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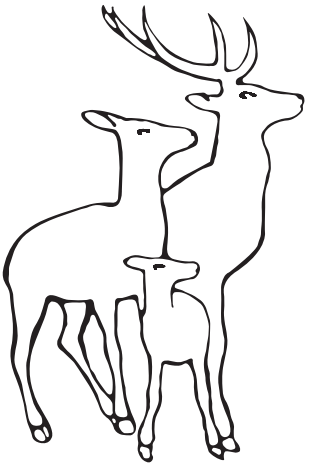

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Review

Systemic treatments in atopic dermatitis in children

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ABSTRACT

Atopic dermatitis (AD) is a very common skin disease caused by inflammatory reactions, in which the main symptoms of severe itching and recurrent eczema diminish quality of life. As epidermal barrier function and the immune system play a critical role in atopic dermatitis, promoting IgE-mediated sensitization can be the main targets of AD treatment. The goal of AD treatment should be to eliminate the symptoms and obtain long-term eczema control with a multi-step approach adapted to the severity of the disease. Basic management for all patients comprises the use of moisturisers and avoiding triggers. While topical therapy is effective for most children diagnosed with AD, there may also be children who require systemic therapy. The aim of this paper was to present an extensive review of the systemic agents commonly used in childhood atopic dermatitis which mainly target cutaneous inflammation.

Key words: atopic dermatitis (AD), children, systemic treatment.

Atopic dermatitis (AD) is a very common skin disease caused by inflammatory reactions, although the mechanism is still not fully understood.[1,2] Severe itching and recurring eczema are the main symptoms of the disease, causing a significant morbidity burden and diminishing the quality of life of patients and their families.

The target of AD treatment should be to eliminate symptoms and obtain long-term eczema control with a multi-step approach adapted to the severity of the disease. Basic management for all patients comprises the use of moisturisers and avoidance of triggers.[3] Oral antihistaminics are not recommended as there is little evidence for the effectiveness of these drugs, so they have no place in the treatment of AD.

Topical corticosteroids are the main drugs for moderate to severe AD. For more severe AD patients, the use of systemic anti-inflammatory drugs may be needed, but because these drugs can have serious side-effects, treatment is sometimes interrupted, which reduces the effectiveness. Therefore, there has recently been increasing interest in therapies with large protein structures to be injected with targeted biological agents, which will act on the pathways directly responsible for AD, without penetrating the lipid bilayer cell membrane.[4]

In cases where topical treatments and phototherapy are not sufficient, it may be necessary to switch to systemic immunosuppressive therapy.[2]

The objective of this paper was to present an extensive review of the systemic agents commonly used in childhood atopic dermatitis which mainly target cutaneous inflammation.

Pathogenesis of Atopic Dermatitis

Any condition that disrupts the epidermal function is predominant in the pathogenesis of AD.[4,5] A proinflammatory microenvironment can sometimes be seen in AD, even in skin without lesions. This proinflammatory microenvironment is created by an increase in Th-2, Th-22 and sometimes Th-17 cells.[6,7] Allergens, irritants and microbes are the most

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important cause of the disrupted skin barrier, leading to local inflammation and related immune responses.[8]

Atopic dermatitis develops due to a complex interplay of factors, which encompass genetic elements, an ineffective epidermal barrier, and type-2 dominant cutaneous inflammation. Individuals who have a genetic mutation in the filaggrin (FLG) gene have an increased susceptibility to AD. Filaggrin is a protein that is involved in the structure of the skin barrier. Non-adaptation of the barrier, which can also be due to mechanical causes, results in enhanced *S. aureus* settlement, susceptibility to cutaneous infections, recurrent stretching, and changes in the skin microbiome.[9,10]

Cutaneous inflammation in AD sends signals to B cells and promotes antigen-specific IgE production with the expression of IL-13 and IL-4 from activated Th2 cells, and thus Th cell-mediated pathways are formed (Fig. 1).

Cytokine-based endotypes in different age or ethnic groups have helped us comprehend atopic dermatitis. New biologics or small compounds can personalise atopic dermatitis treatment.[11] Four different subtypes were defined in the European-American group. These are acute, chronic, intrinsic, and extrinsic (classic). While the extrinsic form is the more common form with elevated IgE and eosinophilia in the atopic background, the intrinsic form does not increase IgE and does

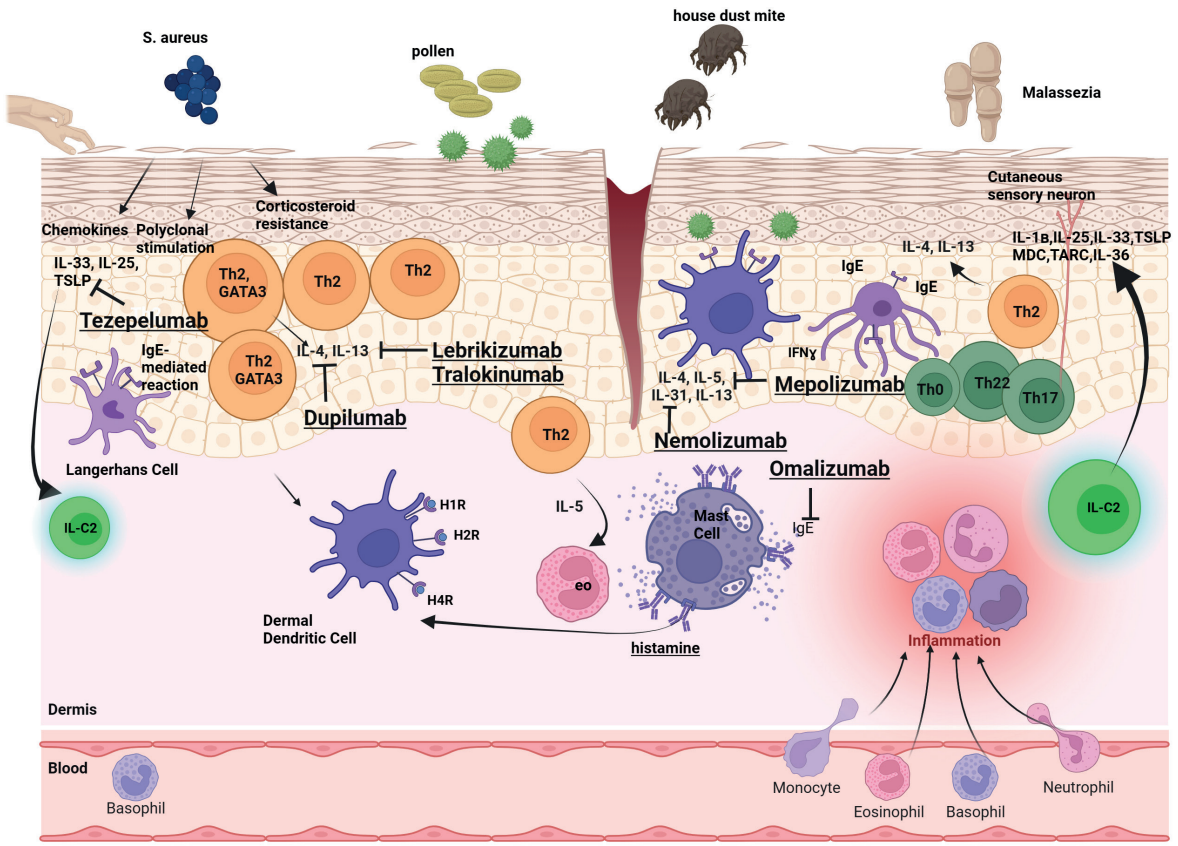

Fig. 1. Pathogenesis and treatment strategies for atopic dermatitis.

It is mediated by epidermal Langerhans cells, inflammatory dendritic epidermal cells and dermal dendritic cells in the lesioned skin of patients with atopic dermatitis.[2] These cells bind to IgE, they can also bind allergens that cause immediate-type allergic reactions and induce delayed-type T cell-mediated reactions. The deterioration of the epidermis and the deterioration of skin integrity are processes that support each other. Chemokines invoking T cells, cytokines that mediate innate immune responses, and Th2 cell and Langerhans cell activation have been demonstrated.[112,113]
(IL-33: Interleukin 33, IL-25: Interleukin 25, TSLP: Thymic Stromal Lymphopoietin, IL-C2: Type 2 Innate Lymphoid Cell, Th:T helper, Eo:Eosinophil).

not show atopic background, but cytokines and epidermal barrier damage are similar.[12] While Th1 is not seen in the pediatric group, Th2 and Th22 are increased in all ethnic groups, and Th17 is increased more in Asians and pediatric groups. Th2-Th22-Th17 are increased more in intrinsic and chronic subtypes.[12]

As a result, epidermal barrier function and the immune system play a critical role in AD and promote IgE-mediated sensitization, and can therefore be the main targets of AD treatment.[13]

Systemic Treatment

Although not safe in the long term and there is a tendency of reversal when discontinued, systemic corticosteroids have long been adopted as the only systemic drug used. Together with phototherapy, immunosuppressants are treatments that can be used as alternatives to steroids and prescribed off-label. However, the use of these drugs is not favored because they require frequent laboratory monitoring, have safety problems, and varying therapeutic benefits. Clarification of the unknown issues about the pathogenesis of AD will enable the development of goal-directed therapy, which may be more effective and safer than the treatments in current use.[14]

Janus Kinase inhibitors (JAKi), which regulate microbial dysbiosis, are new drugs used in the systemic treatment of AD.

Most biological agents for AD treatment are still in the testing phase. Oral JAKis have shown extraordinary efficacy and no serious signs of lack of safety.[15]

Systemic steroids

Corticosteroids are produced by the adrenal gland, which regulates the human stress response and immune system. While these drugs can be given in acute severe eczema flare-ups, they also act as a bridge with other treatments.[16]

The chronic intermittent use of corticosteroids is not recommended in AD, but can be used for transitional therapy in severe, rapidly progressing cases when initiating non-steroidal immunomodulatory agents or phototherapy.[16] Although patients and practitioners may notice immediate improvements in AD with systemic steroids, other systemic medications with fewer side-effects should also be considered.

The main side-effects associated with systemic steroids, which can be seen with prolonged use, include Cushing's syndrome, elevated blood sugar levels, osteoporosis, and gastric complaints.

Cyclosporine (CycA)

Cyclosporine (CycA) shows its effect by inhibiting calcineurin receptors and preventing IL-2 proliferation. This cytokine is vitally important for TH, NK cells, monocytes, and T regulatory lymphocytes. Therefore, inhibiting the activity and proliferation of T lymphocytes is possible by stopping the production of IL-2.[17]

Although it is not approved for use in children under 16 years of age, it is used in patients with refractory and severe AD.[18]

The advised dosage for CycA is 2.5 mg /kg, to a maximum dose of 5 mg/ kg.[17] CycA serum levels should be checked regularly and dose titration should be made according to the increase or decrease of symptoms. When clinical benefit is obtained, the treatment period can be extended up to 12 months.[19]

According to the results of a previous meta-analysis, after 8 weeks of treatment, almost 50% recovery was observed in the disease. The rapid onset of action of CycA allows for short-term use in several 12-week cycles or continuous use for up to 1-2 years.[20]

The most important side-effects include high blood pressure and nephrotoxicity. Therefore, close blood pressure monitoring is required throughout the treatment. In

the follow-up of nephrotoxicity, N-acetyl beta D-glucosaminidase measurements can be performed to determine renal tubular dysfunction, together with plasma creatinine level, and measurement of cystatin c, as in the TReat study.[21]

In a study by Jones et al.[22], in which 27 pediatric patients were treated with CycA for 6 weeks, the patients were followed up for disease activity and side-effects every 2 weeks via visual analog scales and quality of life questionnaires. Significant upgrading in disease activity was detected at all patient visits. Significant improvement or complete clearance was achieved in 22 of the 27 patients, with a significant increase in quality of life for both the patients and their family. The drug was well-tolerated in 25 patients.

In another study, 11 children with severe AD received 8 weeks of treatment with CsA. While 45% of the patients had only *S. aureus* skin colonization, 55% had suppurative *S. aureus* skin infection. All patients had a significant improvement in the clinical findings. The colonized patients showed greater reductions in disease severity and bacterial count with CycA. In conclusion, treatment with CsA in children with severe AD appeared to result in improvement in clinical symptoms.[23]

Choi et al.[24] retrospectively reviewed the use of CycA in dermatology centers. Changes in CycA dose schedules and disease severity were analyzed in 92 (64 eczema, 17 psoriasis) patients. The mean initial dose of CycA was started at 1.53 mg/kg/day and increased to a mean of 2.61 mg/kg/day in 6 months. A response to CycA was observed as early as 2 weeks, and disease control was achieved within 6 months. Although 32 patients used CycA for more than one year, only one patient had a creatinine increase of more than 30%.

In a placebo-controlled randomized study by Jin et al.[25], the SCORAD indices were seen to decrease significantly after the treatment in a population of children with moderate and severe AD unresponsive to topical treatments. Co-administration of glucosamine with CycA did not appear to increase serum CycA levels and there were no adverse events from CycA alone. It was shown that the combination therapy may be beneficial in the treatment of patients with severe AD in order to maintain CycA for a long time.

Permanent use of the drug was found to be more effective than intermittent use, and the dose of the drug should be individualized.

Azathioprine (Aza)

Azathioprine (Aza) is an immunosuppressant used for the treatment of AD, which shows its effect by suppressing proliferating cells. Aza, a purine analog, inhibits DNA-RNA synthesis and inhibits proliferation of B and T cells.[26]

In a study of children with severe AD, Aza was found to have an acceptable effect in the group with a normal thiopurine methyltransferase profile.[27]

Another study of children with a diagnosis of severe atopic dermatitis emphasized that thiopurine methlytransferase activity varied during treatment. Therefore, it was stated that it would be appropriate to make repeated measurements to adjust the dose of Aza.[28,29]

As there are insufficient studies and information about the long-term safety profile, there should be face-to-face discussions with the family if treatment is to be started. Aza has been associated with a number of hematological, hepatotoxic, and long-term cancer-related side-effects, especially non-melanoma skin cancer. As with any systemic therapy, a balance must be struck between the effects on longitudinal growth and neurodevelopment and the need to treat resistant eczema.[30]

The recommended daily dosage is 2-4 mg/kg and this should be checked by performing CBC at regular intervals due to the side-effect of cytopenia.[16]

Although it was stated by the Food and Drug Administration (FDA) in 2011 that there is an increased risk of hepatosplenic T-cell lymphoma, no patient has been diagnosed with this disease to date.[31]

Mycophenolate mofetil (MMF)

Mycophenolic acid (MMF) is a prodrug. MMF, which was used in the treatment of psoriasis in the 1970s and later found use in transplant patients, was also used by dermatologists in other skin diseases with inflammation due to its anti-inflammatory effect. MMF is lymphocyte specific and therefore has a low toxicity profile. Although these features make it a more preferable treatment option, the lack of randomized controlled studies currently limits its use due to potential unknown side-effects and high treatment costs.[32]

In a study which examined 140 patients with AD, there was seen to be a significant decrease in SCORAD scores with the use of MMF. The time of observed first effects was reported to be a mean of 6.8 weeks and relapses occurred in 8.2% of patients.[33]

In a retrospective study, it was determined that 42.8% of the patients switched from Aza treatment to MMF treatment due to intolerance and/or unresponsiveness to the drug. It was stated that 2/3 of the patients showed a significant improvement with the use of MMF and that significant side-effects occurred at a much lower rate.[34]

Methotrexate (MTX)

Methotrexate is an anti-inflammatory agent that acts by inhibiting cell division and lymphocyte proliferation. This effect is shown through the inhibition of the dihydrofolate reductase enzyme, resulting in the prevention of DNA/RNA synthesis and cell division.[35] Low-dose methotrexate is an alternative therapeutic method for severe AD unresponsive to topical treatments. The onset of the effect is slower than that of CycA and systemic corticosteroids. Treatment response typically begins after at least 1.5 months. Dosing in children with AD is 0.2-0.7 mg/kg once a week, and it can be administered orally or subcutaneously.

Purvis et al.[36] reported that it was well tolerated and effective in the results of a study in which 0.33 mg/kg MTX was administered to 43 children aged 2-16 years for 17 months, and improvements in AD were seen in 36 of the 43 patients. A decrease of 50% was determined in patients hospitalized for AD after MTX treatment was started, and the average follow-up period after MTX was two years. Of the children who benefited from MTX, 16% relapsed, and it was stated that MTX should be restarted.

There are few studies on MTX for pediatric AD patients. Only one small study compared MTX with CycA, and the data obtained in the comparison results were generally similar. Previous reports have shown that MTX is cost-effective and clinically effective in pediatric AD patients.[37]

Studies have shown that a low dose (5-15 mg/week orally) is effective for AD.[38]

The most important side-effects of methoteraxate are nausea, elevated liver enzyme levels, and bone marrow supression.[21] It has also been shown that pulmonary fibrosis, although extremely rare, can also be a complication.

Other Therapies

Phototherapy

Phototherapy is an alternative treatment for AD patients, which has been widely used as a proven second-line therapy. However, it has only been evaluated for short-term control and has only been tested in intensive programs of two or three sessions per week. Long-term control of the disease requires a new phototherapy regimen that balances the risks from ultraviolet (UV) exposure and patient acceptance.

In a study by Clayton et al.[39], 50 children (83%) received at least 10 narrow-band UVB treatments. In 40% of these children, complete or minimal recovery was obtained. It was stated that good improvement was obtained in 23% of the children, while a moderate improvement was obtained in 26%. The mean remission period of the treatment was found to be 3 months, which was accepted as an indication that the treatment was easily tolerated.

Narrow-band UVB centered at 311-313 nm is accepted as the first choice agent for some photosensitive dermatoses because it is safer and easier to apply than psoralen-UVA. Studies have shown that narrow-band UV phototherapy is much more effective and less erythematogenic than broadband phototherapy.

Phototherapy may be a suitable treatment alternative for AD patients who do not benefit adequately from topical treatments. Wavelength and treatment plan should be determined according to the patient and the severity of the disease.[16] Although home phototherapy application methods have been shown to reduce the treatment burden for other diseases, no clinical studies have been found on AD.

Extracorporeal photochemotherapy is not recommended for the routine treatment of AD because it varies considerably between patients.[16]

Allergen immunotherapy

Atopy is present in 70% of AD patients, and exposure to aeroallergens is one of the major causes of acute exacerbations.[40]

The preventative function of allergen immunotherapy to prevent atopic march, which is a serious problem in children with AD, has not been proven as yet.[41] There are studies indicating that allergen immunotherapy can be used in the treatment of patients with severe AD if they also have allergic rhinitis and/or asthma.[42,43]

There are many studies showing the efficacy and safety of AIT in AD. Although it is generally seen as a risk that may cause worsening of the disease when used in AD, it can be said to be an option that improves the course of AD when used in appropriate cases.[44]

Biological Agents and New Treatment Strategies

Dupilumab

The development of biological therapies targeting AD is very important. Dupilumab is the only biological agent that received FDA approval for the following indications in 2019: adolescents aged 12 years and above; children aged 6 years and above; and most recently, neonates aged 6 months and above (as of June 2022). It provides patients with a safe and long-lasting alternative.[45] Accelerated FDA approval was obtained after comparing dupilumab in combination with topical corticosteroid (TCS) and TCS alone in terms of efficacy and safety (Table I).[46,47]

Dupilumab is a monoclonal antibody that inhibits the production of IL-4 and IL-13 while maintaining immune system functionality.[48] Patients aged 12 years and over, diagnosed with moderate-to-severe asthma or patients with chronic sinusitis with nasal polyps are suitable patients for the use of dupilumab. There are extended reports of sustained benefits in adolescent patients continuing dupilumab therapy, resulting in greater enhancement in EASI scores at 1 year.[49]

In a study of combined dupilumab + TCS treatment for 16 weeks in patients with severe AD who did not benefit sufficiently from topical treatments, there was a significant and rapid enhancement in clinical findings. As most measures of efficacy show improvement at week 16, greater benefit is possible with a longer treatment duration. A significantly greater improvement in the treatment group compared to the placebo group was demonstrated at week 16 (73% and 18%, respectively).[50]

In a study by Bosma et al.[51], adults and children who started treatment with dupilumab were evaluated with the aim of comparing dupilumab with other immunosuppressant treatments. Although efficacy was similar to other systemic immunomodulatory drug treatments, dupilumab was found to be the most preferred treatment for severe disease requiring systemic agents. This was suggested to be due to a lack of availability or responsiveness to other immunomodulatory therapies rather than access to new systemic agents and disease severity.

In a study of dupilumab in an adolescent patient population, conducted by Simpson et al.[52], dupilumab monotherapy was found to result in statistically and clinically noteworthy improvements in disease signs and symptoms. Dupilumab has an adequate safety profile.

Omalizumab

Omalizumab is another monoclonal antibody produced by recombinant technology, which binds free IgE. It inhibits the binding of IgE to IgE receptors on cells such as mast cells and basophils.[53-55]

In addition to premedication in allergen-specific immunotherapy, it has been used in the treatment of many allergic diseases.

In a randomized controlled trial (RCT) by Iyengar et al.[56] of 8 patients with severe, treatment-resistant AD, 50% of the patients received omalizumab and the other 50% received a placebo. Previous eczema medications were standardized among the patients. Basal blood IgE levels were documented. All medications were discontinued one week before the start of the study. AD scoring was performed using the SCORAD index at monthly visits. A 20-50% decrease in SCORAD values was noted in the omalizumab group, compared to a 45-80% decrease in the placebo group. Both small RCTs failed to demonstrate the superiority of omalizumab over a placebo in AD.[57]

In the ADAPT study by Chan et al.[58], 62 children were recruited and evaluated. The difference between the groups in the improvement of the SCORAD index measured at week 24 was -6.9. Children's Dermatology Life Quality Index in the omalizumab group was improved. Although less potent topical corticosteroids were used in the group receiving omalizumab, a greater decrease in the severity of the disease was determined. Considering the positive side-effect profile, further studies on omalizumab are required to investigate its use in this difficult-to-treat patient group (Table I).

The benefit of the treatment became more pronounced towards the 24th week and it was observed that the effects continued after the treatment was stopped. More studies are needed on the optimal duration of treatment.[59]

Mepolizumab

Mepolizumab is a monoclonal IL-5 antibody, which reduces eosinophils in the blood. In a study of 40 patients diagnosed with AD, it was shown that mepolizumab was not effective. Although a decrease was detected in the amount of eosinophils in the blood, when the skin biopsy was examined, it was not found to have caused any change in the number of eosinophils in the tissue (Table I).[60]

Rituximab

It is known that CD20 is predominant in the pathogenesis of AD. Rituximab is a monoclonal anti-CD20 antibody developed against CD20. In a study using rituximab in patients with AD, 2 doses of 1000 mg of rituximab were given at 2-week intervals, and the EASI score, which was 29.4 at baseline was measured as 8.4 in the 8th week.[61]

Interferon- gamma (IFN- ɣ)

It is known that in AD, IgE levels increase while IFN gamma (IFN- ɣ) production decreases.[53,62] IFN- ɣ is one of the cytokines that has an important place in both the innate and acquired

immune systems. While the production of natural killer cells increases with the effect of IFN-ɣ, it also increases the oxidation of macrophages.

Studies have shown that IFN-ɣ is moderately effective in the treatment of AD, so IFN-ɣ should only be considered as an alternative drug in refractory patients who do not respond to other systemic treatments or phototherapy, or in patients with contraindications.[63] Fatigue, fever, nausea, vomiting, and myalgia are side-effects that can be seen after use. There is no specific recommendation for the pediatric age group.[16]

Biological Agents in Trials

Nemolizumab

Nemolizumab is another monoclonal antibody that blocks IL31 receptors.[64] IL31 is an important cytokine that mediates the formation of pruritus, which is known to occur during the itch-scar cycle in AD that causes disruption of the skin barrier.[65]

In a randomized controlled study, patients were separated into 3 groups, given 0.1 mg/kg, 0.5 mg/kg and 2 mg/kg nemolizumab treatment for 4 weeks, respectively. When the results were compared with the placebo in the 12th week, although the SCORAD-50 and SCORAD-75 results of the patients receiving 0.5 mg/kg were found to be better, no superiority was determined over the placebo when the EASI-50 and EASI-75 results were examined. There was no difference between the placebo group and other groups in terms of patients who dropped out of the study due to adverse events (Table I).[66]

Ongoing Phase 2/3 studies are examining the effects of nemolizumab on infants and adolescents (NCT03921411, NCT04921345, NCT03985943, NCT03989349, NCT03989206). Current research suggests that in conjunction with nemolizumab (rescue therapy), topical treatments including moisturisers, topical corticosteroids, and calcineurin inhibitors may have a synergistic effect in the treatment of AD and associated pruritus.[18]

Tezepelumab

Tezepelumab is an IgG2 monoclonal antibody that binds to TSLP.[67] When the lesions of acute or chronic AD patients were examined, it was observed that there was TSLP over-expression in keratinocytes.[68] At the same time, high levels of TSLP were detected in the blood of AD patients.[69]

TSLP is a key AD molecule, according to research. TSLP produced by epidermal keratinocytes in response to stimuli interacts with a subpopulation of sensory neurons, enhancing Th2 itch responses. For AD prevention or improvement, TSLP is a prospective therapeutic target.[70,71] Tezepelumab is a human monoclonal IgG2λ antibody. Tezepelumab circulates TSLP by binding to the receptor and disrupting TSLP's interaction with it, suppressing downstream inflammatory processes.[72] Tezepelumab is used in phase 1 (NCT00757042) and phase 2a (NCT02525094) randomised, double-blind, placebo-controlled AD investigations.[73]

A phase II study on this has been conducted on an adult population, but as yet there is no pediatric study in the literature (Table I).

Tralokinumab- lebrikizumab

These drugs are monoclonal antibodies which have been developed against IL-13. By binding to free IL-13 with high affinity, they are involved in the prevention of factors that cause damage to the epidermal barrier.[74] Consequently, IL-13 cannot bind with IL-4Ra, and thus IL-4Ra / IL-13 receptor alpha 1 heterodimerization cannot be established[75] Il-13 also inhibits the production of the filagrin protein.[76]

Tralokinumab works in much the same way, but by inhibiting IL-13 from binding to both IL-13 receptor alpha 1 and IL-13 receptor alpha 2.

The FDA approval of tralokinumab is "indicated for the treatment of adults with moderate-to-severe AD, when adequate control is not achieved with topical prescription treatments or the use of these treatments is not recommended".[77] The European Medicines Agency (EMA) approval of tralokinumab is "indicated for the treatment of adolescent and adult patients 12 years of age and older with moderate-to-severe AD who are candidates for systemic therapy".[78]

Lebrikizumab is a high affinity humanized IgG4 mAb that inhibits IL-13 signaling by blocking IL-13Rα. Adolescent and adult studies of lebrikizumab are under submission for FDA approval.

In a randomized controlled study, it was shown that lebrikizumab was effective on many clinical signs in adults with moderate-to-severe AD, and had a favourable safety profile. If these results are also obtained in phase 3 studies, then the drug can be approved for use in the treatment of AD. However, no study has been started in the pediatric age group (Table I).[79]

ISB-830

ISB-830 is another monoclonal antibody developed to inhibit OX40.[80] OX40 (CD 134) is a co-stimulatory molecule of the TNF family, predominantly expressed in T cells. The interaction of this molecule with OX40L increases cytokine production by bridging the Th2 and Th1 pathways. It has been shown that there is greater expression of OX40 in AD lesions and the number of OX40L +DCs is greatly increased in AD patients.[81]

An evidence-based clinical trial by Yassky et al.[80] was the first to target a co-stimulatory immunomodulatory molecule to treat AD patients. It was found that anti-OX40 antibodies administered 1 month apart provided significant improvements in clinical scores and cutaneous findings, lasting up to day 71 (Table I).

Anti IL-17 therapy

IL17 and its associated cytokines have some functions in the inflammatory process of AD. Expression of IL-17 in the skin is thought to cause the skin to form a defence mechanism against foreign substances.[82]

Small Molecules "Janus Kinase Inhibitors"

The small molecules of these drugs make them ideal for topical or oral use. Small molecule therapies are reported to be safer in terms of side-effects when compared to other systemic immunosuppressant agents. The most important reason for this is that these drugs suppress the immune pathways more selectively. Janus kinase inhibitors prevent the signal created by the activation of certain cytokine receptors, and have the advantage of oral use with flexible dosing regimens.[83,84]

The JAK family includes four molecules (Fig. 2) that are necessary for intracellular signaling via multiple cytokine receptors, including for type 2 interleukins.[83,85]

JAK inhibitors can be used orally as well as in topical forms. The fact that they do not increase immunosuppression and their pharmacokinetics are clear may make them superior to other drug groups.

Abroticinib

Abrocitinib inhibits JAK1 in a selective inhibitor. For the multicenter JADE-MONO study, patients older than 12 years with an EASI score greater than 16 and an IGA score greater than 3 were randomised into 100 mg, 200 mg, or placebo groups. Monotherapy with once-daily oral abrocitinib for patients with moderate to severe AD was demonstrated to be effective and well tolerated.[86]

In another RCT (JADE TEEN), two groups of adolescent patients were given 100 mg and 200 mg of abrocitinib, respectively, and the results were compared with a placebo. When

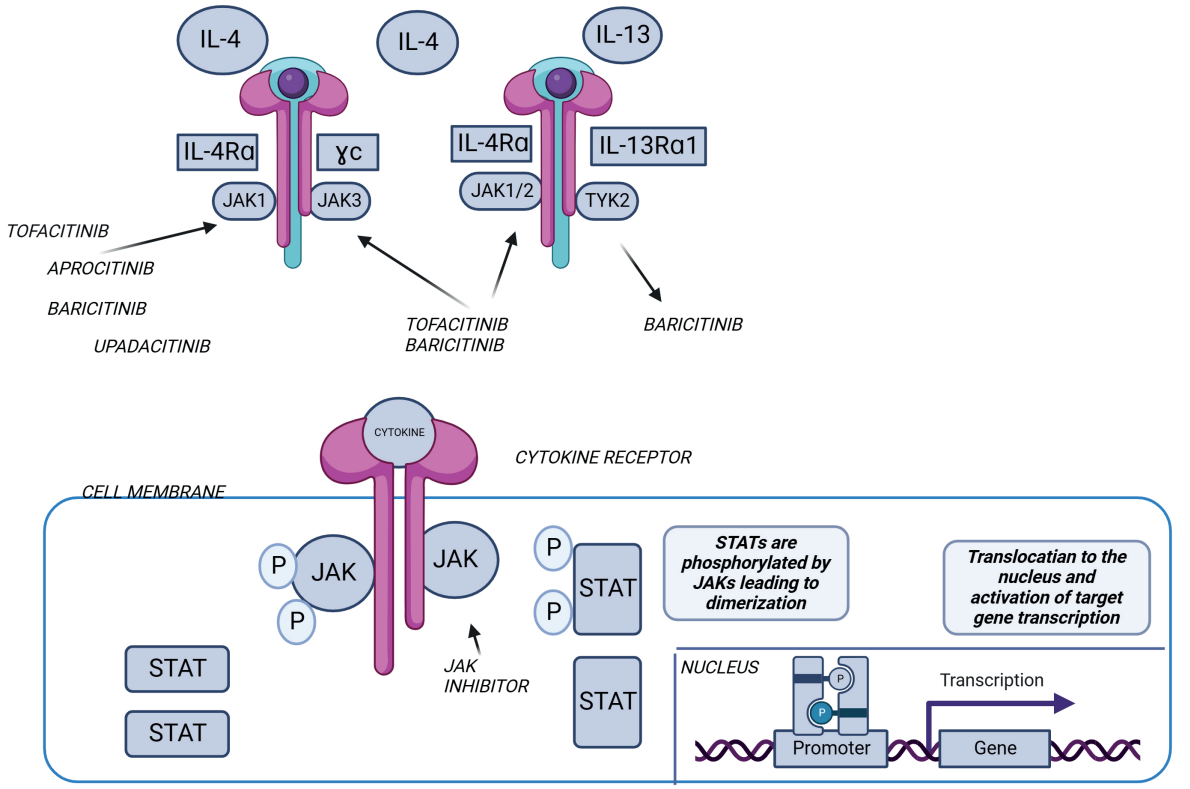

Fig. 2. JAK-STAT Signaling Pathway and JAK Inhibitors Treatment for Atopic Dermatitis.
IL: Interleukin, STAT: Signal transducer and activation of transcription, JAK: Janus kinase.

primary outcomes were evaluated, a greater improvement was found in the patients receiving abrocitinib therapy (Table II).[87]

In another study, which compared dupilumab with abroticinib, no significant difference was found between the outcomes of patients receiving abroticinib and dupilumab at week 16.[88]

Abrositinib FDA approval is "indicated in the treatment of children aged 12 years or older with moderate to severe atopic dermatitis and adults, when adequate control cannot be achieved with other systemic treatments (including biologics) or the use of other treatments is not recommended".[89]

Abrositinib EMA approval is for adult patients aged 18 years and older with moderate to severe atopic dermatitis who are candidates for systemic therapy.[89]

Nasopharyngitis, nausea and headache are the most common complaints in patients receiving abroticinib treatment. In addition, transient thrombocytopenias have been observed depending on the drug dose in patients using abroticinib, but these changes were not considered significant. Inhibition of JAK also affects the hematopoietic system and platelet homeostasis.[90]

Upadacitinib

The selective JAK1 inhibitor upadacitinib has been approved for the treatment of moderate-to-severe AD in patients 12 years and older.[91] The recommended dose is 15-30 mg once a day, depending on the severity of the disease.[92]

In a study comparing patients treated with 15 mg and 30 mg upadacitinib to a placebo group, both upadacitinib groups achieved primary goals for EASI and IGA scores at

week 16. According to the results at 52 weeks, upadacitinib was determined to be sufficiently effective and safe.[93]

In a similar study involving patients older than 12 years, 15 mg and 30 mg upadacitinib doses were administered to patients and compared with a patient group receiving topical corticosteroids. The most significant decrease in EASI and IGA scores at week 16 was observed in both upadacitinib groups (Table II).[94,95]

In another study of adult patients, when the adverse effects seen in patients treated with dupilumab and upadacitinib were evaluated, eczema herpeticum, herpes virus infections and other infections were more common in the upadacitinib group, and conjunctivitis and wound infections were more common in the dupilimab group.[96]

Upadacitinib, which has received approval from the Food and Drug Administration (FDA), is prescribed for the management of moderate-to-severe atopic dermatitis in children and adults aged 12 years or older and weighing a minimum of 40 kg. This treatment is indicated when other systemic treatments (including biologics) fail to achieve satisfactory control or when the use of alternative therapies is not advised.[89]

Systemic therapy is authorised by the European Medicines Agency (EMA) and is available to patients with moderate to severe atopic dermatitis who are 12 years of age or older.[97]

Baricitinib

JAK1 and JAK2 are selectively inhibited by baricitinib.[91] Baricitinib's pharmacokinetic efficacy in paediatric patients with moderate to severe AD is the subject of an ongoing Phase III study that has not yet reached a conclusion (Table II).[98]

According to the results obtained from previous studies conducted on adults, upper respiratory tract infections and herpes simplex infection were stated as the most common side-effects, and the most serious side-effects were evaluated as eczema herpeticum, cellulitis and pneumonia. When the results of studies on baricitinib were evaluated in general, cardiovascular events and thromboembolic events were stated as the two major side-effects.[15,99,100]

Conclusion

A better understanding of the pathogenesis of AD has provided a step-by-step approach supporting the use of targeted therapies with biological agents in treatment. However, there are still many issues that need to be clarified, such as the definition of treatment response, strategies to increase the response rate, the duration and regimen of treatment (in-clinic or at home), cost-effectiveness, and long-term safety.[101]

In order to demonstrate how safe the drugs described above are, long-term follow-up of the patients is required after the treatment. Therefore, there is a need for RCTs to be conducted in this way. It is not only very difficult to elucidate the pathogenesis of AD, but the treatment of the disease is also just as difficult and complex.

Ethical approval

No ethical approval was required.

Author contribution

The authors confirm contribution to the paper as follows: DIG, OS made the literature search and wrote the whole paper, UMS supervised the whole process and prepared the structure of the review.

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Conflict of interest

The authors declare that there is no conflict of interest.

Table I. Biologics for moderate to severe atopic dermatitis.

Molecule	Target	Clinical Development Phase	Advantages	Disadvantages
Dupilumab (Regeneron/Sanofi)	IL-4Ra	Phase 4	Decrease pruritus, eruption[102] and TARC, periostin, IL-22[103] Does not affect pharmacokinetics of medications metabolized by CYP enzymes[104]	Injection site reaction Conjunctivitis[52]
Omalizumab (Xolair) Novartis Pharmaceuticals	Anti-IgE	Phase 4	Bind and neutralized free circulating IgE58 Decrease basophil and dendritic cell FcεRI expression[105]	Anaphylactic reactions[105]
Mepolizumab (GlaxoSmithKlein)	IL-5	Phase 2	No meaningful differences were observed.[106]	Diarrhea Impetigo[106] No study in the pediatric age group
Rituximab	Anti -CD20	Not applicable	Histological alteration (Hyperkeratosis, spongiosis, acanthosis Depletion B cell and T cell activation in blood[61]	Further clinical studies are needed[61]
Tezepelumab (Astra Zeneca Amgen)	TSLP	Phase 2b	No significant change in EASI50 from baseline[107]	No study in the pediatric age group
Lebrikizumab (Eli Lily and Company)	IL-13	Phase 3	Decrease pruritus Well tolerated in adults No efficacy and safety data results[108]	Conjuctivitis Upper respiratory tract infection Headache Nasopharyngitis İnjection site reaction[109] No efficacy and safety data results There are currently ongoing phase 3 trials in pediatric patients[108]
Tralokinumab (LEO Pharma)	IL-13	Phase 3	Decrease pruritus No efficacy and safety data results[108]	Upper respiratory tract infections Conjuctivitis Headache Nasopharyngitis[109] Currently undergoing phase 3 trials in pediatric patients[108]
Nemolizumab (Galderma)	IL-31	Phase 2	Decrease pruritus[110,111] No efficacy and safety data results[108]	Upper respiratory infection Nasopharyngitis Injection site reaction Triggered asthma symptoms (in patients with a history of asthma) No efficacy and safety data results Currently undergoing phase 3 trials in pediatric patients[108]
ISB-830 Ichnos Sciences SA Glenmark Pharmaceuticals SA	OX-40	Phase 2b	Well tolerated Changes in epidermal hyperplasia and gene expression Reduce keratin, ki67, epidermal thickness, mRNA expression[72]	Intravenous administration Nasopharyngitis[72] No study in the pediatric age group

CYP: cytochrome P450, TARC: thymus- and activation-regulated chemokine.

Table II. Janus Kinase Inhibitors for the treatment of moderate to severe atopic dermatitis.

Molecule	Target	Clinical Development Phase	Advantages	Disadvantages
Abrocitinib (Pfizer)	JAK 1	Phase 3	Selective inhibitor Orally once daily Decrease pruritus	Acne Nasopharyngitis Headache Upper respiratory tract infection Herpes Zoster Conjunctivitis[88] Currently ongoing phase 3 trials in pediatric patients
Upadacitinib (AbbVie)	JAK 1	Phase 3	Selective inhibitor Orally once daily Decrease pruritus[96]	Acne Conjunctivitis Transaminase elevation Egzema herpeticum and Herpes zoster One death reported (due to influenza associated pneumoniae)[96] Currently ongoing phase 3 trials in pediatric patients
Baricitinib (Eli Lily and Company)	JAK1/2	Phase 3	Orally once daily Rapid and sustained reduction in itch sensation[99]	Viral infections Herpes Simplex, eczema herpeticum, Headache Venous thrombosis[13] Currently ongoing phase 3 trials in pediatric patients

JAK: Janus kinase.

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Review

A scoping review of the management of acute mastoiditis in children: What is the best approach?

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ABSTRACT

Background. Acute mastoiditis (AM) is a severe infection of the mastoid air cells that occurs in cases of acute, sub-acute, or chronic middle ear infections. No definitive consensus regarding the management of AM has been identified. The current guidelines include a conservative approach (parenteral antibiotics alone, antibiotics plus minor surgical procedures such as myringotomy with a ventilation tube inserted or drainage of the subperiosteal abscess through retro-auricolar incision or needle aspiration) or surgical treatment (mastoidectomy). The main aim of this review was to evaluate and summarize the current knowledge about the management of pediatric AM by analyzing the current evidence in the literature.

Methods. We examined the following bibliographic electronic databases: Pubmed and the Cochrane Library, from the inception date until February 2023. The search was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISM). The key words used for the search across electronic databases were: 'mastoiditis' and 'management'; 'mastoiditis' and 'surgery'; 'mastoiditis' and 'conservative'; 'mastoiditis' and 'antibiotics'; 'mastoiditis' and 'myringotomy'; 'mastoiditis' and 'grommet'; 'mastoiditis' and 'drainage'; and 'mastoiditis' and 'mastoidectomy'.

Results. We selected 12 articles involving 1124 episodes of mastoiditis. Some of these studies considered medical therapy alone as a valid first step, whereas others considered a minor surgical intervention as an initial approach along with antibiotic therapy. Considering the studies that evaluated medical therapy as the initial sole treatment option, the success rate of antibiotics alone was 24.6%. Overall, the success rate of minor surgical procedures, excluding mastoidectomy, was 87.7%, whereas the mastoidectomy success rate was 97%.

Conclusions. Overall, there is no shared consensus on the diagnostic or therapeutic approach to mastoiditis. Conservative therapy has gained considerable ground in recent times, quite limiting the predominant role of mastoidectomy. Further studies will be necessary to definitely develop standardized protocols shared in the scientific community.

Key words: mastoiditis, children, management, antibiotics, mastoidectomy.

Acute mastoiditis (AM) is a severe infection of the mastoid air cells that occurs in cases of acute, sub-acute or chronic middle ear infections.[1] Even though the introduction of antibiotics has reduced the incidence of AM, it remains the main intratemporal complication of acute otitis media (AOM).[2] It involves about 1/400 cases (0.24%) of AOM, with an incidence varying from 1.2 to 6.1 per 100.000 children aged 0–14 years and a peak occurring at 2–3 years.[3,4] Clinical signs and symptoms are the first tool to identify AM and most authors agree that its diagnosis is clinical.[1] The diagnostic clinical criteria include characteristic findings: postauricular tenderness, erythema, swelling with loss of the postauricular crease, fluctuance and protrusion of the auricle, tympanic membrane modifications.[5-7] Ear

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pain is sometimes replaced by irritability or lethargy in younger children, and fever and ear discharge may also occur.[8,9] Radiological imaging should be considered in patients with compromised general conditions and altered laboratory findings, in patients not responding to conservative treatment within 48 hours, if surgery is considered, or in the suspicion of complications.[3,9-11] Computerized tomography (CT) has traditionally been the initial imaging technique for AM, especially for bony structures, while magnetic resonance imaging (MRI) is more commonly applied to evaluate soft tissues and intracranial structures.[12-14] CT examinations are widely used because of their speed, accessibility, and high quality. Generally, they do not require sedation. MRI is more expensive, requires longer time to execute, and is not available in all medical centers.[10] Despite these drawbacks, MRI is the gold standard for suspected intracranial complications (ICCs), especially for extra-axial fluid collections and associated vascular lesions. Furthermore, it is useful to evaluate the equivocal lesions found on a CT scan.[8,15,16] In the last few years, several studies have investigated the necessity of imaging in diagnosing AM versus solely using clinical criteria. No definitive consensus regarding the role of imaging in the diagnosis of AM has been identified.[17] No definitive consensus on the treatment of AM has been established either. The current guidelines include a conservative approach (parenteral antibiotics alone, antibiotics plus minor surgical procedures such as myringotomy with a ventilation tube inserted or drainage of the subperiosteal abscess through retro-auricolar incision or needle aspiration) or surgical treatment (mastoidectomy).[4] AM generally requires parenteral antimicrobial therapy for 7 to 10 days and then transition to oral antibiotics to complete a four-week course.[7,17] Empiric therapy is based on epidemiological data, a history of recurrent AOM or recent antibiotic therapy. When cultures and microbiologic results are obtained, antimicrobial therapy should be adjusted consequently.[4] Drainage procedures consist of myringotomy, with or without placement of tympanostomy tubes (TTs), and retro-auricular needle aspiration or incision. Myringotomy implicates a surgical perforation of the tympanic membrane, along with the TTs placement, allowing effective drainage over a longer duration than myringotomy alone. Retro-auricular needle aspiration or incision is an effective modality for the drainage of subperiosteal abscesses. Mastoidectomy is the surgical removal of the mastoid cortical bone and underlying air cells.[7] In the last few years, a conservative approach has been preferred over surgical treatment, even if surgery remains the main choice in the management of AM, especially if no improvement is observed within 48 hours.[4,18-20] The main aim of this review was to evaluate and summarize the current knowledge about the management of pediatric AM by comparing and analyzing the current evidence in the literature.

Materials and Methods

Data sources

We examined the following bibliographic electronic databases: Pubmed and the Cochrane Library, from the inception date until February 2023. The search was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISM) and was limited to English-language papers that focused on AM in pediatric patients.[21] To be considered eligible for the review, papers had to include the following criteria: (1) children with a diagnosis of AM; (2) who received conservative or surgical treatment; and (3) outcomes based on follow-up assessment. We excluded: 1) non-English language papers; 2) studies in which "conservative" or "surgical" options were not specified; and 3) studies involving patients with co-existing cholesteatoma, chronic mastoiditis, or cochlear implants. The key words used for the search across electronic databases were: 'mastoiditis' and 'management'; 'mastoiditis' and 'surgery'; 'mastoiditis' and 'conservative'; 'mastoiditis' and 'antibiotics'; 'mastoiditis' and 'myringotomy'; 'mastoiditis' and 'grommet';

'mastoiditis' and 'drainage'; and 'mastoiditis' and 'mastoidectomy'. The abstracts of the papers were assessed by two reviewers (LDS and IC), who strictly applied the inclusion and exclusion criteria mentioned above in order to decide whether a paper was eligible for full review. Each paper that met the eligibility criteria was reviewed and analyzed in full text by two authors (LDS and IC), and any discrepancies among them were solved by debate.

Study selection

Overall, we identified 480 records through database searching. As a first step, we excluded 50 articles not in English, 10 records whose related articles were not available, 5 articles concerning ongoing trials, and 200 duplicate papers. As a second step, we eliminated 175 records by evaluating only titles and abstracts because they did not match the inclusive criteria mentioned above. Of the remaining 40 studies, we excluded 28 through a further discussion among authors regarding the reliability of the data. Thus, 12 selected articles were included in the review.

The detailed selection of literature is shown in Fig. 1.

A wide and extensive summary of the results is shown in Tables I and II.

Data extraction

The data extracted from each eligible paper included: study population sample, mean age, sex prevalence, study design, radiological imaging performed, type of AM treatment, type of complications, microbiological cultures, and antibiotics adopted. Studies were grouped into those that evaluated medical therapy as the initial sole treatment and those that evaluated surgery as the initial treatment option along with medical therapy.

In this review, we analyzed the current literature on the management of AM in children. Thus, ethical approval was not required.

Results

The papers included 1124 episodes of mastoiditis. The CT scan examination rate greatly differs among the different papers, varying from 0.4% to 100%, with a mean percentage of 29.09%. MRI was used only in four studies, with a percentage of 29%, 8.5%, 7.7%, and 0.9%, respectively.

Some of these studies considered medical therapy alone as a valid first step, whereas others considered a surgical intervention, either drainage or mastoidectomy, since the beginning along with antibiotic therapy.[11,20,22-31] In the first group, the percentage rate of episodes treated with antibiotic therapy alone as the first step was 48% (278 episodes). Focusing on the first therapeutic approach, the overall percentage rates concerning different drainage procedures (including myringotomy or myringotomy with placement of the tympanostomy tube and retro-auricolar needle aspiration or incision) and mastoidectomy were respectively 38.6% (434 episodes) and 36.4% (409 episodes).

Considering the studies that evaluated medical therapy as the initial sole treatment option, the success rate of antibiotics alone was 24.6%. Overall, the success rate of minor surgical procedures, excluding mastoidectomy, was 87.7% whereas the mastoidectomy success rate was 97% among the included papers.

Concerning the microbiological cultures obtained, *Streptococcus pneumoniae* was the most common bacterium identified (31.3%), alone or in association with other pathogens. At lower rates, *Streptococcus pyogenes (9.8%), Staphylococcus aureus (2.6%), Haemophilus influenzae (3.9%)* and *Pseudomonas aeruginosa (4%)* were commonly detected as well. Considering the high incidence of *Streptococcus pneumoniae* and its susceptibility to beta-lactam antibiotics, cephalosporins such as ceftriaxone sodium and cefotaxime were mostly adopted.[23,28] Subsequent changes or the addition of another type of antibiotic, especially in polymicrobial infections, were made according to cultures and antibiograms.

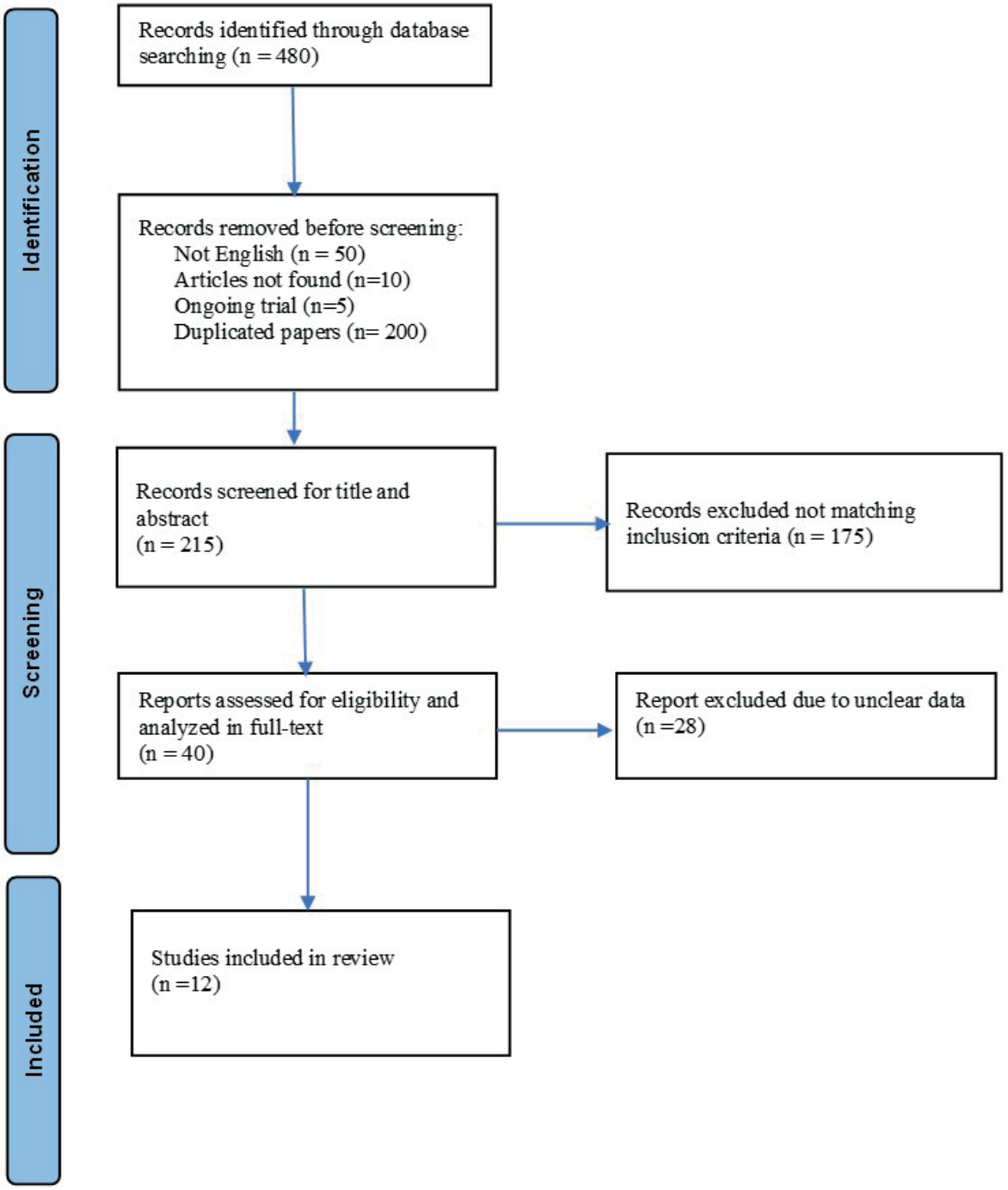

Fig. 1. The detailed summary of the literature search.

Table I. Studies that evaluated medical therapy as the initial sole treatment option.

Study	Sample size and sex (N) Mean age Imaging, n (%)	Treatment	Cultures	Antibiotics adopted	Complications
Gorphe et al. (2011)*	36 31.8 mo CT 36 (100%)	1 IV AB (2.8%), 8 IV AB + TT (22.2%), 3 IV AB + TT + puncture (8.3%), 24 IV AB + TT + mastoidectomy (66.7%)	13 *S. pneumoniae* (36.1%), 5 *S. pyogenes* (13.9%), 5 *S. epidermidis* (13.9%), 3 *P. aeruginosa* (8.3%), 3 *F. necroforum* (8.3%), 1 *H. influenzae* (2.8%)*	CTX and FOS (+ AG if anaerobic infection; + MET in case of *F. necroforum*)	26 subperiosteal abscess (72.2%), 1 Bezold syndrome (2.7%), 4 lateral sinus thromboses (11.1%), 2 subdural empyema (5.5%)
Quesnel et al. (2010)	188 (M: 108, F: 80) 15 mo CT 68 (36.2%)	120 AB alone (monotherapy in 74.5% and a bitherapy in 25.5%), 68 surgery (36.2%): 62 Mastoidectomy (91.2%), 6 Simple drainage of retro auricular abscess (8.8%)	87 *S. pneumoniae* (51%), 20 *S. pyogenes* (11.5%), 3 Other streptococci (1.8%), 11 Anaerobes (6.5%), 13 CNS (7.6%), 8 *H. influenzae* (4.5%), 8 *P. aeruginosa* (4.5%), 7 GNB (4%), 6 *S. aureus* (3.5%), 4 Corynebacteria (2.5%), 4 *T. otitidis* (2.5%)	186 β-lactam AB (100%): 159 CTR (85.5%), 24 CTX (13%), 1 CTZ (0.5%), 2 AMC (1%); 119 AG (64%), 50 FOS (27%), 34 MET (19%), 12 CLI (6.4%), 8 RIF (4.3%), 1 VAN (0.5%)	6 lateral sinus thrombosis (3.2%), 1 Transient balance disorder (0.5%)
Duygu et al. (2020)	28 (M: 16, F: 12) 80.4 mo CT 28 (100%), MRI 8 (29%) in suspected intracranial complications	3 Medical (10.7%), 8 Ventilation tube (28.6%), 8 Ventilation tube + Drainage (28.6%) , 9 Ventilation tube + Mastoidectomy (32.1%)	9 None (32.1%), 12 *S. pneumoniae* (42.9%), 2 *S. pyogenes* (7.1%), 1 *S. maltophilia* (3.6%), 1 *P. aeruginosa* (3.6%), 1 *P. mirabilis* + *E. coli* (3.6%), 1 Enterococci (3.6%), 1 Aspergillus spp. (3.6%)	16 CTR as monotherapy (57.1%); VAN, depending on clinical symptoms or culture results.	8 Subperiosteal abscess (28%), 7 Facial paralysis (25%), 5 Meningitis (17%), 2 Meningitis and sigmoid sinus thrombosis (7.1%), 1 Meningitis and cerebellar abscess (3.5%)

All listed studies were retrospective except for that by Shrestha et al. (2021), which was a prospective observational study.
*Antimicrobial susceptibility was mentioned in the study by Gorphe et al (2011) as follows: *S. pneumoniae* PEN G: 9 sensitive (69%) 4 resistant (31%); AMO: 13 sensitive (100%); CTX: 13 sensitive (100%); ERY: 3 sensitive (23.1%), 10 resistant (76.9%); PRI: 10 sensitive (77%), 3 resistant (23%). *S. pyogenes* Sensitive to: PEN G, AMO, RIF, VAN; Resistant to: TMP-SMX. *S. epidermidis* Sensitive to: AG, RIF, FOS, OFL, VAN, TMP-SMX; Resistant to: PEN G and INN. *F. necroforum* Sensitive to: AMO, AMC, MET. Resistant to: VAN. *P. aeruginosa* Sensitive to: PIP, CTZ, AG, TIC and TIM. Resistant to: FOS.
AB: antibiotics, AG: aminoglycosides, AMC: amoxicillin-clavulanic acid, AMO: amoxicillin, CLI: clindamycin, CN: cranial nerve, CNS: coagulase-negative staphylococci, CRX: cefuroxime, CTR: ceftriaxone, CTX: cefotaxime, CT: computerized tomography, CTZ: ceftazidime, Dexa: dexamethasone, ERY: erythromycin, F: female, FOS: fosfomycin, GEN: gentamycin, GNB: Gram-negative bacilli, INN: methicillin, IV: intravenous, M: male, MET: metronidazole, mo: months, MRI: magnetic resonance imaging, OFL: ofloxacin, PEN: penicillin, PIP: piperacillin, PRI: pristinamycin, RIF: rifampicin, TIC: ticarcillin, TIM: ticarcillin-clavulanic acid, TMP-SMX: trimethoprim-sulfamethoxazole, TT: tympanostomy tube, VAN: vancomycin.

Table I. Continued.

Study	Sample size and sex (N) Mean age Imaging, n (%)	Treatment	Cultures	Antibiotics adopted	Complications
Shrestha et al. (2021)	79 (M: 49, F: 30) 111.84 mo CT 13 (16.4%)	41 Parenteral AB alone (51.9%), 25 Myringotomy or incision and drainage (31.6%), 13 Mastoidectomy along with injectable antibiotics and myringotomy or incision and drainage (16.5%)	16 *S. pneumoniae* (53%), 8 *P. aeruginosa* (26%), 3 *S. pyogenes* (10%), 3 *S. aureus* (10%)	3rd-gen cephalosporin with or without MET	21 Subperiosteal abscess (26%), 1 Facial nerve palsy (1.2%)
Mierzwiński et al. (2019)	73 (M: 35, F: 38) Mean age and imaging numbers not mentioned. Imaging only in case of suspicion of intracranial complications.	9 Medical therapy (11%), 10 Tympanostomy / Myringotomy (12%), 56 Mastoidectomy + Tympanostomy (67%), 8 Mastoidectomy only (10%)	28 *S. pneumoniae* (33.7%), 13 *S. pyogenes* (15.7%), 3 *P. aeruginosa* (3.6%), 3 *H. influenzae* (3.6%), 2 *C. albicans* (2.4 %), 1 *E. coli* (1.2%), 1 *A. baumannii* (1.2%) 1 *E. faecalis* (1.2%), 1 *S. aureus* (1.2%), 1 *M. catarrhalis* (1.2%)	3rd-gen cephalosporin (CTR or CTX) with CLI or MET	1 Epidural abscess (1.2%), 4 Sigmoid sinus Thrombophlebitis (5%), 1 Petrositis (1.2%), 1 Facial paralysis (1.2%)

All listed studies were retrospective except for that by Shrestha et al. (2021), which was a prospective observational study.
*Antimicrobial susceptibility was mentioned in the study by Gorphe et al (2011) as follows: *S. pneumoniae* PEN G: 9 sensitive (69%) 4 resistant (31%); AMO: 13 sensitive (100%); CTX: 13 sensitive (100%); ERY: 3 sensitive (23.1%), 10 resistant (76.9%); PRI: 10 sensitive (77%), 3 resistant (23%). *S. pyogenes* Sensitive to: PEN G, AMO, RIF, VAN; Resistant to: TMP-SMX. *S. epidermidis* Sensitive to: AG, RIF, FOS, OFL, VAN, TMP-SMX; Resistant to: PEN G and INN. *F. necroforum* Sensitive to: AMO, AMC, MET. Resistant to: VAN. *P. aeruginosa* Sensitive to: PIP, CTZ, AG, TIC and TIM. Resistant to: FOS.
AB: antibiotics, AG: aminoglycosides, AMC: amoxicillin-clavulanic acid, AMO: amoxicillin, CLI: clindamycin, CN: cranial nerve, CNS: coagulase-negative staphylococci, CRX: cefuroxime, CTR: ceftriaxone, CTX: cefotaxime, CT: computerized tomography, CTZ: ceftazidime, Dexa: dexamethasone, ERY: erythromycin, F: female, FOS: fosfomycin, GEN: gentamycin, GNB: Gram-negative bacilli, INN: methicillin, IV: intravenous, M: male, MET: metronidazole, mo: months, MRI: magnetic resonance imaging, OFL: ofloxacin, PEN: penicillin, PIP: piperacillin, PRI: pristinamycin, RIF: rifampicin, TIC: ticarcillin, TIM: ticarcillin-clavulanic acid, TMP-SMX: trimethoprim-sulfamethoxazole, TT: tympanostomy tube, VAN: vancomycin.

Table I. Continued.

Study	Sample size and sex (N) Mean age Imaging, n (%)	Treatment	Cultures	Antibiotics adopted	Complications
Mather et al. (2020)	47 (sex not mentioned) 42 mo CT alone 10 (21.2%), MRI alone 1 (2.12%), CT and MRI 3 (6.38%), Ultrasound alone 1 (2.12%) (CT not tolerated)	20 Pharmacological treatment alone (40%), 9 Cortical mastoidectomy with myringotomy and grommet insertion (18%), 9 Cortical mastoidectomy alone (18%), 6 incision and drainage (12%), 2 myringotomy and grommet alone (4%), 3 combined approaches with neurosurgical input (6%)	3 *P. aeruginosa* (6%), 4 *S. pyogenes* (8%), 3 *S. pneumoniae* (6%), 3 *F. necrophorum* (6%), 2 *H. influenzae* (4%), 1 *S. haemolyticus* + *S. mitis* (2%), 1 *S. aureus* alone (2%), 1 Group A streptococci in blood culture only (2%)	14 AMC alone, 5 AMC & Dexa drops, 2 AMC & GEN drops, 2 AMC and MET with OFL drops, 18 Other combination, 8 Not documented	3 Sigmoid sinus thrombosis (6.1%), 2 Extradural abscess (4.1%), 1 7th CN palsy (2%), 1 Internal jugular vein thrombus (2%), 1 Cerebellar abscess (2%), 1 Masseter abscess (2%), 1 Wound granulation (2%), 1 Wound dehiscence (2%), 1 Wound infection, 1 Meningitis (2%), 1 Temporal lobe abscess (2%), 1 Submastoid empyema (2%), 1 Sensorineural hearing loss (2%)
Katz at al. (2003)	101 (M: 61, F: 40) 25 mo CT 54 (47 %)	72 Pharmacological therapy alone (62%), 32 Mastoidectomy (27.5%), 12 Ventilation tube placement (12%)	13 *S. pneumoniae* (38%), 9 *S. pyogenes* (26%), 6 *H. influenzae* (18%), 4 *P. aeruginosa* (12%), 2 *E. coli* (6%)	CRX (49%), CTR (30%), AMC (15%), MET (8%), CTZ (7%)	8 Subperiosteal abscess (7%), 2 Lateral sinus thrombosis (2%)

All listed studies were retrospective except for that by Shrestha et al. (2021), which was a prospective observational study.
*Antimicrobial susceptibility was mentioned in the study by Gorphe et al (2011) as follows: *S. pneumoniae* PEN G: 9 sensitive (69%) 4 resistant (31%); AMO: 13 sensitive (100%); CTX: 13 sensitive (100%); ERY: 3 sensitive (23.1%), 10 resistant (76.9%); PRI: 10 sensitive (77%), 3 resistant (23%). *S. pyogenes* Sensitive to: PEN G, AMO, RIF, VAN; Resistant to: TMP-SMX. *S. epidermidis* Sensitive to: AG, RIF, FOS, OFL, VAN, TMP-SMX; Resistant to: PEN G and INN. *F. necroforum* Sensitive to: AMO, AMC, MET. Resistant to: VAN. *P. aeruginosa* Sensitive to: PIP, CTZ, AG, TIC and TIM. Resistant to: FOS.
AB: antibiotics, AG: aminoglycosides, AMC: amoxicillin-clavulanic acid, AMO: amoxicillin, CLI: clindamycin, CN: cranial nerve, CNS: coagulase-negative staphylococci, CRX: cefuroxime, CTR: ceftriaxone, CTX: cefotaxime, CT: computerized tomography, CTZ: ceftazidime, Dexa: dexamethasone, ERY: erythromycin, F: female, FOS: fosfomycin, GEN: gentamycin, GNB: Gram-negative bacilli, INN: methicillin, IV: intravenous, M: male, MET: metronidazole, mo: months, MRI: magnetic resonance imaging, OFL: ofloxacin, PEN: penicillin, PIP: piperacillin, PRI: pristinamycin, RIF: rifampicin, TIC: ticarcillin, TIM: ticarcillin-clavulanic acid, TMP-SMX: trimethoprim-sulfamethoxazole, TT: tympanostomy tube, VAN: vancomycin.

Table II. Studies that evaluated surgery as the initial treatment option along with the medical therapy.

Study	Sample size and sex (N) Mean age Imaging, n (%)	Treatment	Cultures	Antibiotics adopted	Complications
Bakhos et al. (2011)	50 (M: 29, F: 21) 32 mo CT 43 (86%)	16 Mastoidectomy (34%), 34 AB combined with retroauricular puncture and grommet insertion (37%)	6 No growth (19%), 19 *S. pneumoniae* (61%), 4 *S. pyogenes* (12%), 1 *S. chromogenes* (3%), 1 *F. necroforum* (3%)	CTR was the most used (64%) combined with FOS or MET	31 subperiosteal abscess (62%), 3 sigmoid sinus thrombosis (6%), 1 subdural empyema (2%), 1 facial palsy (2%)
Anthonsen et al (2013)*	214 (M: 112, F: 102) 25.2 mo CT 1 (0.4%)	183 explorative myringotomy (simple drainage) (86%), 66 insertion of a ventilation tube (31%), 67 mastoidectomy (31%)	63 No growth (29%), 46 *S. pneumoniae* (21.6%), 29 Skin flora (13.6%), 15 *P. aeruginosa* (7%), 15 *S. pyogenes* (7%), 10 *S. aureus* (4%), 10 *H. influenzae* (4%), 9 Corynebacteria (4%), 3 Other streptococci (1.4%), 1 Aspergillus (0.5%), 12 Other (5.5%)	62 PEN (29%), 55 AMP (26%), 43 CRX (20%)	68 subperiosteal abscesses (32%), 1 vertigo (0.5%), 1 facial nerve paresis (0.5%), 1 spreading of the infection to the eye and facial region (0.5%), 1 larger perforation of the tympanic membrane (0.5%)
Enoksson et al (2015)	115 34.5 mo, 38.7 mo and 49.5 mo in Group 1, 2, and 3 resp. CT 45 (39%)	115 AB during hospitalization, 0 AB alone Group 1 – 33 needle aspiration (17 only needle aspiration, 10 aspiration and incision, 6 only incision) and/or incision of the abscess (28%) Group 2 – 67 mastoidectomy (47 with previous myringotomy) (58%) Group 3 - 15 needle aspiration and mastoidectomy (13%)	16 *S. pyogenes* (19%), 46 *S. pneumoniae* (56%), 26 Negative (32%)	Not mentioned	3 perforations of the eardrum (2.6%), 3 mild sensorineural hearing loss (2.6%)

All listed studies were retrospective.
*Anthonsen et al (2013) reported antibiotic sensitivity as: 94% sensitive to penicillin, 93% sensitive to Amp
**Zavras et al (2020) reported antibiotic sensitivity of *S. pneumoniae* as PEN-sensitive in 4 (80%) samples, Pen-resistant and cefotaxime-sensitive in 1 (20%) sample.
AB: antibiotics, AMC: amoxicillin-clavulanic acid, AMP: ampicillin, AMS: ampicillin-sulbactam, AZT: aztreonam, CLI: clindamycin, CNS: coagulase-negative staphylococci, CRX: cefuroxime, CT: computerized tomography, CTR: ceftriaxone, CTX: cefotaxime, F: Female, FOS: fosfomycin, M: male, MET: metronidazole, mo: months, MRI: magnetic resonance imaging, MRV: magnetic resonance venography, PEN: penicillin.

Table II. Continued.

Study	Sample size and sex (N) Mean age Imaging, n (%)	Treatment	Cultures	Antibiotics adopted	Complications
Psarommatis et al. (2012)	155 (M: 106, F: 49) 36.7 mo CT 18 (11.6%), MRI 7 (4.5%), CT and MRI/MRV 5 (3.2%)	112 Myringotomy alone (72.3%), 21 abscess drainage (13.5%), 22 Myringotomy + simple mastoidectomy (14.1%), 43 Mastoidectomy as further intervention due to poor response to myringotomy or drainage (37%)	63 *S. pneumoniae* (40.6%), 25 CNS (16.1%), 16 *S. pyogenes* (10.3%), 14 *H. influenzae* (9%), 9 Anaerobes (5.8%), 8 *S. aureus* (5.2%)	149 3rd-gen cephalosporin: (CTX or CTR) and CLI (The 3rd-gen cephalosporin was replaced by AZT in three penicillin allergic children and the combination AMC + CLI was used in two children.), 3 AMC, 2 AMS,1 2nd gen cephalosporin	34 Subperiosteal abscesses (29.5%), 3 Epidural abscess (1.9%), 1 Brain abscess (0.6%), 2 Sigmoid sinus thrombosis (1.2%), 1 Brain abscess plus epidural abscess (0.6%), 1 Brain abscess plus perisinus abscess/ sigmoid sinus thrombosis (0.6%), 1 Epidural abscess plus sigmoid sinus thrombosis (0.6%), 4 Facial nerve paralysis (2.4%)
Zavras et al. (2020)**	11 (M: 6, F: 5) 4.7 mo	11 Myringotomy and ipsilateral needle aspiration of the post-auricular area (100%), 4 Antrotomy (18.2%), 4 Incisional drainage (18.2%)	5 *S. pneumoniae* (45.5%), 4 *S. pyogenes* (36.3%)	CTX and CLI	8 Subperiosteal abscess (36.4%)

All listed studies were retrospective.
*Anthonsen et al (2013) reported antibiotic sensitivity as: 94% sensitive to penicillin, 93% sensitive to Amp
**Zavras et al (2020) reported antibiotic sensitivity of *S. pneumoniae* as PEN-sensitive in 4 (80%) samples, Pen-resistant and cefotaxime-sensitive in 1 (20%) sample.
AB: antibiotics, AMC: amoxicillin-clavulanic acid, AMP: ampicillin, AMS: ampicillin-sulbactam, AZT: aztreonam, CLI: clindamycin, CNS: coagulase-negative staphylococci, CRX: cefuroxime, CT: computerized tomography, CTR: ceftriaxone, CTX: cefotaxime, F: Female, FOS: fosfomycin, M: male, MET: metronidazole, mo: months, MRI: magnetic resonance imaging, MRV: magnetic resonance venography, PEN: penicillin.

Discussion

Acute mastoiditis is a complication of acute AOM, in which there is inflammation of the mastoid periosteum and air cells.[32] Despite the widespread use of antibiotics and vaccines, AM is still a growing complication in children because of rising antibiotic resistance. The introduction of a pneumococcal conjugate vaccine in 2000, rapidly substituted by a polyvalent version, has widely lowered the rate of pneumococcal AOM.[33] In contrast to these data, no reduction has been recorded in the rate of pneumococcal AM.[3] As a matter of fact, *Streptococcus pneumoniae* remains the main pathogen detected in this review as well. Koutouzis et al.[34] in a retrospective study involving 334 children, compared the rate of pneumococcal mastoiditis before and after the introduction of PCV7 and PCV13, documenting no significant differences, probably due to a possible pneumococcal serotype replacement. In the papers analyzed in this review, no specific data on the pneumococcal vaccination status of patients was available. Since these studies were set in different timeframes and countries, we were not able to assess the impact of pneumococcal vaccination on the prevention of mastoiditis.

As mentioned above, there is no gold standard for the management of pediatric AM in terms of the radiological examination performed and treatment. The use of CT scans in the diagnostic process is still a controversial issue today. In the studies we analyzed, the CT scan examination rate greatly differed among papers, implying a heterogeneous radiological approach. Pediatric patients are particularly susceptible to radioactivity, and a CT scan enhances the risk of developing a tumor in a considerable way. This is why some pediatricians, especially in Denmark, desist from using this examination as a routine.[29] Other authors suggest that CT should be a standard procedure in the diagnosis of every AM. In fact, Vassbotn et al.[35] showed in their retrospective study in 2002 that clinical examination revealed only 50% of the cases with surgically proven subperiostal abscesses, recommending a CT scan of every patient treated conservatively. The majority of authors, however, support the opinion that imaging exams should be limited to selected circumstances: neurologic signs, deterioration of the general state, suspicion of intracranial complications, and unresponsiveness to conservative treatment.[28]

Third-generation cephalosporins have been the main antibiotics prescribed among the mentioned papers.[23,28,36] These data are in line with others reported in the literature.[37,38] Edwards et al.[39] in a retrospective study in 2022, emphasized how broad-spectrum antibiotics have been used in this clinical scenario in contrast with microbiological evidence. As a matter of fact, in their paper, vancomycin was widely adopted, even though methicillin-resistant *Staphylococcus aureus* was detected in a negligible number of cultures. In this regard, appropriate antibiotic stewardship with microbiological cultures is an essential tool to treat the underlying pathogen and avoid antibiotic resistance. A major contribution to antibiotic resistance could be due to AOM home therapy. Despite the fact that amoxicillin alone is considered the first line in patients with AOM, Balsamo et al. pointed out how the majority of cases are treated at home with amoxicillin plus clavulanic acid. The abuse of amoxicillin and clavulanic acid in non-hospitalized patients might have triggered a rise in resistant pathogens. However, more studies are needed to confirm this correlation.

As regards treatment, historically, the surgical approach in terms of mastoidectomy has been the most applied, but more recently, many authors have supported the conservative approach considering the surgical complications amongst children.[35] Parenteral antibiotic therapy alone is reported to be successful in some reports.[18,23] In other patients, antimicrobial therapy alone may not be sufficient, especially in the later stages of the disease, when it becomes more challenging to reach adequate antibiotic levels in deep bony tissues.

By tradition, there seems to be an established consensus on performing mastoidectomy if a subperiosteal abscess is encountered, but it still remains a matter of controversy.[28-31] Shrestha et al.[25] and Psarommatis et al.[11] in their studies, treated subperiosteal abscesses mostly by adopting a conservative approach.

Furthermore, there is no solid evidence regarding factors predicting the severity or evolution of AM complications.[32] Subsequently, management decisions cannot be based on a specific validated algorithm. Actually, treatment is based on the decision of the pediatrician; some of them predilect surgery right away, whereas others opt for medical therapy before considering surgery. In this review, medical therapy as the initial sole treatment showed a success rate of 24.6%. In this cluster of studies, medical therapy alone was probably preferred because of a less severe disease, and this could have overestimated this percentage. The success rate of minor surgical procedures, excluding mastoidectomy, was 87.7% in line with other data reported in the literature.[32] Mastoidectomy had almost a 100% success rate, both as a first-line treatment and as a second step after pharmacological therapy or minor procedures. One crucial issue is which treatment is best for AM in terms of efficacy and safety. The current data is not sufficient to answer this question. There is not enough evidence on efficacy to recommend for or against any of these techniques.

Although a validated standardized protocol is not available, according to our data, medical therapy with or without myringotomy as an initial treatment seems to be appropriate in good clinical conditions. As soon as the patient's conditions get clinically worse, a mastoidectomy can be performed.

The drawbacks of our review are included in the limits of the articles involved. The main limitations are the heterogeneity of study designs, the divergence among the different types of interventions, and the different durations of follow-up.

Overall, there is no shared consensus either on the diagnostic approach nor on the therapeutic one of mastoiditis. Conservative therapy has gained considerable ground in recent times, quite limiting the predominant role of mastoidectomy.

Further studies including a wide range of patients will be necessary to definitely develop standardized protocols shared in the scientific community.

Ethical approval

No ethical approval was required.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: Lorenzo Di Sarno, Ignazio Cammisa and Antonio Chiaretti, data collection: Lorenzo Di Sarno and Ignazio Cammisa, analysis and interpretation of results: Lorenzo Di Sarno and Ignazio Cammisa, draft manuscript preparation: Lorenzo Di Sarno, Ignazio Cammisa, Gemma Eftimiadi, Antonio Gatto, Antonietta Curatola, Valeria Pansini. All authors reviewed 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

Neonatal risk factors for functional gastrointestinal disorders in preterm infants in the first year of life

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ABSTRACT

Background. An assessment of functional gastrointestinal disorders (FGIDs) in premature infants in their first year of life and neonatal factors influencing the progression of FGIDs was conducted in this research.

Methods. Subjects selected for the retrospective study involved preterm infants being hospitalized in the neonatal department of Northern Jiangsu People's Hospital from September 2018 to September 2021. Data on neonatal risk factors such as gestational age, gender, birth weight, mode of delivery, feeding pattern, antibiotic administration and addition of probiotics, duration of hospitalization, maternal history of smoking, and mental health status, were all collected and analyzed. FGIDs were diagnosed according to Rome IV criteria.

Results. This study included 988 preterm infants, with 725 (73.4%) having at least one FGID, 449 (45.4%) with infant colic, 411 (41.6%) with infant regurgitation, 237 (24.0%) with infant dyschezia, 190 (19.2%) with functional constipation, and 34 (3.4%) with functional diarrhea throughout the first year of life. In total, 263 infants (26.6%) without FGID symptoms were included in the control group. Further, a higher prevalence of FGIDs was observed in preterm infants with infant colic as well as infant regurgitation in particular as being characterized by a low gestational age (<32 w), low birth weight (<1.5 kg), Cesarean section, formula feeding, neonatal antibiotics use, hospitalization longer than 7 days, and maternal history of smoking. It was found from association analyses that infants exclusively breastfed in their first month of life were at lower risk for regurgitation than those in the control group.

Conclusions. Unnecessary antibiotic use in the neonatal period, Cesarean delivery, passive smoking, lack of breastfeeding along with inappropriate probiotics usage are major risk factors for FGIDs, and their systematic control may be effective in reducing the susceptibility to and prevalence of FGIDs in preterm infants in the first year of life.

Key words: premature, infants, functional gastrointestinal disorders, risk factors.

Functional gastrointestinal disorders (FGIDs) are a group of diseases of the functional digestive tract which are age-related, chronic, or recurring, and are not addressed either by organic lesions or biochemical abnormalities.[1] FGIDs are redefined by the Rome IV criteria where FGIDs in infants include infant colic, infant regurgitation, infant dyschezia, functional constipation, functional diarrhea, rumination as well as cycling vomiting syndrome.[2]

According to multiple studies, around 50% of infants experience FGIDs (including regurgitation, infant colic, and constipation) during the first year of their lives following birth.[3,4] There is a higher rate of medical visits by infants and young children with FGIDs, and consequently, the quality of life is poorer for them[4,5], also causing unnecessary hardship for

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caregivers during early infancy.[2] In children, FGIDs not only affect their growth and development but also deteriorate their quality of life. Additionally, studies show that children with FGIDs are more prone to gastrointestinal illnesses and abdominal migraines as they age than children without FGIDs.[1] This affects the long-term health of children, as well as burdens the family with increasing additional healthcare costs.[6] Most FGIDs are usually treated with inadequate treatment modalities, which adds increased costs to healthcare systems in resource-constrained nations.[7] Possible influencing factors include genetic predispositions, psychological variables, aberrant intestinal motility, visceral hyperalgesia, gut inflammation, intestinal microbiota, early stressful experiences, and trauma, all have been suggested as determinants of FGID susceptibility.[2,8,9] FGIDs, however, remain largely unknown in terms of their pathophysiology. In recent years, the prevalence of FGIDs and disease risk factors have attracted the attention of pediatricians at home and abroad. The prevalence of FGIDs is higher in premature infants, and neonatal life events are crucial in programming later FGIDs in life.[10] Several authors speculate that infants are predisposed to FGIDs depending on the delivery mode, feeding practices, and early administration of antibiotics.[11,12] However, the most recent research subjects of FGIDs were term infants, and few studies have been conducted on premature infants. Therefore, the role of premature birth in FGIDs is less explored. A retrospective analysis concerning preterm infants is conducted in this study to look at the potential link between neonatal factors and the development of FGIDs within the first year of life.

Material and Methods

Study subjects and design

The study was retrospective and non-interventional. Therefore, it was not necessary to obtain informed consent from parents. The ethics committee of Northern Jiangsu People's Hospital approved this research (2022ky291). Preterm newborns at the hospital with gestational ages at birth ranging from 25 to 36 weeks were selected from the tertiary neonatal critical care unit between September 2018 and September 2021.

Premature newborns with severe acute infection or neonatal problems such as inherited metabolic disorders, congenital deformities, and death during hospitalization were excluded.

Data collection

Data on neonatal risk factors such as gestational age, gender, birth weight, mode of delivery, feeding pattern, antibiotic administration and addition of probiotics, duration of hospitalization, maternal history of smoking, and mental health status, were all collected from hospital records and telephone follow-up records. FGIDs were diagnosed as per the Rome IV criteria.[1]

Diagnostic criteria for infant colic:

For clinical purposes, must include all of the following:

1. An infant who is <5 months of age when the symptoms start and stop;
2. Recurrent and prolonged periods of infant crying, fussing, or irritability reported by caregivers that occur without obvious cause and cannot be prevented or resolved by caregivers;
3. No evidence of infant failure to thrive, fever, or illness.

Diagnostic criteria for infant regurgitation

Must include both of the following in otherwise healthy infants 3 weeks to 12 months of age:

1. Regurgitation 2 or more times per day for 3 or more weeks;
2. No retching, hematemesis, aspiration, apnea, failure to thrive, feeding or swallowing difficulties, or abnormal posturing.

Diagnostic criteria for infant dyschezia

Must include in an infant <9 months of age:

1. At least 10 minutes of straining and crying before successful or unsuccessful passage of soft stools;
2. No other health problems.

Diagnostic criteria for functional constipation

Must include 1 month of at least 2 of the following in infants up to 4 years of age:

1. 2 or fewer defecations per week;
2. History of excessive stool retention;
3. History of painful or hard bowel movements;
4. History of large-diameter stool;
5. Presence of a large fecal mass in the rectum.

In toilet-trained children, the following additional criteria may be used:

6. At least 1 episode/week of incontinence after the acquisition of toileting skills;
7. History of large-diameter stool that may obstruct the toilet.

Diagnostic criteria for functional diarrhea

Must include all of the following:

1. Daily painless, recurrent passage of 4 or more large, unformed stools;
2. Symptoms last more than 4 weeks;
3. Onset between 6 and 60 months of age;
4. No failure to thrive if caloric intake is adequate.

Statistical methods

Data were analyzed statistically with the aid of SPSS Statistics (21.0). Continuous variables were presented as the mean ± standard deviation (SD) and analyzed using the Student's t-test. Categorical variables were described as proportions and were analyzed using the chi-square test or Fisher exact probability method. The risk factors were identified by performing univariate as well as multivariate logistic regression analyses. For each risk factor, odds ratio (OR) estimates, 95% confidence intervals, and p-values from the Wald chi-square test were all determined, where $p < 0.05$ indicated a statistical significance level. Multiple logistic regression has been applied if the p-value of any of the factors was less than 0.05.

Results

A total of 988 preterm infants have been involved in this study, and their data for the first year of their lives were evaluated and analyzed. Among a total of 988 subjects, in at least 725 (73.4%), a single FGID was documented within the first year of life. There are 484 (49.0%) cases with a single kind of FGID, 210 (21.3%) with two kinds, 31 (3.1%) with three kinds, and no case with four or more FGIDs. Among the 988 participants, there were 449 (45.4%) and 411 (41.6%) cases of infant colic and regurgitation, respectively, making them frequently occurring disorders. Of the 988 participants, 237 (24.0%) claimed dyschezia, 190 (19.2%) claimed functional constipation, and 34 (3.4%) claimed functional diarrhea. There were no cases of infant rumination syndrome as well as recurrent vomiting syndrome.

The demographic characteristics of subjects are shown in Table I. Of the 988 infants who completed the study, 725 with FGIDs were allotted to the case group and 263 without symptoms associated with FGIDs were allotted to the control group. As expected, all 988 preterm-born infants displayed considerable differences (variations) in gestational age, birth weight, the incidence of cesarean delivery, rate of breastfeeding, antibiotic usage, probiotics intake, and maternal history of smoking, anxiety, and hospital stay (Table I). There were no considerable variations in gender between the case group and the control group.

It was observed that the prevalence of FGIDs among preterm newborns varied considerably

Table I. Baseline demographic characteristics of the enrolled population.

Variables	Case group (n=725)	Control group (n=263)	Test	*P* value
Gestational age, w, mean±SD	31.7±1.3	34.8±0.6	-50.9	<0.001
Male, n (%)	418 (57.7)	139 (52.9)	1.81	0.178
Birth weight, kg, mean±SD	1.6±0.1	2.1±0.2	-38.80	<0.001
Cesarean delivery, n (%)	487 (67.1)	106 (40.3)	58.06	<0.001
Exclusive breastfeeding, n (%)	218 (30.1)	127 (48.3)	28.19	<0.001
Exclusive formula feeding, n (%)	396 (54.6)	85 (32.3)	38.42	<0.001
Antibiotic use, n (%)	479 (66.1)	95 (36.1)	71.10	<0.001
Probiotics use, n (%)	256 (35.3)	145 (55.1)	31.45	<0.001
Maternal anxiety, n (%)	351 (48.4)	63 (24.0)	47.43	<0.001
Maternal smoking, n (%)	327 (45.1)	59 (22.4)	41.67	<0.001
Hospital stay, d, mean±SD	29.4±21.3	6.2±3.5	28.29	<0.001
≤7 d, n (%)	173 (23.9)	153 (58.2)	102.78	
>7 d, n (%)	552 (76.1)	110 (41.8)	102.78	

SD: standard deviation

in terms of gestational age (χ^2 = 21.83; p <0.001), especially in the case of infant colic (χ^2 = 28.10; p <0.001) and infant regurgitation (χ^2 = 33.13; p <0.001) (Table II).

In addition, there were also found differences in infants according to the different weight at birth in at least one FGID (χ^2 = 16.00; p = 0.003), infant colic (χ^2 = 27.18; p <0.001), and infant regurgitation (χ^2 = 18.11; p <0.001) (Table III). There was no significant difference in the others.

There are several neonatal risk factors associated with FGIDs, as shown in Table IV. Univariate analysis revealed that FGIDs were significantly associated with gestational age, birth weight, cesarean delivery, breastfeeding, exclusive formula feeding, use of neonatal antibiotics and probiotics, maternal anxiety, maternal smoking, and hospitalization longer than 7 days (Table IV). The risk from infantile colic ([<28 weeks: OR = 5.28, 95% CI = 1.10-12.4, p = 0.003], [28-32 weeks: OR = 4.16, 95% CI = 1.05-11.5, p = 0.008])

Table II. Proportion (%) of infants born preterm with FIGDs, according to gestational age at birth.

Gestational age (weeks)	Infant colic n (%)	Infant regurgitation n (%)	Infant dyschezia n (%)	Functional constipation n (%)	Functional diarrhea n (%)	At least 1 FGID n (%)
< 28	9	8	5	4	1	12
(n=14)	(64.3)	(57.1)	(35.7)	(28.6)	(7.1)	(85.7)
28 - 32	113	127	79	58	6	206
(n=244)	(46.3)	(52.0)	(32.4)	(23.8)	(2.5)	(84.4)
32 - 34	98	140	74	59	13	238
(n=300)	(32.7)	(46.7)	(24.7)	(19.7)	(4.3)	(71.0)
34 - 36	122	136	79	69	14	269
(n=430)	(28.4)	(31.6)	(18.4)	(16.0)	(3.3)	(68.8)
χ^2 value	28.10	33.13	5.85	6.87	2.05	21.83
P value	<0.001	<0.001	0.119	0.076	0.562	<0.001

FGID: functional gastrointestinal disorder

Table III. Proportion of infants reporting FGIDs, according to type of disorder and birth weight group.

Birth weight (kg)	Infant colic n (%)	Infant regurgitation n (%)	Infant dyschezia n (%)	Functional constipation n (%)	Functional diarrhea n (%)	At least 1 FIGD n (%)
<1	8	7	3	3	1	10
(n=12)	(66.7)	(58.3)	(25.0)	(25.0)	(8.3)	(83.3)
1 – 1.5	89	85	35	30	9	119
(n=148)	(60.1)	(57.4)	(23.6)	(20.3)	(5.4)	(80.4)
1.5 – 2.5	282	280	151	130	21	472
(n=631)	(44.7)	(44.4)	(23.9)	(20.6)	(3.3)	(74.8)
2.5 – 3	60	53	36	21	3	96
(n=152)	(39.5)	(34.9)	(23.7)	(13.8)	(2.0)	(63.2)
>3	10	16	12	6	1	28
(n=45)	(22.2)	(35.6)	(26.7)	(13.3)	(2.2)	(62.2)
χ^2 value	27.18	18.11	0.20	5.00	5.00	16.00
P value	<0.001	0.001	0.995	0.287	0.287	0.003

FGID: functional gastrointestinal disorder

and infant regurgitation ([<28 weeks: OR = 4.12, 95% CI = 1.63-11.3, p = 0.045], [28 - 32 weeks: OR = 3.28, 95% CI = 1.18-12.4, p = 0.049]) was considerably elevated for infants with low gestational age. Moreover, the risk of infantile colic ([<1 kg: OR = 6.84, 95% CI = 2.35-15.6, p = 0.004], [1-1.5kg: OR = 4.21, 95% CI = 1.58-13.2, p = 0.023]) and infant regurgitation ([<1 kg: OR = 2.57, 95% CI = 1.86-5.38, p = 0.012], [1-1.5kg: OR = 1.26, 95% CI = 1.01-6.39, p = 0.035]) was considerably high for infants with lower weight at birth. In addition, if the infant was delivered by cesarean, the risk of functional constipation was higher (OR = 1.99, 95% CI = 1.31-3.18, p = 0 .015). Furthermore, infants exclusively fed with formula following birth displayed a higher risk for infantile regurgitation (OR = 2.02, 95% CI = 1.32-1.38, p = 0.009). Infantile colic was significantly associated with the duration of antibiotic use in the neonatal period ([8-14 days: OR = 2.69, 95% CI = 1.29-4.37, p = 0.006], [> 14 days: OR = 3.24, 95% CI = 1.06-5.45, p <0.001]). The use of probiotics in the first month of life was considerably associated with infantile colic ([age of probiotics initiation ≤14 days, OR = 1.98, 95% CI = 1.06-2.78, p = 0.001], [Duration of probiotics use >14 days, OR = 1.37, 95% CI = 1.24-2.92, p = 0.032]) and functional constipation ([age of probiotics initiation ≤14 days, OR = 1.93, adjusted 95% CI = 1.37-1.29, adjusted p =0.002], [duration of probiotics use >14 days, OR = 1.88, adjusted 95% CI = 1.63-2.19, P = 0.009]). Maternal anxiety (OR = 3.23, 95% CI = 2.83-10.5, p = 0.023), maternal smoking (OR = 2.15, 95% CI = 1.38-3.34, p = 0.005), and hospitalization longer than 7 days (OR = 2.17, 95% CI = 1.32-2.48, p <0.001) were also considerably associated with infantile colic. Furthermore, there were no other significant associations found.

The results of the multivariate logistic regression analysis are shown in Table V. Infantile colic was substantially linked to a gestational age of 32 weeks or less and birth weight of 1.5 kg or less ([gestational age < 32weeks: adjusted OR = 4.08, adjusted 95% CI = 2.37-12.1, adjusted p = 0.013], [birth weight < 1.5kg: adjusted OR = 3.26, adjusted 95% CI =2.48-10.5, adjusted p = 0.026]) and infant regurgitation ([gestational age < 32 weeks: adjusted OR = 3.25, adjusted 95% CI = 2.19–6.84, adjusted p = 0.027], [birth weight < 1.5kg: adjusted OR = 2.78, adjusted 95% CI =1.48–5.25, adjusted p = 0.015]). Cesarean delivery (adjusted OR = 2.74, adjusted 95% CI = 1.28-11.3, adjusted p < 0.001) was considerably linked to functional constipation. Antibiotic use over 8 days (adjusted OR = 2.93, adjusted 95% CI = 1.28-5.39, adjusted p <0.001), maternal smoking (adjusted OR = 2.43, adjusted

Table IV. Neonatal risk factors against FGIDs.

Risk factors		Infant Colic		Infant regurgitation		Infant dyschezia		Functional constipation		Functional diarrhoea	
		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Gestational age (weeks)	<28	5.28 (1.10-12.4)	0.003**	4.12 (1.63-11.3)	0.045*	5.42 (0.92-12.8)	0.468	1.21 (0.32-2.58)	0.362	1.62 (0.91-3.21)	0.796
	28– 32	4.16 (1.05-11.5)	0.008**	3.28 (1.18-12.4)	0.049*	4.26 (0.53-12.3)	0.890	1.19 (0.58-4.97)	0.098	1.43 (0.38-5.46)	0.591
	32– 34	3.56 (0.03-8.23)	0.358	2.89 (0.54-10.7)	0.935	2.03 (0.14-13.6)	0.591	0.99 (0.48-3.74)	0.486	1.83 (0.91-4.62)	0.454
	34– 36	1.24 (0.14-7.84)	0.681	1.02 (0.93-11.8)	0.391	2.08 (0.32-3.51)	0.723	1.15 (0.56-6.23)	0.537	1.16 (0.23-3.28)	0.327
Birth weight (kg)	<1	6.84 (2.35-15.6)	0.004***	2.57 (1.86-5.38)	0.012*	2.83 (0.35-5.83)	0.325	0.99 (0.01-3.57)	0.456	1.58 (0.45-6.72)	0.097
	1 – 1.5	4.21 (1.58-13.2)	0.023*	1.26 (1.01-6.39)	0.035*	2.35 (0.98-4.76)	0.285	1.03 (0.22-3.87)	0.518	1.07 (0.67-6.85)	0.085
	1.5– 2.5	3.26 (0.96-10.8)	0.784	1.13 (0.78-5.67)	0.563	1.78 (0.96-5.98)	0.387	1.19 (0.75-4.93)	0.257	2.23 (0.32-5.75)	0.196
	2.5– 3.0	1.42 (0.89-3.25)	0.256	0.92 (0.46-1.28)	0.359	1.85 (0.21-6.76)	0.149	0.97 (0.35-3.98)	0.768	1.45 (0.91-8.92)	0.799
	>3.0	1.37 (0.85-9.96)	0.478	1.01 (0.35-3.67)	0.192	1.21 (0.45-3.28)	0.293	1.98 (0.92-6.83)	0.358	0.92 (0.03-6.78)	0.596
Mode of delivery	Vaginal	0.89 (0.15-3.65)	0.476	0.96 (0.25-4.28)	0.532	1.03 (0.68-3.86)	0.321	0.32 (0.11-1.71)	0.065	0.08 (0.01-1.28)	0.093
	C-section	2.58 (0.43-10.5)	0.214	1.87 (0.71-3.23)	0.958	1.34 (0.51-2.27)	0.119	1.99 (1.31-3.18)	0.015*	1.56 (0.28-2.95)	0.315
Exclusive breast-feeding		1.65 (0.59-4.01)	0.168	1.67 (1.35-2.28)	0.002**	0.98 (0.03-2.6)	0.437	5.35 (0.78-6.39)	0.309	5.83 (0.25-8.74)	0.538
Exclusive formula feeding		0.98 (0.42-1.78)	0.279	2.02 (1.32-3.38)	0.009**	1.83 (0.67-3.65)	0.735	0.03 (0.01-1.78)	0.537	0.28 (0.15-1.19)	0.546
Duration of antibiotic use(days)	≤7	1.18 (0.54-2.45)	0.345	1.67 (0.27-3.46)	0.231	1.87 (0.54-2.45)	0.549	2.38 (0.84-4.78)	0.768	1.56 (0.26-3.75)	0.467
	8-14	2.69 (0.29-4.37)	0.076**	2.32 (0.14-4.85)	0.675	1.62 (0.43-2.78)	0.337	1.95 (0.25-3.27)	0.573	1.56 (0.26-3.75)	0.498
	>14	3.24 (1.06-5.45)	<.0001***	2.35 (0.01-2.65)	0.062	1.20 (0.94-1.52)	0.144	1.12 (0.85-1.46)	0.426	2.14 (0.51-2.56)	0.749
Age of probiotics initiation (days)	≤14	1.98 (1.06-2.78)	0.001**	1.26 (0.67-2.75)	0.345	0.87 (0.04-1.13)	0.258	1.93 (1.37-2.29)	0.002**	1.01 (0.08-1.69)	0.679
	>14	0.84 (0.01-1.23)	0.067	0.79 (0.12-1.65)	0.289	0.87 (0.25-1.67)	0.323	1.35 (0.65-2.37)	0.856	0.65 (0.02-1.58)	0.007
Duration of probiotics use (days)	≤14	1.36 (0.21-2.89)	0.062	1.84 (0.98-2.63)	0.328	2.36 (0.67-4.56)	0.129	2.36 (0.93-5.47)	0.685	2.36 (0.19-3.68)	0.474
	>14	1.37 (1.24-2.92)	0.032*	2.98 (0.13-3.96)	0.078	1.85 (0.83-2.92)	0.126	1.88 (1.63-2.19)	0.009**	0.92 (0.08-1.67)	0.287
Maternal anxiety		3.23 (2.83-10.5)	0.023*	3.36 (0.52-10.7)	0.058	4.43 (0.54-6.57)	0.374	2.08 (0.62-2.59)	0.708	1.42 (0.65-5.71)	0.657
Maternal smoking		2.15 (1.38-3.34)	0.005**	1.81 (0.27-2.64)	0.176	1.54 (0.83-3.29)	0.096	0.99 (0.27-2.64)	0.384	1.62 (0.77-2.83)	0.753
Hospital stay (days)	≤7	1.29 (0.87-2.58)	0.346	1.15 (0.57-1.94)	0.287	1.49 (0.91-2.03)	0.985	1.15 (0.59-3.86)	0.06	1.15 (0.86-2.74)	0.212
	>7	2.17 (1.32-2.48)	<.0001***	2.13 (0.56-3.49)	0.563	1.85 (0.65-2.56)	0.574	0.97 (0.56-1.49)	0.308	1.36 (0.67-1.99)	0.657

*: p<0.05, **: p<0.01, ***: p<0.001. CI: confidence interval, FGID: functional gastrointestinal disorder, OR: odds ratio.

Table V. Multivariate analysis for risk factors associated with FGIDs.

Risk factors	Infant colic		Infant regurgitation		Infant dyschezia		Functional constipation		Functional diarrhoea	
	aOR (95% CI)	p value	aOR (95% CI)	p value	aOR (95% CI)	p value	aOR (95% CI)	p value	aOR (95% CI)	p value
Gestational age <32 weeks	4.08 (2.37-12.1)	0.013*	3.25 (2.19-6.84)	0.027*	4.21 (0.27-11.5)	0.532	2.74 (0.87-4.82)	0.286	2.62 (0.93-5.27)	0.663
Birth weight <1.5 kg	3.26 (2.48-10.5)	0.026*	2.78 (1.48-5.25)	0.015*	2.56 (0.27-5.92)	0.227	3.25 (0.51-7.62)	0.354	1.88 (0.61-5.63)	0.089
Cesarean delivery	3.58 (0.39-9.05)	0.147	2.08 (0.85-3.99)	0.263	2.38 (0.01-4.72)	0.206	2.74 (1.28-11.3)	<.0001***	4.56 (0.98-8.53)	0.764
Exclusive breast-feeding	0.95 (0.72-5.71)	0.211	1.87 (1.36-2.92)	0.003**	1.56 (0.33-3.26)	0.387	2.35 (0.78-6.29)	0.563	2.74 (0.47-7.35)	0.495
Exclusive formula feeding	1.65 (0.23 -2.86)	0.386	1.84 (1.21-3.83)	0.011*	1.96 (0.67-3.45)	0.564	3.21 (0.86-5.83)	0.485	2.75 (0.32-4.34)	0.381
Duration of antibiotic use ≥ 8 days	2.93 (1.28-5.39)	<.0001***	3.26 (0.28-5.47)	0.547	1.94 (0.37-3.91)	0.229	1.89 (0.35-3.16)	0.672	2.56 (0.89-4.22)	0.502
Duration of probiotics use >14 days	1.85 (1.65-2.23)	0.005**	1.25 (0.84-2.28)	0.108	0.73 (0.23-1.28)	0.227	1.84 (1.46-2.47)	0.021*	0.68 (0.21-0.92)	0.183
Maternal smoking	2.43 (1.57-4.29)	0.004**	2.35 (0.37-3.58)	0.701	2.32 (0.56-5.25)	0.129	1.56 (0.32-4.56)	0.371	2.32 (0.54-3.75)	0.698
Hospital stay > 7 days	2.27 (1.36-5.62)	<.0001***	3.26 (0.73-5.47)	0.335	2.47 (0.53-4.28)	0.229	1.75 (0.47-3.82)	0.834	2.52 (0.43-4.94)	0.469

aOR: adjusted odds ratio, FGID: functional gastrointestinal disorder, *: p<0.05, **: p<0.01, ***: p<0.001

95% CI = 1.57-4.29, adjusted p = 0.004), and hospitalization longer than 7 days (adjusted OR = 2.27, adjusted 95% CI = 1.36-5.62, adjusted p <0.001) were considerably linked to infantile colic. There was a lower prevalence in infantile colic owing to probiotic use lasting more than 14 days (duration of probiotics use >14 days, adjusted OR = 1.85, adjusted 95% CI = 1.65-2.23, adjusted p = 0.005) and functional constipation (duration of probiotics use >14 days, OR = 1.84, adjusted 95% CI = 1.46-2.47, p = 0.02). Following birth, both exclusive breastfeeding (adjusted OR = 1.87, adjusted 95% CI = 1.36-2.92, adjusted p = 0.003) and formula feeding (adjusted OR = 1.84, adjusted 95% CI =1.21-3.83, adjusted p = 0.011) were considerably linked to infant regurgitation.

Discussion

Based on the retrospective evaluation of the preterm infant cohort in their first year of life, a high prevalence (73.4%) of FGIDs were found, which is in line with Salvatore's[1] study (76%). However, a higher prevalence (73.4%) of FGIDs was found than those reported in African (50%), American (24%), and European (25%) populations that were also based on the Rome IV criteria.[5,7,13] There may be an explanation for this situation as a significant portion of the subjects in this study were preterm infants receiving antibiotic treatment within the first month of their lives. This lower incidence in America might be attributed to the small sample size (n = 58). The low prevalence in the European population may be attributed to the fact that the subject's parents were interviewed by a healthcare professional who might interpret the symptoms differently. There are no specific biomarkers or investigations for diagnosing FGIDs. Therefore, clinical criteria are used instead, while organic disease warning signs are excluded. The Rome III criteria and, more recently, the Rome IV criteria give a thorough categorization of distinct FGIDs at various ages. Nevertheless, the likelihood of diagnostic

heterogeneity or misclassification among various physicians, as well as an overevaluation of the incidence of FGIDs, cannot be ruled out.

Infants with FGIDs suffer from a wide variety of disorders.[2] Over 70% of preterm subjects had at least one FGID, and 24.4% had more than one, with infantile colic and regurgitation being the most prevalent to present concurrently. Gastrointestinal infection along with early life experiences like gastric suction, cow milk protein allergies, inflammation, trauma, and stress have all been linked to a higher risk of visceral hyperalgesia and gastrointestinal problems late in life.[2,11,14-20] It is important to consider these results carefully and to replicate them in other preterm populations before drawing a general conclusion, as genetic and environmental factors, parental and physician perceptions, feeding patterns, and pharmacological treatments are all thought to be determinants of infant FGID rates.

The effects of different neonatal factors such as the gestation age, gender, birth weight, delivery type, feed patterns, antibiotics therapy, and probiotics therapy during the first month of life, maternal anxiety, maternal smoking, and duration of hospitalization in developing FGIDs among infants were simultaneously assessed. Both univariate and multivariate statistical analyses were conducted for the purpose of limiting the cumulative effects of various risk factors as well as for identifying the major factors contributing to FGIDs.

According to Milidou[21], infantile colic rates increase with decreasing gestational age. Further, the odds of infantile colic were higher among small-for-gestational-age infants possessing birth weights below the 10th percentile. We also found that infants with a gestational age of lower than 32 weeks have an enhanced risk of infantile colic and infant regurgitation. As reported in Danish and Italian studies, infants with lower birth weights are at double risk (or even higher) for developing infantile colic.[22,23] In line with this, our results indicated that low birth weight (<1.5 kg) was linked to an elevated risk of infantile colic as well as regurgitation.

In recent years, preterm birth as well as neonatal antibiotics usage during the first month of life has been linked with an ever-growing occurrence of FGIDs.[11,24] Premature birth and being exposed to a variety of variables that have been linked to influencing gastrointestinal homeostasis, pain perception, and sensitivity, lead to a higher incidence of preterm infants than term infants.[11,24] Compared with term neonates, the intestinal microflora of preterm infants is more susceptible to dysbiosis or imbalance in gut microbial communities, which is closely related to functional gastrointestinal disorders.[25] In this study, infants delivered by cesarean section were more vulnerable to functional constipation, which was the same as that reported by other studies.[26] Vaginal delivery is linked to considerably lower incidence of functional constipation; this phenomenon may be related to the intestinal microbiota of infants.[26] Several studies have shown that the mode of delivery influences gut microbiota among infants.[27] It is vertically transmitted maternal microbes to infants that contribute significantly to the establishment of the core gut microbiota. Notably, the microbiota of infants delivered via the vagina mimics their mother's vaginal microbiota, whereas those of cesarean-birthed neonates resemble the mother's skin microbiota.[28,29] Another study by Hojsak et al.[25] suggested that the early pattern of infant gut microbial colonization was critical for the suitable development of the human gastrointestinal tract.

It was further observed that infants being exclusively breastfed following birth have reduced risk of infant regurgitation, whereas it is enhanced for formula-fed infants. As part of its pathophysiology, regurgitation can be attributed to limited esophagus volume combined with the immaturity of the lower esophageal sphincter, overfeeding, and infant posture.[2] Parents tend to overfeed in the case of bottle feeding owing to being less likely to respond to the infant's satiety cues.[30] This

might illustrate why formula-fed neonates have a higher proclivity for regurgitation. There is evidence that breastfed infants have different feeding patterns that are associated with reduced reflux due to self-regulation of milk consumption, resulting in increased frequency and decreased volume of feedings.[31] Furthermore, breastfed babies have quick stomach emptying. As a result, a low esophageal pH value, which is more likely to trigger peristalsis, leads to a shorter period of reflux.[32]

Interestingly, it was also found that the prevalence of infantile colic is considerably elevated in the case of antibiotic use over 8 days in the neonatal period. This result was consistent with previous studies.[1,33] Antibiotics have been comprehensively utilized in premature infants, and current evidence also reflects the negative effects of antibiotics on gut microbiota's composition and functions thereof. Antibiotic-induced dysbiosis is an important factor in functional gastrointestinal disorders. Genetically susceptible newborns are at higher risk of allergy and inflammatory bowel disease after being given antibiotics in the early stages of life.[34-36] Infections as well as antibiotic usage during early life can cause immunological dysregulation, abnormal barrier functioning, microbiota change, and altered gut sensory activities, all of which can lead to FGIDs in susceptible individuals.[14,37]

It was also observed that early use of probiotics (less than 14 days after birth) as well as their usage for more than 14 days can reduce the incidence of infantile colic and functional constipation. The use of probiotics can improve the bacterial flora imbalance caused by long-term use of antibiotics, thus relieving infantile colic. Probiotic preparations significantly shorten gastrointestinal passage time, increase defecation frequency, and improve fecal traits, promoting defecation.[38]

This study also revealed that infantile colic was considerably associated with maternal smoking and hospitalization longer than 7 days. A plausible explanation could be that passive smoking is linked to increased plasma and intestinal motilin levels and higher-than-average levels of motilin are linked to elevated risks of infantile colic.[39] The mechanism underlying the length of hospitalization and the incidence of functional gastrointestinal disorders is unknown and requires additional investigation.

This study is of clinical significance since it includes the examination of a large cohort of preterm infants and many neonatal variables along with the utilization of Rome IV criteria for classifying FGIDs. It should be noted that this study has some of the following limitations: First, our study did not have a multi-center design. Second, we attained symptom data from parents, which may be inaccurate. Lastly, parents of infants who experience symptoms are more likely to respond.

Gastrointestinal symptoms are typically stressful to the newborn and parents, resulting in a cascade of events involving infant discomfort, crying, parental concern, diminished quality of life, recurrent healthcare professional visits, and rising healthcare expenditures.[6,40,41] Recognizing the exact incidence pattern of FGIDs is critical for developing a targeted program for parents' education as well as clinical follow-ups. Furthermore, identifying linked neonatal risk factors for FGIDs is a necessary measure for designing potential early-life therapies to reduce FGIDs later in life.[28,42]

Herein, it has been postulated that FGIDs may be prevented by reducing the unnecessary use of neonatal antibiotics, probiotics, and cesarean deliveries as well as by promoting breastfeeding for promoting intestinal homeostasis among at-risk neonates.

In conclusion, the incidence rate of FGIDs among premature infants was higher during the first year. Herein, the data have shown that infantile colic, as well as infant regurgitation, are among prevalent FGIDs, and some infants presented with a variety of FGIDs. It is worth

noting that low gestational age (<32 wks), low birth weight (<1.5 kg), neonatal antibiotic use, cesarean section, hospital stay, formula feeding, and maternal smoking are risk factors for FGIDs in preterm infants. In contrast, postnatal breastfeeding and probiotics supplementation are protective factors against infant FGIDs.

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Ethical approval

The study was approved by the ethics committee of Northern Jiangsu People's Hospital (2022ky291), Jiangsu, China. The survey was conducted in accordance with the guidelines of the Declaration of Helsinki and the International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HJ and GS; data collection: DB and KY; analysis and interpretation of results: DB, KY, TG and LH; draft manuscript preparation: DB, HJ. 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

A common problem in infants: vitamin B_{12} deficiency

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ABSTRACT

Background. Nutritional vitamin B_{12} (VB_{12}) deficiency is characterized by anemia, the inability to gain weight, delay or decline in development. Children of mothers with VB_{12} deficiency have a risk of nutritional VB_{12} deficiency. Prevention and early treatment are necessary to prevent irreversible neurological damage. We aimed to conduct a retrospective study to understand the characteristics of patients with VB_{12} deficiency.

Methods. Our study included patients admitted to Başkent University Faculty of Medicine Pediatric Hematology outpatient clinic between January 2015 - February 2020 for VB_{12} deficiency. Their clinical and laboratory characteristics were retrospectively examined through the hospital automation system.

Results. Vitamin B_{12} deficiency was detected in 129 of the 3198 patients; 100 of them were followed regularly. The mean age at admission of our patients was 10 ± 12 months (1 month - 7.5 years); 98% of these children were aged 0-2 years. The mean VB_{12} level of our patients was 171.63 ± 51.2 pg/ml (83 - 273), mean hemoglobin 11.2 ± 1.37 g/dl (6.3 - 13.9), mean MCV 74.5± 9.1 fl (54-106.5) and mean iron level was 54 ±23 μg/dl (18 - 94). At the end of one month of loading therapy (oral or intramuscular, IM), the average VB_{12} level was 769 ± 537 pg/ml (post loading). One month after the loading therapy (pre-maintenance) the average VB_{12} level was 426 ±156 pg/ml. In seven cases who received IM therapy, the loading treatment was performed for the second time. The mean VB_{12} level of the mothers of 85 cases was 174±127 pg/ml (134 - 650). VB_{12} deficiency was detected in 55% of mothers, VB_{12} level being between 200 - 300 pg/ml in 76%, and below 200 pg/ml in the 24%. The family members of 35% of our patients (including parents) had VB_{12} deficiency.

Conclusions. In our country, routine screening of VB_{12} levels in infants is not performed; however, its early diagnosis and treatment can prevent many adverse effects mainly on the central nervous system. The fact that 98% of patients were 0-2 years old indicates that its deficiency may be quite high in the young age, and routine screening of this age group for VB_{12} deficiency and further studies for prophylaxis may be needed.

Key words: nutritional vitamin B_{12} deficiency, maternal vitamin B_{12} deficiency, vitamin B_{12} treatment, infancy.

Vitamin B_{12} (VB_{12}, cobalamin) is of particular importance for the development of the central nervous system (CNS). It is characterized by anemia, an inability to gain weight, and a delay or decline in development. Children of mothers with VB_{12} deficiency have a similar risk. Prevention and early treatment are necessary to prevent irreversible neurological damage, especially in infants.[1]

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VB_{12} deficiency, may be due to nonconsumption or inadequate consumption of animal foods such as dairy products/ insufficient consumption of meat, milk (because of vegetarianism, poverty) or malabsorption (e.g., pernicious anemia, achlorhydria, ileum damage or gastric bypass surgery).[2]

Mothers who are vegetarians have low levels of VB_{12} in both their serum and breast milk. Symptoms of deficiency can be detected in these infants from 4-6 months after birth.[2]

There is no routine screening of VB_{12} levels in infants, however, its deficiency has significant effects on systems, particularly the central nervous system. The purpose of our study was

to form an opinion regarding the prevalence and characteristics of patients diagnosed with VB_{12} deficiency in our clinic.

Material and Methods

Children between the ages of 0-7 years who were diagnosed with and treated for VB_{12} deficiency at Başkent University Pediatric Hematology outpatient clinic between January 2015 - February 2020 were retrospectively analyzed through the hospital automation system. The study was approved by the Başkent University, Medical and Health Sciences Research Ethics Committee (Project number: KA22/215, 02.06.2022) and informed consent was obtained from the families. Serum VB_{12} level below 300 pg/ml was defined as VB_{12} deficiency. Prior to treatment, other tests such as folic acid levels, urine protein, coeliac auto antibodies, maternal VB_{12} level (serum), parasite in the stool (3 times in a row), *Helicobacter pylori* antigen in the stool were also evaluated. The loading and maintenance therapies were administered either as parenteral (classical therapy) or oral cyanocobalamin therapies. The loading phase of the parenteral therapy protocol included cyanocobalamin administration 100 μg/day every day in the first week; 100 μg/every other day during the 2nd week; 100 μg/twice a week for 3rd and 4th weeks; through intramuscular (IM) route. Maintenance therapy included 100 μg of cyanocobalamin IM once a month, the duration to be determined individually the shortest being three months.[3]

Loading therapy of oral cyanocobalamin (oral therapy) was administered as 1000 μg/day VB_{12} (one ampoule), every day during the first week one ampoule every other day during the 2nd week; one ampoule twice a week during the 3rd and 4th weeks orally before meals. Maintenance therapy was given orally for the next three months, one ampoule per week, on an empty stomach.[3]

For monitoring the response to treatment in patients who received IM therapy, the VB_{12} level was examined immediately after the end of loading treatment (post- loading), 1 month later (before the first maintenance: pre-maintenance) and one month after the third maintenance (post-maintenance) and before the subsequent maintenance treatments if available.

In those who were given oral therapy, the VB_{12} level was examined immediately after the end of the loading therapy (post loading) and immediately after the end of the maintenance therapy (at the end of three months of maintenance therapy: post maintenance).

Serum iron, iron binding capacity, and ferritin were also evaluated in patients if available. In patients who received IM VB_{12} therapy, a second loading therapy was administered if their post-induction VB_{12} level was below 500 pg/ml. This protocol involved 250 μg/week cyanocobalamin by IM route, for four weeks. The aforementioned maintenance therapy was started one month after the end of the second loading therapy.

In the therapy protocol, no second loading therapy was recommended for those who received oral VB_{12} therapy since the maintenance of oral therapy involved more frequent administrations of drugs. The protocol was designed with the goal of keeping the lowest VB_{12} level during treatment at 350 pg/ml and filling up the stores as much as possible.

Statistical analysis

SPSS 21 software was used for statistical analysis. Descriptive statistics (including frequencies and percentages) were calculated for nominal variables. The mean ± standard deviation (SD), and median and range (minimum value-maximum value) were given for continuous variables. The significance of the difference between the groups was evaluated by Student's t-test. $P<0.05$ was considered statistically significant.

Results

VB_{12} deficiency was found in 129 of 3198 patients (4%) admitted to our outpatient clinic during this time period. The mean age at admission was 10 ± 12 months (1 month - 7.5 years); 98% of cases were between the ages of 0-2 years; 61 (47%) were girls and 68 (53%) were boys.

Admission complaints of patients are presented in Table I, and their characteristics and accompanying diseases in Table II. It was striking that a considerable number of patients in our cohort (44.3%) had no complaints or abnormalities on physical examination and were detected coincidentally. However, the majority (55.9%) had complaints or findings like irritability (17.8%), insomnia (11.6%), rejection of complementary foods (9.3%), delay in walking (8.5%), and microcephaly (8.5%) (Table I). Among the 126 patients between the ages of 0 and 2 years (n=126), 2 (1.5%) were receiving formula, 124 (96.1%) received breast milk and complementary food, and the 3 patients older than 2 years of age received only complementary food. Twelve patients (9.3%) refused complementary food.

No patient was solely breast fed. History of food allergy (cow's milk and multiple food) and prematurity were also striking in 10% and 3.2% of patients respectively (Table II). Thirty-five percent of patients (45/129) had a previously known history of VB_{12} deficiency in family members. Mean VB_{12} level of patients was 174 ± 49.6 (83 - 283) pg / ml (both oral and IM), mean hemoglobin (Hb) 11.2 ± 1.37 (6.3 - 13.9) g/dl, mean MCV 74.5 ± 9.1(54-106.5) fl and mean iron level was 54 ±23(18 - 94) μg/dl. Eighty-four mothers could be tested for serum VB_{12}; the mean VB_{12} of the mothers of our cases was 174 ±127 (134 - 650) pg/ml; 55% (46/84) had VB_{12} deficiency. VB_{12} level was between 200-300 pg/ml in 71.7% of the mothers (33/46) with VB_{12} deficiency, and ≤ 200 pg/ml in 28.3% (n: 13). Although none of the mothers were vegetarians, 20% of them consumed limited amounts of animal-based food because of economic conditions and dietary habits. The relationship between VB_{12} levels of the mothers and patients who were or were not breast feeding is presented in Table III. Among the mothers of patients with VB_{12} deficiency, 46 out of 84 mothers (54.7%) had VB_{12} deficiency (VB_{12} < 300 pg/ml). All except 1% of 129 patients were breast fed.

Table I. The symptoms and physical findings of the patients.

Symptoms and physical findings	N	%
Irritability	23	17.8
Insomnia	15	11.6
Rejection of complementary foods	12	9.3
Walking delay	11	8.5
Microcephaly	11	8.5
No symptoms or physical findings	57	44.3
Total	129	100

Table II. Patients' medical history and their comorbidities.

Comorbidities	n (%)
Cow milk allergy/multiple food allergy	13* (10%)
Prematurity/ small for gestational age (SGA) ***	4** (3.2%)

Maternal vitamin B_{12} deficiency in (maternal serum vitamin B_{12} levels <300 pg/ml) was detected in: 38 patients; food allergy (cow milk and multiple food) in 13; celiac disease in the one (both the child and the mother). Known etiology: 52/129 (40.4%); unknown etiology: 77/129 (59.6%).
*The mothers of the 9 out of 13 patients had low vitamin B_{12} level
** The mother of one pair of twins, out of 3, had low vitamin B_{12} level.
***The mean vitamin B_{12} levels of the mothers of premature/SGA babies were 340 pg/ml.

Table III. Vitamin B_{12} levels of patients according to maternal vitamin B_{12} level groups (N=84).

Maternal vitamin B_{12} level groups	N	Vitamin levels of patients (pg/ml)
100 - 150 pg/ml	3	157 + 41
150 – 200 pg/ml	10	160 + 45
200 – 250 pg/ml	16	182 + 49
250 – 300 pg/ml	17	178 + 57
>300 pg/ml	38	186 + 48

* 1 mother was not breastfeeding; her vitamin B_{12} level was 212 pg/ml, her child's vitamin B_{12} level was 189 pg/ml.

Coeliac disease was found in 1 (0.77%) as a coexisting disease with VB_{12} deficiency; the mother of that patient also had coeliac disease, as well. No patient had parasite ova, amoeba, or *Helicobacter pylori* in the stool. No patient had proteinuria suggestive of Imerslund-Grasbek syndrome, although 10% of patients with Imerslund-Grasbek syndrome may not have proteinuria.

Regular follow-up of VB_{12} levels after the loading therapy (oral or IM) could be performed in 85 out of 100 cases. IM therapy was used in 92 of the 100 cases, while oral therapy was used in eight. Detailed characteristics of the response to classical therapy (oral and IM) are presented in Table IV.

Seven cases, who had VB_{12} levels of 314±182 pg/ml (range: 121-402; <100 pg/ml, n=1; 100-150 pg/ml, n=4; 150-200 pg/ml, n=1; 200-250 pg/ml, n=1), were given a second ''loading'' therapy of 250 μg/week for four weeks, according to the therapy protocol. Those who received a second loading therapy attained a mean VB_{12} level of 880±767 pg/ml (range: 334-2000) at post 2nd loading and 452±126 pg/ml (range: 310- 646) at the pre-maintenance time-points (one month after the end of the ''second loading'' therapy, that is just before the ma''intenance therapy).

The VB_{12} levels of patients who had VB_{12} deficiency at admission and achieved a level of >300 pg/ml before first maintenance (n: 78) and <300 pg/ml (n: 7) are included in Table V. A comparison of IM and oral therapy is presented in Table VI.

Discussion

While most adults can tolerate malabsorption or a VB_{12}-insufficient diet without developing any clinical symptoms for several years, newborns may develop VB_{12} deficiency only a few months after birth due to limited liver storage; especially if maternal intake is restricted throughout pregnancy and if predominantly breast milk is given.[2]

Although most of the VB_{12}-deficient cases have only mild hematological findings, in approximately 10% of patients, life-threatening conditions such as symptomatic pancytopenia, severe anemia can be encountered.[4] Since VB_{12} deficiency is a common public health problem,

Table IV. Patients' response to parenteral therapy (before therapy, post-loading and pre-maintenance).

Time	Vitamin B_{12} level (pg/ml)
Before therapy (pre-loading) [n=100]	171.63 ± 51.2 (83-283)
After loading therapy (post-loading) [n=100]	769 ± 537 (147-2000)
Before first maintenance therapy [n=85] (pre-maintenance: 1 month after loading)	426 ± 156 (116-1100)
>350 pg/ml, n (%)	71/85 (83.6%)
<350 pg/ml, n (%)	14/85 (16.4%)

Data are presented as mean ± standard deviation (minimum - maximum) or n (%) as appropriate.

Table V. Distribution of patients according to initial vitamin B_{12} level groups and pre-maintenance level groups (1 month after loading).

Initial vitamin B_{12} level groups	Pre-maintenance level groups	
	>300 pg/ml (n=78)	<300 pg/ml (n=7)
<100 pg/ml (n: 6)	5 (83.3)	1 (16.7)
100-150 pg/ml (n: 28)	24 (85.7)	4 (14.3)
150 -200 pg/ml (n: 30)	29 (96.7)	1 (3.3)
200 - 250 pg/ml (n: 14)	13 (92.9)	1 (7.1)
250 - 300 pg/ml (n: 7)	7 (100.0)	

Data are presented as n (%).

Table VI. Comparison of intramuscular and oral therapy after loading therapy and after 3 months of maintenance therapy (second loading therapy is not included).

		VB_{12} levels (pg/ml)	
Initial VB_{12} level	Therapy	Post-loading level	Post-maintenance level 30 days after third monthly IM maintenance therapy OR 30 days after the end of oral maintenance therapy
200-250 pg/ml	IM cyanocobalamin (n: 12)	600 ±254	392 ± 32
	Oral cyanocobalamin (n: 3)	1085 ± 627	517± 22
	P value	0.083	0.043
250-300 pg/ml	IM cyanocobalamin (n: 7)	1079 ± 565	796 ± 58
	Oral cyanocobalamin (n:5)	1290 ± 598	585 ± 79
	P value	0.062	0.007

IM: intramuscular, VB_{12}: vitamin B_{12}
Data are presented as mean ± standard deviation.

vitamin B_{12} deficient newborns are detected by using markers for methylmalonic and propionic aciduria by tandem mass spectrometry.[5,6] The incidence of VB_{12} deficiency in these screening programs is 1/30000 in Germany, 1/113600 in U.S, 1/3000 in Estonia and 1/5000 in Italy.[7-10] Routine screening of VB_{12} levels in infants is not performed, however, many important consequences can be seen in its deficiency, especially those involving the central nervous system.

Nevertheless, in our cohort, the incidence of refusal of complementary foods was among the complaints of the mothers, in addition to insomnia and irritability. Those who refuse complementary foods, are irritable, and suffer from insomnia or sleep disturbances should be tested for VB_{12} deficiency, according to our findings. However, a large number of patients in our cohort did not have any complaints (44.3%) and found VB_{12} deficient by chance highlights the importance of routine VB_{12} screening of the infants.

Our careful etiological screening tests revealed maternal VB_{12} deficiency (maternal serum VB_{12} levels <300 pg/ml) in 38 patients, food allergy (cow milk and multiple foods) in 13 patients; and celiac disease in one patient (both the child and the mother). No patient had parasites, amoebas, or *Helicobacter pylori* in stool. No patient had proteinuria suggestive of Imerslund-Grasbek syndrome, although 10% of patients with Imerslund-Grasbek syndrome may not have proteinuria.[11] Consequently, no etiological factor could be identified in 59.6% (77/129) of the patients. This indicates that the etiology of many patients with VB_{12} deficiency cannot be determined in a general sense and that the available laboratory tests are insufficient.[2]

Planned long-term follow-up was in place for those who lacked a definitive etiologic factor.

That 10% of patients (who were on a diet at the time of evaluation) had cow's milk or multiple food allergies suggests that disturbance in the ileum probably involving the CUBAM receptor (cubilin/amnionless) was also present, or that the special diet they consume does not contain enough VB_{12}. Therefore, the risk of VB_{12} deficiency was reported to be high in breastfed infants on a diet excluding cow's milk, whereas complementary foods were associated with a higher B_{12} status.[12] These findings suggest that those with food allergies should be examined for VB_{12} deficiency and closely monitored.

Few studies have examined the association between maternal VB_{12} status in breast-fed infants.[13] Tanyildiz et al.[14] observed signs and symptoms of the central nervous system,

particularly in rapidly growing infants between the ages of 2 and 18 months. A remarkable finding was the simultaneous low levels of VB_{12} in the mothers in 55 of the 69 children who presented with neurological symptoms ($p < 0.05$). The fact that, 98% of patients in our cohort were children aged 0-2 years, indicates that the incidence of VB_{12} deficiency in this age group may be quite high in the pediatric population in our country, and therefore VB_{12} levels should be evaluated at an early stage.

The dramatic drop in post-loading VB_{12} levels 30 days later, at the pre-maintenance time-point, is thought to be due to VB_{12} settling into tissues and rebalancing with blood levels over time. Because of this, we believe that the serum VB_{12} level measured immediately after the loading therapy should be evaluated with caution.

The most common cause of cobalamin deficiency in nursing mothers is vegetarianism. In our series, some mothers stated that they consumed animal-based foods in limited amounts However, close to half (46%) of the mothers in our series had VB_{12} deficiency, which indicates the importance of close monitoring of mothers before and after pregnancy. In our study, 96.1% of patients with VB_{12} deficiency were fed with breast milk and additional food. Our findings are consistent with the fact that VB_{12} deficiency in breast milk is the most common cause of VB_{12} deficiency in infants. In addition to many other side effects, VB_{12} deficiency during pregnancy can also result in the birth of premature babies or babies with a low gestational weight.[15]

In order to prevent the development of VB_{12} deficiency, we believe that mothers should be closely monitored throughout and after pregnancy. It should be noted that the normal value of VB_{12} in adults is estimated to be 300 pg/ml. However, the normal range of VB_{12} in all laboratory kits indicates that the lowest normal level is between 160 and 180 pg/ml, which is significantly below the target level. This is the primary reason, in our opinion, for missing VB_{12} deficiency in previously tested patients. It was remarkable that the mothers of a significant proportion of children with VB_{12} deficiency in our cohort (26.3%) had VB_{12} levels between 200 and 300 pg/ml.

In order to avoid this situation, a general awareness of the lowest level of VB_{12} should be established as a level over 300 and even 350 pg/ml not only in children, but also adults and pregnant and nursing women should be given VB_{12} therapy to provide a VB_{12} level over 300-350 pg/ml. Additionally, women who give birth to SGA or premature babies should definitely be screened for VB_{12} deficiency. Breast milk intake alone restricts VB_{12} intake through additional food, interestingly, the duration of breast milk intake in VB_{12}-deficient infants may be longer when infants refuse complementary food. Although the cause of this behavior is unknown, it is thought to be due to hypotonia and difficulty of consuming solid food in VB_{12} deficient infants.[2]

Therapy for children with VB_{12} deficiency is not uniform. The most common therapy protocol used in our country and in our cohort does not involve a definite criteria of response at the end of the loading therapy, duration of therapy, maintenance therapy. In all protocols patient based follow-up is recommended, leaving the initiative to the doctor.

As a result of the studies in the world and our country over the years, it has been shown that oral VB_{12} treatment is as effective as intramuscular therapy in adults. Also sublingual methylcobalamin was determined as effective as oral and intramuscular cyanocobalamin improving vitamin B_{12} levels aged 0–3 years.[16] In order to administer a standard therapy, we administered the same loading therapy to all patients (a total of 1500 μg cyanocobalamin, as loading). However, for those who had not attained a post-loading level >500 pg/ml, we administered a second induction as it was mentioned before (additional 1000 μg of cyanocobalamin, yielding a total of 2500 μg of loading cyanocobalamine). Thus, the maintenance VB_{12} level could be attained over 300-350 pg/ml.

In the therapy protocol we have administered, a post-loading VB_{12} level below 500 pg/ml is arbitrarily deemed inadequate because the level of VB_{12} achieved at the end of the loading declined by nearly half in every patient; therefore, it is recommended that these patients receive a second loading therapy.

Routine therapy with 100 μg may have unfavorable outcomes in some patients. We think that there is no standard therapy but further investigations should be done. In our cohort, all patients benefited from the treatment. The period of maintenance is controversial and recommended for about one year. Our maintenance period was 1 year. Oral VB_{12} therapy, a novel mode of therapy was also administered in our clinic.[3] The results of eight children who received therapy for four months compared to the parenteral group showed contradictory results.

In conclusion, our study showed that the incidence of VB_{12} deficiency in children between the ages of 0-2 years may be high; therefore, evaluation of VB_{12} level in this age group may be necessary. We think that mothers, should be monitored during and after pregnancy for VB_{12} deficiency (their VB_{12} level should be >300 pg/ml) Babies with VB_{12} levels lower than 150 pg/ml - should be treated with a higher loading dose instead of the classical VB_{12} treatment dose (100 μg or less) These results indicate the need for further controlled studies to determine the ideal maintenance period, screening and prophylaxis for newborns.

Ethical approval

The study was approved by the Başkent University, Medical and Health Sciences Research Ethics Committee (Project number: KA22/215, 02.06.2022) and informed consent was obtained from the families.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design, data collection, analysis and interpretation of results, draft manuscript preparation: CK, LO. 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

Interventional cardiac catheterization in neonates and premature infants with congenital heart disease: a single center experience

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ABSTRACT

Background. The increased survival of patients with congenital heart disease over the last three decades has been associated with improvements in diagnosis and treatment. This study aimed to evaluate therapeutic interventional catheterization, outcomes and complications of these procedures in neonates and premature infants.

Methods. In this study, therapeutic catheterization procedures performed on neonates and premature infants with congenital heart disease at a university hospital between February 2000 and October 2019 were retrospectively evaluated.

Results. A total of 322 procedures were performed on 279 neonates and 26 premature infants. Of the patients, 217 (67.4%) were male. The median age of the patients was 8 days (interquartile range [IQR] 2-20) and the median body weight was 3050 g (IQR 2900-3600). The most common procedures were balloon atrial septostomy, balloon aortic angioplasty, balloon pulmonary valvuloplasty and balloon aortic valvuloplasty (35.4%, 20.8%, 18.3% and 12.4% respectively). The most common diagnoses were transposition of the great arteries, coarctation of the aorta, pulmonary stenosis and aortic stenosis (26.7%, 19.3%, 15.2% and 11.5% respectively). Most procedures, 274 (85.1%), were successful. Complications were observed in 74 procedures (23%). Of these complications, 45 (14%) were minor and 29 (9%) were major. The most common complication was transient dysrhythmia (6.9%). There was no significant relationship between body weight, age and the rate of complications. However, longer procedure time and fluoroscopy time were associated with higher complication rates ($p<0.05$). Four procedure-related deaths were observed.

Conclusion. Procedure-related complications are higher in the neonatal period. Although the complication rate varies according to the type of procedure, longer fluoroscopy time and procedure duration are associated with an increased complication rate. Procedures performed with the right indications, appropriate equipment and by experienced teams will play a key role in reducing complication rates.

Key words: children, congenital heart disease, interventional cardiology, complications.

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Severe types of congenital heart disease (CHD) affect 2.5-3.0 per 1000 live births, with 25% of neonatal deaths attributable to severe congenital heart malformations.[1,2] Over the last 30 years, the progress made in the early diagnosis and treatment of CHD has resulted in noticeable improvements in patient survival rates.[3] Cardiac catheterization and angiocardiography have several uses in the preoperative assessment of cardiac diseases. These include determining the anatomy, assessing the presence and size of the shunt, measuring pulmonary vascular resistance, evaluating the response to vasodilator agents and oxygen, conducting postoperative follow-up of congenital cardiac diseases, performing a myocardial biopsy, conducting electrophysiological studies, transcatheter ablation and interventional cardiological treatment applications.[4] Transcatheter treatment methods offer various advantages, including shorter hospitalization duration, no requirement for thoracotomy, reduced need for blood transfusion and general anesthesia, facilitating surgical techniques for complex cardiac anomalies and enabling treatment without a second thoracotomy in postoperative residual anomalies. This study aims to retrospectively evaluate therapeutic interventional cardiologic procedures, their results and complications, carried out on neonates and premature infants and compare the findings with the literature.

Materials and Methods

Among the 10,374 diagnostic or interventional cardiac catheterizations carried out at the Pediatric Cardiology Department's cardiac catheterization laboratory at Hacettepe University hospital between 2000 and 2019 on neonates and preterm infants were included in the study. Age, gender, body weight, diagnosis, echocardiography findings, postoperative follow-up, details of operative complications and management of complications were extracted from the cardiac catheterization laboratory's medical records and patient files.

Complications that arise during the catheterization and angiography or within 24 hours after the procedure are defined as procedure-related complications. Major complications were defined as life-threatening events including death, permanent rhythm abnormalities, bleeding requiring transfusion, respiratory arrest and cardiac perforation. Minor complications included non-life-threatening events such as transient circulatory and rhythm disturbances, bleeding from the surgical site not requiring transfusion, seizures, balloon rupture, etc. Mortality was categorized as either cardiac (procedural or post-procedural) or extra-cardiac (due to other causes, even if potentially aggravated by the cardiac procedure). Mortality resulting from electively planned surgery following a palliative percutaneous procedure was excluded, whereas deaths arising from the surgical rescue of complications or unsuccessful transcatheter intervention were considered procedure-related.[4,5]

Success criteria for procedures

Balloon atrial septostomy (BAS) or atrial septoplasty operations can result in improved arterial oxygen saturation, decreased pressure differences between the atria and an increase in the detectable diameter of the defect or flow with echocardiography.

Valvular pulmonary stenosis (PS): decrease in left ventricular/right ventricular systolic pressure ratio, increase in flow through the valve, or decrease in the need for prostaglandin E1 infusion.[6,7]

Valvular aortic stenosis (AS): 40% or more decrease in peak systolic pressure gradient, lower than 50 mmHg a residual peak gradient in patients with normal cardiac flow rate, decrease in left ventricular end-diastolic pressure, increase in output flow from the valve, increase in oxygen saturation in the lower extremities and decrease in the need for prostaglandin E1 or inotropic support.[8-10]

Coarctation of the aorta (CoA): a systolic gradient of 20 mmHg or less, a higher than 50% increase in the diameter of the coarcted segment or interruption of prostaglandin E1 infusion.[11-13]

Procedures that did not meet the above criteria were considered unsuccessful, while procedures that partially met the criteria were considered partially successful.

IBM SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA) was used for statistical evaluation. Frequency distributions were expressed as numbers and percentages and continuous variables (measurements) were evaluated as median (interquartile range [IQR]). The Shapiro-Wilk test was used to determine whether the data were normally distributed. For statistical evaluation, the independent samples t-test was used to compare paired groups for normally distributed data and the Mann-Whitney U test for non-normally distributed data. This study was approved by the Ethics Committee of Hacettepe University dated decision number LUT 12/42-28.

Results

A review of 10,374 cardiac catheterization procedures was conducted, of which 322 (6.4%) were performed for the treatment and diagnosis of 279 neonates and 26 preterm infants (Table I). Recurrent procedures were performed during the neonatal period; 13 patients (4.2%) underwent catheterization twice and 2 patients (0.7%) three times. Of all patients, 217 (67.4%) were male and 88 (27.3%) were female. The patients' median age was 8 days (IQR 2-20 days), with a median body weight of 3050 g (IQR 2900-3600 g).

BAS was the most frequently performed procedure, accounting for 35.4% (Table I). Transposition of the great arteries (TGA) was the most common diagnosis, followed by CoA, PS and AS (26.7%, 19.3%, 15.2% and 11.5%, respectively) (Table II). CoA was accompanied by AS in 5 patients and isthmus hypoplasia in 9 patients. The procedure lasted for a median of 60 minutes (6-90 minutes) and the median fluoroscopy duration was 12 minutes (7.2-21 minutes). The success rate was 85.1% (274 procedures), while 6.8% (22 procedures) were unsuccessful and 8.1% (26 procedures) were partially successful.

Interventional procedures

One hundred and fourteen BAS procedures were carried out. Two patients received BAS twice, one patient received concurrent aortic

Table I. List of procedures.

Procedure	N:322	%
Balloon atrial septostomy	114	35.5
Balloon aortic angioplasty	67	20.8
Balloon pulmonary valvuloplasty	59	18.3
Balloon aortic valvuloplasty	40	12.5
Ductal stenting	20	6.2
Perforation of pulmonary valve, pulmonary valvuloplasty	12	3.7
Stenting of the right ventricular outflow tract and pulmonary artery	4	1.2
Fistula closure	3	0.9
Closure of major aortopulmonary collateral artery	2	0.6
Balloon pulmonary angioplasty	1	0.3

Table II. List of procedures.

Diagnosis	N:305	%
Transposition of the great arteries	86	28.2
Coarctation of aorta	62	20.3
Pulmonary stenosis	49	16.1
Aortic stenosis	37	12.1
Pulmonary atresia with intact ventricular septum	15	4.9
Hypoplastic left heart syndrome	12	3.9
Pulmonary atresia	10	3.3
Tricuspid atresia	8	2.6
Double outlet right ventricle	6	2.0
Tetralogy of Fallot	6	2.0
Functional single ventricle	5	1.6
Other (coronary artery fistula, pulmonary arteriovenous fistula, interrupted aortic arch, aortic stenosis with coarctation of aorta)	9	3.0

balloon angioplasty and two patients received concurrent stent implantation of PDA with BAS. TGA was the most common diagnosis among patients who underwent BAS, accounting for 86 (76.7%) cases. Other diagnoses comprised hypoplastic left heart syndrome in eight patients (7.2%), tricuspid atresia (TA) in six patients (5.4%), double outlet right ventricle with a restrictive ventricular septal defect (VSD) in six patients (5.4%), pulmonary atresia with an intact ventricular septum in two patients (1.8%), aortic interruption in two patients (1.8%) and single functional ventricle in two patients (1.8%). Out of a total of 114 procedures, 99 (86.8%) were considered successful and 31 (27.2%) were accompanied by complications (Table III).

Sixty-seven aortic angioplasty procedures were performed and three of these were combined with aortic valvuloplasty procedures. Of the remaining 58 procedures (86.6%), the outcomes were successful, while three procedures (4.5%) were unsuccessful due to aortic hypoplasia. Fourteen (20.9%) procedures had complications (Table III). No aneurysms were present at the coarctation site after the procedures. Recoarctation occurred in 34 patients (50.7%), with recurrent angioplasty being performed in seven patients (10.4%). 27 patients (40.3%) survived without requiring surgery.

Fifty-nine procedures for pulmonary valvuloplasty were conducted, with a success rate of 91.5% (n=54) and partial success in four procedures (6.8%). Only one procedure was considered unsuccessful (1.7%), but ten patients (16.9%) had complications (Table III). Of the procedures, 54 (91.5%) were successful, four procedures (6.8%) were partially successful and one procedure (1.7%) was unsuccessful. Subsequently, eight patients needed further balloon valvuloplasty and five patients had surgery for stenosis (giving a total restenosis rate of 23.5%).

Of the 40 procedures carried out for AS using balloon aortic valvuloplasty, 29 (72.5%) were considered successful, one (5%) was

Table III. Distribution of the procedures according to age, body weight, duration of the procedure, duration of fluoroscopy, complications and success rate.

	Balloon atrial septostomy N:114	Balloon aortic angioplasty N:67	Balloon pulmonary valvuloplasty N:59	Balloon aortic valvuloplasty N:40	Stent implantation of patent ductus arteriosus N:20	Perforation of the atretic pulmonary valve and Balloon pulmonary valvuloplasty N:12	p
Age (day)	11.09 (12.46)	15.28(10.76)	14.83 (13.55)	12.60 (12.09)	8.40 (8.98)	2.17 (0.94)*	0.002
Body weight (g)	3188 (581.70)	3352 (637.60)	3119 (838.90)	3249 (755.30)	3045 (537.50)	3042 (264.40)	0.269
Procedure duration (min)	72.36 (30.07)	67.12 (30.15)	74.15 (28.86)	79.25 (31.49)	101.50 (43.71)**	131.25 (59.01)**	<0.001
Fluoroscopy duration (min)	15.48 (12.52)	11.01 (11.74)	16.07 (8.87)	16.52 (12.07)	21.26 (13.50)	38.97 (18.07)	<0.001
Complication	31 (27.20)	14 (20.90)	10 (16.90)	10 (25.00)	3 (15.00)	5 (41.70)	0.260
Success	99 (86.80)	58 (86.60)	54 (91.50)	29 (72.50)	17 (85.00)	8 (66.70)+	0.008

Complications and success are given as n (%) and other variables are given as mean (standard deviation).
*: The statistically significant difference is due to the low mean age of neonates in the marked procedure.
**: The statistically significant difference is due to longer mean fluoroscopy times in marked procedures.
+: The statistically significant difference is due to lower success rate in marked.

unsuccessful and the rest were considered partially successful. In three cases, AS was accompanied by aortic coarctation. However, complications were observed in 10 patients (%25) (Table III) and 11 patients required surgery during follow-up.

Twelve of the fifteen patients with an intact ventricular septum and pulmonary atresia (IVS-PA) underwent pulmonary valvular perforation and balloon pulmonary valvuloplasty, one patient also underwent concomitant pulmonary angioplasty and two patients underwent concomitant stent implantation in the PDA. Eight procedures (66.7%) were successful and four were unsuccessful (33.3%). While three of the unsuccessful procedures were due to technical reasons and the inability to maintain the proper position of the catheter, one procedure was due to right ventricular outflow tract (RVOT) perforation. Complications developed in 5 patients (41.7%). While minor complications were observed in two patients (transient circulatory disorders of the extremity and transient dysrhythmia), major complications were observed in three patients. Bleeding occurred at the intervention site in two patients. The procedures requiring pulmonary valve perforation and balloon pulmonary valvuloplasty had the highest rates of unsuccessful operations, longest operation and fluoroscopy time, and lowest age ($p<0.05$) (Table III).

Complications

Complications were observed in 74 (23%) procedures. Of these, 45 (14%) were minor complications and 29 (9%) were major complications (Table IV). The most common complication was transient arrhythmia (6.9%), mostly transient bradycardia (Table IV). Dysrhythmia was the most common complication in BAS (59%). A transient circulatory disturbance was seen in 11 patients and improved with elevation and warming of the extremity. Heparin infusion therapy was initiated in 5 patients due to the detection of a thrombus in the vascular access site (one femoral arterial and four femoral venous thrombi). All thrombi were resolved with medical therapy. Packed red cell transfusion was required in 6.5% of neonates due to bleeding from the access site during or after the procedure. Dissection of the aortic arch was observed in one patient with hypoplastic left heart syndrome after BAS and ductal stenting. There were no problems in the follow-up of the patient, who had no hemodynamic instability.

One patient had respiratory arrest, requiring short-term positive pressure ventilation without intubation. A 22-day-old neonate with a double outlet right ventricle developed pulseless ventricular fibrillation when the catheter entered the left ventricle during the procedure and responded to defibrillation. The procedure was considered unsuccessful and the patient was taken for emergency surgery. The patient who developed postoperative hemodynamic

Table IV. List of complications during the procedures.

Complications	N:248	%
Minor	45	14.0
Transient dysrhythmia	22	6.9
Bradycardia	8	2.5
Complete atrioventricular block	6	1.9
Atrial flutter	5	1.6
Atrial fibrillation	2	0.6
Tachycardia	1	0.3
Transient circulatory disorders of extremity/extremities in the intervention site	11	3.4
Thrombus	5	1.6
Balloon rupture	4	1.2
Apnea	2	0.6
Convulsion	1	0.3
Major	29	9.0
Bleeding in the insertion site	21	6.5
Vascular injury	3	0.9
Respiratory arrest during the procedure	2	0.6
Massive pulmonary hemorrhage	1	0.3
Perforation of right ventricular outflow tract	1	0.3
Ventricular fibrillation	1	0.3

instability died of cardiac arrest four days after the procedure. A total of 4 procedure-associated mortalities were noted. There was a case of massive pulmonary hemorrhage after ductal stenting in a patient with tricuspid atresia, who died due to cardiopulmonary arrest 48 hours later. A patient with TGA, who underwent BAS, had respiratory arrest in the catheterization room after the successful termination of the procedure and did not respond to resuscitation. There was massive pulmonary hemorrhage after ductal stenting in a patient with tricuspid atresia, who died due to cardiopulmonary arrest 48 hours later. One patient suffered from RVOT perforation during a pulmonary valve perforation and underwent RVOT repair, but died post-operatively. Another patient with hypoplastic left heart syndrome suffered from femoral vein dissection following ductal stenting. Following anticoagulant therapy, the patient's thrombosed femoral vein resolved completely. A neonate weighing 1200 g with IVS-PA experienced femoral vein avulsion during the removal of the 4 Fr sheath after a successful procedure. The patient was sent for surgical repair but suffered cardiac arrest during the operation and did not respond to cardiopulmonary resuscitation.

There was no statistically significant relationship found between body weight, age and complication rate ($p>0.05$). Nevertheless, higher complication rates were associated with longer procedure times and greater fluoroscopy durations (p values 0.029, <0.001, respectively).

Prognosis

Nineteen post-procedural mortalities due to cardiac and respiratory problems occurred within one week of follow-up. Of the patients, 71 (23.3%) died during follow-up (extracardiac mortality), 118 patients (36.6%) were lost to follow-up, and 129 patients (40.1%) continued follow-up. Of the patients who were followed, 74 (57,4%) continued their lives without the need for surgery. In patients requiring surgery, the median follow-up time until the procedure was 62 days (IQR 7-158 days) for the whole patient group, 65 (IQR 36-112) days for aortic angioplasty, 9 (IQR 3-184) days for BAS, 122 (IQR 12-1167) days for balloon aortic valvuloplasty and 401 (IQR 42-708) days for balloon pulmonary valvuloplasty.

Discussion

In this study, interventional cardiac catheterizations for neonates and premature infants were evaluated in a tertiary referral hospital. In recent years, transcatheter interventions for CHD have gained popularity with improvements in technology, devices and pre- and post-intervention care in intensive care units. Catheter based therapies offer a good alternative to surgery for both initial palliation and treatment.[1,14] As shown in the present study, it has a wide profile similar to the literature in terms of procedural diversity.[15-17] In the literature, it has been reported that the mean duration of the procedure in interventional cardiac catheterizations is between 115-122 minutes, with a median fluoroscopy time of 22-35 minutes. Lower durations were observed in this study.[17,18]

In the presented study, the most common procedure was BAS, and it was frequently applied to TGA patients. TGA is the most common cyanotic congenital heart defect in neonates, with an estimated incidence of 1 in 3200 live births.[19] Current medical approaches are medical stabilization and correction of acidosis followed by early arterial switch surgery. Preoperative death in neonates with TGA is due to hypoxemia refractory to prostaglandin E initiation, restrictive atrial septum, persistent pulmonary hypertension, prematurity and very low birth weight.[19] Emergency BAS improves oxygenation until definitive surgical treatment. In another study presented by our center, Celiker et al. reported that patients with TGA, TA, and severe mitral stenosis showed improvement in their clinical status and increased oxygen saturation levels following atrial septostomy. Atrial septostomy is a life-saving and effective intervention.[20]

Although the complication rate in this study was 27.5% and the most common complication was transient arrhythmia, the complication rate for BAS was reported as 47% (the most common complication was a thrombus in the femoral vein) in the literature.[21]

Coarctation of the aorta comprises 5-8% of all CHD.[22] In recent years, aortic balloon angioplasty has become a method that can be preferred to surgery because of its good outcomes.[23] However, this issue is still controversial because of early recoarctation after balloon dilation in newborns. Restenosis rates in balloon angioplasty range from 5% to 33.7% and are higher in neonates.[22,24,25] Postoperative recoarctation rates are similarly 7-30% and are more common in newborns.[11,26] Galal et al. reported that the rate of recoarctation in neonates was 90%, while recoarctatation was seen less by age, respectively, 62% in 30-90 days, 21% in 3-6 months and 6% in 6-12 months of life.[25] Similar to the literature, the rate of recoarctation was found to be high (50.7%) in this study, as it was a study in which newborns were included.

Similar to the presented study, the immediate success rate of balloon valvuloplasty in neonates varies between 87.5-100%. Technical problems and anatomical problems such as hypoplasia in the right ventricle, tricuspid valve and pulmonary valve may also affect this rate.[27, 28] Due to resistance to dilatation or subvalvular stenosis, surgery is required 5-10% of critical PS in neonates.[27,29,30] Karagöz et al.[15] showed that balloon dilatation was a safe and effective method for pulmonary valvular stenosis in neonates that weigh below 3000 gr, there was no procedure related mortality and only 30% of patients required reintervention at the 8th month of the procedure and 76% of patients did not require surgery during the 10-year follow-up. Loureiro et al.[27] showed that of the 24 neonates who underwent percutaneous balloon valvuloplasty, one died (4.17%), six (29.2%) had complications and reintervention rate was 42.9%. In the presented study, lower complication rates (16.9%) and restenosis (23.5%) were found compareble to the literature.

IVS-PA is a ductus-dependent heart disease. Although it has a high mortality rate without intervention, the percutaneous transcatheter approach, including pulmonary valve perforation (laser-assisted or radiofrequency-assisted) and balloon valvuloplasty, may preclude surgery. However, patients treated with the percutaneous approach have been shown to have a 5-25% procedural failure rate, 5-50% risk of cardiac perforation, 17% procedural mortality, 50% hospital mortality, and 76% risk of requiring surgical intervention.[31] This procedure had the lowest success rate, the highest complication rates and the longest procedure and fluoroscopy duration in the presented study. Although complications were common with this procedure the majority of them were hemorrhages requiring blood transfusion and transient dysrhythmia. We thought that this was related to the younger age of the patients, performing two procedures at the same time, and technical difficulties.

Complications range from resolving spontaneously without treatment to requiring open heart surgery. The risk for complications may be related to the age, weight, clinical status of the patient, the type of underlying disease and procedure (diagnostic or interventional), as well as the cardiologist's skills and experience.[32] Mori et al.[33] reported that the rate of complications was 14.7% in the cardiac catheterization of the pediatric age group and the most common complication was arrhythmias. Mehta et al.[16] revealed that the rate of complications was 7.3% and independent risk factors for complications were male gender and being under 6 months of age. In the presented study, no significant relationship was found between the development of complications and age, gender and body weight but procedure time and fluoroscopy duration were associated with higher complication rates. Sutton et al.[18] found that the complication rate was 57% in infants under 1500 g, and 56% in infants between 2000-3000 g. The most common complication was bleeding requiring a blood

transfusion. In our study, the complication rate was 23%. The most common complication was transient dysrhythmia (6.9%) and among these dysrhythmias the most common was bradycardia. The most common major complication was transient bleeding at the insertion site.

This study has some limitations. The study was designed as a retrospective, single-center study. This study did not include long-term longitudinal follow-up data of the patients. Some of the patients were lost to follow-up. Therefore, mortality, restenosis, surgery and prognosis of the whole patient group could only be evaluated in the follow-up patient group. There is a need for prospective studies in which the outcomes of patients are also evaluated.

In conclusion, interventional cardiological procedures in neonates are life-saving and as well as bridging treatment to surgery in order to improve the clinical status of the patient before the operation. Complications related to the procedure are found to be higher in the neonatal period. Although the complication rate varies according to the procedure type, long fluoroscopy time and procedure duration are associated with an increase in the complication rate. Procedures performed with the right indications, appropriate equipment and by experienced teams will play a key role in reducing complication rates. Further prospective studies with a larger series are needed on interventional cardiac catheterization.

Ethical approval

The study was approved by the Ethics Committee of Hacettepe University (no. LUT12, 42-28).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: UTN, KT, AE, AD, EI and CA; data collection: UTN, AHH and UYA; analysis and interpretation of results: UTN, KT and AHH UTN; draft manuscript preparation: UTN, AHH, KT. 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 efficacy of oral motor interventions on feeding outcomes in newborns with hypoxic-ischemic encephalopathy who received therapeutic hypothermia

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ABSTRACT

Background. Feeding difficulties continue to be a serious problem in newborns with hypoxic-ischemic encephalopathy (HIE) undergoing therapeutic hypothermia (TH). The aim of this study was to investigate the efficacy of oral motor interventions (OMI) on feeding outcomes in neonates with HIE/TH.

Methods. This was a prospective randomised control study conducted between January 2022 and September 2022. Premature Infant Oral Motor Intervention (PIOMI) was used as OMI. Newborns with HIE/TH, who underwent PIOMI, constituted the study group, and newborns, who did not receive any feeding exercise, constituted the control group. Transition time to full oral feeding (FOF) was determined as the time between initiation of tube feeding and full oral breastfeeding or bottle feeding. The day per oral (PO) feeding was started was specified as PO first, the day the infants could take half of the volume of the feedings by mouth was PO half, and the day the infants could take all the feedings by mouth was PO full.

Results. There were 50 neonates in each group. Time to FOF was significantly shorter in the study group than in the control group in all stages of HIE/TH (P= 0.008 for stage 1, and <0.001 for stage 2 and 3 HIE). However, times to PO first, PO half, PO full and discharge were shorter in the study group than in the control group only in the neonates with stage 3 HIE (P= 0.003, 0.014, 0.013, 0.042, respectively).

Conclusions. The PIOMI, which could be named as "HIE-OMI" in our study, is an effective intervention in shortening the transition time to FOF in neonates with all stages of HIE undergoing TH. In addition, "HIE-OMI" shortens the length of hospital stay, and improves feeding outcomes in neonates with severe HIE/TH.

Key words: hypoxic ischemic encephalopathy, feeding outcomes, oral motor interventions, therapeutic hypothermia.

Hypoxic-ischemic encephalopathy (HIE) is an important cause of morbidity and mortality in newborns, and therapeutic hypothermia (TH) is the only proven treatment option that is known to decrease the rates of mortality and neurologic sequelae in neonates who are diagnosed as having moderate and severe HIE.[1,2]

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Before the TH era, it was found that almost 50-90% of the neonates with moderate to severe basal ganglia and thalamic hypoxic-ischemic lesions had oral feeding difficulties, and almost all of those neonates subsequently needed a gastrostomy tube or long-term home gavage feedings.[3] After the TH era, it was found that 31% of surviving infants with HIE had persistent feeding difficulty despite TH, and it was demonstrated that persistent feeding difficulty could be predicted by brainstem involvement on post-hypothermia magnetic resonance imaging (MRI).[4] Although there has been a decrease in the frequency of feeding difficulties after TH,

feeding difficulties continue to be a serious problem affecting one third of newborns with HIE/TH.[4]

It has been shown that the application of "Premature Infant Oral Motor Intervention (PIOMI)" improved oro-motor skills, decreased the transition time from gavage to full independent feeds by mouth with better weight gain rates and reduced hospital stay in preterm neonates.[5,6] The aim of this study was to investigate the efficacy of the PIOMI exercises in infants with different stages of HIE/TH.

Materials and Methods

This was a prospective randomised controlled study. Newborns, who underwent TH between January 2022 and September 2022 with the diagnosis of HIE, were included in the study. Approval for the study was obtained from the Local Clinical Research Ethics Committee under the approval number 22/02/20. The parents of the newborns were informed about the purpose of the study, and verbal and written consents were obtained. The neonates who died before discharge and whose parents did not give consent were excluded from the study.

Neonatal intensive care unit protocol for management of hypoxic-ischemic encephalopathy

The decision to apply TH was made based on the Turkish Neonatal Society Guidelines on neonatal encephalopathy.[7,8] Treatment criteria were as follows: (1) newborns with a gestational age of ≥ 36 w and aged below ≤ 6 h; a pH value of ≤ 7.00 or BE value of ≤ -16 mmol/L in the blood sample collected from the cord or in the blood sample collected from the baby in the first hour of life; (3) a tenth minute APGAR score of < 5 or persisting need for resuscitation; (4) signs of moderate or severe encephalopathy on clinical evaluation. Absolute contraindications for TH used in this study were as follows: (1) babies who were aged over twelve hours; (2) babies under the gestational age of 34 w; (3) babies weighing less than 1800 g; (4) babies with major congenital anomalies; (5) babies with very severe or diffuse parenchymal cranial hemorrhages or very severe life-threatening coagulopathy. The treatment plans for babies, who either did not fully meet the TH criteria or did not have an absolute contraindication for TH, were made according to the decision of the consultant neonatologist in line with our national guidelines. Therapeutic hypothermia was applied with either the Tecotherm TS Med 200 N device (Inspiration Healthcare Ltd., Leicester, United Kingdom) or Arctic Sun 5000 Temperature Management System (Medivance, Inc., Louisville, Colorado, United States). Cooling to a rectal temperature of 33.5 ± 0.5°C was achieved in all infants within the postnatal 6 hours. Rewarming to 36.5°C was started following 72-hour cooling by elevating the temperature with a rate of 0.5°C per hour. The patients underwent detailed neurological examination, and the Sarnat and Sarnat staging system was used to determine the stage of HIE (stage 1/ mild, stage 2/ moderate or stage 3/ severe HIE). The severity of HIE was defined daily during the first 72 hours of life, and the worse stage was recorded. Gestational age (GA) was estimated based on the last menstrual date or the Ballard score was used to estimate GA, if last menustual date was not known.[9]

In our hospital, MRI can be performed and NICU babies were given priority for MRI. Cranial MRI was performed as soon as possible after rewarming in all newborns who were admitted to our NICU with the diagnosis of HIE. However, cranial MRI scans of the newborns who could not be weaned from the ventilator or whose clinical findings were not stable enough to be transferred to the radiology unit were delayed until the patient was stable. Normal MRI findings were defined as follows; increased signal intensity corresponding to myelination seen in the posterior half of the posterior limb of the internal capsule, absence of increased signal intensity in the thalamus, or subtly increased signal intensity that was restricted to the posterolateral quadrant of the thalamus on T1-weighted images.[10] Abnormal MRI findings

were defined as the presence of a HIE-specific brain injury pattern as follows; basal ganglia and thalamus dominant injury pattern, white matter/watershed dominant injury pattern or near-complete injury/global hypoxia pattern.[11] Presence of HIE-nonspecific MRI abnormalities such as intracranial hemorrhage, venous thrombosis, etc were not included in the abnormal MRI group, and were evaluated in the normal MRI group. The postnatal day when cranial MRIs of the neonates were performed was recorded.

Feeding policy of the neonatal intensive care unit

Fluid balance was maintained with 10% dextrose infusion in the first 24 hours of life, and later continued by daily adjusted fluids according to the patient's biochemical values, weight and urine output. During TH, the babies were not fed. Enteral feedings were initiated as < 20 mL/kg/d after rewarming the neonates to 36.5°C with an orogastric tube, and routine residual control was performed. Enteral feeding was terminated, if abdominal distention and large (> 5 mL/kg), bilious or blood stained gastric residuals were observed. The volume of enteral feeding was increased, if the infant tolerated the feeds. Enteral feeding was increased at a rate of 10-20 mL/kg/d as tolerated. If enteral feeding could not be started just after rewarming, parenteral nutrition was started.

We routinely fed term neonates eight times a day with three-hour intervals, and trained neonatal nurses of the unit provided care and feeding. For the oral feeding trials, feeding behavior of infants with respiratory stability/weaned from the ventilator was considered on the basis of cue-based feeding. The speech and language therapist (SLT) evaluated the neonates readiness for oral feeding by observing the presence of the rooting reflex, the pattern, rhythm, speed and strength of non-nutritive sucking, oral motor coordination, and orofacial muscle tone. A multidisciplinary team of neonatologists, pediatricians, neonatal nurses and SLT decided on neonates readiness for oral feeding. If the team agreed that newborns were ready for the transition from tube feeding to oral feeding, neonatal nurses attempted to feed the infants orally with an injector every three hours. Mothers of the neonates who were confirmed to be able to be fed with injectors at least three times successfully were called to the unit to breastfeed, and feeding directly from the mother's breast was attempted. In cases in which the mothers did not have milk or breast milk was contraindicated, mothers were called to the unit to feed their babies with a bottle.

Transition time to full oral feeding (FOF) was determined as the time between initiation of tube feeding and full oral breast feeding or bottle feeding. The day per oral (PO) feeding was started was specified as PO first, the day the infants could take half of the volume of the feedings by mouth was PO half, and the day the infants could take all the feedings by mouth was PO full. Length of hospital stay was specified as the time between admission to the unit and discharge from the unit. The clinical findings that might influence the feeding transition process, including information on the duration of ventilation, inotropic requirements, presence of seizures, number of anticonvulsive medications used, and echocardiographic findings were recorded. The duration of mechanical ventilation was defined as the sum of invasive and noninvasive mechanical ventilation. The APGAR scores were categorized as ≤ 7 and > 7, and APGAR scores were compared between groups.

The number of total participants to be included in the study was specified via *http://www.randomizer.org* programme. Randomization was performed by randomly and equally assigning the babies, who constituted the sample group, to two groups using the random numbers obtained from the programme. Fifty newborns, who received TH with a diagnosis of HIE were applied PIOMI exercises and constituted the study group, while 50 newborns, who received TH with a diagnosis of HIE and were not applied PIOMI exercises, constituted the control group. Postnatal ages (PNA) and weights of

the neonates on the days PO first, PO half, and PO full, when the FOF was provided, and at discharge were recorded.

The Premature Infant Oral Motor Intervention (PIOMI) was adapted from the Beckman Oral Motor Intervention exercises such that it included eight steps, and its application lasts for five minutes.[12] PIOMI exercises were applied by SLTs ten minutes before tube feeding once daily, five times weekly in babies who were initiated enteral feeding by paying attention to sterilization. Before the application, the babies were placed in incubators in supine position in the awake state. The SLTs applied the exercises by opening the incubator windows without taking the babies out of incubators. Sucking was triggered by giving sensory stimuli to the cheeks, upper and lower lips, gingivae and the lateral and middle lines of the tongue, respectively, in accordance with the PIOMI exercise protocol with the objective of increasing oral-motor skills. The babies were monitored during the procedure, if venous saturation reduced by >20% and/or apical heart beat increased by >30% the exercises were terminated. PIOMI exercises were continued in the babies in the study group until FOF was initiated. Premature infant oral motor intervention tool with illustrations is presented in Fig. 1.[12]

Sample size calculation

The required sample size was determined through a power analysis using the G*Power (v3.1.9.4) program. The study power and alpha value was set at 93% and 0.05, respectively and effect size was 0.30. Based on these values, a minimum sample of 100 participants was required, meaning 50 participants in each trial group.

Statistical analysis

The Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The data were assessed for normality using visual and analytic methods. The data were tested for normality with the Shapiro-Wilk test and Kolmogorov Smirnov test. The qualitative variables are expressed as percentages and frequencies, normally distributed continuous variables are expressed as means (standard deviation) and non-normally distributed variables are expressed as medians (interquartile range [IQR], p25-p75). The chi-square test was performed for categorical variables. Differences between two groups were tested using the Student's t-test or Mann-Whitney U test, as appropriate. In 2x2 comparisons between the categorical variables, chi-square test or Fisher's exact test was used, as appropriate. In >2x2 comparisons between the categorical variables, the Fisher-Freeman-Halton test was used, as the expected value was <5. A p value of <0.05 was considered statistically significant.

Results

During the study period, 113 newborns with the diagnosis of HIE were admitted to our NICU, of these newborns, 8 with stage 3 HIE died, and the parents of 5 did not allow for the inclusion of their baby in the study. A hundred neonates who underwent TH with the diagnosis of HIE were included in the study. PIOMI exercises were applied to 50 neonates, who constituted the study group, and no feeding exercises were applied to the other 50 patients (control group). The PIOMI was not terminated in any newborn due to changes in vital signs, and no adverse effects related to the PIOMI were observed. Gestational age, birth weight, 1st and 5 th minute APGAR scores, cord blood gas pH and base deficit, postnatal day of MRI and HIE stages were similar between the groups. The frequency of convulsions, the number of anticonvulsant drugs used, the duration of mechanical ventilation, and the frequency of cardiac anomalies did not differ between the study and control groups. No critical congenital heart disease was detected in any patient. Three patients in the study group received sildenafil treatment for mild to moderate pulmonary hypertension. The demographic, clinical and laboratory characteristics of the neonates are presented in Table I.

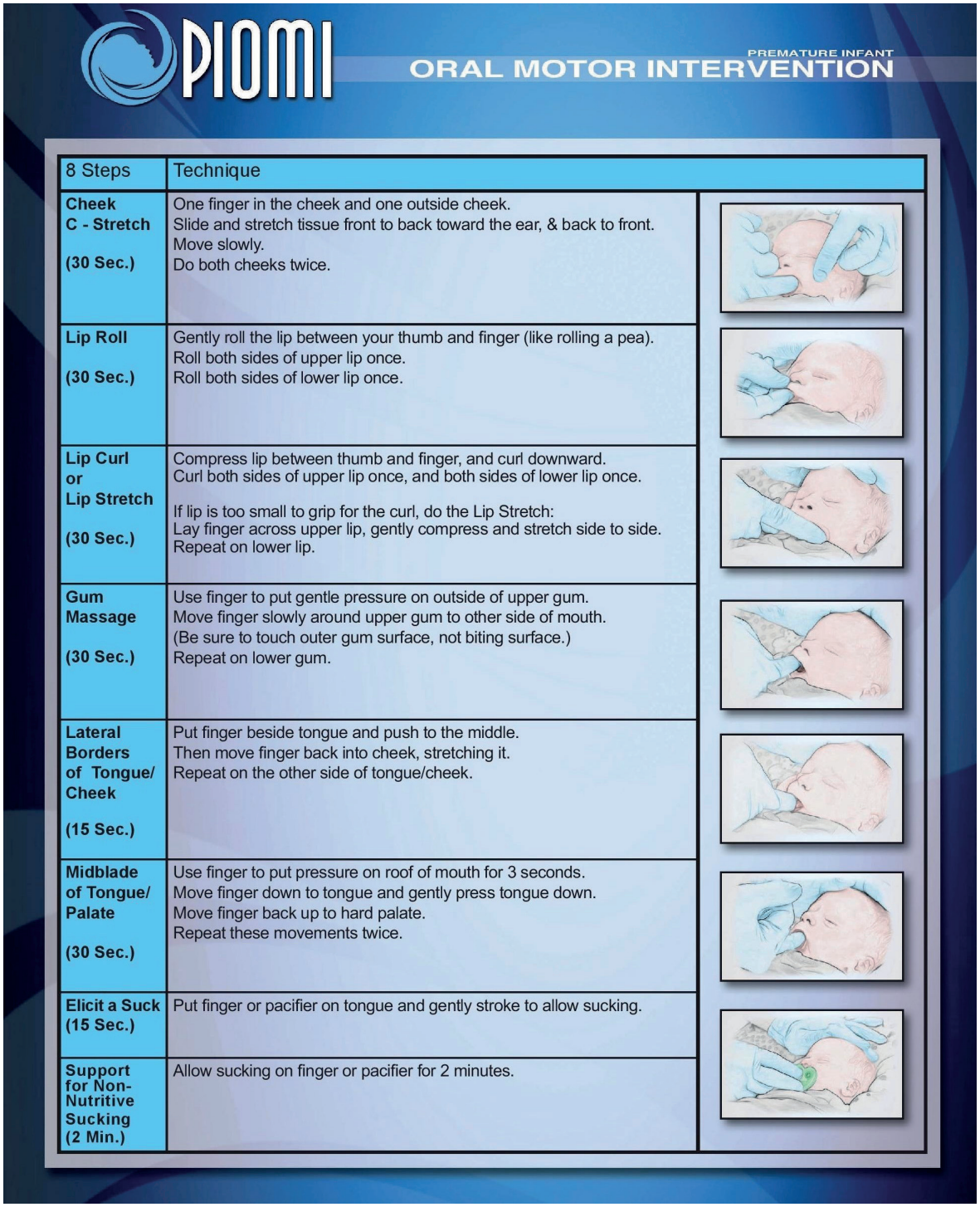

Fig. 1. Premature infant oral motor intervention tool with illustrations.
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Table I. Comparison of demographic, clinical and laboratory characteristics of the neonates by groups.

		Control group (n=50)	Study group (n=50)	P value
Sex, male [a]		37 (74)	24 (48)	0.008
GA, w [b]		37.3 ± 2.0	37.3 ± 2.4	0.978
BW, g [b]		3011± 737	3011 ± 555	0.743
APGAR score, 1 min [c]		4 (1-8)	4 (1-6)	0.094
APGAR 1 min ≤ 7 [a]		4 (8)	0 (0)	0.041
APGAR score, 5 min [c]		6 (3-9)	6 (3-9)	0.050
APGAR 5 min ≤ 7 [a]		15 (30)	8 (16)	0.096
pH [b]		6.9 ± 0.3	6.9 ± 0.1	0.442
Base excess, mmol/L [b]		-18.9 ± 20.9	-17.5 ± 5.1	0.396
MRI, postnatal day [b]		4.8±3.3	4.7±3.5	0.060
Ventilator, day [b]		8.6±8.7	7.6±7.0	0.538
Convulsion [a]		29 (58)	27 (54)	0.687
Anticonvulsive, number [b]		0.9±0.8	0.8±0.8	0.830
Normal ECHO [a]		44 (88)	44 (88)	1.000
HIE stage [d]	1	13 (26)	13 (26)	
	2	22 (44)	18 (36)	0.647
	3	15 (30)	19 (38)	

BW: birth weight, ECHO: echocardiography, GA: gestational age, HIE: hypoxic-ischemic encephalopathy, MRI: magnetic resonance imaging.
[a]Fisher's Exact test (number and percentage), [b]Student's t-test (mean and standard deviation),[c]Mann-Whitney U test (median and interquartile range), [d]Fisher-Freeman-Halton test (number and percentage).

The mean postnatal day of starting PIOMI was 7.6±7 days. Times to PO first, PO half and FOF were found to be shorter in the study group compared to the control group in terms of postnatal day (P= 0.028, 0.011, 0.003, respectively), but the time to start gavage feeding, time to PO full and time to discharge did not differ between the groups (Table II). The weights of the neonates on the days PO first, PO half, PO full and FOF and at discharge did not differ between the groups (Table III).

When the groups were compared according to the HIE stages, times to PO first, PO half, and discharge were similar between the groups in neonates with stage 1 and stage 2 HIE/TH in terms of postnatal day. However, the time to FOF was significantly shorter in the study group compared to the control group in neonates with all stages of HIE/TH in terms of postnatal day (P= 0.008 for stage 1 HIE, <0.001 for stage 2 and 3 HIE). In addition, the times to PO first, PO half, PO full and discharge were shorter in the study group compared to the control group in neonates with stage 3 HIE/TH in terms of postnatal day (P= 0.003, 0.014, 0.013, 0.042, respectively (Table III). All newborns included in the study were discharged by full oral breast feeding or bottle feeding and no tube or gastrostomy feeding was required.

When the groups were compared according to the HIE stages, the frequency of abnormal brain MRI was similar between the groups in all stages of HIE/TH (Table III).

Discussion

In our study, we found that oral motor intervention exercises were effective in shortening the time for FOF in all stages of HIE in neonates with HIE undergoing TH. In addition, we showed that these exercises improved feeding outcomes and shortened the length of hospital stay in neonates with severe HIE/TH. In neonates with HIE, prolonged and poorly coordinated peristaltic responses may result in dysfunction of aerodigestive regulation, as it has been shown that esophageal motility is abnormal in neonates with HIE, and TH can ameliorate these dysmotility issues.[13]

Table II. Comparison of postnatal age and weight of neonates on the specified days by group.

	Control group (n=50)	Study group (n=50)	P value[a]
	Mean ± SD	Mean ± SD	
Gavage feeding start, day	4.3±0.7	4.6±1.1	0.060
PO first, day	11.9± 8.2	9.2± 7.2	0.028
PO half, day	12.2±8.2	9.8± 7.4	0.011
PO full, day	12.4± 8.2	10.4± 7.7	0.213
Time to FOF, day	10.3± 6.8	5.3± 5.2	0.003
Discharge PNA, day	13.6± 8.6	12.5± 8.5	0.948
PO first weight, g	2965± 533	2965± 504	0.997
PO half weight, g	2961± 532	2969 ± 506	0.941
PO full weight, g	2961± 528	2993± 496	0.757
Discharge weight, g	2980 ± 524	3063 ± 515	0.429

FOF: full oral feding, PNA: posnatal age, PO: Per oral, SD: standard deviation
[a]Student's t-test

Table III. Comparison of postnatal age of newborns on specified days and MRI findings according to HIE stages by group.

HIE Stage		Control group (n=50)	Study group (n=50)	P value
1	PO first, day	8.5±2.6	9.1±8.1	0.253[a]
	PO half, day	8.6±2.5	9.5±8.0	0.379[a]
	PO full, day	8.9±2.2	10.0±8.0	0.437[a]
	Time to FOF, day	7.2±1.8	6.2±7.1	0.008[a]
	Discharge PNA, day	10.4±2.9	11.5±7.6	0.814[a]
	Abnormal Brain MRI	6 (12)	4 (8)	0.420[b]
2	PO first, day	8.9±3.6	8.3±6.2	0.184[a]
	PO half, day	9.2±3.6	8.6±6.3	0.139[a]
	PO full, day	9.4±3.7	9.4±6.5	0.293[a]
	Time to FOF, day	8.0±3.3	3.9±4.6	<0.001[a]
	Discharge PNA, day	10.1±3.6	11.7±7.2	0.946[a]
	Abnormal Brain MRI	16 (32)	10 (20)	0.260[b]
3	PO first, day	19.3±11.2	10.1±7.6	0.003[a]
	PO half, day	19.7±11.1	11.2±8.1	0.014[a]
	PO full, day	19.9±11.2	11.6±8.5	0.013[a]
	Time to FOF, day	16.4±9.4	6.0±4.1	<0.001[a]
	Discharge PNA, day	21.5±11.7	14.1±10.1	0.042 [a]
	Abnormal Brain MRI	11 (22)	18 (36)	0.070 [b]

FOF: full oral feding, HIE: hypoxic-ischemic encephalopathy, MRI: magnetic resonance imaging, PNA: posnatal age, PO: Per oral
[a]Student's t-test (mean and standard deviation), [b] chi-square test (number and percentage)

Also, newborns with HIE/TH are at high risk of having oropharyngeal dysphagia, and in this case, early action should be taken to prevent feeding difficulties.[14,15] Relatedly, a considerably high number of neonates with HIE develop long-term feeding problems, such as failure to thrive, impaired growth and development, and increased morbidity and mortality, that are difficult to manage.[16] In a study conducted with 100 NICU infants, the structure and physiology

of the aerodigestive system in at-risk neonates were evaluated with manometric studies of pharynx and esophagus, and swallow studies, and individualized feeding strategies were applied to these neonates. They found that the rate of successfully fed newborns increased approximately fivefold at discharge and nearly twofold at one year of age.[17] Therefore, it is advisable that a team of healthcare providers in NICUs, including neonatologists, SLTs, lactational nurses, and physiotherapists, should always be on hand to prepare at-risk newborns for discharge. It also seems necessary to provide an individualized feeding strategy as early as possible based on pathophysiology to achieve better feeding and long-term outcomes.[16,18]

The PIOMI is known to increase feeding success and efficiency, improve oral-motor skills, sucking capacity and anthropometry, and reduce hospital stay in preterm infants.[12,19-22] Especially, when the PIOMI is applied with breast milk, it has been shown to be more effective compared to PIOMI alone in improving sucking and oral feeding.[21] No study has been found that evaluates the effectiveness of oral motor interventions in neonates with HIE/TH, and a limited number of case-based interventions have been tried to improve feeding in these high-risk neonates. In a case study, nonnutritive sucking, which we apply routinely in all hemodynamically stabile neonates in our NICU, was found to facilitate oral feeding in an infant with stage 2 HIE.[23] In another case with stage 1 HIE, oromotor stimulation was shown to improve effective breastfeeding and led to a steady increase in the baby's weight.[24] Therefore, this subject matter deserves further studies.

In our study, the PIOMI had no effect on the weights of the neonates with HIE/TH, as the weights of the newborns did not differ between the groups at any of the specified time points. The effect of PIOMI on infant weights is unclear in studies conducted with premature infants. Similar to our findings, there was no difference between the weights of premature babies, who did and did not receive PIOMI, in one study[12], whereas the weights of the babies, who received PIOMI, were found to be higher than those who did not in another study, and it was concluded that PIOMI improved anthropometric measurements.[22]

To our knowledge, this is the first study in the literature evaluating the efficacy of oral motor intervention exercises in a large group of neonates with HIE/TH, which was the main strength of the study. On the other hand, the accuracy and appropriateness of applying PIOMI exercises, which is an oral motor intervention with proven effectiveness in preterm infants, to term infants with HIE/TH is debatable. However, feeding problems in these at-risk neonates with HIE/TH should be promptly resolved with every effort. Based on this idea, we showed that PIOMI, which could be named as "hypoxic-ischemic encephalopathy- oral motor intervention (HIE-OMI)" in our study, was an effective intervention in shortening the transition time to FOF in HIE/TH neonates, and also improved feeding outcomes and shortened the length of hospital stay in neonates with severe HIE/TH. The other strength of our study was that the groups were homogeneous in terms of demographic, clinical and laboratory findings that may influence the feeding transition process, such as gestational age, birth weight, APGAR scores, blood gas pH and base deficit, HIE stages, the frequency of abnormal MRI, frequency of convulsions and cardiac anomalies, and duration of mechanical ventilation. Finally, the groups were also homogenous in terms of feeding protocols, although we did not perform minimal enteral feeding (MEN) during TH, which is recommended, as it is safe and has many beneficial effects such as shortening the time to full enteral feeding and increasing the rate of breastfeeding at discharge.[25,26] Furthermore, we changed our feeding protocol in neonates with HIE/TH after the study period, and started to apply MEN during TH to benefit from the advantages of MEN.

Our study has some limitations. The first limitation was that an oral motor intervention developed for premature babies was applied to term newborns for the first time, making

the method of study questionable. The second limitation is that there might be other factors that we did not consider that affected the time to FOF, such as a background abnormality in the electroencephalogram in the first 24 hours of life.[27] The third limitation was that except for the time to PO feeding, FOF and discharge, and the weight of the neonates on those specified days, no other parameters that might be related to oropharyngeal dysphagia based feeding problems were evaluated in this study. Clinician-observed scales that evaluated variables such as problems in feeding behavior, negative effects of feeding incoordination on respiration, and sucking-swallowing-respiratory incoordination could be included in the study. In addition, it would be useful to evaluate newborns with follow-up sessions and to address the negative effects of feeding problems (transition to solid food, breastfeeding at home, etc.) in the first year of life.

PIOMI, which can be named as "HIE-OMI" in our study, is an effective intervention in shortening the transition time to FOF in neonates with all stages of HIE undergoing TH. This intervention improves feeding success and shortens the length of hospital stay in neonates with severe HIE/TH. In the light of our findings, the importance of working as a team with SLTs is obvious in NICUs. Prospective longitudinal studies with larger numbers of neonates with HIE/TH should be conducted to evaluate the effectiveness of "HIE-OMI" exercises.

Ethical approval

Ethics Committee approval was obtained for the study from Harran University Clinical Research Ethics Committee (date: 24.01.2022, number: 22/02/20). Verbal and written informed consent were obtained.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AOG, AB; data collection: AB, HBC, SD; analysis and interpretation of results: AOG, AB; draft manuscript preparation: AOG. 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

Newborn screening for sickle cell anemia in Antalya, Türkiye

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ABSTRACT

Background. In a screening study conducted on adults, the prevalence of sickle cell traits in Antalya was found to be 0.24%. Since no screening studies have been conducted in the neonatal period in our region, the exact incidence has not been determined. In this study, we aim to report our experience of neonatal screening for sickle cell disease in Antalya, Türkiye.

Methods. During a 14-month period, 2562 heel prick blood samples, taken on filter paper from Akdeniz University Hospital, Antalya Education and Research Hospital and Antalya Atatürk State Hospital and four other healthcare centers, were studied using the high pressure liquid chromatography method. Blood samples were studied using the 'Sickle Cell Short Program' test method on a Bio Rad Variant device.

Results. In the study, no patients with sickle cell disease were identified. Four newborns who were sickle cell carriers (0.15%) and two newborns who were Hemoglobin D carriers (0.08 %), were found.

Conclusion. Considering the efficiency and cost calculations made as a result of the data obtained from our study, it was concluded that sickle cell screening would not be effective in newborns. It seems more effective and economical to screen the children of parents, who are found to be at risk for Hemoglobin S carriage as a result of premarital tests.

Key words: newborn screening, sickle cell, hemoglobinopathy, Türkiye.

Sickle cell disease (SCD) is a complex inherited red blood cell disorder with a high worldwide prevalence. SCD are common among people from Africa, Mediterranean countries, Türkiye, Arabian Peninsula, Indian subcontinent, and the United States.[1] Most neonates with SCD are healthy in the early period after birth and they become symptomatic during infancy or childhood with a decrease in fetal hemoglobin (HbF) level and an increase in hemoglobin S (HbS).[2] Delay in the diagnosis of the disease can cause complications that may affect the patient's life. An effective neonatal screening program for SCD will reduce morbidity by providing early diagnosis and treatment of these life-threatening complications of the disease, such as acute splenic sequestration crisis and bacterial sepsis.[3-5] Newborn screening for SCD is being carried out in some countries to prevent morbidity associated with this disease.[6]

In a screening study, including adults, it was established that in Antalya, HbS had the highest prevalence (0.24%) after thalassemia traits (10.2%).[7] We thought that the prevalence of newborns with SCD might be higher than reported. In Türkiye, there is no nationwide

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newborn screening program for SCD. Newborn screening programs in our country are being conducted for phenylketonuria, congenital hypothyroidism, biotinidase deficiency, congenital adrenal hyperplasia, cystic fibrosis and spinal muscular atrophy. In this study, we aimed to identify the prevalence of SCD and HbS carriage among the newborns in Antalya.

Material and Methods

Approval for the study was obtained from the Antalya Provincial Health Directorate. This study was conducted over a 14-month period (in 2004-2005 (as a master's thesis) and 2007-2008 (as a project supported by the Turkish Society of Hematology) at Akdeniz University Hospital. During the study period, the heel prick blood of 2652 newborn babies, including those born in Akdeniz University Hospital, Antalya Education and Research Hospital, Antalya Atatürk State Hospital, and babies, and those who applied to four health centers (Health centers no. 6, 7, 11, and Maternal Child Health and Family Planning Center no. 1), determined by the Antalya Provincial Health Directorate, were collected. Ethical approval was obtained before the start of the study. Before the blood samples were taken, informed consent was obtained from the parents of the newborns. Ninety of the samples were excluded from the study due to improper storage conditions or incompliance with the age range. The results of 2562 newborns were evaluated. These newborns consisted of 1062 males and 1035 females, yet the genders of 465 newborns were not specified. Newborns, who were born preterm or had received red blood cell transfusions, were not included.

Heel prick samples of the newborns were taken onto Guthrie filter paper (Sergio Bianchi). Infants within the first month were included in the study. In general, blood samples were taken just before hospital discharge and usually on day 2 of life. Birth date, sample date, gender, mother's name, address and telephone number information were written on Guthrie paper. The blood samples were left to dry for at least four hours and the samples were stored at 4°C before and after the study. The blood samples were studied within the next two weeks. High performance liquid chromatography (HPLC) was performed using the Bio-Rad VARIANT™ instrument. Experiments were carried out in accordance with the kit procedure (Variant Sickle Cell Short Program Pack, Bio-Rad Laboratories, Milan, Italy). This program is specifically designed to provide a qualitative result for hemoglobins A, F, S, C, D, and E in neonates. No limit value was reported for the hemoglobin variants in the testing procedure. Regardless of the abnormal hemoglobin percentage, all patients showing abnormal hemoglobin were recalled for HPLC study. The families of all babies with hemoglobin variants were requested to be tested in the following months and HPLC analysis was performed using 'thalassemia short program' to confirm the accuracy of the diagnosis. Genetic counseling was offered to the families.

Results

In this study, 2562 babies were screened. As a result of the screening, no homozygous sickle cell patients were detected. HbS carriers were found in four (0.15%) neonates. In addition, Hemoglobin D (HbD) carriage was detected in two (0.08%) cases. Apart from HbS and HbD, no other abnormal hemoglobin was found during our study. The HPLC results and hematological parameters of these four HbS carriers are shown in Table I. The HbS percentage of seven cases was found to be in the range of 0.1-0.3% and when the control electrophoresis of these cases was studied, the results were found to be false positive. In our study, the HbS value in a one-day-old baby who was an HbS carrier was found to be 1.9%. No intermediate value between 0.3% and 1.9% was found in all cases. HbS percentages of all other HbS positive cases were found to be above 2%. The hemoglobin variants of the three families were studied again to confirm the diagnosis, but the family of the fourth baby (HbS 4) refused a retest.

Table I. Hematological parameters of newborns with HbS.

HbS carriers	Age	RBC ($x10^6$mL)	Hb (g/dL)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)	HbF (%)	HbA (%)	HbS (%)
HbS 1	7 days	5.06	17.90	100.10	35.30	35.30	61.2	6.6	4.7
	2 months	3.06	9.50	87.10	31.00	35.60	70.4	16.2	12.5
HbS 2	5 days	4.90	17.50	89.70	35.80	39.90	68.4	3.3	2.7
HbS 3	2 days	4.86	17.20	104.70	35.40	33.80	68.9	5.7	5.2
	4 months	3.76	9.90	76	26.40	34.70	17	42.7	37.2
HbS 4	1 day	NK	NK	NK	NK	NK	55.9	2.6	1.9

Hb: hemoglobin, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, MCV: mean corpuscular volume, NK: not known, RBC: red blood cell.

Table II. Hemoglobin variants of family members.

Newborns	Subjects	Hemoglobin percentages (%)			
		HbA	HbA_2	HbF	HbS
HbS 1	Mother with HbS	53.00	4.10	0.50	42.40
	Sibling with HbS	51.70	2.20	2.20	38.10
	Sibling with SCD	3.10	4.60	4.60	76.20
HbS 2	Father with S/β-thalassemia	11.7	6.9	6.9	74.8
HbS 3	Mother with HbS	43.8	4.40	4.40	41.80
	HbS carier sibling	54.8	4.5	4.5	40.40

Hb: hemoglobin, SCD: sickle cell disease

Hemoglobin variants of the family members are shown in Table II. When the family of the first baby, who was a carrier of HbS, was contacted, it was learned that they already had two more children, one of whom had homozygous sickle cell and the other was a carrier. It was determined that the family had this baby (HbS 1) with the prenatal diagnosis method. When the family of the second HbS carrier baby (HbS 2) was reached, it was learned that his father had Sβ thalassemia. Although these two families were knowledgeable about sickle cell anemia, it was found that the family of the third carrier baby (HbS 3) was not aware of such a disease. As a result of the HPLC analysis of her family, it was seen that her mother and sibling were carriers of HbS. The family was informed about the disease.

Discussion

The main objective of the newborn screening program is to improve outcomes in SCD through early treatment and care. It has been emphasized that knowledge of the diagnosis and parent education will enable the implementation, in the early intervention period, of treatment approaches which change the course and severity of the disease and follow-up of possible complications and therefore, will become life-saving.[8] It will be beneficial to screen newborns for sickle cell anemia in regions where hemoglobinopathies are common, in order to prevent lethal complications of SCD in childhood. Although the benefits of screening for hemoglobinopathies are well known, the most important limiting factor for widespread use is the cost of screening. A sickle cell screening program, including systematic screening of all newborns, particularly in areas where the disease incidence is 0.5 per 1000 or higher, has been proposed.[9] In a screening study on adults, the incidence of HbS was found to be 0.24% in Antalya.[7] Since some of the patients with SCD had died due to intervention problems in the early stages of life, it was thought that this rate may be higher in the neonatal period. However, consequently, we found a carrier rate

that was much lower than we had expected. No SCD patients were found in our study. On the other hand, it has been demonstrated that at a prevalence and incidence of 16 sickle cell traits per 1000 there is no significant identification cost difference between universal and targeted screening programs.[10] In our study, the HbS carrier rate was 1.5/1000 and was 10 times less than the stated rate. In this case, it does not seem very beneficial to perform universal newborn screening for sickle cell anemia in our region. Hemoglobinopathy screening programs in Türkiye are aimed at preventing hemoglobinopathies and are based on premarital screening and prenatal diagnosis. The Hemoglobinopathy Control Program has been implemented in all 81 provinces of the country under the name "Pre-Marital Hemoglobinopathy Screening Program".[11] A premarital hemoglobinopathy test is mandatory and free of charge under this program. A 90.0% reduction has been recorded in the number of affected newborns with hemoglobinopathy as a result of the educational and prevention programs in the previous 10 years.[12] No cases of homozygous sickle cell anemia were found in our study. It can be thought that this situation is a result of social education about hemoglobinopathies and widespread prenatal diagnosis. The reason for her unawareness of sickle cell carriage by the mother of our third case, was that at the time of her marriage, only 33 provinces in our country had mandatory hemoglobinopathy screening programs and their marriage was held in another city, that did not offer this. Since 2019, premarital hemoglobinopathy screening has been carried out throughout the country.[11] However, sickle cell patients continue to be born in our region despite premarital screening tests. This is mainly because families refer to a range of personal, cultural, social and religious beliefs when making decisions. Health professionals have to be sensitive to and respect these beliefs.

Our study has some limitations. The main limitation of our study was that it was conducted only in health institutions in the center of Antalya. The data obtained from this study cannot give a general idea about the frequency of sickle cell disease in Türkiye. A nationwide study is needed to determine whether neonatal hemoglobinopathy screening is suitable for our country. On the other hand, the fact that this study was conducted in a region where hemoglobinopathies are common makes its data valuable. One of the limitations of our study is the time and duration of the study. Although there were no homozygous HbS patients in our study, it is known that children with sickle cell disease have been born in our region in the following years. Therefore, longer-term studies with a larger population are needed.

Newborn screening is an important public health measure to enable early detection of certain diseases for which early treatment is both possible and beneficial. We planned this study with the consideration that it could be necessary to screen newborns for sickle cell anemia due to the high incidence of hemoglobinopathy in our country. However, we did not find any homozygous sickle cell patients and we obtained a lower rate of HbS carriage than the rate found in the study on adults. Consequently, considering the efficiency and cost calculations, we concluded that newborn screening for sickle cell is not necessary in Antalya. We think that it will be more effective and economical to prevent the disease with premarital screening tests and to screen the children of parents who are found to be at risk for HbS carriage as a result of premarital tests.

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Ethical approval

The study was approved by the local Ethics Committee of the Akdeniz University. An administrational approval was received for the study from Antalya Provincial Health Directorate with report number B104ISM4070005/22776 and date 15.10.2004. Another ethnics approval was obtained from Akdeniz University Faculty of Medicine Ethics Committee with report number 264-272 and date 11.04.2006.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AY; data collection: ZO and NO; analysis and interpretation of results: ZO, AK, GK, VU; draft manuscript preparation: Z.O and AY. 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 role of red blood cell distribution width (RDW) in the diagnosis of pediatric sepsis

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ABSTRACT

Background. Early diagnosis of pediatric sepsis is difficult, so it is necessary to find a reliable auxiliary diagnostic method. The purpose of the study was to assess the role of RDW in the diagnosis of pediatric sepsis.

Methods. We did a case control study reviewing pediatric inpatients (≥28 days, <18 years old) who were diagnosed with sepsis between April 2020 and November 2022. According to the sepsis-3 and Pediatric Sequential Organ Failure Assessment (pSOFA) scoring standards, 66 septic inpatients of the pediatric intensive care unit (PICU) were included in the sepsis group and 66 non-septic inpatients of the PICU were included by using the random sampling method during the same period as the control group.

Results. RDW values in the sepsis group were higher than those in the control group (P<0.001). The cut-off value, sensitivity, specificity and area under curve of RDW for sepsis were 39.15, 0.955, 0.758 and 0.943,respectively.

Conclusions. Our study confirms that RDW may have a good value on the early diagnosis of pediatric sepsis.

Key words: red blood cell distribution width (RDW), diagnosis, pediatric, sepsis.

Sepsis is a syndrome of organ dysfunction, is caused by the body's dysfunctional response to infection, and is a common sickness in intensive care units with a high death rate.[1] Globally, about 1.2 million children get the disease every year, and the fatality rate varies due to different medical and sanitary conditions in different countries.[2] Early diagnosis of sepsis is difficult due to factors such as limited admission time and overlapping clinical symptoms of different diseases.[3,4] The new diagnostic criteria for sepsis is Sepsis-3[5], but its Sequential Organ Failure Assessment (SOFA) score is for adults and is not suitable for children. In this case, pSOFA was proposed to cater to the new diagnostic criteria for pediatric sepsis. The pSOFA scores were performed and verified using age-adjusted variables and the results indicated that the use of pSOFA was feasible in children and showed good results.[6]

It is still a difficult problem to obtain a single biomarker with reliable high specificity and sensitivity to rapidly identify sepsis.[7,8] At present, the theory of an uncontrolled inflammatory response is considered to be an important basis for the onset of sepsis. Red blood cell distribution width (RDW), represents the heterogeneity of the volume of red blood cells in peripheral blood. Previous studies revealed that the changes of RDW is closely associated with inflammatory response.[9] The inflammatory response induce the tumor necrosis factor and interleukin-6 receptor expression, the release of inflammatory mediators affects the iron metabolism and hematopoietic function of bone marrow, and the proinflammatory factors could cause a large rise of immature erythrocytes, thus causing the increase of RDW.[10,11] Purtle et al. found that RDW can be used for a inflammatory marker.

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The possible mechanism is that inflammation affects the maturation of erythrocytes, causes myelosuppression, reduces the generating of erythropoietin, decreases iron bioavailability, lead to erythropoietin resistance and apoptosis of the red blood cells, and allows immature erythrocytes release into the bloodstream. The result is an increase in RDW.[12]

Sepsis could obviously cause changes in ion channels and glycoproteins on the erythrocyte membrane, resulting in changes in erythrocyte morphology and thus the RDW level is increased.[13] One of the primary pathological mechanisms of sepsis is oxidative stress, which could decrease rate of survivors of erythrocytes and lead to the release of immature and big erythrocytes into the blood, directly causing an increase in RDW.[14] Sepsis can induce hemolysis and shorten the life span of erythrocytes, which may cause a rise of RDW. The system of renin-angiotensin is significantly activated in sepsis patients, angiotensin II can upregulate the level of erythropoietin, and directly acting on RBC precursors, which may cause an increase in RDW.[15] According to these reasons given above, it could be inferred that the level of RDW in septic patients may be higher than in non-septic patients. Thus, we made the scientific hypothesis that RDW may have efficacy in diagnosing sepsis. But, most studies indicate that RDW is a valuable metric for assessing the prognosis of sepsis. These investigations reveal a correlation between elevated RDW levels and increased fatality rates.[16-19]

At present, few studies have investigated the diagnostic value of RDW in sepsis. The objective of this study was to assess the diagnostic value of RDW in pediatric sepsis.

Material and Methods

Study design

This clinical research was a single center case control study using available electronic medical record data. The hospital ethics committee approved the study with the code JXSETYY-YXKY-20220279 on December 8, 2022. It included inpatients of ≥28 days and <18 years of age who presented to the hospital and were diagnosed with sepsis in the pediatric intensive care unit (PICU) between April 2020 and November 2022 and non-septic inpatients of the PICU who were hospitalized at our hospital during the same period. We divided the study population into a sepsis group and a control group and designed a data collection form, reviewed electronic medical records, and registered the clinical data for the included population.

Study population

Our method for determining the sample size is as follows: We obtained the area under the curve (AUC) (0.658) in the pre-experiment and used the software of PASS (Version 15.0.5, NCSS, LLC) to estimate the sample size. After entering the interface of Tests for One ROC Curve of this software, we had to fill in the following parameter values: "Two-Side Test, Power=0.9, Alpha=0.05, AUC0=0.5, AUC1=0.658" and then calculated the total sample size as 132, with 66 cases and 66 controls. In view of the high clinical mortality rate of pediatric sepsis, highlighting the seriousness of pediatric sepsis, this study adopted the criteria of Sepsis-3 to define pediatric sepsis. According to the criteria of Sepsis-3[5] and pSOFA[6], 66 patients were enrolled in the sepsis group, and 66 non-septic patients were enrolled in the control group by using a random sampling method during the same period. SOFA scores for children were based on the pSOFA developed by Matics et al.[6], and pSOFA were retrospectively calculated based on patients' medical records where possible. We divided all the enrolled patients into two parts: sepsis (patients were diagnosed according to criteria of Sepsis-3: infection plus pSOFA ≥2); controls (patients did not meet the diagnostic criteria of Sepsis-3). Two researchers carried out this process independently by conducting a retrospective review of the electronic medical record. Before data analysis, RDW values were unknown to the researchers during the selection of sepsis and control groups, another researcher

made a final decision on inconsistencies. Infection was defined by clinical symptoms, radiographic and laboratory findings.

Inclusion and exclusion criteria

The inpatients ≥28 days and <18 years of age who were admitted to the PICU of the hospital and were diagnosed with pediatric sepsis based on the criteria of Sepsis-3, and the inpatients of the PICU who did not meet the diagnostic criteria of Sepsis-3 by using the random sampling method during the identical time were enrolled in our research. Both groups included patients who had or had not used antibiotics prior to admission. The exclusion criteria of this study were (i) ≥18 years old; (ii) failure to confirm the parameter of RDW; (iii) incomplete data; (iv) leukemia, lymphoma and other severe hematological diseases.

Data collection

Demographic characteristics and clinical information of all the enrolled patients were collected retrospectively basing on reviewing the electronical hospital medical records. Researchers were unaware of RDW levels in the collection of patients' information. In addition, the RDW values we collected for this study were RDW-SD (the actual width of the red cell volume distribution curve measured at the 20% height of the curve in femtoliters [fL]), and the timing of our collection was the time of the initial admission to the PICU.

Statistical analysis

SPSS statistical software V.22.0 (SPSS, Inc., Chicago, IL) was used for statistical analysis. The numerical data were expressed in mean plus or minus standard deviation ($\bar{\chi}\pm s$), while the categorical data were expressed in absolute values and relative percentages. The differences between the two groups of the measurement data were tested by t-test. The differences between the two groups of the counting data were tested by chi-square test. P<0.05 was considered statistically significant. Receiver operator characteristic (ROC) analysis was used to evaluate the diagnostic efficacy of RDW in sepsis and 95% confidence interval (CI) and AUC were reported. MedCalc (Version 15.2.2, MedCalc Software, Ostend, Belgium) evaluated whether the difference in AUC between RDW, procalcitonin (PCT) and C-reactive protein (CRP) were statistically significant (with Bonferroni's correction). The Youden method was used to estimated the best cut-off value of RDW in the prediction of sepsis, the best cut-off value is chosen under the maximum Jorden index (sensitivity+specificity-1). The sensitivity, specificity, accuracy and Yuden index of RDW for predicting sepsis at the best cut-off value were calculated. Evaluate the predictors of sepsis were basing on univariate and multivariate logistic regression.

Results

The study included 132 pediatric patients. Demographic, clinical, including age-specific vital signs[20], temperature, receiving a transfusion, anemia, purpura, lung disease, intestinal disease, CNS disease, antibiotic use before admission, positive blood culture, admission to the emergency department (ED), various clinical data of the enrolled patients are displayed in Table I. Age (P=0.063) and gender (P=0.363) among the two groups had no statistical differences, tachycardia (P<0.001), temperature (P=0.027), positive blood culture (P=0.006), neutrophil ratio (NE%) (P<0.001) and platelets (PLT) (P=0.001) had statistical differences (Table I) .

RDW, CRP and PCT were significantly higher in the sepsis group compared to the control group (P<0.001 for all) (Table I, Fig. 1A-C).

In ROC curve analysis for predicting sepsis, the AUC of RDW (0.943; 95% CI 0.908-0.978) was greater than that of CRP (0.749; 95% CI 0.667-0.831) and PCT (0.751; 95% CI 0.669-0.834) (Fig. 2). The differences between RDW and PCT (DeLong, P<0.0001) and RDW and CRP (DeLong, P<0.0001) were both statistically

Table I. Demographic, clinical and laboratory data of the sepsis and control groups.

	Sepsis (n=66)	Controls (n=66)	p-Value
Demographic			
Age, days, χ±s	971.56±1459.95	1436.26±1385.57	0.063
Male sex, n (%)	40 (60.6)	45 (68.2)	0. 363
Clinical data, n (%)			
Age-specific vital signs			
Tachycardia	58 (87.9)	33 (50.0)	**<0.001**
Polypnea	47 (71.2)	40 (60.6)	0.199
Hypotension	8 (12.1)	3 (4.5)	0.115
Temperature <36 or >38 °c	28 (42.4)	16 (24.2)	**0.027**
Receiving transfusion	2 (3.0)	1 (1.5)	0.559
Anemia	26 (39.4)	24 (36.4)	0.720
Purpura	5 (7.6)	1 (1.5)	0.071
Lung disease	31 (47.0)	21 (31.8)	0.075
İntestinal disease	15 (22.7)	18 (27.3)	0.546
CNS disease	13 (19.7)	15 (22.7)	0.670
Antibiotic use before admission	47 (71.2)	39 (59.1)	0.177
Positive blood culture	9 (8.2)	0 (0)	**0.006**
Admission in ED	62 (93.9)	65 (98.5)	0.171
Hematological tests, χ±s			
WBC,×10^9/L	11.96±10.06	14.71±7.33	**0.074**
NE%	60.16±20.46	72.63±17.57	**<0.001**
RBC,×10^{12}/L	4.13±0.87	4.31±0.81	0.230
HGB, g/L	104.83±23.26	111.94±18.61	0.055
MCV, fL	82.90±7.99	80.79±5.07	0.072
RDW, fL	47.30±9.04	37.34±2.42	**<0.001**
PLT, 10^9/L	228.12±173.65	313.17±117.70	**0.001**
PDW, fL	12.63±3.20	11.74±2.36	0.073
Biochemical tests, χ±s			
CRP, mg/L	70.80±79. 63	17.79±30.37	**<0.001**
PCT, ng/mL	31.98±37.13	5.69±15.91	**<0.001**

CNS: central nervous system, CRP: C-reactive protein, ED: Emergency Department, NE%: neutrophil ratio, RBC: red blood cell, HGB:hemoglobin, MCV: mean corpuscular volume, PCT: procalcitonin, PDW: platelet distribution width, PLT: platelets, RDW:red blood cell distribution width, WBC: white blood count.
T-test was used to test the differences between the two groups for quantitative variables, Fisher's exact test was used to test the differences between the two groups for categorical variables, and P<0.05 was considered statistically significant.

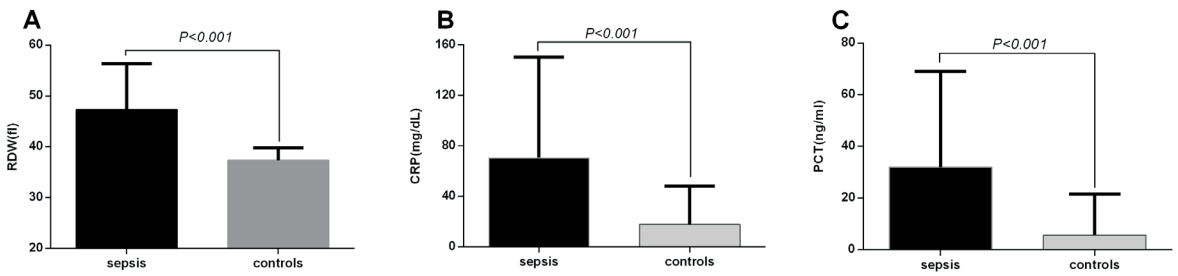

Fig. 1. Comparison of RDW, CRP and PCT levels between the two study groups. (A) Differences in RDW between the two groups. (B) Differences in CRP between the two groups. (C) Differences in PCT between the two groups.

CRP: C-reactive protein, PCT: procalcitonin, RDW: red blood cell distribution width.

significant with Bonferroni's correction, while the CRP: C-reactive protein, PCT: procalcitonin, RDW: red blood cell distribution width, ROC: receiver operating characteristic. difference between PCT and CRP was not significant (DeLong, P=0.9807). The optimal cut-off value of RDW in sepsis diagnosis was 39.15 basing on the Youden index. Sensitivity, specificity, accuracy and Youden index under the cut-off value were 0.955, 0.758, 0.856 and 0.713, respectively. At univariate logistic regression analysis, p-Value of tachycardia (P=0.003), temperature (P=0.045), NE% (P=0.001), RDW (P<0.001), PLT (P=0.002), CRP (P<0.001), and PCT (P<0.001) were less than 0.05, which were thought to be related to sepsis. The correlation factors of P<0.05 coming from the univariate logistic regression analysis were enrolled in the multivariate logistic regression analysis. But, only NE% (P=0.017), RDW (P=0.001) and CRP (P=0.011) were the independent predictors for pediatric sepsis at the multivariate logistic regression analysis (Table II).

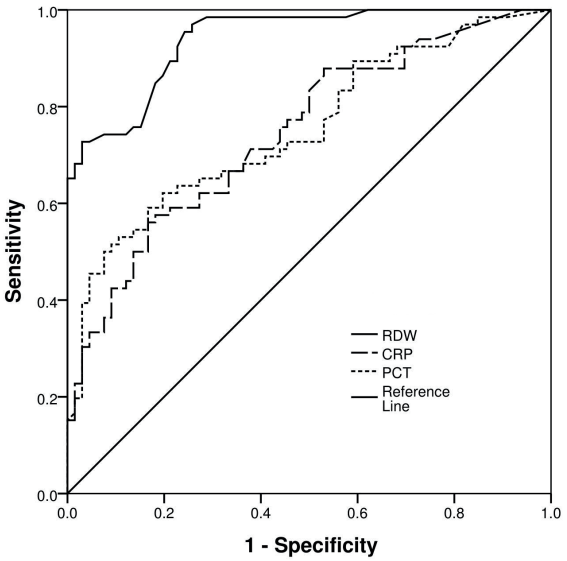

Fig. 2. ROC curve analysis for comparison of RDW, CRP and PCT levels in sepsis prediction.

CRP: C-reactive protein, PCT: procalcitonin, RDW: red blood cell distribution width, ROC: receiver operating characteristic.

Discussion

In this case control study, we assessed the accuracy of using RDW in diagnosing pediatric patients with sepsis. The primary results are summarized in the following aspects: (i) RDW values were higher in the sepsis group than that in the control group; (ii) It revealed that RDW was a strong independent predictor for pediatric sepsis with the univariate and multivariate logistic regression analysis; (iii) It revealed that the best RDW value for detection of pediatric sepsis was 39.15 with the ROC curve analysis,

Table II. Univariate and multivariate logistic regression for sepsis.

Predictor	univariate logistic regression		multivariate logistic regression	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Age	1.000 (1.000-1.000)	0.067		
Tachycardia	3.878 (1.583-9.500)	**0.003**	2.552 (0.249-26.152)	0.430
Temperature	3.429 (1.113-10.570)	**0.045**	0.723 (0.130-4. 033)	0.712
Purpura	5.328 (0.605-46.910)	0.132		
WBC	0.963 (0.924-1.005)	0.081		
NE%	0.966 (0.946 -0.985)	**0.001**	0.938 (0.889-0.989)	**0.017**
RDW	2.538 (1.711-3.764)	**<0.001**	2.733 (1.543-4.842)	**0.001**
PLT	0.996 (0.993-0.999)	**0.002**	0.995 (0.989-1.001)	0.122
PDW	1.120 (0.989-1.269)	0.075		
CRP	1.020 (1.010-1.030)	**<0.001**	1.027 (1.006-1.048)	**0.011**
PCT	1.042 (1.020-1.065)	**<0.001**	1.013 (0.984-1.042)	0.399

CI: confidence interval, CRP: C-reactive protein, NE%: neutrophil ratio, OR: odds ratio, PDW: platelet distribution width, PLT: platelets, RDW: red blood cell distribution width, WBC: white blood cell count.
P<0.05 was considered statistically significant.

the AUC of CRP and PCT were both smaller than that of RDW, and RDW had a good diagnostic accuracy for pediatric sepsis. Overall, our study confirms that RDW may have a good value on early diagnosis of pediatric sepsis. In this study, multivariate analysis suggested that RDW and CRP were independent influencing factors for sepsis. However, PCT was not statistically significant in the multivariate analysis. This is inconsistent with previous research. The possible reason is that PCT could be rised in severe non-septic patients too.[21-23] Therefore, it has certain influences on the research results.

RDW represents the variability of size in circulating red blood cells with the quantitative form. There is accumulating evidence indicating that RDW values are greatly increased in the infection and sepsis patients.[24-26] However, non-infectious factors may also increase the RDW value. One study showed that the RDW of patients could also be obviously increased by the transfusion of erythrocytes and the RDW value mainly reflected the difference between the mean corpuscular volume of patients and the volume of individual erythrocytes, which would be increased by the transfusion of erythrocytes.[27] Fogagnolo et al.[28] found that whether a patient was transfused or not was an important factor determining the RDW value, which may greatly affect the cut-off value of RDW in predicting disease. When they analyzed the clinical variables associated with high RDW, low hemoglobin level was also closely related to high RDW. The increased RDW value may not only reflect the decreased erythrocyte deformability, but may also be secondary to pre-existing chronic anemia or increased reticulocyte production.[29] In our study, the statistical differences in receiving blood transfusions and anemia among the two groups were not significant, so we avoided these factors affecting the diagnostic effectiveness of RDW.

Although most studies suggest that increased RDW may be associated with higher mortality and RDW can be used in evaluating the prognosis of sepsis[16-19], there may also be different views. Sepsis-related organ dysfunction was associated with changes in microcirculation.[30,31] One study found that the change in microcirculation significantly increased the death rate of sepsis patients, but the increase in RDW had no correlation with the change in microcirculation and the change in red blood cell volume did not affect the prognosis of the patients, so the increase in RDW was not a predictor for poor clinical prognosis of sepsis patients.[32] One study discussed the diagnostic efficacy of RDW in neonatal sepsis[33], but the result did not indicate whether the RDW of sepsis was higher than that of other severe patients without sepsis. Currently, few research have discussed the diagnostic efficacy of RDW in the sepsis of older infants or children. The diagnostic efficacy of RDW in pediatric sepsis patients of different ages was investigated in the research. The results are novel and RDW may be expected to be an effective evaluation tool in pediatric clinics. As we know, early detection of sepsis in children is key to hold back the progression and improve the prognosis of the illness. Complete blood count (CBC) is one of the indicators which widely used in clinical diagnosis of diseases. Our study suggests that RDW has good sensitivity and specificity in the diagnosis of pediatric sepsis under the optimal cut-off value. Assessment of RDW in CBC may be able to accomplish early recognition of patients at danger for pediatric sepsis, so we can reduce the missed diagnosis of pediatric sepsis and achieve the purpose of early treatment and improvement of prognosis. However, the diagnostic value of RDW in early pediatric sepsis needs to be confirmed by further study.

The quick sequential organ failure assessment (qSOFA) supported by the criteria of Sepsis-3 revised in 2016 could be used for non-ICU settings to identify patients who are at danger of sepsis.[5] This index is mainly used to identify the possibility of sepsis in patients outside the ICU at an early stage. The criteria of Sepsis-3 stated that patients with suspected infections were considered at high risk for sepsis if they had a qSOFA score ≥2. There was also a

pediatric standard for age-adapted qSOFA.[34] However, patients with sepsis may also have a qSOFA score <2, because organ dysfunction can manifest in various forms and is not limited to the assessment in qSOFA. One study revealed that qSOFA had low sensitivity in diagnosing sepsis, but whether it's sepsis or not, qSOFA score ≥2 may be able to identify these patients with a high danger of death.[35] It should only be used as an early warning value for sepsis and cannot be used to diagnose sepsis. In clinical practice, qSOFA is a simple way to use initially for early recognition of patients who may be at danger for sepsis, but it has also been shown to be inaccurate for use in the ED.[36,37] First of all, the qSOFA can change at short notice. Secondly, it is well known that triage in the ED is dependent on general practitioners rather than sepsis specialists, so there is a need for a easy way to recognize patients with danger of sepsis. Furthermore, as one of the variables of qSOFA, the Glasgow coma scale score cannot be assessed for patients in some clinical situations.[38] Therefore, according to these previous description, RDW may be more valuable than qSOFA in diagnosing sepsis.

This study has a few limitations. Firstly, our study selected a small number of pediatric patients and was a single center retrospective reserch. The randomly selected control group also had a certain sampling error, which could not completely represent the characteristics of all non-septic pediatric patients. Furthermore, in this study, the medical records of children diagnosed with sepsis in our hospital were reviewed, and the sepsis patients were selected basing on the diagnostic criteria of Sepsis-3. This would ensure that all the enrolled sepsis patients met certain diagnostic standard, but some cases that met the diagnostic standard of Sepsis-3 were not actually diagnosed with sepsis might be missed.

In conclusion, due to a lack of accurate screening tools, the diagnosis and therapy for pediatric sepsis are often delayed in pediatric clinics. RDW represents a fast, reliable, low-cost, and readily available indicator for pediatric sepsis. In the study, RDW was proved to be of good value in the diagnosis of pediatric sepsis and the cut-off value was 39.15 under the best sensitivity and specificity. The value of RDW in the early diagnosis of pediatric sepsis needs further study.

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Ethical approval

Informed consent was not required because we did this retrospective study without any intervention in the children. In addition, all patients' data were anonymous. The study was approved by the Ethics Committee of the Jiangxi Provincial Children's Hospital with the code of SKJP220227512 at 2022.03.21, and was performed in accordance with the current revision of the Helsinki Declaration.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: LX, LH; data collection: LX, LY, KC, DZ; analysis and interpretation of results: LX, LH; draft manuscript preparation: LX, LY. 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

Chest pain in children with familial Mediterranean fever

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ABSTRACT

Background. Familial Mediterranean fever (FMF) is the most common and autosomal recessive inherited autoinflammatory disease. The most common signs and symptoms are fever, abdominal pain, chest pain, and arthritis. The aim of this study was to describe the clinical, laboratory and genetic differences between pediatric FMF patients with and without chest pain.

Methods. Between January 2006 and January 2022, 1134 patients with FMF were analyzed retrospectively. Patients were divided into two groups including those with and without recurrent chest pain. These groups were compared in demographic, clinical, treatment, and *MEFV* gene analyses.

Results. A hundred and sixty-two (14.3%) patients had recurrent chest pain. In patients with recurrent chest pain, the age of onset of symptoms was younger (p=0.003), and the family history of FMF was higher (p=0.002). Patients with chest pain had a higher annual attack frequency (p<0.001), a longer attack duration (p<0.001), and higher Pras disease activity scores (p<0.001). The colchicine dose used in the treatment was higher in FMF patients with chest pain (p=0.005), and anti-IL-1treatment was higher (p<0.001). M694V homozygous mutation was found more frequently (p=0.001), whereas M694V/V726A mutation was found less frequently in patients with recurrent chest pain (p=0.017).

Conclusions. Patients with recurrent chest pain seem to have early onset symptoms, often are more likely to have family history, and have a higher disease severity. In addition, the presence of homozygous M694V mutation is more common in patients with chest pain.

Key words: chest pain, familial Mediterranean fever, pleurisy, pyrin.

Familial Mediterranean fever (FMF) is the most common and autosomal recessive inherited autoinflammatory disease, characterized by fever and serositis episodes.[1,2] The disease is common in Turks, Armenians, Arabs, and Jews living in the eastern Mediterranean.[3] The estimated prevalence of FMF in Turkey is 1/1000, and the carrier rate is 1:5.[4,5] It is caused by a Mediterranean FeVer (*MEFV*) gene mutation which is on the arm of the 16th chromosome and encodes a protein called pyrin. The mutated pyrin protein causes hyperinflammation.[6] The most common mutations are M694V, M680I, V726A and M694I mutations, located on the 10th exon of the *MEFV* gene. These mutations are responsible for 85% of cases.[7-9] Some studies have investigated the effects of genetic mutations on clinical outcomes.[10] Clinical signs and symptoms usually manifest in the first decade of life and therefore patients are usually diagnosed in childhood.[4] Inflammatory attacks are often self-limiting within 1-3 days. Typically acute phase reactants rise in attack and return to normal at the end of the attack.[1] The most common signs and symptoms are fever, abdominal pain, chest pain, and arthritis/ arthralgia.[11] Pleuritic chest pain is the third most common symptom after abdominal pain and arthritis. Chest pain is often unilateral and aggravated by inspiration. Chest pain usually ends in 1 to 4 days. The patients may benefit from analgesics and non-steroidal

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anti-inflammatory drugs. Etiology of chest pain in FMF patients is usually pleurisy. However, physical examination and X-ray are usually normal. Transient minimal pleural effusion can be detected in very few patients. The underlying cause may rarely be pericarditis.[12]

The aim of this study was to describe the clinical, laboratory and genetic differences between pediatric FMF patients with and without chest pain.

Material and Methods

Between January 2006 and January 2022, 1134 patients with FMF who applied to the outpatient clinic of Gazi University Faculty of Medicine, Department of Pediatric Rheumatology, were analyzed retrospectively. All patients were younger than 18 years at diagnosis and were selected according to Turkish pediatric FMF criteria.[13] Gender, age of symptom onset and diagnosis, family history of FMF, number of attacks and duration of attacks in the last year with data obtained at the time of diagnosis, and Pras severity score obtained at the last visit.[14] The presence of family history was defined as having an FMF diagnosis in 1st, 2nd, or 3rd-degree relatives. The colchicine dose at their last visit was recorded. Patients with colchicine intolerance or inadequate treatment response are switched to interleukin (IL)-1 antagonist treatment in our clinic. The patients' data using IL-1 antagonists were recorded from the file records. Presence of fever, peritonitis, chest pain, arthralgia, arthritis, erysipelas-like rash, and amyloidosis were recorded. *MEFV* gene analysis results of 1131 patients were available. No mutation was detected in 61 of them. The term "patients without *MEFV* gene mutation" was used for these patients. *MEFV* gene was derived from blood samples in EDTA tubes at the Nephrology and Tissue Laboratory at the Gazi University Medical Faculty. The polymerase chain reaction workup, which was revealed with the pyrosequence DNA analysis system, demonstrated the 2-3-5-10 exons of the *MEFV* gene. Patients were divided into two groups as those with and without recurrent chest pain. These groups were compared in demographic, clinical findings, treatment, and *MEFV* gene analyses. The present study was approved by the local ethics committee of the Gazi University (Decision no: E-77082166-604.01.02-332908 dated 05.04.2022).

Statistical analysis

Data were analyzed with SPSS version 21 software (SPSS, Chicago, USA). Categorical data were expressed by frequency and percentage. Quantitative data were not normally distributed, and the median was presented as an interquartile range. The Mann-Whitney U test was used to compare the quantitative data of patients with and without chest pain. Chi-square or Fisher's exact tests were used for categorical data comparisons. *P* values less than 0.05 were accepted as statistically significant.

Results

One thousand one hundred thirty-four patients with FMF were included in the study. The demographic and clinical characteristics of the patients are presented in Table I. A hundred and sixty-two (14.3%) patients had recurrent chest pain. In patients with recurrent chest pain, the age of onset of symptoms was younger ($p=0.003$), and the family history of FMF was higher ($p=0.002$). Patients with chest pain had a higher annual attack frequency ($p<0.001$), a longer attack duration ($p<0.001$), and higher Pras disease activity scores ($p<0.001$) (Table I). Other clinical findings of patients with and without chest pain were similar. The colchicine dose used in the treatment was higher in FMF patients with chest pain ($p=0.005$), and Anti-IL-1 treatment was higher ($p<0.001$) (Table I). In the patients with chest pain, 92 of the patients (56.8%) had posteroanterior (PA) chest x-ray, and 27 of the patients (16.7%) had electrocardiography (ECG) and echocardiography (ECO). Pleural effusion was detected in 2.2% (2/92) and pericarditis in 3.7% (1/27) was detected.

Table I. Comparison of demographic and clinical characteristics of patients with and without chest pain.

	All patients N=1134	FMF patients with chest pain N=162	FMF patients without chest pain N=972	p
Gender†				0.827
female	593 (52.3)	86 (53.1)	507 (52.2)	
male	541 (47.7)	76 (46.9)	465 (47.8)	
Age of symptom onset, year‡	6 [6]	4 [5]	6 [6]	**0.003**
Age of diagnosis, year‡	7 [7]	6 [6]	7 [6]	0.122
Family history†	579 (51.1)	101 (62.3)	478 (49.2)	**0.002**
Attack count, (Last 6 months before FMF diagnosis)‡	2 [3]	2 [4]	2 [2]	**<0.001**
Attack duration, days‡	2 [1]	3 [2]	2 [1]	**<0.001**
Pras score‡	6 [2]	6 [3]	5 [3]	**<0.001**
Clinical features†				
Abdominal pain	946 (83.4)	131 (80.9)	815 (83.8)	0.344
Fever	873 (77)	120 (74.1)	753 (77.5)	0.342
Arthralgia	644 (56.8)	94 (58)	550 (56.6)	0.732
Arthritis	186 (16.4)	30 (18.5)	156 (16)	0.432
Erysipelas-like lesion	92 (8.1)	18 (11.1)	74 (7.6)	0.131
Amyloidosis	9 (8)	3 (1.9)	6 (0.6)	0.125
Medication				
Colchicine dose, mg/day‡	1 [0]	1 [0.5]	1 [0]	**0.005**
Anti–IL–1 therapy usage†	27 (2.4)	12 (7.4)	15 (1.5)	**<0.001**

† Data are given as numbers and percentages.
‡ Data were given as median (interquartile range).
FMF: Familial Mediterranean fever.

MEFV gene mutation analyzes of 1131 patients were performed. The data of 3 patients were missing. M694V homozygous mutation was found more frequently (p=0.001), whereas M694V/V726A mutation was found less frequently in patients with recurrent chest pain (p=0.017) (Table II).

Demographic, clinical findings, and genetic characteristics of 14 patients with only recurrent chest pain and FMF family history, without fever, peritonitis, and arthritis, are presented in Table III.

Discussion

Recurrent chest pain is observed in approximately 20% of FMF patients and is among the most common clinical findings following abdominal pain and arthritis.[15,16] This study demonstrated that 14% of patients had recurrent chest pain. Patients with chest pain had a younger age at diagnosis. Our previous study evaluated the impact of age at diagnosis in clinical findings; we showed that chest pain complaints were more common in children diagnosed under 2 years of age.[17] Also, in another study by Tanatar et al.[18], chest pain was more common in patients aged three years and older. However, other studies have not shown a relationship between the age of symptom onset and the presence of chest pain.[19,20]

We showed that family history is more common in patients with recurrent chest pain. To the best of our knowledge, there is no detailed study on clinical findings of patients with a family history. However, in a study evaluating the effects of

Table II. Comparison of *MEFV* gene analyzes of patients with and without chest pain.

	All patients* N=1131	FMF patients with chest pain N=161	FMF patients without chest pain N=970	p
M694V/-	321 (28.4)	38 (23.6)	283 (29.2)	0.146
M694V/M694V	218 (19.3)	47 (29.2)	171 (17.6)	**0.001**
M680I/-	82 (7.3)	9 (5.6)	73 (7.5)	0.380
E148Q/-	80 (7.1)	13 (8.1)	67 (6.9)	0.593
M694V/V726A	68 (6)	3 (1.9)	65 (6.7)	**0.017**
M694V/M680I	57 (5)	12 (7.5)	45 (4.6)	0.131
M694V/E148Q	41 (3.6)	10 (6.2)	31 (3.2)	0.058
V726A/-	32 (2.8)	6 (3.7)	26 (2.7)	0.441
M680I/M680I	30 (2.7)	4 (2.5)	26 (2.7)	>0.999
Other mutations**	141(12.5)	12(7.5)	129(13.3)	-
Patients without *MEFV* mutation	61 (5.4)	7 (4.3)	54 (5.6)	0.526

**MEFV* gene analysis data of 3 patients could not be reached
** Other *MEFV* mutation analyzes are mutations that occur less than 20 in total. Among other mutations, there was no statistically significant difference between the two groups. Other *MEFV* mutations; F479L/-, E148Q/E148Q, M694V/R202Q, M680I/R761H, M680I/P369S, M694I/M694I, R202Q/-, A744S/-, M694V/M694I, V726A/V726A, M694V/K695R, M694V/A744S, M694V/P369S, V726A/F479L, R761H/-, K695R/-, E148Q/L110P, E148Q/A744S, E148Q/R202Q, P369S/R408Q, E167D/-, A202G/-, E148Q/P369S, V726A/E148Q, M680I/V726A, M680I/E148Q, M694V/R761H.
FMF: Familial Mediterranean fever.

Table III. Patients with only chest pain complaint.

	Age of diagnosis, year	Sex	*MEFV* gene mutation	Attack count at one year before the diagnosis of FMF	Total attack count before the diagnosis of FMF	Coexistent diseases	Amyloidosis	Anti–IL–1 therapy usage
Patient 1	6	Female	E148Q/-	0	5	IgAV	Absent	Absent
Patient 2	5	Female	M694V/-	2	6	-	Absent	Absent
Patient 3	17	Male	M694V/E148Q	6	6	-	Absent	Absent
Patient 4	15	Male	M694V/-	12	12	-	Absent	Absent
Patient 5	6	Female	M694V/M694V	2	4	-	Absent	Absent
Patient 6	8	Female	M694V/E148Q	2	3	-	Absent	Absent
Patient 7	14	Female	E148Q/-	0	6	-	Absent	Absent
Patient 8	5	Female	M694V/M694V	6	7	-	Absent	Absent
Patient 9	5	Female	M694V/-	2	4	-	Absent	Absent
Patient 10	4	Male	M694V/M694V	4	6	-	Absent	Absent
Patient 11	9	Female	M694V/E148Q	3	3	-	Absent	Absent
Patient 12	15	Male	E148Q/-	2	5	-	Absent	Absent
Patient 13	4	Male	M694V/M694V	2	5	-	Absent	Absent
Patient 14	7	Male	M694V/-	2	4	-	Absent	Absent

FMF: Familial Mediterranean fever, IgAV: Immunoglobulin A vasculitis.

MEFV gene mutations on clinical findings, it was reported that the frequency of chest pain might be less in those with a family history.[21] In addition, it has been reported that family history is more frequently positive in patients with FMF complicated by amyloidosis.[22]

It is known that chest pain is more common in adult and pediatric FMF patients with persistent inflammation.[23,24] Although several studies describe an increased incidence of chest pain in adult FMF patients with amyloidosis, some studies claim vice versa.[22,25] In the current study, we found the frequency of amyloidosis to be similar in both groups. However, Pras scores were higher in patients with chest pain. In our study, the increased frequency of family history, number of attacks, longer duration of attacks, and higher disease severity scores in patients with chest pain suggest that the disease progresses more severely in these patients. For this reason, the need for an IL-1 antagonist was probably more significant in the patients with chest pain.

Many studies have been conducted to assess the effect of *MEFV* gene mutations on clinical findings. In a study based on real-life data from Turkey, chest pain was significantly more common in FMF patients with M694V/M680I, M680I/M680I, M694V/R761H, and M680I/E148Q mutations.[15] Kilic et al.[26] reported an increased frequency of chest pain in patients with M694V homozygous and E148Q heterozygous mutations. There may also be ethnicity differences in the effect of *MEFV* mutations on chest pain. Jewish children with the M694V homozygous mutation have been reported to have more pulmonary symptoms than Arab children.[27] In the presented study, we detected M694V homozygous (29%), M694V heterozygous (24%), E148Q heterozygous (8%), and M694V/M680I (7.5%) compounds heterozygous mutations as the most common mutations in patients with chest pain (Table II). When we compare the groups with and without chest pain, we found that the presence of M694V homozygous mutation is more common, and presence of M694V/V726A compound heterozygous mutation is less common in the group with chest pain (Table II). In a previous study, it was reported that symptoms such as fever and chest pain were less common in patients with the V726A mutation.[28] In the light of this information, it can be said that the V726A mutation may be associated with milder symptoms. However, more comprehensive data are needed for definitive conclusions.

Our study has limitations, such as its single-center retrospective design and lack of detailed pulmonary and cardiac imaging of patients with chest pain. Despite this, the study has its strengths in that it has revealed the characteristics of patients with recurrent chest pain based on data of a relatively large number of patients despite being drawn from a single center.

As a result, patients with recurrent chest pain seem to have early onset symptoms, more often have family history, and have a higher disease severity. In addition, the presence of homozygous M694V mutation is more common in patients with chest pain.

Ethical approval

The present study was approved by the local ethics committee of the Gazi University (Decision no: E-77082166-604.01.02-332908 dated 05.04.2022).

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by ENSY, PŞD, DGY and OS. The first draft of the manuscript was written by ENSY and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Choice and switch of biologic drugs in juvenile idiopathic arthritis

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ABSTRACT

Background. In this study, we aimed to evaluate choices and changes of biologic drugs in juvenile idiopathic arthritis (JIA) patients according to disease subtypes.

Methods. We retrospectively analyzed JIA patients who received biologic treatment between January 2004 and July 2022.

Results. Of 294 JIA patients, 80 (27.2%) had systemic JIA, 68 (23.1%) had oligoarticular JIA, 61 (20.7%) had polyarticular JIA, 79 (26.9%) had enthesitis-associated arthritis (ERA), and six (2.1%) had psoriatic arthritis (PsA). Anakinra (n=66, 82.5%) was the most commonly preferred first line biologic in systemic JIA. Etanercept was the most frequently used biologic drug in patients with ERA (n=69, 87.3%), oligoarticular (n=37, 54.4%) and polyarticular JIA (n=43, 70.5%). Adalimumab was used as a first-line biologic drug in all PsA patients (n=6, 100%). One hundred-fourteen patients (38.8%) were switched to second-line and 29 (9.9%) to third-line biologic drugs. While the most common reason for switching to a second-line biologic was difficulty in usage of daily injections (n=37, 60.6%) in systemic JIA patients, it was an inadequate response to first biologics in non-systemic JIA patients (n=42, 79.2%). Side effects were detected in only seven patients (2.4%) during the follow-up.

Conclusion. In this study, we revealed the biologic drug usage and switch strategies in our JIA patients. Good responses were obtained in most of our patients with a reliable profile. However, studies on larger patient groups are needed to clarify these results.

Key words: adalimumab, anakinra, etanercept, juvenile idiopathic arthritis.

Juvenile idiopathic arthritis (JIA) is the most frequent chronic rheumatic disease of childhood and was classified into seven categories according to the International League of Associations of Rheumatology (ILAR).[1,2] These are systemic, oligoarticular (persistent or extended), polyarticular (rheumatoid factor [RF]-positive, RF-negative), enthesitis-related arthritis (ERA), psoriatic arthritis (PsA), and undifferentiated arthritis.

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Early and efficient treatment is crucial in JIA since the disease causes disability if inadequately treated.[3,4] The prognosis of JIA patients has changed dramatically after the introduction of biologic drugs in the treatment.[5,6] Biologic drugs are highly effective in patients who do not respond or are intolerant to treatment with disease-modifying anti-rheumatic drugs (DMARDs).[7]

In general, tumor necrosis factor-alpha (TNF-α) inhibitors are used in patients with polyarticular JIA and ERA, and biologic drugs targeting interleukin (IL)-1 and IL-6 activity are used in patients with systemic JIA.[8-13] TNF-α inhibitors are divided into two categories, the monoclonal anti-TNF-α antibodies, such

as infliximab (IFX), adalimumab (ADA), golimumab, and certolizumab pegol, and the soluble TNF receptor fusion protein, etanercept (ETN). They are recommended as second- or third-line drugs in polyarticular JIA treatment, often after at least three months of DMARD therapy, and their efficacy has been established in numerous trials.[14,15] Anakinra (IL-1 receptor antagonist), canakinumab (anti-IL-1 antibody), rilonacept (IL-1 receptor antagonist), and tocilizumab (TCZ, IL-6 receptor antibody) are among the biologic drugs frequently used in the treatment of systemic JIA patients.[10,11,16] TCZ is also used to treat patients with polyarticular JIA.[17] Secukinumab, a fully human monoclonal antibody that directly inhibits IL-17A, is also among the drugs of choice in some types of JIA.[18] In addition, tofacitinib, a Janus kinase (JAK) inhibitor, has recently been introduced to the treatment of refractory JIA patients.[19]

Physicians involved in JIA treatment have an increasing number of biologic treatment options, and choosing between them may be challenging. Unfortunately, there is no clear treatment guideline for JIA patients on which biologic to use as first-line neither with regards to biologic switch strategies. The subtype of JIA is the most important factor determining the choice of biologic drug. While the disease pathogenesis, which varies according to JIA subtypes, plays a major role in predicting the efficacy of a biologic drug, the side effect profile should also be taken into consideration. Therefore, it is necessary to carefully weigh the benefits and risks before initiating biological agents. In the present study, we aimed to evaluate biologic drug choices and switching strategies between biologics in our JIA patients.

Materials and Methods

The study was approved by the ethics committee of our center (GO 21/743). The study was performed following the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

Patients

All JIA patients treated with biologics from January 2004 to July 2022 at the Department of Pediatric Rheumatology, Hacettepe University Faculty of Medicine, Ankara, Türkiye, were retrospectively evaluated. All participants met the ILAR classification criteria for JIA.[2] In addition, they had attended at least two visits in our center after the initiation of biologic therapy and their general evaluations and examinations were made by a pediatric rheumatologist. JIA patients who did not use biologic drugs were excluded.

Data collection

The collected data included patients' demographic characteristics, JIA subtypes, laboratory findings, biologic drug used according to JIA subtypes, duration of biologic drug use, reasons for using and switching of biologic drugs, and outcomes. In addition, the Juvenile Arthritis Disease Activity Score-71 (JADAS-71) and Childhood Health Assessment Questionnaire (CHAQ) of the patients were calculated before treatment with first biologic drug and after treatment with last biologic drug.[20,21] Outcomes were determined according to the American College of Rheumatology (ACR) criteria.[22]

Statistical analysis

All data were analyzed using IBM Statistical Package for Social Sciences (SPSS) software v. 24. Descriptive statistics were presented as frequency (n) and percentage (%), median and 1st-3rd quartiles (Q1-Q3), or mean ± standard deviation (SD). The numeric variables were investigated using visual and analytic methods (Kolmogorov-Smirnov / Shapiro-Wilk's test) to determine whether they were normally distributed. Where appropriate, the chi-square test, or Fisher's exact test, was used to analyze relationships between categorical variables. The Mann-Whitney U test or Kruskal-Wallis test was used to test whether the medians between comparison groups were different. A p-value

of less than 0.05 was considered to show a statistically significant result.

Results

General characteristics of juvenile idiopathic arthritis patients treated with various biologic drugs

Among the total 812 JIA patients, 294 patients treated with biologic drugs were included in the study. The median age of 294 patients at diagnosis was 8.9 (3.8-11.4) years, and 153 (52%) were female. Eighty of them (27.2%) had systemic JIA, 68 (23.1%) had oligoarticular JIA, 61 (20.7%) had polyarticular JIA, 79 (26.9%) had ERA and 6 (2.1%) had PsA (Table I). Forty-one (13.9%) patients had uveitis (mostly oligoarticular JIA), 17 (5.8%) had inflammatory bowel disease (IBD) (mostly oligoarticular JIA), and 32 (10.9%) had macrophage activation syndrome (MAS) (all systemic JIA).

Except for patients with oligoarticular JIA, most patients (n=222, 75.5%) had elevated acute phase reactants before biologic therapy. The majority of the patients, especially those with polyarticular JIA, had high JADAS-71 and CHAQ scores before treatment with the first-line biologic drug. JADAS-71 and CHAQ scores significantly decreased in all patient groups after biologic treatment(s) ($p<0.001$ for both). Complete remission was achieved in 242 patients (82.3%) at the last visit.

First-line biologic drugs according to disease subtypes in patients with juvenile idiopathic arthritis

There were several differences between JIA subtypes regarding the selection of biologic

Table I. General characteristics, disease scores and outcome of juvenile idio pathic arthritis patients treated with biologic drugs.

	SJIA (n=80)	OJIA (n=68)	PJIA (n=61)	ERA (n=79)	PsA (n=6)
Female, n (%)	39 (48.8)	54 (79.4)	32 (52.5)	24 (30.3)	4 (66.7)
Age at diagnosis, years, median (Q1-Q3)	5.1 (2.2-6.9)	4.5 (3.1-7.2)	6.4 (3.1-10.2)	9.7 (6.8-12.4)	9.3 (7.1-12.7)
Disease duration, years, median (Q1-Q3)	5.9 (2.3-6.8)	6.4 (3.2-8.6)	4.4 (2.7-6.5)	5.3 (3.9-7.2)	4.7 (3.1-6.4)
Laboratory findings, n (%)					
Elevated APR*	80 (100)	29 (42.6)	49 (80.3)	59 (79.7)	5 (83.3)
ANA	1 (1.3)	47 (69.1)	14 (22.9)	1 (1.3)	2 (33.3)
HLA-B27	0	0	0	54 (68.3)	1 (16.7)
RF	2 (2.5)	0	13 (21.3)	0	0
First JADAS-71*, median (Q1-Q3)	9.3 (4.7-11.9)	12.1 (9.1-16.5)	21.5 (17.1-25.2)	15.2 (9.9-21.3)	16.3 (11.2-21.8)
First CHAQ*, mean ± SD	1.1 ± 1.3	1.3 ± 1.8	1.9 ± 2.4	1.6 ± 2.3	1.5 ± 2.1
Last JADAS-71**, median (Q1-Q3)	0.45 (0.3-0.7)	0.3 (0.15-0.5)	0.7 (0.5-1.0)	0.6 (0.4-0.9)	0.3 (0.1-0.5)
Last CHAQ**, mean ± SD	0.2 ± 0.3	0.1 ± 0.3	0.3 ± 0.6	0.2 ± 0.5	0.1 ± 0.2
Outcome**, n (%)					
Complete remission	68 (85)	63 (92.6)	46 (75.4)	60 (75.9)	5 (83.3)
Partial remission	12 (15)	5 (7.3)	15 (24.6)	19 (24.1)	1 (16.7)

JADAS-71 and CHAQ scores significantly decreased in all patient groups after biologic treatment(s) ($p<0.001$ for both).
ANA: antinuclear antibodies, APR: acute phase reactants, CHAQ: Childhood Health Assessment Questionnaire, ERA: enthesitis-related arthritis, HLA: human lymphocyte antigen, JADAS-71: Juvenile Arthritis Disease Activity Score-71, JIA: juvenile idiopathic arthritis, OJIA: oligoarticular juvenile idiopathic arthritis, PJIA: polyarticular juvenile idiopathic arthritis, PsA: psoriatic arthritis, RF: rheumatoid factor, SD: standard deviation, SJIA: systemic juvenile idiopathic arthritis
*before treatment with first biologic drug
**after treatment with last biologic drug

drugs (Table II). Anakinra (n=66, 82.5%) was the most commonly used first line biologic drug in systemic JIA, especially with MAS. TCZ was used in systemic JIA patients with prominent joint involvement (n=9, 11.3%). ETN was the most frequently used biologic drug in ERA patients (n=69, 87.3%). ETN was also mostly preferred in oligoarticular (n=37, 54.4%) and polyarticular JIA patients (n=43, 70.5%). ADA was used as first-line biologic drug in all PsA patients (n=6, 100%). In addition, ADA was commonly preferred in oligoarticular (n=25, 36.8%) and polyarticular JIA (n=13, 21.3%) patients, especially those who had uveitis or IBD. Biologic drugs were mostly initiated due to the uncontrolled disease activity with NSAIDs/corticosteroids in systemic JIA, however were initiated due to the uncontrolled disease activity with DMARDs in non-systemic JIA subtypes. Concomitant corticosteroid use was frequent in patients who used anakinra or TCZ, and concomitant use of methotrexate (MTX) was prevalent in patients receiving ADA or IFX.

ACR100 responses were achieved by most patients (n=223, 75.9%) after the first-line biologic drug. However, ACR100 responses to the first-line biologics were more frequent among patients with oligoarticular JIA and PsA (p=0.025 and p=0.010) (Table II).

Changes from the first- to second- or third-line biologic drugs

One hundred-fourteen patients (38.8%) were switched to the second-line and 29 (9.9%) to the third-line biologic drugs in the follow-up (Fig. 1 and Fig. 2). In systemic JIA patients, the reasons for switching to a second-line biologic

Table II. First-line of biologic drugs, reasons and treatment responses according to subtypes of juvenile idiopathic arthritis.

	SJIA (n=80)	OJIA (n=68)	PJIA (n=61)	ERA (n=79)	PsA (n=6)
Biologic drugs, n (%)					
Anakinra	66 (82.5)	0	0	0	0
Canakinumab	5 (6.3)	0	0	0	0
Etanercept	0	37 (54.4)	43 (70.5)	69 (87.3)	0
Adalimumab	0	25 (36.8)	13 (21.3)	7 (8.9)	6 (100)
Infliximab	0	6 (8.8)	2 (3.3)	2 (2.5)	0
Tocilizumab	9 (11.3)	0	3 (4.9)	0	0
Reasons to start biologics, n (%)					
Disease not controlled with NSAIDs/corticosteroids	72 (90)	7 (10.3)	6 (9.8)	9 (11.4)	1 (16.7)
Disease not controlled with DMARDs	8 (10)	61 (89.7)	55 (90.1)	70 (88.6)	5 (83.3)
Duration of biologic use, months, median (Q1-Q3)	16 (6-24)	12 (12-18)	18 (12-24)	18 (12-26)	24 (12-30)
Treatment response, n (%)					
ACR30	71 (88.8)	64 (94.1)	55 (90.1)	75 (83.3)	6 (100)
ACR50	65 (81.3)	61 (89.7)	53 (86.9)	72 (91.1)	6 (100)
ACR70	61 (76.3)	58 (85.3)	49 (80.3)	66 (83.6)	6 (100)
ACR90	59 (73.8)	56 (82.4)	45 (73.8)	63 (79.7)	5 (83.3)
ACR100	58 (72.5)	55 (80.9)	43 (70.5)	62 (78.5)	5 (83.3)

ACR100 responses to the first-line biologics were higher among patients with oligoarticular JIA and PsA (p=0.025 and p=0.010), compared to other groups.
ACR: American College of Rheumatology, DMARDs: disease-modifying anti-rheumatic drugs, ERA: enthesitis-related arthritis, NSAIDs:non-steroidal anti-inflammatory drugs, OJIA: oligoarticular juvenile idiopathic arthritis, PJIA: polyarticular juvenile idiopathic arthritis, PsA: psoriatic arthritis, SJIA:systemic juvenile idiopathic arthritis.

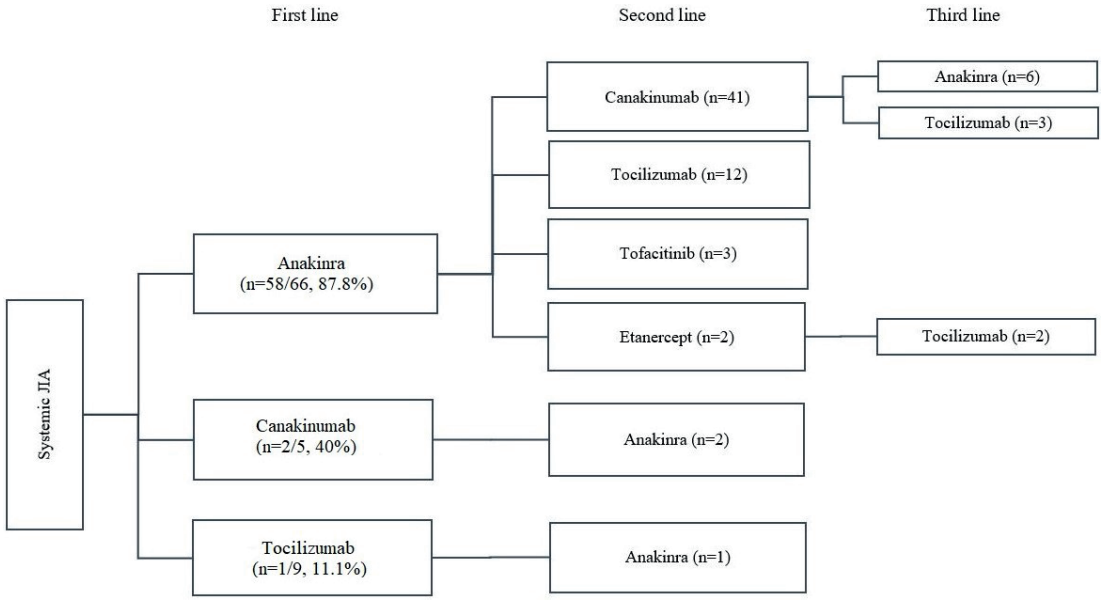

Fig. 1. Biologic drugs used as first-, second-, and third-line in patients with systemic juvenile idiopathic arthritis.

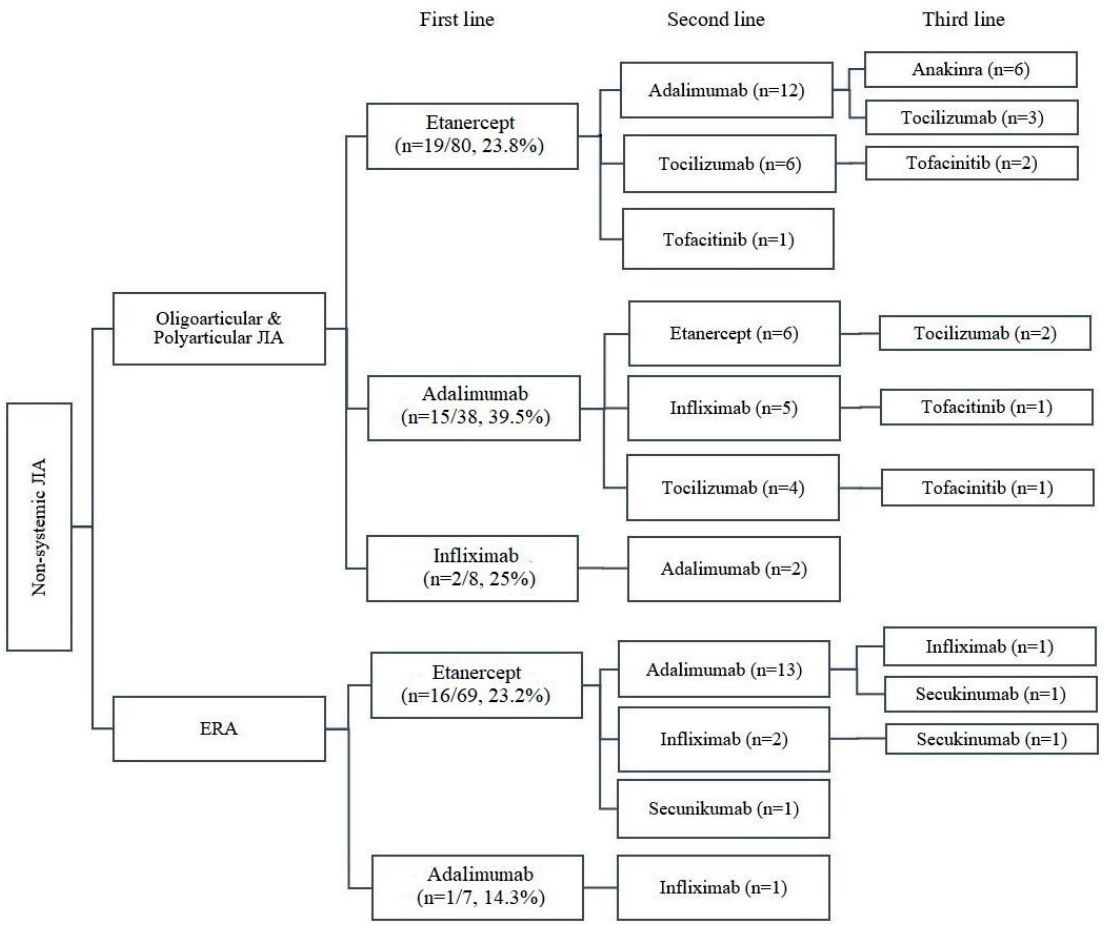

Fig. 2. Biologic drugs used as first-, second-, and third-line in patients with non-systemic juvenile idiopathic arthritis.

were difficulty in usage of daily injections in 37 patients (60.6%), conversion to polyarticular course in 14 patients (22.9%), an inadequate response to the first-line biologic treatment in six patients (9.8%), and side effects in four patients (6.6%). Three of these side effects developed due to anakinra use (local redness and/or urticaria at the injection site) and the other was associated with TCZ (anaphylaxis). In non-systemic JIA patients, the reasons for switching to a second-line biologic were an inadequate response to the first-line biologic treatment in 42 patients (79.2%), development of uveitis in three patients (5.7%), development of IBD in two patients (3.8%), compliance problems in two patients (3.8%), and side effects in two patients (3.8%). One of the side effects was associated with ETN (viral upper respiratory tract infection-like symptoms) and the other was associated with IFX (anaphylaxis). The reason for the transition to the third-line biologic drugs was an inadequate response to the second-line biologic treatment in all patients except one. One patient with polyarticular JIA using TCZ was switched to tofacitinib due to the development of anaphylaxis associated with TCZ.

ACR100 response was achieved in 72% (44/61) of systemic JIA patients who switched to the second-line biologic drug, and 64.2% (34/53) of non-systemic JIA patients.

Discussion

In recent years, biologic drugs have changed the prognosis and treatment of many rheumatic diseases, including JIA.[23] Biologic drug preferences differ according to the JIA subtypes. In our study, anakinra was the most commonly used first-line biologic drug in systemic JIA, while TCZ was preferred in patients with significant joint involvement. While ETN was the most frequently used biologic drug in patients with ERA, oligoarticular and polyarticular JIA, the first-line biologic was ADA in all PsA patients. Biologic drugs were initiated in systemic JIA in cases of uncontrolled disease activity with NSAIDs/corticosteroids, and uncontrolled disease activity with DMARDs in non-systemic JIA subtypes.

Since IL-6 and IL-1β are known to play central roles in the pathogenesis of systemic JIA, biologics targeting these cytokines have also been frequently used in systemic JIA in the literature.[24,25] Likewise, in our study, the most commonly used drug in systemic JIA was anakinra, followed by TCZ. Most of our patients with systemic JIA who received anakinra also had a history of MAS. Successful treatment of patients with MAS associated with systemic JIA with anakinra has been demonstrated in many studies, and anakinra has taken its place in treatment guidelines.[26,27]

Of the TNF-α inhibitors, ETN was most commonly used in our ERA patients (86.1%). Many studies have shown that ETN improves the signs and symptoms of ERA, and remission is achieved with long-term treatment.[28-30] Horneff et al.[28] reported that 24 weeks of ETN treatment reduced the signs and symptoms of ERA, with marked improvement and a high number of patients achieving remission.

Etanercept was also used most frequently in our patients with oligoarticular and polyarticular JIA. TNF-α inhibitors are commonly preferred in patients with oligoarticular (especially in the extended subtype) and polyarticular JIA in the literature.[31,32] In our study, some of the resistant polyarticular JIA patients who did not respond to TNF-α inhibitors were switched to TCZ. Brunner et al.[17] expressed that polyarticular JIA patients treated with TCZ showed a high level of disease control for up to two years. Patients using TCZ also had higher post-treatment JADAS-71 and CHAQ scores than others, possibly due to its use in patients with resistant polyarticular JIA.

Adalimumab was frequently used in JIA patients with a history of uveitis or IBD. There are many studies in the literature reporting that ADA is effective in treating JIA patients with uveitis or IBD.[33,34] In a survey study by

Kotaniemi et al.[34], which evaluated the long-term effects of ADA, successful uveitis control was achieved in two-thirds of 54 JIA uveitis cases who were resistant or intolerant to other immunosuppressive drugs, and corticosteroid treatment was discontinued in 22%.

Adalimumab was also used in all of our PsA patients. Poddubnyy et al.[35] reported that ADA was an effective and generally well-tolerated drug for treating the signs and symptoms of PsA.

In our study cohort, 38.8% were switched to second-line biologics, and 9.9% to third-line biologics. While the most common reason for switching to the second-line biologic therapy in systemic JIA patients were the difficulty in using daily injections and transition to a polyarticular course, an inadequate response to the first-line biologic was the most common cause in non-systemic JIA patients. The main reason for switching to the third biologic drugs was an inadequate response to the second biologics in both groups. There are previous studies on biologic drug switches in patients with JIA.[36,37] In a study evaluating a large cohort including JIA patients, 1152 of 2361 patients were initiated with at least one biologic drug and most of them were treated with TNF-α inhibitors as a first-line biologic (n=1050, 91%).[36] Two hundred seventy (23%) of 1152 patients received a second-line biologic drug, 61 (5%) a third-line biologic, and 11 (1%) a fourth-line biologic. Of 240 patients with polyarticular JIA, 194 (81%) used a second TNF-α inhibitor, and in 46 patients (19%) who did not respond to TNF-α inhibitors, these were switched to a non-TNF-α inhibitor biologic drug. In a study by Mannion et al.[37] which included 1361 patients with JIA using biologic drugs (94% TNF-α inhibitors), biologic drugs were switched in 349 (26%) patients. Among biologic switchers, ineffectiveness/disease flare was the most common reason for switching (n=202, 58%).

In our study, adverse effects related to biologic drugs were detected in seven patients (2.4%). Most of the adverse events were allergic reactions, and one patient had recurrent upper respiratory tract infections. Because of these side effects, the biologic drugs were changed in these patients. Allergic reactions and an increase in the frequency of infections (mostly upper respiratory tract infections) after biologic treatments have been reported in the literature.[38,39] In general, discontinuation and/or switch of the current treatment is recommended in these cases.

The retrospective nature was the main limitation of this study. The study relied heavily on clinical assessments and physical examinations, which can be subjective. Therefore, incomplete or incorrect medical records can lead to erroneous assumptions. In addition, it has a relatively small sample size (especially PsA patients), so it might not capture the full spectrum of JIA presentations. Some rare or atypical forms of JIA may not be adequately represented, limiting the generalizability of the study findings. Finally, only ACR responses could be evaluated after first-line biologic use, as it may have been confusing to assess ACR responses in the end, in patients who had used more than one biologic.

Conclusion

In this study, we demonstrated our preferences of biologic use and biologic switch in JIA patients. Anakinra was more commonly prescribed to systemic JIA patients, while ETN was most frequently used in ERA, oligoarticular and polyarticular JIA patients. A good response with a reliable safety profile was obtained with biologic drugs in most of our patients who had uncontrolled disease activity with NSAIDs/corticosteroids or DMARDs. In general, unresponsiveness to the prescribed biologic treatment was the main reason for switching to a second or third biologic drug, whereas the switch from anakinra to another biologic was frequently due to the challenges associated with daily injections. We believe that understanding the reasons for the use and transition of biologic drugs can help us gain insights into using personalized medicine strategies and

can improve the management of JIA patients. However, studies in larger cohorts are required to assess the efficacy and side-effect profiles of biologic drugs more clearly.

Ethical approval

This study has been approved by the Hacettepe University Ethics Commission (Approval Number: GO 21/743) and was performed following the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SŞ, ÖB, EDB, YB and SÖ data collection: SŞ, MKC, ZB, EA, YB analysis and interpretation of results: SŞ, ÖB, EDB, MKC, ZB, EA, YB, YB and SÖ draft manuscript preparation: SŞ, ÖB, EDB, YB and SÖ. All authors reviewed and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

Ezgi Deniz Batu and Özge Başaran received payment for speakers' bureaus from Novartis. Seza Ozen received consultancy fees and payment for speakers bureaus from Novartis and Sobi. Other authors declare that is there is no conflicts of interest.

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Original Article

Adolescent male soccer players have higher growth rates and risk of injury is associated with biological maturity

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ABSTRACT

Background. The objective of this study was to ascertain disparities in growth and maturation between male adolescents engaged in soccer and their non-athletic counterparts, as well as to examine the injury features specific to young soccer players.

Methods. A total of 206 soccer players between the ages of 11-16 years, and 208 non-athletic peers were enrolled. Height, weight, body mass index (BMI), annual growth rate, and skeletal age evaluated using a left hand-wrist x-ray were determined. Biological and sexual maturation were evaluated using skinfold thickness, body composition, and Tanner stages. The game positions, initial age for playing soccer, the number of games per/week, the number of sports injuries, date of injury, duration for return to activity, the site, nature, mechanism, and rate of injury were recorded for soccer players. Using an injury card, the characteristics of soccer player injuries were recorded.

Results. The mean age of the participants was 13.6 ± 1.5 years. There was no difference in the growth rates between the groups at the ages of 11.0, 12.0, and 15.0 but at the ages of 13.0 and 14.0 years growth rates were higher in the soccer group. The soccer players were taller than the controls. For all Tanner stages, soccer players had a lower BMI and total body fat percentage, as well as a faster growth rate. Injuries occurred at a rate of 39.3% per year among soccer players. The most common being toe injuries, and playing soccer increased the risk of multiple injuries. Additionally, injuries occurred more frequently in soccer players who were taller, heavier, with higher total body fat and/or higher growth rate, and most commonly occurred during Tanner stage 4. Futhermore, Tanner stage 4 had a higher incidence of two or more injuries than the other stages.

Conclusions. Adolescent male soccer players have higher growth rates than their non-athletic peers, and their biological maturity status is associated with an increased risk of injury.

Key words: maturity, tanner stage, soccer, injury, adolescence.

The achievement of physical growth, the development of secondary sexual characteristics and the maturation of psychosocial skills all occur during adolescence. This period of change and development is a vastly individual process, varying considerably.[1] Participating in sporting activities is also one of the factors that affects this variation among individuals. In the literature, an opposite relationship between physical activity and the timing of biological maturation has been reported.[1] Although some studies have shown that male adolescents with insufficient physical activity have an earlier risk of maturation[2], it has similarly been reported that those adolescents who engage in vigorous physical activity may also mature earlier than

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their peers.[3] Regular physical training and participation in sports have not been shown to have an impact on the timing, grade or size of the growth spurt in many studies.[4,5]

In the first decade of the new millennium, the age of participation in sports has decreased, whereas the intensity of training has increased, which in turn is associated with an increase in acute and overuse injuries.[6] An important topic when discussing participation in sports during childhood and adolescence is the injury of the developing musculoskeletal system, especially the epiphyseal or growth plate.[7] Epiphyseal injuries can disrupt normal bone development and growth causing permanent damage. It has been reported that the incidence of injury is high during the growth spurt period because of non-linearity and the complexity of growth with a period of clumsiness in motor coordination, in addition to changes in flexibility and increase in stiffness when growth accelerates and maturation increases.[8-10] Decreased flexibility causes acute or overuse injuries, especially in boys and body mass index (BMI), fat percentage, and muscle mass changes are also reported as anthropometric factors that have an influence on injury risk.[8] On the other hand, no significant relationship between early and late maturation and injury risk has been reported in some studies.[11,12] In adolescent male soccer players, injury range has been reported from 38 to 85%, with 0.4-2.2 injuries per player per season.[13] Soccer is the most popular team sport in Turkey and children start playing at an early age. A large group of young players attend local youth soccer clubs affiliated with professional soccer clubs. In a study investigating the admissions to a sports medicine outpatient clinic in our country, it was reported that injuries in children and adolescents occurred at a rate of 23.3% in soccer, 17.2% in basketball and 14.5% in volleyball.[14]

In the literature, epidemiological studies on soccer injuries among adolescents have been conducted primarily in European settings, resulting in a geographic imbalance.[13] Although in recent years a great deal of focus has been placed on the injury risk of adolescent soccer players in order to reduce medical health care costs, promote talent development, and introduce an adequate prevention program, some areas, such as evaluating biological maturity, are still lacking.[15]

This study aimed to assess the growth, skeletal, and sexual maturation of male soccer players and non-athletic peers, as well as the injury characteristics of the soccer players. To the best of our knowledge, this is the first study evaluating this in Turkey. We hypothesize that biological maturation will influence injury rate, particularly as growth accelerates.

Materials and Methods

This was a prospective, cross-sectional study. It was approved by the institutional review board. Informed consent was obtained from all the participants and their parents or guardians. Male adolescent soccer players from the infrastructure of two Turkish Super League clubs, two from Istanbul and two from Ankara, comprised the study group. The training frequency was usually five days a week, one game day/week (Saturday), one day off/week (Sunday), and an average of 90 minutes a day, including warm-up, cooling, endurance, strength, speed, technical, tactical training, and game form. The control group included male adolescent volunteers who did not regularly participate in any sports with no chronic disease, did not take any medication and volunteered to participate in the study. The initial study group comprised 550 male soccer players and 393 controls. Those with missing data, including a left wrist radiograph, were excluded. Finally, 206 soccer players and 208 healthy controls, between the ages of 11-16, were included in the study. All the analyses were performed between January 2017 and September 2018. Growth rate, biological maturation (somatic assessment), and sexual and skeletal maturation were assessed in both groups. Additionally, for the soccer players, the game positions, initial age for playing soccer, number of games per week, number of sports injuries, date of injury,

duration for return to activity, site of injury, injury type, and mechanism of injury were recorded, and injury rate was calculated.

Growth rate: The participants and their parents were asked for their height assessment from last year to establish one-year linear growth and established growth rate (cm/year). Current height, was measured using a stadiometer, all participants were weighed using TANITA 418 (Tanita, Japan) with an error of 0.1kg and BMI was calculated (weight (kg)/height (m^2).

Body Compositions: The skinfold thickness measurements of five areas (triceps, subscapular, abdominal, thigh, and calf) were performed using the Holtain caliper following the standard procedure. Body composition in terms of total body fat, water, and fat-free mass (FFM) was measured using a TANITA 418.

Standard deviation scores (SDS) of all measurements according to Turkish standards were calculated.[16]

Skeletal maturation: A left-hand wrist radiograph was obtained. Skeletal age assessment was performed independently by the same physician using the Greulich-Pyle method.

Sexual maturation: Sexual maturation status was evaluated by showing the participants the Tanner Pubertal Stages Form and using it as a self-assessment tool by the same physician. This method has been shown to be reliable for determining pubertal staging when the examination is not possible.[17]

All data were collected between 06:00-08:00 am prospectively after 12 hours of overnight fasting. The participants were evaluated as barefoot and wearing only shorts. The Endocrine Calculator (ENDO-C) and CHILD Metrics computer programs were used to establish the parameters.

Injury: The Elite Clubs Injury Form[18] was used to assess sports injuries. All injuries, as well as the time spent in games and/or regular activities and the need for medical care were assessed.

Details concerning the injuries were asked of the parents, participants, and if possible, their physicians. The details of injuries such as mechanism (dribbling, collision, or other mechanisms) and injury type (fractures, strains, and sprains.. .etc) were asked. The injury incidence was calculated as injuries per 1000 hours of exposure (training and games).

Statistical analysis

IBM SPSS for Windows Version 22.0 package program was used. The results were presented as mean +/- SD for normally distributed variables; and as median (IQR) or median (Q1-Q3) for non-normally distributed variables throughout the main document and tables. The methods applied were frequencies, cross-tabulations, descriptive statistics, and means were calculated. When parametric test assumptions were provided, the Mann-Whitney U test was used when the Student's t-test was not provided, and the comparison of numerical variables between more than two groups was investigated using one-way ANOVA or Kruskal-Wallis test. Pearson correlation coefficients were calculated for correlation analyses. Logistic regression analysis was performed for injury risk analysis. Differences between groups were examined using the chi-square test. The level of significance was set at 5%.

Results

Participants were aged 13.6±1.5 years. The skeletal age was 13.7±1.6 years in the study group and 14.3±1.6 years in the control group. Age, anthropometry, and body composition characteristics are shown in Table I.

There was no difference in the growth rates (cm/year) between the groups at the ages of 11.0, 12.0, and 15.0 ($p>0.05$) but at the age of 13.0 years [(7.7±3.3 cm/year in the study group vs 3.9±2.4cm/year in controls and $p=0.033$, respectively)] and 14.0 years [(7.8±3.3 cm/year in the study group vs. 5.5±2.9 cm/year in controls and $p=0.028$)] growth rates were higher in the study group. At the age of 16 years, it was

Table I. Demographic features of participants.

	Study Group		Control Group		Total		
	N	Median (min-max)/ Mean±SD	N	Median (min-max)/ Mean±SD	N	Median (min-max)/ Mean±SD	p
Age	206	13.4±1.5	208	13.8±1.5	414	13.6±1.5	0.019[t]
Skeletal age	206	13.7±1.6	208	14.3±1.6	414	14.0±1.8	<0.001[t]
Height (cm)	204	168.5 (130.0-190.0)	205	165.0 (132.0-185.0)	409	166.0 (130.0-190.0)	<0.001[m]
Height (p)	204	64.9±25.7	205	41.3±28.3	409	53.1±29.5	<0.001[t]
Weight (kg)	206	53.0±12.3	207	56.3±12.8	413	54.6±12.7	0.008[t]
Weight (p)	206	38.2 (1.0-95.3)	207	39.3 (1.0-99.5)	413	38.9 (1.0-99.5)	0.748[m]
BMI (kg/m^2)	204	18.7 (13.4-25.0)	204	20.6 (13.8-33.6)	408	19.6 (13.4-33.6)	<0.001[m]
BMI (p)	204	29.1 (1.0-82.6)	204	44.5 (3.0-99.5)	408	34.0 (1.010.0-99.5)	<0.001[m]
Growth rate (cm/y)	117	5.0 (0.0-19.0)	112	5.0 (0.0-18.0)	229	5.0 (0.0-19.0)	0.424[m]
Growth rate SDS	116	(-0.1)±1.7	112	0.0±1.4	228	0.0±1.6	0.266[t]
Total body fat%	206	12.9 (5.9-21.9)	208	17.2 (9.0-38.9)	414	14.9 (5.9-38.9)	<0.001[m]
Total body water%	193	43.5 (17.1-66.8)	208	33.4 (10.3-53.4)	401	36.7 (10.3-66.8)	<0.001[m]
FFM%	193	59.4 (23.3-91.1)	208	45.6 (10.9-74.4)	401	50.1 (10.9-91.1)	<0.001[m]
Abdominal fat%	206	8.7 (3.0-18.4)	208	13.4 (4.4-33.7)	414	10.9 (3.0-33.7)	<0.001[m]
Triceps (mm)	206	14.1 (5.0-28.0)	207	12.3 (5.0-26.8)	413	13.0 (5.0-28.0)	0.001[m]
Subscapular (mm)	206	12.3±4.5	208	11.3±4.5	414	11.8±4.5	0.024[t]
Abdominal (mm)	206	12.0 (4.04.0-27.0)	208	13.8 (5.0-37.0)	414	13.0 (4.0-37.0)	0.001[m]
Thigh (mm)	206	17.2 (8.4-34.0)	208	13.0 (7.0-28.0)	414	15.0 (7.0-34.0)	<0.001[m]
Calf (mm)	206	14.8 (6.0-33.3)	208	11.4 (5.2-24.6)	414	13.0 (5.2-33.0)	<0.001[m]

BMI: body mass index, FFM: fat-free mass, m: Mann Whitney U test, Mean±SD: mean±standart deviation, p: percentile, SDS: standart deviation score, t=Student t test, [(Measurements that are not noted, are considered as the number of missing data (height (cm) 204, height (p) 204, BMI (kg/m^2) 204, BMI (p) 204, Growth rate (cm/y) 117, Growth rate SDS 116, Total body water% 193, FFM% 193)].

[(2.9±1.6 cm/year in the study group vs. 6.4±3.0 cm/year in controls and (p=0.004)] higher in the controls.

The triceps and subscapular skinfold thicknesses were lower at the ages of 11.0, 12.0, and 13.0 years in the study group when compared to the controls, but at the ages of 14.0, 15.0, 16.0 years they were thicker than the controls. The parameters were compared according to chronological age and are presented in Table II.

The growth rate was evaluated according to the Tanner stage, and although there was no difference, it was found to be higher in the study group for Tanner stage 1-4 (p>0.05), but it was higher in the control group at stage_5 (p=0.012). A comparison of all the parameters according to the Tanner stages is presented in Table III.

In the study group, 8.7% were goalkeepers, 35.9% were defenders, 37.4% were strikers, and 37.4% were midfield players. The initial age for playing soccer was 8.3±1.9 years and the number of games played in the previous season was 23.6±9.4. The amount of time spent with training was 4.7±0.4 days/week and 82.5±13.4 min/day. There were no differences between playing positions and total body fat (p=0.098), FFM (p=0.580), or total body water (p=0.578). Training time (hours/day) had a negative correlation with total body fat (rho= -0.443, p<0.001), total abdominal fat (rho= -0.528, p<0.001), and abdominal skinfold thickness (rho= -0.434, p<0.001), and positively correlated with total body water (rho= 0.749, p<0.001) and FFM (rho= 0.750, p<0.001). A positive correlation was found between the growth rate and hours of training (rho= 0.379, p<0.001), and no correlation was observed with the training time (day) (rho= 0.178, p=0.055).

Table II. The comparison of growth and body parameters according to chronological age.

Age	Group		Skeletal Age (year)	Height (cm)	Weight (kg)	BMI (kg/m²)	Growth rate (cm/year)	FFM%	Total Body water%	Total body fat%	Abdominal fat%	Triceps caliper (mm)	Subscapular caliper (mm)	Abdominal caliper (mm)
11	Study	N	25	25	25	25	21	25	25	25	25	25	25	25
		Median(min-max)/	12.0	151.5	38.0	17.2±1.6	4.0	36.6	26.8	14.4±2.7	9.5±2.9	12.9	9.9±3.1	14.3
		Mean±SD	(11.0-12.0)	(130.0-175.0)	(27.0-50.0)		(0.0-19.0)	(23.3-89.3)	(17.1-65.3)			(6.0-24.4)		(6.0-25.8)
	Control	N	22	22	22	22	8	22	22	22	22	22	22	22
		Median(min-max)/	12.0	145.5	43.0	20.6±4.3	5.0	33.0	24.1	23.5±7.6	19.1±7.7	16.0	13.4±5.7	17.9
		Mean±SD	(10.0-13.0)	(132.0-161.0)	(29.0-71.0)		(3.0-8.0)	(24.9-44.1)	(18.2-32.3)			(7.3-23.0)		(7.1-35.6)
		p	0.865m	0.064m	0.162m	**0.005**	0.79m	0.065m	0.062m	**<0.001**	**<0.001**	0.381m	**0.035**	0.094m
12	Study	N	36	36	36	36	18	36	36	36	36	36	36	36
		Median(min-max)/	12.2±0.8	156.0	41.5±6.2	16.8±1.7	5.0 (0.0-10.0)	68.9±24.8	50.4±18.1	13.0±3.0	8.2±3.3	11.5±4.0	9.0±2.7	12.9±5.3
		Mean±SD		(146.0-175.0)										
	Control	N	25	25	25	25	7	25	25	25	25	25	25	25
		Median(min-max)/	12.7±0.6	153.0	48.4±9.6	20.3±3.7	6.0 (2.0-8.0)	37.7±6.0	27.6±4.4	21.2±5.9	16.8±6.5	15.2±4.8	11.3±4.5	16.6±6.0
		Mean±SD		(146.0-172.0)										
		p	**0.023**	0.143m	**0.004**	**<0.001**	0.363m	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**0.035**	**0.015**
13	Study	N	41	39	41	39	22	41	41	41	41	41	41	41
		Median(min-max)/	13.0	163.0	48.0	18.2±1.9	7.7±3.3	63.3±23.2	46.3±17.0	12.6±2.1	8.2±2.4	12.0	10.0	12.4±4.0
		Mean±SD	(12.0-15.0)	(150.0-180.0)	(34.0-68.0)							(7.0-26.0)	(5.0-24.0)	
	Control	N	24	23	24	23	10	24	24	24	24	24	24	24
		Median(min-max)/	13.0	162.0	51.5	20.2±3.6	3.9±2.4	42.0±7.7	30.8±5.6	18.1±5.3	14.1±4.9	12.8	10.3	15.1±6.2
		Mean±SD	(12.0-14.0)	(147.0-174.0)	(33.0-82.0)							(8.5-26.8)	(5.6-25.2)	
		p	0.083m	0.06m	0.175m	0.033	**0.003**	**0.001**	**0.001**	**<0.001**	**<0.001**	0.743m	0.971m	**0.038**
14	Study	N	47	47	47	47	26	44	44	47	47	47	47	47
		Median(min-max)/	14.0±0.8	173.6±6.2	58.9±7.7	19.3	7.8±3.3	68.8±18.9	50.4±13.8	12.6±2.0	8.4±2.4	17.9±5.3	14.4±4.2	11.0 (4.0-22.5)
		Mean±SD				(16.4-23.8)								
	Control	N	63	61	63	61	37	63	63	63	63	62	63	63
		Median(min-max)/	14.4±0.8	166.0±7.0	58.6±13.3	20.5	5.5±2.9	47.5±8.5	34.5±5.7	18.6±6.1	14.9±6.6	12.9±4.3	11.3±4.8	13.0
		Mean±SD				(14.4-33.6)								(5.0-37.0)
		p	**0.009**	**<0.001**	**<0.001**	0.396m	**0.028**	**0.005**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	0.062m
15	Study	N	36	36	36	36	18	29	29	36	36	36	36	36
		Median(min-max)/	14.9±0.8	175.6±6.8	62.0	20.5	8.5	68.6±19.4	50.2±14.2	13.8±2.9	10.3±3.2	14.0±3.8	12.4±3.1	12.0
		Mean±SD			(30.0-80.0)	(15.3-25.0)	(2.0-13.0)							(6.0-21.6)
	Control	N	46	46	45	45	35	46	46	46	46	46	46	46
		Median(min-max)/	15.3±0.9	168.7±5.9	59.0	20.4	6.0 (0.0-18.0)	50.6±6.8	37.1±5.0	17.4±4.5	13.9±4.8	11.8±3.9	10.5±4.1	13.0
		Mean±SD			(46.0-80.0)	(17.1-29.4)								(5.0-36.1)
		p	**0.016**	**<0.001**	0.449m	0.177m	0.698m	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.017**	**0.024**	0.623m
16	Study	N	21	21	21	21	12	18	18	21	21	21	21	21
		Median(min-max)/	17.0	176.8±6.9	70.0	21.4	2.9±1.6	57.3±6.3	42.1±4.2	15.7	12.5	21.0±3.6	18.6±3.2	13.0
		Mean±SD	(15.0-18.0)		(51.0-81.0)	(18.2-24.9)				(12.2-21.4)	(8.4-18.1)			(8.0-19.0)
	Control	N	28	28	28	28	15	28	28	28	28	28	28	28
		Median(min-max)/	17.0	169.5±7.4	60.0	21.4	6.4±3.0	51.2±10.6	37.6±7.4	15.6	12.6	11.8±3.7	11.3±3.1	14.4
		Mean±SD	(14.0-17.0)		(42.0-87.0)	(17.0-30.5)				(10.7-22.7)	(7.5-19.4)			(7.8-30.1)
		p	0.223m	**0.001**	0.121m	0.832m	**0.004**	**0.035**	**0.024**	0.686m	0.976m	**<0.001**	**<0.001**	0.107m

BMI: body mass index, FFM: fat-free mass, m: Mann Whitney U test, Mean±SD: mean±standart deviation, p: percentile, SDS: standart deviation score, t: Student t test.

Table III. The comparison of growth and body parameters according to chronological age.

Tanner Stages	Group		Height (cm)	Weight (kg)	BMI (kg/m^2)	Growth rate(cm/year)	FFM%	Total body water %	Total body fat %	Abdominal fat %	Triceps calliper (mm)	Subscapular calliper (mm)	Abdominal calliper (mm)
1	Study	N	9	9	9	7	9	9	9	9	9	9	9
		Median(min-max)/ Mean±SD	148.5±5.9	39.0±6.1	17.6±2.1	5.2±2.2	32.5±4.2	23.8±3.0	14.6 (5.9-19.9)	9.8 (6.3-15.0)	16.9±3.6	11.5±3.2	18.4±5.1
	Control	N	16	16	16	7	16	16	16	16	16	16	16
		Median(min-max)/ Mean±SD	144.0±6.3	41.6±9.2	19.9±3.6	4.4±1.9	31.9±5.1	23.3±3.7	22.7 (12.5-30.5)	17.2 (6.7-28.1)	14.5±3.9	12.4±5.1	17.1±6.7
		p	0.091*	0.454*	0.101*	0.469*	0.751*	0.748*	0.015 KW	**0.008** KW	0.149*	0.621*	0.613*
2	Study	N	47	49	47	28	49	49	49	49	49	49	49
		Median(min-max)/ Mean±SD	156.2±8.5	40.0 (27.0-68.0)	17.4 (13.8-21.5)	4.9±4.5	45.4 (23.3-90.3)	33.3 (17.1-66.1)	12.9 (9.4-21.9)	8.5 (3.5-18.4)	11.5 (6.0-24.4)	9.0 (5.0-18.1)	13.0 (6.0-27.0)
	Control	N	27	29	27	12	29	29	29	29	29	29	29
		Median(min-max)/ Mean±SD	154.8±6.6	49.5 (32.0-71.0)	20.5 (14.1-30.7)	5.0±2.2	37.1 (26.5-48.7)	27.2 (19.4-35.7)	19.2 (12.1-38.9)	14.6 (7.5-33.7)	15.0 (7.6-25.1)	11.3 (3.2-23.2)	17.0 (6.8-35.6)
		p	0.462*	**0.004** KW	**<0.001** KW	0.912*	**<0.001** KW	**<0.001** KW	**<0.001** KW	**<0.001** KW	**0.002** KW	**0.001** KW	**0.021** KW
3	Study	N	53	53	53	32	51	51	53	53	53	53	53
		Median(min-max)/ Mean±SD	165.3±9.9	53.0 (30.0-76.0)	18.0±2.3	7.3±3.1	58.9 (26.0-91.1)	43.2 (19.0-66.8)	12.5 (8.9-18.5)	8.1 (3.0-15.1)	14.3±5.6	11.9±4.8	12.0±3.9
	Control	N	58	58	58	32	58	58	58	58	57	58	58
		Median(min-max)/ Mean±SD	163.5±9.5	53.0 (33.0-96.0)	20.2±3.9	5.7±3.4	44.4 (27.8-74.4)	32.5 (20.4-48.8)	15.9 (10.2-34.3)	12.1 (6.4-32.8)	12.2±3.7	10.3±4.3	13.6±6.6
		p	0.317*	**0.049** KW	0.101*	0.052*	**<0.001** KW	**<0.001** KW	**<0.001** KW	**<0.001** KW	0.094*	0.071*	0.408*
4	Study	N	75	75	75	43	67	67	75	75	75	75	75
		Median(min-max)/ Mean±SD	174.0 (153.0-190.0)	59.7±7.9	19.7 (15.6-24.9)	7.3±3.2	85.5 (39.1-90.7)	62.6 (28.6-66.4)	12.8 (9.0-21.4)	8.4 (3.4-18.1)	15.6 (7.0-28.0)	12.7 (7.0-26.0)	11.0 (4.0-22.5)
	Control	N	75	75	74	50	76	76	76	76	76	76	76
		Median(min-max)/ Mean±SD	168.0 (155.0-185.0)	61.6±10.9	21.2 (16.6-32.0)	6.7±3.0	50.3 (10.9-72.9)	36.8 (10.3-53.4)	16.7 (9.0-32.7)	13.4 (4.4-28.4)	11.6 (5.0-25.0)	10.0 (5.0-26.3)	13.2 (5.0-36.1)
		p	**<0.001** KW	0.374*	**<0.001** KW	0.385*	**<0.001** KW	**<0.001** KW	**<0.001** KW	**<0.001** KW	**<0.001** KW	**<0.001** KW	**0.021** KW
5	Study	N	20	20	20	7	17	17	20	20	20	20	20
		Median(min-max)/ Mean±SD	178.0 (164.0-190.0)	70.0 (51.0-81.0)	21.9±1.4	2.0 (1.0-3.0)	59.1 (41.8-89.6)	43.3 (33.6-65.6)	15.7±2.7	12.0±3.2	21.0 (10.8-27.0)	17.5 (11.0-22.0)	12.9±3.0
	Control	N	10	10	10	6	10	10	10	10	10	10	10
		Median(min-max)/ Mean±SD	169.5 (155.0-175.0)	59.0 (53.0-80.0)	21.9±3.3	3.5 (2.0-4.0)	49.2 (44.8-61.8)	36.1 (32.8-45.2)	16.7±3.8	13.7±3.7	10.5 (5.1-22.3)	9.6 (7.0-20.0)	14.5±4.8
		p	**<0.001** KW	**0.038** KW	0.553*	**0.012** KW	**0.011** KW	**0.008** KW	0.418*	0.203*	**0.001** KW	**0.001** KW	0.266*

BMI: Body mass index, FFM: fat-free mass, KW=Kruskall Wallis, Mean±SD: mean±standart deviation, p: percentile, SDS: standart deviation score, *=One way ANOVA.

Injury

The injury rate in soccer players was 39.3% per year, and playing soccer increased the risk of multiple injuries [(OR=0.084, CI 0.018-0.384, p=0.002)]. There was a difference between Tanner stages comparing rates of injury (p<0.05), as the injuries were most common in Tanner stage 4 (16.0%) [(1.5% stage 1, 6.3% stage 2, 9.2% stage 3, and 6.3% stage 5)]. Additionally, two or more injuries were higher in Tanner stage 4 than in the other stages (p=0.031). Injuries occurred more commonly in strikers (86.5%), followed by goalkeepers (33.3%), defenders (32.4%), and midfielders (24.7%) (p<0.001). The most common injuries occurred during the game (53.8%), particularly during the first part (45 min) of the game. The relationship between injury and the study variables is shown in Table IV.

Lower extremity injuries (60.9%) were more common, with toe injuries being the leading cause (28.1%) followed by ankle injuries (14.1%). The most common location of upper extremity injury was the wrist (12.5%). The mean age was reported as 13.6±1.6 for knee injuries, 13.5±1.3 for ankle injuries, and 14.7±1.1 for wrist injuries, and a difference was found between the injury region and chronological age (p=0.001).

The most common injury mechanisms in soccer players were dribbling and collisions with another player (38.4%, p=0.001). The most common injury types were fractures (26.8%), strains (24.4%), and sprains (17.1% (p=0.008). Although there was no significant relationship between injury type and Tanner stage (p=0.160), fractures (37.0%) and sprains (37.0%) were most common in stage 4 and strain in stage 3 (31.8%) (p = 0.160). A difference was found between injury type and skeletal age (fracture 14.1±1.7, sprain 14.4±1.7, strain 13.7±1.7, and p=0.036). Additionally, the injury type differed according to the playing position. Fractures were more common in defenders (40.9%) and strains/sprains in strikers (55.0%) (p=0.027).

Weekly training time (hours/week) was lower in the injury group [(6.1±1.1 hours/week in injury positive group vs 6.7±0.8 hours/week in injury negative group), (p<0.001)] but no difference was detected in the number of training days [(4.7±0.4 day/week in injury positive group vs 4.8±0.3 day/week in injury negative group), (p=0.068)]. The duration of the return to training and/or a game was 26.5±24.2 days. The injury rate was 5.8/1000 h, and a positive correlation

Table IV. Injury and variables in the study group.

Variables	Injury History				p
	Positive		Negative		
	N	Median (min-max)/Mean±SD	N	Median (min-max)/Mean±SD	
Chronological age	81	13.9±1.5	125	13.1±1.4	<0.001
Skeletal age	81	14.0 (11.0-18.0)	125	13.0 (11.0-17.0)	<0.001*
Weight (kg)	81	56.7±12.4	125	50.7±11.8	0.001
Height (cm)	80	168.8±10.6	124	165.4±12.0	0.038
BMI (kg/m^2)	80	19.7±2.4	124	18.3±2.3	<0.001
BMI (p)	80	34.6 (3.2-82.6)	124	24.7 (1.0-82.1)	0.003*
Growth rate (cm/year)	46	6.6±3.8	71	6.1±3.7	0.518
Growth rate SDS	45	0.3±1.3	71	0.5±1.9	0.021
Total body fat %	81	13.9 (9.0-21.9)	125	12.8 (5.9-20.0)	0.104*
FFM%	75	58.1 (27.8-91.0)	118	80.5 (23.3-91.1)	0.972*
Total body water %	75	42.5 (20.4-66.5)	118	58.8 (17.1-66.8)	0.974*
Total abdominal fat %	81	9.7 (3.4-18.4)	125	8.3 (3.0-16.1)	0.043*

BMI: body mass index, FFM: fat-free mass, Mean±SD: mean±standart deviation, p: percentile, SDS: standart deviation score, *Mann Whitney U test.

was found with chronological age (rho= 0.477, p<0.001), skeletal age (rho= 0.457, p<0.001), height (rho= 0.333, p=0.003), weight (rho= 440, p<0.001), and BMI (rho= 0.474, p<0.001), and a negative correlation was observed with training hours/week (rho= -0.486, p<0.001). The injury rate of the training time (days) for five days was 5.4/1000 h, and for the four days was 6.8/1000 h (p=0.041). Regression analysis showed that the growth rate of SDS and total body water had an impact on injury risk (Table V).

Discussion

The purpose of the study was to compare the growth and biological maturation of young soccer players to that of their non-athletic, healthy counterparts, as well as to assess injury characteristics, in particular their relationship to biological maturity. Taller height, lower weight and lower BMI observed in the study group clearly demonstrate the benefits of regular training on the body. Cacciari et al.[19] stated that the soccer players were taller than the controls in the pubertal period (14-16 chronological age), however, they found no significant growth differences in soccer players compared to the control group in the prepubertal period. We found that the anthropometrical findings were better in soccer players in all age groups, but the growth rate was not observed to be different at 11.0, 12.0, and 15.0 years old. Moreover, the growth rate was higher in the study group, especially at the predicted growth spurt ages of 13.0, 14.0 years. It was interesting to find that at the age of 16.0 years, the growth rate was higher in the controls than in the players. This result may suggest that players reached their target height on time or earlier than the controls. Moreover, the growth rate was higher in the study group in Tanner stages 1, 2, 3, and 4, but was higher in the control group only at stage 5. This result might be due to the small number of participants in Tanner Stage 5.

While total body fat and abdominal fat percentages were found to be lower in soccer players, total body water and FFM were higher; moreover, these findings were seen in all Tanner stages. Playing soccer also has beneficial effects on the body's metabolism, as suggested by the positive correlations between total body water, FFM, and the negative correlations between total body fat, total abdominal fat, and abdominal skinfold thickness with training times.

Another important finding was that growth rate had a positive correlation with training time (h/day), but not with the total days of training per week. This result suggests that the training duration is more important than the number of training sessions per week. This shows the importance of adjusting the training time to obtain the maximum benefit by following the growing age.

The peripheral fat thickness, such as triceps, subscapular, thigh, and calf was higher in the study group at the chronological ages of 14.0, 15.0, and 16.0 years old. However, the abdominal skinfold thickness, which is accepted as an indicator of central and visceral obesity, was lower in all ages in the study group. While

Table V. Regression analysis of injury in the study group.

Variables	B	Exp (B)	95% CI for Exp(B)	
			Lower-Upper	p
Age	.358	1.431	.971-2.233	0.114
Growth rate (SDS)	.304	1.356	1.019-1.803	**0.036**
BMI (kg/m^2)	.011	1.011	.691-1.477	0.957
Total body fat%	.268	1.308	1.000-1.711	0.050
Total body water %	-.035	.966	.934-.999	**0.041**
Abdominal skinfold thickness (mm)	-.115	.891	.771-1.031	0.120

B: estimated coefficient, BMI: body mass index, CI: confidence interval, Exp(B): odds ratio, SDS: standart deviation score.

total body fat and FFM continue to increase during the growth spurt, it is known that fat accumulation in the extremities temporarily decreases and fat deposits centrally.[20] Cacciari et al.[19] found that triceps and subscapular skinfold thickness were thinner than controls only in the 12-13.9 years old (chronological age and skeletal age) group, as in the current study. On the other hand, sport-specific training programs increase in intensity and change as age progresses. Akin et al.[21] found that central fatness was higher in wrestling, weightlifting, handball, and taekwondo players, and peripheral fatness was higher in soccer players. These results may suggest that exercises for the upper extremity were neglected in the study group, reflecting the nature of soccer. We believe that to reduce the risk of injury in young athletes, whole-body training programs should be encouraged.

It has previously been shown that playing soccer increases the risk of injury.[22] Soccer is a sport involving movement, speed, frequent changes of direction, and direct contact, with the risk of injury among players.[23] When examining the frequency of sports injuries, a description of the injury is also important. In the literature, sports injuries are classified according to several criteria, such as; emergency admission, medical care needs or reduction of sports activity, and having a time loss of more than 21 days or having a time loss of at least 48 hours in sports activities.[23] In this study, inquiries about the injuries in the last season included hospital or doctor admission and/or the necessity of stopping the activity at the time of injury, the requirement of rest or medical control, and if it occurred during training and/or a game, and/or if it occurred due to another reason. The injury rate in adolescent male soccer players was 5.8/1000 hours. Faude et al.[23] evaluated soccer injuries in children and adolescents and found the injury rate in adolescent soccer players aged between 13 and 19 years to be 2-7/1000 hours.

In the current study, the injury rate was highest in Tanner stage 4. Linder et al.[24] examined the relationship between Tanner stage and injury incidence in junior high school soccer players and found that it was higher in adolescents in Tanner stages 3, 4, and 5 who were more mature. In another study evaluating 122 players, the rate of injury was 21.3% (3.6% in the 10-12 age group, 25.0% in the 13-15 age group, and 28.0% in the 16-18 age group).[25]

More aggressive play and greater risk-taking was associated with maturity and lack of coordination and strength in the males who were mature according to Tanner stage but had a weak grip could also be a factor leading to injury.[26] The expected growth spurt in males coincides with Tanner stages 3 and 4 (13-14 years), and physical growth and puberty which are characterized by many hormonal, emotional, and neurological changes that impact injury.[27] Many studies have reported the high prevalence of sports injuries around the age of growth spurt among adolescent soccer players, especially 6 months before or after the growth spurt age.[28] It was shown that injury risk is higher among taller and heavier adolescent male soccer players. Several studies have reported an increased injury rate among heavier players or players with a high BMI. Higher weights produce greater forces that are supposed to be absorbed through soft tissues and joints.[29] And It was reported that taller players reported more injuries than shorter ones.[30] Our results suggest that maturity could be a risk factor for injury in soccer.

Training time in hours had a more significant relationship with injury rate than the number of training days per week. These results indicate that the planning of training time to conform to the growth rate is essential to reduce the injury rate. Otherwise, specialization in sports at an early age and an increase in training intensity, duration, and frequency may cause overuse injuries.[31] Considering all the findings, growth rate seems to be related to higher injury risk, whereas higher total body water reduces the risk in adolescent male soccer players. It can be deduced that the high range of growth rate increases the risk of injury by increasing the mechanical stress on the musculoskeletal system.[32] The growth rates of at least 0.6 cm/

month, monthly BMI-increase of >0.3 kg/m^2, and decrease in BMI value of at least 0.4 kg/m^2 were found to be potential injury risk factors.[30] Similarly, Rommers et al.[33] found a 15% increase in injury risk per cm of growth per year and identified a greater increase in leg length (cm/year) as an overuse injury risk factor in young soccer players.[30,33]

On the other hand, adequate hydration should be underlined concerning the lower risk of injury in adolescent male soccer players as shown in our study. Williams[34] reported that hydration increases energy, positively affects agility and movement, helps thermoregulation and mental clarity, increases physical performance, and reduces injury risk. However, optimal fluid intake has been studied more extensively in adults than in children and adolescents. This issue needs to be studied further in children and adolescents.

This research has certain limitations. The primary limitation was that the participants' heights from one year ago were self-reported by the participants of parents. However, studies have shown that adolescents' self-reported height and weight are accurate, so although not ideal, we believe this data to be of value.[35] In addition, due to the large number of groups participating at the same time and the participants' reluctance to endure pubertal evaluation due to embarrassment, the evaluation of sexual maturation was also based on self-reported charts rather than physical examination which was another important limitation. Due to the dearth of female professionals in the field, recruiting females from women's soccer teams in Turkey would have been exceedingly difficult. We believe that additional studies evaluating women should be conducted.

In conclusion, playing soccer during the adolescent period is related to better growth and maturity and the risk of injury, and the growth rate of SDS increases this risk. As the impact of injuries varies with maturation status and timing, soccer academies should regularly monitor the maturation status and timing of adolescent soccer players throughout each season with an emphasis on training hours rather than training days, and close monitoring of hydration and nutrition. As adolescents continue to develop physically, their motor and cognitive skills may not yet simultaneously develop, and they are more prone to injury.

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Ethical approval

The study was approved by the Institutional review board of Hacettepe University GO 17/235-04. Informed consent was obtained from all the participants and their parents or guardians.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: RD, SA, BÜ, NK, OD data collection: RD, GD analysis and interpretation of results: RD, GD, SA, BÜ, NK, OD draft manuscript preparation: RD, SA, GD, BÜ, NK, OD. All authors reviewed and approved the final version of the manuscript.

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Conflict of interest

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Original Article

Computed tomography with clinical scoring to differentiate phytobezoar from feces in childhood small bowel obstruction

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ABSTRACT

Background. Identification of phytobezoar in childhood small bowel obstruction (SBO) characterized by small-bowel feces sign (SBFS) is still challenging. The aim of our study was to assess the diagnostic performance of quantitative computed tomography (CT) analysis combined with the Acute General Emergency Surgical Severity-Small Bowel Obstruction (AGESS-SBO) scoring system in determining phytobezoar-related SBO.

Methods. Sixteen phytobezoar-related SBO were categorized as the phytobezoar group and the other 19 SBFS-positive SBO was categorized as the control group. Demographic data, clinical presentation, and laboratory and CT findings were collected and analyzed. Each patient's AGESS-SBO score was determined according to the individual medical record. Multivariate logistic regression analyses were used to identify significant variables associated with phytobezoar-related SBO. Diagnostic performance of key variables was assessed using receiver operating characteristic (ROC) curve analysis.

Results. Compared to the control group, the phytobezoar group showed a significantly shorter debris maximal length (3.0 ± 0.5 cm vs. 3.5 ± 0.7 cm, P<0.05), stronger attenuation (12.6 ± 5.9 HU vs. 8.2 ± 4.0 HU, P <0.05) in CT, and higher AGESS-SBO scores (4.5 [interquartile (IQR): 4–5]) vs. (2 [IQR: 1–4]). With the combination of debris attenuation (with a cut-off of >9.0 HU) and AGESS-SBO score (with a cut-off of >3 points), the positive predictive value (PPV) and negative predictive value (NPV) to diagnose phytobezoar-related SBO were 80% (12/15) and 84% (16/19), respectively.

Conclusions. The diagnostic method of integrating quantitative CT analysis and the AGESS-SBO scoring system can improve the identification accuracy of phytobezoar in SBFS-positive childhood SBO.

Key words: bezoars, feces, intestinal obstruction, differential diagnosis.

The presence of phytobezoar contributes to 6% of unusual etiologies of small bowel obstruction (SBO), and it could cause serious complications, such as bowel bleeding, perforation, and fistula formation.[1] Small-bowel feces sign (SBFS) obtained from morphological assessment of computed tomography (CT) in SBO can imply the existence of phytobezoar, but it is also the common CT manifestation of a series of SBO without phytobezoar which could be treated conservatively.[2,3] Quantitative analysis of CT works effectively in distinguishing phytobezoar from feces in adult SBO[3-6], but whether it is also practically efficient in children's SBO remains unclear.

Recently, the Acute General Emergency Surgical Severity-Small Bowel Obstruction (AGESS-SBO) scoring system was proven effective in categorizing SBO in adults.[7] In this study, by re-evaluating clinical and imaging information of 35 SBFS-positive childhood SBO, we further explored the clinical differences between

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phytobezoar and non-phytobezoar related SBFS-positive SBO using quantitative CT combined with the AGESS-SBO scoring system (qCT+ASSS). We tried to provide effective ways to improve diagnostic accuracy and facilitate appropriate clinical decisions in SBFS-positive childhood SBO especially when the presence of phytobezoar should be considered.

Material and Methods

Patient population

The study protocol was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Dali University (number: 20190098). Two hundred fourteen pediatric patients were treated for SBO between July 2009 and June 2016. Sixteen patients without prior abdominal operation were diagnosed with phytobezoar-related SBO (Fig. 1). For the control group, we included 19 SBO with CT findings of SBFS who had not undergone surgical intervention on the abdomen before. Among these 19 children, 17 incomplete obstructions were resolved after conservative treatment and were not hospitalized again in the following 2 months because of SBO; Vitelline duct anomalies were confirmed in the other 2 cases in the subsequent operation, one was omphalomesenteric cord and another was Meckel's diverticulum.

Clinical data

The following clinical data were recorded for each patient: symptoms, signs, laboratory tests, and intraoperative findings.

Radiological examination and analyses

All children underwent an unenhanced CT scan. Scanning was performed by a 16-slice multidetector CT system (Philips Healthcare Brilliance, Netherlands). CT parameters used were slice thickness, 3 mm; beam collimation, 0.5 mm; pitch, 1.5; tube voltage, 120 kV; and maximum tube current, 250 mA. CT images were analyzed on a picture archiving and communications system (PACS) (Digital Imaging and Communications in Medicine [DICOM] 3.0).

All CT images were reevaluated retrospectively in a blind fashion by an experienced radiologist. The following quantitative CT findings were analyzed: (1) obstruction degree; (2) presence of air-fluid levels in the distended bowel; (3) pneumoperitoneum, defined as the presence of free gas in the peritoneal cavity, intestinal clearance, or subphrenic space; (4) intraperitoneal fluid, defined as the presence of liquid in the hepatorenal recess, splenorenal recess, intestinal clearance, or pelvic cavity; (5) size of food debris sign (in cm), measured as the maximal length of the intraluminal mass

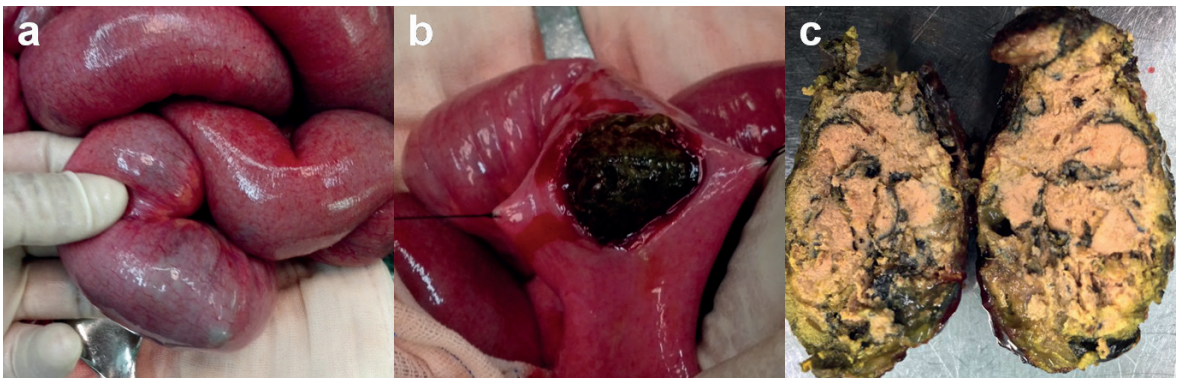

Fig. 1. Intraoperative findings of 6-year-old boy was diagnosed small bowel obstruction leading by phytobezoar. (a). A hard olive-like mass impacted in the lumen of ileum without bowel necrosis and perforation, except to bowel edema, distention, and congestion. (b).The phytobezoar was exposed in the enterotomy. (c). The longitudinal section of phytobezoar.

located at the transitional site of the proximal dilated loop and distal collapsed loop; and (6) debris attenuation (in Hounsfield units [HU]), which was averaged from four measurements for each mass.

AGESS-SBO scoring system

All patients were evaluated according to the AGESS-SBO scoring system.[7] We used on-admission parameters for anatomy and physiology in the current study. The anatomic criteria were scored as 0–5 points. Obstruction degree and perforation of bowel were evaluated by CT imaging.[8] Physiological changes were also scored between 0–5 based on the pathological severity, including systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock, and multiple organ dysfunction syndrome determined by international consensus in pediatric patients (Table I).[9] With individual scores from anatomic and physiological parameters, we assigned patients a total score as follows:

AGESS-SBO scores = anatomic scores^2 *+ physiological scores*^{2}

Statistical analyses

Categorical data were reported as frequencies and percentages and compared between groups by using the chi-squared test; continuous data was reported as means (± standard deviations) or medians (interquartile range [IQR]) and compared between groups by using the independent *t*-test or non-parametric test. Correlation between CT findings and AGESS-SBO scores was tested by spearman rank correlation. Binary logistic regression was used to assess possible associations of several CT findings and AGESS-SBO scores with phytobezoar-related SBO. Logistic regression was used to calculate odds ratios (ORs) of phytobezoar-related SBO, depending on the presence of certain CT findings and certain AGESS-SBO scores. Receiver operating characteristic (ROC) curves were used to determine the optimal cut-off values for CT parameters and the AGESS-SBO score for differentially diagnosing phytobezoar-related SBO. The diagnostic performance by combining these factors was assessed by calculating the area under the ROC curve (AUC).

Table I. AGESS-SBO scoring system.

Component	Scale score
Anatomic	
Normal	0
Incomplete SBO without the need of operation	1
Completed SBO without strangulation	2
Completed SBO with strangulation	3
Perforation with local peritonitis	4
Perforation with diffuse peritonitis	5
Physiological	
Normal physiology	0
SIRS	1
Sepsis	2
Severe sepsis	3
Septic shock	4
Multiple organ dysfunction syndrome	5

AGESS-SBO: Acute General Emergency Surgical Severity-Small Bowel Obstruction, SBO: small bowel obstruction, SIRS: systemic inflammatory response syndrome

Results

Clinical findings

The group with phytobezoar contained 11 boys and 5 girls, with a mean age of 7.2 ± 3.2 yrs. The control group contained 11 boys and 8 girls, with a mean age of 6.0 ± 3.1 yrs. More phytobezoar patients presented with vomiting (P = 0.001), abdominal distention (P = 0.003), and higher white blood cell counts (P = 0.003). Other symptoms and laboratory tests did not differ significantly between the two groups (Table II).

AGESS-SBO scores

The median of anatomic scores in the phytobezoar group was greater than in the control group (P=0.001). A greater median

Table II. Comparative analysis of clinical and laboratory parameters between phytobezoars and feces groups.

Clinical parameters	Phytobezoars group (n=16)	Feces group (n=19)	p value*
Age	7.2±3.2	6.0±3.1	0.254
Sex			0.508
Male	11 (69%)	11 (58%)	
Female	5 (31%)	8 (42%)	
Clinical symptoms			
Fever (> 37.7 °C)	3 (19%)	2 (11%)	0.489
Vomiting	13 (81%)	5 (26%)	0.001*
Abdominal pain	14 (88%)	12 (63%)	0.181
Constipation	10 (63%)	11 (58%)	0.782
Dehydration	7 (44%)	7 (37%)	0.678
Abdominal distention	13 (81%)	11 (58%)	0.003*
Muscle guarding	4 (25%)	3 (16%)	0.497
Abdominal masses	2 (13%)	1 (5%)	0.446
Laboratory tests			
White blood cells count ($\times 10^9$/l)	11.0±2.9	8.1±2.4	0.003*
Netrophil percentage (%)	62.6±24.2	52.8±18.8	0.189
Blood amylase (IU)	67.9±32.1	52.4±15.2	0.07
Urine amylase (IU)	564.1±229	492.2±134.8	0.257
[K+] (mmol/l)	4.4±0.5	4.2±0.5	0.318
[Na+] (mmol/l)	140.3±5.8	139.5±5.9	0.697

[K^+]: serum potassium concentration, [Na^+]: serum sodium concentration, IU: international unit
Categorical data are indicated as number of patients (percentage, %). Measurement data are indicated by mean± standard deviation. *p value <0.05 statistical significance

Table III. The distribution of patients' AGESS-SBO scores.

Components	Score	Phytobezoars group (n=16)	Feces group (n=19)
Anatomic			
Normal	0	0 (0)	0 (0)
Partial SBO	1	6 (38%)	14 (74%)
Complete SBO without rebound tenderness	2	8 (50%)	5 (26%)
Complete SBO with rebound tenderness	3	2 (12%)	0 (0)
Complete SBO with perforation or local muscle guarding	4	0 (0)	0 (0)
Diffuse muscle guarding	5	0 (0)	0 (0)
Physiological		5 (31%)	
Normal	0	11 (69%)	17 (89%)
SIRS	1	0 (0)	2 (11%)
Sepsis	2	0 (0)	0 (0)
Serve Sepsis	3	0 (0)	0 (0)
Septic shock	4	0 (0)	0 (0)
MODS	5	4.8±1.9	0 (0)
AGESS-SBO score	50	4.8±1.9	2.3±1.5

AGESS-SBO: Acute General Emergency Surgical Severity-Small Bowel Obstruction, MODS: multiple organ dysfunction syndrome, SBO: small bowel obstruction, SIRS: systemic inflammatory response syndrome.
Categorical data are indicated as number of patients (percentage, %). Measurement data are indicated by mean± standard deviation.
*p value <0.05 statistical significance

Table IV. Comparative analysis of CT variables between phytobezoars and feces groups.

CT variable	Phytobezoars group (n=16)	Feces group (n=19)	P Value*
Obstruction levels			0.031*
Incomplete	6 (38%)	14 (74%)	
Complete	10 (62%)	5 (26%)	
Air-fluid level	16 (100%)	18 (94%)	0.377
Pneumoperitoneum	0 (0)	0 (0)	
Intraperitoneal fluid	9 (56%)	7 (37%)	0.251
Food debris description			
Size			
Long axis (cm)	3.0±0.5	3.5±0.7	<0.001*
Short axis (cm)	2.5±0.5	2.6±0.6	0.775
Attenuation			
Mean value (HU)	12.6±5.9	8.2±4.0	0.014*
Minimal value (HU)	2.4	2.1	
Maximal value (HU)	28	15.9	

Categorical data are indicated as number of patients (percentage, %). Measurement data are indicated by mean± standard deviation. *p value <0.05 statistical significance. CT: computed tomography, HU: Hounsfield unit.

AGESS-SBO score was seen in the phytobezoar group (P<0.001). Half of the 16 children (50%) in the phytobezoar group scored more than 4 points based on the AGESS-SBO scoring system, whereas 18 of the 19 children (95%) from the control group scored fewer than 4 points (Table III).

CT findings

The primary CT finding in both groups was air-fluid levels in a dilated bowel loop, with children in the phytobezoar group showing a higher frequency of complete obstruction (Fig. 2). Intraperitoneal fluid did not differ significantly between the two groups (P=0.377). Food debris signs were detected in all children in the control group. The maximal length was significantly shorter in children with phytobezoars (P=0.01). Attenuation was significantly higher in children with phytobezoars (P =0.014, Table IV).

Correlation between CT findings and AGESS-SBO scores

Significant variables of CT findings, including obstruction levels, attenuation and maximal length were analyzed with AGESS-SBO scores in terms of correlations. There was a positive correlation between attenuation and AGESS-SBO scores (P=0.001). There were no correlation between the rest of the CT findings (obstruction levels and maximal length) and AGESS-SBO scores (P>0.05, Table V).

Diagnostic performance analysis AGESS-SBO scores

Binary logistic regression identified the following factors as evidence of a significant association with phytobezoar-related SBO: higher AGESS-SBO scores, more serious obstruction, shorter maximal length of debris, and stronger debris attenuation (P <0.05, Table VI). The following factors did not show a significant association with phytobezoar-related SBO: air-fluid level and intraperitoneal

Table V. Correlation between CT findings and AGESS-SBO scores.

CT findings	AGESS-SBO scores	
	Spearman' rank	P
obstruction levels	-0.215	0.115
Long axis (cm)	-0.203	0.242
Attenuation	0.522	0.001*

AGESS-SBO: Acute General Emergency Surgical Severity-Small Bowel Obstruction, CT: computed tomography.

Table VI. Results of multivariate analysis by means of logistic regression.

Effects	OR	95% CI	P Value*
AGESS-SBO score	2.847	1.377,5.888	0.005*
Obstruction level	0.214	0.051, 0.902	0.036*
Air-fluid level	0.360	0.033, 3.805	0.393
Intraperitoneal fluid	0.454	0.117, 1.764	0.254
Debris long axis (cm)	0.190	0.046, 0.781	0.021*
Debris short axis (cm)	0.831	0.244, 2.830	0.767
Debris attenuation (HU)	1.220	1.024, 1.453	0.026*

*p value <0.05 statistical significance. AGESS-SBO: Acute General Emergency Surgical Severity-Small Bowel Obstruction, CI: confidence interval, HU: Hounsfield unit, OR: odds ratio.

Table VII. The area under the curve of correlated effects of phytobezoars-related SBO.

Variable	AUC	95% CI	P Value
AGESS-SBO scores	0.850	0.720, 0.980	<0.001
Obstruction level	0.319	0.137, 0.501	0.049
Debris long axis (cm)	0.266	0.101, 0.432	0.019
Debris attenuation (HU)	0.755	0.590, 0.920	0.010
CT findings	0.819	0.677, 0.961	0.001
Debris attenuation+AGESS-SBO scores	0.896	0.787, 1.000	<0.001
CT findings+AGESS-SBO scores	0.918	0.826, 1.000	<0.001

AGESS-SBO: Acute General Emergency Surgical Severity-Small Bowel Obstruction, CI: confidence interval, CT: computed tomography, HU: Hounsfield unit, OR: odds ratio.

fluid (P >0.05). Based on AUC analysis, phytobezoar-related SBO was weakly predicted by obstruction level and maximal length of food debris (AUC <0.5) and strongly predicted by AGESS-SBO score and food debris attenuation (AUC >0.5). The AUC of certain CT findings (including obstruction level, maximal length of debris, and debris attenuation), the combination of debris attenuation and AGESS-SBO score, as well as the combination of CT findings and AGESS-SBO score were greater than 0.5 (Table VII).

Using a food debris attenuation cut-off of >9.0 HU, we calculated a sensitivity of 81% (13/16), specificity of 68% (13/19), positive predictive value (PPV) of 68% (13/19), and negative predictive value (NPV) of 65% (13/20). The PPV and NPV, using the combination of debris attenuation (with cut-off of >9.0 HU) and AGESS-SBO score (with cut-off of >3points), were 80% (12/15) and 84% (16/19), respectively.

Discussion

Operative intervention would be considered for SBO caused by phytobezoar, which is a trapped mass of undigested food leading to mechanical intestinal obstruction with higher risk of bowel strangulation.[1] On the contrary, a non-operative approach could be effective for treating a series of SBFS-positive SBO without phytobezoar which develops secondary to increased water absorption and delayed transit.[10] Inappropriate or delayed diagnosis of phytobezoar-related SBO may result in bowel bleeding, perforation, or fistula formation, which will threat patients' life, bring about longer hospital stays and increased cost. Therefore, a more effective diagnostic methodology needs to be established to differentiate phytobezoar effectively from feces in childhood SBO. In the present study, we developed a combined diagnostic approach based on qCT+ASSS, which can improve the diagnostic performance to identify phytobezoar-

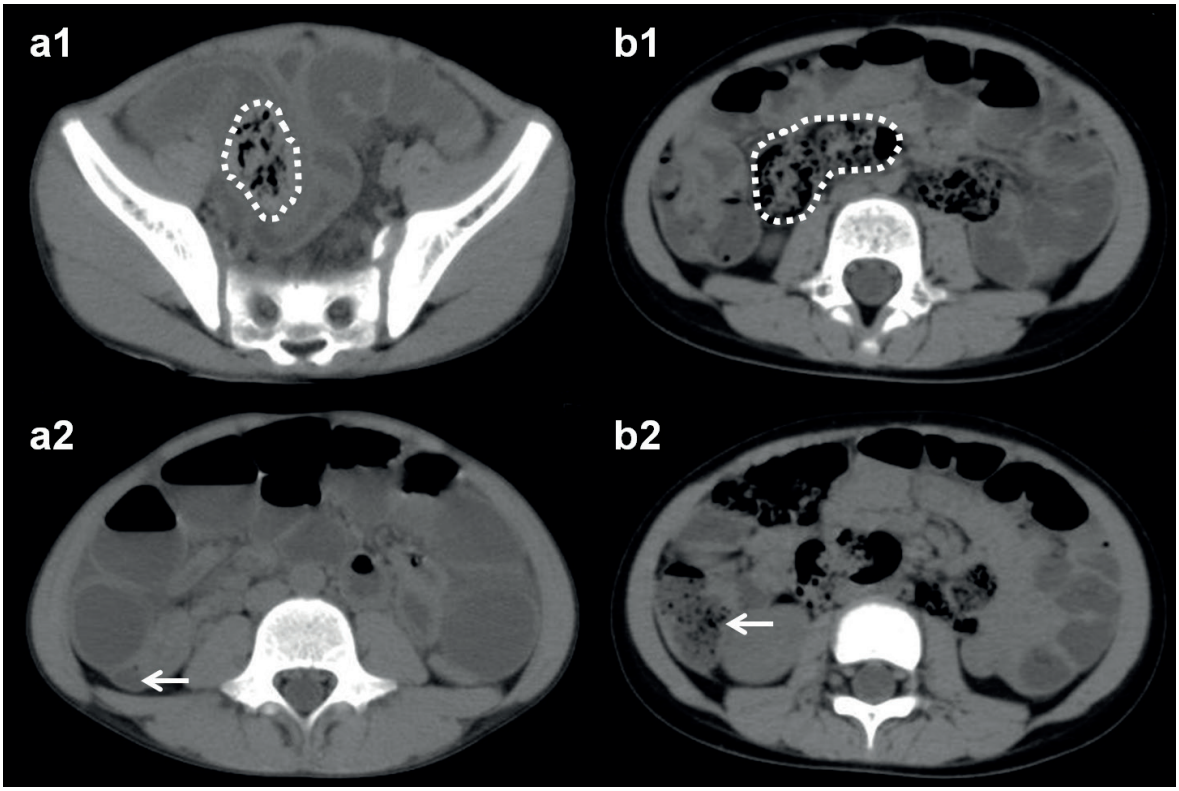

Fig. 2. The differences of unenhanced CT features between phytobezoar and feces in two children with small bowel obstruction. **a1, a2:** A 11-year-old boy was diagnosed with phytobezoar-related obstruction. The size of the intraluminal mass measured about 3.2 × 2.8 cm, and mean attenuation was 11.4 HU (a1, dotted outline). Only minimal liquid was seen in the ascending colon (a2, arrow). **b1, b2:** A 3-year-old girl was diagnosed with incomplete obstruction. The size of the food debris was about 4.8 × 2.6 cm, and mean attenuation was 2.1 HU (b1, dotted outline). A moderate amount of gas and stool was observed in the ascending colon (b2, arrow).

caused SBO, one kind of SBFS-positive SBO that needs to be removed by surgery.

The advent of CT allows surgeons to find visible details, assisting clinical decision making in the management of phytobezoar-related SBO.[3,4,6,11] Early image-based differentiation between phytobezoar and feces was based on morphological characteristics, such as SBFS[5], the presence of an encapsulating wall in the case of phytobezoar[3], or more tubular shape in the case of feces.[4] However, the diagnostic performance of morphological findings from CT was greatly affected by variability between observers. Study of quantitative CT, including measurement of food debris length and mean attenuation may improve diagnostic performance in differentiating phytobezoar from feces.[6] Our study showed that further analysis in children with SBFS-positive SBO is essential for differentiating phytobezoar from feces. Quantitative CT analysis turned out to be helpful in finding radiological differences of childhood phytobezoar and feces. Children with quantitative CT features such as shorter debris maximal length and stronger attenuation are more likely to have phytobezoar-related SBO. Stronger debris attenuation accompanied by presentation of complete bowel obstruction indicates irreversible obstruction occurring in phytobezoar-related SBO.

Although quantitative CT resolution has helped surgeons discriminate phytobezoar from feces in SBO, differential diagnosis of SBO in children is still challenging. On the one hand, single quantitative image-based analysis is not efficient. Our study shows that

only debris attenuation has predictive value and, meanwhile, with the cut-off value of the debris attenuation (>9 HU), the lower PPV (68%, 13/19) and NPV (65%, 13/20) indicate a potentially unrecognized phytobezoar and missed diagnosis. On the other hand, acute SBO is progressive, and variates determined by quantitative CT justly reflect anatomic changes of the SBO, which is one aspect of disease development. Clinical features, patients' pathophysiological responses to SBO caused by different etiological factors, are also critical for judgment. In fact, increased studies in adults have elucidated the preferable performance of a treatment algorithm for SBO based on patients' CT findings and clinical features.[8,12,13] However, these effective clinical guidelines validated in adult studies cannot be applied to childhood SBO because of different causes and various clinical features in two different patient populations. A diagnostic method integrating quantitative CT analysis and clinical features for children with SBO is therefore greatly needed. In the present study, frequent vomiting, progressive abdominal distension, as well as increased white blood cells were more commonly observed in the phytobezoar group. It shows that clinical features could be practical for identify phytobezoar-related SBO in SBFS-positive SBO. In addition, with the advantage of consecutive evaluation, clinical features can reflect the progression of childhood SBO in a timely way. Therefore, the establishment of a more effective diagnostic method of differentiating phytobezoar from feces in SBO should incorporate existing quantitative CT findings in patients' clinical features.

The AGESS-SBO scoring system, a systematic tool consisting of anatomic, physiological, and comorbidity parameters[7], is a relatively practical and comprehensive system that integrates CT findings and clinical features in the management of SBO. Based on quantitative CT findings, we developed a combined method that integrates quantitative CT analysis and the AGESS-SBO scoring system to differentiate phytobezoar from feces in childhood SBO. The criteria for evaluating anatomic parameters in the AGESS-SBO system relied on findings from enhanced CT performed with the use of contrast materials and higher radiation dose to recognize potential bowel ischemia. We noticed that few children with phytobezoar had undergone bowel strangulation in the early stage of hospitalization. Therefore, the prioritized purpose of CT in clinical decision making concerning phytobezoar-related SBO is to obtain imaging features of debris rather than to discover potential bowel ischemia. In fact, the signs obtained from physical examination are workable to identify potential bowel strangulation. Moreover, radiation damage to children, including the risk of radiation-induced cancer should be considered when enhanced CT is applied.[14,15] The comorbidity index, which assessed the relationship between perioperative complications and age-related diseases such as diabetes and hypertension[16], was limited by the sample size in this study. Appropriate modifications of the AGESS-SBO scoring system could be more suitable for management of childhood SBFS-positive SBO.

Assessment of results showed that the higher AGESS-SBO scores presented in the phytobezoar group were correlated with higher degree of obstruction and development of SIRS. In fact, phytobezoar-related SBO in children was more likely associated with dramatic anatomic and physiological changes.[17] However, SBFS-positive CT findings can appear in patients without SBO.[18,19] Increased PPV (80% (12/15)) and NPV (84% (16/19)) by the use of qCT+ASSS we developed in the present study could potentially decrease missed diagnosis or misdiagnosis. Differentiation based on high-performance diagnostic methods is efficient and can further guide appropriate clinical decision making in the management of phytobezoar-related SBO.

There are some limitations in our study. First, our diagnostic assessment is limited by its retrospective design and small sample size. In addition, patients selected in the control group had no abdominal operation history,

which were to be comparable with the history of affected children with phytobezoar-related SBO. It might not reflect the overall situation of SBFS-positive SBO. Ultimately, more SBO-related parameters could be considered in future studies to improve the differential diagnosis of pediatric SBO further.

The present study developed an effective diagnostic method, the combination of the AGESS-SBO scoring system and quantitative CT analysis, to identify phytobezoar-related SBO in childhood SBFS-positive SBO. This method is effective and practical to identify the unrecognized phytobezoar, make appropriate clinical decisions for phytobezoar-related SBO. Thus, we suggest this new method be applied in the clinical management of childhood phytobezoar-related SBO.

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Ethical approval

The study was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Dali University (number: 20190098). Research work was performed in accordance with the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: analysis, interpretation and draft manuscript preparation: NW, data collection: XL, WS, cases scoring: SZ, revised manuscript: XW. All authors reviewed and approved the final version of the manuscript.

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The authors declare that there is no conflict of interest.

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Case Report

A novel homozygous mutation in the *USP53* gene as the cause of benign recurrent intrahepatic cholestasis in children: a case report

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ABSTRACT

Background. Benign recurrent intrahepatic cholestasis (BRIC) is a rare cause of cholestasis with recurrent episodes of jaundice and pruritus without extrahepatic bile duct obstruction. A mutation in the *USP53* gene is known to cause BRIC-like cholestasis with normal serum gamma-glutamyltransferase (GGT) levels.

Case. We report a 16-year-old boy with recurrent episodes of cholestasis since 6 months of age with normal serum GGT levels. The liver biopsy showed ballooning degeneration of hepatocytes which is typical for BRIC, and intrahepatic and canalicular cholestasis with bilirubinostasis. We performed whole exome sequencing (WES) and identified a novel homozygous variant (NM_001371399.1:c.1558C>T) of the *USP53* gene at exon 14 as the cause of BRIC.

Conclusion. This is the first case of USP53 disease from Türkiye with a novel mutation in the *USP53* gene. This novel identification of the mutation of c.1558C>T at exon 14 can provide elucidative data for those who work in the field of intrahepatic cholestasis. Our case suggests that USP53 disease must be kept in mind in patients with recurrent intrahepatic cholestasis with normal serum GGT levels.

Keywords: cholestasis, children, *USP53* gene, benign recurrent intrahepatic cholestasis (BRIC).

Familial intrahepatic cholestasis (FIC) is a heterogeneous group of liver disorders in which the excretion and production of bile acids from hepatocytes are impaired.[1] Progressive familial intrahepatic cholestasis (PFIC) and benign recurrent intrahepatic cholestasis (BRIC) are considered to be part of a spectrum of intrahepatic cholestasis. The difference between the two diseases is based on phenotypic presentation.[2] BRIC is an autosomal recessive cholestatic disease characterized by recurrent episodes of cholestasis manifested by jaundice, pruritus, fatigue, and steatorrhea with variable severity and duration.[3] Episodes can be triggered by an infection, and can last from weeks to months.[4]

Genes associated with FIC include *ATP8B1* (FIC1), *ABCB11* (FIC2), *ABCB4* (FIC3), *TJP2* (FIC4), *NR1H4* (FIC5), *SLC51A* (FIC6), *KIF12* (FIC8), *MYO5B* (MYO5B-PFIC).[5,6] Among these, mutations of the *ATP8B1* (BRIC1), *ABCB11* (BRIC2), *TJP2* and *MYO5B* genes are previously defined to be related to BRIC.[6-8] USP53 (Ubiquitin specific peptidase 53), a member of the deubiquitinating enzyme family, is expressed in the liver, brain, kidney, and inner ear.[9] As it was previously reported in a mouse-deafness model, USP53 colocalizes and interacts with tight junction proteins, TJP1 and TJP2, suggesting that USP53 is a part of the tight junction complex and essential for the stability of tight junctions.[10] *TJP2* gene is known to be associated with a spectrum of cholestatic

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hepatobiliary disease.[11] It was preliminarily reported in three Saudi children that asserted the association with the mutation in *USP53* and low-GGT cholestasis in 2019.[12] In addition, in recent studies, the USP53 disease has been included in the PFIC group, and specified as PFIC type 7.[13] Here, we report a Turkish child manifesting as BRIC with a novel *USP53* mutation.

Case

A 16-year-old boy was referred to our department with jaundice and pruritus refractory to antihistaminic treatment. He also reported a loss of appetite, dyspepsia, and right upper quadrant pain. His stool was loose and fatty, and he had lost 3 kg in three weeks. He was born to second-degree consanguineous parents. There was no family history of liver disease.

According to his complaints, the jaundice occurred for the first time 6 months ago. After 6 months of follow-up, his symptoms were completely relieved. After symptom-free 9 years, at the age of 10, his parents reported a similar episode characterized by jaundice, pruritus, and loss of appetite, which recovered within a month.

On examination, there was no abdominal tenderness, hepatosplenomegaly, or ascites. Scleral and skin icterus, and scratch marks on his trunk and extremities were noted. Liver enzymes showed cholestatic hepatitis with markedly elevated total and direct bilirubin and mildly elevated aminotransferases; total bilirubin 11.3mg/dl, direct bilirubin 8.9 mg/dl, ALT 56 U/L, AST 46 U/L, gamma-glutamyl transferase (GGT) 15 U/L. Alkaline phosphatase level and international normalized ratio were normal. Total serum bile acids were 242 μmol/L (reference range; 0-10 μmol/L). Other biochemical parameters including amylase, lipase, serum electrolytes, and renal function tests were normal. Hepatitis B surface antigen and antibodies to hepatitis C and A virus were negative. Immunoglobulin G and subtypes including immunoglobulin G4 were normal. Liver-kidney microsomal antibodies, antinuclear antibodies, antimitochondrial antibodies, anti-smooth muscle antibodies were negative and ceruloplasmin, 24-hour urine copper excretion test, and alpha 1 antitrypsin were normal.

Abdominal ultrasonography revealed mild hepatosplenomegaly with grade 1 hepatosteatosis. Magnetic resonance cholangiopancreatography showed normal intra- and extra-hepatic biliary tree and pancreatic ductal system.

He was prescribed ursodeoxycholic acid, rifampicin, vitamin D, E, and A. Given the progression of the cholestasis (total bilirubin 23.4 mg/dl, direct bilirubin 17.41 mg/dl) and refractory pruritus, endoscopic retrograde cholangiopancreatography (ERCP) was performed; and a nasobiliary drainage catheter and a 5Fr stent were inserted into the pancreatic duct. Remarkable improvement of the cholestasis and pruritus was noted after 48 to 72 hours of nasobiliary drainage catheter insertion. Two days after the ERCP, acute pancreatitis developed with right upper quadrant pain, elevated serum lipase, and amylase levels and heterogeneity of the parenchyma, and increased volume of the pancreas in ultrasonography. During the follow-up he had three episodes of acute pancreatitis in total, one of which was after the ERCP, that resolved with supportive treatment, including fluid resuscitation in the first 48 hours and without any complications.

The liver biopsy showed ballooning degeneration of the hepatocytes, and intrahepatic and canalicular cholestasis with bilirubinostasis without any significant inflammation. There was no evidence of fatty change, portal tract fibrosis, or hepatitis (Fig. 1).

Mutation analysis of cholestasis-related genes, *ATP8B1, ABCB11, ABCB4, TJP2,*

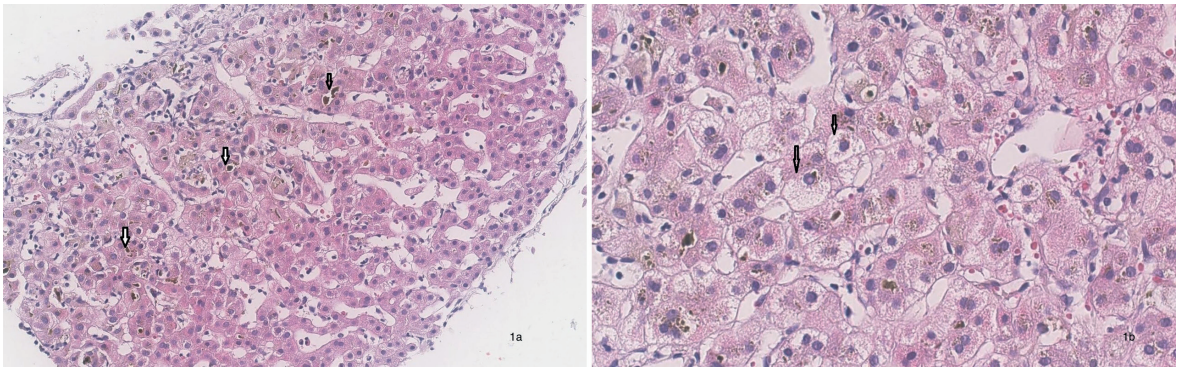

Fig. 1. Histological findings of the patient's liver. Intrahepatic and canalicular cholestasis with bilirubinostasis (1a, arrows), and ballooning degeneration of hepatocytes (1b, arrows). (H&E stain)

NR1H4, MYO5B were negative. Whole exome sequencing was performed at Ankara City Hospital, Medical Genetics Laboratory. IDT xGen Exome Research Panel v2 was performed using the Nextseq 550 next-generation sequencing platform (Illumina, San Diego, CA) according to the manufacturer's instructions for the whole-exome sequencing. FASTQ files were analyzed on QCIAU1.6 and the annotation of VCF files was completed by using Qiagen Ingenuity Variant Analysis and Clinical Insight Interpret. For the variant filtering process, we considered only nonsense and missense variants, indels, and variants at canonical splice sites, excluding variants with minor allele frequency greater than 0.01 in different public and local resources. We identified a novel homozygous variant (NM_001371399.1: c.1558C>T) of the *USP53* gene at exon 14. The variant causes premature termination of the protein at p.Arg520Ter. Autosomal recessive inheritance was confirmed by segregation analysis of mutated alleles within parents. We also searched for genes causing recurrent pancreatitis and identified a heterozygous mutation (NM_000492.4: c.3154T>G) on the *CFTR* gene during the WES analysis, which has been implicated in recurrent pancreatitis.

Currently, the patient is still on ursodeoxycholic acid and rifampicin therapy and for 3 months he did not have any cholestatic flares accompanied by jaundice and pruritus. Informed consent was received from the patient and the family.

Discussion

BRIC, which was first described in 1950, is part of a spectrum of familial intrahepatic cholestasis with recurrent episodes of cholestasis.[14] Luketic and Shiffman have since proposed the following diagnostic criteria for BRIC: 1) at least two episodes of jaundice separated by a symptom-free interval lasting several months to years, 2) laboratory data consistent with intrahepatic cholestasis, 3) a normal or minimally-elevated GGT level, 4) severe pruritus secondary to cholestasis, 5) centrilobular cholestasis evident on liver biopsy, 6) normal intra- and extrahepatic bile duct on cholangiography, and 7) an absence of factors known to be associated with cholestasis.[15] Our patient fulfilled these criteria and had been having cholestatic flares since the age of 6 months with a completely asymptomatic period in between.

Mutations in several genes have been reported to cause FIC. Among these *ATP8B1* (BRIC1), *ABCB11* (BRIC2), *TJP2* and *MYO5B* are previously defined mutations known to be related to BRIC.[2,4,6-8,11,15] By sequencing all coding exons of known cholestasis-related genes, *ATP8B1, ABCB11, ABCB4, TJP2, NR1H4, MYO5B*, we did not detect any mutation. Because of the typical presentation compatible with BRIC, further investigation was needed and a novel homozygous variant (NM_001371399.1: c.1558C>T) of the *USP53* gene at exon 14 was identified. The relation between a mutation

in the *USP53* gene and cholestasis was first reported in three Saudi children, two sisters and a cousin.[12] All three patients had cholestasis with normal GGT, very high ALP, and hypocalcemia. Unlike the reported cases, there was no confirmed hypocalcemia or elevation in ALP level in any of the cholestatic flares in our patient.

It is reported that USP53 protein gets involved in the tight junction-associated protein family, which maintains the stability of tight junctions and takes part in auditory hair cell and hearing. To date, deafness has been detected in 4 of 22 cases with a known *USP53* mutation.[9,16,17] As it has been reported that it may develop later in life in patients with normal hearing at the time of diagnosis, our patient is being followed up for possible hearing loss.[12]

Pathological findings of USP53 disease can vary on a patient basis; some of which solely have intrahepatic and canalicular cholestasis, and others have fibrosis with parenchymal nodularity. The liver biopsy of our patient showed ballooning degeneration of hepatocytes, which is typical for BRIC, and intrahepatic and canalicular cholestasis without inflammation, steatosis or fibrosis. Unfortunately, we did not have the opportunity to evaluate the liver tissue on transmission electron microscopy, which could provide more detailed information about typical changes in tight junctions. It is reported that in the tissues of the *USP53*-mutated patients, tight junctions elongate and extend deeper into the paracellular or lateral space, which resembles those in TJP2 disease.[17] Hepatocellular carcinoma may occur in TJP2 disease.[18,19] Thus it was speculated that USP53 related disease appears not to be entirely benign and patients must be monitored for malignancy.[17]

Among the cases described so far, only one patient had liver transplantation. She had a living-related liver transplantation at the age of 6 because of intractable itching that was not responsive to medical treatment.[9] Our patient was under traditional medical management with ursodeoxycholic acid and rifampicin. He retains his native liver with normalized bilirubin levels.

Acute recurrent pancreatitis has been previously reported in BRIC patients and this situation has been related to the expression of *ATP8B1*, the responsible gene in BRIC1, in the pancreas.[4] As a result of genetic analysis to investigate the etiology of recurrent pancreatitis in our patient, we determined a heterozygous mutation in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*). CFTR is an ion channel regulating the movement of chloride and bicarbonate across cell membranes. If the *CFTR* gene mutates severely it causes a complete loss of CFTR function which results in cystic fibrosis (CF). If the mutation in the *CFTR* gene is selective and only affects the bicarbonate-preferring channel, it does not cause typical CF but has some effects on the pancreas, nasal sinus, and vas deferens in variable degrees.[20] Severe, mild-variable, and compound heterozygous *CFTR* mutations are known to be associated with recurrent acute pancreatitis and chronic pancreatitis.[20,21] Besides these causalities between trans-heterozygous mutations in both *CFTR* and *SPINK1* and pancreatitis had been previously reported.[22] The association between pancreatitis and heterozygous mutation in the *CFTR* gene has not been reported yet. And no data has shown that the *USP53* mutation causes pancreatitis as well. Thus, in the patient, the relevance between recurrent pancreatitis, and USP53 disease, and heterozygous mutation in *CFTR* is unclear. In the last instance, the ERCP procedure performed on the patient seems like the most potential cause of the pancreatitis.

Here we report the first case of USP53 related disease from Türkiye. The novel identification of the mutation c.1558C>T at exon 14 can provide elucidative data for those who work in the field of intrahepatic cholestasis. USP53 disease should be considered in patients who present with normal GGT levels and recurrent episodes of cholestasis.

Ethical approval

This study was conducted in adherence to the Declaration of Helsinki and the participation involved informed consent. Informed consent was received from the patient and the family.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BBA, GH; data collection: FD; analysis and interpretation of results: BBA, ACC, HTD; draft manuscript preparation: BBA, SH. All authors reviewed the results and approved the final version of the manuscript.

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Case Report

The first case of combined oxidative phosphorylation deficiency-1 due to a *GFM1* mutation in the Serbian population: a case report and literature review

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ABSTRACT

Background. Combined oxidative phosphorylation deficiency-1 (COXPD1) resulting from a mutation in the G elongation factor mitochondrial 1 (*GFM1*) gene is an autosomal recessive multisystem disorder arising from a defect in the mitochondrial oxidative phosphorylation system. Death usually appears in the first weeks or years of lifespan.

Case. We report a male patient with ventriculomegaly diagnosed in the 8th month of pregnancy. The delivery was done by caesarean section and respiratory failure occurred immediately after birth. Hypoglycemia, lactic acidosis, elevated gamma-glutamyl transferase and hepatomegaly were confirmed. The brain MRI detected hypoplasia of the cerebellar hemispheres, dilated lateral ventricles, and markedly immature brain parenchyma. Epilepsy had been present since the third month. At 5 months of age, neurological follow-up showed his head circumference to be 37 cm, with plagiocephaly, a low hairline, a short neck, axial hypotonia and he did not adopt any developmental milestones. A genetic mutation, a missense variant in the *GFM1* gene, was confirmed: c.748C>T (p.Arg250Trp) was homozygous in the *GFM1* gene.

Conclusions. To the best of our knowledge, 28 cases of COXPD1 disease caused by mutations in the *GFM1* gene have been described in the literature. COXPD1 should be considered due to symptoms and signs which begin during intrauterine life or at birth. Signs of impaired energy metabolism should indicate that the disease is in the group of metabolic encephalopathies.

Key words: elongation factor G1, mutation, hepatoencephalopathy, lactic acidosis, mitochondrial disorder.

Cellular bioenergetics relies heavily on the mitochondria, which operates the oxidative phosphorylation system (OXPHOS) to produce energy in the form of adenosine triphosphate. Through the numerous signaling pathways and cellular functions, mitochondria and OXPHOS, are involved in neuronal development, connectivity, plasticity and differentiation.[1] Malfunctions in the mitochondrial translation apparatus, stemming from mutations in either mitochondrial or nuclear DNA, have the potential to result in various mitochondrial-related disorders.[2] Coenen et al.[3] described two siblings, born to consanguineous parents, who died at 27 days and 5 months, and were found to have a severe defect in mitochondrial translation, reduced levels of OXPHOS and progressive hepatoencephalopathy (HE). A postmortem analysis indicated significant liver necrosis, corpus callosum hypoplasia, and widespread brain atrophy.

Combined oxidative phosphorylation deficiency-1 (COXPD1) is an autosomal

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recessive multisystem disorder resulting from a defect in the mitochondrial OXPHOS. In particular, COXPD1 is caused by a homozygous or compound heterozygous mutation in the G elongation factor mitochondrial 1 gene (*GFM1*). Instructions encoded in this gene guide the production of a mitochondrial translation elongation factor, specifically elongation factor G1 (EGF1). Hammarsund et al.[4] discovered and isolated the entire coding sequence of the human EFG (GFM1) gene on chromosome 3q25 and later authors mapped the *GFM1* gene to chromosome 3q25.1-q26.2. The GFM1 gene, encompassing 18 exons and spanning no less than 40 kb, is specifically involved in facilitating the translocation of peptidyl-tRNA from the ribosomal acceptor aminoacyl site to the peptidyl site once peptide bond formation has occurred.[5] The deficiency in the protein produced by GFM1 leads to a compromised capacity of the mitochondria to generate energy required for cellular functions.

COXPD1 is a severe, progressive disorder with variable manifestations and fatal outcome. The onset occurs soon after birth, and features may include growth retardation, microcephaly, spasticity, axial hypotonia, encephalopathy and liver impairment. Death usually occurs in the first weeks or years of lifespan.[2]

Here we describe the clinical features and diagnostic workup of a patient with neonatal hepatoencephalopathy due to recessive mutations in the nuclear gene *GFM1*, and compare these findings with other reports of this rare disease. To the best of our knowledge, a total of 28 patients have been reported so far, and this is the first case of COXPD1 due to a mutation in *GFM1* in a Serbian patient.

Case Report

Our patient is a 7-year-old male, born as the first child of young and healthy parents without consanguinity. Pregnancy was monitored regularly and in the 8th month of pregnancy ventriculomegaly was diagnosed by ultrasound (US) and then fetal brain magnetic resonance imaging (MRI) revealed unilateral left ventriculomegaly. The delivery was done by caesarean section due to the impending fetal asphyxia at 39 weeks of gestation. The birth weight (BW) was 3150 g, the birth length (BL) was 54 cm, the birth head circumference (HC) was 31 cm and Apgar score was 9/10. Hypospadias was observed during the physical examination. Due to respiratory failure immediately after birth, the child was transferred to the Department of Neonatology. A neurological examination at birth showed a dysmorphic face (hypertelorism and microcephaly). Hypoglycemia and lactic acidosis (LA) were detected in laboratory analyzes. Metabolic screening of urine and serum and genetic screening were normal. In laboratory analysis, there was an increased gamma-glutamyltransferase (γGT) of 217 IU/L, and the patient is currently being followed for hepatomegaly by gastroenterologists-hepatologists, even though liver enzymes were normal afterwards. After birth, the US of the central nervous system (CNS) showed that there was a dilated interhemispheric fissure, moderate dilation of the frontal horns bilaterally, wide plexuses, asymmetric, wide subarachnoid spaces, and poorly differentiated brain parenchyma. At that time, the brain MR verified marked expansion of the retrocerebellar space, hypoplasia of both cerebellar hemispheres, dilated lateral ventricles, and markedly immature brain parenchyma more prominent on the left. Since the third month, epileptic seizures have been present, which are understood as infantile spasms. The boy had about 30 seizures per day. Seizure semiology was an extension of his arms and legs, with a fixed gaze whereby the boy cries, all lasting only up to 10 seconds, and occasionally turns his head and eyes to the left side for a few seconds. The child also had multiple episodes of head-twitching with rapid blinking. Night attacks have occurred several times a year, and semiology corresponds to daytime seizures. After being transferred to another facility, phenobarbital and valproate were introduced into therapy, followed by transaminase and

γ-GT elevation. As a result, valproates were discontinued and phenobarbital was gradually reduced, leading to the normalization of liver enzymes. Echocardiography revealed a patent ductus arteriosus.

A control brain MR (at 4 months of age) revealed a decreased cerebral parenchyma volume, an expense of white mass (WM) with zones of gliosis, a dilated ventricular system and cerebellar hemisphere hypoplasia with partial vermis agenesis (Fig. 1).

At 5 months of age, neurological follow-up showed a HC of 37 cm, plagiocephaly, a low hairline, a short neck, and axial hypotonia, and he did not adopt any developmental milestones.

The control video electroencephalogram (EEG) examination confirmed the epileptic nature of the spasms but not the etiology. EEG revealed localized epileptiform changes bilaterally anteriorly at slow baseline activity. A hypsarhythmic EEG pattern was not recorded. Vigabatrin was introduced into therapy, and favorable but not complete seizure control was observed, which was the reason why lamotrigine was also introduced.

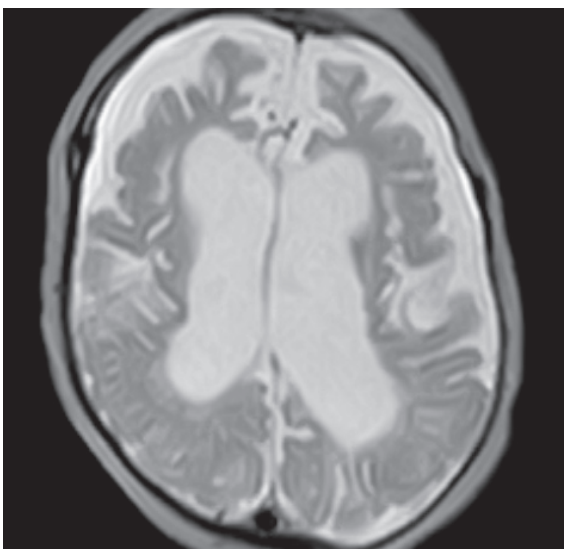

Fig. 1. Brain magnetic resonance imaging. Control brain MRI (at 4 months of age) demonstrating decreased cerebral parenchyma volume, the expense of WM, with gliosis zones and dilated ventricular system and cerebellar hemisphere hypoplasia with partial vermis agenesis.

The parents gave written consent for the publication of this case report.

Genetic findings

Due to respiratory weakness that existed at birth, LA, impaired liver function, seizures, pathological neurological findings, and slow psychomotor development, a metabolic disorder (MD) was suspected. With next generation sequencing, a homozygous missense variant in *GFM1*, was confirmed: c.748C>T (p.Arg250Trp), at 3q25.32. Both parents are asymptomatic carriers of the mutation. A diagnosis of neonatal mitochondrial disease was established. At the age of 3.5 years, he was again hospitalized for frequent seizures and slow psychomotor development. Neurological examination revealed, axial hypotonia and spastic tetraparesis, and it was noticed that he has not functionally adopted a single milestone. At the age of 4, he had pancreatitis, which was treated by antibiotics and symptomatic therapy (June 2019). The last control was in 2021, when it was concluded that there was no progression of the disease, the patient had unchanged neurological findings, and he did not have repeated epileptic seizures.

Discussion

Combined oxidative phosphorylation deficiency-1 disorder occurs as a consequence of a mutation in *GFM1* gene leading to impaired translation function in the mitochondria resulting in death in early childhood. Here we report a 7-year-old male patient with a homozygous c.748C>T (p.Arg250Tyr) mutation in *GFM1* gene. Since 2004, a total of 28 cases of children with a mutation in *GFM1* have been reported (Table I).

Most of the children were female (63.0%).[2,3,6-8,10-12,15] As in our case, consanguinity was not present in most cases (68.0%) [6-8,11,12,14,15], although both parents of our patient were healthy

Table I. The basic demographic and clinical characteristic of patients with GFMS mutations.

No	Reference	Year of publication	Consanguinity	Gender	Preterm birth	Onset of disease	Mode of birth	Death	Survival
1	Coenen[3]	2004	Yes	F[a]	NA[b]	*In utero*	C/S	Yes	27 d
2		2004	Yes	M[c]	Yes	*In utero*	NA	Yes	5 mo
3	Antonicka[6]	2006	No	F	No	*In utero*	NA	Yes	9 d
4		2006	No	F	Yes	*In utero*	C/S	Yes	45 min
5	Valente[7]	2007	No	F	No	7.d	NA	Yes	16 mo
6		2007	No	F	No	2.d	Ordinary	Yes	14 mo
7	Smits[2]	2011	Yes	F	No	2.d	Ordinary	Yes	2 yr
8	Balasubramaniam[8]	2011	No	F	No	*In utero*	C/S	Yes	8 mo
9	Galmiche[9]	2012	Yes	M	No	At birth	Ordinary	Yes	4 yr
10		2012	Yes	M	Yes	NA	C/S	Yes	20 mo
11	Calvo[10]	2012	NA	M	NA	1.wk	NA	NA	NA
12		2012	NA	F	NA	1.year	NA	No	NA
13	Brito[11]	2015	No	F	Yes	*In utero*	C/S	NA	NA
14	Ravn[12]	2015	Yes	M	No	*In utero*	Ordinary	Yes	4 mo
15		2015	Yes	F	No	*In utero*	Ordinary	Yes	14 d
16		2015	No	F	No	*In utero*	Ordinary	Yes	3 mo
17	Kohda[13]	2016	NA	NA	NA	NA	NA	NA	NA
18	Simon[14]	2017	No	M	Yes	3 mo	NA	No	7 yr
19		2017	No	M	NA	*In utero*	NA	Yes	10 mo
20	Barcia[15]	2019	No	F	No	At birth	Ordinary	No	9 yr
21		2019	No	F	NA	At birth	NA	No	2.5 yr
22		2019	No	F	No	At birth	Ordinary	Yes	3 yr
23		2019	No	M	No	8. mo	Ordinary	No	5 yr
24		2019	No	M	No	At birth	NA	No	2 yr
25		2019	No	F	No	At birth	Ordinary	No	16 mo
26		2019	No	M	No	At birth	Ordinary	Yes	9 mo
27		2019	Yes	F	No	At birth	Ordinary	No	15 mo
28		2019	No	F	No	At birth	Ordinary	No	5 yr

[a]Female [b]Not available [c]Male, C/S: Caesarean section

heterozygous carriers of the same mutation. Premature birth was reported in 20.0% of cases.[3,6,9,11,14] The birth of our patient was at the 39th week of pregnancy. The onset of signs and symptoms was most common in utero (38.5%)[3,6,8,11,12,14], but symptoms were also present at birth in 34.6%[9,15] during the neonatal period in 15.4%[2,7,10] and during infancy in 11.5%.[10,14,15] Intrauterine growth delay was observed in 10 cases.[2,3,6,8,9,12,14] All cases from the study by Barcia et al.[15] had parameters at birth that were either normal or within 1 SD. Our patient, born shortly before term and by caesarean section, had a normal BW, BL, HC, at birth. In a quarter of cases (27.8%), the pregnancy ended with a caesarean section[3,6,8,9,11], as in our case.

One patient from the study by Barcia et al.[15] was born without respiratory movements and was intubated for the first 6 hours.[15] Our patient was transferred immediately after birth due to respiratory distress syndrome. All of the published cases had slow psychomotor development. Other common elements of physical findings were spasticity of the arms and legs in 40.7%[2,3,7,9,11,12,14,15], microcephaly

in 37.0%[2,3,6,7,9,12], axial hypotonia in 37.0%[2,7,15], dystonic movements in 33.3%[9-11,15], a dysmorphic face in 25.9%[6-10,12,14], and feeding problems in 25.9%.[2,14,15] Spasticity of the arms and legs and axial hypotonia, which we also observed in our patient, were the most common neurological findings that have been reported in the published cases. Hypospadias at birth has been reported in 3 cases in the literature[9,12,14], as was the case with our patient.

Ten patients[2,10-12,14,15] had seizures, with 5 being diagnosed with West syndrome[15], 2 with infantile spasms[10,14], or evolution of Lennox-Gastaut syndrome.[2] Our patient had epileptic seizures from 3.5 months, and at 5 months, an EEG confirmed infantile spasms. In other cases, the EEG findings detected have been reported as follows: global, severe disorganization with complete lack of sleep spindles[7] or multifocal spikes and waves, without clear hypsarrhythmia[2] and multifocal polyspike paroxysms.[11] Initial therapy with phenobarbital and valproate in our patient resulted in an increase in transaminase values, so vigabatrin and lamotrigine were included. Bracia et al.[15] reported that they used a ketogenic diet, levetiracetam, vigabatrin, and hydrocortisone in West's syndrome therapy.

Our patient had a persistent ductus arteriosus, Antonicka et al.[6] also presented a patient who had a large ductus arteriosus. However, cardiac function is spared in this disease, and concentric left ventricular hypertrophy was shown in only one patient.[14]

Developmental delay was observed in all of the 19 patients in the literature[2,3,6-12,15], similarly our patient did not adopt a single developmental milestone. The most important and most common laboratory parameter was the elevated lactate (lactic acid) present in 21 patients[2,3,6-12,14,15], while 14 patients were reported to have acidosis (metabolic or lactic).[3,6-12,14,15] Although the disease is also known as neonatal mitochondrial HE[12], or infantile progressive hepatoencephalomyopathy[8], liver dysfunction has been reported in nine patients[3,9,12,13,15], while another four had hepatomegaly[2,8,14] and three cases had elevated transaminases.[7,8,14] Hypoalbuminemia[6,8,14] and coagulopathy[6,8,12] were reported in three patients each. Hypoglicemia[9,12] was observed in two patients, and one[8] had elevated γ-GT similar to our patient. Our patient had elevated transaminase levels due to phenobarbital and valproate therapy, but enzyme levels returned to normal after these two drugs were discontinued. Encephalopathy has been reported in 10 cases from the literature[2,3,7-10,15], although slow psychomotor development was present in all.

A change in signal intensity was verified on brain MRI in 13 patients.[7,8,11,15] For two patients, bilateral symmetric T2 enhancement and T1 decrease in signal intensity were revealed[7,8], while for three patients the authors did not specify what the change in signal intensity was.[7,11] An increase in T2 in the basal ganglia was detected in all patients presented by Barcia et al.[15] WM periventricular abnormalities[15] were shown in eight patients, ventricular dilatation in four[8,11,14,15] and thin corpus callosum in three patients.[2,11,15] Normal brain MRI on day 5 from birth for one patient was verified.[6] In our patient, decreased brain parenchyma volume, enlarged ventricular system, cerebellar tonsil hypoplasia, and partial vermis agenesis were revealed on MRI.

A fatal outcome was reported in 16 patients from the literature.[2,3,6,8,9,12,14,15] The mean age at death was 12.4 ± 13.8 months. For the 9 patients who were reported to be alive, the mean age was 4.1 ± 2.9 years[10,14,15] (data was available for 8). The oldest child who was reported to be alive was a 9-year-old girl.[15] In 4 patients, the same c.748C>T (p.Arg250Trp) mutation was present as in our patient[2,13,14] with only one patient being homozygous as our patient[2], and three patients (from two families) harbored the c.748C>T (p.Arg250Trp) mutation in compound heterozygosity with c.170C>A (p.Ser59Tyr) or c.689+908G>A (p.Gly230_231Glnins19).[13,14] There was no survival data available for

the patient presented by Kohda et al.[13] The patient who was homozygous for c.748C>T (p.Arg250Trp) passed away at 16 months of age[2], while one patient presented by Simon et al.[14] was 7 years old at the time of publication and the other who carried the identical combined heterozygous mutation died at 10 months. The longest survival of 9 years was reported by Barcia et al.[15] for a patient who had a combined heterozygote with the c.2011C>T (p.Arg671Cys) inherited from their father and the mutation c.1297_1300del (p.Asp433Lysfs*20) inherited from the mother.

We conclude that neonatal HE deserves special attention because of the symptoms and signs that begin during intrauterine life, either at birth or in the first few days of life. Signs of impaired energy metabolism (acidosis, respiratory problems, liver dysfunction, and encephalopathy) should indicate that the disease is in the group of metabolic encephalopathies. Additional features such as spasticity of the arms and legs, axial hypotonia, microcephaly, dystonic movements, a dysmorphic face, seizures, and MRI findings, such as changes in intensity of T1 and T2 signals, ventriculomegaly, could guide the testing process toward genetic analysis and this diagnosis.

Ethical approval

The parents gave written consent for the publication of this case report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DA MGJ, ST, MK, MB; data collection: DA, MGJ, ST, MK, MB; analysis and interpretation of results: DA, MGJ, ST, MK, MB; draft manuscript preparation: DA, MGJ, ST, MK, MB. 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

Novel sphingosine-1-phosphate lyase mutation causes multisystemic diseases: case report

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ABSTRACT

Background. Sphingosine phosphate lyase insufficiency syndrome (SPLIS) caused by inactivating mutations in the human *SGPL1* gene results in congenital nephrotic syndrome, adrenal insufficiency, ichthyosis, immunodeficiency, and a wide range of pathological neurological features. We present a novel mutation in the *SGPL1* gene causing hypocalcemia, primary adrenal insufficiency (PAI), nephrotic syndrome, subclinical hypothyroidism, lymphopenia, ptosis, and pathologic neuroimaging findings.

Case. A Turkish male infant presented with bruising at 2 months of age and was diagnosed with hypocalcemia, PAI, and subclinical hypothyroidism. At the age of 15 months, he was admitted to the hospital with ptosis. Other systemic manifestations included persistent lymphopenia and nephrotic syndrome. Magnetic resonance imaging (MRI) of the brain and orbit demonstrated asymmetric contrast enhancement in the left cavernosal sinus, orbital apex, and thinning at the bilateral optic nerve. Whole exome sequencing (WES) revealed a homozygous c.1432C>G (p.Gln478Glu) variant in the *SGPL1* gene (NM_003901.4), which has not previously been reported in the literature.

Conclusions. Novel mutations in *SGPL1* are still being identified. This case reminded us that SPLIS should not be considered for patients with nephrotic syndrome alone. Still, PAI may also include patients with neurological disorders, hypocalcemia, and pathological neuroimaging findings such as thinning at the bilateral optic nerve.

Key words: sphingolipids, Sphingosine phosphate lyase insufficiency syndrome, adrenal insufficiency, Nephrotic syndrome, Ptosis.

Sphingolipids (SLs) were discovered by Thudichum in 1884 and are considered normal components of the plasma membrane, myelin sheath, and plasma.[1,2] SLs are degraded into bioactive intermediates that can join in signal transduction pathways that play a role in the regulation of cell survival, migration, programmed cell death, and intracellular functions.[3] In the degradation and recycling of SLs, a highly preserved group of enzymes are involved. Sphingolipidoses, a storage disorder, is caused by the accumulation of different classes of SLs due to the deficiency of these enzymes.[4]

Ceramide and sphingosine 1 phosphate (S1P) are two important bioactive SLs.[5] S1P can be degraded into two non-SL products, hexadecanal and ethanolamine phosphate, by sphingosine 1 phosphate lyase (SGPL1).[6] The only known exit pathway of SL metabolism is the production of these two compounds. SGPL1, the last enzyme in the sphingolipid degradation pathway, catalyzes the irreversible division of long-chain base phosphates.[7]

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In reports between 2017 and 2018, a new childhood syndrome called sphingosine phosphate lyase insufficiency syndrome (SPLIS) was defined. SPLIS is caused by inactivating mutations in the human *SGPL1* gene that encodes SGPL1.[8-10] SPLIS includes a combination of ichthyosis/acanthosis, steroid-resistant nephrotic syndrome, hypothyroidism, primary adrenal insufficiency (PAI), gonadal dysgenesis, lymphopenia and/or neurological disorders including microcephaly, cranial nerve defects, and peripheral neuropathy. The pathogenesis of findings other than lymphopenia has yet to be fully elucidated. In the literature, it has been reported that clinical findings may be caused by excess intracellular S1P, accumulation of other SLs, abnormal S1P receptor signaling, or loss of SGPL1 products.[11] Accumulation of the species S1P, sphingosine, and ceramide, have been associated with the induction of cytotoxicity and apoptosis.[12,13] S1P functions as a ligand for a family of 5 specific G-protein coupled receptors (S1PR1-5).[14] S1PR1, the prototype of S1PR, regulates the outflow of T lymphocytes from the thymus and peripheral lymphoid organs.[15] While S1P levels are extremely low in most tissues other than blood and lymph, they are kept at low concentration levels in tissues by SGPL1.[10] When SGPL1 activity is disrupted, this gradient cannot occur, and increased S1P level reduces the S1P chemotactic gradient or the ability of the lymphocyte to detect it, which leads to lymphopenia.[16]

Herein, we present a novel mutation in *SGPL1* causing multi-systemic disease.

Case Report

A male Turkish patient was the fourth child of first-degree consanguineous healthy parents without a family history of chronic diseases. After an uneventful pregnancy, he was born at 39th weeks of gestation, with a birth weight of 2800 g. His elder siblings were healthy. He was referred to an external center with a complaint of bruising at two months of age. Biochemical tests revealed calcium: 5.3 mg/dL (9–11), phosphorus: 7.3 mg/dL (3.7–6.5), magnesium: 1.7 mg/dL (1.3-2.7), alkaline phosphatase: 658 IU/mL (122–469), parathyroid hormone: 157 pg/mL (15–65), and 25OH vitamin D: 6.72 ng/mL (20-30) levels. Complete blood count, electrolyte, glucose, liver, and kidney tests were normal. Treatment with calcium and vitamin D was started. High doses of intravenous and oral calcium were required to treat his hypocalcemia. Thyroid hormone replacement was started due to the results of the thyroid stimulating hormone (TSH): 10.2 IU/mL (0.27–4.2) and fT4: 14.25 pmol/L (12-22). No problems were detected on the thyroid ultrasound. When cholestasis developed during his hospitalization, an evaluation was conducted and resulted in an adrenocorticotropic hormone (ACTH) level of 722 pg/mL and cortisol level of 2.2 ug/dL; thus, he was diagnosed with PAI, and hydrocortisone treatment was started. Cholestasis was improved after hydrocortisone treatment.

At the age of 15 months, he was admitted to our hospital with a preliminary diagnosis of periorbital cellulitis and complaints of ptosis and eyelid swelling. Body weight was measured as 8 kg (–2.73 standard deviation score [SDS]), height as 72 cm (–2.79 SDS), and head circumference as 44 cm (–2.76 SDS). The cranial nerve and ophthalmological examination revealed nearly complete ophthalmoplegia of the left eye, without direct light reflex. Ophthalmological examination of the right eye was normal, and there was no other motor or sensory neurological deficit. The patient was transferred to the ophthalmology department for further assessment and management. Upon ophthalmological and neurological examination there was severe ptosis on the left, and when the right eye was fixed, the left eye was in an abducted position, indicating oculomotor and trochlear nerve palsies on the left side (Fig. 1). This exotropia was at a large angle in the primary gaze position. When the right eye was manually closed, the patient was unable to bring his exotropic left eye to the midline. Direct pupillary light reflex was not obtained

Fig. 1. The patient at 15 months of age presenting with ptosis.

from the left eye but it was normal for the right eye. Biomicroscopic and fundus examinations were normal for both eyes. Other system examinations including the genitourinary system were normal. The laboratory results of the patient are given in Table I.

The echocardiography was normal. He had nephrotic range proteinuria (2.5 g/day, 200 mg/m²/h) and hypoalbuminemia (26 g/L). He was administered captopril first. A hearing test was normal. Malignancy was excluded. Investigations for infectious diseases were normal. No thrombus was detected on orbital venography. Abdominal computed tomography imaging was normal. No adrenal calcification was detected. Immunological evaluation was performed due to the detection of lymphopenia (650–1500/mm³), and the number of B cells and CD4+ T cells were found to be low. Trimethoprim-sulfamethoxazole, fluconazole and monthly IVIG treatments were started prophylactically. Metabolic investigations including very long chain fatty acids, acyl carnitines, urinary organic acids, urine and plasma amino acids, lactic and pyruvic acids were all normal.

T1 weighted (T1W) magnetic resonance imaging (MRI) of the brain and orbit demonstrated asymmetric contrast enhancement in the left cavernosal sinus and orbital apex (Fig. 2-3). MRI also showed thinning at the bilateral optic nerve (Fig. 2, 4). His imaging findings were discussed with neuroradiology and assumed to likely represent an underlying inflammatory process. Methylprednisolone treatment was started. After 10 weeks of steroid treatment, no

Table I. The results of the initial laboratory investigations (at 15 months)

Parameter	Value	Unit	Reference range	Parameter	Value	Unit	Reference range
Corrected Ca	7.1	mg/dL	9.1-10.3	Hgb	8.2	g/dL	10.2-13.4
Phosphorus	3.8	mg/dL	4.1-6.5	PLT	198	x10⁹/L	220-490
ALP	192	U/L	142-336	WBC	3.34	x10⁹/L	5.4-13.8
PTH	108	ng/L	18.4-80.1	Lymphocytes	0.76	x10⁹/L	3-10
25OH-D	19	nmol/L	75-375	ALT	8	U/L	0-32
Creatinine	0.1	mg/dL	0.1-0.4	AST	46	U/L	0-46
Urea	9	mg/dL	11-39	Na	138	mEq/L	132-146
ACTH	903	pg/mL	<46	K	3.0	mEq/L	3.5-5.5
Cortisol	8.2	μg/dL	5.2-22	Cl	109	mEq/L	99-109
TSH	15.3	mU/L	0.5-4.9	Glucose	86	mg/dL	<100
fT4	0.97	ng/dL	0.83-1.43	Albumin	26	g/L	32-48
FSH	1.9	U/L	0.3-10.1	Prot. (urinalysis)	++++	-	negative
LH	0.1	U/L	<0.6	Prot./cre (urine)	21.4	mg/mg	<0.5
Renin	0.47	ng/mL/h	1.7-11.2	24h urine prot.	200	mg/m²/h	<4
Aldosterone	12	pg/mL	10-160				

25OH-D: 25-hydroxyvitamin D, ACTH: adrenocorticotropic hormone, ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate transaminase, cre: creatinine, FSH: follicle-stimulating hormone, fT4: free thyroxine, Hgb: hemoglobin, LH: luteinizing hormone, PLT: platelet, Prot.: protein, PTH: parathyroid hormone, TSH: thyroid-stimulating hormone, WBC: white blood cell.

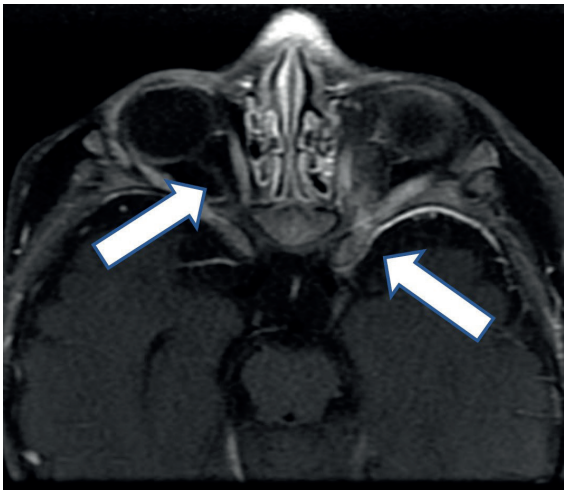

Fig. 2. Axial contrast enhancing T1W MRI shows asymetric contrast enhancement at the left orbital apex, thinning of the optic nerve.

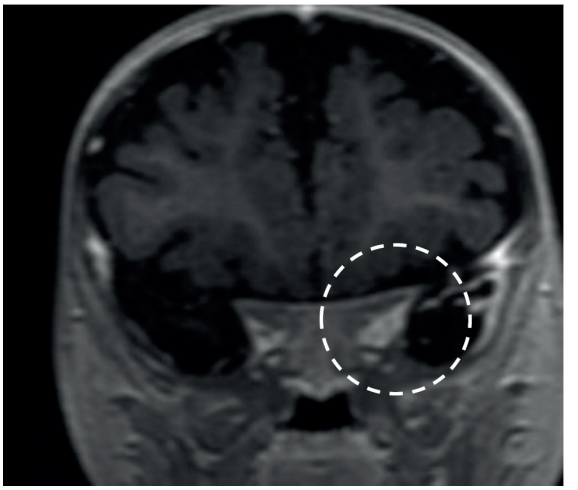

Fig. 3. Coronal contrast enhancing T1W MRI shows asymetric contrast enhancement at the left orbital apex.

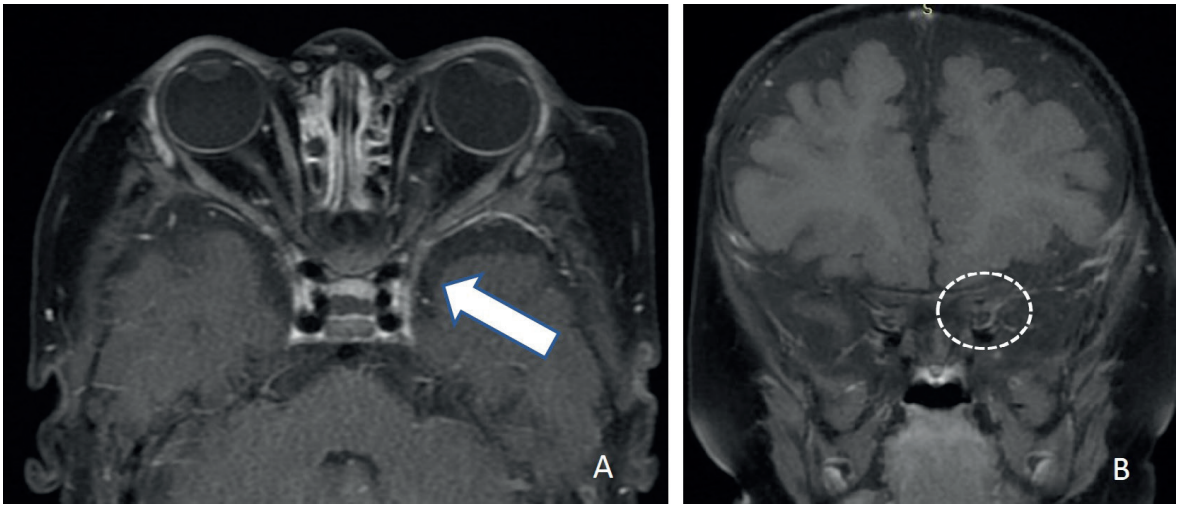

Fig. 4. There is no asymmetric contrast enhancement at left orbital apex.

asymmetric contrast enhancement in the left cavernosal sinus and orbital apex was detected on the MRI (Fig. 4). Despite the enhancement in MRI findings, there was no improvement in ptosis.

With the findings of hypocalcemia, PAI, nephrotic syndrome, subclinic hypothyroidism, lymphopenia, and ptosis, a homozygous mutation was found in the *SGPL1* gene, confirming SPLIS.

Whole exome sequencing (WES) was performed using the TWIST Comprehensive Exome Kit and MGI DNB SEQ G400. WES revealed a homozygous c.1432C>G (p.Gln478Glu) variant in the *SGPL1* gene (NM_003901.4), which was not previously reported in the literature. The variant was not found in the gnomAD genomes, 1000G and ExAC databases. This variant is classified as VUS according to the American College of Medical Genetics and Genomics (ACMG) guidelines and estimated to be deleterious by in silico pathogenicity prediction tools such as MutationTaster, SIFT, and Polyphen-2 (score 0.999). The detected variant resides in a highly conserved protein region according to the

Fig. 5. Schematic representation of the evolutionary conservation of the SGPL1 protein region involved in c.1432C>G (p.Gln478Glu) variant among various species.

GERP++ in-silico prediction (GERP score= 5.78) (Fig. 5). Sanger sequencing was carried out for validation and segregation analysis showed that his parents were heterozygous carriers for the same variant. Informed consent was obtained from the patient's family for the publication of this case report, including photographs.

Discussion

We described the phenotypic features and molecular diagnosis of SPLIS in a Turkish male patient who had a novel homozygous variant discovered in the *SGPL1* gene using WES. In the current case, hypocalcemia, PAI, and subclinic hypothyroidism were found in the first year of life. Lymphopenia, ptosis, and nephrotic syndrome were detected between one and two years of age. Also, an MRI showed thinning of the bilateral optic nerves. To date, less than 70 confirmed cases of SPLIS have been reported, and 13 patients stated in the literature were of Turkish origin. All cases reported from Turkey had homozygous variants, and all of the patients had consanguineous parents, just like our patient. Although the most common initial clinical manifestations of reported patients were kidney disorders; our patient presented with endocrine disorders at the age of two months. In our patient, who is currently 2.7 years old, there were no new findings other than the clinical manifestations we reported above.

In a recent review, 55 patients with SPLIS from 19 articles were identified. Endocrine disorders, especially PAI, were found to be the most prevalent clinical features.[17] While most patients affected by adrenal insufficiency present with signs of glucocorticoid deficiency, cases of mineralocorticoid deficiency and adrenal androgen deficiency have also been reported.[18,19] It was reported that disrupted adrenocortical zonation and defective expression of steroidogenic enzymes may cause adrenal insufficiency in Sgpl1 null mice.[20] Ceramide, sphingosine, and sphingosine 1-phosphate are modulators of the steroidogenic pathway.[18]

While S1P plays a role at multiple levels in the steroidogenic pathway to upregulate cortisol biosynthesis, ceramide and sphingosine play a role in reducing steroidogenesis.[21,22] The study by Maharaj et al.[23] reported that sphingolipid accumulation may impair steroidogenesis by impairing mitochondrial morphology and function. Elevated ceramide levels in the mitochondria may lead to inner mitochondrial membrane dysfunction.[24] In addition, adrenal calcification detected in many of the SPLIS patients suggests that adrenal insufficiency may occur with lipid accumulation in the adrenal gland.[18] Expression of SGPL1 in the testes and thyroid gland explains thyroid dysfunction and/or testosterone deficiency in such cases.[18,20,25] To date, no endocrinopathy other than hypocalcemia, adrenal insufficiency and subclinic hypothyroidism has been detected in our patient.

S1P signaling is also known to regulate bone metabolism. The effect of S1P on bone homeostasis is associated with bone remodeling by regulating the circulation of osteoclast progenitors.[26] In the study of Weske et al., it was revealed that raising S1P levels in adult mice through SGPL1 inhibition markedly increased bone formation, mass, and strength, and significantly reduced white adipose tissue. It was reported that S1P signaling via S1PR2 strongly stimulates osteoblastogenesis and inhibits osteoclastogenesis by simultaneously inducing osteoprotegerin.[27] In the literature, hypocalcemia in SPLIS was reported in one case.[12] The patient herein was admitted for the first time with hypocalcemia at the age of 2 months old, and his hypocalcemia continued despite the treatment of vitamin D and calcium. The calcium level was in the normal range, with intravenous calcium, calcitriol and vitamin D treatment. This case may be important in terms of raising awareness about hypocalcemia in patients with SPLIS.

It was reported that kidney disorders were the most common initial manifestations of SPLIS.[17] Damage to glomerular podocytes is reported as one of the causes of kidney pathology in SPLIS. Immunofluorescence experiments in mice detected that SGPL1 is localized in the podocyte, mesangial and endothelial cell endoplasmic reticulum of renal glomerular cells.[12,28] Renal involvement varies from non-immune fetal hydrops to the absence of renal involvement in long-term follow-up. Patients usually present with steroid-resistant nephrotic syndrome that progresses to end-stage renal disease, whose histological findings on renal biopsy are focal segmental glomerulosclerosis (FSGS) and diffuse mesangial sclerosis.[29] This form of congenital nephrotic syndrome is called nephrotic syndrome type 14.[12] Tastemel Ozturk et al.[30] from Turkey reported six patients with homozygous *SGPL1* mutations. The median age at which kidney symptoms manifested in this study was five months, and all of the patients developed chronic kidney disease. The patient herein had nephrotic range proteinuria, hypoalbuminemia and edema accompanied by slightly increased serum cholesterol levels.

The complex biological effects of S1P affect the nervous system as well as many other systems. In patients with SGPL1 deficiency, pathological neurological disorders, such as Charcot-Marie-Tooth neuropathy, neurodevelopmental delay, sensorineural hearing loss, microcephaly, seizures, cranial nerve deficits, strabismus, ptosis, and encephalopathic neurodegenerative disease, have been reported.[8,9,31] Vertebrate and invertebrate models of SGPL1 insufficiency have been shown to cause neurotoxicity.[32] Nevertheless, the underlying mechanisms responsible for the molecular pathogenesis of neurotoxicity remain unresolved. The neuroimaging results encompass a spectrum of observations, including loss of the corpus callosum, progressive cortical atrophy, cerebellar hypoplasia, as well as notable involvement of the globus pallidus, thalamus, and dentate nucleus.[29,33] In the literature, MRI findings are not specific and may show similarities with other toxic, metabolic, mitochondrial, infectious, and post infectious

disorders. Our patient showed asymmetric contrast enhancement in the left orbital apex and cortical atrophy with thinning at the optic nerve. After steroid treatment, no asymmetric contrast enhancement was detected. The cause of this finding is not known. To the best of our knowledge, thinning of the optic nerve has not been reported in SPLIS in the literature. It is not known whether this condition is associated with SPLIS.

In conclusion, SPLIS patients may present with a wide spectrum of findings. Hypocalcemia, adrenal insufficiency and subclinic hypothyroidism were the earliest findings in our case. Early diagnosis can allow early identification of other comorbidities of the disease. As such cases are reported, it will also assist in determining the appropriate genotype-phenotype correlations in patients suffering from SGPL-related pathogenesis.

Ethical approval

Informed consent was obtained from the patient's family.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: GB, KTA, FG; data collection: GB, KTA, GÜD, ED, ŞBE, ÖYA; analysis and interpretation of results: GB, KTA, ÇSK; draft manuscript preparation: GB, KTA, ÇSK, MB, FG. All authors have reviewed 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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Attitudes of parents with children aged 12-18 to COVID-19 vaccines for themselves and their children: vaccine hesitancy in Türkiye

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Dear Editor,

We read with great interest the article entitled "Attitudes of parents with children aged 12-18 to COVID-19 vaccines for themselves and their children" submitted by Şahin et al.[1] We congratulate the authors for their study about COVID-19 vaccine hesitancy. However, we have the following comments and concerns.

In the article, the definition of vaccine hesitancy (VH) was provided, but the definition of vaccine refusal (VR) was not, and the unvaccinated children for other reasons were not considered. Defining these concepts enables a more accurate evaluation of the results. Vaccine refusal and VH are emerging problems all over the world. In a study examining 80 provinces in Türkiye, 8,977 VR cases were detected in 2016 (VR rate 3.5‰) and 14,779 cases in 2017 (VR rate 5.9‰; $p<0.001$).[2] The data were also reported on a provincial basis. VR rate was very low in İzmir; 1.3 ‰ in 2016 and 1.9 ‰ in 2017. The same study demonstrated that the percentage of family health units with at least one VR case was 14% in the Aegean region in 2016-2017.[2] However, it is known that the percentage of unvaccinated children in our country decreased from 3.2 to 0.9% between 1990 and 2018.[3]

The percentage of participants who refused to participate in the study should be stated in the article. The high percentage of vaccine refusal may be attributed to these individuals. Conditions such as hospitalization or death, including chronic illness in the child and the severity of infections such as COVID-19, also affect vaccination status. This situation was emphasized in two studies conducted on the experiences of health personnel throughout Türkiye.[2,4] Vaccine refusal is not alone in some cases, but also in the form of denial of health care and other interventions. Since hospital admissions formed the study population, no information could be obtained about these cases.[2,4]

Şahin et al.[1] revealed that concerns about side effects and children not being eager to be vaccinated were the most common causes of COVID-19 vaccine refusal. Since this study was conducted in only one province (İzmir) of Türkiye, the data were limited. Many different factors influence parents' decisions to vaccinate their children. In the national study in Türkiye, the most common reasons for VH were "Concerns about the vaccine content (66.3%), harmfulness (51.2%), and fears about adverse effects (50.0%)".[2] Similarly, lack of information about vaccines, fear of side effects, concerns about vaccine efficacy and safety, the thought that vaccines are harmful, anti-vaccine publications on the internet and social media, belief in natural immunity, and religious beliefs are reported to be the most common reasons for VH and VR in a qualitative study from Türkiye.[2,4,5] In evaluating the study results, it should be considered that the factors that are related to VH and VR in a normal state in the community, would also affect the vaccination rate during the pandemic.

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Response to "Attitudes of parents with children aged 12-18 to COVID-19 vaccines for themselves and their children: vaccine hesitancy in Türkiye"

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Dear Editor,

We would like to thank the authors for their interest in our article.[1] Below, we have provided our response to the comments and concerns raised by the authors.[2]

As the authors note, rates of vaccine hesitancy vary between regions. Since our research was conducted in only one province of Türkiye, it has limitations in reflecting the situation throughout the country.[1] However, when the research was conducted, the rate of adults in Izmir (%86) receiving at least two doses of the COVID-19 vaccine was similar to the general average in Türkiye (%85.6), unlike the refusal of routine childhood vaccines.[3] In our study, 8% of participants had routine childhood vaccination hesitancy. However, we did not detect a statistically significant relationship between routine childhood vaccine hesitancy and COVID-19 vaccine hesitancy ($p = 0.611$).[1] With this, it can be said that there is a need to conduct multi-center research and include a larger number of participants in research on COVID-19 vaccine refusal in our country.

As a result of our study, 11.4% of the children had a history of COVID-19, but in the logistic regression analysis, there was no statistically significant relationship between parents' acceptance of the COVID-19 vaccine and their children's history of COVID-19.[1] However, in our study, the relationship between conditions such as hospitalization or death, including chronic illness in the child, and parental acceptance of the COVID-19 vaccine was not evaluated. This can be considered among the limitations of our study. As mentioned in the letter, since the research population consisted of those who applied to our hospital, it does not include parents who refuse health services or other interventions along with vaccine refusal. Therefore, the rate of COVID-19 vaccine refusal by parents for their children may be higher than we found.

Studies conducted before the pandemic evaluated the reasons for refusing routine childhood vaccines, which have been around and administered longer than COVID-19 vaccines. In these studies, "Concerns about vaccine content and harmfulness" were found to be more prominent.[4,5] However, since COVID-19 vaccines are new and use different technologies, the reasons for refusal of the COVID-19 vaccine will likely be different from those of other childhood vaccines. Indeed, the main concerns reported by parents in our study were the side effects and the vaccine's safety.[1] Various studies on accepting COVID-19 vaccines have also shown that parents' main concerns

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about vaccines are safety and effectiveness.[6] In conclusion, the results of studies suggest that COVID-19 vaccine refusal may have its own reasons.

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