ultrasound demonstrated increased parenchymal echogenicity in both kidneys, consistent with grade 1 changes. Shiga toxin was tested in the stool sample, confirming STEC infection, and the patient tested positive for STEC with *stx1* and *stx2* genes detected by reverse transcription polymerase chain reaction. Other exclusion tests, including ADAMTS-13 activity, homocysteine, and vitamin B_{12} levels, were all within normal limits (Table I).

Based on the clinical presentation of hemolysis, acute renal failure, and thrombocytopenia, the patient was diagnosed with HUS secondary to STEC infection (STEC-HUS).

The patient was transferred to our pediatric intensive care unit for further management. Upon admission, the patient's immunoglobulin levels were found to be IgG 5.4 g/L (normal range: 7.41-15.13 g/L) and IgA 0.04 g/L (normal range: 0.57-3.5). He was administered IVIG at a dose of 1 g/kg and intravenous albumin at 1 g/kg, followed by diuretic therapy. Despite initial treatment, the patient showed persistent oliguria (urine output: 0.4 mL/kg/hr), necessitating continuous renal replacement therapy (CRRT). Prior to catheter insertion, erythrocyte suspension and platelet suspension transfusions were provided due to anemia (hemoglobin < 6 g/dL) and active bleeding associated with severe thrombocytopenia (< 30,000 /μL).

After 72 hours of CRRT, the patient's urine output improved to an adequate level, allowing for the discontinuation of dialysis. By the ninth day, the patient was fully enterally fed, remained stable on room air with normal vital signs, and had adequate urine and stool output. He was subsequently transferred to the clinic for continued follow-up related to his A-T. At discharge, laboratory tests revealed a hemoglobin level of 10 g/dL, proteinuria of 0.4 mg/mg, urea of 24 mg/dL, and creatinine of 0.6 mg/dL.

Table I. Laboratory values at admission, peak, and discharge with normal ranges in brackets.

Parameter, normal range	Admission	Peak value	On 9th day of admission
Renal function			
Urea (mg/dL, 11-36)	176	198	64
Creatinine (mg/dL, 0.24-0.41)	2.57	4.06	1.1
GFR (mL/min/1.73m^2, >60)	22	46	
Uric acid (mg/dL, 3.4-7)	6.6	7.5	2.5
Phosphate (mg/dL, 2.9-5.1)	5.5	5.8	4.5
Calcium (mg/dL, 9.4-10.2)	7.8	7.5	8.9
Liver function & inflammation			
AST (U/L, <40)	78	80	38
ALT (U/L, <40)	65	70	24
GGT (U/L, <60)	13	50	15
LDH (U/L, 120-300)	1571	1743	1070
CRP (mg/L, <5)	172	172	8.5
Procalcitonin (μg/L, <0.5)	3.6	3.6	0.5
Albumin (g/dL, 3.5-5.5) lowest	2.35	2.2	3.0
Hematologic parameters			
WBC (x10^3/μL, 4-16)	19.4	19.4	6.6
Neutrophils (x10^3/μL, 1.7-5.3)	15.8	15.8	2.9
Lymphocytes (x10^3/μL, 0.8-7.1)	1.0	0.6	0.9
Hemoglobin (g/dL, 10-12.5)	8.7	5.8	5.9
Platelets (x10^3/μL, 150-400)	39	39	66
Haptoglobin (g/L, 0.3-2)	0.02		
Homocysteine (μmol/L, <12)	8.6		
Vitamin B_{12} (pg/mL, >400)	394		
Immunologic parameters			
Complement 3 (g/L, 0.9-1.8)	0.81		
Complement 4 (g/L, 0.1-0.4)	0.06		
IgA (g/L, 0.57-3.5)	0.04		
IgG (g/L, 7.41-15.13)	5.14		
IgM (g/L, 0.35-2.39)	2.07		
Total IgE (g/L, <200)	2.06		

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, GFR: glomerular filtration rate, GGT: gamma-glutamyl transferase, Ig: immunoglobulin, LDH: lactate dehydrogenase, WBC: white blood cells.

During the first week, the patient's clinical condition stabilized. Two weeks later, thrombocytopenia, hypertension, and anemia had resolved, and at the two-month follow-up, no proteinuria was detected. At the two-month follow-up, hemoglobin was 12 g/dL, urinary protein was 0.10 mg/mg creatinine, urea was 22 mg/dL, and creatinine was 0.5 mg/dL, all within normal limits for age and sex, and no proteinuria was detected. The pediatric nephrology outpatient clinic recommended follow-up every 3 months to 1 year to monitor the patient's renal function and overall progress. The patient's clinical and laboratory data for the third month following discharge are unavailable.

Table II. Review of cases involving immunodeficiencies complicated by HUS, including clinical, and treatment data.

Case #	Reference	Age, sex	Underlying immunodeficiency	Hematologic findings	Renal findings	Neurological findings	Other clinical findings	Initial treatment	Ongoing treatment	Outcome
1	Nikolajeva et al. [8]	2 mo, male	ADA deficiency	Red cell fragmentation, thrombocytopenia	AKI with nephritic syndrome, required plasmapheresis and peritoneal dialysis	Lethargy, irritability	Multi-organ failure; Prior pneumonia (no pathogen isolated)	Plasmapheresis, peritoneal dialysis	None	Exitus
2	Nikolajeva et al.[8]	16 mo, female	ADA deficiency	Evolving HUS, mild anti-complement factor H autoantibodies	Persistent proteinuria, treated with hemofiltration and plasma exchange	Seizures, visual loss	Hypertension, CKD, vomiting, lethargy (no pathogen isolated)	Hemofiltration, plasma exchange, PEG-ADA	PEG-ADA, gene therapy, enzyme replacement	Alive
3	Nikolajeva et al.[8]	6 yr, male	ADA deficiency	HUS with red cell fragmentation, schistocytes, low fibrinogen	Failure to improve with eculizumab, remained plasmapheresis-dependent	Lethargy, irritability	Reduced Hb	PEG-ADA, eculizumab, plasma exchange	None	Exitus
4	Nikolajeva et al.[8]	9 mo, male	ADA deficiency	No schistocytes or coagulopathy	Renal failure requiring RRT for 30 days (hemofiltration, peritoneal dialysis)	None	*Streptococcus pneumoniae*-positive, two-week diarrhea and fever	Supportive care, PEG-ADA	Gene therapy with lentiviral vector	Mild residual renal impairment
5	Keller et al.[9]	2.5 mo, male	SCID	Megaloblastic anemia, leukopenia, thrombocytosis	Atypical HUS	Seizures, mild hearing loss, intellectual disability	Eczema, recurrent infections	OHCbl, folate, betaine, IgG, TMP-SMX	OHCbl, MeCbl, folate, methyl folate, betaine, IgG	Alive
6	Sudhakar et al.[10]	8 yr, male	X-linked agammaglobulinemia	No hematologic abnormalities	HUS associated with *Citrobacter freundii*	None	Suspected enteroviral myelitis, recurrent sinopulmonary infections, *Giardia lamblia* gastroenteritis, pneumococcal septic arthritis	Hemodialysis, IV methylprednisolone, IVIG, ceftriaxone	IVIG (400 mg/kg/month), azithromycin prophylaxis, tapering prednisolone	Alive

ADA: adenosine deaminase, AKI: acute kidney injury, aPTT: activated partial thromboplastin time, CKD: chronic kidney disease, CMV: cytomegalovirus, CVID: common variable immunodeficiency, EBV: Epstein-Barr virus, Hb: hemoglobin, HUS: hemolytic uremic syndrome, HSV: herpes simplex virus, IgG: immunoglobulin G, IV: intravenous, IVIG: intravenous immunoglobulin, MeCbl: methylcobalamin, mo: months, OHCbl: hydroxocobalamin, PEG-ADA: PEG-ylated adenosine deaminase, PJP: Pneumocystis jirovecii pneumonia, PT: prothrombin time, RRT: renal replacement therapy, SCID: severe combined immunodeficiency, STEC: Shiga toxin-producing Escherichia coli, TMP-SMX: trimethoprim-sulfamethoxazole, VZV: varicella-zoster virus, yr: years.

Table II. Continued.

Case #	Reference	Age, sex	Underlying immunodeficiency	Hematologic findings	Renal findings	Neurological findings	Other clinical findings	Initial treatment	Ongoing treatment	Outcome
7	Bogdał et al.[11]	15 mo	ADA deficiency	Megaloblastic anemia, thrombocytopenia	Atypical HUS	None	VZV (3 months), HSV (9 months), CMV, PJP (15 months)	Peritoneal dialysis (29 days), 8 cycles plasmapheresis, antihypertensives	None	Exitus
8	Milošević et al.[7]	5 yr	CVID	Microangiopathic hemolytic anemia, thrombocytopenia	Acute renal failure, atypical HUS	None	Generalized lymphadenopathy, hepatosplenomegaly, gallop rhythm, recurrent bacterial respiratory infections	Peritoneal dialysis (3 months), corticosteroids	IVIG (400 mg/kg/ month)	Exitus
9	Akcay et al. (Our case)	12 yr, male	Ataxia-telangiectasia	Megaloblastic anemia, thrombocytopenia, uremia	STEC-HUS, 72 hours dialysis	None	Recurrent sinopulmonary infections	Dialysis (discontinued after urine output improvement)	IVIG (400 mg/kg/ month)	Alive

ADA: adenosine deaminase, AKI: acute kidney injury, aPTT: activated partial thromboplastin time, CKD: chronic kidney disease, CMV: cytomegalovirus, CVID: common variable immunodeficiency, EBV: Epstein-Barr virus, Hb: hemoglobin, HUS: hemolytic uremic syndrome, HSV: herpes simplex virus, IgG: immunoglobulin G, IV: intravenous, IVIG: intravenous immunoglobulin, MeCbl: methylcobalamin, mo: months, OHCbl: hydroxocobalamin, PEG-ADA: PEG-ylated adenosine deaminase, PJP: Pneumocystis jirovecii pneumonia, PT: prothrombin time, RRT: renal replacement therapy, SCID: severe combined immunodeficiency, STEC: Shiga toxin-producing Escherichia coli, TMP-SMX: trimethoprim-sulfamethoxazole, VZV: varicella-zoster virus, yr: years.

Informed consent for the publication of this case report was obtained from the patient's parents.

Discussion

This case exemplifies the complex interplay between STEC and the immune vulnerabilities associated with ataxia telangiectasia (A-T). The patient's immunoglobulin deficiencies and history of recurrent infections significantly heightened the risk of severe complications, including STEC- HUS. Notably, this case represents the first documented instance of STEC-HUS in a patient with A-T, emphasizing the unique challenges faced by this population.

In the context of A-T, the patient's immune dysregulation—particularly the low levels of IgA and IgG—complicated the clinical presentation. As A-T predisposes patients to recurrent infections due to immunodeficiencies, as described by Amirifar et al.[2] and Rothblum-Oviatt et al.[4], this patient's immunocompromised state necessitated regular IVIG therapy to mitigate the risks associated with infections.

Talukder et al.[6] proposed that Shiga toxin plays significant role in celluler damage by activating the ATM/p53-dependent DNA damage signaling pathway. In A-T patients with muations in ataxia-telangiectasia (*ATM*) gene, the ATM pathway is already impaired. The ATM protein is essential for DNA repair and the maintenance of genomic stability, playing a crucial role in the cellular response to stress, such as DNA damage and oxidative stress.

When Shiga toxins are presented, they trigger the formation of reactive oxygen species that activate the ATM/p53 pathway, leading to DNA damage. In healthy individuals, the ATM protein facilitate DNA repair, but in A-T patients, ATM dysfunction impairs this repair process. This dysfunction can lead to increased DNA damage, increased cellular stress, and potentially promoting more severe clinical course of HUS.

When cellular damage combines with Shiga toxins-induced endothelial damage, the clinical course of STEC-HUS may worsen in patients with A-T. This interaction between Shiga toxin exposure and ATM dysfunction may increase microangiopathy, and renal and systemic complications in A-T patients, compared to those with normal ATM function.

Therefore, our case highlights the necessity of close monitoring of A-T patients at risk for infections like STEC, because any cellular and vascular that may develop could lead to critival outcomes. Taking this situation into account, developing management strategies is important to improve patient outcomes and reduce the risk of irreversible organ damage.[6]

Comparative analyses with other immunodeficiency cases complicated by HUS provide valuable insights (Table II).[7-11] Notably, four of these patients succumbed to their conditions following HUS, underscoring the serious importance of complications in immunocompromised patients. For example, Nikolajeva et al.[8] described patients with adenosine deaminase (ADA) deficiency, who developed atypical HUS (aHUS), presenting with acute kidney injury and requiring prolonged CRRT. Similar to our case, advanced therapeutic interventions, such as plasmapheresis and replacement therapy are often required to address both immunological and renal issues with ADA deficiency. Furthermore, Bogdał et al.[11] discussed the bidirectional exacerbation of ADA deficiency and aHUS, further emphasizing the need for individualized management strategies in primary immunodeficiencies.

Likewise, Sudhakar et al.[10] reported HUS in a patient with X-linked agammaglobulinemia (XLA), which involves profound B-cell deficiency. The clinical manifestations of HUS in XLA share similarities with those observed in A-T and ADA deficiencies, emphasizing the broader implications of immune dysregulation

in the pathogenesis of HUS. These comparisons highlight the necessity of integrating immune-modulatory therapies, such as IVIG, alongside renal support for optimal patient outcomes.

Our patient's successful recovery following three days of hemodialysis demonstrates the potential for positive outcomes when early and targeted interventions are employed, despite underlying immunodeficiency. As emphasized by Joseph et al.[1], timely initiation of supportive therapies, including dialysis when indicated, is critical in reducing morbidity and preventing long-term renal sequelae.

One of the limitations of our study is that it is a single case report. Additionally, cobalamin levels were not measured, nor was mutation analysis conducted to investigate a potential defect in the alternative complement system and the patient's clinical and laboratory data for the third month following discharge are unavailable.

In conclusion, this case underscores the importance of recognizing the heightened risks and unique complications associated with primary immunodeficiencies, such as A-T, in patients presenting with infectious diseases. Early detection of clinical signs, along with a multidisciplinary approach, is critical in mitigating severe complications and improving patient outcomes. Further research is needed to better understand the complex interactions between immune dysregulation and infectious diseases in high-risk populations.

Ethical approval

The informed consent form was obtained from the patient.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: NA, DT; data collection: İB; analysis and interpretation of results: NA, DT, İB; draft manuscript preparation: NA, DT, İB. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

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Case Report

An unusual case of musculoskeletal graft-versus-host disease mimicking dermatomyopathies

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ABSTRACT

Background. Musculoskeletal manifestations of graft-versus-host disease (GVHD) are rare but often result in mobility impairments, reducing the patient's quality of life. Typically, such diagnoses are made based on clinical findings without the need for performing a muscle biopsy.

Case Presentation. A 12-year-old boy diagnosed with acute myeloblastic leukemia (M2 subtype) underwent allogeneic hematopoietic stem cell transplantation (HSCT) due to a molecular relapse before his last chemotherapy cycle. Cyclosporine prophylaxis was stopped three months after transplantation, but the patient developed ocular, cutaneous, and oral chronic GVHD at four, five, and seven months after transplantation, respectively, for which intermittent steroid treatment and mycophenolate mofetil were given. All signs of GVHD resolved by one year after transplant, and immunosuppressive treatment was stopped; however, three months later, he experienced muscular weakness in bilateral upper and lower extremities. Subsequently, immunosuppressive treatment was restarted following a muscle biopsy.

Conclusion. Diagnosing musculoskeletal GVHD is challenging due to the lack of reliable parameters for histopathological diagnosis, and initial clinical findings can be mistaken for steroid-induced myopathy or inflammatory dermatomyopathies. We applied methylprednisolone, mycophenolate mofetil and extracorporeal photopheresis for treatment, and the clinical findings completely improved with these treatments.

Key words: graft-versus-host disease, allogeneic bone marrow transplantation, musculoskeletal symptoms, inflammatory dermatomyopathy.

Graft-versus-host disease (GVHD) is an immune-mediated condition often encountered after allogeneic bone marrow transplantation when the donor's immunocompetent T-cells deem the recipient's antigens, known as human leukocyte antigen, as foreign. GVHD represents a significant issue for patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Typically, acute GVHD (aGVHD) commonly affects the skin, liver, and gastrointestinal tract, while chronic GVHD (cGVHD) can affect nearly any organ system, with the gastrointestinal tract, oropharynx, skin, eyes, urogenital tract, lungs, and lymphohematopoietic system being the most frequently involved.[1]

Musculoskeletal GVHD is a rare manifestation of cGVHD with an incidence rate of 0.6% (49 cases per 1,000,000,000 people/year).[1] It is extremely rare in children, with no prevalence data available, and only a few pediatric cases have been reported so far.[2] However, the

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condition can be potentially life-threatening and causes significant disability, impairment, and a severe decline in the patient's quality of life.[2-11] This case report describes the histopathological findings of musculoskeletal GVHD in a pediatric patient with acute myeloblastic leukemia (M2 subtype; AML-M2).

Case presentation

A 12-year-old boy presented with eye swelling and pancytopenia. Bone marrow aspiration cytology showed 40% myeloblastic cells, and flow cytometry revealed CD13, CD33, CD117, and myeloperoxidase positivity. Further bone marrow genetic analysis revealed trisomy-8 and t(8;21) (AML-ETO-1) positivity on polymerase chain reaction (PCR) testing, confirming the diagnosis of AML-M2. Accordingly, the AML Berlin-Frankfurt-Münster (BFM) 2019 chemotherapy protocol was initiated. After induction treatment, initially, the AML-ETO-1 PCR turned negative but eventually returned to positive before the fifth and final chemotherapy cycles. Consequently, he was transferred to a stem cell transplantation unit. As no suitable donor was found within the family, HSCT was performed from a 10/10 matched unrelated donor following the FLAG-IDA (fludarabine, cytarabine, granulocyte colony stimulating factor - idarubicin) protocol. The following myeloablative conditioning regimen was included – busulfan (3.8 mg/kg/day, days -7 to -4; cyclophosphamide 60 mg/kg/day, days -3 to -2; and melphalan 140 mg/m^2/day, last day before HSCT). Additionally, cyclosporine and methotrexate were used for GVHD prophylaxis.

Cyclosporine prophylaxis was stopped three months after transplantation. In the fourth month, there were signs of ocular GVHD, and at five months post-transplant, the patient developed grade III skin GVHD. Accordingly, mycophenolate mofetil and steroids were added to his regimen. At this time, he had 100% chimerism, and the bone marrow was in molecular remission. Although his skin and eye symptoms resolved, he developed oral grade II GVHD in the seventh month during the steroid tapering phase, necessitating an increase in the steroid dose.

By the end of the first year, the GVHD had resolved, and immunosuppressive therapies had been discontinued; muscle, joint, and neurological examinations remained normal during this period. However, three months after immunosuppressive therapy cessation, he exhibited muscular weakness in all four limbs, primarily in the lower limbs. Biochemical tests and blood counts, including creatine kinase (CK: 34 IU/L), were normal, and he tested negative for Epstein-Barr virus and cytomegalovirus PCR, Lyme disease serology, rheumatoid factor, antinuclear antibody, anti-dsDNA, anti-cardiolipin antibodies immunoglobulin G, and immunoglobulin M. The patient was uncooperative; hence, an electromyography (EMG) could not be performed, but nerve conduction tests were within normal limits. A muscle biopsy confirmed muscle involvement in cGVHD. Eventually, extracorporeal photopheresis was planned, and steroid (2 mg/kg/day) and mycophenolate mofetil (1500 mg/m^2) were reintroduced. During the treatment, he developed a cough, which was diagnosed as pulmonary GVHD based on pulmonary function tests and thoracic computed tomography. All symptoms resolved after receiving two months of extracorporeal photopheresis.

Histopathological findings

Microscopic examination of the muscle biopsy specimen showed distortions in the diameter and shape of the muscle fibers. Scattered atrophic fiber structures were seen in the periphery of the fiber bundles, with no fiber grouping. However, the internal architecture of the muscle fibers exhibited normal nuclear retraction (< 3%), with no evidence of vacuoles or accumulations. There was no increase in the endomysial fibrous tissue, while the perimysial connective tissue appeared edematous and degenerated (Fig. 1). An inflammatory cellular reaction, comprising of macrophages, lymphocytes, and a few

plasma cells, was predominant in the perimysial area and noticeable in the endomysial area, particularly around the capillary vessels (Fig. 2). Histochemical and enzyme staining revealed that the distribution and ratio of type I and type II fibers were preserved, and no abnormal glycogen or fat accumulation was detected. Cytochrome *c* oxidase (COX) and succinate dehydrogenase enzyme staining displayed no loss of enzymatic activity or mitochondrial aggregates. Immunohistochemical staining revealed sarcolemmal upregulation of major histocompatibility complex (MHC)-1, which was slightly more pronounced in the perifascicular fibers. Acid phosphatase and C5b9 staining exhibited characteristic features of inflammatory myopathy, with positive staining in macrophages and capillary structures (Fig. 3 and Fig. 4). Finally, staining of the cluster of differentiation 3 (CD3), CD4, CD8, CD20, and CD68 cells showed a cellular reaction rich in CD8-positive T cells and macrophages. When combined with the patient's history, the histopathological findings were consistent with muscle involvement in cGVHD.

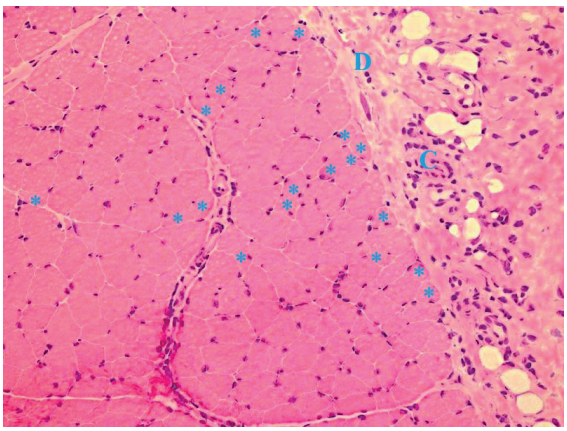

Fig. 1. In the striated muscle tissue sample, diameter and shape difference in the fibers, scattered atrophic fibers (*), as well as degeneration (D) and cellular reaction (C) in the perimysial connective tissue are observed (H&E, original magnification x200).

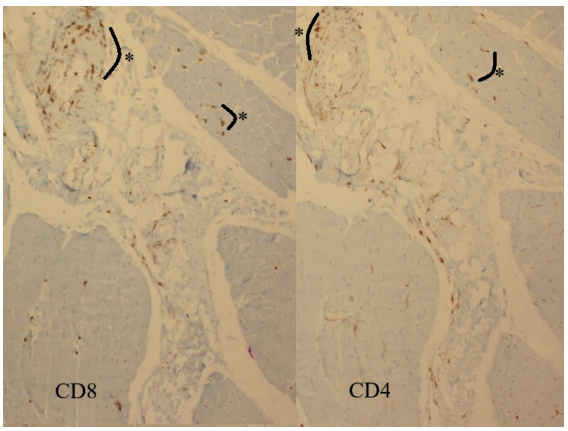

Fig. 2. T cell reaction (*) is observed via CD8 (left) and CD4 (right) staining in the perimysial connective tissue and around the endomysial capillaries (original magnification x200).

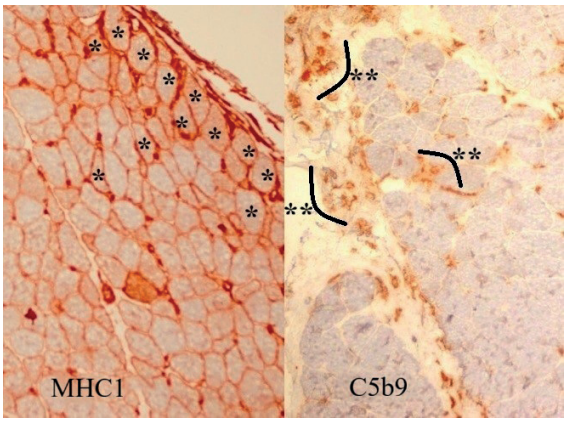

Fig. 3. Left: In MHC-1 staining, a positive sarcolemmal reaction (*) is observed in the fibers. Right: In C5b9 staining, a positive reaction is observed in macrophages (**) and around the capillary (original magnification x200).

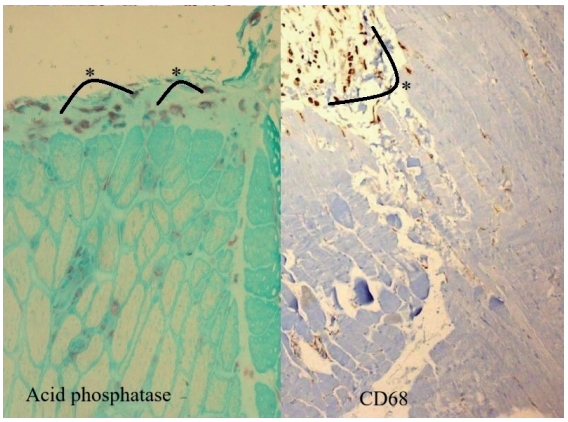

Fig. 4. In the striated muscle tissue sample, a positive reaction is observed in macrophages (*) in acid phosphatase and CD68 staining (original magnification x200).

Informed consent was obtained from the patient's parents, who agreed to the publication of the case findings.

Discussion

This case report underscores the importance of recognizing rare manifestations of cGVHD, particularly in pediatric patients. The existing literature affirms that musculoskeletal involvement in cGVHD implies a unique pathological process. In the present case, positive MHC-1 and C5b9 staining supported the diagnosis of inflammatory myopathy associated with cGVHD, highlighting the need for careful interpretation of the diagnostic findings in such cases.

Previous studies by Shono et al.[4] contribute to the understanding of bone marrow GVHD, while Shulman et al.[3] focus on the neurologic and cardiac complications associated with GVHD. Lehky et al.[6] investigated the prevalence of neuropathy and muscle cramps in patients undergoing HSCT and found that 2.6–8.1% of patients exhibiting cGVHD symptoms may develop neuromuscular complications. Myositis was the most common neuromuscular disorder, followed by neuropathy and, less frequently, myasthenia gravis, and 55% of patients with neuromuscular symptoms experienced muscle cramps, while 65% demonstrated a muscle weakness phenotype.[6]

Kvinge et al.[2] conducted a comprehensive review of the literature on musculoskeletal manifestations of cGVHD and reported that cutaneous (52%) and oropharyngeal (37%) complications were the most common complications of cGVHD, while musculoskeletal complications (0.5%–3%) were among the rarer findings. They also emphasized that muscle pain is a grave and debilitating condition that should be considered in patients exhibiting muscle weakness, joint stiffness, and tissue inflammation.

Rørvik et al.[7] provided an overview of different clinical presentations of cGVHD, categorizing fasciitis and joint stiffness as musculoskeletal findings, and myositis and polymyositis as diagnostic indicators; notably, they described cardiac myositis and pericardial involvement as uncommon. Likewise, Tan et al.[11] reported a case of polymyositis following stem cell transplantation in a 6-year-old patient with thalassemia. The researchers underline that approximately 40 comparable cases have been acknowledged worldwide, the treatment of which is intricate. Moreover, Allen et al.[12] provided clinical, pathological, and molecular examination details of three patients with cGVHD who developed myopathy post-allogeneic HSCT. In each instance, Allen et al.[12] noticed perifascicular atrophy, a histological hallmark of dermatomyositis (DM).

There are no reliable benchmarks for the histopathological confirmation of muscle involvement in either aGVHD or cGVHD; however, excluding potential causative factors leading to similar morphological appearances may help determine a confirmed diagnosis. The upregulation of MHC-1 is a significant finding in inflammatory/immune-related myopathies; this is particularly noteworthy for dermatomyopathies, where it is more pronounced in perifascicular regions. However, our patient did not exhibit other typical features of dermatomyopathies, such as perifascicular atrophy, cell reactions with CD20-positive lymphocytes (especially pronounced in the perimysial region), COX deficiency in enzyme stains, and a serological increase in CK. Hence, inflammatory dermatomyopathy was not considered a potential diagnosis.

Other potential differential diagnoses of musculoskeletal manifestations of GVHD include hereditary myopathies (facioscapulohumeral muscular dystrophy [FSHMD], limb girdle muscular dystrophy [LGMD] 2A and 2B, hereditary inclusion body myopathy [HIBM]). In such cases, dystrophic findings from the distribution of cellular reactions in the biopsy specimen, combined with the patient's personal and family history, may facilitate differentiation.

Table I. Differential diagnosis in immune dermatomyopathy syndromes.[14]

Features		IM + perimysial pathology	Dermatomyositis + vascular pathology	Regional ischemic immune myopathy	DM: pauci-myopathic	cGVHD	Myositis + Mi-2 antibody	Systemic sclerosis
Clinical	Age at onset	Adult > child	Child or adult	Older adult	Adult	Adult	Child or adult	Adult
	Weakness	Proximal > distal	Proximal > distal	Proximal > distal	Uncommon	Proximal ± distal (50%)	Proximal (98%)	Proximal
	Myalgia	Common	Common	Common	None or Mild	Some		
	Skin pathology	Raynaud	Rash	Rash (70%)	Palmar: papules & ulcers	Sclerosis	Rash	Scleroderma
		Rash	Heliotrope		MxA + Keratinocytes	Dyskeratosis	Raynaud	Raynaud
		Mechanic's hands	Limbs: Extensor surface		Type I IFN			Capillary telangiectasia
		Psoriasiform	Capillary Δ Tif1γ: Photosensitive		Alopecia			
		Eczematous	Palm hyperkeratosis					
		Dyskeratosis	NXP2: Edema					
	Interstitial lung disease	Common	Uncommon	No	Common	Common	Rare	Some
	Neoplasm association	No	Adults	Common; 72%, > 60 yrs.	No	Prior, hematologic	No	No
	Systemic, other	Joints	Calcification; GI			GI; mucous membranes	Joints; GI	Esophagus, small bowel
								Calcinosis
Serum	Myositis-associated antibody	Jo-1	TIF1γ	TIF1γ	MDA5	Varied	Mi-2	HEp-2 IIF nuclear
		tRNA synthetase	NXP-2	NXP-2				SMN
	CK > 1,000 U/L	Some	Few	Most	No	No	Most	Some

AP: Alkaline phosphatase, cGVHD: Chronic graft versus host disease, CK: Creatine kinease, COX: Cytochrome oxidase, C5b9: complement 5b-9, DM: Dermatomyositis, GI: Gastrointestinal, IM: Immune myopathies, MHC-1: Major Histocompatibility Complex class I, NOS: Nitric oxide synthase.

Table I. Continued.[14]

Features		IM + perimysial pathology	Dermatomyositis + vascular pathology	Regional ischemic immune myopathy	DM: pauci-myopathic	cGVHD	Myositis + Mi-2 antibody	Systemic sclerosis
Muscle pathology	Inflammation			No	No	Uncommon	Yes (50%)	No
	Focal	Some	Yes (80%)					
	Location	Perimysium	Perivascular				Perimysial & Endomysial	
	Type	Histiocytic	Lymphocyte (B-cell)				Lymphocyte	
	Perimysium pathology							No
	Fragmentation	Common	Occasional	Necrotic regions	Some	Common		
	Immune cells	Histiocytic Scattered	Lymphocyte (B-cell) perivascular	Histiocytic	Histiocytic Scattered	Histiocytic	Histiocytic	
	AP	Some	Uncommon	Yes		Some	Common	
	Myofibril pathology							
	General				Mild or no	Glycosylation ↓		
	Perifascicular	Often	Yes	No	NOS2	No	Yes	MHC-1 upregulated
	COX staining reduced	Never	Most patients		No			
	Necrosis & regeneration	Common	Unusual					
	LC-3 aggregates	Rare	Common				Common 60%	
	Atrophy	Uncommon	Common				Perifascicular	
	Necrosis			Regional	No	No		
	Capillaries near myofiber	Normal	Reduced		Normal	Reduced	Normal	Thick walls
	C_{5b-9}	Normal	Large	Large				
	AP stain	Absent	Few	Common				
	Large vessel pathology	No	Artery + vein	Vein	No	No	No	No

AP: Alkaline phosphatase, cGVHD: Chronic graft versus host disease, CK: Creatine kinease, COX: Cytochrome oxidase, C5b9: complement 5b-9, DM: Dermatomyositis, GI: Gastrointestinal, IM: Immune myopathies, MHC-1: Major Histocompatibility Complex class I, NOS: Nitric oxide synthase.

On the other hand, in toxic and drug-induced myopathies, pathological findings attributed to the causative agent are crucial. Notably, steroids, commonly utilized in treating GVHD, can cause steroid-induced myopathy, which is characterized by painless proximal muscle weakness in the bilateral upper and lower extremities. The diagnosis is primarily clinical, with fatty alterations in proximal muscles seen on magnetic resonance imaging. Unlike other myopathies, steroid-induced myopathy is less likely to be detected via EMG[5]; muscle biopsy often reveals selective atrophy of type II muscle fibers.[13] In cases of polymyositis and DM linked to connective tissue diseases, both clinical history and serologic findings play a significant role (Table I).[14]

In conclusion, this case report describes the features of a rare case of musculoskeletal GVHD with DM-like manifestations in a pediatric patient undergoing allogeneic HSCT for AML-M2. Through this unique case, we aim to enhance the understanding of atypical GVHD presentations and highlight the need for a comprehensive diagnostic approach for prompt and effective treatment.

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Ethical approval

Informed consent was obtained from the patient's parents. They also agreed to the publication of this case.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BE, ZCÖ; data collection: BE, Hİ; analysis and interpretation of results: BE; draft manuscript preparation: BE, ZCÖ, ÖB. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Successful management of sudden cardiac arrest in an adolescent with arrhythmogenic right ventricular cardiomyopathy

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ABSTRACT

Background. Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a fatal, genetically transmitted cardiomyopathy that can cause unpredictable malignant life-threatening arrhythmias. Arrhythmias that may be hemodynamically insignificant in healthy persons, yet may be fatal in patients with cardiomyopathy and end-stage heart failure. Thus, urgent and prompt management of arrhythmias in these patients is essential to achieve favorable outcomes.

Case Presentation. Here, we present a 13-year-old male who was referred to our institution with a prediagnosis of ARVC and had sudden cardiac arrest on the second day due to ventricular tachycardia / fibrillation. Successful extracorporeal cardiopulmonary resuscitation (E-CPR) was performed. A successful endo-epicardial ablation of ventricular tachycardia and implantable cardiac defibrillator insertion were performed under extracorporeal membrane oxygenation (ECMO) due to recurrent malignant ventricular arrhythmias. On the fourth day, he was weaned from ECMO without any sequelae. Although the patient did not experience any hemodynamically significant or sustained tachycardia after catheter ablation, he underwent a successful transplantation due to progressive heart failure.

Conclusion. Appropriate and urgent management of life-threatening arrhythmias and when necessary high-quality resuscitation measures including E-CPR and a multidisciplinary coordinated approach is crucial in the management of patients with cardiomyopathy and end-stage heart failure.

Key words: arrhythmogenic right ventricular cardiomyopathy, implantable cardiac defibrillator, ablation, transplantation.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an inherited disorder characterized by progressive fibro-fatty replacement of the myocardium, and ventricular tachycardia (VT) with left bundle branch block (LBBB) morphology. It usually presents with symptomatic arrhythmias or sudden death.[1]

Diagnosis of arrhythmogenic cardiomyopathy is made using revised Padua criteria (2020) that are based on a multi parametric approach encompassing functional and structural ventricular abnormalities, tissue characterization findings, depolarization and repolarization alterations, ventricular arrhythmias and familial/genetic background.[2]

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The most striking clinical features that must alert physicians are signs and symptoms of right heart failure, VT originating from the right ventricle, negative T waves and epsilon waves in leads V1-V3. Other diagnostic tools include electrocardiography, echocardiography, magnetic resonance imaging (MRI), and genetic tests.

Several concurrent processes have been proposed as responsible for the arrhythmogenicity in ARVC, including triggered activity linked to sympathetic activity, re-entrant mechanisms caused by myocardial fibrosis, myocardial inflammation, and/or ion channel failure.[3,4] These variables could account for the occurrence of both hemodynamically stable monomorphic VT and rapid, unstable rhythms such as polymorphic VT or ventricular fibrillation in patients with ARVC.[5-7] Roudijk et al. reported that male sex, the quantity of T-wave inversions in the precordial leads, ventricular ectopy on Holter monitoring, and a lower biventricular ejection fraction on cardiac imaging are all linked to arrhythmic occurrences.[7]

Here, a 13-year-old male diagnosed with ARVC, who experienced sudden cardiac arrest due to malignant ventricular arrhythmia (VA) and successfully bridged to heart transplantation is presented.

Case presentation

A 13-year-old male patient was admitted with complaints of chest pain and shortness of breath with exertion for 2 months. He was referred to our institution with a prediagnosis of ARVC and heart failure. Physical examination findings were as follows: Mild tachycardia (120-130 bpm), tachypnea (38/min), a 2-3/6 pansystolic murmur best heard at the left lower sternal border, jugular venous distension, a liver palpable 3 cm below the costal margin and mild ascites. Electrocardiogram showed; sinus tachycardia (110 bpm), a widened QRS (120 msec) and the corrected QT interval (QTc) of 480 msec (because of widened QRS) (Fig. 1). Echocardiography revealed significantly reduced biventricular function (left ventricle M-mode ejection fraction: 15%), enlarged right heart chambers with a RV diameter of 53.8 mm (z score: + 4.08) in apical 4 chamber view and severe tricuspid regurgitation (Fig. 2). Cardiac MRI showed marked dilation of the right ventricle (RV) with an indexed volume of 149 mL/m^2 and hypokinesia in the free and inferior walls, as well as segmental wall thinning and focal late gadolinium enhancement (Fig. 3). The ejection fraction was 10% and 15% for the right and left ventricles, respectively. Heart failure treatment was administered immediately and a 24-hour-rhythm Holter was planned. On the second day of hospitalization, the patient had a sudden cardiac arrest and during cardiopulmonary resuscitation, it was recognized that the patient had VT exhibiting LBBB morphology with an intermittent transition to ventricular fibrillation (VF). Multiple antiarrhythmics including lidocaine, amiodarone, and magnesium were administered and the arrhythmia was converted to sinus rhythm after 5 cardioversions and 2 defibrillations. The patient was transferred to the pediatric intensive care unit (PICU). During follow-up in the PICU, the patient had episodes of LBBB morphology VT with intermittent transition to VF despite amiodarone and lidocaine infusion and was re-arrested. During cardiopulmonary resuscitation (CPR), venoarterial extracorporeal membrane oxygenation (ECMO) was initiated in 35 minutes. As drug-resistant VT storm continued during ECMO support, a single staged endocardial and epicardial approach for ablation was planned along with the assistance of cardiothoracic surgery. Even though the epicardial scar was much larger than the endocardial scar, a clinical VT isthmus was present at the endocardial site where ablation terminated the VT quickly (Fig. 4). An implantable cardiac defibrillator was implanted for secondary prevention, amiodarone was administered, and the patient was decannulated on the 4th day. The patient was discharged without any sequelae meanwhile autosomal dominant mutation that is linked to ARVC

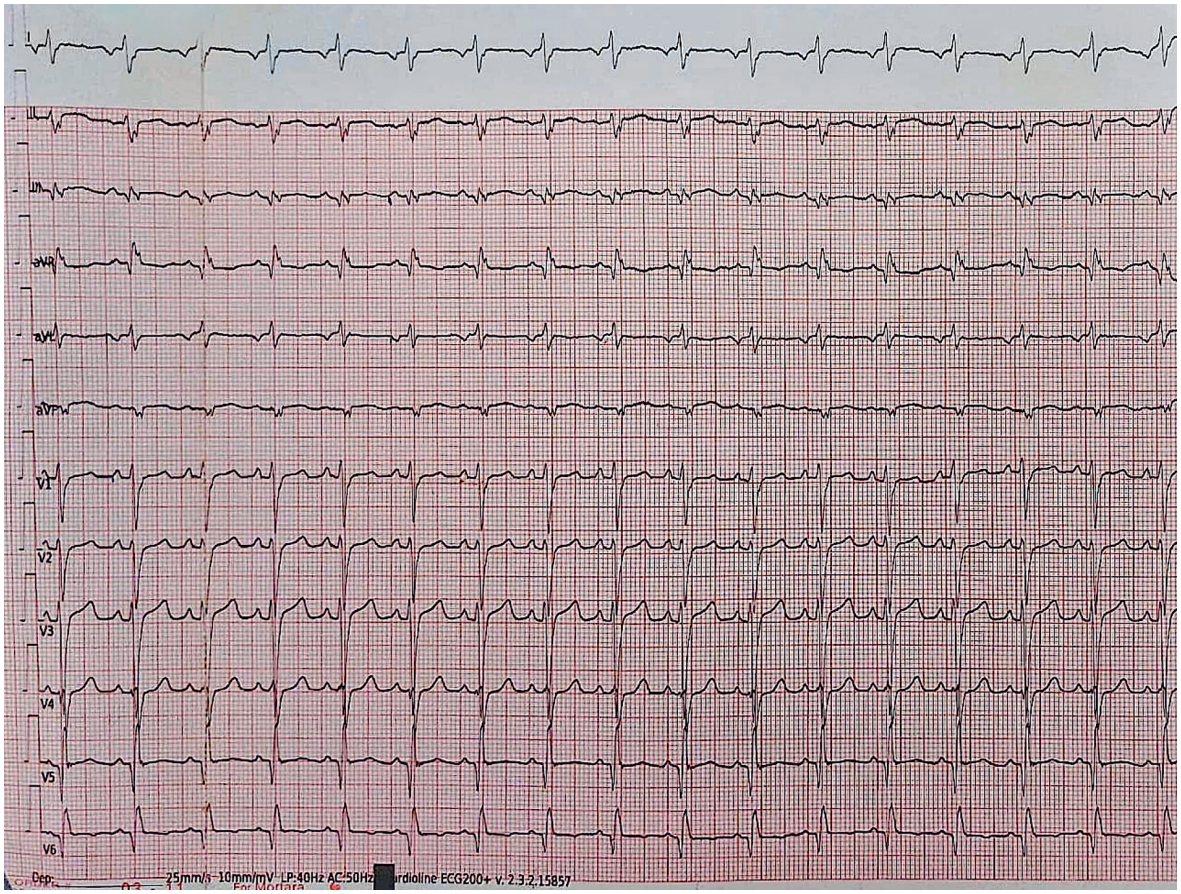

Fig. 1. Electrocardiography of the patient showing; sinus tachycardia (110 bpm), a widened QRS (120 msec) and the corrected QT interval (QTc) of 480 msec.

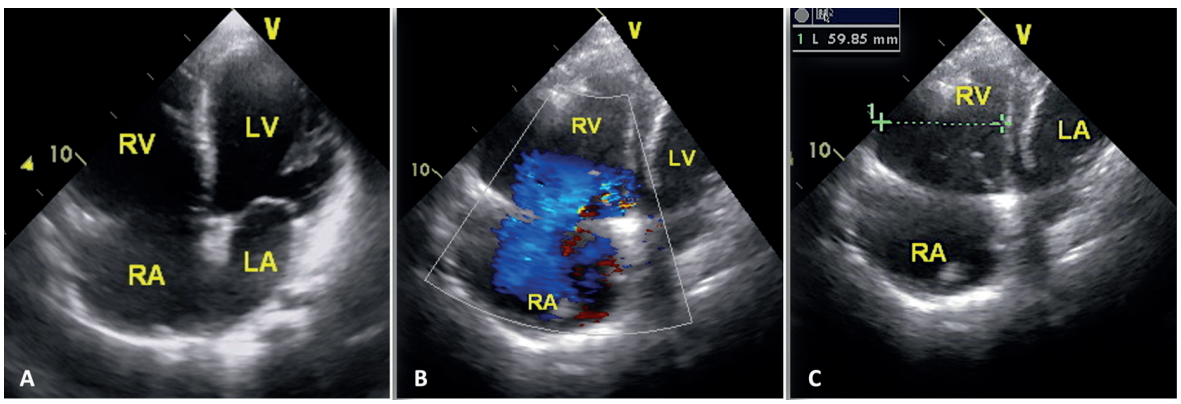

Fig. 2. Echocardiography of the patient: A: apical 4-chamber view with enlarged right cardiac chambers; B: severe tricuspid regurgitation; C: apical 4-chamber view with RV dimension.

LA: left atrium, LV: left ventricle, RA: right atrium, RV: right ventricle.

was detected in *DSC2* gene at 18q12.1. Three months later the patient was rehospitalized for decompensated heart failure (NYHA class III-IV). Following this, his clinical status deteriorated due to multidrug refractory and inotrope-dependent heart failure. His kidney functions worsened, he became symptomatic even at rest, and developed massive

hepatomegaly and ascites which required multiple paracentesis but never experienced sustained and hemodynamically significant arrhythmia. He could not be discharged and was on inotropes for 12 months till he underwent a successful heart transplantation. Pathological analysis of the explanted heart revealed: diffuse degenerative changes characterized by nuclear hyperchromasia, centralization and pleomorphism, areas of mucinous degeneration and oedema under the endocardium, mucinous degeneration in the valves, fibrosis between muscle fibers, congestion and fibrin accumulation sites in the pericardium and also a quite thin right ventricle wall. The patient has been followed up for 9 months and shown no symptoms post-transplantation.

The patient's family gave their informed consent for this case report to be published.

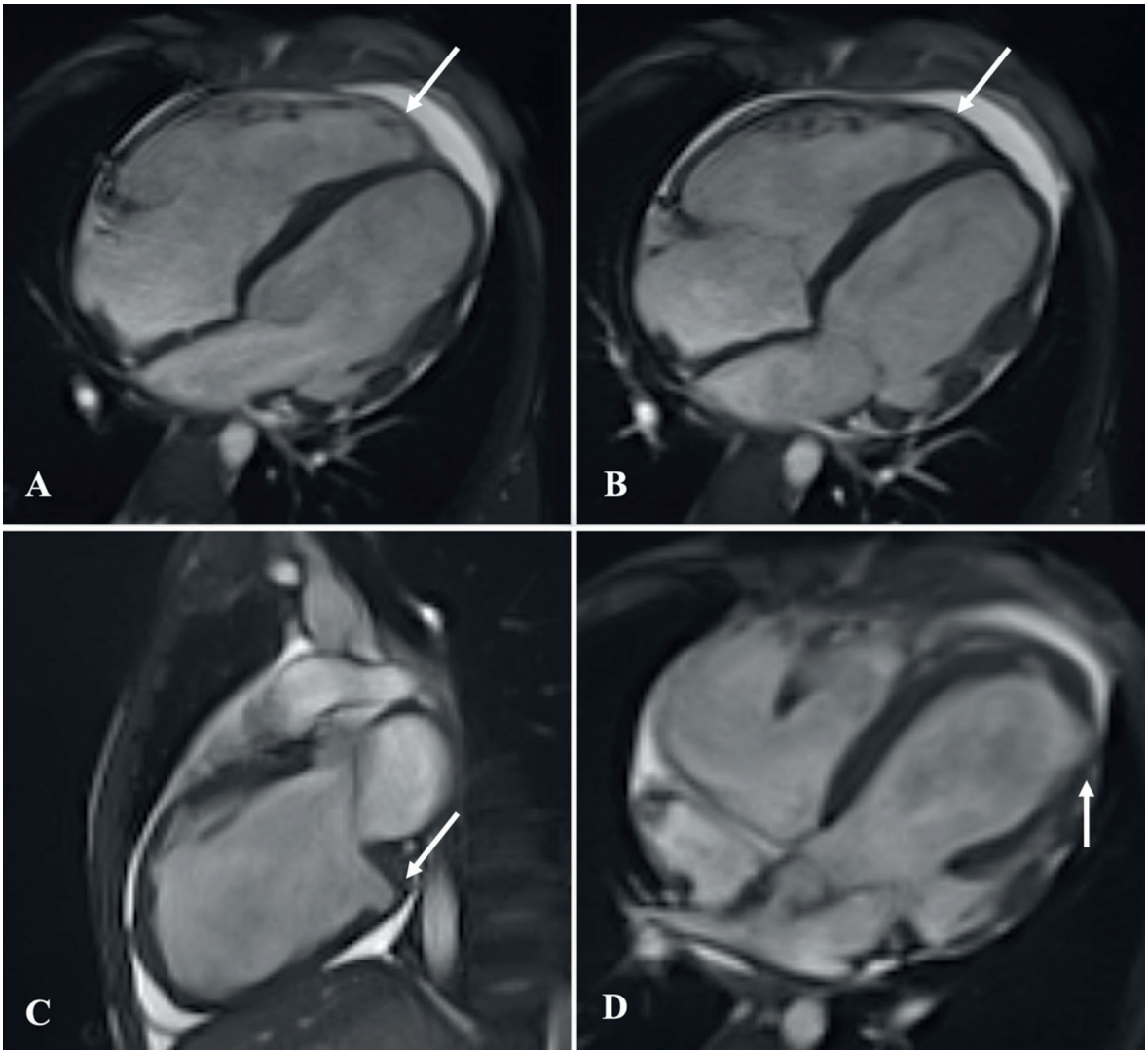

Fig. 3. Cardiac magnetic resonance imaging of the patient showing hypokinesia: A: bulging in the RV at the end of systole; B: bulging in the RV at the end of systole; C: bulging in the RV at the end of systole; D: Bulging in the LV at the end of systole. Arrows indicate the point of bulging.
LV: left ventricle, RV: right ventricle.

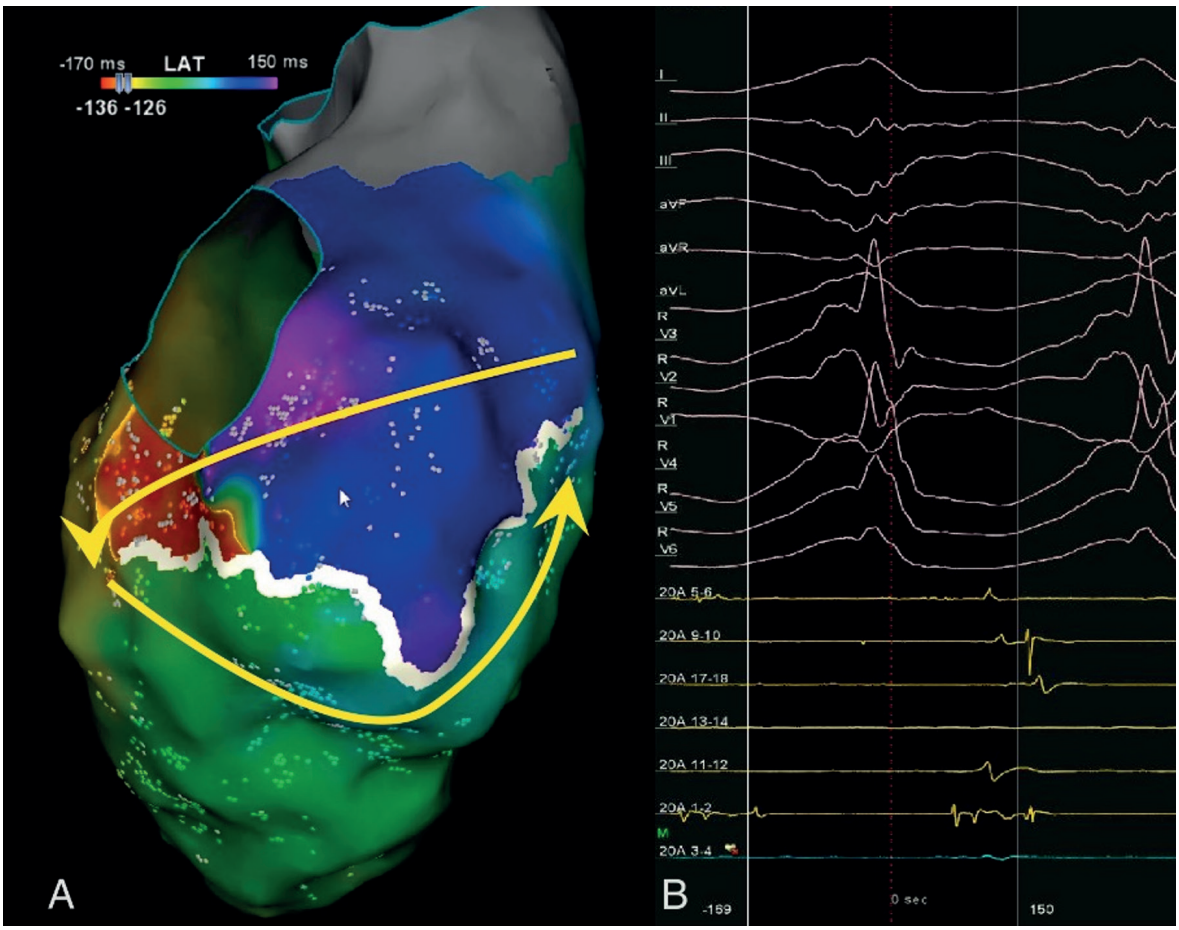

Fig. 4. A: Three-dimensional activation map demonstrating critical isthmus of the ventricular tachycardia. B: Twelve-lead electrocardiogram of clinical ventricular tachycardia. Below the electrocardiogram local mid-diastolic bipolar electrograms are present, obtained from critical isthmus of the tachycardia.

Discussion

A case of ARVC who had sudden cardiac arrest due to VT and successfully bridged to heart transplantation is presented here. We aimed to emphasize life-threatening arrhythmias and sudden cardiac arrest that may occur in patients with cardiomyopathy and end-stage heart failure (HF), as well as the prompt management of these patients with high-quality resuscitation measures and a multidisciplinary coordinated approach to achieve favorable outcomes.

Our patient fulfilled the diagnosis of ARVC according to the Padau criteria and had experienced sudden cardiac arrest (SCA) due to VT / VF in the absence of a prior history of syncope and palpitation. Consistent with the literature, as a risk factor for life-threatening arrhythmias, our patient was male and had biventricular failure.

The main goals of treatment are to prevent SCA, slow the rate of disease progression and reduce VA. In patients with cardiomyopathy and heart failure, malignant arrhythmias are the first cause to rule out in case of rapid clinical deterioration or sudden cardiac arrest as in our patient. Although implantable cardioverter – defibrillators (ICDs) can be used as primary or secondary prevention in these patients, they are not beneficial and may cause electrical storms in the presence of uncontrolled ventricular arrhythmias. In cases experiencing VA in the presence of appropriate medical treatment catheter ablation of VA may be necessary in

addition to ICD implantation.[8,9] Even though traditional endocardial ablation is quite effective, some patients do not respond well to ablation due to the existence of epicardial reentrant circuits. Up to 30% of the substrates of aberrant ventricular activity are intramural or subepicardial.[10] Pokushalov et al. reported that epicardial ablation may be necessary and that it increases overall success in adolescent patients with ARVC in whom endocardial ablation of VT has failed.[11] Several recent publications indicate that simultaneous epicardial and endocardial ablation is superior to endocardial ablation alone in means of VA recurrence without a significant difference in all-cause mortality or acute procedural complications and may even result in the permanent elimination of this arrhythmia.[12]

Epicardial catheter ablation has been demonstrated to be safe and effective in adults, but there are limited reports in the pediatric population.[13] Both endocardial and epicardial ablation may be challenging in hemodynamically unstable patients. In infants with incessant tachyarrhythmias, extracorporeal membrane oxygenation provides a hemodynamically stable and safe platform for antiarrhythmic drug therapy and ablation.[13] Our patient was already under ECMO support because of aborted cardiac arrest and was still clinically deteriorating due to multidrug refractory VT / VF storm. At this point, ablation under ECMO was our only option. Similarly, Thomas et al performed epicardial ablation in a 13-month-old infant and reported that venoarterial ECMO provides hemodynamic stability in the face of unstable arrhythmias. In cases with hemodynamic instability, ablation may be performed with ECMO support to achieve hemodynamic stability.

Our patient who suffered SCA was successfully discharged from the hospital without any sequelae after prompt treatment of arrhythmia with epi-endo mapping and endocardial ablation, ICD implantation and amiodarone treatment. He had never had clinically significant or sustained arrhythmia up until he underwent a successful heart transplantation due to refractory heart failure. As in our patient, in cases with refractory heart failure, the sole treatment option is heart transplantation.[14]

In cases with uncontrollable life-threatening arrhythmias, E-CPR and ECMO may bridge to successful recovery and transplantation.

In conclusion, malignant life-threatening arrhythmias and sudden cardiac arrest may occur in patients with ARVC. High-quality resuscitation measures and a multidisciplinary coordinated approach including E-CPR and ECMO play a crucial role and have a life-saving potential for survival during in-hospital sudden cardiac arrest. Successful endo/epicardial ablation may be the choice of treatment in patients with recurrent VT despite appropriate antiarrhythmic treatment and in cases with hemodynamic instability, ECMO support may be a successful strategy to sustain hemodynamic stability.

Ethical approval

The patient's family gave their informed consent for this case report to be published.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BM, MGR, EB, TA, TK, TU; data collection: BM, MGR, EB; analysis and interpretation of results: BM, MGR, TU; draft manuscript preparation: BM, MGR, EB, TA, TK, TU. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Prognostic value of early treatment response to craniospinal irradiation in diffuse leptomeningeal glioneuronal tumors: a case series

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ABSTRACT

Background. Diffuse leptomeningeal glioneuronal tumors (DLGNTs) are rare pediatric central nervous system neoplasms with poorly defined treatment strategies and prognostic factors. Although chemotherapy (CHT) is frequently used, the role of radiotherapy (RT), particularly craniospinal irradiation (CSI), remains unclear.

Case Presentations. We present a case series of three pediatric patients diagnosed with DLGNTs and treated with CSI at an initial dose of 36 Gy, with an additional boost to 54 Gy. Patients were evaluated for early radiological response post-CSI and its potential prognostic implications, alongside their clinical and histological features. Two patients demonstrated significant radiological regression after 36 Gy of CSI, with continued improvement 1.5 months post-treatment. These patients remained stable for 88 and 27 months, respectively, without further disease progression. The third patient exhibited disease progression despite CSI and concurrent temozolomide, ultimately succumbing to the disease within 10 months. Notably, this patient had a Ki-67/MIB-1 index of 70%, while surviving patients had lower proliferation indices.

Conclusions. Our findings suggest that an early favorable response to 36 Gy of CSI may serve as a prognostic indicator in DLGNTs. This study highlights the potential value of CSI in managing these tumors and underscores the need for further research to establish standardized treatment approaches.

Key words: craniospinal irradiation, diffuse leptomeningeal glioneuronal tumor, pediatrics, central nervous system neoplasms, prognosis.

Diffuse leptomeningeal glioneuronal tumors (DLGNTs), previously known as primary diffuse leptomeningeal oligodendroglioma or disseminated oligodendroglial-like leptomeningeal tumors of childhood, have gained recognition over the past decade.[1-3] First introduced in the 2016 World Health Organization (WHO) classification, DLGNTs are now included in the 2021 WHO classification under glioneuronal and neuronal tumors.[4] Although some molecular alterations associated with the disease such as KIAA1549-BRAF fusion and 1p deletion have been identified, a formal grading system for these tumors has yet

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to be established[2,3], and additional molecular changes are still under investigation.[5]

DLGNTs are more commonly observed in the pediatric population.[4] Patients often present with hydrocephalus and may require ventriculoperitoneal (VP) shunt surgery.[6] Other symptoms include headache, confusion, ataxia, focal neurological deficits, and symptoms related to spinal cord compression.[7] Typical magnetic resonance imaging (MRI) findings in DLGNT include diffuse leptomeningeal enhancement along the surfaces of the brain and spinal cord, often described as a "sugar-coating" or "cobweb-like" pattern.[5] T2-hyperintense cystic or nodular deposits are frequently found along the parenchymal surface, particularly in areas such as the cerebellum, brainstem, temporal lobes near the Sylvian fissures, hippocampi, and medial occipital lobes.[3,5]

DLGNT lacks established treatment guidelines, with approaches varying across cases.[8-10] Surgical resection is mainly for biopsy or symptom relief, not cure, due to the disease's disseminated nature.[8,10] Chemotherapy (CHT) is first-line, but responses are often partial or stable.[8] Radiotherapy (RT) has unclear efficacy, with mixed outcomes reported[9,11-14], and is typically reserved for progressive disease, especially in pediatric patients, due to potential side effects.[1,3,6,8,15] Given DLGNT's slow progression, RT is rarely used initially.

Here, we aim to present three cases of DLGNT treated with craniospinal irradiation (CSI) as the primary approach, with an additional tumor bed boost. This is anticipated to be the first publication of its kind in literature.

Case Presentations

The present study was approved by the Ege University Medical Research Ethics Committee (approval number: 24-8T/52). All participants provided informed consent. The three cases of DLGNT presented here were managed at the Ege University Faculty of Medicine, Department of Radiation Oncology between 2017 and 2023. They were treated with craniospinal irradiation (CSI) as the primary approach, with an additional tumor bed boost, reaching a cumulative dose of 54 Gy. Radiological response to CSI was assessed based on the Response Assessment in Neuro-Oncology (RANO) criteria, which evaluates changes in contrast enhancement, non-enhancing lesions, and clinical status.[16] CHT was specifically selected for the patient based on the tumor board's decision. We evaluated the patients' responses to treatment and believe that the early favorable response to 36 Gy of CSI carries a potential prognostic significance.

The overview of clinical presentations and interventions for 3 cases of DLGNTs is summarized in Supplementary Table S1.

Case 1

A 3-year-old girl was referred to our hospital with a 5-month history of imbalance and gait disturbances. During the neurological examination, she presented with lethargy, bilateral nystagmus, ataxia, and exaggerated deep tendon reflexes. A computed tomography (CT) scan revealed hydrocephalus, cisternal dilatation, and hypodense nodular lesions in the posterior fossa. VP shunt was performed, and a cerebrospinal fluid (CSF) sample was collected. - CSF analysis was unremarkable. Subsequent cranial MRI revealed extensive T2-hyperintense cystic lesions along the subarachnoid spaces in the posterior fossa, as well as in the inferior frontal and temporal lobes (Fig. 1A). Spinal MRI exhibited similar T2-hyperintense and T1-hypointense cystic lesions, accompanied by leptomeningeal thickening and enhancement (Fig. 1B, Fig. 1C).

A cerebellar biopsy was performed. Histopathological examination revealed low to moderate cellularity, consisting of uniform tumor cells (Fig. 2A). Immunohistochemistry was consistent with DLGNT, with a Ki-67/MIB-1 index of 10% (Supplementary Table S2).

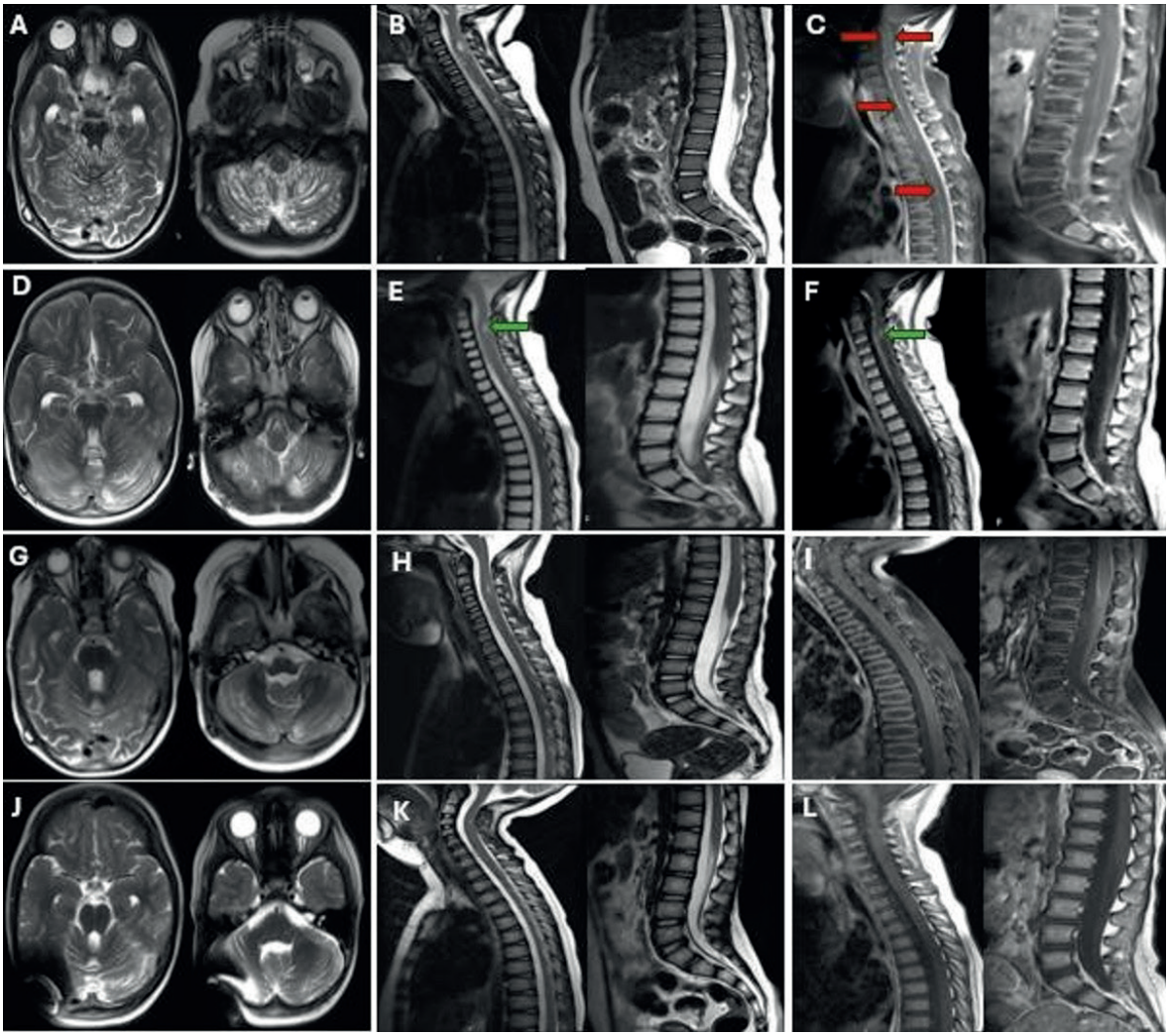

Fig. 1. Initial radiological images and post-treatment radiological changes for Case 1.

Axial cranial magnetic resonance imaging (MRI) revealed extensive T2-hyperintense cystic lesions located along the subarachnoid spaces in the posterior fossa, inferior frontal lobes, and temporal lobes (A). The sagittal T2-weighted images of the spinal cord displayed hyperintense cystic lesions (B), while the sagittal T1-weighted post-contrast sequences indicated basal and diffuse leptomeningeal enhancement (C, red arrows). Following 36 Gy of craniospinal irradiation, significant regression of the lesions was observed in most areas of the brain (D) and similarly in the T2-hyperintense spinal cystic lesions (E). However, a nodule at the C3 level remained visible on both the sagittal T2-weighted and T1-weighted post-contrast images (E, F, green arrows). Imaging conducted 1.5 months after radiotherapy (RT) demonstrated further regression of leptomeningeal enhancement in both the cranial and spinal regions (G, H, I). The C3 spinal nodule showed regression in the sagittal T2-weighted images (H) and disappeared in the sagittal T1-weighted post-contrast images (I). The most recent MRI images indicate stable disease (J, K, L).

The patient received RT (36 Gy CSI and 54 Gy to the tumor bed) without concurrent CHT, showing significant cranial and spinal lesion regression on MRI (Fig. 1D-I). Post-RT, she underwent carboplatin and vincristine chemotherapy for 6 years and 3 months, which was stopped due to stable disease. She has remained off treatment and stable for the past 8 months, with a total survival of 88 months (Fig. 1J-L). Follow-up revealed short stature and mild periventricular leukoencephalopathy, but no other clinical or neurological issues.

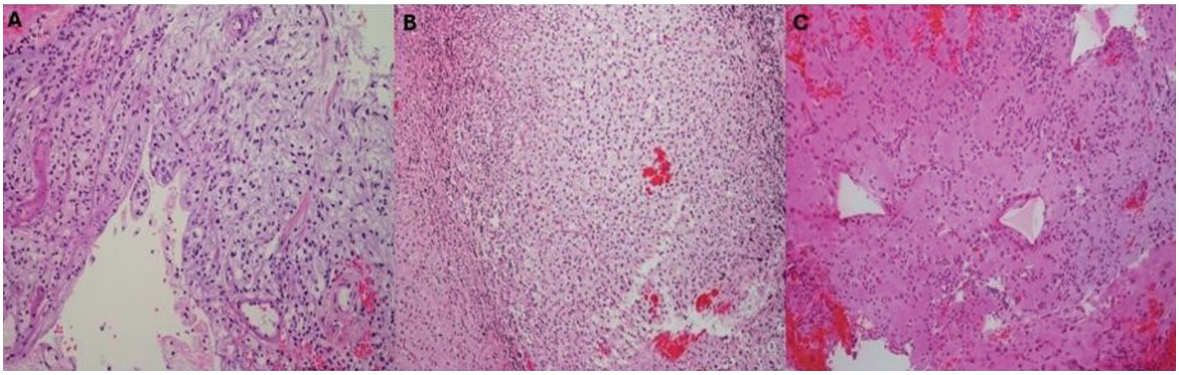

Fig. 2. Tumor histopathology (hematoxylin & eosin, original magnification x10.

(A) Case 1, cerebellum. Oligodendrocyte-like tumor cells with perinuclear haloes in nested pattern. (B) Case 2, cerebellum. Uniform, oligodendrocyte like tumor cells with perinuclear haloes in diffuse pattern. (C) Case 3, lumbar spine. Monomorphous tumor showing low to moderate cellularity, cells with hyperchromatic nuclei and scant cytoplasms. No neurocytic rosettes, mitotic figures, necrotic foci, or microvascular proliferation were observed in any of the patients.

Case 2

A 4-year-old boy presented to our hospital with symptoms of seizure, lethargy, nausea, and vomiting, which had started 1.5 months prior to admission. Neurological examination revealed inward deviation of the right eye and bilateral lower extremity weakness. It was learned that he had been diagnosed with hydrocephalus and underwent VP shunt placement at the age of 3, approximately one year ago. The cranial MR images from one year ago showed no additional findings aside from hydrocephalus. Current imaging shows no signs of VP shunt dysfunction. Current MRI demonstrated multiple T1 hypointense/T2 hyperintense cystic lesions in the posterior fossa, frontal, and temporal lobes, with leptomeningeal thickening and enhancement in the brainstem, cerebellum, and hemispheres (Fig. 3A). Spinal MRI revealed diffuse leptomeningeal enhancement and multicystic lesions, the largest 1 cm at T5 (Fig. 3B, Fig. 3C).

Cerebellar biopsy showed oligodendrocyte-like cells with perinuclear halos with low to moderate cellularity (Fig. 2B). Immunohistochemistry was consistent with DLGNT (Supplementary Table S2), with a Ki-67/MIB-1 proliferation index of 1%, and benign CSF cytology.

Radiotherapy (36 Gy craniospinal irradiation and 54 Gy to the tumor bed) was administered without concurrent CHT. Significant regression was observed in both cranial and spinal lesions after 36 Gy (Fig. 3D-F). An MRI performed 1.5 months post-RT revealed reduced leptomeningeal enhancement and decreased size and number of cystic lesions (Fig. 3G-I). The patient's neurological symptoms at the time of diagnosis have since completely resolved, and there is no history of seizures. Subsequently, he received carboplatin and vincristine for 1-year post-RT with the disease stabilizing.

At the latest follow-up, 27 months from diagnosis, he remains stable off therapy for 8 months, seizure-free, with no neurological deficits, developmental delay, or endocrinological abnormalities (Fig. 3J-L).

Case 3

A 4-year-old boy presented with a 6-month history of seizures and headaches. Cranial MRI findings revealed leptomeningeal thickening and enhancement around the brainstem, in the basal cisterns, within the cerebellar folia, and in the sulci of both cerebral hemispheres, accompanied by occasional nodular tumor implants (Fig. 4A). Along the spinal cord,

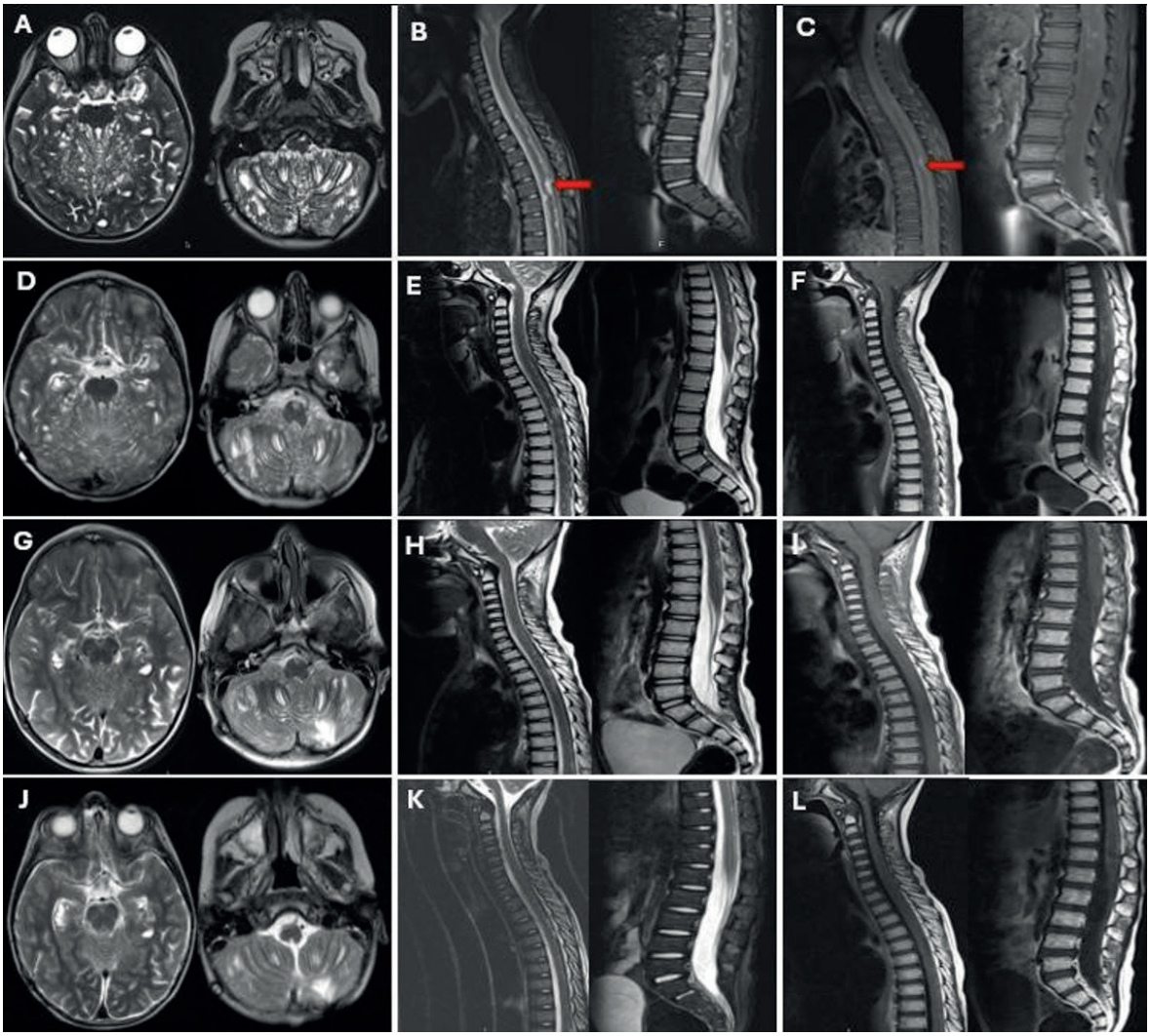

Fig. 3. Initial radiological images and post-treatment radiological changes for Case 1.

Axial cranial magnetic resonance imaging (MRI) revealed extensive T2-hyperintense cystic lesions located along the subpial areas and perivascular spaces in the posterior fossa, inferior frontal lobes, and temporal lobes. Leptomeningeal thickening and enhancement were observed in the brainstem, cerebellar region, and cerebral hemispheres (A). In the spinal cord, T2-hyperintense nodular leptomeningeal contrast enhancements with multicystic lesions were identified at all levels (B). The largest nodular lesion measured 1 cm in the long axis at the T5 vertebral body level, as seen in both sagittal T2-weighted (B) and T1-weighted post-contrast images (C, red arrows). Following 36 Gy of craniospinal irradiation (CSI), significant regression of lesions was noted in most areas of the brain (D) and the T2-hyperintense spinal cystic lesions (E). Additionally, regression of leptomeningeal enhancement was observed in the sagittal T1-weighted post-contrast images (F). Imaging conducted 1.5 months after radiotherapy (RT) demonstrated further regression of leptomeningeal enhancement in both the cranial and spinal regions, as well as in the cystic lesions (G, H, I). The most recent MRI images indicate stable disease (J, K, L).

particularly at the L4-5 level, nodular tumor implants with leptomeningeal enhancement were identified (Fig. 4B, Fig. 4C).

Lumbar spinal biopsy revealed low to moderate proliferative activity, with a focal tumor area exhibiting a Ki-67/MIB-1 proliferation index of 70%, characterized by hyperchromatic nuclei and scant cytoplasm (Fig. 2C). Immunohistochemistry was consistent with DLGNT (Supplementary Table S2). CSF cytology was benign.

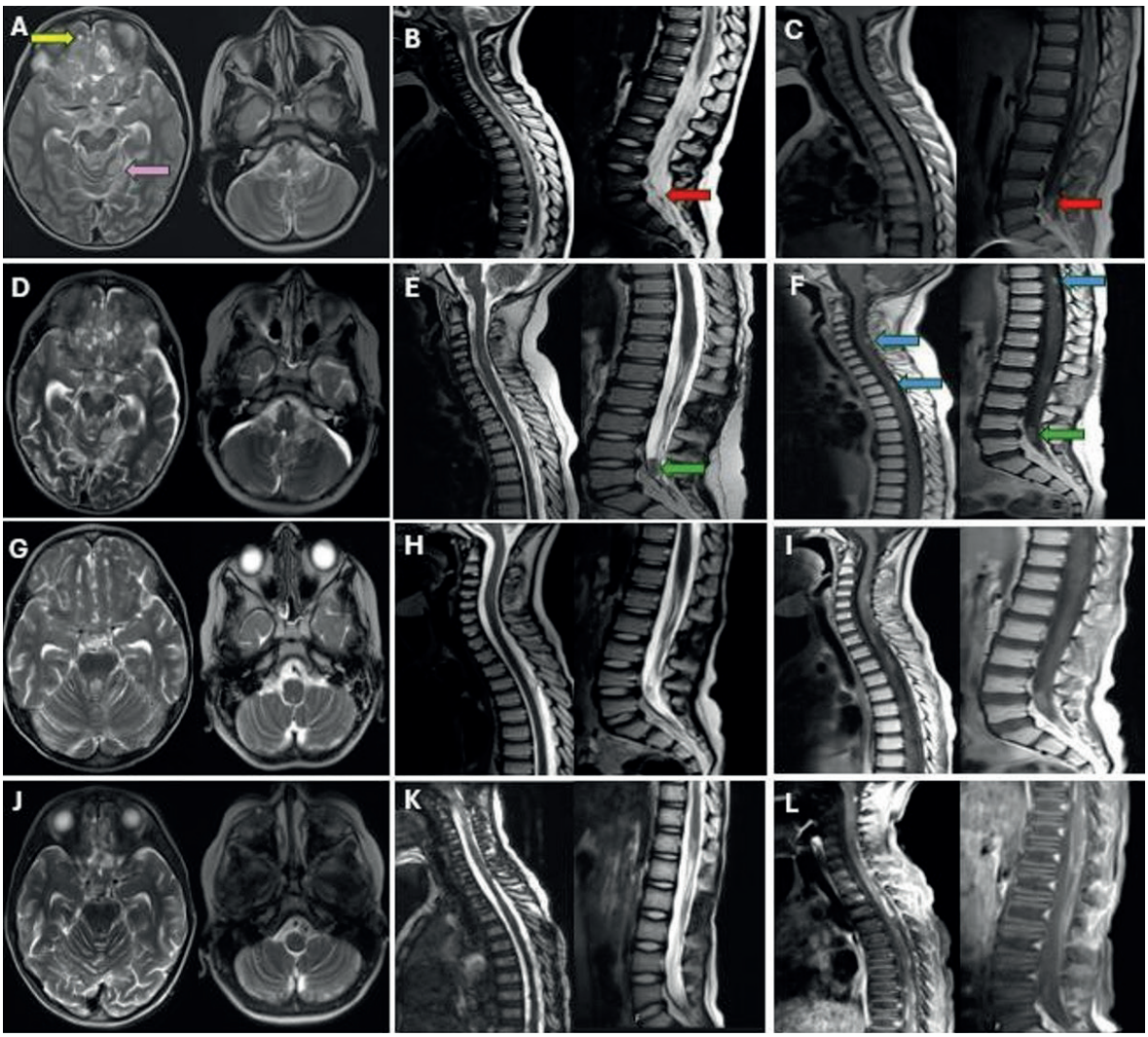

Fig. 4. Initial radiological images and post-treatment radiological changes for Case 3.

Axial cranial magnetic resonance imaging (MRI) revealed leptomeningeal thickening and enhancement surrounding the brainstem, in the basal cisterns, within the cerebellar folia, and in the sulci of both cerebral hemispheres. These findings were accompanied by occasional cystic lesions (yellow arrow) and nodular tumor implants (A, pink arrow). Along the spinal cord, particularly at the L4-5 level (red arrows), nodular tumor implants with leptomeningeal enhancement were identified (B, C). After administering 36 Gy of craniospinal irradiation, progression was observed in both the leptomeningeal thickening and enhancement in the brain (D) and spine (E, F), as well as at the L4-5 level nodules in both sagittal T2-weighted and T1-weighted post-contrast images (E, F, green arrows). The sagittal T1-weighted post-contrast images also showed the occurrence of spinal nodular lesions (F, blue arrows). At 1.5 months post-radiotherapy, MRI indicated slight regression of the leptomeningeal enhancement and the nodular and cystic lesions (G-I). However, the last follow-up MRI, taken 7 months after completing RT, showed significant progression in the thoracic and lumbar regions (J-L).

The patient was treated with RT consisting of 36 Gy CSI and a 54 Gy boost to the tumor bed, administered concurrently with temozolomide. Unfortunately, after the initial 36 Gy of CSI, MRI scans showed progression in both cranial and spinal regions (Fig. 4D-F). Treatment continued with a boost to the tumor bed up to 54 Gy alongside temozolomide. At 1.5 months post-RT, MRI revealed slight regression of leptomeningeal enhancement and cystic lesions (Fig. 4G-I). Despite ongoing temozolomide therapy, the patient experienced clinical

and radiological progression 7 months after completing RT, particularly characterized by increased leptomeningeal thickening and enhancement in the thoracic and lumbar regions on post-contrast sequences (Fig. 4J-L). He was switched to ICE (ifosfamide, carboplatin, and etoposide) chemotherapy but died of febrile neutropenia after the first cycle. Survival after the diagnosis was 10 months.

Discussion

We report three cases of DLGNT initially treated with CSI. Two showed significant radiological response after 36 Gy without concurrent CHT, while the third progressed despite treatment and died within seven months. To our knowledge, this is the first report indicating that early radiological response to 36 Gy CSI may represent a prognostic marker in DLGNT.

The biological nature of DLGNT remains poorly understood. Consistent with several studies[3,17], cytological evaluation of CSF in our series was not informative, with all CSF samples negative for tumor cells despite extensive radiological evidence of leptomeningeal involvement. DLGNTs are typically classified as low-grade gliomas (LGG) and often follow an indolent course characterized by stability or slow progression.[3] In some instances, stability has been observed even without treatment.[1,18] A study by Lu et al., involving 54 cases, reported a 10-year survival rate of 69%, with rates reaching 78% and 75% for patients treated with CHT alone and RT alone, respectively.[19] Despite these favorable outcomes, DLGNT differs markedly from other LGGs due to its disseminated nature and unique clinical spectrum. Aggressive cases are also commonly reported, often associated with significant morbidity and long-term functional impairments.[1,20] Xiao et al. noted that untreated cases had a survival range of 4 to 6 weeks, primarily due to cranial hypertension, underscoring the need for early intervention.[21] Gardiman et al. reported 9 fatalities among 36 patients, with survival times ranging from 3 months to 21 years post-biopsy.[1] Furthermore, a systematic review by Wiśniewski et al. reported a median overall survival (OS) of just 19 months, emphasizing the poor prognosis in certain cases.[22] These findings underscore the heterogeneous nature of DLGNT, with outcomes influenced by early treatment and individual tumor characteristics.

There is currently no standardized treatment guideline for DLGNTs. Prior studies have highlighted CHT as a cornerstone in management, showing statistically significant improvements in OS.[19] Common treatment protocols incorporate carboplatin, vincristine, temozolomide, cyclophosphamide, cisplatin, and/or etoposide—agents typically used in treating LGG.[9,12] While CHT appears crucial, it may not suffice as monotherapy, often necessitating lifelong, multi-regimen treatment. Reports of radiological regression following CHT alone are limited, with most patients either remaining stable[1,9,19,23] or showing progression.[6,9,13,20,24,25] This variability underscores the need for additional or combined treatment approaches to optimize outcomes for DLGNT patients.

The role of RT in treating DLGNTs is complex, with mixed findings on its clinical and radiological efficacy. In a case series by Schniederjan et al., a patient who received CSI with temozolomide remained stable at 137 months, while another case treated with CSI alone showed progression.[17] Lyle et al. reported a 14-year-old girl who, after receiving CSI with temozolomide as a first-line treatment, showed complete clinical and radiological response within six weeks, except for a residual nodule in the spine.[26] Conversely, other studies suggest limited RT efficacy. Policicchio et al. indicated that the mean OS was comparable between patients treated with CHT and RT and those who were not treated (51 months vs. 53 months). Their findings also highlighted that CHT was more commonly administered in patients with better outcomes, whereas RT was slightly more prevalent in those with poorer prognoses. Among RT treated patients, 25% had poor prognostic factors, compared to only

8% in untreated patients, suggesting that RT may often be selected for patients with worse clinical characteristics.[8] Supporting this, many studies employ RT primarily in cases of disease progression.[12,13,27] This may be due to limited data and the perception of DLGNTs as LGGs, which has led to an expectation of limited response to RT. Furthermore, RT for DLGNT typically involves CSI, which raises concerns about potential long-term side effects, particularly for pediatric patients. In a series by Rebella et al., one case initially showed regression following CSI but subsequently developed severe leukoencephalopathy 1.5 years after treatment completion.[11] - Our surviving patients, Case 1 and Case 2, exhibited a favorable response to RT and did not experience any significant side effects. Although Case 1 exhibited slight periventricular leukoencephalopathy, the patient remains asymptomatic.

Concurrent CHT with RT may be considered for aggressive cases, though risks should be individualized. Lyle et al. combined CHT and CSI based on rapid, sustained responses.[26] In contrast, our Case 3 progressed despite concurrent temozolomide with CSI, highlighting the need for biomarkers to identify aggressive subtypes.

To better identify patients at risk for aggressive disease, preliminary data suggest prioritizing molecular features over traditional clinical approaches. A review by Policicchio et al., covering both adult and pediatric populations, found that Ki-67/MIB-1 levels in pediatric studies typically ranged from 1% to 30%. Karlowee et al. reported a Ki-67/MIB-1 index of 40%, while Swetye et al. observed focal Ki-67/MIB-1 levels reaching 53% in this review. A cutoff of 5% has also been suggested. An average OS of 46 months was observed for patients with Ki-67/MIB-1 levels between 0% and 5%. In contrast, those with Ki-67/MIB-1 levels exceeding 5% experienced a mortality rate of 36% (7 out of 19 patients) and an average OS of only 8.8 months.[8] Additionally, Wiśniewski et al. identified a Ki-67/MIB-1 index greater than 7% as the most significant prognostic factor for OS in patients with DLGNT.[22] Rodriguez et al. further identified that a Ki-67/MIB-1 index over 4%, mitotic activity exceeding 4 mitoses per high-power field, or the presence of glomeruloid vasculature correlated with poor survival, collectively suggesting anaplasia when any of these criteria are present.[2] Schniederjan et al. described aggressive histologic features, including necrosis, mitotic figures, nuclear pleomorphism, and microvascular proliferation, as indicators of anaplastic behavior.[17] Another study also noted polar spongioblastoma patterns and tumor invasion into brain parenchyma as indicators of anaplasia, though identified only in postmortem tissue.[28] According to Swetye et al.'s case, with a Ki-67/MIB-1 proliferative index of 53% and increased mitotic activity, aligned with the anaplastic features defined by Rodriguez et al. and demonstrated rapid progression within 6 months.[24] It can be inferred from this literature that a reported Ki-67/MIB-1 level of 4% does not reliably indicate anaplasia, particularly when compared to studies that use differing cutoff values. Also, it remains uncertain whether these anaplastic features are linked to more aggressive clinical behavior. In our study, - Case 3 did not exhibit glomeruloid vasculature, high mitotic activity, necrosis, or microvascular proliferation, though a focal Ki-67/MIB-1 index of 70% —the highest reported in the literature— might suggest anaplasia. The patient showed only a transient response after CSI, with progression by 7 months and eventual death. This suggests a link between anaplastic features, lack of early radiological response, and poor prognosis.

Currently, additional immunohistochemical and molecular markers for DLGNTs are still under investigation. While MAP-2 has been linked to poor survival, our Cases 1 and 2 showed favorable outcomes, highlighting variability among patients.[22] The role of molecular alterations, such as BRAF mutations, in prognosis remains under examination[2]; notably, our Case 1 and Case 3 did not demonstrate these mutations. Deng et al. proposed two methylation subtypes (DLGNT-

MC-1 with 1p/19q codeletion and DLGNT-MC-2 with 1p deletion/1q gain), suggesting a grading system resembling CNS WHO grades II and III.[5] However, data on these classifications remain limited, and our inability to perform such analyses is a key limitation, emphasizing the need for further research to refine prognosis and treatment strategies in DLGNT.

Our study emphasizes the importance of CSI in treating DLGNTs. Although the small sample size of three cases limits statistical power, this study serves as a valuable reference for future research in this rare tumor entity. We believe that an early favorable response to RT may be associated with a better prognosis. We emphasize the need for future research to establish more reliable prognostic markers and to refine therapeutic strategies.

Supplementary materials

Online supplementary materials are available for this article at https://doi.org/10.24953/turkjpediatr.2025.6139

Ethical approval

The study was approved by Ege University Medical Research Ethics Committee (date: 22.08.2024, number: 24-8T/52).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BBT, SK; data collection: TBV, MÖİ, EA, EB; analysis and interpretation of results: BBT, TBV, SK, CE, YE, TA; draft manuscript preparation: BBT, SK. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Primary B-cell non-Hodgkin lymphoma of the larynx in children: report of two cases and a review of the literature

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ABSTRACT

Background. Non-Hodgkin lymphoma of the larynx in children is a rare condition. Diagnosis is difficult as its symptoms are usually attributed to respiratory tract infections and pubertal voice changes.

Case Presentations. We report two children diagnosed with laryngeal B-cell lymphoma based on imaging and histopathological findings. We also review other pediatric cases of laryngeal lymphoma documented in the literature, detailing tumor locations, lymphoma types, stages, etiological factors, and treatment regimens of these patients.

Conclusion. Diagnosis of laryngeal lymphoma is challenging. Although certain imaging features can be suggestive of the disease, a definitive diagnosis requires histopathological examination. Surgery is not required for the treatment, and chemotherapy is the main treatment approach. Early diagnosis is important.

Key words: laryngeal lymphoma, non-Hodgkin lymphoma, children.

Primary lymphoma of the larynx accounts for less than 1% of laryngeal tumors and is extremely rare in childhood.[1,2] The classic presenting symptoms of laryngeal tumors -dysphonia, dysphagia, dyspnea, and cervical lymphadenopathy- can often be mistaken for acute inflammatory diseases such as croup, acute epiglottitis, and retropharyngeal abscess.[1,2] Diagnosis of primary laryngeal lymphoma is challenging because systemic symptoms are uncommon and symptoms typically remain localized for an extended period without progression.[3] The primary treatment is chemotherapy.[4] Early diagnosis is crucial as the prognosis is favorable in the early stages. Here, we present two pediatric cases of laryngeal lymphoma and provide a comprehensive review of previously reported cases of laryngeal lymphoma in children.

Case Presentations

Case 1

A 6-year-old male was admitted to the otolaryngology department with complaints of hoarseness, dyspnea, and wheezing for three months. There was no accompanying complaint of fever, weight loss, or night sweats. Firstly, a 10-day course of amoxicillin-clavulanic acid was administered by a local doctor. Since symptoms persisted for three months, magnetic resonance

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imaging (MRI) of the neck was performed. A 1.5x1.4 cm mass with markedly restricted diffusion was shown at the left aryepiglottic fold (Fig. 1). During direct laryngoscopy, a convex, regularly circumscribed mass was detected in the left arytenoid of the supraglottic area, and multiple biopsies were performed. Histopathological findings revealed discohesive medium/large-sized cells with eosinophilic cytoplasm. Immunohistochemistry staining showed LCA, CD20, CD19, CD43, CD30, MUM-1, and BCL-2 positivity. However, the staining with CD3, MPO, CD23, BCL-6, CD10, CD34, S100, synaptophysin, desmin, TdT, and pan-keratin were negative. A translocation involving c-MYC and IRF4/DUSP22 was not detected in neoplastic cells. Based on the immunohistochemical findings, the diagnosis of laryngeal mature B-cell non-Hodgkin lymphoma was made. Positron emission tomography - computed tomography (PET-CT) scan showed increased fluorodeoxyglucose (FDG) uptake only in the laryngeal mass, and low FDG uptake in bilateral cervical level 2 lymph nodes. There were no malignant cells in bone marrow and cerebrospinal fluid examinations. Serum biochemistry and LDH levels were within normal limits. He was classified as stage-II non-Hodgkin lymphoma and the FAB/LMB96 group B chemotherapy protocol was started. After completing treatment, he has been followed up without disease for the past two years.

Case 2

A 15-year-old girl with serine/threonine kinase 4 (STK4) deficiency presented with progressively worsening hoarseness for one month. Initially seen in the immunology department and referred to the otolaryngology department, where she was prescribed oral gargle and clarithromycin. As she did not benefit from this treatment, a flexible laryngoscopy was performed, revealing a polypoid structure in the supraglottic area, filling the ventricular band and extending through the arytenoid to the aryepiglottic fold. Multiple punch biopsies were taken from the mass. Histopathological examination showed a neoplastic tissue having a wide necrotic area under the epithelium. Pleomorphic neoplastic cells had vesicular nucleus with multiple nucleoli and had high mitotic activity. Immunohistochemical staining for CD20, Bcl-2 and MUM1 were positive while CD3, CD10, BCL-6, PAX5, ALK, granzyme B, and Tia-1 staining were negative. Additionally, neoplastic cells were strongly positive in the

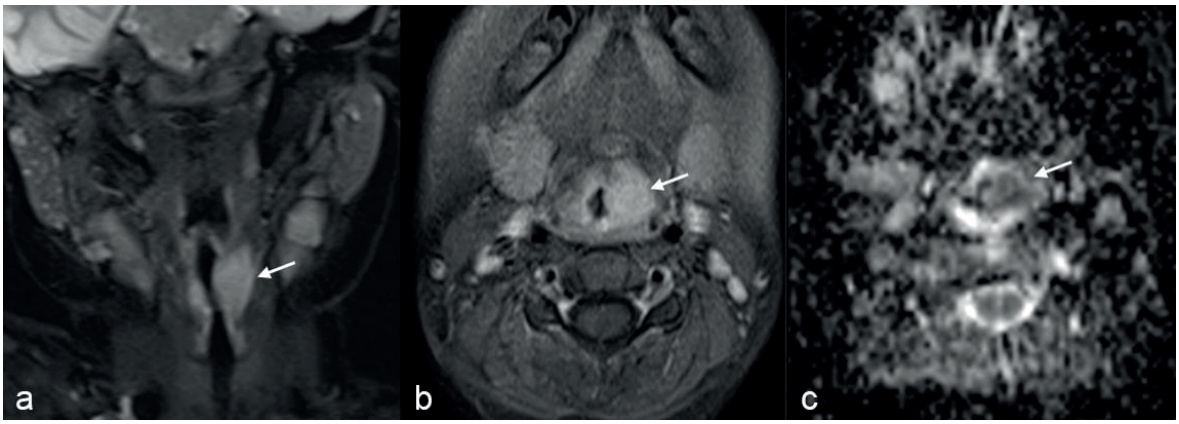

Fig. 1. Magnetic resonance imaging of Case 1 at disease onset. (a) Coronal fat saturated T2-weighted images show the hyperintense soft tissue thickening at the left side of the supraglottic larynx. (b) Axial fat saturated post-contrast T1-weighted images show diffuse enhancement of the soft tissue thickening. (c) Apparent diffusion coefficient (ADC) map demonstrates restricted diffusion in the lesion.

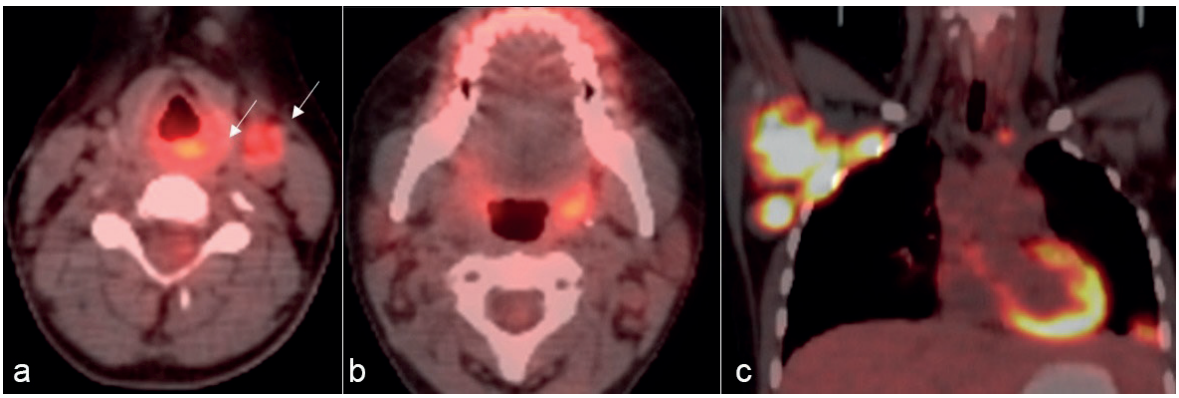

Fig. 2. Positron emission tomography - computed tomography of Case 2 shows increased fluorodeoxyglucose (FDG) uptake in the posterior part of the left ventricular band and the lymph node at level 3 (arrows in image a). Increased FDG uptake can also be seen in the left palatine tonsil (b), the right axillary, mediastinal lymph nodes, and the left pulmonary nodule (c).

Epstein-Barr encoding region (EBER) *in situ* hybridization test. She was diagnosed with Epstein-Barr virus (EBV)-positive diffuse large B cell lymphoma and referred to the pediatric oncology department. Although there was no peripheral lymphadenopathy at first admission, lymphadenopathy with a diameter of 1.5 cm in the left middle cervical chain and a diameter of 3x3 cm in the right axilla developed during the diagnostic investigation process. PET-CT scan showed increased FDG uptake in the posterior part of the left ventricular band and in the left cervical lymph nodes. Increased FDG uptake was also demonstrated in the nasopharynx, left palatine tonsil, bilateral axillary lymph nodes, mediastinal lymph nodes, and left pulmonary nodule (Fig. 2). Bone marrow biopsy and cerebrospinal fluid analysis were negative for tumor involvement. FAB/LMB96 group B chemotherapy protocol was started with the diagnosis of stage III B-cell lymphoma. At the end of 5 cycles of chemotherapy, a new lymph node was detected at the left supraclavicular region and excised. The histopathological examination confirmed the recurrence. Rituximab combined with chemotherapy including ifosfamide, carboplatin, and etoposide (ICE) was started as second-line treatment. Remission was achieved after 6 cycles of chemotherapy. Two months after remission, she underwent allogenic hematopoietic stem cell transplantation from an HLA-matched unrelated donor, for the treatment of her underlying immunodeficiency. She has been followed up without disease for four years.

Discussion

Head and neck malignancies are rare in the pediatric age group and represent 5-12% of all pediatric cancers. The most common pediatric head and neck malignancies are lymphoma, rhabdomyosarcoma, thyroid carcinoma, nasopharyngeal carcinoma, and salivary gland malignancies.[5,6] Arboleda et al. evaluated 367 head and neck tumors among the 7181 pediatric cancers diagnosed within 30 years. Lymphomas, carcinomas (nasopharynx, thyroid), and sarcomas (soft tissue and bone) constitute 52.8%, 22.9%, and 19.1% of them, respectively.[6] No tumors of laryngeal origin, including lymphoma, were detected in this large pediatric head and neck tumors series.

Approximately 13% of pediatric lymphoma cases occur in the head and neck region and most commonly involve cervical lymph nodes. However, involvement of the extranodal regions such as Waldeyer's ring, nasal cavity, paranasal sinuses, maxilla, and mandible are

not unusual.[1,5] Roh et al. reported that 40.5% of 37 head and neck lymphoma cases were located in extranodal sites and half of them were in Waldeyer's ring.[1] In a Brazilian series of 104 head and neck lymphoma patients, half of the cases were located in extranodal sites. There were no cases with larynx lymphoma in these series.[6]

Lymphoma of the larynx is extremely rare. A total of 200 cases have been recorded in the Surveillance, Epidemiology and End Results (SEER) database in 40 years. The average age of onset is the 6th decade and there is a slight male predominance.[2] Laryngeal lymphoma is even rarer in the pediatric age group. Ayyaswamy et al. found only 7 pediatric cases in the literature published between 1987-2022.[3] We found 11 cases of laryngeal lymphoma diagnosed under 18 years of age in the English literature (Table I).[3,4,7-14] Including our patients, there were a total of 9 male and 4 female cases (M/F: 2.25). The median age of patients was 10.5 (4-15) years. Hoarseness, dyspnea, and dysphagia are often the initial symptoms of laryngeal lymphoma.[4] Neoplastic infiltration is mostly located in the supraglottic region and may extend to the glottis and subglottic area.[3,4,7-14] Similar to the literature, the location in our cases was a supraglottic area. Immunodeficiency is one of the strongest risk factors for non-Hodgkin lymphoma especially originating from atypical sites.[1,15] Diagnosis of immunodeficiency diseases was found in 2.5% of cases with non-Hodgkin lymphoma.[16] Mayor et al. examined a group of 3658 patients with primary immunodeficiency and found that 171 (4.6%) cases had malignancy. The highest increase in cancer incidence was observed in lymphoma, with a 10-fold increase in men and an 8.34-fold increase in women.[17] An article reporting an increased risk of EBV-associated lymphoproliferative diseases in STK4 deficiency, including our cases, has been published previously.[18]

A detailed family history and systemic examination are essential for the diagnosis of immunodeficiencies. These assessments help clinicians make a differential diagnosis, guide further testing, and improve prognosis by enabling timely treatment.[19-21] Once a diagnosis has been made, genetic counseling and prenatal diagnosis may be offered to at-risk family members.[22] Likewise, in our patient with progressive hoarseness, who was diagnosed with STK4 deficiency, it was determined that a 3x3 cm lymphadenopathy had developed in the right axilla during this period. Following diagnostic evaluation, the patient was diagnosed with stage IIIB diffuse large B-cell lymphoma. After chemotherapy, physical examination revealed regression of cervical and axillary lymph nodes, while a new lymphadenopathy measuring 1.5x1 cm was detected in the supraclavicular region. The biopsy performed due to suspicion of the disease was found to be consistent with lymphoma. The patient was started on chemotherapy once again and, during follow-up, underwent a bone marrow transplant and recovered. If a systemic evaluation had not been performed at the end of treatment, the disease could have progressed, and the patient could have died. In conclusion, we can conclude that a systemic examination is necessary for early diagnosis and treatment.

Although laryngeal lymphoma is rare, certain imaging features suggest this diagnosis. A homogeneously growing supraglottic tumor without central necrosis is the characteristic finding of lymphoma and it shows limited diffusion on diffusion-weighted imaging.[23] There was no necrosis in the imaging findings of our first case, and a soft tissue lesion at the supraglottic level with diffusion restriction was detected in the diffusion-weighted images. Although imaging methods are helpful, a definitive diagnosis must be confirmed by biopsy.[10]

The current standard treatment of laryngeal lymphoma is chemotherapy, although some previous studies have shown that it can be successfully treated and remission can be achieved with radiotherapy.[1] There is an increase in treatment success in high-risk patients with the addition of monoclonal antibodies such as rituximab to existing treatment regimens.[24]

Table I. Laryngeal lymphoma in children and adolescents reported in the literature.

Case	Ref / year	Age(y) / Sex	Site	Stage	Histopathologic subtype	Therapy	Follow-up
1	Wang[7], 1972	14/M	Supraglottis (aryepiglottic fold, epiglottis, arytenoid, and the interior of the larynx)	I	NHL, NOS	RT	Alive wod, 8.5 y
2	Cohen[8], 1987	4/F	Supraglottis, glottis, subglottis (entire supraglottic area, the right true vocal cord and false vocal cord, extending into the immediate subglottic area anteriorly along the anterior commissure)	I	NHL, NOS	CT, RT	Alive wod, 2 y
3		9/F	Supraglottis and subglottis (epiglottis, glossoepiglottic ligaments, pharyngoepiglottic ligaments, arytenoids, aryepiglottic folds, false vocal cords, subglottic area)	I	NHL, NOS	CT, RT	Alive wod, 2 y
4	Palenzula[9], 2002	15/M	Supraglottis, glottis, subglottis (left supraglottis, left vocal cord, the subglottic area from the level of the cricoid cartilage to the left vocal cord)	I	DLBCL, EBER+	CT, RT, tumor debulking by laser	Died
5	Naik[4], 2012	10/M	Supraglottis, glottis (lesion in the right pyriform fossa extending to the right true and false vocal cords, aryepiglottic folds)	II	DLBCL	CT	Alive wod, 13 mo
6	Rodriguez[10], 2014	8/M	Supraglottis, glottis (epiglottis, ventricular bands, glottis)	I	T-LBL	CT	Died, 16 mo
7	Martin[11], 2017	14/F	Supraglottis (epiglottis, left arytenoid, post cricoid area)	I	LBCL, IRF4 (+)	Surgery, CT	Alive wod, 1 mo
8	Perez[12], 2019	13/M	Supraglottis (right aryepiglottic fold, right supraglottic mass)	I	DLBCL	Surgery, CT	NA
9	Tsur[13], 2021	13/M	Supraglottis, glottis (right false vocal cords, aryepiglottic folds, a cystic lesion on the right vocal cord bulging into the trachea and causing partial narrowing of the larynx)	II	DLBCL	CT	NA
10	Munjal[14], 2021	7/M	Supraglottis, glottis (swelling of the glottis and supraglottic soft tissues with patent airway)	IV	DLBCL, EBV+	Oncologic treatment	Died, within a few mo
11	Ayyaswamy[3], 2022	9/M	Supraglottis (a single globular mass with an irregular surface arising from epiglottis extending till vallecula and base of tongue)	I	DLBCL	Surgery, CT	Alive wod, 12 mo
12	Present cases	6/M	Supraglottis (left aryepiglottic fold)	II	Mature B-cell NHL	CT	Alive wod, 24 mo
13			Supraglottis (false vocal cords, arytenoid, aryepiglottic fold)	III	Mature B-cell NHL, EBER+	CT	Alive wod, 30 mo

CT: chemotherapy, DLBCL: diffuse large B-cell lymphoma, EBER: Epstein-Barr encoding region, EBV: Epstein-Barr virus, F: female, IRF4: interferon regulatory factor 4, LBCL: large-B-cell lymphoma, M: male, mo: month, NA: not available, NHL: non-Hodgkin lymphoma, NOS: not otherwise specified, RT: radiotherapy, T-LBL: lymphoblastic T-cell lymphoma, wod: without disease, y: years.

Surgery may be necessary only in the case of laryngeal obstruction and massive bleeding.[10] Among pediatric patients that we reviewed in the literature, four received radiotherapy before 2003, 11 received chemotherapy, and three underwent surgical procedures. Detail of the oncologic treatment was not present for one patient.

The prognosis of patients with laryngeal non-Hodgkin lymphoma is generally good. In the study of Zhao et al., the overall survival rate was 69.4%.[22] One of the prognostic risk factors is the stage.[24,25] All but one of the pediatric cases with laryngeal lymphoma in the literature had stage 1-2 disease. Another prognostic risk factor is the cellular subtype (B or T-cell). T cell subtype is associated with a worse prognosis.[24,25] One patient among children diagnosed with laryngeal lymphoma in the literature was of the T cell subtype and died despite receiving chemotherapy.

The feature of this article as a case report naturally carries some limitations. However, we compiled and evaluated cases from the literature to reduce these limitations by increasing the number of cases.

In conclusion, symptoms such as dysphonia, dysphagia, and dyspnea in children are primarily attributed to infections, inflammatory conditions, or pubertal voice changes. Flexible or direct laryngoscopy should be performed in all patients with persistent dysphonia to exclude laryngeal tumors. Differential diagnosis in pediatric cases should include lymphoma.

Ethical approval

Written informed consent was obtained from the parents for this publication.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ÇC, NK, TK, TY, EB, AÜ; data collection: ÇC, NK, TK, TY, EB, AÜ; analysis and interpretation of results: ÇC, NK, TK, TY, EB, AÜ; draft manuscript preparation: ÇC, NK, TK, TY, EB, AÜ. All authors reviewed the results and approved the final version of the manuscript.

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