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Review

Neuroblastoma-targeted Anticancer Drug Delivery

Nöroblastomda Hedeflenmiş Anti Kanser İlaç Taşınımı

Safiye Aktas
Özde Elif Gökbayrak
Aylin Erol
Hatice Nur Olgun

ABSTRACT

Neuroblastoma (NB) is the most common solid tumor in pediatric cases. NB accounts for about 8% of malignancies in patients younger than 15 years, and since 50% of newly diagnosed cases metastasize to regional lymph nodes, bone marrow, bone, liver, and skin, the disease is usually diagnosed in its late stages. Overexpression of N-MYC, which is characterized by poor prognosis in NB, changes the progression of the disease and the course of the treatment. Targeting these pathways may be a treatment option in NB, since the mTOR and AURKA pathways interact with N-MYC and directly cause protein stabilization. The widespread use of conventional chemotherapeutics has some limitations associated with their common side effects and the bioavailability of the drugs. New therapeutic approaches have focused on nanoparticle (NP) -based therapies, and chemoimmunoagents in the form of many NPs are being tried in NB. Here, we review new therapeutic approaches that would help to treat NB.

Keywords: *Neuroblastoma, nanoparticle, signaling pathways*

ÖZ

Nöroblastom (NB), pediatrik olgularda en yaygın görülen solid tümördür. NB, 15 yaşından genç pediatrik hastaların %8'inde malignansi göstermekle birlikte, tanı konanlarda %50'sinde bölgesel lenf noduna, kemik iliğine, kemiğe, karaciğere ve deriye metastaz görülmektedir. Hastalık genelde geç evrelerde tanı almaktadır. N-MYC'nin aşırı ekspresyonu NB'de kötü prognoz ile karakterize olmuştur ve tedavi ve hastalığın ilerleyişini değiştirmektedir. mTOR ve AURKA yolakları N-MYC ile ilişkili olarak protein stabilizasyonuna neden olduğundan dolayı, NB'de bu yolakları hedeflemek yeni bir tedavi stratejisi oluşturabilir. Günümüzde kullanım alanı geniş olan konvansiyonel kemoterapötik ilaçlar, bilinen yaygın yan etkileri ve ilacın biyoyararlanımı gibi sebeplerden dolayı bazı sınırlayıcı faktörlere sahiptir. Yeni terapötik ajanlar, nanopartikül (NP) bazlı tedavilere yönelmiştir ve NP formunda birçok kemoimmünoajan NB'de denenmektedir. Bu makalede NB tedavisinde kullanılan yeni yaklaşımları özetledik.

Anahtar kelimeler: *Nöroblastom, hedeflendirilmiş tedavi, nanopartikül*

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INTRODUCTION

Neuroblastoma (NB) is an embryonic tumor originating from the neural crest. The best defined genetic change in NB is the overexpression of the MYCN protein, which occurs in approximately 20% of all NB cases and is associated with its high-risk phenotype. Although targeting MYCN is quite challenging due to the lack of suitable surfaces on the DNA binding domain to which drugs can bind, indirect targeting method has been shown to be the most effective approach to inhibit or control the regulation of MYCN. Overexpression of Myc dramatically changes the trajectories of gene expression. Everolimus, a hydroxyl ester of rapamycin, is an agent specific to the mTOR pathway and inhibits the nuclear pathway. AURKA is critical for the assembly and stability of mitotic spindle strands, as well as the regulation of centrosome and kinetochore formation. It stabilizes MYCN by direct protein-protein interaction, making it less degradable by the nuclear proteosome complex [(1)].

Although inhibition of Myc is an FDA-approved therapeutic strategy, the development of clinical compounds that directly target proteins (IDPs) is disrupted by natural mutations, such as Myc, and lack of stable and well-defined molecular packings probed with small molecules has created new therapeutic challenges. Conventional chemotherapeutics, although toxic to cancer cells,

also damage healthy cells. Encapsulating drugs in nano-carrier systems targeting cancer cells is an effective method in presenting drugs in combinations and reducing toxicity. Hybrid lipid-polymer nanoformulations combine the advantages of both models, providing controlled drug release and enhanced bio-functionality. In recent studies, it has been shown that nanoformulations, which are being studied as a new therapeutic method, provide advantages in drug delivery and imaging in many molecular cancer therapies and conventional chemotherapy.

Neuroblastoma

Neuroblastoma (NB) is a pediatric solid tumor involving the sympathetic nervous system. The incidence rate of NB in children <15 years of age is 1.2 cases per 100,000 and accounts for approximately 15% of cancer-related pediatric deaths. The clinical picture and outcomes of NB are highly heterogeneous, due to disease regression, its multifocality and multidrug resistance [1]. N-MYC amplification has been reported in approximately 25% of NB cases, and N-MYC amplification has been considered the strongest marker associated with a poor prognosis and rapid progression of the tumor in approximately 40% of high-risk patients. Other anomalies seen in NB are loss of 1p heterogeneity (30%), 11q deletions (45%) and 17q gains (60%) which are associated with diploid or tetraploid karyotypes. In addition, ALK amplification encoding anaplastic lymphoma kinase (ALK) receptor tyrosine kinase is seen in 1-2% of the cases and has been mostly evaluated together with N-MYC amplification. Recently, a large genomic rearrangement known as chromotripsis has been observed in 18% of advanced stage tumors; Therefore, NB can be considered a type of cancer that is predominantly dependent on copy number. Approximately 8-10% of sporadic NB tumors are seen with point mutations in ALK. In addition, 1-2% of NB tumors show ALK amplification that actually occurs only in the presence of N-MYC amplification, given their proximal association on chromosome 2p23-24. Therefore, ALK amplification is also a predictive marker of poor prognosis. ALK variants serve as important biomarkers in NB, as they confer sensitivity to small molecule kinase inhibitors currently under clinical evaluation in phase I and II trials [2].

Current Treatment Approaches in Neuroblastoma

The treatment algorithm for NB depends on the risk stratification defined using parameters such as age, disease stage, tumor histopathology, coefficient of MYCN amplification and DNA ploidy. Since spontaneous regression is often observed in this risk group, low-risk patients are usually evaluated only intraoperatively or with close follow-up. In contrast, intermediate-risk patients need both surgery and chemotherapy. High-risk patients are treated with high-intensity chemotherapy, radiotherapy, surgery and autologous hematopoietic stem cell transplantation. In addition, high-risk patients receive immunotherapy with anti-GD2 antibodies and cytokines, and differentiation therapy with 13-cis-retinoic acid to eliminate minimal residual disease (MRD) [2]. NB contains a large number of genetic and protein aberrations. Most of the potential therapeutic targets for NB have been evaluated in preclinical studies. Since epigenetic factors also contribute to the disease, in addition to targeting signaling pathways, and treatments with tyrosine kinase inhibitors, several agents also target epigenetic regions. DNA methyltransferases (DNMTs), enzymes responsible for histone modifications (acetylation, methylation and deacetylation), can be also targeted [3].

The anti-GD2 monoclonal antibody dinutuximab (Unituxin) is the first treatment approved by the Food and Drug Administration (FDA) in the treatment of a high-risk NB. Multiple immunotherapy strategies continue to be developed for the treatment of NB and may have a place in the treatment of relapsed high-risk NB disease [4].

Aurora Kinases

Aurora kinases belong to a small family of proteins

made up of triple serine-threonine kinases (Aurora A, B and C). They are very important molecules for the stage of mitosis, as they play a role in the maturation of centrosomes, separation of chromosomes and cytokinesis. Aurora kinases play a role in the initiation and progression of many tumorigenic processes by dysregulating the phosphorylation of H3, a histone protein, and tumor suppressor p53. They are overexpressed in many human tumors, including 50% of colorectal, ovarian, and stomach cancers. With the overexpression of aurora kinases, multiple centrosomes and multipolar spindle filaments are formed in the cell. Thus, genetic instability occurs, which contributes to tumor formation [3].

In humans, the aurora kinase family consists of 3 members; Aurora A, B, and C. Each of these shares a conserved C-terminal catalytic domain but differ in their intracellular localization, substrate specificity, and functions during mitosis. Aurora A binds to TPX2 at the start of mitosis and is activated by autophosphorylation at threonine 288 (T288). Immediately after its activation, Aurora A phosphorylates multiple substrates that regulate centrosome maturation and mitotic spindle strands. Aurora B is part of the chromosomal passenger complex (CPC) together with the centromere protein, survivin and borealin. Aurora B is activated through binding and phosphorylation of the centromere protein in a positive feedback mechanism. Since phosphorylation of histone H3 by Aurora B at serine 10 (S10) and serine 28 (S28) is required for condensation of the chromosome, inhibition of S10 phosphorylation is widely used as a biomarker for Aurora B inhibition in vitro and in vivo settings. Aurora C also plays a role in chromosomal structure like Aurora B, but its expression site is limited to the testicles [4].

AURKA gene encodes Aurora kinase enzymes. It has been shown that AURKA is widely overexpressed in various tumors, including NB. Overexpression of AURKA has been associated with a poor prognosis. In addition, overexpression of AURKA is closely related to overexpression of MYCN. Studies have shown that AURKA can form a complex with MYCN to stabilize the MYCN structure and prevent its degradation, while inhibition of AURKA activity can promote the disruption of the stabilization of MYCN. Thus, targeting AURKA therapeutics not only improves the effect of NB therapy by inhibiting the activity of AURKA, but also achieves the goal of lowering the expression levels of the MYCN protein [5].

An agent named MLN8237 (alisertib), an Aurora Kinase A inhibitor, has been studied in preclinical models and in phase-I of relapsed/refractory solid tumors. In studies using single and double doses, dose-limiting toxicities stemming from mucositis and myelosuppression were noted with double dosing. Recommended dosage for Phase-II has been found to be 80 mg/m^2/day. Although the response rate seems low when used as a single agent, based on preclinical data, MLN8237 works synergistically with irinotecan and temozolomide and therefore this combination is studied in relapsed or refractory cases. This is a significant combination with an overall response rate of 31.8% in phase 1 trials, but currently the use of this agent in NB has not been investigated in any ongoing study [6].

Thus, instead of targeting Aurora A alone, the use and combination of pan-Aurora inhibitors can provide a more comprehensive antitumor effect. The potent activity of Tozasertib (VX680,MK-0457), a pan-Aurora inhibitor, has been revealed in drug-resistant NB cell lines The findings, supported by the studies conducted, reveal that Aurora inhibitors are effective as monotherapy or in combinations with other agents, in the stabilization and post-translational inhibition of N-MYC. Anti-tumor effects have also been tested in in vivo NB models. While inducing permanent tumor regression in multiple tumor xenograft models, even more significant results have been observed with anti-tubule chemotherapeutics [7].

PI3K / AKT / mTOR Pathway

Abnormal activation of the PI3K/AKT/mTOR pathway has been demonstrated in NB which plays an important role in stabilization of N-MYC. The mTOR signaling pathway is critical for cell growth, proliferation and survival. It is also known to have a

central role in a signaling pathway consisting of many components involved in tumorigenesis including mTOR, PI3K, AKT, PTEN, TSC1/TSC2, p53, LKB1 and downstream proteins S6K1, eIF4E, FOXO. Many studies have revealed that the genetic regulation of mTOR may also play an antitumor role in pediatric NB through upregulation of tumor suppressor proteins PTEN, FOXO, TSC1/TSC2, p53 and LKB1 or downregulation of oncogenes in the pathway [8].

Phosphorylation (55.2%) of the S6 ribosomal protein, which is the target of mTOR, was observed in NB samples [8]. This evidence of mTOR activity in cellular processes that contribute to the development and progression of NB has established mTOR as the main link in tumorigenesis in NB.

Preclinical data support the essential role of mTOR in cancer; Thus, agents targeting the activity of key proteins in the mTOR signaling pathway could be a potential therapeutic method in the treatment of NB. As a result, mTOR inhibitors, mTOR kinase inhibitors, and inhibitors of mTOR regulators have been developed and evaluated for their safety and efficacy in patients with cancer, including NB. Analogues targeting mTOR, including rapamycin, temsirolimus, everolimus, and ridaforolimus, affect mTORC1 with a similar mechanism of action [9,10].

A New Treatment Perspective with Drug Delivery Systems in NB

Drug delivery systems have long been studied as a way of delivering cytotoxic chemotherapy directly to a tumor or other site of action, while potentially reducing systemic side effects [11]. Nanodrug delivery systems provide promising strategies for cancer chemoimmunotherapy because they are easily internalized by cells of the immune system and can rearrange the tumor microenvironment due to their specific physical and chemical properties, thereby reinforcing the immune system. These systems can maximize the solubility of the chemotherapeutic agents used and their bioavailability to the body, prolong the circulation time of the agents in the body with passive or active targeting, increase the accumulation of agents in tumor tissue, and improve in vivo pharmacokinetic behavior, leading to increased therapeutic effects and decreased side effects [12].

In drug delivery systems applied in chemoimmunotherapy, basically several approaches exist for delivering more than one agent to the target tissue in combination. If more than one agent is to be administered in combination, one agent can be administered in free form and the others can be administered with a drug (Free Drug + Nano) absorbed in the nanoparticle (NP) or both can be administered with similar or different NP (Nano + Nano) or both agents are co-encapsulated (co-encapsulation). “Free drug + Nano” approach is one of the most up-to-date approaches in the current treatment of cancer in nanomedicine. “Free drug + Nano” approach has shown favourable properties such as adjustable dose, controllable application range, easy preparation, facilitated industrial preparation and clinical orientation. These approaches basically involve two strategies. The first is that the immunotherapeutic agent can be administered in appropriate NPs and the chemotherapeutic drug is administered in its free form. Yong Taik Lim et al. [13] designed two poly lactic-co-glycolic acid (PLGA) NPs combined with a chemotherapeutic agent, paclitaxel (PTX). One of them is CpG- loaded PLGA-NPs (PCNs), the other is PLGA NPs (PINs) loaded with IL-10 miRNA designed primarily to suppress IL-10. In this study by Yong Taik Lim et al, it was found that PTX treatment followed by PCNs and PINs can increase the antitumor effect

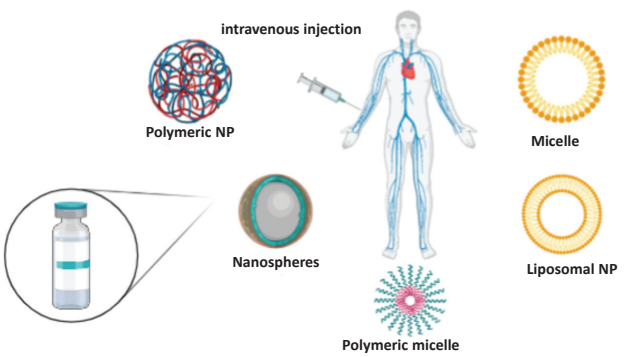

Figure 1. The most commonly used NPs in cancer immunotherapy and intravenous administration.
It is designed in the BioRender application.

and survival rate of the chemotherapeutics used in murine melanoma models carrying B16 F10 compared to PTX alone ($p<0.05$). Another "Free drug + Nano" approach is designed to give the immunotherapeutic agent in its free form and to load the chemotheraprutic drug onto the NP. The "Nano + Nano" approach can be flexible in the design and formulation of NPs. Besides, the application dosage can be adjusted and the coordinated distribution of the two agents can be realized [14].

Engineered NPs can be in different forms. Of these; chitosan is a natural carbohydrate polymer Derived from deacetylation of chitin, it is considered suitable for pharmaceutical applications due to its low price, high biocompatibility, low toxicity and ability to be degraded by chitinases in the body. During the production of chitosan-based NPs, toxic organic solvents or heat are not needed and they can be dissolved in acidic aqueous solutions. Small molecules, proteins and polynucleotides can be added to chitosan/NP. Chitosan can release the encapsulated drug in a controlled manner. Silica xerogels are in the class of inorganic molecules used in drug transport. They are biocompatible and also easily modifiable. Polylactide-co-glycolic acid (PLGA) is a copolymer of polylactic acid (PLA) and polyglycolic acid (PGA) is synthesized by ring-opening polymerization of lactide (LA) and glycolide (GA). The use of PLGA in drug transport in biomaterials provides many advantages. First, it has already been approved by the US Food and Drug Administration (FDA) and the European Medical Agency (EMA) for use in the human body. Also, as a synthetic polymer, it has higher purity, suitable molecular weight, and higher reproducibility than many natural polymers. In addition, PLGA is biodegradable, when it undergoes hydrolytic cleavage in the body. It is reduced to lactic acid and glycolic acid and metabolized through the Krebs cycle into CO_2 and water. Compared to PLA and PGA, the copolymer is more stable to hydrolysis and can therefore be used for drug release that lasts for days, weeks or months [14].

Polymer micelles are thermodynamically stable colloidal solutions formed by combining amphiphilic block copolymers. Drugs in the form of hydrophobic small molecules can be encapsulated in the hydrophobic core of micelles. In addition, hydrophilic drugs can be loaded onto the NP through physical interactions or chemical conjugation. Paclitaxel loaded with Genexol® and docetaxel loaded with Nanoxel (DTX) are NPs approved for cancer treatment. Polymer micelles are widely used in cancer chemoimmunotherapy. Dendrimer NPs are hyperbranched spherical polymers composed of a hydrophobic central core, branched monomer, and functional peripheral groups. Small molecular drugs can be loaded onto the nucleus. In addition, the functional peripheral group in the dendrimer chemically binds immunotherapeutic agents such as therapeutic antibodies. Currently, commonly used dendrimers are polyamidoamine (PAMAM), polypropyleneimine (PEI) and peptide dendrimers [12].

Nanogels are another structure in which chemotherapeutic agents are frequently loaded. Nanogels containing a nano-sized hydrogel scaffold are biocompatible and have a high water content. They are compatible with a variety of therapeutic agents and considered as promising toosl in chemoimmunotherapy [14].

Treatment Strategies with NB Targeted NPs and Using Nano-Technology

Drug delivery strategies play an important role in the treatment of NB. Being able to deliver the drug more effectively, earlier and with a longer-lasting effect can be an effective role in preventing the disease. Today, in liposomal systems, which are one of the most important drug delivery systems, different applicable liposomal drug delivery strategies for NB treatment are available All contain a drug or compound encapsulated in a liposome embellished with different molecules or ions capable of targeting a particular molecule on or in the NB.

Apart from liposomal systems, CNTs, drug loaded silk films, amphiphilic diblock polymers and different types of NPs, especially PEGylated PLGA co-polymers, can be engineered by targeting ligands common in the NB and drug delivery, which can efficiently

deliver the chemotherapeutic agent to the target site. systems. Different drug transport systems have been tried in NB. Among these trials in a study on encapsulation of siRNAs, it was aimed to encapsulate MYCN siRNA in a liposome coated with folate molecules. As it is known, overexpression of MYCN is associated with poor prognosis in NB. Therefore, a specific siRNA has been used to silence this gene to a certain extent. Folate is a low- weight molecule that is highly absorbed by tumor cells to aid in division compared to normal cells.

In this case, due to the surface conjugation of the engineered NPs with folate, the tumor can enter more easily through overexpressed folate receptors. With this method, researchers have been more successful in silencing MYCN and apoptosis and have shown that this is an effective method for drug transport in NB. TNF-α is an inflammatory cytokine that can affect vascular permeability and angiogenesis. With this feature, a treatment strategy has been developed by targeting TNF-α in drug loaded nano liposomes to be used before chemotherapy.

CD13 is highly expressed in NB. The NGR peptide is a suitable ligand for CD13 targeting tumor vascularization. Studies have shown that the combination of TNF - α and NGR can cross the barriers to drugs, access into the tumor and help deliver a higher drug concentration to the tumor. Therefore, designing a nano-liposome targeting CD13 by NGR containing a chemotherapeutic agent (eg doxorubicin) would result in better drug delivery and greater drug uptake. GD2 is expressed in neural crest-derived tumors and peripheral nerve cancer cells, and targeting this molecule with antibodies so as to deliver drugs to the target more effectively has been the subject of recently performed studies.

Coating liposomes with hydrophilic agents reduces cellular drug uptake outside the target sites, slows down the escape from the immune system, and decreases the total drug dose needed, resulting in a higher level of free drug in the bloodstream compared to the conventional drug delivery systems. Therefore, the strategy to engineer a nano - immunoliposome, typically 10-200 nm in diameter, that encapsulates the chemotherapeutic drug, coated with a hydrophilic agent such as polyethylene glycol (PEG), and decorated with an antibody against a tumor target such as anti-GD2, has high efficacy in the treatment of NB, and can be considered as an effective complex [15].

Inhibitory agents specific to mTOR and Aurora pathways, which are directly related to the N-MYC pathway and are involved in N-MYC stabilization, can be targeted to GD2, an antigen receptor on the surface of the NB cell, with PEGylated co-polymers. Although there is information in the literature regarding the use of both agents together in different cancer types, there is no study conducted in NB [16].

CONCLUSION

Apart from all the results obtained in NB treatment, based on clinical and preclinical knowledge, treatment of neuroblastoma is managed by combined use of chemotherapy and immunotherapy. Molecules used in immunotherapy are being combined with drugs used in conventional chemotherapy. It is anticipated that combined use of agents will maximize the anti-tumor effects of the agents via a synergistic mechanism. Nano-carriers are being used with the aim to increase the effectiveness of the agents used in targeted NB treatment strategies. Chemotherapeutic and chemoimmunotherapeutic agents loaded onto NPs created by using many molecules have positive effects such as increasing solubility of the drugs and prolonging their bioavailability in the body. This treatment strategy has also several advantages as increased circulation time in the body, targeting to a specific site, and improved pharmacokinetic behavior of the drug.

All of these treatment strategies are critical to improving outcomes in high-risk NB patients. Research areas in rapidly expanding cancer genomics, immunotherapy fields, nano-carriers and targeting with nano-technological molecules are promising possibilities for the future treatment of NB.

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Research Article

Somatic Growth Features of Moderate-Late and Early Preterm Infants at Early Childhood

Erken Çocukluk Döneminde Orta ve Geç Prematüre Bebeklerin Somatik Büyüme Özelliklerinin Erken Prematüre Bebeklerle Karşılaştırılması

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ABSTRACT

Objective: *The aim of this study was to compare the physical growth features of preterms infants at early childhood and to evaluate the perinatal and postnatal risk factors that affect growth.*
Method: *Somatic growth features including weight, height, and target height, of moderate-late and early preterm infants followed-up in our unit were evaluated at a mean age of 42 months. The effects of intrauterine growth characteristics (IUGC) on current growth as well as the effects of perinatal and early neonatal problems were investigated.*
Results: *A total of 232 moderate-late preterm infants (Group I) (mean gestational age [GA]: 34.9±1.2 weeks) and 112 early preterms (Group II) (mean GA: 29.9±1.6 weeks) were evaluated. The ratio of retardation in height was 2.6 % and 6.3% in Group I and Group II, respectively. The rate of failure in achieving the target height was higher in Group II (3.9% vs 8%). Growth differences in terms of height and weight were not significantly different between the two groups (p>0.05). Severe intraventricular hemorrhage (IVH), hydrocephalus and invasive mechanical ventilation requirements negatively affected the growth in height and reaching the target height (p<0.05).*
Conclusion: *The moderate-late and early preterm infants had similar growth features in terms of weight, height, and target height when evaluated at 42 months of age.*

Keywords: *Moderate-late preterms, early preterms, growth, target height, early childhood*

ÖZ

Amaç: *Çalışmamızın amacı çocukluk çağında orta-geç ve erken prematürelerin fiziksel büyüme özelliklerini karşılaştırmak ve büyümeyi etkileyen perinatal ve postnatal risk faktörlerini değerlendirmek idi.*
Yöntem: *Ünitemizde takip edilen orta-geç ve erken preterm bebeklerin kilo, boy ve hedef boy gibi somatik büyüme özellikleri ortalama 42 ayda değerlendirildi. İntrauterin büyüme özelliklerinin mevcut büyüme üzerindeki etkileri ile birlikte perinatal ve erken neonatal problemlerin etkileri de araştırıldı.*
Bulgular: *232 orta-geç preterm bebek (Grup I) (ortalama gestasyonel yaş: 34.9±1.2 hafta) ve 112 erken preterm bebek (Grup II) (ortalama gestasyonel yaş: 29.9±1.6 hafta) değerlendirildi. Büyüme kısıtlılığı oranı Grup I ve Grup II'de sırasıyla %2.6 ve %6.3 idi. Hedef boya ulaşamama oranı Grup II'de yüksekti. (%3.9 & %8). Her iki grup arasında boy ve kilo açısından büyüme farklılıkları istatistiksel olarak anlamlı değildi. Ağır intraventriküler hemoraji (IVH), hidrosefali ve invaziv mekanik ventilasyon gereksinimi boy uzamasını ve hedef boya ulaşmayı olumsuz etkilemekteydi.*
Sonuç: *Orta-geç ve erken pretermler ortalama 42 ayda değerlendirildiğinde kilo, boy ve hedef boy açısından benzer büyüme özelliklerine sahipti.*

Anahtar kelimeler: *Orta-geç preterm bebekler, erken preterm bebekler, büyüme, hedef boy, erken çocukluk dönemi*

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INTRODUCTION

Prematurity is the most important issue in the current neonatal care system and remains a long-term health and neurodevelopmental problem. An increasing proportion of preterm newborns, especially moderate-late preterms ($32^{0/7}$-$36^{6/7}$ weeks), have higher rates of neonatal unit admissions compared to full-term newborns. The major problem for this risk group is a high mortality rate in the short-term and neurodevelopmental and somatic growth retardation in the long-term.

Preterm newborns are not a homogeneous group. Moderate (delivery between $32^{0/7}$ and $33^{6/7}$ weeks) and late preterm babies (delivery between $34^{0/7}$ and $36^{6/7}$ weeks) are associated with adverse short-term and long-term outcomes [1-3]. Moderate-late preterms babies constitute 4.4%-10% of all deliveries [4]. The groups that faces more risks in both aspects include early preterms (born prior to 32 weeks), including very early preterms (delivery between 28 and 32 weeks) and extremely early preterms (delivery before 28 weeks). Most early preterms also have a very low-birth-weight (VLBW). Compared to early preterms, moderate-late preterm infants are at a lower risk of numerous medical problems. However, somatic growth retardation, neurodevelopmental retardation and behavioral disorders are observed in the long-term follow-up of moderate-late preterms [5-7]. Some studies have focused on the somatic growth in early preterms and their neurodevelopment [8-11]. Moreover, there are a lot of studies on the neurodevelopment of late-moderate preterms [12]. However, as far as we know, there are no study in the literature that compare the long-term somatic growth between moderate-late preterms and early preterms in our country.

In this study, we aimed to compare the somatic growth features of moderate-late preterms and early preterms, where the adjusted age was not used, and instead, a chronological age of 42 months was used. Additionally, the risk factors, including the early and late neonatal morbidities that affect their growth features were compared between both groups.

MATERIAL and METHOD

This study was conducted on preterm infants who were born as moderate-late preterms (range of gestational age was $32^{0/7}$-$36^{6/7}$ weeks), according to the Ballard score [13], and early preterms (born prior to 32 weeks). The moderate-late preterms were included in Group I and the early preterms in Group II.

All subjects were born in our hospital and discharged from our tertiary level neonatal intensive carrier unit (NICU). All the discharged infants continued to have follow-ups in our out-patient clinics. Both the groups' growth features were evaluated at a mean age of 42 months.

The patients' data including prenatal risk factors and early neonatal morbidities were obtained from the NICU records in our hospital. The intrauterine growth properties were evaluated by the intrauterine growth curve for Turkish children [14]. A percentile value of 10-90th percentile on the growth chart was considered Appropriate for Gestational Age (AGA) and a percentile value below the 10th percentile was considered Small for Gestational Age (SGA). The perinatal risk factors that have influence on growth in both groups were identified as maternal history of smoking, premature rupture of membranes (PROM), receiving antenatal steroids, newborns' history of resuscitation in the delivery room, and a low APGAR score (at the 5th minute <6). Early neonatal morbidities during NICU stay admission, including respiratory distress syndrome (RDS), invasive mechanical ventilation, early and/late onset sepsis, hypoglycemia, anemia, meningitis, bronchopulmonary dysplasia (BPD), seizure, surgical necrotizing enterocolitis with perforation (advanced stage NEC), moderate-severe intraventricular hemorrhage (grade III and IV IVH) with/without hydrocephalus and retinopathy of prematurity (ROP) were recorded.

A written informed consent was taken from all parents of the participants' and approval for the study was granted by the local ethics committee. (Bakırköy Women's and Children's Diseases Education and Research Hospital Ethics Committee, Decision no:25/06/09/270).

The infants' data of growth features were

obtained from the outpatient follow-up clinic records. A Harpenden Stadiometer device was used to measure the subjects' height. The subjects were weighed as naked on a digital infant scale. The actual height (cm) and weight (kg) measurements of the subjects were recorded and marked on the percentile curves for the Turkish children [15]. During this process, the adjusted age calculation was not made, because all the subjects were older than 36 months. To predict the final height of the subjects and the familial effects on it, we used the target height formula prepared for the Turkish population [15-18]. The subjects who had congenital anomalies, died after discharge, and/or did not continue follow-up regularly were not included in the study.

Statistical Analysis

Statistical analyses were made with the SPSS (Statistical Program in Social Sciences) version 15.0 package program. To test the normal distribution of variables, visual (histogram and probability graphics) and analytic methods (Kolmogorov-Smirov/Shapiro-Wilk tests) were used. Clinical characteristics of the infants are described by mean values and standard deviation, or rates and percentage. Univariate statistical analysis was performed using the Student t test for parametric continuous variables, Mann-Whitney U test test for nonparametric continuous variables, and Fisher exact test or Chi-square test for categorical variables. A p-value <0.05 was considered statistically significant.

RESULTS

Group I and Group II consisted of 232 and 112 infants, respectively. The gestational age was 34.9±1.2 (range 32-36) weeks in Group I and 29.9±1.6 (range 26-31.6) weeks in Group II. The chronological ages of the groups were 42.8±1.6 (range 37–45) months and 42.9±2.2 (range 37-46) months. The demographic data of the subjects, including gender, intrauterine growth properties, birth weight, actual height and weight, target height, and their parents' height are summarized in Table 1.

Among the perinatal risk factors, receiving antenatal steroid, being underwent resuscitation, and low APGAR scores were significantly high in Group II, in accordance with our expectation. Furthermore, early neonatal morbidities, such as anemia, RDS, invasive mechanical ventilation support requirement, sepsis, hypoglycemia, meningitis, seizure, surgical NEC, moderate-severe IVH, BPD, and ROP were significantly higher in Group II, as expected (Table 2).

The height growth remained below the 3rd percentile in 6 (2.6%) of the Group I subjects and in 7 (6.3%) of the Group II subjects. However, the difference between groups was not statistically significant (Table 3). Nine (3.9%) of the late preterm infants and 9 (8%) of the early preterm infants could not achieve the target height percentile, but the difference was not statistically significant (Table 4). Additionally, the effects of gender and intrauterine

Table 1. Demographic data of the subjects in both groups.

	Group I (n=232)	Group II (n=112)	p
Female [n (%)]	120 (51.7%)	64 (57.1%)	0.32
Male [n (%)]	112 (48.3%)	48 (42.9%)	0.12
Gestational Age (w) [mean±SD (range)]	34.9±1.2 (32-36)	29.9±1.6 (26-31.6)	0.56
Birth Weight (g) [mean (range)]	1826.3±373.5 (840-3550)	1191.8±264.5 (740-1980)	0.02
AGA [n (%)]	194 (83.6%)	83 (74.1%)	0.63
SGA [n (%)]	38 (16.4%)	29 (25.9%)	0.42
Chronological Age (months) [mean±SD (range)]	42.8±1.6 (37-45)	42.9±2.2 (37-46)	0.74
Current Height (cm) [mean±SD (range)]	100±3.5 (92-106)	97.7±5.9 (85-106)	0.45
Current Weight (kg) [mean±SD (range)]	16.5±1.4 (13-19)	15.1±2.7 (10-19)	0.55
Maternal Height (cm) [mean±SD (range)]	160±4.7 (149-175)	160.8±4.7 (149-176)	0.76
Paternal Height (cm) [mean±SD (range)]	175.5±5.3 (155-192)	176.2±5.6 (160-192)	0.81
Target Height (cm) [mean±SD (range)]	168.4±7.3 (148.5-187)	167.6±7.4 (151-186.5)	0.86

AGA: Appropriate for Gestational Age, SGA: Small for Gestational Age.

Table 2. Distribution of the neonatal morbidities of the subjects.

	Group I (n=232) n (%)	Group II (n=112) n (%)	p value
Hyperbilirubinemia	46 (19.8)	29 (25.9)	>0.05
Anemia	72 (31)	63 (56.3)	<0.05
RDS	33 (14.2)	33 (29.5)	<0.05
Sepsis	41 (17.6)	28 (25)	<0.05
Hypoglycemia	29 (12.5)	21 (18.8)	<0.05
Meningitis	19 (8.2)	16 (14.3)	<0.05
Post hemorrhagic hydrocephalus	2 (0.9)	4 (3.6)	<0.05
BPD	6 (2.6)	13 (11.6)	<0.05
Convulsion	9 (3.9)	11 (9.8)	<0.05
>Stage III ICH	4 (1.7)	7 (6.3)	<0.05
>Stage III NEC	15 (6.5)	12 (10.7)	<0.05
>Stage III ROP	2 (0.9)	7 (6.3)	<0.05

RDS: Respiratory distress syndrome BPD: Bronchopulmonary dysplasia, NEC: Necrotizing enterocolitis, ROP: Retinopathy of prematurity, ICH: Intracranial Hemorrhage.

Table 3. Comparison of the somatic growth of the subjects according to the percentile curve.

	Group I (n=232)		Group II (n=112)		
	≤3rd Percentile n (%)	>3rd Percentile n (%)	≤3rd Percentile n (%)	>3rd Percentile n (%)	p value
Height	6 (2.6)	226 (97.4)	7 (6.3)	105 (93.8)	>0.05
Weight	13 (5.6)	219 (94.4)	11 (9.8)	101 (90.2)	>0.05

growth features on the target height were not significant in both groups (Table 4).

The effects of SGA, AGA and gender on weight and height at 42 months were similar between the groups. There was no significant difference between the groups in terms of the relationship between the diagnosis of RDS, BPD, sepsis and hypoglycemia and reaching the target height (Tablo 5). However, the

Table 4. The effect of intrauterine growth characteristics and gender on growth in height and target height achievement in both groups.

	Group I (n=232) Cases That Could Not Achieve the Target Percentile n (%)	Group II (n=112) Cases That Could Not Achieve the Target Percentile n (%)	p value
Height	9 (3.9)	9 (8)	>0.05
Female	5 (2.2)	5 (4.5)	>0.05
Male	4 (1.7)	4 (3.6)	>0.05
AGA	3 (1.3)	4 (3.6)	>0.05
SGA	6 (2.6)	5 (4.5)	>0.05

AGA: Appropriate for Gestational Age, SGA: Small for Gestational Age.

Table 5. The effects of the main neonatal morbidities on the target height in both groups.

	Group I (n=232) Cases That Could Not Achieve the Target Percentile n (%)	Group II (n=112) Cases That Could Not Achieve the Target Percentile n (%)	p value
RDS	5 (2.2)	8 (7.1)	>0.05
BPD	4 (1.7)	7 (6.3)	>0.05
Sepsis	3 (1.3)	5 (4.5)	>0.05
≥Stage III NEC	3 (1.3)	4 (3.5)	>0.05
Hypoglycemia	4 (1.7)	8 (3.5)	>0.05
≥Stage III ICH	3 (1.3)	7 (6.3)	<0.05
Hydrocephalus	2 (0.9)	3 (3.6)	<0.05
Follow-up in ICU	5 (2.2)	8 (7.1)	>0.05
Mechanical Ventilatory Support	3 (1.3)	9 (8)	<0.05

RDS: Respiratory Distress Syndrome, BPD: Bronchopulmonary Dysplasia, NEC: Necrotizing Enterocolitis, ICH: Intracranial Hemorrhage, ICU: Intensive Care Unit.

subjects who could not achieve the target height had a significantly higher rate of invasive ventilation, and moderate to severe IVH and hydrocephalus in Group II compared to Group I (Table 5). In addition, the need for mechanical ventilation, and the frequency of IVH, BPD and hydrocephalus were higher in the infants with a height and a weight below the 3th percentile at 42 months. When growth in weight was evaluated, 13 (5.6%) of the late preterm infants and 11 (9.8%) of the early preterm infants were found to be below the 3rd percentile; however, the difference between the groups was not significant (Table 3).

DISCUSSION

To the best of our knowledge, this is the first study from our country that investigates and compares the growth characteristics of infants who were older than 3 years and those who were born as early and moderate-late preterms and also prenatal, postnatal and genetic factors affecting on them. According to our results, the growth features, including weight, height, and achieving target height were similar between the late and early preterms at postnatal 42 months of age. Moderate-severe IVH, hydrocephalus and invasive mechanical ventilation support had negative effects on the target height in both groups.

There are many studies that support the negative effects of early neonatal morbidities on the growth characteristics of preterms who have perinatal risks, especially when they were assessed according to their corrected age. The effects of intrauterine growth retardation on postnatal growth has been emphasized in some studies. On the contrary, there are some authors who advocate that intrauterine growth characteristics do not affect the growth features of preterms. They suggest that preterms achieve the normal growth percentile up to the age of two years if there are no perinatal complications and no severe congenital anomalies and if optimal postnatal care was given [8,19]. Since the growth properties of preterms, especially after the age of 3 years, are shaped by the effects of genetic and environmental factors, the effects of early neonatal problems, gender, and being AGA and SGA are limited. In support of this knowledge, the present study revealed that gender and intrauterine growth features do not affect all the growth features, including those of both moderate-late and early preterms when they are 42 months old.

According to the studies covered in the earlier period on first and second years of corrected age [20], the growth properties of preterms and term/late preterm newborns are different [21-23]. Koc et al. [11] reported growth retardation as 6% in height and 7% in weight in school-age premature children. Severe neurodevelopmental problems were described by 80% of the cases with growth retardation in height, while there was no such a relationship in retardation in weight. In a study by Sütçüoglu et al. [10], growth retardation was reported as 20.5-27.8% in height and 32.8%-24.6% in weight in VLBW premature infants at 18-20 months. In the same study, neurodevelopmental retardation was stated to be the most common cause of retardation in height. Toome L et al. [27] compared premature infants born before the 32th week of gestation with 2 years old term infants and emphasized that somatic growth was a statistically significant problem in premature infants. In a study from our country by Yesinel S et al. [9], in which adjusted age was used, the results showed that the intrauterine growth characteristics, being SGA or AGA, and gender did not affect the growth properties of VLBW preterms at the mean age of 36 months. Their results showed that invasive mechanical ventilation support, presence of chronic diseases, severe IVH, posthemorrhagic hydrocephalus, and lack of breastfeeding negatively affected the growth in height and target height achievement as well. The effect of gender on preterms' growth features have also been studied in the literature. A study that focused on the growth features of SGA babies showed that male preterms had a higher rate of failing to catch-up on growth (for both weight and height) postnatally up to the age of 4 years (boys 11%, girls 5%) [21]. Some of the factors mentioned above, especially severe IVH, hydrocephalus, and invasive mechanical ventilation support requirements, have negative effects on the growth in height and

achievement of target height in preterms. The effect of gender on growth was not observed in both groups in our study. Problems with reaching the target height in very small premature babies are more common because they are more exposed to such problems related to prematurity. One of the important problems that affect growth is the presence of chronic disease, especially BPD and hydrocephalus. They cause chronic hypoxia, recurrent hospitalizations and surgery, infections, nutritional problems, etc. Finally, growth is affected negatively [24,25]. The growth retardation rate can reach to 10% in cases with BPD and hydrocephalus [9,26]. In our study, BPD did not affect the growth in both groups, but an advanced stage IVH and hydrocephalus negatively affected the growth in height in both the groups.

One of the most important factors that affect physical growth is inheritance. A child who grows up normally reaches the percentile appropriate for his/her genetic potential after 6-12 months and the growth in height after 2-3 years and correlates with parental height. Therefore, it is important to determine the percentile of the child's growth in height and its compatibility with the target height to calculate the final height [16-18]. Our results showed that moderate-late and early preterms have similar growth features in terms of height catch-up and also achievement of the target height. The findings of our study suggest that growth after the age of 2-3 years shows correlation to genetic and environmental factors, as mentioned before. Severe IVH, hydrocephalus and mechanical ventilation support requirements have a negative effect on the growth in height and achievement of target height in preterms of both groups.

We can conclude that moderate-late preterms and early preterms have similar growth features, including weight, height, and target height, when they reach the early childhood period. The prenatal risk factors, including intrauterine growth features and postnatal morbidities, especially IVH and/or hydrocephalus, and invasive mechanical ventilation, affect the growth features of preterms when they are evaluated at the early childhood period.

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Research Article

A New Experimental Allergic Rhinitis Model in Mice

Farelerde Oluşturulan Yeni Bir Alerjik Rinit Modeli

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ABSTRACT

Objective: *Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa mediated by IgE after exposure to an allergen. The most well known related comorbidity of AR is asthma. This study was planned due to the need for an animal model for studies on AR-asthma coexistence. In this study, the frequency of AR accompanying in the asthma model created in mice,and the usability of the related model in AR studies will be investigated.*
Methods: *In our study, 6-8 week-old, 18-20 g BALB/c mice were used. Chicken egg ovalbumin (OVA Grade V, Sigma) was administered through intraperitoneal (IP) route at doses of 10 µg on days 0 and 14. Mice were exposed to aerosolized 2.5% ovalbumin solution in sterile saline for 30 minutes 3 days a week for 8 weeks, starting 7 days after the last IP administration (21st day). After exposure to OVA, mice were observed for typical signs of AR including sneezing, runny nose, and nasal itching. The final diagnosis of AR was made by histopathological examination of the rhinotracheal tissues of mice.*
Results: *In our study, all mice exposed to ovalbumin received histopathologic diagnosis of AR. Increased number of capillaries lymphocytes, polymorphonuclear leukocytes and eosinophilsper square millimetre of rhinotracheal tissues were calculated in the murine model of AR compared to the the control group.*
Conclusion: *This study introduced a new AR model, not cited in the literature, and induced with the longest-term ovalbumin exposure in the literature. It was concluded that this model, known as the asthma model, can also be used to induce an AR model and can be used in studies investigating coexistence of allergic rhinitis and asthma.*

Keywords: *Allergic rhinitis, asthma, mouse*

ÖZ

Amaç: *Alerjik rinit (AR), allerjenle karşılaştıktan sonra IgE aracılığı ile oluşan burun mukozasının inflamatuvar hastalığıdır. AR' in en iyi bilinen ve üzerinde en fazla durulan ilişkili komorbiditesi astım'dır. AR-astım birlikteliği üzerine yapılacak çalışmalar için hayvan modeli ihtiyacından dolayı bu çalışma planlanmıştır. Bu çalışmada farelerde oluşturulan astım modelinde AR'in ne sıklıkta eşlik ettiği araştırılacak olup, ilgili modelin aynı zamanda AR çalışmalarında da kullanılabilirliği araştırılacaktır.*
Yöntem: *Çalışmamızda 6-8 haftalık 18-20 gr ağılığında, BALB/c fareler kullanılmıştır. Farelere 0. ve 14. günlerde 10 µg intraperitoneal tavuk yumurtası ovalbumini (OVA Grade V, Sigma) uygulanmıştır. Son uygulamadan 7 gün sonra (21. gün) başlamak üzere, günde 30 dakika süre ile haftanın 3 günü, 8 hafta boyunca steril serum fizyolojik içindeki %2,5'lik ovalbumin solüsyonundan oluşan aerosol inhale ettirilmiştir. İnhalasyon uygulamaları tüm vücut inhalasyon sistemi ile yapılmıştır. OVA'ya maruz kaldıktan sonra fareler hapşırma, burun akıntısı, burun kaşınması dahil AR'nin tipik belirtileri açısından gözlenmiştir. AR kesin tanısı farelerin rinotrakeal materyallerinin histopatolojik incelenmesi ile konulmuştur.*
Bulgular: *Çalışmamızda ovalbumine maruz bırakılan tüm fareler histopatolojik olarak AR tanısı almıştır. Bu farelerin rinotrakeal dokusunda kontrol grubundaki farelerin rinotrakeal dokusuna kıyasla AR lehine milimetrekarede artmış kapiller sayısı, lenfosit, polimorfonükleer lökosit ve eozinofil sayısı saptanmıştır.*
Sonuç: *Bu çalışmanın literatürde en uzun süreli ovalbümin maruziyeti ile oluşturulan yeni bir AR modeli olduğu saptanmıştır. Astım modeli olarak bilinen bu modelin AR oluşturulmak için de kullanılabileceği ve astım-AR birlikteliği üzerine yapılacak çalışmalarda bu modelin kullanılabileceği sonucuna ulaşılmıştır.*

Anahtar kelimeler: *Alerjik rinit, astım, fare*

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INTRODUCTION

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa occurring after exposure to the allergen and mediated by immunoglobulin E (IgE) [1]. The prevalence of AR in the world is 10-25% in adults and up to 40% in children, and millions of people have allergic rhinitis [2]. The most well known and emphasized related comorbidity of AR is asthma, and up to 85% of asthmatic patients have AR [1]. AR and asthma are comorbidities that occur as a result of inflammation of the respiratory tract mucosa associated with similar cells and mediators, but the mechanisms by which AR and asthma occur in the same patient are still unclear.

As far as is known, in AR, the antigen binds to the IgE receptors and causes the release of histamine, prostaglandin, leukotriene and protease from the mast cells, revealing inflammation that will cause runny nose, sneezing, nasal itching, and congestion [3]. This binding to IgE receptors in asthmatic patients creates acute bronchoconstriction in smooth muscles of the lower respiratory tract.

Animal models of AR are extremely important for research studies on AR. To date, guinea pigs, mice, rats and rabbits have been used in animal models of AR [4-8]. Firstly, guinea pigs were used, and BALB/c type mice have been used mostly in recent years [4]. The reasons why mice are used in AR models are that they produce human-like IgE and are relatively cheap [9]. In various studies ovalbumin, pollen, dust mites, fungi, and schistosoma antigens have been used as allergens [4,6]. These agents were also administered as nasal drops, inhalation or multipoint subcutaneous injections to increase sensitivity [4].

In our study, the animal model, which was created by Temelkovski J. et al. [10] in BALB/c type mice and defined as an asthma model, was used. Our aim was to investigate whether AR also coexisted in these asthma-induced mice and whether the asthma model induced can also be used as an AR model in future studies. At the end of this study, the frequency of coexistence of AR in the murine model of asthma model, and the usability of the relevant model in AR studies and/or studies on asthma-AR coexistence will be investigated.

The aim of this study is to contribute to the literature by using a new murine model of AR. If at the end of the study, AR is induced in mice, then this animal model of AR will be defined as an AR model with the longest-term ovalbumin exposure in the literature.

MATERIAL and METHOD

A total of 22 BALB/c female 6-8- week- old mice weighing 18-20 g were used in the study. Before starting the study, ethics committee approval (decision no: 40/2018 date: February 24, 2021) was obtained from Dokuz Eylül University Experimental Animals Local Ethics Committee. Mice were kept individually in plastic cages on a 12 hour light/12 hour dark cycle, at 22±2°C room temperature and 40-50% relative humidity.

Mice acclimated to the environment for one week were fed ad libitum with standard pellets and tap water under optimum conditions throughout the study. The asthma model developed by Temelkovski J. et al. [10] in BALB / c mice was used.

To induce mice model of asthma, chicken egg ovalbumin (OVA Grade V, Sigma) was administered through intraperitoneal (IP) route at doses of 10 µg on days 0 and 14. Female BALB/c mice inhaled an aerosol solution consisting of 2.5% OVA in sterile saline for 8 weeks, 3 days a week for 30 minutes a day, starting 7 days after the last IP injection of OVA (day 21). Inhalation applications were performed with the whole- body exposure inhalation system.

OVA was given in a closed glass chamber through a jet nebulizer using whole-body exposure inhalation system and the particle size was below 4 µm with a concentration of 10-20 mg/m^3. After the mice models were created, the mice were sacrificed and the rhinotracheal tissue samples were evaluated histopathologically.

Randomly selected mice were divided into 3 groups.

Group 1: Sham (control) group (n=6).

Group 2: Mice model of asthma was induced, and the group was sacrificed on the 74th day (n=7).

Group 3: The asthma model was formed and the group was sacrificed on the 81st day (n=9).

Evaluation of AR Symptoms

After sensitization following exposure to OVA, mice were observed for typical signs of AR including sneezing, runny, and itching nose. Nasal itch was defined as perinasal rubbing with one or both forelimbs.

Histopathological evaluation

Rhinotracheal tissue samples were evaluated histopathologically after the mice were sacrificed. All tissue samples were first fixed in a 10% formaldehyde solution for 24 hours. Following this process, the tissue samples were treated with conventional grades of alcohol (70%, 80%, 90%, and 100%) to remove the water within the tissues. The tissue samples were then passed through xylol and embedded in paraffin. Four-to-five micron sections were cut from the paraffin blocks and stained with hematoxylin-eosin. An experienced pathologist examined the histological preparations under a light microscope (Olympus BX51), and photographs were taken with an Olympus DP72 camera. Histopathological evaluation was performed by this pathologist blinded to the allocation of study groups.

Number of capillaries, lymphocytes, polymorphonuclear leukocytes (PMNLs), eosinophils per mm^2 were calculated in histopathological examination.

RESULTS

Clinical evaluation

In all mice exposed to OVA, increased sneezing, rubbing perinasal area with one or both forelegs, significant increase in nasal discharge were observed compared to the control group, all of which suggested clinical symptoms of AR.

Histopathological findings

In our study, diagnosis of AR was made histopathologically in all mice exposed to OVA in Groups 2 and 3. In Groups 2 and 3, increased number of capillaries, lymphocytes, PNLs and eosinophils were found per mm^2 of the rhinotracheal tissue of mice in favor of AR compared to the control group (Table 1). The increase in lymphocyte, PNL and eosinophil counts in Group 3 compared to Group 2 could be attributed to the longer exposure to ovalbumin. Although the number of capillaries in Group 3 decreased compared to Group 2, there was no statistically significant difference between the two groups. All mice that were sacrificed on the 74th and 81st days after exposure to OVA received the histopathologic diagnosis of AR. Considering the cost of the experiment, we thought that it will be

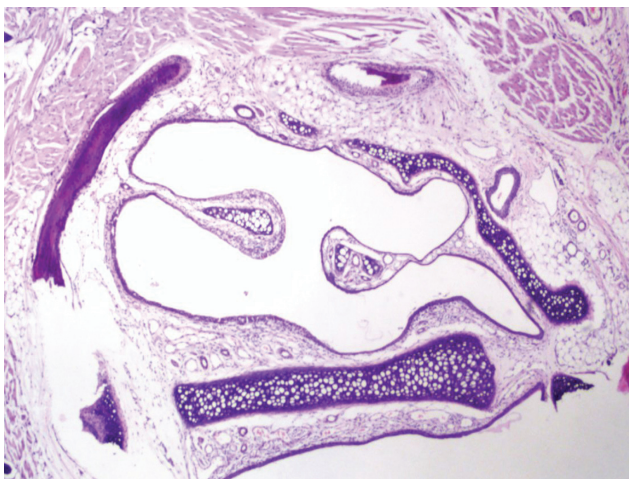

Figure 1. Cross-sectional area of nasal cavity (HEx40).

Table 1. Number of capillaries, lymphocytes, PNLs and eosinophils calculated per mm^2 of rhinotracheal tissue samples of mice in Groups 1, 2 and 3.

	Number of capillaries/HPF mean (min-max)	Lymphocytes/mm^2 mean (min-max)	PNLs/mm^2 mean (min-max)	Eosinophils/mm^2 mean (min-max)
Group 1	5.3 (4-7)	18.3 (10-30)	0.5 (0-2)	0.16 (0-1)
Group 2	9.8 (7-13)	57.2 (35-70)	6.2 (4-10)	3.7 (2-5)
Group 3	8 (6-10)	64.4 (40-90)	8.2 (4-15)	4 (3-5)

HPF: high - power field; PNL: polymorphonuclear leukocyte.

appropriate for the AR model to terminate the experiment on the 74th day.

Full-thickness sections were prepared from nasal specimens obtained from mice (Figure 1). In the control, and AR groups, capillary vessels in the nasal tissue samples were countedper high-power field

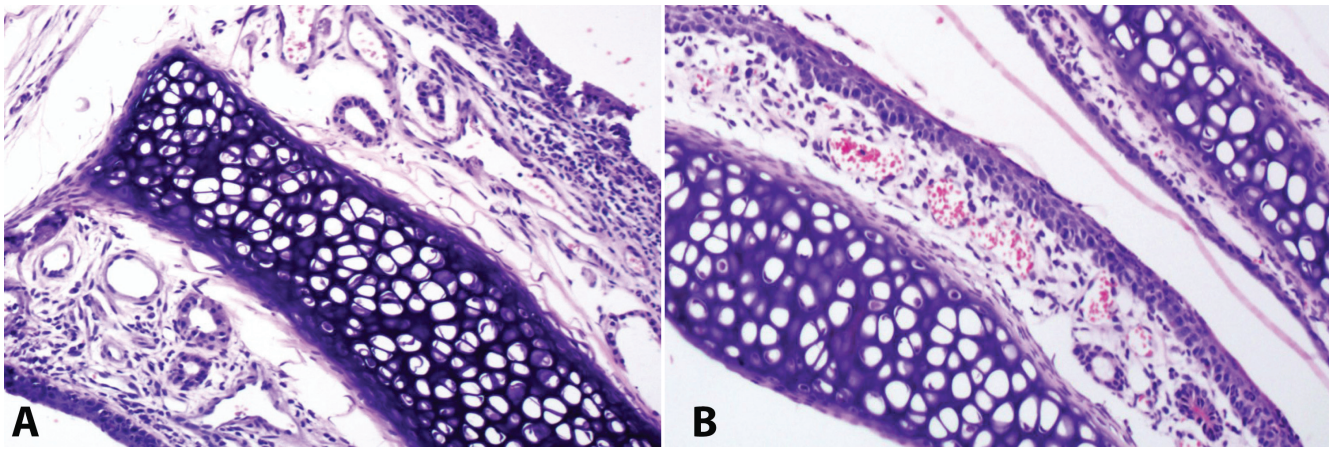

Figure 2. Capillary vessels per high power field (HPF) were counted. A) A rat from the control group with 3-4 capillary vessels per HPF and B) A rat from the allergic rhinitis group with 13 capillary vessels per HPF (HEX200).

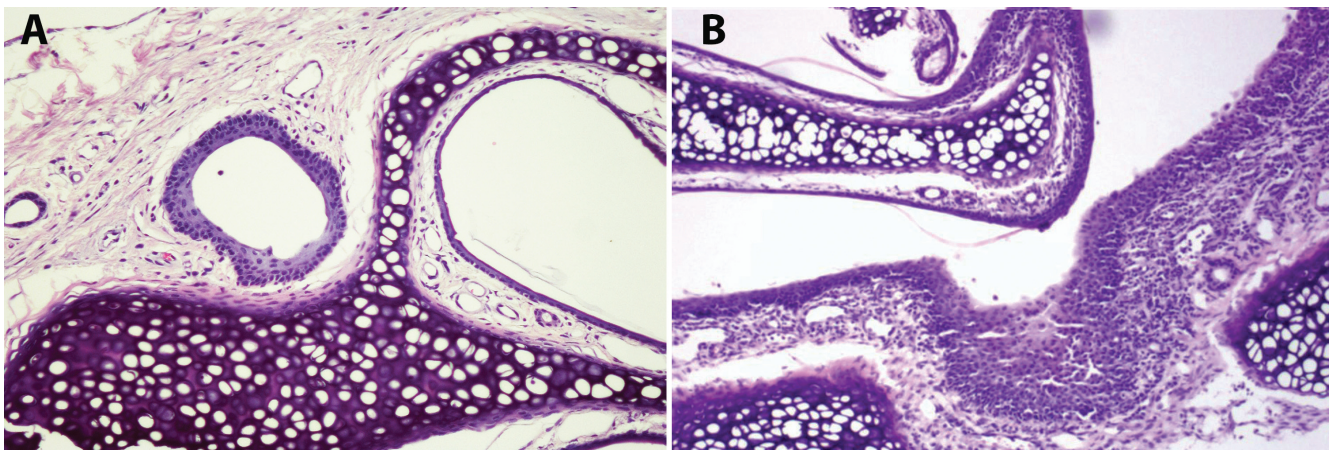

Figure 3. A) A case from the control group with mild inflammation B) A case from allergic rhinitis group with diffuse inflammation (Hex 200).

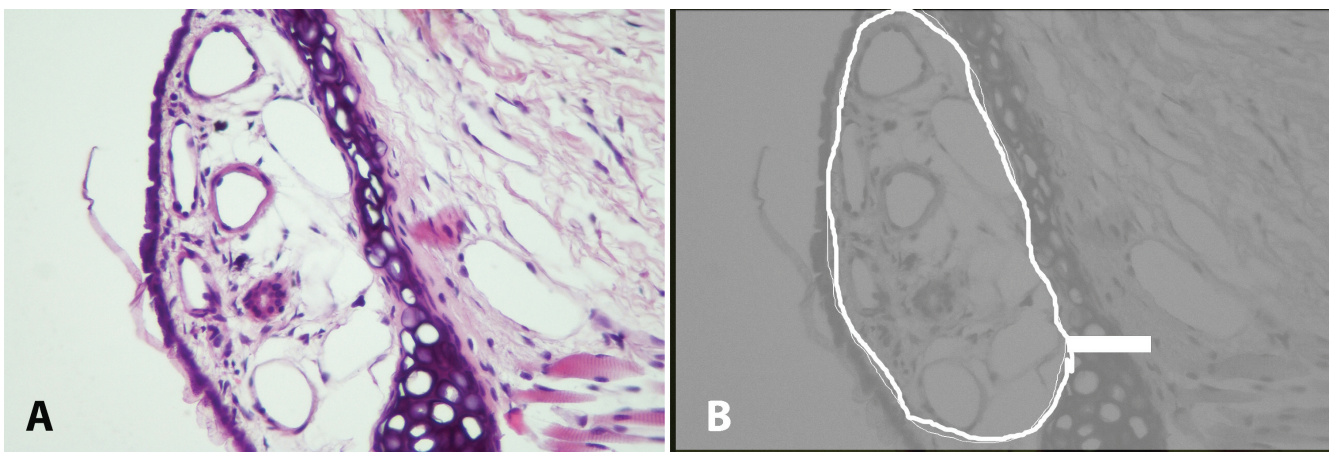

Figure 4. In all samples, a demonstrative area was selected, marked, and the inflammatory cells per 0.1 mm² were calculated. A) A microscopic area with minimal inflammation B) Demonstration of the same area in dark-field microscopy (HEX400).

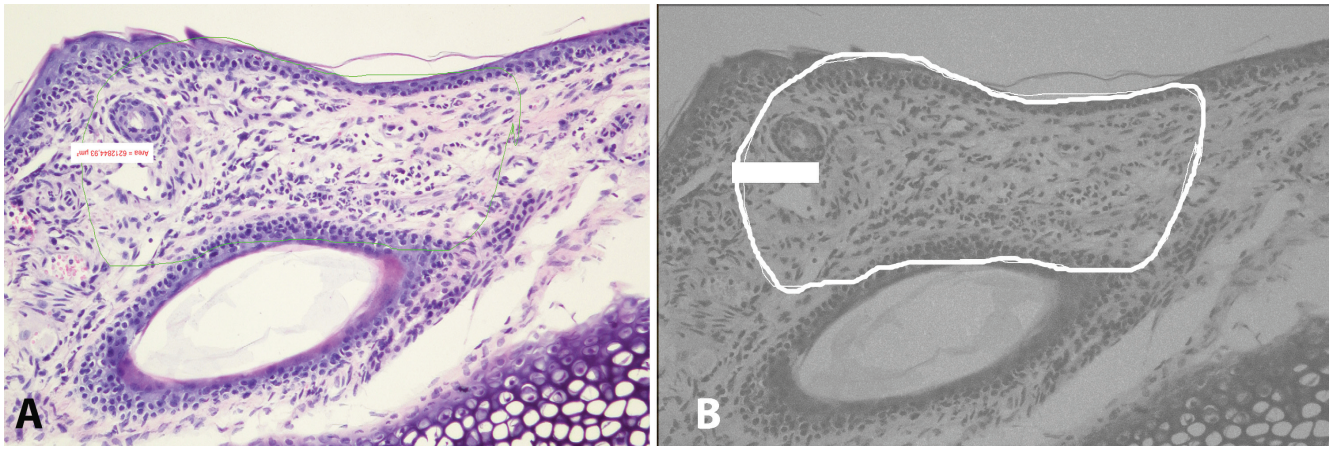

Figure 5. A) A microscopic area with severe inflammation B) Demonstration of the same area in dark-field microscopy (HEX200).

(Figure 2). A case from the control group with mild inflammation in the rhinotracheal tissue and the case from the allergic rhinitis group with intense inflammation are shown in Figure 3. In all samples, a demonstrative area was selected and marked, then inflammatory cells per mm^2 were counted. Cross-sectional areas with minimal (HEX400), and diffuse (HEX200) inflammation are shown in dark-field microscopy (Figures 4, and 5, respectively).

DISCUSSION

In our study, all mice exposed to OVA were clinically and histopathologically diagnosed with AR. AR models have been defined using different living species in the literature. AR has been induced by Nabe et al. [11] by making guinea pigs to inhale the pollen of Japan cedar flower [12]. In this study, AR had been induced by dropping pollen extracts into the nostrils of guinea pigs twice a day for a total of 7 days [11]. The researchers used lidocaine inhalation anesthesia to prevent the antigen from being removed by nasal ciliary activity [11]. Chen et al. [5] reported that AR was induced by IP injection of OVA on days 1, 3, 5, 7, 14 and 21, and then instillation of intranasal OVA for seven consecutive days.

In the rabbit model of AR defined by Güneş et al. [7] 30 mg of OVA in 100 mL of saline was administered intraperitoneally to rabbits every other day for 14 days. Then AR was induced in rabbits by intranasal application of OVA twice a day between the 14th and 18th days. In another rabbit model of AR, AR was induced by IP administration of OVA on days 0, 2, 4 and 6, followed by its intranasal application for seven days [13]. In the literature, AR models have been described in rats induced by a total of 7 IP injections of OVA once a day on days 1, 3, 5, 7, 9, 11 and 13, followed by its once daily intranasal applications for 14 days [6,14-16].

AR models are important for experimental studies performed on diagnosis and treatment of AR. Mice have been the most widely used animal species in AR models because they can be easily raised for the purpose, and often produce IgE allergens similar to humans at almost no expense [19]. AR has been induced in mice after IP injections of OVA on days 0, 7 and 14, followed by its intranasal applications twice a day for three weeks [8,17]. Bisphenol A (BPA) has been reported to exacerbate allergic inflammation in an ovalbumin-dependent AR mouse model [18]. In this study, BPA was given to mice after they received ovalbumin IP on days 0, 7 and 14. it was reported that allergic symptoms of AR were exacerbated and serum levels of OVA-specific IgE levels increased in mice [18]. In a study that investigating the effect of mesenchymal stem cells of human umbilical cord in AR treatment, IP ovalbumin was given to mice on the 0,7, and 14th days to induce AR [19]. In this study, AR was induced by instilling nasal ovalbumin in mice on days 15 and 21 [19]. In our study, 10 µg chicken egg ovalbumin (OVA Grade V, Sigma) was administered intraperitoneally to BALB/c mice on days 0 and 14.

Then the mice were exposed to inhaled aerosolized 2.5% OVA solution in sterile saline for 30 minutes 3 days a week for 8 weeks, starting 7 days after the last IP administration (21st day). As a result, AR was induced in all mice. It has been concluded that this murine model of AR can be used in experimental studies on asthma-AR coexistence.

We think that the model we defined can be used in experimental treatment studies on AR. Kan et al. [19] used the AR model that created in mice while investigating the effect of stem cells of the umbilical cord in the treatment of AR. In this model, 25 µg IP OVA was given to Balb/c strain mice on days 0, 7 and 14, followed by intranasal OVA was administered on days 15, and 21 [19]. Another study investigated the potential therapeutic effect of Metagonimus yokogawai extract on the OVA-induced allergic rhinitis model [20]. In this study, 25 µg OVA were given IP 4 times on days 0, 7 and 14 to Balb/c type mice [20]. Then, AR was induced by giving intranasal OVA every day from the 25th to the 29th day [20]. In another study investigating the role of topical red onion extract in AR treatment, the murine AR model was used [21]. In this model, Balb/c strain mice received 25 µg OVA IP on days 0,7 and 14 [21]. Intranasal 100 µg of OVA was administered five times a week between the days 21 and 41 [21]. In another study, an AR mouse model was used to investigate the therapeutic effect of Astragalus membranaceus plant on AR [22]. In this model, IP 75 µg OVA was given on days 0,7 and 14 [22]. AR model was created by intranasal administration of 200 µg IP OVA on days 21 and 42 [22].

In our study, 10 µg chicken egg ovalbumin (OVA Grade V, Sigma) was administered to BALB/c type mice via intraperitoneal route on days 0 and 14. Mice were made to inhale aerosolized 2.5% OVA solution in sterile saline for 30 minutes 3 days a week for 8 weeks, starting 7 days after the last IP administration (21st day). As a result of this application, which was used to create a murine model of asthma in the literature, AR diagnosis was made in all mice based on clinical and histopathological findings. It was concluded that this model can also be used to create an AR model whichcan be used in studies investigating asthma-AR coexistence.

Ethics Committee Approval: The study protocol was approved by the Dokuz Eylül University Experimental Animals Local Ethics Committee (decision no: 40/2018 date: February 24, 2021).

Conflict of Interest: The authors have stated that they have no conflict of interests.

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Research Article

The Effects of Low FODMAP Diet on the Quality of Life and Gastrointestinal Symptoms in Children with Irritable Bowel Syndrome. A Pilot Study

İrritabl Bağırsak Sendromu (İBS) Tanılı Çocuklarda Uygulanan Düşük FODMAP Diyetinin Yaşam Kalitesi ve Gastrointestinal Semptomlar Üzerine Etkisi. Pilot Çalışma

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ABSTRACT

Objective: *Irritable bowel syndrome is a disease that negatively affects life. Recently, diet therapies have been emphasized. Our study, the aim was to investigate the effect of low FODMAP (fermented oligo-, di-, monosaccharide and polyols) diet on the frequency of gastrointestinal symptoms and the effects on quality of life in patients with IBS.*
Method: *18 children aged between 7-18 years, who were diagnosed with IBS, followed by University of Health Sciences Izmir Dr. Behçet Uz Children's Diseases and Surgery Training and Research Hospital the Child Gastroenterology, Hepatology and Nutrition Clinic were included in the study. The appropriate KINDL scale was applied at the time of application and 2 weeks after the end of the low FODMAP diet. GIS symptoms of the week 0 and 6 KINDL results were compared. KINDL scale was applied to the families before and after dieting and the results were compared.*
Results: *The study was completed with 10 patients. The most common symptom was abdominal pain and it was present in all patients. All symptoms were found to decrease after diet but it was not significant. There was a significant increases in emotional well-being, family divisions and total KINDL results at the 6th week of diet in the children In parent KINDL scales, the results were not considered significant.*
Conclusion: *Despite there was a decrease in GIS related complaints and increase in quality of life in IBS patients who underwent low FODMAP diet, it has been found appropriate to continue the study with larger patient groups for longer follow-up periods.*

Keywords: *Irritable bowel syndrome, low FODMAP diet, KINDL scale*

ÖZ

Amaç: *İrritabl bağırsak sendromu yaşamı olumsuz etkileyen bir hastalıktır. Son zamanlarda diyet tedavileri üzerinde durulmaktadır. Çalışmamızda, İBS tanılı hastalara düşük FODMAP (fermente oligo, di, monosakkaritler ve polyoller) diyeti uygulanarak, gastrointestinal semptomların sıklığı ve yaşam kalitesi üzerine etkilerinin incelenmesi amaçlandı.*
Yöntem: *Sağlık Bilimleri Üniversitesi İzmir Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim Araştırma Hastanesi, Çocuk Gastroenteroloji, Hepatoloji ve Beslenme Kliniği tarafından takipli, İBS tanılı 7-18 yaş aralığında 18 çocuk çalışmaya dahil edildi. Olgulara başvuru anında ve düşük FODMAP diyeti bitiminden 2 hafta sonra KINDL (Çocuklar İçin Genel Amaçlı Sağlıkla İlgili Yaşam Kalitesi Ölçeği) uygulandı. 0. hafta ve 6. hafta KINDL sonuçları ve haftalık GİS semptomları karşılaştırıldı. Ailelere de diyetten önce ve sonra KINDL ölçeği uygulanarak sonuçlar karşılaştırıldı.*
Bulgular: *Çalışma 10 hasta ile tamamlandı. Hastaların 6'sı erkek (%60), 4'ü kız (%40) idi. 7-12 yaş arası 4 (%40), 13-17 yaş arası 6 (%60) hasta vardı. Semptom olarak en sık karın ağrısı eşlik etmekte olup tüm hastalarda mevcuttu. Semptomların tamamının diyet sonrası azaldığı tespit edildi ancak anlamlı p değeri elde edilemedi. Çocukların duygusal iyilik, aile bölümleri ve toplam KINDL sonuçlarında 6.hafta sonunda anlamlı artış saptandı. Ebeveyn KINDL öllçeklerinde ise sonuçlar istatistiksel olarak anlamlı değildi.*
Sonuç: *Hastalarda diyet ile genel iyilik hali ve bulgularda azalma sağlandığı görülse de, diyetin etkin ve geçerli olduğunun kanıtlanabilmesi için daha büyük gruplar ile daha uzun izlem süresini kapsayan çalışmalara ihtiyaç olduğunu düşünmekteyiz.*

Anahtar kelimeler: *İrritabl bağırsak sendromu, düşük FODMAP diyeti, KINDL ölçeği*

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§ Bu makale, Ecem İpek Altınok'un uzmanlık tezinden üretilmiştir.

INTRODUCTION

Irritable bowel syndrome (IBS) that causes abdominal discomfort is a common gastrointestinal system disease associated with changes in stool shape and frequency in defecation, which affects 20% of school- age children. Its pathophysiology can be explained partially with abnormal gastrointestinal motility, visceral hypersensitivity, low level inflammation, and psychological factors. Its etiology is complex and poorly understood, and there are many treatment methods developed, although very few are actually effective [1-4].

Gastrointestinal symptoms encountered most frequently and causing discomfort in patients include abdominal pain, bloating, diarrhea, constipation, vomiting, and a sudden feeling of the need to defecate. Recently, the effect of diet on the development of symptoms has been understood in more detail, and different dietary treatments have begun to be applied. In randomized controlled studies, the Low FODMAP (fermented oligo-, di-, monosaccharide and polyols) diet has been shown to be effective on alleviation of gastrointestinal symptoms and for the treatment of IBS [5,6].

Quality of life is a broader concept that includes personal well-being rather than personal health. The Generic Health-Related Quality of Life Scale for Children (KINDL) is used in children with various chronic illnesses and developmental problems to determine which aspects of life are more deeply affected due to disease or its treatment. There are three versions of the KINDL scale: Kiddy-KINDL (version implemented through the interviewer) for children 4-7 years old, Kid-KINDL for children 8-12 years old, and Kiddo-KINDL for adolescents 13-16 years old. In addition, there are two parent forms in which the quality of life of young children (4-7 years) and older children and adolescents (8-16 years) can be indirectly evaluated by their families. KINDL consists of six domains (physical well-being, emotional well-being, self-esteem, family, friends, and school) with the evaluation of a total quality of life [7].

Studies have shown that children with IBS have a quality of life worse than healthy children. However, there is little data on the quality of life of these children during the posttreatment follow-up period [8].

In this study, we aimed to examine the effects of low FODMAP diet on the frequency of gastrointestinal symptoms and quality of life in patients with IBS.

MATERIALS and METHOD

Because IBS does not have a specific biological marker, diagnosis of these patients is made based on symptoms. First of all, some examinations, which are the first step to rule out organic pathologies are performed. Rome III criteria are used for the diagnosis. To establish the diagnosis of IBS two of the following criteria associated with at least 3 attacks of abdominal pain per month persisting for the previous 3 months should be present: relief of pain with defecation, change in the frequency of defecation, and feçes.

A total of 18 children between 7-18 years of age diagnosed with IBS according to the Rome III criteria and followed up by University of Health Sciences Behçet Uz Pediatrics and Surgery Training and Research Hospital Pediatric Gastroenterology, Hepatology and Nutrition Clinic were included in the study. These children were all IBS patients who were followed up by this clinic during the study period extending from December 2016 to December 2017.

Patients who were followed up with a diagnosis of IBS but used medication within the previous week were not included in the study. Six of the 18 study patients on dietary treatment were excluded due to non-compliance with the diet plan. One patient was excluded from the study because of a diagnosis of FMF, and symptoms of another patient increased and required further investigation. The study was completed with the remaining ten patients.

Subjects were queried about family history, medication use, diets, presence of abdominal pain, bloating, diarrhea, constipation, nausea-vomiting, need for urgent defecation, loss of appetite, weight loss, abdominal pain at night, nocturnal defecation and fever. Hemogram, liver-kidney function tests,

electrolytes, calcium-phosphorus levels, stool tests, abdominal ultrasonography findings, and standing direct abdominal radiographs were evaluated.

Our study was conducted based on the hypothesis "With a low FODMAP diet, the gastrointestinal symptoms of IBS patients will decrease and the quality of life of the IBS patients will improve." All patients diagnosed with irritable bowel syndrome and their families were informed about the study and their consent was obtained. Existing symptoms were identified and age-adjusted KINDL scales were applied to patients before starting dietary therapy for four weeks. Follow-up visits were implemented at 1, 3, 4, and 6 (two weeks after the end of dietary treatment) weeks after the initiation of a low FODMAP diet. The symptoms were reevaluated at the follow-up visits so as to make comparisons. KINDL scale scores were calculated again two weeks after termination of the dietary therapy (sixth week). In our study, GIS symptoms and KINDL scale results were compared before implementation of the dietary plan, between one, four, and also six weeks after termination of the dietary therapy. Similarly, the changes in the results were evaluated by applying the KINDL scale scores to the parents before and after the treatment in the sixth week.

The low FODMAP diet is based on the removal of oligo-di-monosaccharides and polyols that can be fermented by intestinal bacteria. The low FODMAP diet does not contain monosaccharides (glucose, fructose, galactose, xylose, arabinose), disaccharides (sucrose, lactose, maltose, isomaltose) ,oligosaccharides (maltodextrin, refined sugars, fructo-oligosaccharide, soy), and polyols (sorbitol, mannitol, isomalt, lactitol(. Luminal distension and visceral hypersensitivity caused by these small molecules are prevented [9,10]. The adjustment of the diet together with collection of information about the patients was performed by a single dietician. Food lists containing low or high FODMAP were given to the patients. Full low FODMAP diet was applied for the first two weeks. Then in the remaining two weeks, food from the high FODMAP groups consumed in the daily routine were added to the diet at three-day intervals. The FODMAP dietary therapy was terminated at the end of four weeks.

SPSS (Statistical Package for Social Sciences- IBM Inc, Chicago, Illinois, USA) 22 Windows programs were used for statistical analysis. Mean, standard deviation and percentage distribution data were used for descriptive findings and KINDL scores. The McNemar test was used to evaluate the symptoms and the weekly controls were made using chi- square test. Use of average and standard deviation for parametric, and median and minimum-maximum values for nonparametric data was preferred. The t-test was used for parametric and Wilcoxon rank-sum test for nonparametric measurements in the comparison of KINDL scores and the factors affecting the KINDL scores in independent groups,. Test results with $p<0.05$ were considered statistically significant. A post-hoc power analysis was calculated using the G*power computer program. The result of the post-hoc power analysis was found to be 32% for our study, which has a one tailed hypothesis.

RESULTS

Six (60%) male, and 4 (40%) female 10 patients completed the study. Although the number of patients was low in our study, it was found that 30% of the patients had mixed type IBS, 30% had constipation predominant IBS, 10% had diarrhea predominant IBS and type of IBS of 30% of the patients were not classified.

The complaints of the patients were urgency (60%), constipation (50%), anorexia (50%) and diarrhea (40%) in decreasing frequency. At the follow-up visits performed at the first and fourth weeks after the dietary therapy was started, it was found that the complaints of abdominal pain, bloating, diarrhea, constipation, vomiting, urgent defecation, anorexia, weight loss decreased but the p value could not be obtained due to the limited number of study patients (Table 1).

The results observed at follow-up visits two weeks after the end of the dietary therapy were compared with the symptoms of GI before the initiation of the dietary therapy. Abdominal pain decreased in 50%, and constipation in 40% of the

Table 1. Comparison of symptoms of gastrointestinal tract week 1 and week 4 (n=10).

	Week 1		Week 4		
	Yes n (%)	No n (%)	Yes n (%)	No n (%)	p value
Abdominal pain	7 (70)	3 (30)	4 (40)	6 (60)	NA
Diarrhea	2 (20)	8 (80)	0 (0)	10 (100)	NA
Constipation	4 (40)	6 (60)	3 (30)	7 (70)	NA
Vomiting	2 (0)	8 (80)	0 (0)	10 (100)	NA
Urgent feeling of defecation	5 (50)	5 (50)	2 (20)	8 (80)	NA
Anorexia	2 (20)	8 (80)	1 (10)	9 (90)	NA
Weight loss	1 (0)	9 (90)	0 (0)	10 (100)	NA

Table 2. Comparison of symptoms of gastrointestinal tract before and after low FODMAP diet.

	Before Diet (Week 0)		After Diet (Week 6)		
	Yes n (%)	No n (%)	Yes n (%)	No n (%)	p value
Abdominal pain	10 (100)	0 (0)	5 (50)	5 (50)	NA
Bloating	7 (70)	3 (30)	1 (10)	9 (90)	0.07
Diarrhea	4 (40)	6 (60)	0(0)	10 (100)	NA
Constipation	5 (50)	5 (50)	2 (20)	8 (80)	0.25
Vomiting	2 (0)	8 (80)	0 (0)	10 (100)	NA
Urgent feeling of defecation	6 (60)	4 (40)	3 (30)	7 (70)	0.25
Anorexia	5 (50)	5 (50)	0 (0)	10 (100)	NA
Weight loss	1 (0)	9 (90)	0 (0)	10 (100)	NA

Table 3. Comparison of Child-Adolescent KINDL scores before and after diet.

	Department Score Mean±Standard Deviation		
KINDL Subgroups	Before diet (Week 0)	After Diet (Week 6)	p value
Physical well-being	46.25±15.92	53.75±15.36	0.2
Emotional well-being	65.62±14.80	79.32±13.19	0.01
Family Relationships	45.62±8.86	75.00±21.65	0.01
Friend Relationships	72.50±16.97	70.00±22.97	0.76
School	63.75±17.12	71.25±15.36	0.14
Total score	59.58±9.69	68.12±12.97	0.039

As the T-test failed to meet the conditions, the Wilcoxon signed-ranks test, a nonparametric measurement, was used to evaluate the self-esteem section. There were negative findings at the end of the 6th week in the results of the self-esteem section, but without any statistically significant intergroup difference (p=0.63).

Table 4. Comparison of parental KINDL scores before and after diet.

	Department Score Mean±Standard Deviation		
KINDL Subgroups	Before diet (Week 0)	After Diet (Week 6)	p value
Physical well-being	51.87±19.77	61.25±11.33	0.14
Self-esteem	61.87±20.50	66.25±20.87	0.34
Family Relationships	75.62±23.28	75.00±19.09	0.92
Friend Relationships	73.75±19.72	66.25±15.92	0.18
School	68.12±16.78	68.75±22.43	0.93
Total score	65.62±14,96	67.60±13.61	0.65

cases, and urgency described in 50% of the patients persisted. While bloating was present in only one case (10%), the complaints of diarrhea, vomiting, anorexia and weight loss were not observed in any case at the end of the dietary therapy (Table 2).

There was a significant increase in emotional well-being, family subscale and total KINDL scores after termination of the dietary therapy (p=0.01, p=0.01, and p=0.039). Though not statistically significant, there was a positive increase in the physical well-being and school subscale scores (p=0.2, and p=0.14). The KINDL scores of the patients related to peer relationships were lower after the dietary therapy without statistically significant intergroup difference. Although there were negative findings at the end of the sixth week in the self-esteem section results, again the intergroup difference was not statistically significant (p=0.63) (Table 3).

The KINDL scale scores of the parents estimated following dietary therapy showed a nonsignificant increase in physical well-being, self-esteem, school subscale and total scores compared to baseline (p>0.05). KINDL subscale scores of the parents concerning family and peer relationships were lower after dietary therapy without any significant intergroup difference (p>0.05) (Table 4). As the test for normality failed to allow for a t test, as a nonparametric test, Wilcoxon signed-ranks test was

used in the evaluation of the emotional well-being dimension. There was a positive, but a nonsignificant increase in emotional well-being subscale scores after the dietary therapy (p=0.49).

DISCUSSION

IBS is a functional disease of the intestines characterized by chronic abdominal pain, which cannot be explained by an organic pathology, and changes in bowel habits which resolve with defecation. It forms a large part of the group known as functional gastrointestinal diseases. The main symptoms of this disease, whose pathophysiology has not yet been fully clarified, are chronic abdominal pain, changes in stool character and frequency, and bloating, together with abdominal distention [11].

Sample size was calculated as 30 patients. However, this goal was not achieved in IBS patients followed up between December 2016 and December 2017. The number of patients may have been low due to the non-compliance to a strict dietary therapy, and the lower incidence of functional gastrointestinal disorders (FGID) in childhood. In the literature, there are still few studies performed with pediatric patients and the number of patients recruited is around 30 [12]. According to the result of the power analysis of the study based on a one tailed hypothesis , statistical power of the study was found to be 32 percent. Since no other study cited in the literature used the FODMAP diet in pediatric IBS patients, our study is particularly important as being a pilot study.

As a general application of diet in the studies, the patients did not consume high-FODMAP foods for 2 weeks and and these food types were added to the diet gradually at the end of 2 weeks [10]. In adult studies, the duration of the full high FODMAP diet was longer (about 21 days). The reason for this may be the concerns about the deterioration of dietary continuity in pediatric patients. Especially in childhood, the duration of the diet cannot be extended. In addition, unfortunately, there is no information about the duration of the diet even in our classic textbooks. Total duration of dietary therapy is around 6-8 weeks in adult reviews.

Most of the studies with irritable bowel syndrome and FODMAP diet consist of adult studies [5,6,13,14]. In these studies, when the new dietary therapy was used in patients with IBS, there was a decrease in GIS symptoms primarily including abdominal pain and urgent defecation. Similar to the results of the present study. Halmos et al. have found positive responses using the low FODMAP diet in the adult patient group, particularly complaining with nausea and intestinal passage, with a concomitant significant improvement in the frequency and form of defecation in IBS, where diarrhea was at the forefront [5]. In our study, consistent with findings in the literature, 40% of the patients initially complained of diarrhea that did not persist after dietary therapy.

In our study, the rates of symptomatic improvement were 50% for abdominal pain, 100% for diarrhea and 80% for constipation. In a study conducted in 29 patients with functional intestinal disease in the pediatric age group, improvement was observed in 77% of patients with abdominal pain, 87% of those with diarrhea, 85% of those with constipation after the FODMAP diet [12]. In another randomized controlled double-blind study performed with 33 pediatric IBS patients, clinical responses of the patients to the FODMAP diet and the relationship between this response and intestinal microbiome biomarkers were investigated [9]. In this study as in ours, it was observed that abdominal pain episodes decreased after the FODMAP diet. In addition, response to the diet has been shown to be better in patients with a high intestinal microbiome, and it has been suggested that microbiota may be important in the pathophysiology of and clinical response in IBS.

Irritable bowel disease, like many other chronic diseases, is thought to negatively affect the quality of life. In many studies, the quality of life has been shown to be lower in children with both functional and organic GIS disease compared to healthy children. In this study, the KINDL scale, which was validated in Turkish and developed as a general-purpose quality of life assessment for children, was preferred [7]. In a study performed by Pederson et al. with 19 adult IBS cases, cases were evaluated with IBS-SSS (Irritable Bowel Syndrome Symptom Severity Scale) and IBS-

QoL (Irritable Bowel Syndrome Quality of lifeScale), and a decrease in symptoms during the dietary therapy was observed, along with a concomitant increase in the quality of life scores [15]. In our study, results similar to those of this pilot study were obtained as a result of querying about symptoms and evaluating them using the KINDL scoring system

The KINDL scoring made two weeks after the end of dietary therapy demonstrated that physical well-being, emotional well-being, family, school and total KINDL scores and averages increased, and a significant p value was observed in the results related to emotional well-being, family relationships and the total scale ($p < 0.05$). In the self-esteem and friendship sections, the repeatedly lower KINDL scale scores were not found to be significant. This fact may suggest that more time should be spent for the treatment of these patients so as to estimate the the impact of dietary therapy on their social lives. In addition, a conclusion could be drawn that aspects of physical well-being are partly more objective findings and that other subscales may be affected by the duration of the dietary therapy. The problems experienced during the day and mood changes that took place should be taken into consideration when evaluating results.

In a study using the Pediatric Quality of Life Questionnaire (PedsQL), which also covered functional GIS patients, the quality of life of patients was found to be lower for GIS patients than for the healthy group [16]. In our study, general quality of life scores, which were lower before the diet, increased after the dietary therapy, and the increases in the subscale scores related to emotional well-being and family dimensions were statistically. The negative relationship between age and the total KINDL scale scores may be attributable to a better ability to understand complaints and to respond to questions asked by the older people.

The first KINDL scale scores calculated at the beginning of treatment were higher in parents than in children. From a study using PedsQL on 25 functional GIS patients, when a questionnaire was applied to the family and the patient, the quality of life scores assessed by the parents were shown to be significantly worse than those reported by the children [17]. Our study did not show similarity with the literature in this aspect. At the end of the diet, the total KINDL scale scores and their averages calculated in the mothers were higher than in the first questionnaires. While increases were observed in the subscale scores of physical well-being, emotional well-being, self-esteem and school performance, relatively lower values were observed in dimensions of the family and friend relationships. We concluded that the results were not significant due to the fact that they were very close to each other and that there were few patients.

Adaptation and uninterrupted compliance to diet in the pediatric patient group is more difficult than in the adult group. This fact, and the scarcity of patients are the limitations of the study. In addition, non-randomization of the patients is another limitation of the study.

CONCLUSION

After use of the low FODMAP diet in our study, an improvement was noticed in gastrointestinal system symptoms and in the affected quality of life of the patients. However, we think that studies involving larger groups with longer follow-up periods are needed to determine the effects of low FODMAP diet therapy on duration and long-term symptoms in children with the diagnosis of IBS.

As the T-test failed to meet the conditions, the Wilcoxon signed-ranks test, a nonparametric measurement, was used to evaluate the self-esteem section. There were negative findings at the end of the 6th week in the results of the self-esteem section, but without any statistically significant intergroup difference ($p=0.63$).

Ethics Committee Approval: For the study, İzmir Güney Public Hospitals Union, University of Health Sciences. Approval was obtained from University of Health Sciences Behçet Uz Pediatrics and Surgery Training and Research Hospital Clinical Research Et-

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Research Article

Central Venous Catheter Types and Association with Bloodstream Infection in the Pediatric Intensive Care Unit: Experience of two Years

Pediatrik Yoğun Bakım Ünitesinde Santral Venöz Kateter Tipleri ve Kan Dolaşımı Enfeksiyonuyla İlişkisi: İki Yıllık Deneyimimiz

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ABSTRACT

Objective: *Central venous catheters (CVC) provides great convenience in pediatric intensive care units (PICUs). In this study, we aimed to prospectively examine patients who underwent CVC in the PICU in terms of catheter types and infections*
Methods: *We conducted our monocentric, prospective, and cohort study by including patients between January 2019 and December 2020, involving all central catheters temporarily inserted, except port-line catheters, PICCs, indwelling catheters (cuffed and uncuffed tunnel catheters), and arterial catheters. The main issue we focus on is the rate of catheter-associated bloodstream infection (CLABSI). We analyzed the relationship between infection and risk factors using binary logistic regression analysis.*
Results: *We included 26 CLABSIs with 196 CVCs. The incidence rate was 6.2/1000 catheter days. We found that the incidence of CLABSI increased in femoral catheters (OR: 0.04 p: 0.035, 95% CI: 0.49-3.49). Moreover, the incidence was increased in catheters with 3 lumens (OR: 0.06, p: 0.031, 95% CI: 0.34-1.69). The prolongation of the catheter also increases the risk of infection (OR: 0.06, p: 0.028, 95% CI: 0.56-2.36). Also, we found that the frequency of CLABSI increased in patients with underlying immunodeficiency (OR: 0.19, p: 0.007, 95% CI: 0.85-1.39) and in patients who were given total parenteral nutrition (OR: 0.02, p: 0.041, 95% CI: 0.063-2.38).*
Conclusion: *The number of studies that directly compare catheter types in pediatric patients and their relationship with CLABSI is limited. Moreover, the comparison of unrelated studies is difficult because of heterogeneity in study populations. Multicenter pediatric prospective studies focused on identifying catheter-associated infections are needed.*

Keywords: *Catheter-associated bloodstream infections, central venous catheter, pediatric intensive care unit*

ÖZ

Amaç: *Santral venöz kateterler (SVK), çocuk yoğun bakım ünitelerinde (ÇYB) büyük kolaylık sağlar. Bu çalışmada, ÇYB'de SVK uygulanan hastaları kateter tipleri ve enfeksiyon oranları açısından ileriye dönük olarak incelemeyi amaçladık.*
Yöntem: *Port-line kateterler, periferik olarak yerleştirilen santral kateterler, kalıcı kateterler (kaflı ve kafsız tünelli kateterler) ve arteryel kateterler hariç geçici olarak takılmış olan santral keteterleri içeren Ocak 2019 ile Aralık 2020 arasındaki hastaları dahil ederek monosentrik, prospektif ve kohort olarak tasarladığımız çalışmamızı yürüttük. Odaklandığımız ana konu, SVK tiplerine göre kateterle ilişkili kan dolaşımı enfeksiyonu (Kİ-KDE) oranıdır. Enfeksiyon ve risk faktörleri arasındaki ilişkiyi ikili lojistik regresyon analizi ile inceledik.*
Bulgular: *Çalışmamıza toplam 26 Kİ-KDE ile 196 SVK dahil ettik. İnsidans oranı 6.2/1000 kateter günü idi. Kateter yerleşim yerlerinden femoral kateterlerde Kİ-KDE insidansının arttığını saptadık (OR: 0.04, p: 0.035, 95% CI: 0.49-3.49). Ayrıca 3 lümenli olan kateterlerde daha az lümeni olanlara göre Kİ-KDE insidansı artmaktaydı (OR: 0.06, p: 0.031, 95%, CI: 0.34-1.69). Santral kateterin takılı kaldığı sürenin uzaması da hastalarda enfeksiyon riskinde artışa neden olmaktadır (OR: 0.06, p: 0.028, 95% CI: 0.56-2.36). Bunun yanısıra altta yatan immün yetmezliği olan hastalarda (OR: 0.19, p: 0.007, 95% CI: 0.85-1.39) ve total parenteral nutrisyon verilmiş olan hastalarda Kİ-KDE sıklığının arttığını saptadık (OR: 0.02, p: 0.041, 95% CI: 0.063-2.38).*
Sonuç: *Pediyatrik hastalarda kateter tiplerini ve bunların Kİ-KDE ile ilişkisini doğrudan karşılaştıran çalışma sayısı sınırlıdır. Dahası, çalışma popülasyonlarındaki heterojenlik nedeniyle çalışmaların karşılaştırılması zordur. Kateter ilişkili enfksiyonlara odaklanmış, çok merkezli pediatrik ileriye dönük çalışmalara ihtiyaç vardır.*

Anahtar kelimeler: *Kateter ilişkili kan dolaşımı enfeksiyonları, santral venöz kateter, çocuk yoğun bakım ünitesi*

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INTRODUCTION

Safe and effective venous access is essential for providing care to children in pediatric intensive care units (PICUs). Central venous catheters (CVCs) are usually applied for long-term treatments such as blood transfusion, inotropic agents, and parenteral nutrition in intensive care units [1,2]. Catheter-associated bloodstream infections (CLABSIs) are the most common healthcare-associated infection reported in a network of 1003 hospitals in the United States between 2011 and 2014 [3]. In previous studies, CLABSIs have been shown to significantly increase mortality, morbidity, length of hospital stay, and cost [4-7]. In general, studies involving risk factors of CLABSIsmostly include adult patients. The risk of CLABSI depends on many factors: choice of the device, technique of insertion, the technique of management, and prompt removal [1,2,8]. In the previous studies; risk factors such as neutropenia, prolonged mechanical ventilation, total parenteral nutrition have been identified for the development of CLABSI [8,9].

When we review the literature on this topic; no specific studies related to the type of catheter have been observed in pediatric intensive care patients. In our study; we aimed to prospectively examine the relationship between the particular subtype of the CVCs and the frequency of CLABSIs and the type of infections (frequency and microorganisms) in our clinic.

MATERIAL and METHODS

We conducted a prospective cohort study of CLABSI incidence and association with the characteristics of catheters. This study includes all CVCs, except port-line catheters, permanent catheters, and arterial catheters, inserted from a month to 18 years of age between January 2019 and December 2020 in our tertiary care hospital's PICU.

The study was conducted by the ethical standards stated in the 'Declaration of Helsinki'. The local ethics committee approved the study (protocol number: 2020/05-09).

Population

Patients between a month to 18 years of age who needed to be admitted to the PICU of our hospital and who were followed up with CVCs for at least 48 hours between January 2019 and December 2020 were included in the study. Patients with CLABSI for the second time during the study period were included in the study only once.

Catheters

All of the central catheters temporarily inserted during the study period in our tertiary care hospital's PICU in two years were included. Port-line catheters, permanent catheters (cuffed-tunneled and non-cuffed tunneled catheters), peripherally inserted central catheters (PICCs), and arterial catheters were excluded in this study. Also, those catheters which have been removed in less than 48 hours were excluded.

Protocols for insertion of the central venous catheters

During the study period, catheters were placed by pediatric intensivists or pediatric intensive care fellows. We used a solution of alcoholic 4% chlorhexidine gluconate as an antiseptic solution to clean the site before applying. Practitioners cleaned the surgical area with antiseptics after they are prepared according to full barrier protection measures (sterile gloves, mask, bone, and long-sleeved sterile box). Central venous catheters were inserted by ultrasound-guided Seldinger method to the internal jugular, femoral and subclavian veins. After the application of the catheter, a transparent semipermeable dressing is made around it. It is replaced when the dressings are loose, moist, and get dirty. Also, even if nothing happens, dressing is renewed every 48 hours period [10]. In our study, only catheters inserted using a percutaneous route were included. Catheters inserted with cut down or implanted were not included. We implement a bundle care program to reduce the CLABSI incidence in our intensive care.

Definition of central catheter-associated bloodstream infection

Clinical Practice Guidelines for the Diagnosis and Management of Intravascular Catheter-Related Infection: 2009 Update by the Infectious Diseases Society of America is adopted for the definition of CLABSI, in our clinic [11]. If bacteremia or fungemia is detected in a patient who has been using a central venous catheter for more than 48 hours and has multiple positive blood cultures from a peripheral vein and clinical signs of infection (such as fever, chills, and/or hypotension), and a bloodstream infection is excluded, except for a visible central catheter. defined as CLABSI in the absence of an other resource.

To diagnose CLABSI, one of the specified criteria must be present: a positive result of a semi-quantitative (>15 CFU per catheter segment) or quantitative (>10^2 CFU per catheter segment) catheter culture in which the same organism is isolated from a blood culture from a catheter and from a peripheral blood sample; simultaneous quantitative blood cultures (catheter versus peripheral blood) with >3:1 CFU/mL blood ratio; different time to positivity (growth in a blood culture obtained through a catheter hub should be detected at least 2 hours before an equal volume of a peripheral blood sample taken simultaneously).

Study process and data collection

A case report form was prepared. The form started to be filled as soon as the catheter was inserted. Participants were followed from catheter insertion to removal only until one course of CLABSI. Data were prospectively collected by clinicians. About the catheter; an indication of insertion, type, diameter, number of lumens, vein to which it is inserted (jugular, femoral, subclavian), whether it is inserted urgently or electively, whether it is the first catheter, the length of stay (in days), and the presence of a microbial agent in the blood and catheter culture, the microorganism type were prospectively recorded. About the patient; age, gender, weight, the primary diagnosis at admission if any the condition and duration of stay in the invasive mechanical ventilation for longer than 48 h, whether or not total parenteral nutrition was given from the catheter and its duration, duration of the hospitalization in days, were recorded prospectively. If several catheters were inserted in the same patient, a catheter form was completed for each one.

Statistical analysis

Statistical analyses were performed using SPSS 20 software (IBM, Armonk, NY, USA). First of all, numerical and categorical data were evaluated by descriptive statistical methods. Distributions of numerical variables were examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov). The mean and standard deviation were used as the distribution was homogenous. Non-homogeneous data were shown with median and interquartile ranges.

The incidence rate was defined as the number of new CLABSIs relative to the total number of catheter days in our study. The definition of the number of catheter days was specified as the number of days between insertion and removal of CVCs. The confidence interval for each incidence was 95%.

We examined binary logistic regression analysis to determine independent predictors of CLABSI. Hosmer-Lemeshow goodness of fit statistics was used to assess model fit. A 5% type-I error level was used to infer statistical significance. The results were interpreted and reported by the researchers. Statistically, a p-value of less than 0.05 was considered significant.

Sample size

The sample size of our study was determined based on the total number of CVCs in the intensive care. Because the aim of us was to obtain an overview of CVCs of PICU patients. All of them were therefore included in our study.

RESULTS

Characteristics of the patients

We included a total of 196 patients. The patients' median age at placement days was 13 (IR-interquartile

range-: 7-26) months; median weight was 8 (IR: 7-15) kg; median height was 72 (IR: 62-103) cm. The median length of hospitalization was 61 (IR: 15-132) days and the median duration of CVCwas 22 (IR:11-33) days. No relationship was found between the height and weight of the patients and the frequency of CLABSI.Underlying diseases at admission was respiratory deficiency (76; 39%), sepsis (59; 30%), cardiovascular deficiency (37; 19%) and neurologic diseases (24; 12%). On the other hand, some of the foremost underlying chronic diseases of patients' were neurologic and genetic diseases (74; 42%), hematologic diseases (34; 17%), metabolic diseases (12; 6%), and immunodeficiency rate at admission (23; 13%) is specified in Table 1. Also, a total of 62 (32%) of the patients had defined immunodeficiency. Immunodeficiencies that have been identified were; neutropenia (39; 62%), Severe combined immunodeficiency (2; 3%), Common variable immunodeficiency (3; 5%), T cell deficiency(6; 10%), Hypogammaglobulinemia (10; 16%), Griscelli syndrome (1; 2%), and DiGeorge syndrome (1; 2%). Immune deficiency accompanied all of our patients with underlying hematological and oncological diseases. Neurological diseases were spinal muscular atrophy, congenital muscular dystrophy, and neurodegenerative diseases. There is no defined immunodeficiency in these patients. The patients with neurological, genetics, and metabolic diseases and by identified immunodeficiency are included in the immunodeficiency group. Immunodeficiency was identified in all of our patients who received TPN and they were included in both groups for statistical analysis.

Table 1. Demographic and clinical characteristics of the children with cental venous catheters.

Gender (F; %) (M; %)	(88; 45%) (108; 55%)
Age (months)- median (IR)	13 (7-26)
Weight (kg)- median (IR)	8 (7-15)
Height (cm)- median (IR)	72 (62-103)
Length of hospitalization (days) median (IR)	61 (15-132)
Duration of CVC (days) median (IR)	22 (11-33)
Underlying diseasesat admission (N/%)	
- Respiratory deficiency	76 (39)
- Sepsis	59 (30)
- Cardiovascular deficiency	37 (19)
- Neurologic diseases	24 (12)
Immunodeficiency rate at admission	23 (13)
One of the foremost underlying chronic diseases	
- Neurologic and genetic diseases	74 (42)
- Hematologic diseases	34 (17)
- Metabolic diseases	12 (6)
- Immune deficiency	23 (13)

CVC: Central venous catheter, IR: Interquartile range, N: Number.

Characteristics of the catheters

Indication of CVC were no vascular access (120; 61%), required inotropic treatment (35; 18%), extracorporeal treatment (41; 21%) (Table 2). Characteristics of catheter types are given detailed in Table 2.

Table 2. Description of the catheters.

CVC type (N; %)	
- Simple CVC	(154; 79%)
- Hemodialysis catheter	(42; 21%)
CVC placement (N; %)	
- Jugular	(150; 77%)
- Subclavian	(24; 12%)
- Femoral	(22; 11%)
Indication of CVC (N; %)	
- No vascular access	(120; 61%)
- Required inotropic treatment	(35; 18%)
- Extracorporeal treatment	(41; 21%)
Number of lumens (N; %)	
- 1	(16; 8%)
- 2	(100; 51%)
- 3	(80; 41%)
CVC diameter (F-French)-median (IR)	5 (4-5)
CVC length (cm)-median (IR)	8 (8-12)

CVC: Central venous catheter, IR: Interquartile range, N: Number.

The incidence rate

We included a total of 196 CVCs and 26 CLABSIs. The total time of catheter use was 4180 days. The incidence rate(Number of CLABSIs/Total catheter days x1000)was 6.2/1000 catheter-days (95% CI: 0.92-7.91). The confidence interval was determined 95%.

Characteristics of the CLABSI

Microbial agentsin catheter infections was determined as gram-negative (18; 69%), fungus (5; 19%), gram-positive (3; 12%). Identified microorganisms were; *Klebsiella pneumonia* (7;

28%), *Pseudomonas aeruginosa* (5; 20%), *Klebsiella oxytoca* (2; 7%), *Escherichia coli* (2; 7%), *Serratia marcescens* (1; 3%), *Burcholderia cepacia* (1; 3%), *Proteus mirabilis* (1; 3%), *Candida albicans* (2; 7%), *Candida parapsilosis* (2; 7%), *Candida glabrata* (1; 3%) and Coagulase negative staphylococcus (*Staphylococcus epidermidis*, *Staphylococcus hemolyticus*, ...) (3; 12%).

The infection rate of femoral catheters was higher than the other placements (OR: 0.04 p: 0.035, 95% CI: 0.49-3.49). The rate of CLABSI was increasing in patients with three catheter lumens (OR: 0.06, p: 0.031, 95% CI: 0.34-1.69) and in whom catheters were placed for longer than 10 days period (OR: 0.06, p: 0.028, 95% CI: 0.56-2.36). Also, the rate of CLABSI was higher in patients with immunodeficiency (OR: 0.19, p: 0.007, 95% CI: 0.85-1.39). In addition, neutropenia (one of the immunodeficiency subgroups) increased the rate of catheter infection (OR: 0.08, p: 0.022, 95% CI: 0.77-2.72). No effect of other immunodeficiency subgroups on the frequency of CLABSI was detected. In addition, we determined that the underlying chronic diseases have increased the catheter infections rate (OR: 0.15, P: 0.009, 95% CI: 0.91-2.11). For instance, the incidence of CLABSI was higher in patients with underlying neurological and genetic diseases(OR: 0.09, P: 0.028, 95% CI: 0.82-1.79). The hematological disease of the underlying chronic diseases was found to increase the CLABSI incidence (OR: 0.02, P: 0.045, 95% CI: 0.59-2.58). However, the underlying metabolic diseases did not show any statistical effect on CLABSI incidence. The fact that total parenteral nutrition was given through the catheter increases the risk of CLABSI (OR: 0.02, p: 0.041, 95% CI: 0.63-2.38). Furthermore; receiving mechanical ventilation support, being the first catheter, urgent insertion of the catheter did not have any statistical effect on the CLABSI rate (Table 3).

Table 3. Logistic regression analysis of risk factors associated with catheter-related infections.

	OR (95% CI)	p
CVC type		1.0
CVC placement (Femoral)	*0.04 (0.49-3.49)	0.035
CVC duration (Longer than 10 days)	**0.06 (0.56-2.36)	0.028
Count of lumens (3)	***0.06 (0.34-1.69)	0.031
CVC diameter (F-French)		0.109
CVC length (cm)		0.591
TPN infusion through the CVC	****0.02 (0.63-2.38)	0.041
Administration of invasive MV		0.658
Immune deficiency	*****0.19 (0.85-1.39)	0.007
Neutropenia	0.08 (0.77-2.72)	0.022
SCID		0.678
CVID		0.701
T cell deficiency		0.059
Hypogammaglobulinemia		0.067
Griscelli syndrome		0.564
DiGeorge syndrome		0.094
Underlying chronic diseases	******0.15 (0.91-2.11)	0.009
Neurologic and genetic diseases	0.09 (0.82-1.79)	0.028
Hematologic diseases	0.02 (0.59-2.58)	0.045
Metabolic diseases		0.866
First catheter		0.707
Urgent catheter		0.999

CI: Confidence interval, CVC: Central venous catheter, CVID: Common variable immunodeficiency, MV: Mechanical ventilation, SCID:Severe combined immunodeficiency, TPN: Total parenteral nutrition
**Logistic Regression; Cox&Snell R Square: 0,376, Nagelkerke R Square: 0,717.*
***Logistic Regression; Cox&Snell R Square: 0,429, Nagelkerke R Square: 0,855.*
****Logistic Regression; Cox&Snell R Square: 0,415, Nagelkerke R Square: 0,781.*
*****Logistic Regression; Cox&Snell R Square: 0,233, Nagelkerke R Square: 0,507.*
******Logistic Regression; Cox&Snell R Square: 0,641, Nagelkerke R Square: 0,978.*
*******Logistic Regression; Cox&Snell R Square: 0,634, Nagelkerke R Square: 0,932*

DISCUSSION

In our study, the CLABSI incidence was calculated as 6.2/1000 catheter days. We only examined the effect of non-permanent catheters. The infection rate of femoral catheters, three catheter lumens, and in whom catheters were placed for longer than 10 days period was higher than the others. The CLABSI rate was higher in patients with immunodeficiency (neutropenia), underlying chronic diseases, and receiving total parenteral nutrition. On the other hand; receiving mechanical ventilation support, being the first catheter, urgent insertion of the catheter did not show any statistical effect on the CLABSI rate.

The incidence of CLABSI in our clinic was higher than in other studies on this topic [12,13]. When the studies conducted were examined and when the

CLABSI incidence was looked into, it was seen that Carter et al. [13] were found 3.87 per 1,000 in-hospital line days. This incidence rate includes neonatal patients and catheters placed in general pediatrics and surgical services, as well as in pediatric intensive care. Also, in this study, the inclusion of totally implantable catheters, PICCs, and tunneled catheters, as well as temporary CVCs may cause the incidence rate to be lower. In the study of Broudic et al. [14], CLABSI incidence was determined 4.6/1000 catheter days for general hospitals and it was 2.4/1000 catheter days specifically for the pediatric intensive care. Unlike our study, in this study PICCs and tunneled catheters inclusion with temporary catheters may be the reason for the lower incidence of infection.

Besides, in the study which is designed in a tertiary care children's university hospital by Venturini et al. [12]; port catheters, PICC line catheters, and indwelling catheters in all departments of the hospital are included. A total of 388 children between October 2014 and April 2015 with all catheters under the age of 18 were included. Catheter-associated bloodstream infections rate was determined 3.73/1000 (95% CI: 2.54–5.28) central line-days. The results of the International Nosocomial Infection Control Consortium surveillance study from January 2007 to December 2012 in PICUs showed a CLABSI rate of 6.1/1000 (95% CI: 5.7-6.5) central line-days [15].

To obtain reliable data on this topic, a continuous prospective study should be conducted. A prospective study performed in 29 NICU in the United States and found that the risk of CLABSI is very low during the first week of catheterization and especially with lines inserted in the jugular vein [12,16]. In the study conducted by Ergul et al. [17], it was determined that CLABSI incidence increased with the catheter duration. Similarly in our study, there was a relation between CLABSI and catheter duration days, femoral catheters, and three-lumen catheters.

Moreover, studies are difficult to compare because of the diversity in study populations and study methods [18-20]. Larger prospective pediatric studies are needed to identify CVC types and their association with infection rates. Point prevalence studies are easier to perform than long prospective studies but may underestimate the real risk. Pediatric studies which focusing on catheter infection in such patients should be conducted to deepen our understanding of the associated risk factors.

In a prospective pediatric cohort study which was conducted by Carter et al. [13], a total of 5648 patients, 385 who developed CLABSI between 1995 and 2013 were examined. Over time, the incidence of catheter-associated infection has decreased, but in the process, the hand hygiene campaign to the risk of CLABSI has been launched. The time in this study is very long, and in this process, there is inevitably expansion and change in the infection prevention packages. This suggests that other mixing factors cannot be standardized while evaluating the effect of the type of catheter on CLABSI in the process. In this study, it was also found that the risk of catheter infection increased with increasing the number of lumens [13]. In our study, the rate of CLABSI increased in 3-lumen catheters compared to those with fewer lumens. This result shows us that choosing catheters with fewer lumens can reduce the CLABSI rate. Carter et al. [13] reported that patients' comorbidities and underlying chronic diseases increased the incidence of CLABSI. Underlying chronic comorbidities were present in most of our patients who needed catheter insertion. In our study, we determined that the underlying chronic diseases have increased catheter infections. As; genetic and neurological diseases have increased the incidence of CLABSI. This may also be associated with the need to be a longer hospitalization period. In addition, the underlying hematological diseases have also increased the incidence of CLABSI. This may be associated with the accompanying immunodeficiency of those with hematological disease. On the other hand, we determined the underlying metabolic diseases did not affect the CLABSI incidence. Also, the rate of catheter infection was higher in immunocompromised patients in our study. Similar to the results in other studies [8,9], the rate of catheter infection was increasing in patients with neutropenia. Since the intensive care follow-up of critical

hematology and oncology patients is also performed in our clinic, we think that our immunodeficiency especially the neutropenia rate is high and this may lead to an increase in susceptibility to infection and a high rate of CLABSI.

Our study has some limitations. The most important of these is the single-centered design of the study and therefore the inability to generalize the results. Also, more cases can be included by keeping the cohort research longer and the duration can be extended. Since there was a problem with the intake process of PICCs in our hospital, a limited number of cases could be inserted and CVCs were inserted to patients who could not have vascular access. For this reason, the number of catheter insertions was high with the indication of no vascular availability. Moreover, the change of CLABSI incidence over the years and factors can be specifically studied.

The incidence of CLABSIs in children hospitalized in our PICU is higher than reported in the literature. The results of our study show that choosing a catheter location other than femoral, preferring fewer lumen catheters instead of 3-lumen catheters, and removing the catheters as soon as possible can reduce the incidence of CLABSI.

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Ethics Committee Approval: The study was conducted by the ethical standards stated in the 'Declaration of Helsinki'. The local ethics committee approved the study (protocol number: 2020/05-09).

Conflict of Interest: The authors declare that they have no conflict of interest.

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Research Article

Prevalence of Ocular Abnormalities in Term Infants

Term İnfantlarda Oküler Anormalliklerin Prevalansı

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ABSTRACT

Objective: *To exhibit the results of routine ophthalmologic screening in infants between 0-1 years of age referred to the ophthalmology clinic from the departments of pediatrics and family medicine.*
Method: *Referred to the ophthalmology clinic between August 2014 and November 2019, 11196 eyes of 5598 term infants were retrospectively investigated in the study, and all participants were ophthalmologically examined at 1st, 6th, and 12th months of age. Infants' pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine. On examination, eye and face symmetries were evaluated with inspection, fixation, and ocular tracking. Pupil responses and motility were evaluated with the light source. While the red reflex test was evaluated using a direct ophthalmoscope, fundus was assessed through an indirect ophthalmoscope.*
Results: *Congenital cataract (6), congenital glaucoma (3), strabismus (81), epiphora (426), non-specific retinal hemorrhages (42) and retinal pigmentation changes (10), coloboma (4) (one eyelid, four iris, one optical disc and three chorioretinal), optic disc abnormalities (3), congenital ptosis (13) (unilateral in 12 patients and bilateral in one patient), corneal dysgenesis (2) and microphthalmia (3) were determined in 11196 eyes of 5598 infants (2709 females, 2889 males).*
Conclusion: *Perinatal ophthalmologic screening program is likely to diagnose several diseases earlier, such as congenital cataracts, congenital glaucoma, strabismus, corneal opacities, causing vision losses in infants. Treatment options are available, and some diseases can be treated due to early intervention. Early treatment can also eliminate the problems precluding the development of complex visual ability continuing in perinatal period. Consequently, final visual acuity may be increased.*

Keywords: *Amblyopia, congenital cataract, congenital glaucoma, vision loss*

ÖZ

Amaç: *Pediatri ve Aile Hekimliği kliniklerinden rutin göz taraması açısından yönlendirilen 0-1 yaş arası infantlardan elde edilen verileri değerlendirmek.*
Yöntem: *Ağustos 2014-Kasım 2019 tarihleri arasında Göz kliniğine yönlendirilen 5598 term infantın 11196 gözü retrospektif olarak çalışmaya dahil edildi. Tüm infantların 1. ay, 6. ay ve 12.ayda detaylı oftalmolojik muayeneleri yapıldı. Bebeklerin pupilleri %0,5 tropikamid ve %2,5 fenilefrin ile dilate edildi. Muayenede inspeksiyon ile göz ve yüz simetrisi, ışık kaynağı ile fiksasyon ve takip, pupil cevabı ve motilite, direkt oftalmoskop ile kırmızı refle testi, indirekt oftalmoskop ile fundus değerlendirildi.*
Bulgular: *5598 infantın (2709 kız, 2889 erkek) 11196 gözünde, 6 konjenital katarakt, 3 konjenital glokom, 81 şaşılık, 426 epifora, 42 nonspesifik retinal hemoraji, 10 nonspesifik retinal pigmentasyon değişikliği, 4 kolobom (1 göz kapağı, 4 iris, 1 optik disk, 3 koryoretinal), 3 optik disk anomalisi, 13 konjenital pitoz (12 hasta tek taraflı, 1 hasta iki taraflı), 2 korneal disgenezi, 3 mikroftalmi tespit edildi.*
Sonuç: *Perinatal dönemde yapılacak oftalmik tarama programı ile bebeklerde görme azlığına neden olabilecek konjenital katarakt, konjenital glokom, şaşılık, kornea opasiteleri gibi hastalıklara erken tanı konulabilmekte, tedavisi mevcut olanlara müdahele edilebilmekte ve perinatal dönemde gelişimi devam etmekte olan kompleks görme işlevinin kazanılmasını engelleyebilecek durumlar ortadan kaldırılarak nihai görme keskinliği oranlarında artış sağlanabilmektedir.*

Anahtar kelimeler: *Konjenital katarakt, konjenital glokom, görme kaybı, ambliyopi*

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INTRODUCTION

Ocular problems, such as poor vision in infants, should be determined passively because babies cannot express their discomfort [1]. Therefore, the American Academy of Pediatrics (AAP) recommends managing the red reflex test for newborns shortly after birth [1,2]. However, there are no convincing data on the sensitivity and false negativity rates of the red reflex test [3]. Such pathologies as congenital cataracts, corneal opacities, and retinoblastoma, which can be detected with the red reflex test, are of great importance due to potential threats to vision and even life [4]. Based on the literature, this age segment has not yet to be studied adequately, and the true prevalence of temporary or permanent ocular abnormalities remains unknown [3].

Since 2016, ophthalmologic screening has been launched in 81 provinces of Turkey by family physicians. The present study aims to assess the prevalence of ocular abnormalities in term infants aged between 0-1 year by evaluating the results of ophthalmologic screening guided by the departments of pediatrics and family medicine. The study also intends to determine the features that may show demographic changes and compare our study results with those reported in previous studies.

MATERIAL and METHOD

In our study, 11196 eyes of 5598 infants were screened between August 2014 and November 2019 in our hospital referred to the ophthalmology clinic from the pediatric and family medicine clinics, and the data were evaluated retrospectively. Approval was obtained from the ethics committee of the institution before the study. Detailed ophthalmological examinations were performed at 1st, 6th, and 12th months of age by the researchers (Daldal H, Turkyılmaz M, and Salis O). Infants' pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine. In performing examinations, the eyes, eyelids, and areas around the eyelids were first inspected, and eye and face symmetries were evaluated. Then, pupil responses and ocular motilities were checked with a light source. The red reflex test was performed using a direct ophthalmoscope, and the motility was also evaluated. On the other hand, fundus examination was performed with an indirect ophthalmoscope with the help of a 20 D lens. Abnormal ocular findings were recorded, and all infants were followed up. The Statistical Package for Social Sciences for Windows 22.0 program (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses by using descriptive statistics (percentages).

RESULTS

We included 5598 infants (2709 females, 2889 males) in our study. Abnormal ocular findings were detected in 593 (10.59%) cases of infants involved in the study. Among the abnormal ocular findings, six congenital cataracts, three congenital glaucomas, 81 strabismus, 426 epiphoras, 42 non-specific retinal hemorrhages, 10 non-specific retinal pigmentation changes, four colobomas (as one eyelid, four iris, one optical disc, and three chorioretinal), three optic disc abnormalities, 13 congenital ptoses (unilateral in 12 patients and bilateral in one patient), two corneal dysgeneses and three microphthalmia were diagnosed (Table 1). Of 81 infants having strabismus, two and 79 were exotropia and esotropia, respectively. Those with exotropia had also cerebral palsy. Two of

Table 1. Distributions of the pathologies determined among patients

Pathologies	Patients (n)	Percentage of all infants (%)	Percentage of determined pathologies (%)
Epiphora	426	7.61	71.83
Strabismus	81	1.44	13.66
Retinal hemorrhages	42	0.75	7.08
Congenital ptosis	13	0.23	2.19
Retinal pigmentation changes	10	0.18	1.69
Congenital cataracts	6	0.11	1.01
Coloboma	4	0.07	0.67
Congenital glaucomas	3	0.05	0.51
Optic disc abnormalities	3	0.05	0.51
Micro-ophthalmia	3	0.05	0.51
Corneal dysgenesis	2	0.04	0.34

those with esotropia had cataracts, and the other two had glaucoma, and the remaining 75 patients were with hypermetropia above three diopters. Of 593 ocular abnormalities, 115 (19.39%) were assessed as important findings threatening the visual acuity.

DISCUSSION

Ophthalmologic screening, as well as the tests used to diagnose such diseases as phenylketonuria, hypothyroidism, and developmental hip dysplasia, is performed through the red reflex test as a newborn screening in our country. The red reflex test is an indispensable part of the neonatal examination and is used to determine many important pathologies, including cataract, corneal opacity, retinoblastoma, and retinal detachment that may be located in the visual axis [4]. In the study, the red reflex test performed in the eye without pathology is seen in Figure 1. Whether to be decreased, white, or asymmetrical, the presence of dark spots in the red reflex creates an indication for the referral of the baby to an ophthalmologist [5].

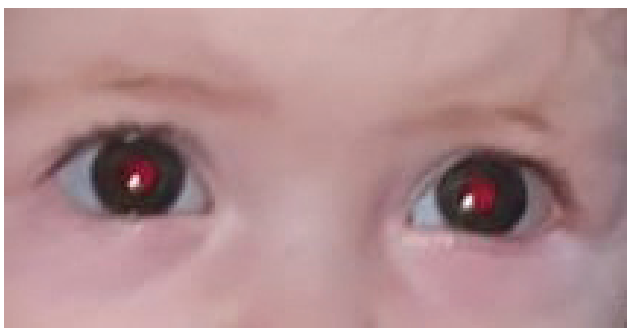

Figure 1. Red reflex test.

Ocular abnormalities can be classified into three groups. In the first group, there are non-clinical findings, such as subconjunctival hemorrhages, retinal pigmentation, and non-specific retinal hemorrhages. The second group, including findings such as the immature retina, has less clinical significance and requires the follow-up of the patients. The third and the last group consists of clinically important diseases, such as premature retinopathy, congenital cataract, congenital glaucoma, and ptosis requiring advanced treatment [1].

Retinal hemorrhages are one of the common conditions at birth and seen more commonly in vacuum-assisted births, followed by spontaneous vaginal deliveries, unlike those encountered rarely in cesarean deliveries [6,7]. Most birth-related retinal hemorrhages are intraretinal and typically resorbed within seven to 10 days, but can last up to 30 days [6-8].

In two previous studies, the most common occurring abnormality was reported as retinal hemorrhages with a prevalence of 21.52 and 20.3%, respectively [3,9]. In our study, the frequency of retinal hemorrhages was determined in 42 patients (0.75%). The low prevalence in our study was probably since our first examination at the first month of age was performed at a later period, compared to previous studies. Additionally, non-specific retinal pigmentation was observed in 10 patients (0.18%).

In a cohort study of 4792 infants by MacEwen et al., it was found out that the prevalence of epiphora was about 20% in the first year of life, and nearly 95% of the study population showed symptoms at the age of one month [10]. In our study, epiphora was detected in 426 (7.61%) infants. Three of 426 cases were due to congenital glaucoma, while the rest were due to nasolacrimal duct obstruction.

Congenital cataracts are one of the common and treatable ocular pathologies leading to childhood blindness [11]. The incidence of congenital cataracts is 1.2-6 per 10.000 cases [12]. The studies in the USA reveal that 10-38% of childhood blindness is due to congenital cataracts [13]. In our study, congenital cataracts were observed in six patients (0.11%), and one patient with congenital cataracts had galactosemia. There was no drug use and radiation history in the mothers of infants with congenital cataracts. Additionally, the parents of another patient had also a history of consanguineous marriage. The cases with congenital cataracts are presented in Figures 2, 3, and 4.

Among the most important reasons bringing families to the healthcare centers are leukocoria, strabismus, suspicion of visual decreasement, and

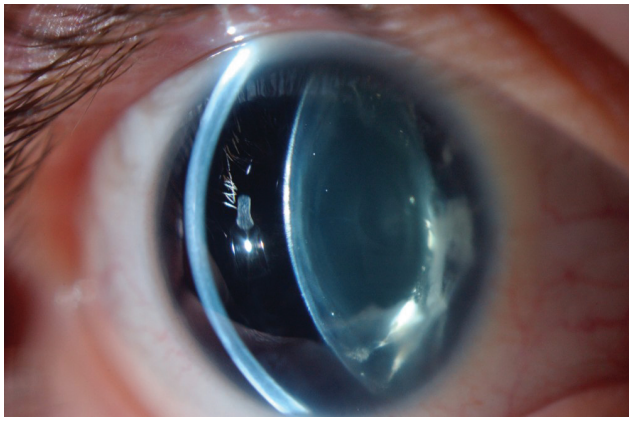

Figure 2. Congenital cataract.

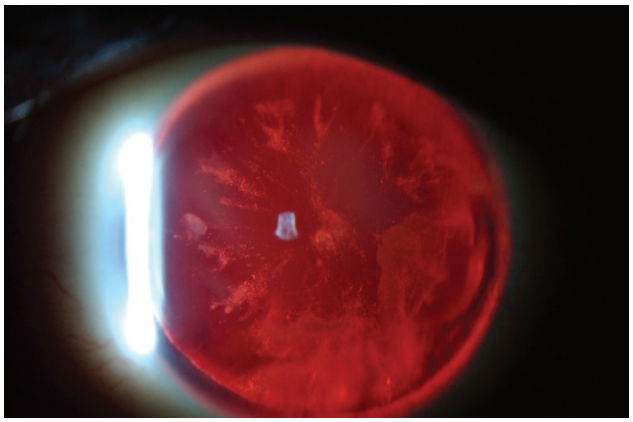

Figure 3. Congenital cataract with retroillumination.

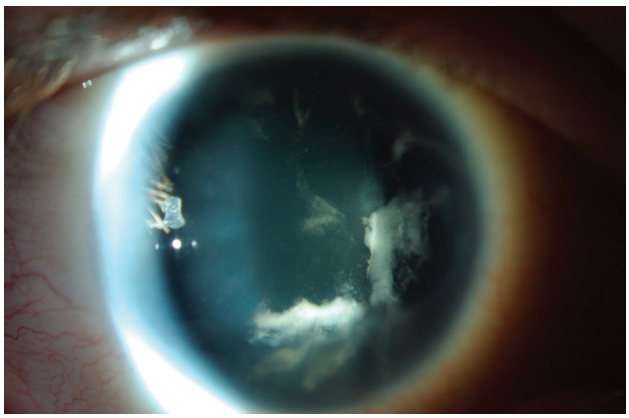

Figure 4. Congenital cataract and aniridia.

nystagmus. Visual acuity is expressed by parents as "not pursuing the objects" in the early childhood period. The cases with congenital cataracts are mostly admitted to hospitals at a late stage, and the length of time between the deterioration of the image in the retina and the elimination of the cause may lead to the irreversible deprivation of amblyopia [14]. Here, the point at which healthcare professionals should be meticulous is that pediatric cataracts may not always be seen with leukocoria. Besides ophthalmologists, therefore, family physicians and pediatricians should also examine children with the red reflex test so that cataracts can be detected more easily and treated at an early period.

Developmental glaucomas are associated with developmental ocular abnormalities at birth as a result of insufficient development of the anterior segment structures of the eyes and seen in approximately 1 out of 10000 live births [15]. Primary congenital glaucomas are rare cases and usually affect less than 0.05% of ophthalmology patients; however, the rate of blindness is high in such patients, ranging between 2-15% [16]. Glaucoma is the second most common cause of blindness across the world and can be prevented when diagnosed and treated at an early period [17]. While the risk of congenital glaucomas does not exceed 3% in the second child of the couples if one child has glaucoma, the risk rises to 25% in the third child if there are two children with glaucoma [18]. For this reason, all family members should be questioned meticulously in terms of congenital glaucomas. When congenital glaucoma is detected in a member of the family, other children should also be examined. The probability of developing bilateral congenital glaucoma is 75%, and congenital glaucomas are encountered more often among the male population [15]. In our study, congenital glaucomas were determined in three (0.05%) male cases, one was unilateral while the two were bilateral. Epiphora, photophobia, and blepharospasm are frequently seen as the first ophthalmologic symptoms in infants, and such symptoms are caused by irritation due to corneal epithelial edema due to the increased intraocular pressure. The blurred appearance of the cornea may be intermittent at the early stages and may result from tears in Descemet's membrane. The rupture of Descemet's membrane (Haab's striae) is seen in Figure 5. The abnormal growth of the globe (buphthalmos) occurs due to the increased intraocular pressure, and such a growth develops primarily at

the corneoscleral junction. Accordingly, while the anterior chamber is deep, the anterior chamber is narrow in healthy babies [15]. In our study, all congenital glaucoma patients had epiphora and photophobia.

Family physicians or pediatricians should suspect congenital glaucomas, especially whenever buphthalmos, epiphora, photophobia, and corneal haze are encountered in screening the baby. Early diagnosis and treatment are of crucial importance as congenital glaucoma can lead to life-long blindness.

Congenital ptosis is the congenital myogenic dystrophy of the levator muscle [19]. In the general population, the incidence of amblyopia due to congenital ptosis is reported between 3-5% [20,21]. Refractive errors, strabismus, and amblyopia are more common in those with congenital ptosis than those in the general population [20,22,23]. Therefore, it is important to perform examinations for strabismus and refraction to prevent the development of amblyopia in those with ptosis. In our study, congenital ptosis was detected in 13 patients (0.23%).

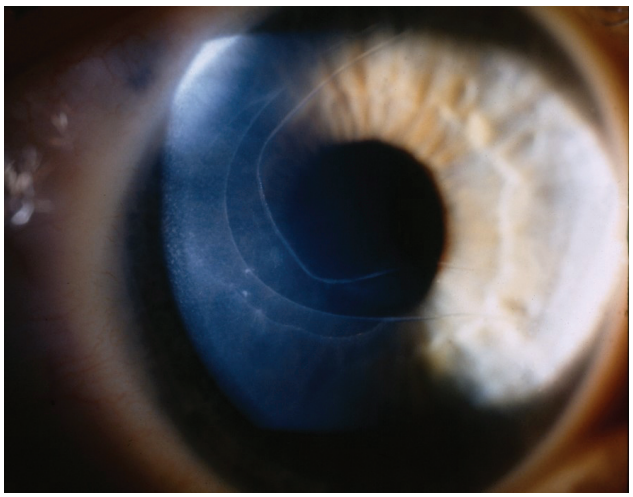

Figure 5. Congenital glaucoma, Haab striae.

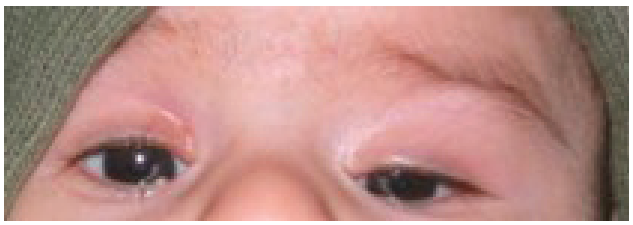

Figure 6. Congenital ptosis.

One of the patients had bilateral ptosis, while unilateral ptosis was found among the rest. Blepharophimosis syndrome was present in the bilateral case. Blepharophimosis syndrome is characterized by the narrowed horizontal palpebral opening, ptosis, epicanthus inversus, and telecanthus [24]. The case of congenital ptosis is seen in Figure 6.

While strabismus is seen between 2-4 % of the general population [25], esotropia is seen between 1-3% of the children, and infantile esotropia constitutes 28-54% of all childhood esotropias [26]. Based on a recent study, esotropia was reported to affect an average of 0.25% of the society [27]. The critical period for the development of stereopsis is between the 4th and 6th months of age [28]. Therefore, when the exposure of the infant to stereoscopic visual stimuli stops in the first 12th and 24th months of life, the development of stereopsis will be defective [29]. In a strabismic patient, irreversible sensory abnormalities may develop due to delayed examination. Amblyopia treatment with refractive error should be carried out at the earliest period [30]. It was suggested that if the strabismus was corrected before the 6th month of age, the sensory and motor outcomes would become superior to those operated within 18 months [31].

In our study, strabismus was detected in 81 infants. Of 81 infants having strabismus, two and 79 were exotropia and esotropia, respectively. Those with exotropia had also cerebral palsy. Two of those with esotropia had cataracts, and the other two had glaucoma, and the remaining 75 patients were with hypermetropia above three diopters.

Ocular coloboma is a rare malformation developing due to the closing defect of the embryonic optic cleft [32,33]. Ocular colobomas may involve the iris, ciliary body, choroid, retina, and optic nerves [34]. In population-based studies, the prevalence of coloboma is stated to vary from 3.7 to 8 per 100.000 births [35-37]. In our study, four (0.07%) patients had colobomas (one eyelid, four iris, one optical disc, and three chorioretinal). In our study, optic disc coloboma, optic disc hypoplasia, and morning glory syndrome were also observed in three patients (0.05%) as optic disc abnormalities. There was no history of infection,

diabetes mellitus, or drug use in the mothers of these patients. In the light of literature, the frequency of optic nerve hypoplasias was reported between 7-10/100,000 [38,39]. In our study, however, two patients had corneal dysgenesis (0.04%); one had posterior embryotoxone, and the other had corneal opacity with iridocorneal adhesions.

The prevalence of some diseases appears to be lower than that in similar studies screening ocular conditions in infants. Such a situation can be explained by two reasons: The first is that because there are three hospitals in our region, the other infants could be diagnosed in other hospitals, and the second is that some babies are referred to ophthalmologists after 12 months of age, or at a later period.

In conclusion, such diseases as congenital cataracts, congenital glaucoma, strabismus, corneal opacities, retinal pathologies, and optic disc pathologies that may cause poor vision in infants should be diagnosed at an early period. Due to an easy test in detecting problematic eye disorders at the early stages of infants' lives, the red reflex test is of vital importance. We consider that with the early treatment, various situations that may prevent the acquisition of complex vision function in the perinatal period can be eliminated, and so an increase in final visual acuity can be obtained.

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Research Article

The Role of Early Family-Centered Therapy Program in Infants With Brachial Plexus Birth Palsy

Duygu Çubukçu
Ünsal Yılmaz

Doğumsal Brakiyal Pleksus Felçli Bebeklerde Aile Odaklı Erken Terapi Programının Rolü

ABSTRACT

Objective: *The purpose of this study is to evaluate the effectiveness of a family-centered early exercise program in infants with Narakas Stage 1 brachial plexus birth palsy.*
Method: *The data of the infants with brachial plexus birth palsy followed up at the physical medicine and rehabilitation outpatient clinic of a tertiary pediatric research center were retrospectively investigated. Infants with Narakas Stage 1 brachial plexus birth palsy according to the Narakas classification were included in the study. Sixty infants were assessed using a passive-active range of motion (ROM) and active movement scale at first clinic visit and every month until they became 12 months old.*
Results: *Cases were divided into two groups (Group I, n:32) or (Group II, n:28) according to their referral times to the outpatient clinics of physical medicine and rehabilitation. In both groups, a significant improvement was observed in the ROM and muscle strength of shoulder, elbow flexion, and forearm supination at the 3rd, 6th and 12th-month assessments. At 12th months intergroup differences were detected in shoulder abduction muscle force assessments ($p<0.05$) and active ROM measures of shoulder abduction-internal rotation-external rotation and forearm supination ($p<0.05$).*
Conclusion: *Family-centered early therapy program is effective against complications that may occur in the first year of infants with Narakas Stage1 brachial plexus birth palsy. In infants with brachial plexus birth palsy, not only neurological improvement should be focused, but also regular follow-ups should be made regarding complications.*

Keywords: *Brachial plexus birth palsy, brachial plexus, physiotherapy*

ÖZ

Amaç: *Bu çalışmanın amacı, aile odaklı erken terapi programının Narakas evre 1 olan doğumsal brakiyal pleksus felçli infantlar üzerindeki etkinliğini değerlendirmektir.*
Yöntem: *Üçüncü basamak pediyatrik araştırma merkezinin fiziksel tıp ve rehabilitasyon polikliniğinde takip edilen doğumsal brakiyal pleksus felçli infantların verileri retrospektif olarak incelendi. Çalışmaya Narakas sınıflamasına göre Narakas Evre 1 brakiyal pleksus doğum felçli bebekler dahil edildi. Altmış bebek, pasif-aktif hareket açıklığı (EHA) ve aktif hareket ölçeği ilk klinik ziyareti ve sonrasında 12 aylık olana kadar her ay değerlendirildi.*
Bulgular: *Olgular, fiziksel tıp ve rehabilitasyon kliniğinde polikliniğine sevk zamanına göre iki gruba (Grup I, 32 olgu) (Grup II, 28 olgu) ayrıldı. Her iki grupta da 3., 6. ve 12. ay değerlendirmelerinde omuz, dirsek fleksiyonu ve önkol supinasyonunun EHA ve kas gücünde anlamlı iyileşme gözlendi. Omuz abdüksiyon kas kuvveti değerlendirmelerinde ($p>0,05$) ve omuz abdüksiyon-iç rotasyon-dış rotasyon ve önkol supinasyon aktif EHA ölçümlerinde 12. ay grupları arasında farklılıklar vardı ($p<0,05$).*
Sonuç: *Aile odaklı erken terapi programı Narakas evre 1 doğumsal brakiyal pleksus felçli infantlarda hayatın ilk 1 yılında oluşabilecek komplikasyonlara karşı etkilidir. Doğumsal brakiyal pleksus felçli bebeklerde sadece nörolojik iyileşmeye odaklanılmamalı, aynı zamanda komplikasyonlar ile ilgili düzenli takipler yapılmalıdır.*

Anahtar kelimeler: *Doğumsal brakial pleksus felci, brakial pleksus, fizyoterapi*

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INTRODUCTION

Brachial plexus birth palsy (BPBP) is an upper extremity paralysis that occurs due to traction injury of the brachial plexus during birth [1]. Various data were obtained at different times and locations in terms of its incidence. Its incidence has been reported at a rate ranging from 0.42-4 per 1000 live births [2,3]. As per Narakas' classification, the most obstetrical brachial plexus injuries are classified as type 1: Erb's palsy involving the spinal levels of C5 and C6. Studies have shown that the course of narakas type 1 BPBP leads a better course [4,5]. Howover some of these infants with a seemingly complete neurologic recovery will develop a shoulder contracture or subluxation during growth [6]. Several studies submitted their functional results however scheduling-planning of exercise programs and their contributions have not been presented in detail [4, 7, 8]. For this reason, this study was planned to investigate the effectiveness of the early exercise program applied on infants with narakas grade 1 brachial plexus birth palsy.

MATERIAL and METHODS

Newborns with diagnosis of the BPBP newborn infants referred to the physical medicine and rehabilitation (PMR) outpatient clinic of Izmir Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital over a three-year period were included in this study. The infants were identified by retrospectively investigating a prospective BPBP record at a single tertiary pediatric research hospital.

Patients were classified using Narakas classification [9]. All nonsurgical candidates (Narakas class 1) were included in the study. The patients who had another comorbid neurological problem, fracture on the affected arm, lost to follow-up (<12 mo) and surgical candidates were excluded from the study (*n*=4). Sixty-four infants constituted the study sample. At each clinical visit, patients underwent standardized physical examination every month with use of the Active Movement Scale (AMS) and measurements of passive and active range of motion of the elbow and shoulder. Recovery of antigravity elbow flexion was defined as AMS ≥5 for elbow flexion. Our work was followed up regularly every month until one year but was given statistical data for the first, 3. months, 6. months and 12. months of age.

Since 4 patients had biceps muscle force <3 AMS when they were 4-5 months old, these children were referred to surgery and excluded from the study. A retrospective search of this prospective database identified 60 patients.

Demographic information including age at the time of referral for PMR outpatient clinic for initial functional evaluation, gender and affected side was recorded. Subjects were divided into two groups (group I, n:32) or (group II, n:28) according to PMR referral time. The group I received a family-centered therapy program starting from the 3 weeks of life (Early referred to PMR), whilst the group II received this program the after 3 weeks of life (Delayed referred to PMR). Thirty two (%53) patients were referred within the 3 weeks after delivery, while 28 (%47) applied after the first 3 weeks of life.

The families were trained by a physiotherapist at the hospital through a visual and applied training program, and then the parents were tested. The patients are evaluated by the same physiatrist for sustained. Within the scope of this program, a few days after birth, the patients were recommended to support the arm in the neutral position for 10 days, and after 10 days the joint movements of the paralyzed limb were initiated. Parents were taught a home-based range of motion exercise program, and guided by monthly therapy sessions. Very gentle passive range of motion (PROM) were given every waking hour for 10 repetitions for shoulder flexion, abduction-external rotation, elbow flexion and extension, forearm supination and pronation and wrist flexion and extension. One month aftery delivery passive joint movements were first shown to the parents and progressed towards active reinforcement, active positioning and strengthening exercises against gravity in the periodic evaluator lights. Passive joint movements were first shown to the parents, and proceeded towards active assistive

and strengthening exercises against gravity in the periodic evaluative directions. In order to increase the awareness of the child's paretic extremity, the family were taught initiatives that could induce tactile stimulation, such as contacting materials with different tissue surfaces along the child's affected arm and transferring weight to the affected arm. In order to increase the awareness of the child's paretic extremity, the family were taught initiatives that could induce tactile stimulation, such as contacting materials with different tissue surfaces along the child's affected arm and weight-bearing exercises. After the 4 th; the exercises in the previous stages were continued. Bimanual activities were encouraged to avoid neglect of the limb involved [10].

Assessment criteria: Narakas classification; this classification consists of four groups: Group I C5-6 shoulder and biceps paralysis, Group II C5-7 shoulder, biceps and forearm extremities, Group III C5-Th1 complete paralysis of the extremity and Group IV C5-Th1 complete paralysis of the extremity accompanying Horner syndrome [9]. The Active Movement Scale (AMS): AMS is a method recommended for the evaluation of patients with brachial palsy from the newborn period to the age of one [11,12]. At each visit functional recovery is assessed with a special grading system based on" the AMS" as below. The Active Movement Scale (AMS) is used to objectively examine the activation of UE muscle groups. Scores for UE joint motions are graded on a 0-7 point scale based on the percent of active motion observed within the available PROM. Active motions performed with minimize impact of gravity scored from 0-4 and motions performed against gravity are scored from 5-7 [11,12] (Table 1). Range of motions (ROM) involving flexion-extension and abduction adduction and internal and external rotation of the shoulder, flexion, extension and supination of the elbow are measured by goniometer and then recorded.

Ethical Approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee as well as the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the medical local research ethics committee of Izmir Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital (26.12.2013/No:1339918/80).

Statistical Analysis: Statistical evaluation was performed using PASW Statistics for Windows, Version 18.0. (SPSS Inc. Chicago, USA, Released 2009). The Student t-test was used for independent groups to analyze data that complied with a normal distribution. A paired sample t-test was used for dependent group analysis $p<0.05$ was considered to be statistically significant.

RESULTS

According to Narakas classification, 64 patients asessed as grade I cases were included in this study. Since biceps muscle strenght of 4 patient was AMS <3 when they were 4-5 months old, these children were referred to surgery and excluded from the study. So a total of 60 patients were included in the study. There were a total of 32 patients in Group I (15 girls, 17 boys; mean age 7.4±day, mean birth weight 3420±287 grams) and 28 patients in group II (13 girls,15 boys; mean age 37.8±0.5 day, mean birth weight 3485±216 grams). The general characteristics of both groups are presented in Table 1. At baseline, there were no significant differences between two groups.

İntragroup statistical analysis inside the groups: In terms of active ROM, while a statistically significant improvement was achieved in both groups between

Table 1. Hospital for sick children muscle grading system.

Observation Muscle grade	
Gravity eliminated	0
No contraction	1
Contraction, no motion	2
Motion<1⁄2 range	3
Motion> 1⁄2 range	4
Full motion	
Against gravity	5
Motion<1⁄2 range	6
Motion> 1⁄2 range	7
Full motion	

Table 2. Characteristics of patients of BPBP.

n=60	Group I (n=32)	Group II (n=28)	p
Age (days)			
Mean±SD	7.4±2.0	37.8±5.0	<0.001
Sex, n (%) *			
Girl	15 (46.9)	13 (46.4)	
Boy	17 (53.1)	15 (53.1)	0.603[2]
Affected side, n (%) *			
Right	17 (53.1)	13 (46.9)	
Left	15 (46.9)	15 (53.1)	0.603[2]
Birt weight (g)			
Mean±SD	3420±287	3485±216	0.515[1]

**Row percentage, [1]t-test, [2]chi square-test*

1st-3rd months, 3rd-6th months and 6th-12nd months in shoulder abduction-flexion, external-internal rotation and elbow flexion and forearm supination (p<0,001). Active ROM measures of the patients are shown in Table 3. A significant muscle strength improvement was observed in both groups between 1st, 3rd, 6th, and 12nd months in shoulder abduction, shoulder flexion, elbow flexion and forearm supination (p<0.001). The active movement scale (AMS) measures of the patients are shown in Table 4.

Statistical analysis between the groups: statistically significant difference was observed between the

Table 3. Hospital for sick children muscle grading system in patients with BPBP.

	Birth		3 months		6 months		12 months	
n=60	Group I (n=30)	Group II (n=30)	Group I (n=30)	Group II (n=30)	Group I (n=30)	Group II (n=30)	Group I (n=30)	Group II (n=30)
Shoulder								
Abduction[1]	2.5±0.5	2.7±0.4	3.8±0.4	3.8±0.5	5.3±0.5	5.0±0.6	6.5±0.5[2]	6.2±0.4[2]
Flexion[1]	2.4±0.6	2.6±0.3	4.6±0.7	4.5±0.7	5.6±0.4	5.5± .5	6.6±0.4	6.4±0.5
Elbow								
Flex[1]	0.8±0.6	1.1±0.7	3.5±0.5	3.4±0.5	5.5±0.5	5.5±0.5	6.6±0.4	6.5±0.5
Forearm								
Supination[1]	0.5±0.5	0.7±0.6	2.8±0.4	2.9±0.5	5.5±0.7	5.4±0.5	6.5±0.5	6.4±0.5

[1]: Statistically significant differences according to the birth value inner group I and II evaluations (p<0.001).
[2]: p<0.05 for each between group evaluations.

Table 4. Measurements of active range of motion in patients with BPBP.

	Birth		3 months		6 months		12 months	
n=60	Group I (n=32)	Group II (n=28)	Group I (n=32)	Group II (n=28)	Group I (n=32)	Group II (n=28)	Group I (n=32)	Group II (n=28)
Shoulder								
Abduction[1]	13.7±3.3	15.7±5.4	37.0±4.4	36.6±4.7	62.2± .0	59.2±7.4	103.7±27.6[2]	88.0±21.6[2]
Ext rot[1]	0	0.3±2.6	18.1±1.9	17.7± .3	24.9±1.9	23.9± .1	33.4±9.1	30.5± 6.5[2]
Int rot[1]	36.0±4.2	39.2± .2	59.1±3.4	57.5± .0	76.3±3.5[2]	73.5±4.5[2]	79.0±2.4[2]	76.0±4.5[2]
Flexion[1]	20.3±3.6	23.7±3.0	48.8±4.3	46.6±9.4	67.3±12.7	73.3±22.1	92.8±21.4	87.1±2.5
Elbow								
Flexion[1]	0.9±1.5	1.9±3.0	65.7±4.1	63.2±5.4	78.2±3.8	76.4±3.6	103.7±5.6	101.0±6.8
Forearm								
Supination[1]	1.1±2.0	1.6±2.3	31.6± .6	29.2±5.3	68.1±7.2	65.4±8.6	79.8±6.6[2]	74.2±7.4[2]

[1]: Statistically significant differences according to the birth value inner group I and II evaluations (p<0.001).
[2]: p<0.05 for each between group evaluations.

groups At 12nd months in shoulder abduction muscle force assessments and (p>0.05) shoulder abduction-internal rotation-external rotation and 6th months in shoulder internal rotation (p<0,05).

All infants regained antigravity elbow flexion (AMS≥5) at a median of 6 months. Elbow outcomes remained unaffected by delayed referral to PMR. Early referral PMR clinic were associated with an improved shoulder outcome. As for range of passive motion two children had reduced shoulder external

rotation and observed revealed posterior glenohumeral dislocation, two supination contracture and the trumpet sign was present in 2 children at 12 months of age in group II.

DISCUSSION

The Group I received a family-centered early therapy program starting from the 3 weeks of life, whilst the Group II took this program after 3 weeks of life. The parents of 28 children were unable to early bring their children to the hospital, because they were living in the rural or remote areas. This study proved that regarding Narakas 1 BPBP patients, any significant difference did not exist between the groups who had, and had not started exercise programme at an early stage as for functional improvement in elbow. In all the patients who early initiate exercise program nearly normal shoulder functions were found 12 months old. Shoulder complications developed in the patients during their follow-ups who did not the initiate exercise program early. Early referral for physical therapy has been observed to be beneficial to children with BBPI.

Meta-analysis-based results revealed that the prognosis of Narakas stage 1 cases have a more improved prognosis [13]. However, it should be kept in mind that approximately 20-30% of patients with C5-C6 root involvement may develop permanent deficiencies on the upper limb [1]. A physical therapy program is the recommended treatment for patients with BPBP at the first stage. The main goal of physical therapy program is to prevent the development of contractures in the period when improvement of passive joint movement, the flexibility of joints and muscle strength are expected and healing of the plexus. In many studies; the effectiveness of primary conservative treatment in infants with BPB has been investigated, but a complete conclusion could not be achieved [8,14]. Some authors suggested that exercises did not have much effect on recovery [5]. However, there are limited studies about the time-planning of exercise programs for the patients with brachial plexus birth palsies.

It is generally believed that most BPBP patients will recover spontaneously [15]. However, many studies have overestimated the spontaneous functional recovery rate, as reported in the systematic review of Pondaag et al [16]. Hamzat et al. [17], reported that some infants with BPBP were referred for physiotherapy more than 6 months after birth. In this study, it was observed that some narakas 1 cases were neglected and were not directed in the early period. Parents who may not have appreciated the significance of the disorder can delay their appointments for various family-social-economic reasons and difficulties in accessing PMR clinic. In some cases, the movements of the biceps and deltoid muscles that could never reach a sufficient level; cause complaints induce optimistic expectations in the parents of the patient and the infant is not brought for control.

DiTaranto et al [18] evaluated 91 infants with BPBP treated conservatively for 2 years. In 69% of the patients with a typical Erb's palsy functions of biceps, deltoid, triceps, and wrist recovered well by 6 months, and by 2 years, only mild shoulder range of motion was only mildly restricted. Therapy consisted of active, active-assisted and passive range of motion exercises. Azzi et al reported an association between delayed referral Narakas class 1 BPBP the the obstetric brachial plexus clinic and shoulder outcome. They stated that the early referral to the PMR clinic crucially prevents joint contractures. However, the frequency and intensity of physical therapy were not taken into account in this study [19].

Ultrasound is recommended to detect infantile shoulder dislocation [20]. Therefore, in our study, we evaluated suspicious patients with ultrasound. Dahlin et al; described six of 82 patients with brachial plexus birth palsy who developed a posterior dislocation of the shoulder during the first year of life [21]. In our study, 5 patients were referred for baseline shoulder ultrasonography at 12 months of age and posterior glenohumeral dislocation was revealed in 2 patients aged> 3 weeks who applied to PMR clinic.

A patient who detected internal rotation contracture of the shoulder in the 9th months of age has used a SUP-ER orthosis for three months. An

increase in the ROM of the passive shoulder external rotation was observed at the 12th month. Weekly kinesiology taping added to stretching exercises was applied to 2 patients who developed supination strain in 9 months. The kinesiotype of the patients was renewed every week until 12 months. The supination strain of these patients was resolved at 12 months. However, supination contracture and trumpet sign were observed in 2 different patients at 12 months. No relationship was found between rotation movements of the forearm and the onset time of biceps recovery. The age at which biceps function recovers is not a reliable predictor of forearm rotation.

Exercises increase the sensory input which results in increasing plasticity. The development of plasticity can be precipitated with excercises initiated at an early stage and repeated frequently, and regularly [22,23]. In the first months of neonatal life, passive joint movement exercises and stretches performed depending on the number and set of motion repetitions performed with the affected upper extremity may play a role in preparing the environment for regeneration by preventing secondary changes that may ocur during joint movements. In our study, it was shown that secondary deformities were prevented by shoulder positioning and range of motion exercises taught to the family from the birth. It is important to perform regular measurements in children with birth-related brachial plexus palsy in order to follow the clinical course adequately and detect and take precautions against future deformities.

Our results indicate significant improvement in ROMs, and the function to the upper extremity functions by means of an early exercise program. The newborn with BPP should be referred to PMR at an early stage and then followed-up regularly. Focus should not be placed only on neurological improvement but the patients should also be monitored regarding complications. Since the rate of spontaneous recovery tends to be less than the previously reported literature data, early diagnosis and regular follow-up should be performed by a qualified healthcare provider who is aware of the complications and long-term effects of this condition.

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Conflict of Interest: There is no conflict of interest among the authors.

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Research Article

The Prevalence and Risk Factors of Allergic Diseases in School-Age Athletes in Southwest of Turkey: A Cross-Sectional Questionnaire Study

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Türkiye'nin Güney Batısında Okul Çağındaki Atletlerde Alerjik Hastalık Prevalansı ve Risk Faktörleri: Kesitsel Anket Çalışması

ABSTRACT

Objective: *The prevalence and the risk factors influencing allergic disorders in amateur athletes are still poorly defined. The aim of this study was to evaluate the prevalence and risk factors of the common allergic disorders in school aged athletes compared with the general population in southwest of Turkey.*
Methods: *Using the "International Study of Asthma and Allergy in Childhood (ISAAC) Phase I" questionnaire, 714 athletes aged 7-18 and 325 age-matched healthy controls were examined. Risk factors that would affect the prevalence were evaluated with the questions given in addition to this questionnaire.*
Results: *In the athlete group, the prevalence of existing asthma, allergic rhinitis, and eczema was lower than controls [(3.8%) and (16.3%), respectively, p<0.001], [(18.7%) and (42.1%)], respectively, p <0.001] and [(5.5%) and (10.5%), respectively, p<0.001]. Multivariate logistic regression analysis revealed that in the athlete population, a previously known allergy increased risk of curent wheezing (odds ratio [OR] =5.3; confidence interval, [CI]=1.8-15.4), current allergic rhinitis ([OR]=2.8; [CI]=1.3-6.2), and current eczema [OR]=4.5; [CI]=1.1-17.1). Familial atopy increased risk of current allergic rhinitis (OR=2.2; CI=0.9-4.9), and current eczema ([OR] = 6.6; [CI]=1.7-25.7).*
Conclusion: *This study is the first prevalence study using the ISAAC method in school-age sports children in southwestern Turkey. Unlike adults, the prevalence of asthma, allergic rhinitis and eczema was found to be lower than controls of the same age. It is thought that sports and spending more time outdoors in children reduce allergic inflammation.*

Keywords: *School age, children, allergic disease, sports, prevalence, athlete*

ÖZ

Amaç: *Spor yapan çocuklarda alerjik hastalıkların sıklığı ve bu hastalıklara yol açan risk faktörleri tam olarak tanımlanmamıştır. Bu çalışma,Türkiye'nin güneybatısında okul çağındaki sporcularda alerjik hastalıkların sıklığını ve risk faktörlerini araştırmayı amaçlamaktadır.*
Yöntem: *Kesitsel, prospektif bu çalışmaya 7-18 yaş arası 714 sporcu ve yaş uyumlu 325 sağlıklı kontrol grubu alınmıştır. Alerjik hastalıkların prevalansı için "International Study of Asthma and Allergy in Childhood (ISAAC) Phase I"anketi kullanılmış, prevalansı etkileyecek risk faktörleri ise bu ankete ek olarak verilen sorular ile değerlendirilmiştir.*
Bulgular: *Okul çağındaki sporcu çocuklarda; astım (%3,8), alerjik rinit (%18,7) ve egzama (%5,5) sıklığı kontrol grubundaki astım (%16,3), alerjik rinit (%42,1) ve egzama (%10.5) sıklığına göre daha düşük idi (sırasıyla p<0,001, p<0,001, p<0.001). Çok değişkenli lojistik regresyon analizinde okul çağındaki sporcu çocuklarda daha önce bilinen bir alerjik hastalık olmasının, mevcut hışıltı(odds oranı [OR]=5.3; güven aralığı, [CI]=1.8-15.4), alerjik rinit ([OR]=2.8; [CI]=1.3-6.2) ve egzama ([OR]=4.5; [CI]=1.1-17.1) için önemli bir risk faktörü olduğunu gösterdi. Ailede atopi varlığının alerjik rinit ([OR]=2.2; [CI]=0.9-4.9) ve egzama ([OR]=6.6; [CI]=1.7-25.7) ile istatistiksel olarak anlamlı ilişkisi olduğu saptandı.*
Sonuç: *Bu çalışma Türkiye'nin güneybatısında okul çağındaki sporcu çocuklarda ISAAC yöntemi kullanılarak yapılan ilk prevalans çalışmasıdır. Çalışmada erişkinlerden farklı olarak astım, alerjik rinit ve egzama prevalansı aynı yaştaki kontrollere göre daha düşük bulunmuştur. Çocuklarda sporun ve dış ortamda daha fazla zaman geçirmenin alerjik inflamasyonu azalttığı düşünülmüştür.*

Anahtar kelimeler: *Okul çağı, çocuklar, alerjik hastalıklar, spor, prevalans, atlet*

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INTRODUCTION

Estimating the incidence and prevalence of allergic disorders such as asthma, allergic rhinitis and eczema, is challenging worldwide. Allergies are affected by environmental factors, including diet, infections, exposure to air pollutants and occupational exposure [1]. A large proportion of the young population undertake exercise and amateur sports in various disciplines [2].

Recent epidemiological studies have reported that the prevalence of asthma and rhinitis in athletes is higher than in the normal population [3,4]. In contrast, some studies have found that doing sport does not affect allergic symptoms, including asthma and rhinitis [5,6]. The relationship between sports activities and allergies in school children is not yet fully understood. It is seen that the problems regarding the management and prevention of allergic symptoms in this age group continue [7].

Allergic diseases can decrease sports performance through deteriorations in quality of life, sleep disturbances and inadequate training [8-10]. Exercise-induced (EI) allergic disorders are complex, frustrating and distressing for both patients and physicians. The respiratory (asthma, bronchospasm and rhinitis), cutaneous (urticaria and angio-oedema) or cardiovascular (anaphylaxis) systems are usually targeted [11].

Asthma and rhinitis are more common in competitive athletes because of airway dehydration from hyperpnea, increased exposure to aeroallergens and airway injury caused by irritant chemicals and the environment [3,4,11].

The prevalence of allergic diseases shows a great variability and differs not only between countries but also among various regions of the same country [12] (The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee). Worldwide variations have been reported in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema [13]. The inconsistencies in rates can also be attributed to methodological differences between studies, such as variations in the age range of study participants [12]. In recent years, there are many studies on the prevalence of asthma and allergic diseases in our country. Prevalence studies have reported a frequency of 2.8% to 13.4% for asthma, 3.4% to 13.3% for wheezing, 4.5% to 17.3% for allergic rhinitis, and 0.9% to 4.6% for atopic dermatitis [12,14-20].

To the best of our knowledge, there is no epidemiological study about allergic diseases in young athletes in the southwest of Turkey. Therefore, the aim of this survey-based study was to investigate the prevalence and risk factors of asthma, allergic rhinitis and eczema in young Turkish athletes, and to compare the results with an age-matched control group.

MATERIAL and METHODS

Study Population and Design

This cross-sectional study was conducted between June 2019 and January 2020, using the ISAAC Phase I method on a group of children aged 7-18 years (n:714), who presented at the Sports Medicine Department of our hospital requesting a medical certificate testifying to their suitability to practise a sport (mandatory by Turkish law for participation in sports). The control group was formed of sex-matched volunteer children aged 7-18 years, randomly selected from the general population (n:325). Parents were asked to complete the International Study of Asthma and Allergies in Childhood (ISAAC) phase I questionnaire, which has been previously validated for the Turkish population [13,21]. An additional questionnaire was prepared and used to identify related risk factors for allergy. The research was reviewed and approved by the Institutional Review Board, (2019-222, 16/11) and informed consent was provided by a parent of each subject.

Questionnaires

Parents were questioned about allergic diseases of their child such as asthma, allergic rhinitis and atopic dermatitis, using the International Study of Asthma and Allergies in Childhood (ISAAC) phase I questionnaire. Risk factors that would affect the

prevalence were evaluated with the questions given in addition to this questionnaire. The additional questionnaire included items about the income level of the family, number of persons in the household, the presence of a separate bedroom for the child, home heating system, educational levels of the parents, employment status of the parents, prematurity, duration of breastfeeding, the time of starting supplementary foods, pet ownership, and tobacco smoke exposure at home and during pregnancy. Income was classified in three levels as 'lower than 300 USD, between 300-750 USD and higher than 750 USD. Famial atopy was defined as a positive history or diagnosis of asthma, rhinitis, and/or eczema in one or both of the parents.

Definitions and terms

Individuals were requested to reply to questionnaire which contained questions regarding the terms below [22].

- Wheeze ever: Have you ever had wheezing or whistling in the chest at any time in the past?
- Current wheezing: In the past 12 months, have you had wheezing or whistling in the chest?
- Severe wheeze in the past year: In the past 12 months, has wheezing ever been severe enough to limit your speech to only 1 or 2 words at a time between breaths?
- Asthma ever: Have you ever had asthma?
- Exercise-induced wheeze in the past year: In the past 12 months, has your chest sounded wheezy during or after exercise?
- Nocturnal cough in the past year: In the past 12 months, have you had a dry cough at night, apart from a cough associated with a cold or a chest infection?
- Allergic rhinitis ever: Have you ever had a problem with sneezing, or a runny, or a blocked nose when you did not have a cold or the flu?
- Current allergic rhinitis: In the past 12 months, have you had a problem with sneezing, or a runny, or a blocked nose when you did not have a cold or the flu?
- Current rhinoconjunctivitis: In the past 12 months, has this nose problem been accompanied by itchy/watery eyes?
- Hay fever ever: Have you ever had hay fever?
- Itchy rash ever: Have you ever had an itchy rash which came and went for at least 6 months?
- Current itchy rash: Have you had this itchy rash at any time in the past 12 months?
- Itchy flexural rash in the last year: Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears or eyes?
- Eczema ever (skin allergy ever): Have you ever had eczema (skin allergy)?

Statistical Methods

Data obtained in the study were analysed statistically using SPSS vn. 23.0 software. Descriptive statistics were presented as mean, standard deviation, median, IQR, minimum and maximum values, or number (n) and percentage (%). Conformity of the data to normal distribution was assessed using the Kolmogrov- Smirnov test. The Chi- square test, Fisher Exact Test and Mann Whitney U-test were used to compare groups. For the multivariate analysis, the possible factors identifed with univariate analyses were then entered into the logistic regression analysis to determine independent predictors of asthma, allergic rhinitis and eczema outcome. Hosmer-Lemeshow goodness of fit statistics were used to assess model fit. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The athletes group comprised 67.9% males with mean height of 155±17 cm (range, 110 -197 cm) and the control group comprised 54.8% males with mean height of 152±18 cm (range, 110 -187 cm) ($p<0.0001$, $p=0.019$). The demographic characteristics of the study participants are summarized in Table 1.

The sports practised most frequently in the

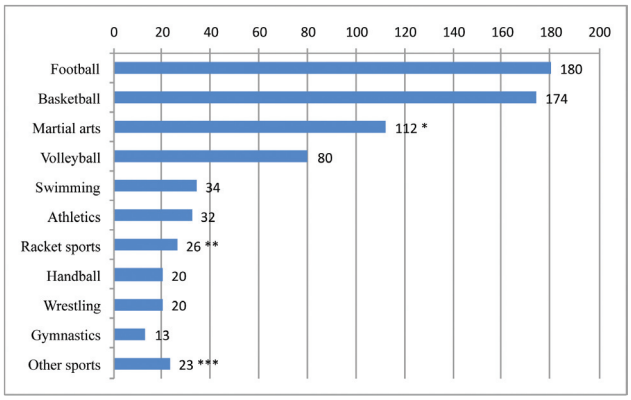
Table 1. The demographic characteristics of the groups.

	AG	CG	p value
No of subjects (%)	714 (68.7%)	325 (31.3%)	0.801
Age (years) (mean±SD)	12.65±2.88	12.72±3.34	<0.0001*
Male/Female	485/229	178/147	0.019
Height (cm) (mean±SD)	155±17	152±18	0.198
BMI (mean±SD)	19.29±3,53	19.69±3,83	0.484
Previously known allergy n (%)	69 (9.7)	36 (11.1%)	<0.0001
Familial atopy n (%)	65 (9.1)	80 (24.6)	
Income level n (%)			
<300 USD	139 (20.1)	42 (13.3)	0.004*
300-750 USD	446 (64.5)	204 (64.6)	
>750 USD	107 (15.5)	70 (22.2)	
Total	692 (100)	316 (100)	
Maternal education n (%)			
Primary school	171 (24.1)	75 (23.3)	0.68
Secondary school	107 (15.1)	52 (16.1)	
High school	220 (31)	90 (28)	
University	211(29.8)	105 (32.6)	
Total	709 (100)	322 (100)	
Paternal education n (%)			
Primary school	145 (20.4)	53 (16.6)	0.002*
Secondary school	99 (13.9)	40 (12.5)	
High school	241 (33.8)	85 (26.6)	
University	227 (31.9)	142 (44.4)	
Total	712 (100)	320 (100)	
Gestational age (≤37 weeks)	34 (4.9%)	18 (5.9%)	0.52
Breast feeding (mean±SD)	16.49±9.25	15.31±8.43	0.17
Starting supplementary food (mean±SD)	6.17±1.6	6.12±1.6	0.84
Smoking at home n (%)	177 (25.5)	105 (33.5)	0.008*
Smoking during pregnancy	42 (6.1)	41 (12.9)	<0.0001*
Home type n (%)			
Apartment	624 (87.8)	248 (76.5)	<0.0001*
Detached house	60 (8.4)	58 (17.9)	
Slum area dwelling	27 (3.8)	18 (5.6)	
Total	711(100)	324(100)	
Duration of doing sports (years) mean±SD	2.68±2.29		

*Values in parentheses are percentages; *p<0.05 is statistically significant, AG: Athletes Group, CG: Control Group, SD: Standard Deviation.*

athletes group were football (25.2%) and basketball (24.4%). The athletes had been playing sports for an average of 2.68±2.29 years. The sport disciplines and categories are summarized in Figure 1. There was a positive association between doing sports and income level below 300 USD per month and above 750 USD per month (p=0.004). In the athlete group, there was a higher rate of fathers with college level education (p=0.002).

Maternal education status, prematurity, a separate bedroom, number of people living in the home, home heating system, and pet ownership



**Taekwondo, Kick-boxing, Muay-thai, Karate, Judo*
***Tennis, Table tennis, Squash, Badminton*
****Archery, Cycling, Orienteering, Weightlifting, Hockey, Skating*

Figure 1. Distribution of the athletes according to sport disciplines.

Table 2. Responses to the key questions of the questionnaire.

Question	AG	CG	p
Wheeze ever	45 (6.3)	86 (26.5)	<0.0001*
Current wheeze	27 (3.8)	53 (16.3)	<0.0001*
Attacks of wheezing in last year			
• 1-3	21 (2.9)	41 (12.6)	<0.0001*
• 4-12	6 (0.8)	10 (3)	
• <12	0(0)	1(0.3)	
Sleep disturbed by wheeze in last year			
• Never woken with wheezing	15 (2.1)	24 (7.3)	<0.0001*
• Less than one night per week	11 (1.5)	24 (7.3)	
• One or more nights per week	2 (0.3)	5 (1.5)	
Severe attacks of wheeze limiting speech in last year	3 (0.4)	21 (6.7)	
Doctor-diagnosed asthma	32 (4.5)	35 (10.8)	<0.0001*
Wheeze after exercise in last year	13 (1.8)	27 (8.3)	<0.0001*
Waking with cough in last year	37 (5.2)	78 (24)	<0.0001*
Allergic rhinitis ever	167 (23.4)	158 (48.6)	<0.0001*
Current rhinitis	134 (18.8)	137 (42.2)	<0.0001*
Current rhinoconjunctivitis	46 (6.4)	65 (20)	<0.0001*
Doctor-diagnosed allergic rhinitis	36 (5)	44 (13.5)	<0.0001*
Itchy rash ever	29 (4.1)	42 (12.9)	<0.0001
Current itchy rash	18 (5.5)	34 (10.5)	<0.0001*
Itchy flexural rash in the last year	13 (1.8)	24 (7.4)	<0.0001*
Doctor-diagnosed eczema	17 (2.4)	12 (3.7)	0.23

*Values in parentheses are percentages; *p<0.05 is statistically significant, AG: Athletes Group, CG: Control Group.*

were not correlated with doing sports. The prevalence of ever wheeze, current wheeze, doctor diagnosed asthma, ever rhinitis, current rhinitis, current rhinoconjunctivitis, doctor diagnosed conjunctivitis, itchy rash ever, current itchy rash and doctor

diagnosed eczema was lower in the athlete group (6.3%, 3.8%, 4.5%, 23.4%, 18.8%, 6.4%, 5.0%, 4.1%, 5.5%, 2.4%) compared to the control group (26.5%, 16.3%, 10.8%, 48.6%, 42.2%, 20.0%, 13.5%, 12.9%, 10.5%, 3.7%, p<0.001, respectively). The prevalence rates of atopic eczema, allergic rhinitis and asthma are shown in Table 2. Differences were determined in sex, height, family income level, and paternal education level. After adjustment for potential confounders, the athletes showed no statistically significant increased risk for asthma, allergic rhinitis or eczema. When the influences were evaluated with multivariate logistic regression analysis, in the athlete population, a previously known allergy was associated with a statistically significant increased risk of curent wheezing (OR=5.3; CI=1.8-15.4), current allergic rhinitis (OR=2.8; CI=1.3-6.2), and current eczema (OR 4.5; 95% CI 1.1-17.1). Familial atopy was associated with a statistically significant increased risk of current allergic rhinitis (OR=2.2; CI=0.9-4.9), and current eczema (OR=6.6; CI=1.7-25.7). A seasonal difference in complaints was associated with a statistically significant increased risk of current allergic rhinitis (OR=7.7; CI=2.9-20.5) in comparsion with the control group.

DISCUSSION

Epidemiologic studies have demonstrated that the prevalence of allergic diseases have increased progressively over the last few decades in worldwide. Likewise, a rising trend of asthma and allergy have also been observed among competitive athletes [4,23,24]. The studies demonstrated that the prevalence of rhinitis in elite athletes was highly variable, ranging from 16.8 % to 56.0% and the and the prevalence of asthma between 3.7% and 22.8%, depending on the study population and methods used for diagnosis [25-29]. Therefore, asthma and asthma-like symptoms occur very frequently in elite athletes compared with age-matched control subjects. It has been shown in ongoing studies that the frequency of the disease continues in comparable population samples [24,29-31]. Most of the studies examining the relationship between allergy and sports have been conducted on elite or Olympic athletes who are quite heterogeneous in terms of age, gender, geographical origin and type of sport. The training programs of these athletes differ considerably from those of non-professional young amateur athletes [32].

Unlike previous elite athlete studies, we showed that there is no increased risk of allergic diseases in school-age athlete children in our study. In Turkey, school-age children in must obtain a physical fitness certificate from the Sports Medicine Center in order to participate in any sports activity. Since almost all the residents of Antalya who are involved in sports are examined at the Sports Medicine Center, it can be considered that the study sample is probably quite representative of the entire athlete population in the region. Macucci et al. [33] assessed the prevalence of asthma and related respiratory symptoms in a sample of the Siena pediatric population engaged in sport. The subjects were 460 young athletes, aged 7-14 years, and the lifetime prevalences of asthma, allergic rhinitis and atopic dermatitis were found to be 17.33%, 22.16% and 11.08%, respectively. Kurowski et al. [24] found the prevalence of diagnosed asthma to be 5.9% and allergic rhinitis 21.0% among Olympic athletes, which was similar to the prevalence in the current study .

The prevalence of self reported asthma in the athletes of the present study was roughly similar to the findings of previous studies of amateur endurance-trained athletes [5,6,34]. Tardivo et al. [5] reported the prevalence of ever asthma and current asthma to be lower in athletes (4.0%, 3.0%) than in the general population (11.0%, 4.8%), asthma was found to be less common in amateur athletes than in the general population and the risk of asthma and respiratory symptoms was associated more with outdoor sports than indoor, but not with any particular type of exercise. Ventura et al. [32] studied 194 soccer players in age groups of beginners (8-11 years), juniors (12-16 years) and under 21 (17-20 years). The prevalence of allergic diseases was 34.5% in the soccer players and 31.6% in the control subjects, skin sensitization to inhalant allergens was detected in 14.4% of symptomatic soccer players and in 19.2% of the control subjects, and patch tests were positive in 35.7% of the soccer players and

23.0% of the control subjects with allergic dermatitis (p>0.05). The prevalence of allergic diseases did not significantly change in relation to the intensity of training. Although the relative prevalence of sensitization to perennial allergens and asthma was less frequent in the soccer players than in the control group, sensitization to pollens was relatively (p<0.05) more frequent in the soccer players than in the control group (64.3% vs 20.0%) while sensitization to dust mites occurred relatively more often in the control group (50.0% vs 14.3%). In the current study, when the groups were separated into age groups of 7-12 years and 13-18 years to exclude the age effect, no significant change was determined in the prevalence of allergic disease.

In another study in Trabzon, northern Turkey, which investigated the prevalence of exercise-induced bronchospasm (EIB) in football players aged 8-18 years, the prevalence of a history of doctor-diagnosed asthma or allergic rhinitis was 0.9% and 2.1% respectively and a reduction in FEV1 of ≥10% was observed after exercise in 22 (9.6%) children [35]. In the current study, EIB was not diagnosed in any of the children with doctor-diagnosed asthma or allergic rhinitis. No difference was determined in the prevalence of EIB between children who reported or did not report ever wheezing, current wheezing, a whistle in the chest after running and playing in the last 12 months, and symptoms of allergic rhinitis. None of the children reported symptoms of atopic dermatitis or had doctor-diagnosed atopic dermatitis. Exercise testing was not performed in this study, but the prevalence of wheeze after exercise was reported to be 1.8%. This may be due to a belief that a child with a chronic respiratory disease such as asthma should avoid any activities including strenuous exercise.

The current study results showed that seasonal complaints were a risk factor for allergic rhinitis. It can be speculated that outdoor activities might increase the airway exposure to environmental factors, such as air pollutants or pollen allergens [36,37]. Asthma and allergy are caused by complex mechanisms and their interaction. Personal factors (familial atopy) and environmental factors play an important role on an individual basis [18,38].

The main result of this study was that the prevalence of self-reported asthma and allergic diseases was lower in athletes than in the control group. It should be taken into account that many differences such as genetic variability, geographical conditions, differences in exercise intensity and duration, and other lifestyles factors may have an effect on this [39]. Although intense regular training over long periods of time may contribute to the development of asthma and other respiratory allergies, regular aerobic training promotes health and may prevents allergic disease by decreasing the systemic inflammation. Physical exercise has been shown to regulate chronic allergic inflammation by modulating cytokines [40,41].

Limitations of this study were primarily the low number of control group children from the general population, the questionnaire-based design and the fact that information bias could not be completely ruled out as there were no face-to-face interviews.

In conclusion, based on the results of this study, the prevalence of self-reported asthma, allergic rhinitis and eczema were seen to be lower in Turkish school-age athletes than in age-matched control subects. Unlike professional athletes, the frequency of allergic diseases is less common among amateur athletes. Previously known allergy and family atopy can be considered to increase the risk of allergic diseases. This study can provide a basis for studies that will have broader involvement, as there is a need for further more extensive studies on this subject using standard survey forms.

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Research Article

Morphometric Analysis of Corpus Callosum with Magnetic Resonance Imaging in Children; Correlation with Age and Gender

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Çocuklarda Korpus Kallozumun Manyetik Rezonans Görüntüleme ile Morfometrik Analizi; Yaş ve Cinsiyetle Korelasyon

ABSTRACT

Objective: *Corpus Callosum (CC) is a dynamic organ that undergoes morphological modifications throughout life which must be taken into account for appropriate evaluation of CC. The objectives of this study were to provide normative data about the morphological modifications in CC during childhood and to evaluate possible differences related to age and sex.*
Methods: *In this retrospective study, cranial MRI studies of 320 children (160 boys, and 160 girls) between 2 to 17 years old with normal neurologic examination findings were reviewed. We divided the age groups as increments of 1 year, and included 10 girls and 10 boys, in each age group. The following parameters were measured on midsagittal T1-weighted images: area of CC (CCA), supracallosal area (SCA), length of CC (LCC), genu thickness (GT), body thickness (BT), splenium thickness (ST), ratio of genu thickness to splenium thickness (GT/ST) and a novel parameter termed callosal index (CCA/SCA). These parameters were correlated with age and sex.*
Results: *All parameter values increased with age. After age 10, there was no evident age or gender-related changes between groups, except for the 15-year-old group. When we appraised all age groups, we determined that CCA and SCA were larger in boys but the callosal index did not differ between genders.*
Conclusion: *This is the first study which used the modified callosal index in the pediatric age group. Callosal index gives the opportunity to make brain size corrections according to the individual and allows a more accurate and personal measurement in the follow-up of the same individual. This study offers an objective assessment measure.*

Keywords: *Corpus callosum, magnetic resonance imaging, biometry, callosal index, pediatrics*

ÖZ

Amaç: *Korpus Kallozum (KK), yaşam boyu morfolojik değişiklikler gösteren dinamik bir organdır. Günlük pratikte KK değerlendirmesi subjektif yapılsa da daha doğru değerlendirme yapabilmek yaş ve cinsiyete göre hazırlanmış referans değerlere ihtiyaç vardır. Bu çalışmanın amacı, çocuklarda yaş grubuna göre referans değerleri sağlamanın yanı sıra ve yaş ve cinsiyet farklılıklarını değerlendirmektir. Ek olarak kişinin kendi KK'si ile beyin alanını oranlayarak kişiselleştirilmiş ölçüm yapılmasına imkan veren Modifiye Kallozal İndeksi çocuklarda değerlendirmeyi amaçladık.*
Yöntem: *Retrospektif dizayn edilmiş çalışmamızda, nörolojik muayene bulguları normal olan 2-17 yaş arası 320 çocuğun (160 erkek, 160 kız) kraniyal MRG'leri incelendi. Midsagittal planda T1 görüntülerde: KK alanı (CCA), suprakallozal alan (SCA), KK uzunluğu (LCC), genu kalınlığı (GT), gövde kalınlığı (BT), splenium kalınlığı (ST), genu kalınlığının splenium kalınlığına oranı (GT / ST) ve callosal indeksi (CCA / SCA) olarak adlandırılan yeni bir parametre değerlendirildi. Bu parametrelerin yaş ve cinsiyet ile ilişkisi değerlendirildi.*
Bulgular: *Değerlendirilen tüm parametreler yaşla artış göstermiştir. 10 yaşından sonra 15 yaşındaki grup dışında, gruplar arasında yaş veya cinsiyetle ilgili istatistiksel olarak anlamlı farklılık saptanmadı. Tüm yaş gruplarını değerlendirdiğimizde, erkek çocuklarda CCA ve SCA'nın daha büyük olduğunu ancak kallozal indeksin cinsiyetler arasında istatistiksel olarak anlamlı farklılık göstermediğini belirledik.*
Sonuç: *Çalışmamız, KK değerlendirilirken yaşa ve cinsiyete göre gösterdiği değişikliği göz önünde bulundurarak objektif ölçüme olanak sağlayan bir referans değer belirlemesi açısından önemlidir. Ayrıca kantitatif modifiye kallozal indeksi çocuklarda kullanan ilk çalışmadır.*

Anahtar kelimeler: *Korpus kallozum, Manyetik rezonans görüntüleme, biyometri, kallozal indeks, pediatri*

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INTRODUCTION

The corpus callosum (CC) is the most significant forebrain commissure, a true evolutionary modification only seen in placental mammals [1,2]. The first role of CC is to assemble and transfer data between the two hemispheres. The CC processes sensory, motor, and high-level cognitive signals [3]. The posterior part of the CC transfers visual, auditory, and somatosensory information. Higher cognitive functions are processed in the anterior part of the CC [3]. As the brain structures evolve, the CC improves motor movements and develops cognitive functions [3,4]. Fiber composition of the CC changes with differing proportions of small and large diameter fibers across callosal subregions. The splenium and genu have high density of short fibers; however, the corpus contains large diameter fibers. Morphological differences and morphological changes in the CC throughout infancy lead to assessment and measurement challenges for each segment and size [5].

A large number of studies have demonstrated that the callosal abnormalities are related with neurological disorders, learning disabilities, aphasia, schizophrenia, dyslexia and spastic cerebral palsy [6-10]. Because of these adverse conditions, and in consideration of changes associated with age and gender, it is very important to distinguish the appearance of normal CC from the pathological one, and only visual evaluation is not sufficient to make this distinction.

Several different imaging modalities can be used in the assessment of CC. Ultrasound is an advantageous method, in that it is an easily available, cost-effective, radiation-free bedside procedure which provides real-time information. Unfortunately, ultrasound can only be used for CC screening in the prenatal and only in the early postnatal period until the anterior fontanel closes. Although computed tomography (CT) appears to be advantageous over magnetic resonance imaging (MRI) with shorter procedural time, it is not recommended for the evaluation of CC due to the high radiation exposure and low soft tissue resolution relative to MRI. MR images in the midsagittal plane are useful for measurement of CC length and evaluating subsegmental anatomy. However, in many reference centers, CC is assessed visually without measuring the dimensions of CC. Failure to obtain standard measurement data for fellows or residents who have just started pediatric neuroradiology practice may lead to difficulties in structured reporting. To date, there are limited number of studies in the literature about biometric evaluation of the size and thickness of CC in children [5,6,11,12]. There is a need for standard normative biometric data about the CC that are easy to use in daily practice.

The aims of this study were to provide reference biometric data about the CC with MR imaging in children and to evaluate possible impact of age and gender on biometric and morphologic characteristics of CC. Normal values may be used to differentiate and evaluate developmental diseases or diseases that may lead to changes in CC structure in the future.

MATERIAL and METHODS

Patient Selection

Cranial MR imaging data were selected from the hospital Picture Archiving Communication Systems (PACS) encompassing the period extending from March 2018 to January 2020. Local ethics committee approval was obtained prior to initiation of the study (02.19.2020/file number: 2020/77). We assessed a total of 320 cases including 160 males and 160 females, with ages ranging from 2-17 years (median age: 9.5 years). The MR imaging studies were performed for a variety of symptoms like seizures, myoclonus, dizziness, balance disorders, precocious puberty, headache, abnormal visual findings, deafness, facial palsy. Patients without cerebral pathology identified on MRI were included the study.

Children with metabolic disorders, cranial trauma, intracranial hemorrhage, intracranial mass, cerebral edema, hydrocephalus, cranial malformation, cerebral atrophy and multiple extracerebral malformations, any pathologic cranial findings,

premature birth and suboptimal image quality were excluded from the study.

Cases were stratified by age in increments of 1 year and each age group was evaluated separately. Each group contained 10 male and 10 female cases with a total of 20 individuals in every age group. Evaluation of MR images was performed by a pediatric radiologist with more than 8 years of experience in pediatric neuroradiology.

MR imaging and measurements

MR imaging was performed on a 1.5 T scanner (Magnetom Aera; Siemens AG Healthcare Sector, Erlangen, Germany) and midsagittal T1-weighted images which best illustrated the CC were selected for evaluation. Parameters for T1-weighted images were as follows: FOV: 230 mm, matrix: 256x256, slice thickness: 5 mm, interslice gap: 1 mm, NEX: 2, and TR/TE: 562/14 msec. We have chosen T1 sequence, because it allows the best morphologic evaluation.

On midsagittal T1-WI, thickness of CC was measured at the level of genu, body and splenium

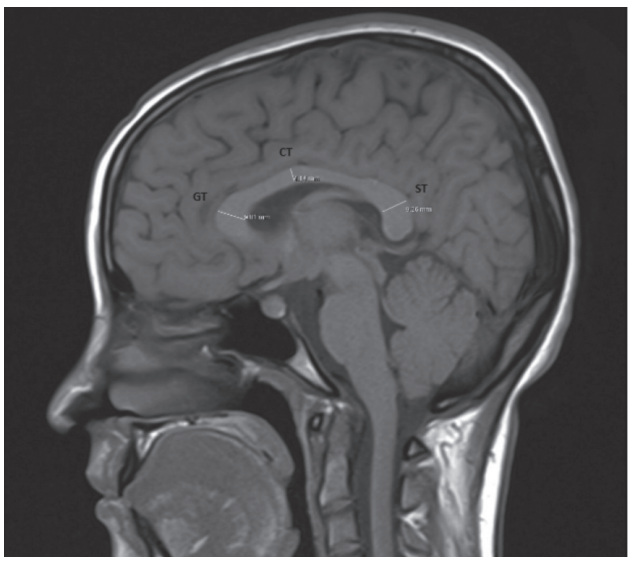

Figure 1. Measurement of the thickness of the CC, at the level of the genu (GT), corpus (CT), and splenium (ST).

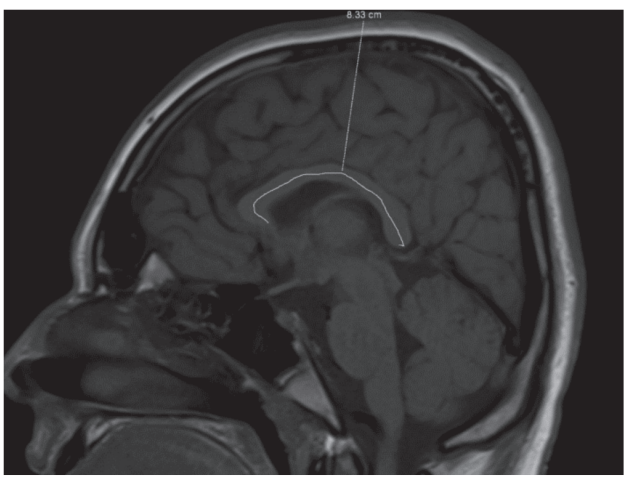

Figure 2. Measurement.

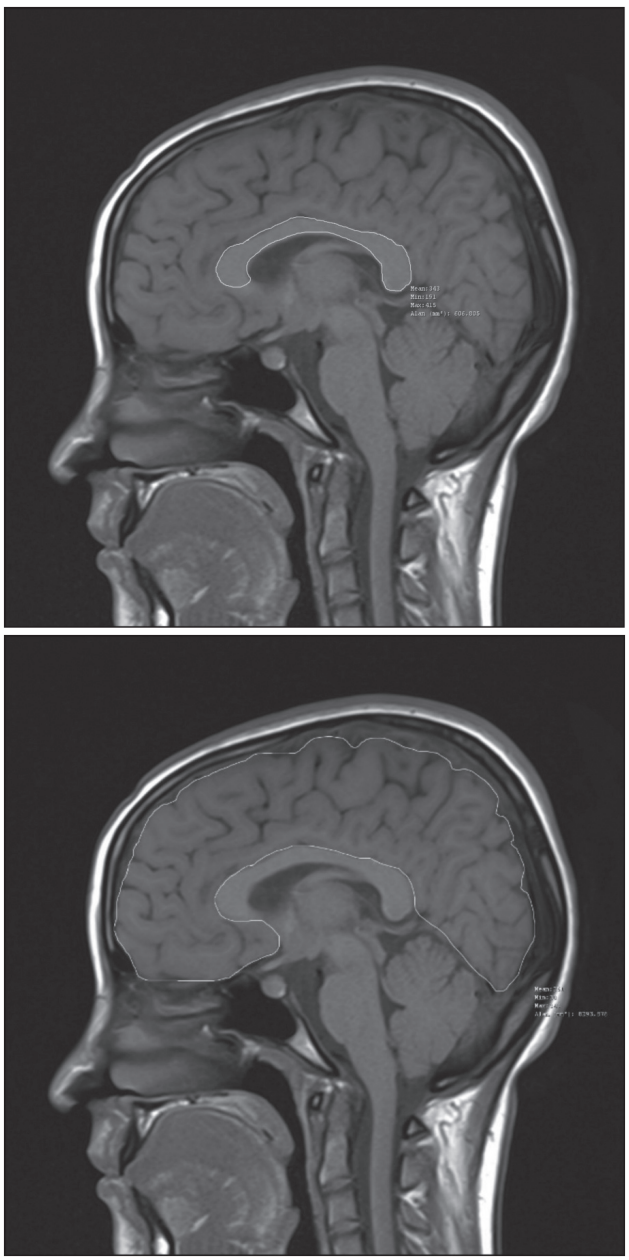

Figure 3-4. Sagittal T1-weighted MR image demonstrates the area measurements for index calculation. Area of CC (Figure 3) and area of supracallosal cerebral parenchymal area (Figure 4).

Table 1. Biometric parameters evaluated in the study.

Abbreviation	Parameter	Definition
LCC	True length of CC	Curvilinear distance between the rostrum and the splenium at mid-thickness of the CC
GT	Genu Thickness of the CC	Thickness of the CC measured at the level of the genu
BT	Body Thickness of the CC	Thickness of the CC measured at the level of the body
ST	Splenium Thickness of the CC	Thickness of the CC measured at the level of the splenium
CCA	Area of CC (mm^2)	The area inside of a closed line crossing the CC
SCA	Supracallosal area (mm^2)	The area of supracallosal brain parenchyma
GT/ST	Genu-splenium thickness ratio	Ratio of genu thickness to splenium thickness
CCA/ SCA	Callosal Index	Ratio of callosal area to supracallosal area

CC: Corpus Callosum, LCC: True length of CC, GT: genu thickness, BT: body thickness, ST: splenium thickness, GT / ST: the ratio of genu thickness to splenium thickness, CCA: area of corpus callosum, SCA: supracallosal area, Callosal index: Ratio of callosal area to supracallosal area

(Figure 1). True length of CC was measured as the curvilinear distance between the rostrum and the splenium at midthickness of the CC (Figure 2). Ratio of GT to ST was calculated.

A region of interest was placed around the CC and area of CC at midsagittal plane was measured (Figure 3). Subsequently, the supracallosal area at the same level was measured by placing a region of interest on it (Figure 4). Area measurements were calculated automatically by the device. Callosal index indicating ratio of CC area to supracallosal area was calculated. Analysis of morphological parameters is detailed in Table 1.

Statistical analysis

Kolmogorov-Smirnov test was used to examine whether the quantitative variables had a normal distribution. For independent groups, variables with normal distribution were compared with t test one-way analysis of variance (ANOVA), and variables that were not normally distributed were compared with Mann-Whitney U test or Kruskal-Wallis H test. The relationship between quantitative variables and age was examined with the Spearman correlation analysis. Descriptive statistics of variables with normal distribution are presented as mean ± standard deviation, and descriptive statistics of non-normally distributed quantitative variables are shown as median (25^{th}-75^{th} percentile). Descriptive statistics for qualitative variables are expressed as frequency (%). $p<0.05$ values were considered statistically significant. Accordingly, descriptive statistics for BT and GT/ST variables are shown as median (25-75 percentile) and descriptive statistics for GT, BT, ST, CCA, SCA, LCC, CCA/SCA and GT/ST variables are stated as arithmetic mean ± standard deviation. Accordingly, descriptive statistics for SCA and CCA/SCA variables are given as arithmetic mean±standard deviation.

RESULTS

Descriptive statistics for quantitative variables are given in Table 2. In the 2, 3, 7 and 15 year-age groups, SCA was larger in boys than in girls, GT in the 4 year-age group and GT/ST ratio in the 5 year-age group was higher in girls, and the GT/ST ratio was greater in boys in the 12-year age group. Under the normality assumption, descriptive statistics for quantitative variables of 320 individuals are given in Table 2. Descriptive statistics for quantitative

Table 2. Descriptive statistics of quantitative variables.

GT (mm)	8.25±1.50
BT (mm)	4.10 (3.59-4.74)
ST (mm)	8.08±1.49
CCA (mm^2)	521.65±95.90
SCA (mm^2)	8029.24±891.47
LCC (mm)	79.85±7.48
CCA/SCA	0.07±0.01
GT/ST	1.02 (0.89-1.14)

CC: Corpus Callosum, LCC: True length of CC, GT: genu thickness, BT: body thickness, ST: splenium thickness, GT / ST: the ratio of genu thickness to splenium thickness, CCA: area of corpus callosum, SCA: supracallosal area, Callosal index: Ratio of callosal area to supracallosal area.

Table 3. Descriptive statistics of quantitative variables in gender groups.

	GENDER		
VARIABLES	**Girls (n=160)**	**Boys (n=160)**	**P**
GT (mm)	8.24±1.43	8.26±1.57	0.905
BT (mm)	4.06 (3.59-4.72)	4.25 (3.59-4.80)	0.346
ST (mm)	8.06±1.48	8.10±1.50	0.849
CCA (mm^2)	508.74±91.21	534.56±98.99	0.016
SCA (mm^2)	7782.83±849.91	8275.66±865.96	0.001>
LCC (mm)	79.10±6.89	80.61±7.99	0.072
CCA/SA	0.07±0.01	0.06±0.01	0.443
GT/ST	1.03 (0.89-1.14)	1.02 (0.89-1.14)	0.762

CC: Corpus Callosum, LCC: True length of CC, GT: genu thickness, BT: body thickness, ST: splenium thickness, GT / ST: the ratio of genu thickness to splenium thickness, CCA: area of corpus callosum, SCA: supracallosal area, Callosal index: Ratio of callosal area to supracallosal area.

variables according to gender are given in Table 3. Accordingly, descriptive statistics for BT and GT/ST variables are shown as median (25-75 percentile), descriptive statistics for GT, ST, CCA, SCA, LCC and CCA/SCA (callosal index) variables are stated as arithmetic mean±standard deviation. According to the results of the correlation analysis, SCA and CCA values were significantly higher in boys than girls. But the callosal index was identical for girls and boys. Descriptive statistics for quantitative variables by age groups are given in Table 4.

In our study, we found a moderate positive correlation between age and CCL, GT, ST, CCA, and SCA. A weak positive correlation was present between age, BT and CCA/SCA. Results of correlation analysis for quantitative variables with age is given in Table 5. All parameters increased with age.

Table 4. Descriptive statistics of quantitative variables in age groups.

	AGE GROUPS							
Variables	**2 (n=20)**	**3 (n=20)**	**4 (n=20)**	**5 (n=20)**	**6 (n=20)**	**7 (n=20)**	**8 (n=20)**	**9 (n=20)**
GT (mm)	6.23 (5.43-7.15)	7.23 (6.37-7.75)	6.75 (5.91-7.61)	7.40 (6.73-8.81)	7.57 (6.41-8.52)	8.76 (7.63-9.52)	8.68 (8.07-9.71)	8.73 (8.06-9.84)
BT (mm)	3.28 (3.13-3.76)	4.20 (3.52-4.51)	3.75 (3.24-4.46)	3.85 (3.62-4.96)	4.04 (3.59-4.51)	4.39 (3.59-4.67)	4.39 (4.04-4.74)	4.04 (3.55-4.51)
ST (mm)	6.27 (5.86-6.92)	7.23 (6.10-8)	6.89 (6.18-7.43)	6.89 (6.30-7.68)	8 (6.63-8.37)	7.77 (6.53-8.50)	8.17 (7.57-9.29)	8.09 (7.29-9.03)
CCA (mm)	352.45 (329.15-429.78)	454.55 (397.13-489.80)	423.95 (400.50-475.40)	480.10 (437.03-566.40)	462.10 (428.85-558.85)	520.50 (479.55-583.13)	552.80 (504.33-576.10)	496.10 (460.43-588.30)
SCA (mm^2)	6460.58±774.06	7393.70±649.76	7329.70±851.82	7708.68±528.60	7873.07±953.39	8226.74±796.95	8081.92±670.85	8098.13±719.07
LCC (mm)	71 (66.75-73)	75 (70.50-76.75)	80 (70-82)	78 (73-82)	74 (71.25-81.75)	78 (71.25-86.50)	80.50 (77-85)	78.50 (76-82)
CCA/SA	0.059±0.011	0.061±0.009	0.059±0.007	0.064±0.011	0.064±0.010	0.065±0.011	0.068±0.008	0.064±0.010
GT/ST	0.98 (0.86-1.12)	0.97 (0.87-1.13)	1 (0.91-1.15)	1.08 (0.98-1.21)	0.96 (0.89-1.11)	1.16 (0.94-1.40)	1.06 (0.97-1.14)	1.07 (0.92-1.24)

Table 4. continued .

	AGE GROUPS							
Variables	**10 (n=20)**	**11 (n=20)**	**12 (n=20)**	**13 (n=20)**	**14 (n=20)**	**15 (n=20)**	**16 (n=20)**	**17 (n=20)**
GT (mm)	8.68 (7.96-9.91)	8.65 (7.47-9.42)	8.59 (7.28-9.42)	8.56 (7.75-9.16)	9.44 (7.90-10.02)	8.41 (7.51-9.11)	9.13 (8.27-9.91)	9.28 (8.64-10.16)
BT (mm)	4.30 (3.64-5.28)	4.01 (3.59-4.51)	3.96 (3.59-4.77)	4.06 (3.93-5.16)	4.21 (3.64-5.28)	4.50 (3.70-4.96)	4.63 (4.08-5.16)	4.93 (3.74-5.39)
ST (mm)	7.66 (7.38-8.54)	8.32 (7.43-8.72)	8.26 (7.40-9.55)	8.46 (7.43-10.40)	8.88 (8.27-9.94)	9.49 (8.56-10.49)	9.11 (8.35-9.68)	9.06 (8.14-10.09)
CCA (mm)	539.45 (466.28-624.10)	528.05 (490.13-573.45)	512.55 (463.35-592.55)	568.78 (523.63-643.78)	558.40 (514.50-607.95)	584.65 (496.90-647.50)	576.90 (527.13-598.10)	593.26 (531.83-652.63)
SCA (mm2)	8533.51±782.69	8404.92±575.02	8349.02±657.68	8408.04±750.42	8410.13±770.59	8415.43±744.49	8310.82±582.91	8463.52±631.40
LCC (mm)	79.50 (74-84.25)	83 (79-88)	82 (77.25-86.75)	82.50 (79.25-87)	83.50 (78-87)	84.50 (77.75-87)	82.50 (80-84)	85.50 (81-87)
CCA/SA	0.064±0.008	0.065±0.008	0.063±0.011	0.069±0.009	0.068±0.011	0.068±0.010	0.069±0.009	0.070±0.010
GT/ST	1.11 (1.01-1.28)	1.06 (0.91-1.17)	0.94 (0.88-1.13)	0.94 (0.81-1.17)	1.02 (0.82-1.14)	0.94 (0.80-1.02)	1.04 (0.93-1.12)	1.05 (0.87-1.13)

CC: Corpus Callosum, LCC: True length of CC, GT: genu thickness, BT: body thickness, ST: splenium thickness, GT / ST: the ratio of genu thickness to splenium thickness, CCA: area of corpus callosum, SCA: supracallosal area, Callosal index: Ratio of callosal area to supracallosal area.

Table 5. Results of correlation analysis of quantitative variables with age.

	GT	BT	ST	CCA	SCA	LCC	CCA/SA	GT/ST
Age	r=0.498 p<0.001	r=0.269 p<0.001	r=0.596 p<0.001	r=0.541 p<0.001	r=0.491 p<0.001	r=0.466 p<0.001	r=0.288 p<0.001	r=-0.059 p=0.294

r : Correlation coefficient
CC: Corpus Callosum, LCC: True length of CC, GT: genu thickness, BT: body thickness, ST: splenium thickness, GT / ST: the ratio of genu thickness to splenium thickness, CCA: area of corpus callosum, SCA: supracallosal area, Callosal index: Ratio of callosal area to supracallosal area.

DISCUSSION

To our knowledge this is the largest MR imaging study to investigate the efficiency of age and gender on multiple measurements of CC morphology and its subsegments in healthy children, and the first study which used modified callosal index in pediatrics.

The corpus callosum is the most important commissure in the human brain, connecting cortical regions of both hemispheres. The CC is the largest commissure, existing of more than 200-300 million fibers of various lengths, connecting both hemispheres. Myelination of this commissure, which is present from birth, continues throughout puberty [13]. Although there is no anatomically obvious border in the CC, it is topographically divided into 5 sections from anterior to posterior as rostrum, genu, body, isthmus, and splenium. Callosal length and width displayed significant differences based on sex and age of the individuals [13-16]. Variations among studies may be caused by being without brain size corrections, differences in patient groups or devices and the method of measurement. In this study we added a new parameter of callosal index to evaluate the CC more objectively, accounting for variations in cerebral size.

Callosal Index

Callosal index is estimated by proportioning CC area with the supracallosal area. Callosal index gives the opportunity to make individualized brain size corrections and allows more accurate and personal measurement in the follow-up of the same individual.

Erdogan et al. [17] evaluated callosal index in adults and found no significant difference between genders. Erdoğan et al. used midsagittal T1-weighted images. First, they calculated the area inside of a closed line crossing the CC. Later, supratentorial areas were calculated and proportioned by them. Like their study, we also found that callosal index did not show differences between girls and boys in all pediatric age groups. In the literature, there are very old two studies in which callosal index was used in children [11,18]. But they are different from our study due to the measurement technique and developments in MR technology.

The measurement technique of Rauch and Jinkins is different from ours. They proportioned the CC area to the average of the cerebral areas calculated in the axial and sagittal planes [11]. In line with the study of Rauch and Jinkins, we observed that the supracallosal area grows with age and the callosal index does not differ between genders.

Schaefer et al. [18] measured the total cerebral parenchyma by dividing it into 4 different parts using MR images and photo printing technique. It is quite different from our study in terms of both measurement technique and possible errors that may arise from using different measurement tools.

Since dura mater is a good reference line for distinction of the supratentorial area, in our study we used ST for the calculation of the ratio. The method we used to estimate callosal index is different from previously used ones, and seems to be a method with high ease of application and reproducibility. It is also a parameter that can be used confidently in follow-up of the same cases by minimizing variations among individuals.

CCA and SCA

In our study consistent with Erdogan et al. [17] the supratentorial area and CC area of boys were found

to be significantly larger than girls. Clarke et al. [19] performed brain examination in the postmortem period on children aged 28 weeks to 14 years. They found that CCA was larger in boys than girls, as in our study. Clarke et al. stated that the difference in CC size may have been solely related to the differences in brain size.

In support of this, the disparity reported between boys and girls in terms of CC size was about 15 percent.

The length of CC

To the best of our knowledge, very few studies in the literature evaluated true curvilinear length of CC [3]. In our study, we preferred to examine, and use curvilinear measurement technique, even though it was more demanding and meticulous. We did not find a significant difference in LCC measurements among age and sex groups, except the 15-year-old group, which could be called preadults. In this age group LCC in boys was significantly longer than girls. Also, we found that supracallosal area was larger in boys than girls in the 15-year-old group. The difference between sexes in this age group in LCC may be the result of the diffuse growth of CC due to hormonal changes. As shown in functional MRI and brain mapping studies, testosterone has a beneficial effect on the microstructure of white matter. Moreover, positive associations have been reported between testosterone and white matter fractional anisotropy in boys [20,21].

There is a conflict in the literature in terms gender-related differences in measurements. These discrepancies have been attributed to the differences in the age range of the sample group or in the number of male, and female subjects included in the studies [22-24].

Effect of Age and Sex

In our study all parameters related to the size and length of CC showed increases with age in accordance with the literature, [5,17]. This study was performed on healthy children and subregions of CC showed proportional growth in healthy children. The present study has not demonstrated significant gender difference in age subgroups as for morphometry of the CC in children after 10 years old, which is consistent with the results of many studies cited in the literature. This finding suggests that CC acquires its adult form by about age 10 [4,6,12,23]. However, we found some differences between the sexes in the 15-year-old group for supracallosal area and CC length which were larger in boys than girls. The correlation we found between these parameters is different from other studies, which may be due to ethnic, and hormonal factors, or the small number of patients included per age groups.

The size and degree of myelination of callosal fibers depends on their location. The large fibers are mostly placed in the isthmus which is responsible from connection between somatosensory, motor, auditory cortex. We also encountered large fibers in the posterior splenium (visual cortex). Thin fibers are localized in the genu and in the anterior splenium which connects prefrontal and temporoparietal lobes [4]. We also investigated correlations of age and gender with the ratio of anterior to posterior fibers (GT/ST). The sex-related differences were seen in the 4-year age group for GT values and in the 5-year age group for GT/ST ratio, with higher values in girls compared with boys. In the 12-year age group, GT/ST ratio was significantly higher in boys than in girls. In the literature, some studies have found significant relationships between the thickness of posterior body of the CC and academic success in language and mathematics. Ng et al. [12] investigated the relationship between CC morphometry and gender, academic performance in Chinese children and concluded that language and mathematic skills are related to the shape and size of the fibers connecting both posterior parietal and temporal lobes.

When all age groups were evaluated separately, any significant gender differences were not found in ST and BT values. Allen et al. also found no gender difference in the area of CC or its subsegments in children from 2 to 16 years old. Nevertheless, they suggested that the shape of the splenium was more bulbous in girls than boys which makes it difficult to accurately measure and evaluate the corpus callosum [12,23].

Prendergast et al. [25] observed different age-associated changes through the lifetime of males and females in the subsegments of CC corresponding to the genu. Their findings implicate gender-related differences localized to the genu in CC morphology through the lifetime which apparently mediates neuropsychological functions.

Morphologic and size differences of CC can be seen in autism, dyslexia, attention deficit, hyperactivity disorders and other neuropsychiatric disorders. Hence morfologic and size determination of CC is important and beneficial in pediatric population with neuropsychiatric disorders. [5,25-27]. Therefore, it is important to identify the size and morphology of CC according to age and gender in childhood.

Limitations

Many factors implicated in CC growth and morphology have been reported. Various studies have indicated environmental factors, ethnicity, prematurity, mother's alcohol intake in prenatal period, and protein-poor diet that are effective in the development of CC [10,28].

We did not question variables other than age, and gender which might be one of the limitations that affected the results of our study. Another limitation is that although we included a large number of participants in our survey, we evaluated each age group separately and the number of people per subgroup was relatively low. BMI values could not be obtained due to the retrospective design of the study. Lastly, images were assessed by a single radiologist. Therefore, interobserver agreement could not be evaluated. Handedness was not deliberately investigated because previous studies have shown that left-handed people's both hemispheres acting equal role in language, or that right-handed people have 1-5% right hemisphere dominance [29].

In conclusion; we provided reference normative data about the CC using multiple parameters on MR imaging in patients from 2 to 17 years of age and showed differences between age and gender. Modified callosal index did not display any gender-related changes. When we evaluated all age groups in combination, we determined that CCA and SCA are.

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Research Article

What is the Role of Mannose-Binding Lectin Gene Polymorphism in the Development of Acute Post-Streptococcal Glomerulonephritis?

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Akut Post Streptokokal Glomerülonefrit Gelişiminde Mannoz-Bağlayıcı Lektinin Rolü Nedir?

ABSTRACT

Objective: *This study aims to determine the effects of the mannose-binding lectin (MBL) gene polymorphism on the clinical and laboratory findings, response to treatment, and progress of patients with acute post-streptococcal glomerulonephritis (APSGN).*
Methods: *Codon 54 polymorphism found in exon 1 of the MBL gene was investigated by polymerase chain reaction-restriction fragment length polymorphism method in 110 children followed up with the diagnosis of APSGN and compared with healthy control group.*
Results: *The normal allele AA and, the variant alleles AB and BB gene frequencies were determined within the APSGN group as 74.5%, 20% and, 5.5%, respectively. No statistically significant difference was found with concerning to the gene polymorphism in the APSGN group when compared with the control group (p>0.05). No correlation was found in the patient group between gene polymorphism and the presence of hematuria, edema, central nervous system findings, and blood pressure (p>0.05). Concerning laboratory findings during the diagnosis, no correlation existed between the gene polymorphism and high levels of urea, creatine, total cholesterol, and triglycerides, low levels of albumin, and the presence of proteinuria (p>0.05). Within the first years following the diagnosis, no statistically significant difference was found in the glomerular filtration rates, blood creatine levels, proteinuria levels, duration of microscopic hematuria and proteinuria between the patients with the gene polymorphism and those without the gene polymorphism (p>0.05)*
Conclusion: *Our study determined that the MBL gene polymorphism was not important in the development, the laboratory and clinical findings, or the progression of the patients with APSGN.*

Keywords: *Acute post-streptococcal glomerulonephritis, mannose-binding lectin, gene polymorphism, prognosis*

ÖZ

Amaç: *Bu çalışma, mannoz bağlayıcı lektin (MBL) gen polimorfizminin, akut post-streptokokal glomerülonefrit (APSGN) klinik ve laboratuvar bulguları, tedaviye yanıtı ve prognozu üzerindeki etkilerini belirlemeyi amaçlamaktadır.*
Yöntem: *MBL geninin ekson 1'inde bulunan Codon 54 polimorfizmi, APSGN tanısı ile takip edilen 110 çocukta polimeraz zincir reaksiyon-restriksiyon fragman uzunluğu polimorfizmi yöntemi ile araştırıldı.*
Bulgular: *APSGN grubunda normal alel AA ve varyant allel AB ve BB gen frekansları sırasıyla %74,5, %20 ve %5,5 olarak belirlendi. Kontrol grubuna göre APSGN grubunda gen polimorfizmi açısından istatistiksel olarak anlamlı ilişki bulunmadı (p>0,05). MBL gen polimorfizmi ile tanı sırasındaki laboratuvar bulguları olan; yüksek üre, kreatin, total kolesterol ve trigliserid düzeyleri, düşük albümin düzeyleri ve proteinüri varlığı arasında ilişki yoktu (p>0,05). Tanıyı takip eden ilk yılda gen polimorfizmi olan ve olmayan hastalar arasında glomerüler filtrasyon oranları, kan kreatin düzeyleri, proteinüri düzeyleri, mikroskobik hematüri ve proteinüri süresi açısından istatistiksel olarak anlamlı fark bulunmadı (p>0,05)*
Sonuç: *Çalışmamız, MBL gen polimorfizminin APSGN gelişiminde, laboratuvar ve klinik bulgularında ve hastalığın ilerlemesinde önemli olmadığını göstermiştir.*

Anahtar kelimeler: *Akut post-streptokokal glomerülonefrit, mannoz bağlayıcı lektin, gen polimorfizm, prognoz*

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INTRODUCTION

Acute post-streptococcal glomerulonephritis (APSGN) is the most common non-suppurative and immune-mediated disease of the kidneys that is related with the nephritogenic strains of group A streptococci that cause throat and skin infections [1]. APSGN can present as asymptomatic microscopic hematuria or a full-blown acute nephritic syndrome with red to brown urine, proteinuria, edema, hypertension, and acute kidney injury. The prognosis is generally favourable, particularly in children, but the long-term prognosis is not always favorable [1].

Streptococcal contents and products are known to trigger this process, while the process has not been precisely defined. Although the mechanism of renal damage is not known, theories, such as glomerular localization of circulatory immune complexes, the molecular similarity between streptococcal and renal antigens, immune complex formation between *in situ* anti-streptococcal antibodies and glomerular antigens, and complement activation directly induced by deposit streptococcal antigens inside the glomerulus have been proposed [1-4].

Many morphologic, clinical and serologic findings support the idea of APSGN is an immune complex disease. Even though the mechanism of the antigen-antibody complexes in APSGN has not been delineated, it is thought that complement activation takes place through the alternative rather than the classic pathway. None of the studies have addressed the role of the lectin pathway (LP) [1]. Renal biopsies performed in typical cases involving APSGN within the 4th week following the onset of symptoms have demonstrated MBL, C4d, and MAPS-1-positivities inside the mesangium [2]. It has been proposed that the LP, which is the third pathway of the complement system, can activate APSGN which takes place by the recognition of glucosamine residues on the bacterial wall by MBL; however, patients with defects in MBL may also develop glomerulonephritis [3]. By studying nephritogenic *Streptococcus pyogenes* serotype M1 in APSGN patients, it has been shown that the lectin pathway of the complement system plays a role in the development of hypocomplementemia in APSGN [4]. Based on these findings, it appears that the LP is essential in the pathogenesis of APSGN [5]. This study aimed to analyze the effect of the MBL gene polymorphism on the development of APSGN, relevant laboratory, clinical findings, and disease progression.

MATERIAL and METHODS

Diagnosis

A total of 110 patients diagnosed with APSGN (39 females, 71 males) and 100 healthy controls without hypertension, renal, and/or cardiac disease were enrolled in the study. The diagnosis of APSGN was defined as the occurrence of edema, hypertension, hematuria, oliguria, decreased C3 in addition to increased plasma creatine levels following streptococcal upper respiratory tract or skin infection within previous 1-3 weeks. Hematuria was defined as the presence of 5 or more erythrocytes in the microscopic analysis of urine with high magnification. Hypertension was defined as systolic blood pressure being higher than 95 percentile according to appropriate age, gender and height; measured multiple times, at three visits or more. Proteinuria was defined as the presence of protein at a rate of 4 mg/m^2/hour in 24-hour urine. Glomerular filtration rate (GFR) was evaluated by Schwartz formula. The medical records of patients were evaluated retrospectively for the following parameters: age at the time of diagnosis, history of infection, presence of macroscopic or microscopic hematuria, edema, central nervous system findings, hypertension, blood urea nitrogen (BUN) at the first visit, creatine, total cholesterol, triglycerides, C3, C4 levels, GFR. The duration of hematuria and proteinuria, GFR, BUN and creatinine levels, and blood pressure percentiles within the first year were evaluated.

Molecular Analysis

In the DNA obtained from the peripheral blood of the patient and control groups, the codon 54 polymorphism of exon 1 of the MBL2 gene was investigated by the restriction fragment length polymorphism method (separation of DNA into fragments of

different sizes using restriction enzymes). The first exon of the MBL gene was amplified by PCR (349 bp). Primer sequences were 5'-TAGGACAGAGGGCATGCTC-3' (F) and inverse 5'-CAGGCAGTTTCCTCTGGAAGG-3' (R). The PCR product was obtained at 94°C for 30 seconds after denaturation at 94°C for 10 minutes, 57°C for 30 seconds, and 72 cycles of 72°C for 45 seconds with final holding at 7°C for 7 minutes. The PCR product thus obtained was kept at 50°C for 60 minutes with 5 IU of BanI restriction enzyme. The normal allele (allele A) of BanI was cut into two parts of 260 bp and 89 bp, while the variant allele (allele B) remained uncut. The products obtained were visualized by electrophoresis on 2% agarose gel.

Statistical Analysis

Statistical analysis was performed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). MBL genotype frequencies were compared by chi-square test, where $p<0.05$ was considered significant. ANOVA testing was used for comparison of numerical parameters between variables with multiple groups, and the Kruskal-Wallis test was used for those variables that did not have multiple groups. MBL gene polymorphism allele distribution was studied with the Hardy-Weinberg equation in the patient and control groups.

RESULTS

The study population consisted of 71 male and 39 female patients. The mean age of patients at the time of diagnosis was 7.8±3 years (min-max: 2-15). A history of streptococcal upper respiratory tract infection was present in 83 (75.5%) and a history of streptococcal skin infection was present in 4 (3.6%) patients. Macroscopic hematuria was present in 77 (70%), edema in 91 (82.7%), and central nervous system findings in 5 (4.5%) patients. The systolic blood pressure at the time of the first visit was between 50 -90 percentiles in 31 (28.2%), 90-95 percentiles in 7 (6.4%), 95-99 percentiles in 21 (19.1%), and >99 percentile in 51 (46%) patients when evaluated with respect to appropriate age, gender and height. Having evaluated the diastolic blood pressure with respect to appropriate age, gender and height at the time of first visit, there were 47 (42.7%) patients between 50-90 percentiles, 16 (14.5%) between 90-95 percentiles, 11 (10%) between 95-99 percentiles, and 36 (32.7%) >99 percentile. The mean (min-max) values for some laboratory parameters were as follows: BUN, 36.09±38.356 g/dL (min-max:3-252); creatine, 1.133±1.0566 mg/dL (min-max: 0.5-9.8); C3, 23.739±14.7712 (min-max: 0.1-77); C4, 17.005±6.4751 (min-max: 6-34); total cholesterol, 147.25±38.462 mg/dL (min-max: 86-304); triglycerides, 116.31±57.072 mg/dL (min-max: 20-414); total protein, 5.921±0.8132 g/dL (min-max: 3.7-8.2); albumin, 3.210±0.6279 g/dL (min-max: 1.1-5); GFR 77.6±524.734 mL/min/1.73 m^2 (min-max:11-158); and proteinuria in 24-hour urine, 24.399±42.0391 mg/m^2/hour (min-max:1.5-260) (Table 1).

When the patients were examined again at the end of the 1st year, the following values were measured; GFR, 142. 62±27.267 mL/min/1.73 m^2 (min-max: 83-212); and blood creatine, 0.518±0.1277 mg/dL (min-max: 0.3-1). The systolic blood pressure with respect to appropriate age, gender and height after 1 year was <50 percentile in 92 (83.6%), and between 50-90 mmHg in 18 (16.4%) patients; the diastolic blood pressure was <50 percentile in 12 (10.9%), and between 50-90 percentiles in 98 (81%) patients. The duration of macroscopic hematuria (1.26±0.470 weeks [min-max: 1-3]), microscopic hematuria (2.75±1.213 months [min-max:1-6]), and proteinuria (3.68±5.553 weeks [min-max:1-40]) was also determined (Table 1).

While AA (normal allele), AB (homozygous allele), and BB (variant allele) MBL codon 54 polymorphism were determined in exon 1 at rates of 82 (74.5%), 22 (20%) and 6 (5.5%) in children with APSGN, these rates were 73 (73%), 26 (26%) and 1 (1%) in the control group. Thus, in terms of codon 54 polymorphism in exon 1, no statistically significant difference was found between the patient and the control group (p=0.318) (Table 2).

Among children who were diagnosed with APSGN, when those with, and without MBL gene polymorphism (AA and BB) were compared in terms of their

Table 1. Demographic, clinical and laboratory findings, and disease progression in children diagnosed with APSGN.

Parameters	APSGN cases(n=110)
Female/Male*	39/71
Age of onseta	7.825±3.027 years (min-max:2-15)
Upper respiratory tract infection*	83 (75.5%)
Skin infection*	4 (3.6%)
Macroscopic hematuria*	77 (70%)
Edema*	91 (82.7%)
Central nerve system findings*	5 (4.5%)
First visit systolic blood pressure*	
50-90 percentiles	31 (28.2%)
90-95 percentiles	7 (6.4%)
95-99 percentiles	21 (19.2%)
>99 percentiles	51 (46.4%)
First visit diastolic blood pressure*	
50-90 percentiles	47 (42.7%)
90-95 percentiles	16 (14.5%)
95-99 percentiles	11 (10%)
>99 percentiles	36 (32.7%)
First visit blood urea/nitrogen (g/dL)[a]	36.09±38.356 (min-max:3-252)
First visit creatine (mg/dL)[a]	1.133±1.0566 (min-max:0.5-9.8)
First visit C3[a]	23.739±14.7712 (min-max:0.1-77)
First visit C4[a]	17.005±6.4751 (min-max:6-34)
First visit total cholesterol (mg/dL)[a]	147.25±38.462 (min-max:86-304)
First visit triglycerides (mg/dL)[a]	116.31±57.072 (min-max:20-414)
First visit total protein (g/dL)[a]	5.921±0.8132 (min-max:3.7-8.2)
First visit total albumin (g/dL)[a]	3.210±0.6279 (min-max:1.1-5)
First visit GFR (mL/min/1.73 m^2)[a]	77.6±524.734 (min-max:11-158)
First visit proteinuria in 24-hour urine (mg/m^2/hour)[a]	24.399±42.0391 (min-max:1.5-260)
First year GFR (mL/min/1.73 m^2)[a]	142. 62±27.267 (min-max:83-212)
First year creatine (mg/dL)[a]	0.518±0.1277 (min-max: 0.3-1)
First year systolic blood pressure*	
50-90 percentiles	92 (83.6%)
90-95 percentiles	18 (16.4%)
First year diastolic blood pressure*	
<50 percent	12 (10.9%)
50-90 percentiles	98 (81%)
Duration of macroscopic hematuria (weeks)[a]	1.26±0.470 (min-max:1-3)
Duration of microscopic hematuria (months)[a]	2.75±1.213 (min-max:1-6)
Duration of proteinuria (weeks)[a]	3.68±5.553 (min-max:1-40)

*Values [a]: mean±SD; *:number of patients and percentiles. The blood pressure evaluated according to appropriate age, gender and height; measured multiple times, three visits or more.*

Table 2. Distribution of MBL gene polymorphisms in children who were diagnosed with APSGN and the control group.

Gene Polymorphisms	APSGN (n=110)	Control (n=100)	P Value
AA	82 (74.5%)	73 (73%)	
AB	22 (20%)	26 (26%)	0.318[a]
BB	6 (5.5%)	1 (1%)	

Values indicate the numbers of patients and those within the parentheses are percentiles. A chi-square test was performed between the patient and control groups. A p<0.05 was assumed to be statistically significant.

genders, no statistically significant difference was found. The mean age at onset was 7.7927±3.02964 years in the AA, 7.8182±2.92178 years in the AB, and 8.4±4.03733 years in the BB group without any statistically significant intergroup difference (p>0.05). No relationship existed between history of streptococcal upper respiratory tract or skin infection and MBL gene polymorphism (p>0.05). No correlation existed between a MBL gene polymorphism and following findings: macroscopic hematuria; edema;

Table 3. Demographic, clinical and laboratory findings, and disease progression in children diagnosed with APSGN according to distribution of MBL gene polymorphisms.

Parameters	AA	AB	BB
Male* (n=71)	48 (69.6%)	19 (26.8%)	4 (5.6%)
Female* (n=39)	34 (87.2%)	3 (7.7%)	2 (5.1%)
Age of onset*	7.7927±3.02964	7.8182±2.92178	8.4±4.03733
Upper respiratory tract infection* (n=83)	62 (74.4%)	16 (19.3%)	5 (6%)
Skin infection (n=4)	4 (100%)	0 (0%)	0 (0%)
Macroscopic hematuria* (n=77)	61 (79.2%)	13 (16.9%)	3 (3.9%)
Edema* (n=91)	67 (73.6%)	19 (20.9%)	5 (5.5%)
Central nerve system findings* (n=5)	5 (100%)	0 (0%)	0 (0%)
First visit systolic blood pressure*			
50-90 percentiles (n=31)	22 (71%)	7 (22.6%)	2 (6.5%)
90-95 percentiles (n=7)	4 (57.1%)	3 (42.9%)	0 (0%)
95-99 percentiles (n=21)	18 (85.7%)	2 (9.5%)	1 (4.8%)
>99 percentiles (n=51)	38 (74.5%)	10 (19.6%)	3 (5.9%)
First visit diastolic blood pressure*			
50-90 percentiles (n=47)	38 (80.9%)	8 (17%)	1 (2.1%)
90-95 percentiles (n=16)	11 (68.8%)	5 (31.3%)	0 (0%)
95-99 percentiles (n=11)	7 (63.6%)	2 (18.2%)	2 (18.2%)
>99 percentiles (n=36)	26 (72.2%)	7 (19.45)	3 (8.3%)
First visit blood urea/nitrogen (g/dL)[a]	37.96±41.178	34.68±30.84	15.67±6.218
First visit creatine (mg/dL)[a]	1.125±1.1448	1.232±0.8357	0.883±0.2317
First visit C3[a]	24.483±14.5684	19.455±12.1370	29.283±23.9341
First visit total cholesterol (mg/dL)[a]	145.76±39.704	144.73±28.345	176.83±47.131
First visit triglycerides (mg/dL)[a]	114.91±53.813	114.14±33.165	143.33±136.180
First visit total albumin (g/dL)[a]	3.330±0.6253	3.195±0.5420	2.983±0.9786
First visit GFR (mL/min/1.73 m^2)[a]	77.55±23.725	75.32±29.912	87.67±17.773
First visit proteinuria in 24-hour urine (mg/m^a/hour)[a]	24.362±43.5361	27.109±42.0249	24.399±42.0391
First year GFR (mL/min/1.73 m^2)[a]	143.52±27.713	139.73±26.157	140.83±28.958
First year creatine (mg/dL)[a]	0.518±0.1304	0.509±0.1192	0.550±0.1378
First year systolic blood pressure*			
50-90 percentiles	68 (73.9%)	19 (20.7%)	5 (5.4%)
90-95 percentiles	14 (77.8%)	3 (16.7%)	1 (5.6%)
First year diastolic blood pressure*			
<50 percentiles	73 (75.4%)	20 (20.4%)	5 (5.1%)
50-90 percentiles	9 (75%)	2 (16.7%)	1 (8.3%)
Duration of macroscopic hematuria (weeks)[a]	1.26±0.444	1.15±3.76	1.67±1.155
Duration of microscopic hematuria (months)[a]	2.68±1.206	2.68±1.125	3.33±1.633
Duration of proteinuria (weeks)[a]	4.03±6.417	2.80±1.135	2.50±1.732

*Values [a]: mean±SD; *:number of patients and percentiles. The blood pressure evaluated according to appropriate age, gender and height; measured multiple times, at three visits or more.*

central nervous system findings; high systolic and diastolic blood pressures; high levels of BUN; creatine; total cholesterol and the triglycerides; the level of proteinuria in the 24-hour urine sample; low levels of C3 and albumin; GFR at the time of first visit (p>0.05) (Table 3).

GFR and blood creatine levels of children who were diagnosed with APSGN were evaluated again at the end of the first year, and no statistically significant correlation existed between APSGN, and the presence of a MBL gene polymorphism (p>0.05). At the end of first year proteinuria was observed in 3 patients. Each one of these patients had AA, AB or BB allele. No statistically significant correlation was found between the MBL gene polymorphism and ongoing proteinuria (p>0.05). At the end of the first year, there were no elevations in systolic and diastolic pressures in any of patients. MBL gene polymorphism did not predispose to the development of APSGN and the presence of the polymorphism did not cause severe laboratory abnormalities or clinical symptoms and it is not important in terms of disease progression (Table 3).

DISCUSSION

MBL is a crucial component of innate immunity [6-8]. Studies related to MBL have reported that a MBL deficiency increases the risk of infection, especially in lower and upper respiratory tracts [9,10]. The higher MBL levels are crucial in terms of protection from sepsis and septic shock when different alleles compared with each other [11]. A wide variety of bacteria, fungi, viruses, and parasitic organisms have connections to MBL [12]. MBL plays an important role in host defense against *N. meningitis, H. influenza, Human Immunodeficiency Virus (HIV), Influenza A, Herpes simplex virus, Candida Albicans, Saccharomyces Cerevisiae, Aspergillus Fumigatus* infections [6,13]. The frequency of the MBL variant allele was also evaluated in pediatric patients with infections and suspected immunodeficiency.

The MBL defect was first described in 1989 as a major opsonization defect [14]. MBL deficiency and low MBL levels have been strongly associated with three missense mutations in codons 52, 54, and 57 of exon 1 in the human MBL gene [11,13]. These mutations cause impairment in MBL multimerization, decrease in ligand binding and, inactivation of complement. Polymorphism has been detected in the promoter region of MBL. These polymorphism are called H/L, X/Y and, P/Q, and they are in positions 550, 221, +4. HYP leads to medium to high MBL production, LXP to low MBL production. While 5% of people are homozygous or heterozygous for these three types of point mutations and they have MBL deficiency [11,13,14]. In this defect, MBL levels are less than 100 ng/ml. MBL deficiency is not a classic primary immunodeficiency, it has various regulatory mutations, and its clinical penetrance is significantly low [2,14].

An increase in MBL levels has been correlated with autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), Celiac disease, Sjögren's syndrome and, Crohn's disease [15,16]. MBL deficiency increases an individual's potential vulnerability to infectious and autoimmune diseases. Considering that the MBL pathway causes a tendency for autoimmune diseases, the role of MBL has been investigated in the development of glomerulonephritis in human beings. MBL manifests itself with immunoglobulin (Ig) G deposits in the kidney biopsy materials of patients with lupus nephropathy, membrane-proliferative glomerulonephritis, anti-glomerular basement membrane nephritis, focal segmental glomerulosclerosis, and IgA nephropathy [7]. It has been suggested that a MBL genetic defect can be related to the development of SLE; lower MBL levels serve as a risk factor in terms of SLE development [18,19]. In membranous nephropathy (MN) and Henoch-Schönlein Purpura nephritis, complement activation through the lectin pathway may play a role in the development of advanced glomerular injuries [20-22]. The higher levels of MBL are associate with a higher risk of acute kidney allograft rejection and the decrease in graft life expectancy [23]. Studies have investigated the role of higher levels of MBL in the pathogenesis of cryoglobulinemic glomerulonephritis [24]. MBL deficiency and excess can both slow the progression of Ig A Nephropathy [25]. Higher MBL levels have been correlated with the development of persistent micro-macro albuminuria and microvascular complications in early onset type-1 diabetes patients [26]. Increased MBL activation has been proposed to trigger organ damage that develops as a result of acute renal deficiency and ischemia-reperfusion and can cause a tendency for the development of atherosclerosis in patients with chronic renal insufficiency [27,28]. LP can also activate APSGN, and patients with MBL defects may develop glomerulonephritis [1,5]. MBL and C4d staining intensity in the glomerular mesangium in post-streptococcal GN determine the degree of LP activation [29,30].

The polymorphism in the MBL gene is also associated with diseases [31]. The MBL exon 1 polymorphisms may play a role in the predisposition to SLE, progression of RA, development of leprosy and tuberculosis [31-34]. Preterm infants with MBL2 gene polymorphisms are at an increased risk of developing respiratory distress syndrome and sepsis [35]. The MBL genotypes AA for rs180040 (G/A), GG for rs1800451 (G/A), and CC for rs5030737 (T/C) have a higher prevalence in patients infected with Coronavirus Disease 2019 (COVID-19). The patients

with these polymorphisms and COVID-19 have a worse outcome due to the extreme activation of the lectin pathway, with a focus on the MBL pathway [36].

APSGN is known as immune-mediated disease that occurs following skin and pharyngeal infections which are related to nephritogenic strains of group a streptococci; however, the pathologic process has not been clarified yet [1]. The role of lectin cascade, which is triggered by MBL (the third pathway of complement system) in the development of APSGN has received considerable attention [2-5]. MBL recognizes high levels of mannose and N-acetylglucosamine (G1cNAc) derivatives, which are located on the surface of micro-organisms. Cell wall polysaccharides bear G1cNAc as an antigenic determinant and it is thought that MBL activates the complement system by recognizing this molecule on the pathogen [3-5]. MBL can also bind to galactosamine radicals, which in turn can bind glomeruli that contain these carbohydrates covered with streptococcal neuraminidase. The lectin pathway of the complement system leads to renal damage by activation of C3 directly by MAPS-1 and C4 by MAPS-2 [37]. Recognition of lectin may be important in early pathogen invasion. Antigen is independent of the antibody system and hence can illuminate the development of APSGN [5,37]. However, there is no study in the literature investigating the relationship between MBL gene polymorphism and APSGN. With these interactions in mind, patients who developed APSGN and healthy controls were compared in terms of MBL gene polymorphism and the importance of the lectin pathway in the pathogenesis of APSGN was investigated. In our study, it was observed that MBL polymorphism did not lead to the development of APSGN by increasing the tendency for streptococcal infections.

The studies have suggested that MBL can play a harmful as well as a beneficial role in renal diseases, sometimes it does not cause any tendency to disease progression based on clinical and laboratory entities. Because of the association between MBL and APSGN, we suspected that MBL gene polymorphism could lead to a higher risk of APSGN development in children. In this study, we looked at the codon 54 (allele B) polymorphism in the first exon of the MBL gene, its distribution, and its impact on clinical laboratory results, prognosis of APSGN and other related factors were evaluated. AA (normal allele), AB, and BB (variant allele) gene frequencies were 74.5% (n:82), 20% (n:22) and 5.5% (n:6), respectively in the patient group. No significant difference was found in terms of gene polymorphism when patients were compared with the control group ($p>0.05$). The infectious and autoimmune origin of the disease activation was investigated because those with MBL gene polymorphisms could be predisposed to defect-related development; however, there was no statistically significant difference in terms of development of APSGN between the AB (heterozygous) and BB (homozygous) groups with polymorphism and the AA (normal allele) group without polymorphism ($p>0.05$). In patients with APSGN, no significant difference was found in terms of susceptibility to MBL gene polymorphism.

CONCLUSION

Due to association between MBL and APSGN; we investigated the role of MBL gene polymorphism in the development of APSGN. Being consistent with the literature, our study also revealed that MBL gene polymorphism does not cause tendency for the development of APSGN and the presence of the polymorphism did not cause severe laboratory or clinical abnormalities and it is not important in terms of disease progression. Since the number of patients has increased and the rate of mutation increases as the level of MBL decreases, it would be valuable to perform new studies that elucidate the role of MBL mutation in the etiology of APSGN in children.

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Research Article

Efficacy of Lacosamide Therapy in Focal Onset Refractory Epilepsy of Childhood: A Single Center Experience

Çocukluk Çağının Fokal Başlangıçlı Dirençli Epilepsisinde Lakozamid Tedavisinin Etkinliği: Tek Merkez Deneyimi

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ABSTRACT

Objective: *Treatment of childhood refractory epilepsy is a challenge for clinicians. Lacosamide is a new generation antiepileptic drug which is being used for focal onset seizures of adults and children. Efficacy and safety of the drug for adults have been demonstrated in various studies. The aim of this retrospective cross-sectional study is to evaluate the efficacy and safety of lacosamide in childhood refractory focal seizures in our clinic.*
Methods: *We examined the medical records of 14 patients treated with lacosamide in our clinic between January 2016 and January 2020 in terms of demographic, etiological, neuroimaging findings, responses to treatment, adverse effects and drug-drug interactions. We evaluated the patients as responders to treatment whose seizure frequency decreased ≥%50 after 6 months of lacosamide treatment.*
Results: *In 12 patiens (%85.7) seizure frequency decreased ≥%50 (p<0.001) while 5 of them (%35.7) was seizure free. Despite to the long term treatment one patient did not response to lacosamide treatment, and 1 patient's treatment stopped due to aggravation of seizure after initiation of lacosamide treatment. Clinical adverse effects were observed in 3 (%21.4) patients. Cardiac adverse effects or drug-drug interactions were not observed in any patient.*
Conclusion: *As a result of our study, we think that lacosamide is an effective and reliable treatment option for refractory focal seizures of childhood similar to the results of the studies cited in the literature. We also think that further investigations are needed to evaluate its efficacy in focal and different type of seizures of childhood.*

Keywords: *Childhood, focal epilepsy, lacosamide*

ÖZ

Amaç: *Çocukluk çağının refrakter nöbetlerinin tedavisi klinisyenler için zorluk oluşturmaktadır. Lakozamid, yetişkinlerde ve çocuklarda fokal başlangıçlı nöbetler için kullanılan yeni nesil bir antiepileptik ilaçtır. İlacın yetişkinler için etkinliği ve güvenliği çeşitli çalışmalarda gösterilmiştir. Bu retrospektif kesitsel çalışmanın amacı refrakter fokal nöbetleri olan ve lakozamid tedavisi başlanan hastalarda tedavinin etkinliğini ve güvenilirliğini değerlendirmektir.*
Yöntem: *Ocak 2016 ve Ocak 2020 tarihleri arasında kliniğimizde lakozamid tedavisi alan 14 hastanın tıbbi kayıtlarını demografik, etiyolojik, görüntüleme bulguları, tedaviye yanıtları, tedavi yan etkileri ve ilaç-ilaç etkileşimleri açısından inceledik. Tedavinin 6. ayında nöbet sıklığında ≥%50 azalma olan hastaları tedaviye yanıtlı olarak değerlendirdik.*
Bulgular: *On iki hastada (%85,7) nöbet sıklığında ≥%50 azalma izlenirken (p<0,001) bunlardan 5 tanesinde (%35,7) tam nöbet kontrolü sağlandı. Bir hastada uzun dönem lakozamid kullanımına rağmen tedaviye yanıt alınamadı, bir hastamızda ise tedavi sonrası nöbet sıklığında artış olması nedeni ile ilaç kesildi. Toplam 3 hastamızda (%21,4) klinik yan etki izlendi. Hiçbir hastamızda kardiak yan etki veya ilaç-ilaç etkileşimi izlenmedi.*
Sonuç: *Çalışmamızın sonucunda lakozamid tedavisinin etkinliği literatürdekine benzer şekilde yüksek olarak izlendi. Lakozamidin çocukluk çağı refrakter fokal nöbetlerinde etkili ve güvenilir bir tedavi seçeneği olduğunu düşünüyoruz. Çocukluk çağının fokal nöbetlerinde ve diğer nöbet tiplerinde etkinliğini ve güvenilirliğini değerlendirmek için daha fazla araştırmaya ihtiyaç olduğunu düşünüyoruz..*

Anahtar kelimeler: *Çocukluk çağı, fokal epilepsi, lakozamid*

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INTRODUCTION

Epilepsy is the most common chronic neurological disease in childhood. With a properly selected monotherapy, seizures can be controlled in approximately 2/3 of patients, while approximately 30% of patients have refractory seizures despite proper use of multiple antiepileptic drugs [1]. Since most antiepileptics used in the treatment of refractory epilepsy have similar mechanisms of action, decreased activity or increased toxic effects of antiepileptics as a result drug-drug interactions in polytherapy are common problems. Non-pharmacologic treatments such as epilepsy surgery, vagal nerve stimulation (VNS) and ketogenic diet have limited indications, in addition to difficulty of administration and variable treatment response rates among patients. Because of these treatment difficulties in patients that have refractory epilepsy, several studies on the development of new antiepileptic drugs evaluation of their post-marketing effectiveness and safety are ongoing [2].

Lacosamide (LCM) is a new-generation antiepileptic drug that reduces neuronal membrane excitability with slow inactivation of sodium channel [3]. LCM also modulates collapsin response mediator protein (CRMP-2), which is an intracellular messenger effective in neuronal growth, axonal sprouting and myelinization [4]. However, the clinical effect of this mechanism is not fully understood. Thanks to its mechanism of action different from other sodium channel blockers, lack of its induction or inhibition by hepatic enzymes, low rate of binding to serum proteins, high renal clearance rates, and linear pharmacokinetics, LCM has low drug-drug interaction and advers effect profile [5]. LCM was first approved by the US Food and Drug Administration (FDA) in 2008 for the treatment of focal seizures in adult patients. In our country, it was approved for use in resistant focal seizures in adults in 2012, and pediatric patients with refractory focal seizures over 4 years old in 2016 [6].

In this retrospective cross-sectional study, we evaluated efficacy and the safety of LCM in children with refractory focal seizures.

MATERIAL and METHODS

The records of patients who were treated with LCM and followed-up at our clinic between January 2016 and January 2020 were retrospectively examined. Patients with ≥50% reduction in their seizure frequencies after 6th month of treatment were accepted as responders to treatment. Patients were evaluated as for age, gender, seizure etiology, and semiology; Electroencephalography (EEG), Magnetic Resonance Imaging (MRI) Electrocardiography (ECG) findings; types, numbers and serum levels of antiepileptic drugs used concomitantly or before LCM treatment; non-drug antiepileptic therapies such as VNS, ketogenic diet, and epilepsy surgery. After obtaining these information, all patiens evaluated in our clinic in terms of seizure frequency, adverse effects of the treatment, serum drug levels, control EEG and ECG findings.

Statistical analysis was performed by using IBM SPSS 20 package program. Descriptive statistics were expressed as percentages, mean ± standard deviation, or median (minimum-maximum) according to the normality distribution. McNemar chi-square test was applied for categorical variables. Type 1 error value was evaluated as 5%, and a p-value of <0.05 was considered statistically significant. Ethical approval for our research was obtained from local ethics committee (2020/66).

RESULTS

Patients' Demographic Data: Fourteen children (female n=3 21.4%, male n=11 78.6%, and mean age: 8.64 years) on LCM add-on therapy included in the study (Table 1). Cranial MRI was normal in 3 patients (21.4%), while 11 patients (78.6%) had various pathological findings in their cranial MRI. The average number of antiepileptic drugs used by patients after being diagnosed with epilepsy was 3.29 (range: 2-6). All patients used at least two antiepileptic drugs (max: 4, mean: 2.4) at the beginning of treatment. The most commonly used antiepileptics in decreasing order of frequency were levetiracetam (n=13),

Table 1. Demographic, clinic informations and treatment responses of patients

Patient	Male	Age (year)	Etiology	Concomitant AED	Duration of Treatment (Month)	≥%50 Seizure Reduction	Seizure Free	Adverse Effects
1	M	13	İdiopathic	LEV, VPA, CLB	16	Yes	Yes	None
2	M	16	HİE	LEV, VPA	26	Yes	Yes	Aggression, behavior change
3	M	9	Choroid plexus papilloma	VPA, CBZ	25	Yes	Yes	None
4	M	12	Epileptic encephalopathy	LEV, VPA	1	No	No	Seizure aggravation
5	M	16	İdiopathic	LEV, VPA	34	Yes	Yes	Nausea and vomiting
6	M	10	HİE	LEV, TPR, OXC	14	Yes	No	None
7	F	16	Neurodegenerative disease	LEV, VPA, TPR	14	Yes	No	None
8	M	7	Trauma/ICB	LEV, VPA	16	Yes	Yes	None
9	F	13	HİE	LEV, CBZ	13	Yes	No	None
10	M	9	Meningitis sequelae	LEV, VPA, CBZ, TPR	12	No	No	None
11	M	14	HİE	LEV, VPA	11	Yes	No	None
12	F	13	Structural/CCA	LEV, VPA	15	Yes	No	None
13	M	12	Structural/Cerebellar athropy	LEV, VPA	12	Yes	No	None
14	M	8	Trauma/ICB	LEV, VPA	8	Yes	No	None

AED:Antiepileptic drug, HİE Hypoxic ischemic encephalopathy, İCB:Intracranial bleeding, CCA: Corpus callosum agenesis, LEV:Levetiracetam, VPA:Valproic acid, CLB:Clobazam, CBZ:Carbamazepine, OXC:Oxcarbazepine:, TPR:Topiramate.

sodium valproate (n=12), carbamazepine (n=3), topiramate (n=3), oxcarbazepine (n=1), and clobazam (n=1) respectively. Our four patients (28.6%) were using sodium channel blocking antiepileptics along with LCM. None of the patients had received non-drug antiepileptic treatments. When the laboratory values were examined in the 6th month of the treatment, liver and kidney function tests of all our patients were found to be normal. Serum concentrations of valproic acid, carbamazepine and phenobarbital were examined in the 6th month of the treatment. Serum drug concentrations of all of our patients who used these antiepileptics were within normal range. Serum levels of other antiepileptics could not be tested in our clinic.

Etiology: Etiological examination revealed that 2 patients (14,3%) were classified as idiopathic epilepsy, while 12 patients (85,7%) had symptomatic epilepsy. The most common etiology was hypoxic-ischemic encephalopathy (HIE) (n=4, 28.6%), trauma-related bleeding and brain damage (n=2, 14.3%), congenital structural anomalies of brain (n=2, 14.%), choroid plexus papilloma (n=1), meningitis sequelae (n=1), neurodegenerative disease (n=1) and epileptic encephalopathy (n=1) respectively.

Seziure semiology: All of fourteen patients had the same seizure semiology (focal-onset seizures). Isolated focal seizures were observed in 6 patients (42.9%), while 8 patients (57.1%) had secondary generalized seizures with focal onset. The mean age at the onset of seizures was 35.5 months (range: 1 month-10 years).

EEG findings: Several epileptiform anomalies were present in all patients' EEGs before initiation of the treatment. Focal epileptiform anomalies were observed in 8 patients (57.1%), while 6 patients (42.6%) had generalized epileptiform anomalies. Eleven (78.6%) patients had moderate-to-severe mental-motor developmental delay.

Efficacy: The mean age of initiating LCM treatment was 12 years (median: 12-13). LCM has given in an average dose of 9.57 mg/kg/day (lowest: 8, highest: 12 mg/kg/day) after three weeks titration period. The mean duration of treatment for all our patients was 15.5 months (range: 1-34 Months). When ignoring the patient whose treatment was terminated in the 1th month due to aggravation of the seizure. the average duration of treatment was 16.62 months (Range: 8-34 Months).

In the 6th month of the treatment, in 12 patients (85,7%) seizure frequency decreased by ≥50% compared with the beginning of the treatment ($p<0.001$). Five patients (35.7%) were seizure-free. LCM treatment was discontinued at the 1th month

after the number and frequency of seizures increased in the patient with the diagnosis of electrical status epilepticus of slow sleep (ESES). The patient is still being followed-up with a diagnosis of epileptic encephalopathy. In one patient, despite using lacosamide for 12 months, there was no significant decrease in the frequency of seizures. When the total number of seizures of our patients was evaluated, the average number of seizures before treatment was 18,86/month while it was found to be 7.14/month in the first year of treatment (p<0.001) (Table 2).

When we examined the effect of LCM therapy by gender and etiology, the p-value could not be calculated because the data did not meet the statistical assumptions due to the low number of units in females (3/11), and patients with idiopathic epilepsy etiology (2/11). Clinical Treatment-related clinical adverse effects were observed in 3 patients (21.4%) The symptoms of a patient with complaints of nausea and vomiting, and another patient with complaints of hyperactivity and aggressiveness were disappeared after dose regulation and did not require drug discontinuation. However, there was a significant increase in the number and frequency of seizures after treatment in the patient we followed with the diagnosis of ESES. The treatment of this patient was discontinued in the 1th month. Subsequently the patient diagnosed as epileptic encephalopathy with unknown etiology. No abnormalities were found in the QT/QTC or PR intervals in the follow-up. No major change was observed in EEG of any patient in the 6th month of their treatment.

Table 2. Seizure frequencies at the first year of treatment.

	Before LCM treatment mean±sd (25P-75P)	At the first year of LCM treatment mean±sd (25P-75P)	p
Average seizure frequency (number of seizures per month)	18.86±30.69 (4.00-15.00)	7.14±17.11 (0.00-1.75)	<0.001

LCM: Lacosamide, sd: Standard deviation.

DISCUSSION and CONCLUSION

LCM therapy in refractory childhood focal epilepsies was approved, and several researches regarding efficacy and safety of the drug is ongoing. Recently, in a broad review including 26 studies and 797 patients (refractory epilepsy and epileptic syndromes n=757, status epilepticus n=40) demonstrated that 50.07% of the cases had a decreased frequency of seizures at a rate of ≥50%, and 23.62% of the patients were seizure-free during an average follow-up period of 10.23 months after LCM treatment. The drug efficacy rates among the studies included in the review were quite different (0%-100%) [7]. The reason for these different treatment response rates seem to be due to the highly heterogeneous age ranges, seizure types, seizure etiologies, drug dose, and duration of drug use of the patients in the studies. But in studies performed with only groups of patients with focal-onset seizures, the efficacy of LCM was found to be higher [8-10].

The first randomized, double-blind, placebo-controlled study for efficacy and safety of LCM in childhood focal seizures was published in 2019. The study included 306 patients (LCM=152, placebo=154, mean age=10.7), and ≥50% decrease in seizure frequency on the 28th day of treatment was found to be statistically significant (p=0.0006) in the LCM group, compared to the placebo group [11]. LCM treatment seems to be more effective in focal-onset seizures of childhood in literature. In our study, efficacy of LCM in focal-onset seizures was quite high (85,7%), like those cited in the literature. However, the number of patients in our study was small. The age range, gender (female/male: 3/11) and etiologies (idiopatic/symptomatic: 2/12) of our patients were comparable to each other. We think that high response rates to LCM treatment in our research are likely due to these factors.

Studies on the efficacy of LCM in special epileptic syndromes of childhood are limited. In a study of 18 patients with Lennox-Gastaut syndrome -a specific epileptic encephalopathy, efficacy of LCM treatment was low (33%) with a higher seizure aggravation rate (17%) [12]. In our study, seizure aggravation was observed in one male patient with a diagnosis of

ESES, who was diagnosed as epileptic encephalopathy in his follow-up. The average follow-up period of our cases was more than 1 year (mean=15.5 months) in line with the literature. In some studies seizure control rates at the end of the first year of treatment was significantly lower than the seizure control on the 28th day [8,13]. In our study, seizure frequency of the patients significantly decreased ($p<0.0001$) at first year of treatment compared to their seizure frequency before treatment (Table 2).

There is no common consensus on the dose of lacosamide in childhood, however, the recommended dose is 8-12 mg/kg/day [14]. In the literature, various dose ranges (2.4-19.4 mg/kg/day) were used, but any difference in dose-related efficacy was not reported [7]. In our study, the drug was used according to the dose range recommended by the manufacturer (mean=9.57mg/kg/day). In our patients no relationship was found between dose and clinical response.

The most common adverse effects during treatment are dizziness and somnolence, similar to those in adult patients. Headaches, tremors, ataxia, behavioral disorders and aggression have been also observed. These adverse effects are usually seen in the titration phase of the drug and can be controlled by dose adjustments. Most commonly adverse effect which requires discontinuation of treatment is the aggravation of seizures [3,7,11,14,15]. The mechanism of seizure aggravation is unknown. Although it is stated that the frequency of adverse effects increases with concomittant use of a classical sodium channel blocker, there are no findings to support this suggestion [16]. LCM treatment apparently has a favourable safety profile as for cardiac system and no arrhythmias or QT/QTc and PR interval changes have been detected during treatment in childhood patients [3,17]. No pathological ECG findings were found in any of our patients who used drugs for more than 6 months. The adverse effects such as dizziness and somnolence, which are the most common adverse effects seen in the literature, were not observed in our study. Behavioral change and aggression observed in one of our patients, and nausea-vomiting symptoms in one patient were controlled by dose titration. Initially we could not understand whether the increase in seizures in our patient with epileptic encephalopathy was due to medication or the course of the disease. Since seizures decreased after drug withdrawal, we thought that this was an adverse effect of the drug. Serum levels of sodium valproate, carbamazepine, and phenobarbital were within normal ranges in patients receiving these treatments. In our clinic serum levels of other drugs could not be analyzed. Most of our cases (78.6%) were unable to state their subjective complaints due to having moderate-severe mental and motor developmental delays. Parents and caregivers might not remember the symptoms at the beginning of treatment. We think that these two factors are mainly causes of low adverse effect rate (21.4%) detected in our study distinctively different from literature data.

In our country, permission from the national drug agency is required for LCM treatment in patients younger than 16 years old. Therefore, the number of patients in our study was small. Another limitation in our study was the inability to compare statistical assumptions because gender (female/male=3/11) and etiological (idiopathic/symptomatic=2/11) distributions were not close to each other.

In conclusion, results of our study and other studies in the literature suggest that LCM is an alternative treatment with high efficacy and safety in the treatment of focal-onset refractory seizures in childhood. We think that more prospective randomized, placebo-controlled studies with a wider number of patients are needed to evaluate its efficacy and safety in focal and other type of seizures and epileptic syndromes of childhood.

Ethics Committee Approval: Manisa Celal Bayar University Faculty of Medicine Clinical Research Ethics Committee approval was obtained (27.01.2020/66).
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Research Article

A Preliminary Report on the Efficacy of The Lateral T-Stoma: Solution for Functional Obstruction and Short Bowel Sydrome in High Jejunoileal Atresia

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Yüksek Jejunoileal Atrezide Fonksiyonel Tıkanma ve Kısa Bağırsak Sendromu için Çözüm: Lateral T-Stoma'nın Etkinliğine İlişkin Bir Ön Rapor

ABSTRACT

Objective: *This study is a preliminary report in the efficacy of lateral T-stoma (LTS), which we developed as a solution for anastomosis dysfunction and/or short bowel syndrome in the jejunoileal atresia (JIA).*
Method: *The primary pathologies and results of all cases who underwent lateral t-stoma between July 2017-2020 were evaluated retrospectively.*
Results: *Case 1 with meconium pseudocysts secondary to intrauterine volvulus had Type-3A JIA at 50 cm from the Treitz, and end-oblique anastomosis was performed. The LTS was created proximal to the first anastomosis. on Day 15 due to abdominal distension with 5/1 diameter ratio. The patient was discharged 13 days later. The LTS was closed in the 10th month. In Case 2 with Type-2 JIA at 60 cm from the Treitz, primary LTS was created proximal to the anastomosis with 5/1 diameter ratio. Patient was discharged 15 days later. The LTS was closed in the 10th month. End-to-side anastomosis was performed in Case 3 with Type-2 JIA located 70 cm from Treitz. Anastomotic leakage occurred on Day 36, and the LTS was created with 6/1 diameter ratio. The LTS was closed in the 5th month. Prenatally diagnosed Case 4 was postnatally intubated due to pulmonary hypertension, and surfactant was applied. Intrauterine volvulus, meconium pseudocyst and intestinal perforation were detected at 50 cm from the Treitz, and jejunoileal anastomosis together with primary LTS was performed with 5/1 diameter ratio. Nasogastric feeding started on the 10th day, but patient was lost on Day 44 without extubation. Primary repair and end-stoma were performed in Case 5 with gastroschisis/intestinal atresia with perforation. Due to high-flow stomal discharge/weight loss, the ileo-colic anastomosis and LTS were performed proximal to anastomosis with 3/1 diameter ratio at 60 cm from Treitz on Day 68. Patient was discharged on Day 90. In Case 6 with prenatal diagnosis as meconium cyst with Type-2 JIA (at 65 cm from Treitz), primary LTS was created proximal to the anastomosis with 4/1 diameter ratio. The LTS was closed in the 14th month due to high-flow stomal discharge.*
Conclusion: *Creation of lateral T-stoma proximal to the anastomosis in JIAs with large differences in diameters may shorten hospitalization time and relieve functional obstruction/short bowel syndrome.*

Keywords: *Jejunoileal atresia, meconium ileus, neonatal obstructions, enterostomy, bishop-koop prosedure, santulli procedure*

ÖZ

Amaç: *Bu çalışma, jejunoileal atrezide (JIA) anastomoz disfonksiyonu ve/veya Kısa Barsak Sendromu (SBS) için bir çözüm olarak geliştirdiğimiz lateral T-stomanın (LTS) etkinliğine ilişkin bir ön rapordur.*
Yöntem: *Temmuz 2017-2020 tarihleri arasında lateral T-stoma yapılan olguların birincil patolojileri ve sonuçları geriye dönük olarak değerlendirildi.*
Bulgular: *İntrauterin volvulusa sekonder mekonyum psödokistli ilk olguda Treitz'den 50 cm uzaklıkta Tip-3a JIA mevcuttu, uç oblik anastomoz yapıldı. Abdominal distansiyon nedeniyle 15. gün 5/1 çap farkı nedeniyle anastomoz proksimalinden LTS oluşturuldu. Hasta 13. gün taburcu edildi, 10. ayda stoması kapatıldı.*
Treitz'den 60 cm uzaklıkta Tip-2 JIA'li ikinci olguda, 5/1 çap farkı nedeniyle anastomoz ve proksimalinde primer LTS oluşturuldu, 15. gün taburcu edildi, 10. ayda stoması kapatıdı. Treitz'e 70 cm uzaklıkta bulunan Tip 2 JIA'lı 3. olguda uç-yan anastomoz yapıldı. 36. gün anastomoz kaçağı nedeniyle yeniden opere edildiğinde 6/1 çap farkıyla LTS oluşturuldu, 5.ayda stoması kapatıldı. Prenatal tanılı 4.olgu pulmoner hipertansiyon nedeniyle doğum sonrası entübe edildi, sürfaktan uygulandı. İntrauterin volvulus, mekonyum psödokisti ve Treitz'den 50 cm'de barsak perforasyonu ve 5/1 çap farkı nedeniyle primer LTS yapıldı. Nazogastrik beslenme 10. gün başladı, ancak hasta 44. gün ekstübe edilemeden kaybedildi. Gastroşizis, intestinal atrezi ve barsak perforasyonu olan 5.olguda primer onarım ve end-stoma yapıldı. Yüksek debili stomadan kilo kaybı nedeniyle, 68. gün Treitz'den 60 cm'de 3/1 çap farkıyla ileo-kolik anastomoz proksimalinden LTS oluşturuldu, 90.gün taburcu edildi. Prenatal intrauterin volvulus/mekonyum kisti tanılı 6. olguda Treitz'den 65 cm'de Tip-2 JIA'ya 4/1 çap farkı nedeniyle anastomoza proksimalde primer LTS oluşturuldu. Yüksek debili stoma gelenleri nedeniyle 3,5. ayda stoması kapatıldı.
Sonuç: *Büyük çap farklılıkları olan JİA'lerde anastomozun proksimalinde lateral T-stoma oluşturulması hastanede kalış süresini kısaltabilir, fonksiyonel obstrüksiyon ve kısa bağırsak sendromu bulgularını hafifletebilir.*

Anahtar kelimeler: *Jenunoileal atrezi, mekonyum ileus, yenidoğan tıkanıklıkları, enterostomi, bishop-koop prosedürü, santulli prosedürü*

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INTRODUCTION

Jejunoileal atresia (JIA) is a congenital gastrointestinal defect that occurs in one of 5,000 live births [1]. The type of atresia, remaining bowel length, and accompanying anomalies are among the factors affecting the transition to enteral feeding and the duration of hospital stay in cases with jejunoileal atresia [1]. In recent years, improvements have been made in the treatment of patients with intestinal atresia due to the improvements in neonatal intensive care units, the development of anesthesia methods and the widespread use of total parenteral nutrition [2,3].

Basic treatment methods for cases of jejunoileal atresia include primary anastomosis or the creation of intestinal stoma. The Mikulicz double-barreled enterostomy was defined firstly by Gross et al. [4]. Bishop and Coop created a distal chimney enterostomy in 1957 [5]. The opposite of the Bishop-Koop enterostomy was the proximal enterostomy described by Santulli and Blanc four years later [6]. In 1970, O'Neill et al. [7] described tube enterostomy with or without resection. A similar tecnique as a T-tube enterostomy was created by Harberg [8]. A method of the appendiceal stump for irrigation and evacuation of impacted meconium was described by Fitzgerald and Conlon [9]. Despite all of these technological advances in medicine; being of great diameter difference between the atretic tips, functional obstruction of adynamic enlarged intestinal loop in the proximal, anastomotic leak and short bowel syndrome (SBS) are still the most important complications in cases with high jejunoileal atresia.

The intent of this study is to show the preliminary results of the lateral T-stoma (LTS) technique which was developed as a solution to the anastomotic dysfunction and short bowel syndrome in cases with jejunoileal atresia by Aysenur Celayir.

MATERIAL and METHODS

After the approval of the ethics committee of our hospital, retrospective study was performed in cases that had undergone lateral T-stoma procedures. Informed consent of the families was obtained both during the hospitalization and before this procedure. The parents of all cases had been informed about the disease, all procedures performed before the treatment and surgery, and their consent was obtained.

All patients who had undergone the LTS between July 2017 and January 2020 were evaluated retrospectively. The lateral T-stoma was used in some cases with high jejunal atresia and large difference between luminal diameters of proximal and distal loops.

The success and effectiveness of the LTS were revealed by evaluating the findings in terms of age, gender, prenatal diagnosis, diagnosis, intraoperative findings, clinical condition before and after opening the LTS and its outcomes. Stool passage from the lateral T-stoma and the rectal route, transition to the enteral feeding, weight gain, duration of the total parenteral nutrition and enteral nutrition were evaluated. The results were evaluated with percentage distributions, statistical analysis was not performed because the number of cases was not sufficient yet.

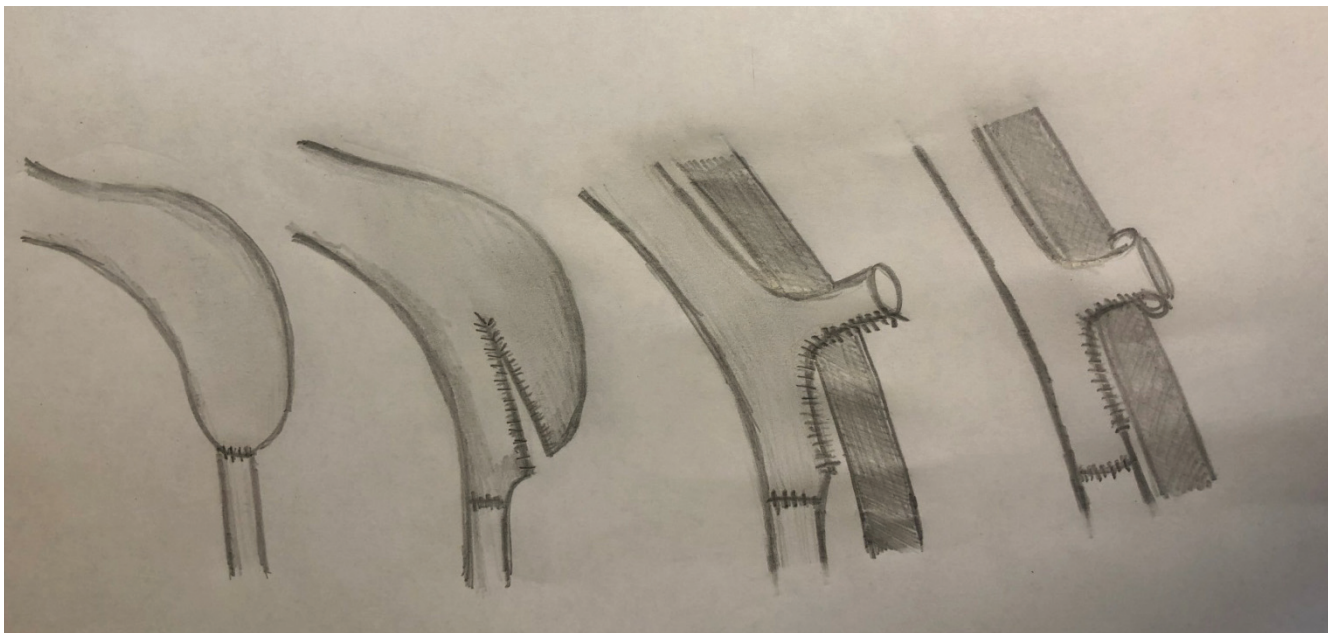
Surgical technique of the Lateral T-Stoma:

Firstly, the wide jejunum at the proximal and the narrow ileum at the distal end were anastomosed from antimesenteric edge (Figure 1A).

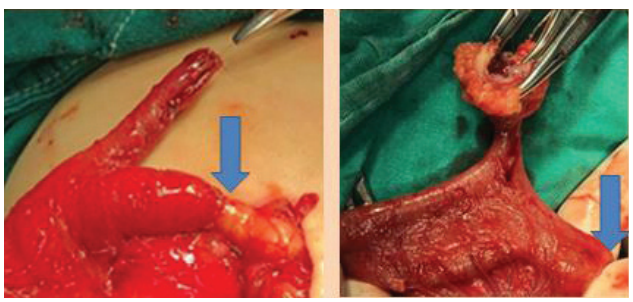
Secondly, approximately 4cm-long middle incision between the trouser legs was made along the antimesenteric edge of the enlarged jejunal loop at the proximal of the anastomosis leaving an equal-sized bowel lumen on both sides (Figure 1B).

Thirdly, the incision was sutured starting from the anastomosis as a single line continuously with 5/0 Vycril or PDS, and approximately 7-8 cm- suture line was created along the antimesenteric edge of the proximal loop. Thus, the diameter of the upper large loop was equal to that of the lower loop and the continuity of the bowel was provided with an anastomosis (Figure 1C).

Finally, an approximately 3-4 cm-long tubularized intestine extending laterally from the antimesenteric



Figures 1A, B, C, D. (A) Diagram of the first step of this technique; end-to-end anastomosis of the intestinal loops from the mesenteric edge. (B) Diagram of the second step of this technique; 4-cm long middle incision on trouser legs was made along the antimesenteric edge of enlarged proximal loop. (C) The incision made between trouser legs was sutured starting from the anastomosis side through the other side as a single line with 5/0 Vycril or PDS continuously, and approximately 7-8 cm-long suture line was created on the antimesenteric edge of the proximal loop. The continuity of the bowel was provided with an anastomosis made with the same caliber. Thus, the diameter of the anastomosed bowel was equalized. (D) Finally, an approximately 3-4 cm-long tubularized intestine extending laterally from the antimesenteric edge of the proximal loop at 4 cm above the anastomosis was anastomosed to the abdominal wall in the form of a lateral end stoma. Thus, the anastomosis and suture line remained under the stoma.



Figures 2A, B. (A) Intraoperative photo (B) Closure of the lateral T-stoma is demonstrated. Anastomosis line is marked with blue arrow in each picture.

edge of the proximal loop at 4 cm above the anastomosis was anastomosed to the abdominal wall in the form of a lateral end stoma. Thus, the anastomosis and suture line remained under the stoma (Figure 1D).

In all cases, ganglion cells in the edge of lateral T stoma, and all removed intestinal tissues were evaluated.. Before the creation of LTS, the stoma mouth was closed with a dressing, and complete stool passage was observed rectally for fifteen days. Rectal biopsies of all patients were performed before the stoma closure; and the stoma edge biopsies were evaluated.

RESULTS

The lateral T-stoma was created in six cases over a 2.5 years period; 4 of them were created as primary and 2 of them as secondary procedures.

Case 1: A Type 3a JIA at 50th cm from the Treitz was detected during the operation in a male neonate who was born with a prenatal diagnosis of meconium pseudocysts secondary to intrauterine volvulus. Distally a 35 cm ileum was detected, and end-to-oblique anastomosis was performed with a diameter difference of 5/1. Due to the development of abdominal distention and the absence of stool passage, an explorative laparotomy was performed on the 15th day. It was observed that the intact anastomosis did not work due to the diameter difference; and in the dilated jejunum loop LTS was created from the proximal of the anastomosis. A little amount stool came out from the stoma at the

third day, and the stool passage started on the 6th day. Enteral feeding was started on the 8th day, and full dose oral feeding was passed on the 11th day, TPN was tapered gradually and terminated three days later, and the patient was discharged on the 13th day. The stoma was closed due to the fact that the majority of the stool passage was achieved rectally at the 10th month. The 3.5 years old child is still devoid of any relevant complications.

Case 2: An abdominal exploration was performed on the postnatal 3rd day in a girl baby born 3900 g with the prenatal diagnosis of an intestinal dilatation. A Type 1 JIA atresia was found at 60th cm from Treitz. Primary anastomosis of the dilated jejunum and ileum was performed, and at the same session the LTS from the proximal to the anastomosis was created primarily. Discharges from the stoma and rectal route started on the 4th day, and the oral feeding was started on the 6th day as a trophic feeding, and full feeding by mouth was initiated on the 12th day. She was discharged on the 14th day. At the 10th month, the stoma was closed due to the large amount of rectal discharge. An intraoperative photo taken during the surgery of the LTS and closure of the stoma closure is demonstrated in Figures 2A, and B. Now she is 3.5 years old and is still devoid of any relevant complications.

Case 3: A 3250 g male neonate was born with the prenatal diagnosis of intestinal obstruction. In the abdominal surgery, end-to-side anastomosis was performed due to Type 2 JIA at 70th cm from the Treitz. On the 36th day, a second laparotomy was made due to intolerance of the feeding, incomplete gastrointestinal passage, abdominal distension, and a closed perforation on the anastomosis line was detected. The LTS was opened from dilated proximal loop because of the diameter difference as 6/1. In this case full-dose oral feeding was initiated on the 4th day after stool discharge from the stoma and rectal route, and the stoma was closed after observing high flow rate of rectal discharge at the 5th month.

Case 4: A male neonate with prenatal diagnosis as intestinal dilatation and suspected cystic fibrosis was born at the 36th gestational week as 2680 g. He was intubated due to low APGAR scores, and pulmonary hypertension in delivery room and the surfactant was applied. The meconium pseudocyst secondary to the intrauterine volvulus and perforation of jejunum at 50th cm from the Treitz were detected in the exploration performed on the second day after birth. All proximal and distal intestines were filled up with sticky meconium and meconium beads suggestive of cystic fibrosis. The end-to-end anastomosis with 5/1 diameter difference and primary LTS from the proximal dilated loop were performed. The patient tolerated feeding on the postoperative 8th day, and he had complete stomal and rectal passage, but still intubated infant died on the 44th day due to sepsis because he was unresponsive to treatment of severe pulmonary hypertension and heart failure.

Case 5: This male neonate was born as 2220 g with gastroschisis at 32 weeks of gestation without prenatal diagnosis. During the primary abdominal repair of gastroschisis at the postnatal first hour; an end-stoma from the site of the intestinal perforation was also created. The short bowel syndrome developed due to a very high output stoma, and the patient was not able to gain weight despite total parenteral nutrition. Breast milk was definitely not given to the patient because of bacterial growth in the breast milk sample taken from his mother, and the patient suffered from the sepsis caused by Serratia Marcencens. During the laparotomy performed on a 3380 g, and 68-day-old male newborn, it was determined that the end ileostoma was 60 cm from Treitz and only 30 cm of unused colon remained distally. These operative findings suggested that the patient had short bowel syndrome secondary to a possible volvulus in the intrauterine period together with high type jejunoileal atresia and gastroschisis. The end-stoma was excised from the abdominal wall, and an end-to-end jejunocolic anastomosis was performed with 3/1 diameter difference at both bowel ends. Then the lateral T-stoma was created from the enlarged proximal jejunum. The patient still had short bowel syndrome. Stomal and rectal passage started from the 6th day, oral feeding on the 8th day, and increased to full dose at 14th day. Total parenteral nutrition was gradually

reduced and ceased at an average of 80th days. The patient was fed with 75 cc oral formula containinig medium chain fatty acid at every three hours with 75 cc oral water intake before each feeding. Weight gain was between 10-20 g per day and he was discharged on the 90th day after treatment of second episode of sepsis caused by Serratia Marcescens completed. However, it was learned that a 17-year-old mother who was admitted to our department again one week later did not feed her baby with severe dehydration and weight loss. Rotavirus was grown in his stool. Total parenteral nutrition and enteral nutrition were started again due to the patient's excessive dehydration and weight loss. Despite the complete passage through the stoma and rectal route and weight gain, he was lost at the age of 4.5 months due to severe sepsis.

Case 6: A male neonate with prenatal diagnosis as intestinal dilatation, suspected volvulus and meconium cyst was born at the 36th gestational week as 3000 g. The meconium pseudocyst secondary to the intrauterine volvulus and jejunoileal atresia Type II at 65th cm from the Treitz was detected in the exploration on the first day of his life. Volvulated intestine was excised aproximately for 25 cm, and end-to-end anastomosis with 4/1 diameter difference and primary LTS from the proximal dilated loop were performed. The patient tolerated feeding after the postoperative 5th day, and he was discharced on the 30th day when he had complete stomal and rectal passage. At the 3.5th month, the stoma was closed due to the large amount of rectal discharge.

Fecal discharge from the stoma and rectal route was completely achieved in 6 patients at the time of discharge from the hospital. The mean time to rectal and stomal passage was 5.6±1.66 days, and to the initiation of full oral feeding was 8.4±1.41 days. All stomas worked without any complications. Amount of rectal discharge was lower than the stomal passage in all cases initially, but during the discharge of patients amount of the rectal discharge was higher than the discharge from the stoma The mean time to discharge was 21.6±13.01 days. The causes of death of two cases were not related with their stomas, and parents of both cases were second-degree relatives (both of them were cousins). These cases had jejunal

Table 1. The demographic characteristics, diagnoses, operations and final results of our 6 patients.

n	Gender	Diagnosis	1. operation and anastomosis	From Treitz (cm)	Diameter Difference	Lateral T-Stoma	Oral İntake (day)	Before Stoma/ Discharge (day)	Stoma Closure
1	Male	Meconium cyst, Type3aJIA	end-to-oblique	50.	5/1	secondary	22./7.	30./13.	10. months
2	Female	Type1 JIA	end-to-end	60.	5/1	primary	7.	14.	10. months
3	Male	Type2 JIA	end-to-end	70.	6/1	secondary	44./8.	56./15.	5. months
4	Male	Meconium Cyst: Meconium İleus, Volvulus, Perforation	end-to-end	50.	5/1	primary	10.	44. exitus	exitus
5	Male	Gastroschisis with İntrauterin (Jejunum) Perforation	primary repair with end-stoma	60.	3/1	secondary	78./10.	98./22. sepsis exitus	exitus
6	Male	Meconium Cyst: Volvulus, Tip2 JIA	end-to-end	65.	4/1	primary	5.	30.	3.5 months

n=6	Stool passage from	Oral intake
Mean day	5,6	8,4
Standart deviation	±1,66	±1,41

perforations secondary to intrauterine volvulus, and possibly cystic fibrosis disease.

Biopsies obtained from stoma edge and rectum were reported as ganglion-positive in all cases. The demographic characteristics, diagnoses, operations and final results of the patients are summarized in Table 1.

DISCUSSION

In Type 1 and Type 2 atresia where the length of the distal intestine is sufficient, removal of the adynamic dilated bowel is recommended for anastomotic safety and passage. However, the slightest resection in Type 3b and Type 4 jejunoileal atresia, or complicated intrauterine atresia, may aggravate the short bowel condition. Atresia, such as the presence of meconium peritonitis with Type 3b and Type 4 jejunoileal atresia called severe intestinal atresia requires long-lasting parenteral nutrition with higher mortality rates and re-operation [10,11].

Treatment of cases with intestinal atresia include primary anastomosis and/or the stoma opening. Both of these techniques have its potential complications as well as variable success rates [12-15]. In cases where a stoma needs to be opened, different stoma opening methods have been defined in consideration of the length of the intestine up to the stoma as well as facilitating stool passage distally. The Mikulicz double-barreled enterostomy is preferred by some because complete evacuation of thickened meconium is not necessary and intra-abdominal anastomosis is avoided, thereby preventing the risk of anastomotic leakage [4]. In addition, the bowel can be opened after complete closure of the abdominal wound, thereby reducing the risk of intraperitoneal contamination. A distal chimney enterostomy, described by Bishop and Koop, involves resection with anastomosis between the end of the proximal segment and the side of the distal segment of bowel. The opposite of the Bishop-Koop enterostomy is the proximal enterostomy described by Santulli and Blanc [4-6]. Like the distal chimney enterostomy, catheter inserted into distal limb is pulled out through the stoma, thus providing means of irrigating the distal bowel. Performing a Santulli or Bishop-Koop type enterostomy allows progressive transanastomotic enteral feeding by slight flow of luminal nutrients, which has a trophic influence on the distal intestinum and stimulated maturity of bowel motility and function [4-6,10-15]. Bishop-Koop ileostomy shortened the median hospital stay and TPN duration in severe intestinal atresia [13]. Bishop-Koop or Santulli ileostomies can be preferred as a more effective method than divided stoma in patients with meconium ileus, intestinal atresia and necrotizing enterocolitis [13]. Despite medical and surgical management, cases with short bowel syndrome (SBS) and discrepancy between the luminal diameters of proximal small bowel and distal colon, have persistent feeding intolerance; and the apparent disadvantage with these techniques is the presence of a high output stoma and the inherent risk of dehydration [4].

The lateral T-stoma procedure developed by Aysenur Celayir. involves end- to- end anastomosis between proximal and distal segments and tapering a part of the antimesenteric side of the dilated proksimal segment as a stoma. Actually LTS can be considered as a modification of the Santulli procedure. Lateral T-stoma is more advantageous than the Santulli procedure; since the anastomosis and tapering suture line remain under the stoma in the lateral T-stoma technique, and also complications such as anastomotic leak or detachment are not observed.

In the lateral T-stoma procedure, since the disadvantages of adynamic ileal loop are eliminated by tapering the large adynamic loop at the proximal of the lateral T-stoma as inspired by Santulli or Bishop-Koop ileostomy, the possibility of leakage from sutures decreases. Thus sutures of the tapering and anastomosis are located approximately 3-4 cm distally from the stoma which reduces the risk of anastomotic leakage from sutures. This tecnique provides the enteral continuity and anastomotic line and tapering line retain its distal position for treatment or inspection. Secondarily since the diameter of the intestine proximal to the anastomosis created by the tapering is larger at least as wide as

the distal bowel, easy transition to the distal part is achieved. This method, which was applied for the first time in a secondary case, was thought to be useful in the treatment of high jejunal atresia with a large diameter difference between the blind ends. It was thought that this procedure can be applied primarily in cases with an increased difference between luminal diameters.

A high-output stoma occurred in all patients after creation of a lateral T-stoma. In all patients with LTS, functions of stomas were good after stomas matured, with regression of the mucosal edema and restart of bowel movement, and gastrointestinal passages under the level of stoma were opened, and rectal outputs were much more than the stomal outputs in all patients. No patient with lateral T-stoma required premature closure of the stoma thanks to achievement of a high-output stoma. Surgical choice for lateral T-stoma procedure allowed maintenance of enteral continuity with the distal anastomosis and tapered suture lines, therefor were provided in reducing the risk of short bowel syndrome and the duration of TPN and hospitalization even in “severe” cases.

Anastomotic leaks are serious complications occurring after repair of intestinal atresia. The high incidence of anastomotic leaks in apple peel atresia (14%) compared with the other types of intestinal atresia (4%) is due to inadequate blood supply at the anastomotic site owing to its retrograde blood supply provided by only a single artery [11]. Due to the large diameter difference in high jejunoileal atresia and the long suture line in tapering, anastomotic leaks can be seen at very high rates. Some studies have reported that half of the cases of sepsis were caused by anastomotic leakage, and therefore a functional anastomosis appears to be a key prognostic factor for the early survival of these children [11]. Since the patency of the passage could not be achieved due to the adynamic bowel loop at the proximal of the anastomosis because of the high difference in luminal diameters, the LTS technique was applied in two cases as a secondary procedure. Anastomotic leakage did not develop in any patient after the creation of the LTS as primary or secondary procedure.

According to preliminary results of our six cases; LTS can be applied as a primary alternative in appropriately selected cases, and it can be safely applied as a secondary procedure to all other cases in functional obstruction with greater difference in luminal diameters. Although the number of our cases is not sufficient yet, according to our preliminary results, the opening of gastrointestinal passage was achieved in cases in which LTS was applied as primary or secondary procedures. In these patients, the use of a lateral T-stoma in continuity as a salvage method to decompress the proximal intestine with or without maintaining maximal intestinal length can be proposed. In addition, a rectal biopsy was performed in all cases to exclude a possible Hirschsprung disease or neuronal intestinal dysplasia before closing the neonatal stoma. In cases with lateral T-stoma, it is also possible to close the lateral T-stoma without biopsy in cases where the stoma is closed with dressing and the rectal passage is seen to be uneventful. Therefore, lateral T-stoma seems to be more advantageous compared to other methods in neanatal intestinal obstructions with increasd difference in luminal diameters in terms of ease of application and decreasing the need for rectal biopsy.

In selected cases of candidates carrying higher risk for developing short bowel syndrome such as jejunoileal atresia, total colonic aganglionosis or meconium ileus where difference between the diameters of proximal distal ends is greater than 5/1, the lateral T-stoma procedure seems to be a promising option. LTS is a feasible and safe surgical chois for the management of congenital anomalies resulting in SBS. LTS provides early intestinal continuity, creates intestinal tapering from congenitally dilated intestinal loop, and appears to prevent the need for creating interval stomas and their associated loss of intestinal length in newborns with congenital SBS. However, additional studies with recent changes in SBS treatment emphasizing intestinal rehabilitation are needed to assess long-term impact on bowel adaptation of the lateral T-stoma performed in neonates prior to adoption of this method.

Ethics Committee Approval: Zeynep Kamil Gynecology and Pediatrics Training and Research Hospital Clinical Research Ethics Committee approval was obtained (18.09.2019/91).

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Conflict of Interest: The authors declare no conflict of interest.

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Informed Consent: Obtained.

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Research Article

Long-Term Follow-up Results of Children Undergoing Achalasia Surgery

Akalazya Nedeniyle Ameliyat Edilen Çocukların Uzun Dönem Sonuçları

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ABSTRACT

Objective: *Achalasia is a disease characterized by lower esophageal sphincter motility disorder. Whereas there is no clear algorithm in treating achalasia in children, Heller myotomy is known to be as the most effective method. Gastroesophageal reflux after myotomy is a frequently reported complication. Therefore, the fundoplication procedure should be added to the myotomy simultaneously. Our study aimed to present the results of patients who underwent Heller myotomy and fundoplication for achalasia.*
Materails and Methods: *Twelve patients who underwent laparoscopic Heller myotomy with the diagnosis of achalasia between the 2006 and 2019 have been included in the study.*
Results: *There were 12 children. 75% of them were male, and 25% were female. Laparoscopic Heller myotomy and antireflux procedure were applied to all patients. The average nasogastric withdrawal time was 1.75 days, the average time to start feeding was 2.25 days. The average hospital stay was 6.75 days. Dysphagia persisted in 3 patients who underwent Dor fundoplication and in 1 patient who underwent Toupet fundoplication at postoperative 3rd-week controls. It was observed that the symptoms improved after the one-time endoscopic dilatation procedure.*
Conclusion: *Heller myotomy is gold standard method in the treatment of achalasia in children.We believe that partial fundoplication added to myotomy reduces the risk of GER.*

Keywords: *Esophageal achalasia, heller myotomy, fundoplication*

ÖZ

Amaç: *Akalazya, alt özofagus sfinkterinde motilite bozukluğuyla karaterize bir hastalıktır. Çocuklarda akalazya tedavisinde net bir algoritma olamamakla birlikte Heller miyotomisi en etkili yöntem olarak kabul edilmektedir. Miyotomi sonrası gastroözofageal reflü sık bildirilen bir komplikasyondur. Bu nedenle miyotomiye eş zamanlı olarak fundoplikasyon prosedürü eklenebilir. Çalışmamızda akalazya nedeniyle Heller myotomi ve fundoplikasyon yapılan hastaların sonuçlarının sunulması amaçlanmıştır.*
Yöntem: *Kliniğimizde 2006-2019 yılları arasında akalazya tanısı ile laparoskopik Heller miyotomisi uygulanan 12 hasta çalışmaya dahil edildi.*
Bulgular: *Olguların %75'i erkek, %25'i kızdı. Hastalara laparoskopik Heller miyotomisi ve antireflü prosedürü uygulandı. Ortalama nazogastrik çekilme süresi 1,75 gün, ortalama beslenmeye başlama zamanı 2,25 gündü. Ortalama hastane yatış süresi 6,75 gündü. Dor fundoplikasyonu uygulanan 3 hastada ve Toupet fundoplikasyonu uygulanan 1 hastada postoperatif 3. hafta kontrollerinde disfajinin devam ettiği görüldü. Tek seferlik endoskopik dilatasyon işlemi sonrası semptomların düzeldiği izlendi.*
Sonuç: *Heller miyotomi çocuklarda akalazya tedavisinde altın standart yöntemdir. Miyotomiye eklenen parsiyel fundoplikasyonun GÖR riskini azalttığını düşünüyoruz.*

Anahtar kelimeler: *Özofageal akalazya, heller miyotomi, fundoplikasyon*

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INTRODUCTION

Achalasia is a pathology characterized by degeneration of myenteric neurons that innervate the lower end of the esophagus and characterized by a motility disorder in the esophagus [1,2]. It is a rare disease in children and its annual incidence is 0.11/100,000 [2,3]. Although its etiology is not fully elucidated, it is thought to be autoimmune-mediatedsituation connected with viral infections [4]. It has also been shown to be associated with trisomy 21 and Triple-A Syndrome (Achalasia, Alachymia, and Adrenal Insufficiency) [4,5]. Vomiting, loss of appetite and chronic cough may be the initial symptoms in children under five years of age, and it progressive dysphagia, regurgitation, chest pain, and growth retardation in older children [2,3,5]. The diagnosis may usually be made clinically. However, since the symptoms are generally interpreted in favor of gastroesophageal reflux (GER) due to its rarity, diagnosis is often delayed [4]. The definitive diagnosis is made by esophageal manometry, contrast studies and endoscopic examinations [3].

The treatment of achalasia aims to relieve the symptoms by eliminating the obstruction in the lower esophageal sphincter with relaxation disorder [5]. For this purpose, pharmacological treatment such as calcium channel blocker and botulinum toxin injection, mechanical or pneumatic dilatation, open or laparoscopic esophagomyotomy (Heller Myotomy) can be used in treatment [4–6]. Pharmacological treatment options are not preferred in the pediatric population because of their short-term effectiveness, and calcium channel blockers have side effects such as headache and dizziness [4]. Although publications are reporting that pneumatic balloon dilatation is as effective as Heller myotomy in adult achalasia patients, it is not recommended for use in children because it is not designed for pediatric use [7]. Heller myotomy is considered as the most effective method in the treatment of achalasia [4,6]. GER is a frequently reported complication after Heller myotomy. Therefore, the fundoplication procedure is usually added to this Heller myotomy [4].

Our study aimed to present the results of patients who underwent laparoscopic Heller myotomy and fundoplication due to achalasia.

MATERIAL and METHODS

Twelve patients with a diagnosis of achalasia who underwent laparoscopic Heller myotomy between 2006 and 2019 were included in the study. This study was conducted in accordance with the Helsinki Declaration and with the permission of the local ethics committee (No: I11-682-20) Demographic data, symptoms at the time of application, methods used in diagnosis, operation details (antireflux surgery added to myotomy, intraoperative complications), length of hospital stay, surgery complications, recurrence and follow-up results were reviewed retrospectively.

Preoperative Management

Patients who presented with a pre-diagnosis of achalasia were diagnosed by preoperative contrast studies and endoscopy (Figure 1). With the induction of anesthesia, a nasogastric catheter with a size suitable for the patient's age was inserted, and prophylaxis at a dose of 100 mg/kg/day of ceftriaxone was administered.

Surgical Technique

The patients were placed in a supine position under general anesthesia and prepared for the laparoscopic approach. A 5 mm (millimeter) camera trocar was inserted through the umbilicus and intraabdominal space was inflated with carbon dioxide up to 10-14 mmHg according to the age of the patient. 5 mm working trocars were inserted from the right and left hypochondriac region from the 3 cm superolateral of the umbilicus under camera guidance. A 5 mm incision was made for liver retraction in the epigastric region, and a Nathanson retractor was inserted. The adequate vision was provided by retracting the liver. In esophageal dissection, only the anterior surface was dissected, preserving the posterior and lateral physiological adhesions. Appropriate abdominal esophagus was obtained and myotomy was performed from the

gastroesophageal junction towards the distal esophagus. Myotomy was completed by cutting the muscle tissue up to the mucosa with the help of hook cautery. Saline was given to the area where myotomy was performed. At the same time, possible perforation controls were made by blowing air through a nasogastric catheter. Afterward, according to the surgeon's preference, an anti-reflux procedure was performed with Dor (Anterior partial fundoplication) or Toupet (Posterior partial fundoplication). Dor fundoplication is performed by wrapping the stomach 180 degrees around the anterior of esophagus. If Toupet procedure is performed, short gastric vessels are divided minimmaly, posterior part of the esophagus is dissected for fundus mobilization and fundus is wrapped 270 degrees around the posterior part of the esophagus. The procedure was completed by suturing the port incisions.

Postoperative Management

Generally, the patients' nasogastric catheters were removed on the first postoperative day, and oral fluid intake was allowed on the second day. Patients were recommended to feed a soft food diet for three weeks. Children were recommended to have outpatient visits at postoperative 3rd week, 6th and 24th months. Gastrointestinal tract contraswt studies and/or esophagoscopy was performed in case of residual symptoms.

RESULTS

Between 2006-2019, 12 patients underwent surgery due to lower esophageal achalasia. Nine (75%) of the patients were male, and 3 (25%) were female. The median age of the patients was 12 years (1-17 years). Triple-A syndrome was found in 1 patient. All patients applied with symptoms of dysphagia. Additionally, 3 patients had vomiting, and 2 patients had retrosternal painsymptoms. Endoscopic balloon dilatation was performed initiallyfor 4 children in different centers. Laparoscopic Heller myotomy and antireflux surgery were performed in all of the patients. Dor fundoplication was performed in 4 patients and Toupet fundoplication in 8. There were no intraoperative complications. The average nasogastric withdrawal time was 1.75 days (1-3 days). The average feeding start time was 2.25 days (2-5 days). The average hospital stay was 6.75 (3-19 days) days. The average follow-up time was 6.25 years (1-14 years). It was observed that dysphagia symptoms persisted in the postoperative 3rd-week control in 3 patients who underwent Dor partial fundoplication and in 1 patient who underwent Toupet partial fundoplication. The children underwent one time endoscopic dilatation and did not have any further symptoms.

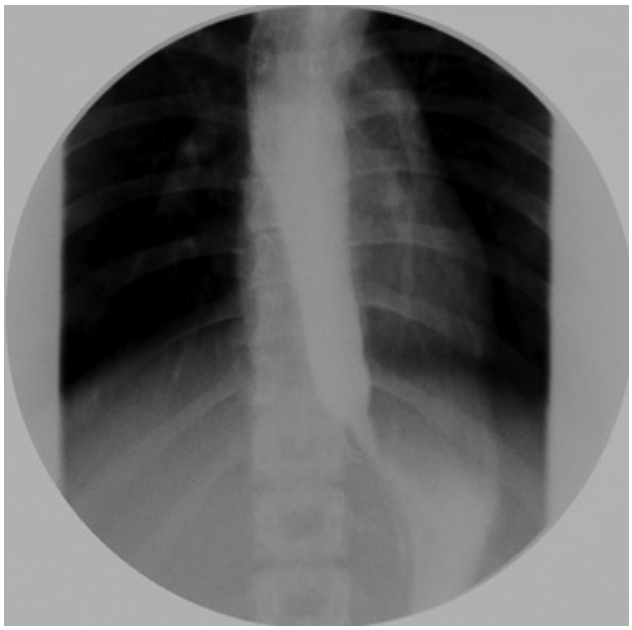

Figure 1. Preoperative esophageal stomach image taken for the diagnosis of achalasia.

DISCUSSION

Achalasia is an esophageal motility disorder that results in the inability to relax and peristalsis in the lower esophagus sphincter due to the pathology in the inhibitory ganglion cells in the myenteric plexus [8,9]. The study aims to show the results of patients who underwent Heller myotomy and antireflux surgery due to achalasia.

There are options such as calcium channel blockers such as nifedipine, botulinum toxin injection, endoscopic balloon dilatation, and surgical myotomy

in the treatment of achalasia [3,5,6]. Although there is no consensus about which is the gold standard treatment, the treatment option with the highest success rate is Heller myotomy [8,9]. The short duration of calcium channel blocker and botulinum toxin injection effectiveness is seen as a disadvantage of pharmacological treatment [4,10,11]. In addition, although the short-term success rate in the treatment of achalasia is high, there are not enough studies in the literature regarding the optimal dose of botulinum toxin injection in pediatric patients [8]. Calcium channel blockers are not preferred in pediatric patients in the treatment of achalasia due to their side effects such as headache and dizziness [4].

Although successful results have been published for endoscopic balloon dilatation used in adult series treatment, there are not enough publications about the success rate in pediatric patients [11]. Besides, it is not recommended to be used for treatment purposes in children under 5 years of age because of the technical difficulties [7,11]. Surgical myotomy becomes a priority in the treatment of pediatric patients due to complications such as recurrence of symptoms within the first year after balloon dilatation and the risk of perforation at a rate of 4-12% [9,11,12]. In the first 4 patients in our series, endoscopic dilatation was applied primarily as a treatment. The recurrence of the patients' symptoms after the treatment led to myotomy as the first choice of treatment. Also, laparoscopic Heller myotomy is the preferred method in achalasia surgery because it gives early nutrition opportunity, the advantage of less analgesic need, it shortens the length of hospital stay, has better cosmetic results and shorter return to normal life [13].

GER is a common complication after Heller myotomy in patients with achalasia. In the literature while some authors argue that an antireflux surgery should generally be added to the Heller myotomy to prevent this complication, some authors argue that dysphagia will recur, and therefore antireflux surgery should not be added [4,8,14,15]. The antireflux surgery procedure was performed simultaneously with myotomy in the present study. Dysphagia presented in four patients, and it resolved with endoscopic balloon dilatation in one session. There were not postoperative GER findings in any patient. A partial antireflux procedure may respond these arguments since it may prevent reflux and also not cause dysphagia. Dor and Toupet fundoplications may both be performed but the authors' prefer Dor fundoplication in case of intraoperative esophageal perforations since it may prevent esophageal leakage. This suggestion may only be hypothetical since we did not encounter any esophageal perforations in our series.

Another controversial issue is that which fundoplication procedure will be added to Heller myotomy simultaneously [14]. Considering the literature, the most preferred surgical procedure has been reported as 360 ° total Nissen fundoplication, 270° Toupet posterior partial fundoplication, and 180° Dor anterior partial fundoplication [14]. Some authors argue that the presence of motility impairment and total Nissen fundoplication in achalasia patients will increase esophageal emptying more than partial fundoplications. Therefore, there will be a symptom of permanent dysphagia [14]. In their 10-year series published by Topart et al., it was reported that there were 82% recurrent dysphagia [16]. In the 5-year series published by Rebecchi et al., it was reported that the recurrent dysphagia symptom was higher in patients who underwent Nissen total fundoplication compared to patients who underwent Dor anterior partial fundoplication [17]. The advantages of toupet posterior partial fundoplication and Dor anterior partial fundoplication over each other have not been demonstrated in the literature [14]. Therefore, we think that surgeons should choose one of these procedures according to their experience and preference.

The study's limitation is that the two groups that underwent partial fundoplication were not statistically compared because of the small number of patients.

Heller myotomy is gold standard method in the treatment of achalasia in children. Partial fundoplication added to myotomy may reduce the risk of GER and dysphagia.

Ethics Committee Approval: Ankara University Ethics Committee approval was obtained (24.12.2020/682-20).

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Informed Consent: Informed consent was obtained from the patients family with regards they could be used in the scientific study before the operation.

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Research Article

Retrospective Analysis of Hashimoto's Thyroiditis in Children and Adolescents: A Single Center Experience

Selma Tunç
Behzat Özkan

Hashimoto Tiroiditinin Çocuklarda ve Ergenlerde Geriye Dönük Analizi: Tek Merkez Deneyimi

ABSTRACT

Objective: *Hashimoto's thyroiditis is an organ-specific autoimmune disease and the most common cause of goiter and acquired hypothyroidism in children and adolescents in regions devoid of endemic iodine deficiency. In this study, it was aimed to examine the epidemiological, clinical and laboratory features of Hashimoto's thyroiditis and autoimmune diseases accompanying Hashimoto's thyroiditis in children and adolescents.*
Methods: *We retrospectively examined thyrotropin, free thyroxin, thyroid autoantibodies (thyroid peroxidase and thyroglobulin antibodies), immunoglobulin A (IgA), anti-tissue transglutaminase antibodies (IgA-tTG), and thyroid ultrasonography findings of 108 cases aged 5-18 years with positive anti-thyroid antibodies.*
Results: *The female/male ratio was 80/28 and 68% of the patients were adolescents. The most common admission complaint was goiter. At the time of diagnosis, the cases had euthyroidism (44.4%), subclinical hypothyroidism (35%), overt hypothyroidism (16.6%), and hyperthyroidism (3.7%). Goiter was detected in 58 (53.7%) cases by thyroid ultrasonography. During the follow-up, overt hypothyroidism developed in 6 patients who had subclinical hypothyroidism and subclinical hypothyroidism developed in 8 patients who had euthyroidism. All patients with hyperthyroidism became euthyroid. Levothyroxine treatment was administered to 47 (43.5%) patients. Celiac disease was detected in 2 and type 1 diabetes mellitus in 1 patient.*
Conclusions: *The prevalence of Hashimoto's thyroiditis increases with age both in childhood and adolescence, and thyroid functions tend to deteriorate over time. Therefore, close follow-up and appropriate treatment are important. Although the prevalence of celiac disease is higher in children and adolescents with Hashimoto's thyroiditis compared to healthy children, the true prevalence of autoimmune diseases accompanying Hashimoto's thyroiditis will be revealed in studies to be conducted in larger patient populations.*

Keywords: *Hashimoto's thyroiditis, children and adolescents, thyroid function, goiter*

ÖZ

Amaç: *Hashimato tiroiditi, organa özgü otoimmun bir hastalık olup çocuk ve ergenlerde endemik iyot eksikliğinin bulunmadığı bölgelerde guatr ve kazanılmış hipotiroidinin en sık nedenidir. Bu çalışmada çocuk ve ergenlerde Hashimato tiroiditinin epidemiyolojik, klinik ve laboratuvar özellikleri ve en sık eşlik eden otoimmun hastalıkları irdelemek amaçlandı.*
Yöntem: *Tiroid otoantikorları (tiroid peroksidaz ve tiroglobulin antikorları) pozitif olan 5-18 yaş arası 108 olgunun tirotropin, serbest tiroksin, doku transglutaminaz Ig A antikoru (dTG-igA) ve tiroid ultrasonografi bulguları retrospektif olarak incelendi. Tiroid fonksiyon durumuna göre gruplandırılan olgular karşılaştırıldı.*
Bulgular: *Bu çalışmada olguların %68'i ergen ve kadın/erkek oranı 80/28 idi. En yaygın başvuru şikayeti guatr idi. Tanı anında vakaların %44,4'ünde ötiroidizm, %35'inde subklinik hipotiroidizm, %16,6'sında aşıkar hipotiroidizm ve yaklaşık %3,7'sinde hipertiroidizm saptandı. Olguların 58'inde (%53,7) tiroid ultrasonografi (US) ile guatr tespit edildi. Takip sırasında, ötiroidisi olan 8 hastada subklinik hipotiroidi ve subklinik hipotiroidisi olan 6 hastada aşıkar hipotiroidi gelişti. Hipertiroidili tüm hastalar takipte ötiroid hale geldi. Olguların %43,5'i (n=47) levotiroksin tedavisi alıyordu. Hashimato tiroiditli 2 hastada çölyak hastalığı, 1 hastada tip 1 diabetes mellitus eşlik ediyordu.*
Sonuç: *Çocukluk ve ergenlik döneminde Hashimato tiroiditi prevelansı yaşla birlikte artarken, tiroid fonksiyonları zamanla bozulma eğilimindedir. Bu nedenle bu hastalarda yakın takip ve uygun tedavi önemlidir. Hashimato tiroiditli çocuk ve ergenlerde çölyak hastalığı prevalansı sağlıklı çocuklara göre daha yüksek saptanmakla birlikte Hashimato tiroiditine eşlik eden otoimmün hastalıkların gerçek prevalansının belirlenmesi, daha geniş katılımlı çalışmalar ile mümkün olabilecektir.*

Anahtar kelimeler: *Hashimato tiroiditi, çocuk ve ergenler, tiroid fonksiyonu, guatr*

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INTRODUCTION

Hashimoto's thyroiditis is an organ-specific autoimmune disease associated with other autoimmune diseases. It is the most common cause of goiter and acquired hypothyroidism in children and adolescents in the regions where there is no endemic iodine deficiency [1]. Although the prevalence of Hashimoto's thyroiditis varies from region to region, it occurs in 3% of children aged 6-18 years. The female/male ratio is 2-9/1 in children and adolescents and 9/1 in adults. It is most common in the pediatric age group in early and middle adolescence and peaks in girls in the pubertal age group [2].

The etiology of Hashimoto's thyroiditis is multifactorial and occurs under the influence of environmental and genetic factors. The fact that it occurs in more than one member of the same family and is more common in monozygotic twins than dizygotic ones in twin studies points to the presence of genetic factors. In Hashimoto's thyroiditis, cellular and humoral responses play a role in the pathogenesis; lymphocytic infiltration of the thyroid gland progresses with fibrosis and leads to increased tissue hardness [3]. In the families of 23-46% of children and adolescents affected by Hashimoto's thyroiditis, history of either Hashimoto's thyroiditis or other autoimmune disease has been reported [1-2]. The most common clinical finding in Hashimoto's thyroiditis is goiter. Hashimoto's thyroiditis is the most common cause of acquired goiter in children and adolescents. Hashimoto's thyroiditis accounts for 40% of goiters found in adolescents. Affected children may be asymptomatic or present a wide variety of complaints. Thyroid function in Hashimoto's thyroiditis can range from euthyroidism to hypothyroidism or in rare instances to hyperthyroidism [4].

Thyroid is the most frequently affected organ in autoimmune diseases. Hashimoto's thyroiditis, an autoimmune disease, may accompany other autoimmune diseases such as alopecia, vitiligo, celiac disease, Addison's disease, type 1 diabetes and polyglandular syndromes [5].

The aim of this study is to evaluate clinical, epidemiological and laboratory findings in children and adolescents with Hashimoto's thyroiditis and to determine the prevalence of autoimmune diseases accompanying Hashimoto's thyroiditis.

MATERIAL and METHODS

A total of 108 patients aged 5-18 years who were followed up with the diagnosis of Hashimoto's thyroiditis in the pediatric endocrinology outpatient clinic from 2017 to 2020 were included in the study. The diagnosis of Hashimoto's thyroiditis was made with increased levels of anti-thyroglobulin (anti-TG) and/or anti-thyroid peroxidase (anti-TPO) autoantibodies. Age, complaints at presentation, family history, body weight, height, body mass index (BMI) and laboratory findings of all subjects were recorded retrospectively from the medical records. Body weight and height of all subjects were measured by the same physician (S.T.) using a digital scale (sensitive to 10 grams) (Densi GL 150) with automatic measurement of the height. Body mass index was calculated by dividing body weight by the square of height in meters. Body weight, height and BMI standard deviation scores (SDSs) were obtained using national database [6].

Thyroid stimulating hormone (TSH), free thyroxine (fT4), thyroid autoantibodies (anti-TG and anti-TPO) and findings of thyroid ultrasonography (US) performed considering autoimmune thyroiditis were recorded. The tissue transglutaminase IgA (dTG -A) and serum total IgA levels measured considering accompanying autoimmune diseases were recorded. Thyroid volume measured with thyroid US was calculated using the formula; {(R1 x R2 x R3 x 0.5) / 1000} + {(L1 x L2x L3 x 0.5) /1000}.

Note: R's and L's indicate right, and left thyroid lobes, from right to left side, respectively.

Values above the 97th percentile were accepted as goiter [7]. Serum fT4 and TSH levels were measured using the electro chemiluminescence method (Cobas 6000 Roche). Patients whose serum TSH and fT4 levels within normal reference values (fT4: 0.9-1.67 ng/dL, and TSH: 0.6-4.9 µIU/ml, respectively) were

accepted as euthyroidism. Elevated TSH, but decreaed fT4 were considered as hypothyroidism while elevated TSH but normal fT4 levels as subclinical hypothyroidism [8]. Patients with large goiter accompanied by either overt or subclinical hypothyroidism, or even normal thyroid hormones received levothyroxine treatment in case of globus and/or compression findings. Serum anti-TPO and anti-TG levels were measured using the electro chemiluminescence method (Cobas 6000 Roche). The normal reference range was considered as 0-13 IU/mL for anti-TPO and 0-38 IU/mL for anti-TG.

Serum dTG-IgA was studied by micro ELISA method and 0-10 RU/mL was considered the normal reference range. Serum IgA was studied by immunoturbidimetric method using AU5800 autoanalyzer. Normal reference intervals were determined by age and gender. The cases were divided into groups according to the thyroid functions at admission (euthyroidism, subclinical hypothyroidism, hypothyroidism, hyperthyroidism). Ethics committee approval was obtained for this study.

Statistical analysis

SPSS 21.0 package program was used for all statistical analysis. Kolmogorov- Smirnov and Shapiro-Wilk tests were used to determine the normal distribution. Descriptive statistics were used. Mean (± SD) values of continuous, and categorical variables were calculated. Student's t-test was applied for comparing two groups and Anova test was applied for comparing more than two groups. The chi-square test was used to compare percentages. A p value of <0.05 was considered statistically significant.

RESULTS

The study group consisted of 108 patients including 80 girls (74%) 28 boys (26%), of whom 68% were pubertal. Sixty-four patients (59%) had a family history of autoimmune thyroid disease. The mean age at diagnosis was 12.2±2.8 years. The mean height SDS was 0.39±0.98 and mean BMI SDS was 0.34±1.2. In our study, the most common complaint at presentation was swelling in the neck, followed by weight gain, weakness and fatigue, dry hair, muscle weakness and intolerance to cold. Fifteen percent of the cases were referred from other clinics due to impaired thyroid function tests with an accompanying goiter in 25% of them (n=4). The complaints of the cases at admission are summarized in Table 1.

Anti-TPO was positive in 95%, and anti-TG in 87% of the cases. At the time of diagnosis, the patients had euthyroidism (n:48; 44.4%) had, 38 (35%) subclinical hypothyroidism (n:38; 35%), overt hypothyroidism (n:18; 16.6%) and hyperthyroidism (n:4; 3.7%). Goiter was detected in 47 (43.5%) cases by physical examination, and in 58 (53.7%) cases by thyroid US. In 88% (n=95) of the cases, the thyroid parenchyma was heterogeneous due to fibrosis and

Table 1. Complaints of patients with Hashimoto's thyroiditis.

Complaints	n	%	Complaints	n	%
Swelling in the neck	49	43.5	Hair loss	16	15
Fatigue-weakness	42	39	Thyroid dysfunction	16	15
Increased weight	38	35	Loss of appetite	15	14
Dry hair	25	23	Decreased academic success	14	13
Muscle weakness	24	22	Constipation	13	12
Intolerance to cold	17	16	Nervousness	11	12
Dry skin	16	15	Easy nail splitting	9	8.3

Table 2. Comparison of the groups according to thyroid function status.

	Euthyroidism n=48	Subclinical hypothyroidism n=38	Overt hypothyroidism n=18	Hyperthyroidism n=4	p
Mean (±SD) age (years)	12.1±2.5	12.6±2.1	13.3±1.8	12.5±2.2	0.620
Sex(female/male)	38/10	30/8	14/4	3/1	0.340
Pubertal status (%)	67%	63%	64%	66%	0.165
Goiter (PE) (n)	21 (43.7%)	16 (42%)	8 (44.4%)	2 (50%)	0.324
Goiter (US)	26 (54%)	20 (52.6%)	10 (55.5%)	2 (50%)	0.850
anti-TPO (IU/mL)	114±91	482±123	774±226	561±109	0.018

PE: physical examination, US: ultrasonography.

hypoechogenic areas, and in 12% (n=13) of the cases it was homogeneous. Moreover, with US, pseudonodular appearance was detected in 90 (83%), and true nodules (13 solitary, 5 multinodules) in 18 (17%) cases. The patients with thyroid nodules had euthyroidism (n:10; 55.5%), subclinical hypothyroidism (n:5; 27.7%), and overt hypothyroidism (n:3; 16.6%). Thyroid receptor antibody (TRab) was negative in all patients with hyperthyroidism. The characteristics of the cases grouped according to thyroid functions are shown in Table 2. The mean age, gender and pubertal status of the cases were similar in both groups (p=0.620, p=0.340, and p=0.165, respectively). There was no statistical difference between the groups in terms of prevalence of goiter detected by both physical examination and thyroid US (p=0.324, and p=0.85, respectively). The patients were compared according to the presence of goiter detected by ultrasound (Table 3). There was no significant difference between the groups in terms of anthropometric, clinical and laboratory parameters according to the presence of goiter. Mean serum TPO-ab titer was higher in patients with overt/subclinical hypothyroidism and hyperthyroidism than in patients with euthyroidism (mean values: 774±226, 561±109, 482±123, and 114±91 IU/ml, ρ=0.018, respectively). After 3 years of follow-up, while 15.7% (n=6) of the patients who had subclinical hypothyroidism at presentation developed overt hypothyroidism, 8 patients with euthyroidism developed subclinical hypothyroidism. All patients with hyperthyroidism became euthyroid during follow-up. These patients are still being followed up. While 47 (43.5%) cases included in the study were receiving levothyroxine treatment, the mean levothyroxine dose to reach euthyroid state was 1.62±0.53 µg/kg/day. When other accompanying autoimmune diseases were evaluated, celiac disease was detected in two, and type 1 diabetes mellitus in one patient.

Table 3. Comparison of the groups according to the presence of goiter.

	Goiter (+) n=58	Goiter (-) n=50	p
Mean age (years)	12.8±2.6	11.9±2.4	0.323
Female/male (n)	36/22	30/20	0.816
BMI SDS	0.28±0.9	0.24±1.1	0.160
Pubertal / prepubertal (n)	33/25	28/22	0.620
Anti-TPO (IU/mL)	642±113	581±99	0.265
Thyroid function status (n)			
Euthyroidism	26	22	0.920
Subclinical Hypothyroidsm	20	18	0.625
Overt hypothyroidsm	10	8	0.720
Hyperthyroidism	2	2	-

BMI: Body mass index, SDS: standard deviation score, Anti-TPO: anti-thyroid peroxidase.

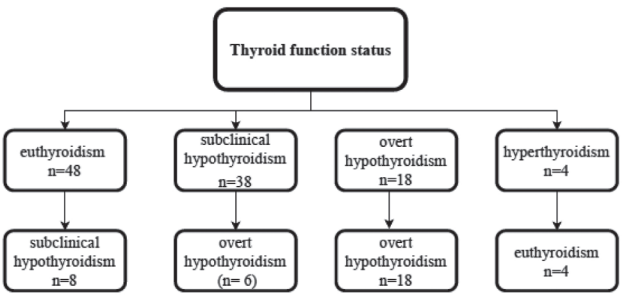

Figure. Thyroid functions of patients with Hashimoto thyroiditis at baseline and follow-up.

DISCUSSION

Hashimoto's thyroiditis is more common in women, and a female/male ratio varying between 2.1-9.7/1 in childhood and adolescence has been reported [9]. In our study, the female/male ratio was 2.8/1. Hashimoto's thyroiditis is more common in adolescence. In this study, 68% of the cases were between the ages of 12-15 years. In the literature, age peaks similar to our results have been reported [9-12]. Hashimoto's thyroiditis is very rare under 5 years of age. However, rare cases in early childhood have been reported and even in infancy [9-12]. In our study, there were no patients younger than 5 years of age. Euthyroidism is the most common thyroid pattern associated with Hashimoto's thyroiditis (20-80%). Dundar et al. [13] reported euthyroidism in 62.8% of children and adolescents with Hashimoto's thyroiditis, Özen et al. [14] and Tuhan et al. [15] reported euthyroidism in 51.2% and 36.7% of these cases, respectively.

Wasniewska et al. [16] found euthyroidism in 52.1%, SH in 19.2%, and overt hypothyroidism in 22.2% of the cases in a study involving 608 children

and adolescents with Hashimoto's thyroiditis. In our study, the most common thyroid pattern was euthyroidism (44.4%) followed by subclinical and overt hypothyroidism, and hyperthyroidism. The frequency of subclinical hypothyroidism in our study was 35%, which was similar to the rates reported in other studies (26.5%-39.4%) [11,17-20].

It has been reported that thyroid functions in children with Hashimoto's thyroiditis are mainly associated with the age of the cases and the possibility of subclinical and overt hypothyroidism increases with age [9]. Contrarily, it has been reported in the literature that patients with thyroid dysfunction are younger, and this condition has been explained with the view that early onset HT could have a worse prognosis [1,15,16]. However, in our study, no significant difference was found between age and thyroid functions which may be due to the small number of cases in our study population. Initially, thyroid function of the children with Hashimoto's thyroiditis presenting with both euthyroidism and SH tend to gradually worsen [9]. In addition, children and adolescents with SH and underlying Hashimoto's thyroiditis have a higher risk of developing hypothyroidism over time compared to children and adolescents with SH without preexisting thyroid disease [9]. In this study, 15.7% of the patients with SH developed overt hypothyroidism over time during follow-up, while SH developed in 8 cases with euthyroidism.

Although a specific genetic transition has not been identified in Hashimoto's thyroiditis, there is strong evidence that it has a familial inheritance. It has been reported that thyroid antibodies were found to be positive in the first-degree relatives in about half of the cases, and therefore autosomal dominant inheritance was considered. Tuhan et al. [15] reported that the history of thyroid disease in the first degree relatives of cases with Hashimoto's thyroiditis had been revealed in 47.5% of the cases, Wasniewska et al. [16] reported its incidence as 31.6%. In our study, a family history of thyroid disease was detected in 59% of the cases. This result is similar to the rates in the literature and has been evaluated as a finding supporting genetic predisposition for Hashimoto's thyroiditis.

In the literature, it has been stated that the most common complaint of the cases is neck swelling [4,14,21-23]. In a study conducted by Demirbilek et al. [9], 54.9% of the cases with a diagnosis of Hashimoto's thyroiditis had asymptomatic goiter and in a study conducted by Matsuura et al. [24] goiter was detected in 71% of the cases. In this study, asymptomatic goiter was the most common complaint in 45.3% of the cases which was consistent with the literature.

Hashimoto's thyroiditis is the most common cause of acquired hypothyroidism in children and adolescents. Its incidence varies between 13-52.4% in different series [17,19,20,25]. The frequency of overt hypothyroidism in our study was 16.6%. In studies on children with a diagnosis of Hashimoto's thyroiditis, Alos et al. [11] reported overt hypothyroidism in 21.2% and Tuhan et al. [15] in 17.5% of their cases. The differences in the frequency of hypothyroidism reported in the literature were explained by dietary iodine intake, female/male ratio, average age of the patients, diagnostic criteria, and the time elapsed between the onset of the disease and its diagnosis. Increasing iodine intake with diet, using higher TSH threshold levels for the diagnosis of the disease, and delay in diagnosis contribute to the increase in the estimated frequency of hypothyroidism [9].

Hyperthyroidism can also be seen at presentation and it is usually mild. However, some patients may present with thyrotoxicosis (Hashitoxicosis). In this study, hyperthyroidism was detected in 4 cases (3.7%). This rate was slightly lower than the previous reports (7.8-11.7%) [9,19,20]. This situation may be due to the small number of cases in our study population.

In the literature, it has been reported that patients with thyroid dysfunction are younger [1,15,16]. The younger age of the patients with thyroid dysfunction has been explained with the view that early-onset Hashimoto's thyroiditis may have a more severe course [16]. However, when the cases in our study were compared according to thyroid function status, there was no difference between the groups in terms of age. Again, in this study, there was no significant difference between the groups in terms of

gender, adolescence and the presence of goiter. Consistent with our study, Tuhan et al. [15] found that there was no difference in terms of gender, adolescence and presence of goiter between the groups that were compared according to thyroid function status. Dündar et al. [13] reported a higher rate of goiter in euthyroid cases.

In the literature, rates of thyroid nodules up to 34.4% have been reported in cases with Hashimoto's thyroiditis [13,15,26]. Dündar et al. [13] reported thyroid nodules in 7% of children and adolescents with Hashimoto's thyroiditis and Tuhan et al. [15] found thyroid nodules in 7.5% of their patients. In our study, true nodules (13 solitary, 5 multinodules) were detected in 17% of the cases. The patients with thyroid nodules had euthyroidism in 55.5%, SH in 27.7% and overt hypothyroidism in 16.6% of the cases.

In children with Hashimoto's thyroiditis, L-thyroxine treatment is given to the subjects with overt and subclinical hypothyroidism. L-thyroxine treatment is also recommended in euthyroid subjects who have large goiter leading to compression and/or tenderness [23,26]. In the literature, the rate of using L-thyroxine in cases with Hashimoto's thyroiditis varies between 40-70% [13,26-28]. In our study, 43.5% of the cases were receiving levothyroxine treatment, while the average L-thyroxine dose to reach euthyroid state was calculated as 1.62±0.53 µg/kg/day in accordance with the literature [14].

As an autoimmune disease Hashimoto's thyroiditis is frequently associated with other autoimmune diseases. In our study, 2 patients had celiac disease and 1 patient had type 1 diabetes. In studies conducted with adults, the prevalence of celiac disease in patients with autoimmune thyroid disease was reported to be 1.8-3.3% [29-31]. In children and adolescents with Hashimoto's thyroiditis, the frequency of celiac disease is between 1.3-6.5% [32,33]. Studies should be conducted with greater number of patients. In studies conducted with children and adolescents diagnosed with Hashimoto's thyroiditis, Sarı et al. [32] found celiac disease in 4.9% of 101, while Tuhan et al. [15] in only one (1.25%) of 80 cases. In another study, Sattar et al. [33], found dTG-IgA positivity in 14 (4.6%) of 302 children and adolescents with Hashimoto's thyroiditis, and celiac disease was diagnosed by small intestine biopsy in half of these cases (2.3%).

As a result, Hashimoto's thyroiditis is more common in women, and its incidence increases with age during childhood and adolescence. Moreover, thyroid functions tend to deteriorate over time. Therefore, close follow-up and appropriate treatment are important. Although the prevalence of celiac disease in children and adolescents with Hashimoto's thyroiditis is higher than in healthy children, the true prevalence of autoimmune diseases accompanying Hashimoto's thyroiditis will be revealed by studies to be conducted in a larger patient population.

Ethics Committee Approval: S.B.U. Gazi Yaşargil Training and Research Hospital Clinical Research Ethics Committee approval was obtained (05.03.2021/693).

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Research Article

Molecular Epidemiology and Clinical Risk Factors of Rotavirus Diarrhea: Single Center 5-Year Experience

Rotavirüs İshalinin Moleküler Epidemiyolojisi ve Klinik Risk Faktörleri: 5 Yıllık Tek Merkez Deneyim

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ABSTRACT

Objective: *Acute gastroenteritis due to the rotavirus is one of the common causes of morbidity and mortality in children under five years of age. The objective of this study was to evaluate the epidemiological, clinical, and laboratory data of rotavirus diarrhea in hospitalized children under five years of age.*
Methods: *All children between one month and 60 months old ages who were hospitalized in Health Sciences University Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital during September 2013 to August 2017 and diagnosed as acute gastroenteritis with rotavirus antigen test positive in feces were included in the current study. Data of the patients were collected retrospectively from medical records.*
Results: *A total of 100 patients were evaluated.The median age of the patients was 13 months (IQR 5-45 months) and 54 patients were male and 46 were female.The highest hospitalization rate was in December, followed by November, and in October. The most common type of G9P (8) serotype was detected in rotavirus serotype analysis by PCR. Breastfeeding infants had milder clinic findings in comparison to the older ones. It was found that clinical findings were milder and Vesicari score was lower in infants who had breast milk. Vesikari score was found to be high in children with severe clinical findings.*
Conclusion: *Rotavirus infection is important for all ages.In case of fever, increased numbers of vomiting and diarrhea, and higher Vesikari system scores may be associated with the severe clinical forms. Determination of rotavirus serotypes and clinical monitoring of genotypic changes are required.*

Keywords: *Gastroenteritis, rotavirus, rotavirus serotype, Vesikari score*

ÖZ

Amaç: *Rotavirüs gastroenteriti tüm dünyada, beş yaş altı çocuklarda önemli morbidite ve mortalite nedenidir. Bu çalışma ile rotavirüs ishali nedeni ile hastaneye yatan beş yaş altı çocukların epidemiyolojik özelliklerinin, klinik ve laboratuvar verilerinin değerlendirilmesi amaçlandı.*
Yöntem: *Sağlık Bilimleri Üniversitesi Dr. Behçet Uz Çocuk Hastalıkları ve Cerrahisi Eğitim ve Araştırma Hastanesi'ne Eylül 2013 - Ağustos 2017 tarihleri arasında akut ishal nedeni ile yatırılan ve dışkısında rotavirüs antijeni pozitif saptanan 1 ay - 60 ay aralığındaki hastalar çalışmaya dahil edildi. Hastaların verileri, geriye dönük olarak "hasta yatış dosyaları" ve "hastane elektronik bilgi sistemi" kullanılarak elde edildi.*
Bulgular: *Çalışmada toplam 100 hasta değerlendirildi. Medyan yaş 13 ay (IQR 5-45 ay) ve 54'ü erkek, 46'sı kızdı. En sık yatış Aralık ayında yapılmış, bunu Kasım ve Ekim ayları takip etmiştir. PCR ile bakılan rotavirüs serotip analizinde en sık G9P(8) serotipi saptandı. Anne sütü alan bebeklerde klinik bulguların daha hafif olduğu ve vesikari skorunun daha düşük olduğu saptandı. Ağır klinik bulguları olan çocuklarda da Vesikari skorunun yüksek olduğu bulundu.*
Sonuç: *Rotavirüs enfeksiyonu,anne sütü alan bebeklerde daha hafif klinik bulgulara neden olmaktadır. Başvuru sırasında Vesikari skoru ciddi hastalığı ön görmede etkili olabilir. Rotavirüs serotiplerinin belirlenmesi ve genotipik değişimlerin klinik olarak izlenmesi gereklidir.*

Anahtar kelimeler: *Gastroenterit, rotavirüs, rotavirüs serotip, Vesikari skoru*

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INTRODUCTION

Rotavirus associated with diarrhea is the most common cause of mortality and morbidity in children under 5 years of age worldwide. In United States, there are 55,000-70,000 hospitalizations each year due to rotavirus diarrhea [1,2]. In a report published between 2000 and 2013 on rotavirus, it was stated that the number of deaths with rotavirus gastroenteritis under the age of 5 was 529,000, 215.000 in 2013 [1]. By the studies conducted in different countries, it has been reported that rotavirus is the agent in 10-70% of viral gastroenteritis cases [2].

Rotavirus is a double-stranded ribonucleic acid (RNA) virus and divided into 7 serogroups from A to G (9). The most common rotavirus types in the world are G1P[8], G3P[8], G4P[8] and G2P[4], and G9P[8]. The frequency of these five strains makes up more than 90% of the strains compared to the other strains. The dominant serotypes vary from year to year and from region to region [11].

Rotavirus infections are more common in winter and spring in studies [3]. Sudden-onset watery diarrhea is followed by vomiting, which usually lasts 1-3 days. Diarrhea, which can occur 10-20 times a day, can last 5-7 days. It can cause severe dehydration, especially in children younger than 2 years old. It has been reported that the severity and duration of the disease are low in children fed with breast milk [5].

Altough the rotavirus associated diarhea has a usually self-limiting milder course, it may cause severe syptoms aspecially in infants. A scoring system was developed to determine the course of the disease and the treatment approach. Parameters used in clinical scoring; The number of daily diarrhea, the number of daily vomiting, the number of days for vomiting, the highest degree of fever, the degree of dehydration, and treatment requirements are included [7].

In this study, the clinical, laboratory, and epidemiological characteristics of children under the age of five who were hospitalized for rotavirus gastroenteritis between 2013-2017 were examined.

MATERIAL and METHODS

Patients between the ages of 1 month and 60 months who were hospitalized in the **** Hospital due to acute diarrhea between September 2013 and May 2017 and who were found to have positive rotavirus antigen in their stool were included in the study. The study was initiated after it was approved by the Ethics Committee of our hospital (2017/17-06).

The data of the patients were recorded retrospectively in the case report form using “patient admission files” and “hospital electronic information system”. Patients whose diarrhea complaint lasted longer than 14 days were accepted as “prolonged diarrhea” and excluded from the study. Besides, patients over the age of five and under onemonth, patients with chronic diseases associated with the gastrointestinal system, immune system disorders, and patients whose rotavirus antigen test was found to be positive, were not included in the study.

A qualitative immunochromatographic assay test (Ameritek Inc, Everett, WA, USA), which is a rapid antigen test, was used to detect rotavirus antigen in stool. This examination was carried out in the microbiology laboratory of our hospital following the test procedure recommended by the manufacturer. After the stool sample was treated with 1 mL of buffer solution, approximately 150 microL of the mixture was dropped onto the strip in the kit. Waiting for 10 minutes, the reaction of the liquid was evaluated. The sensitivity of the test was 98,9% and the specificity was 99,6% [13].

The age, gender, number of days of hospitalization, month and season of hospitalization, breastfeeding history, previous antibiotic use, and complications developed during the follow-up of the patients with positive rotavirus antigen in stool samples were recorded. Vital signs, body weight, and dehydration degrees of the patients at admission were also recorded. Dehydration due to rotavirus gastroenteritis is classified as mild, moderate and severe [14].

The rotavirus diarrhea clinical severity score was applied to all patients. Clinical findings were scored between “0-20 points”. Parameters used in clinical

scoring; It includes the number of daily diarrhea, the number of daily vomiting, how many days it has been vomiting, the highest fever, the degree of dehydration, and treatment requirements (Table 1).

According to this scoring system, those who scored <7 points were considered mild, 7-10 points moderate, and 11 points as severe disease. The patients were divided into two groups according to their breastfeeding and not breastfeeding at the time of admission and compared in terms of Vesikari score, presence of vomiting, duration of diarrhea, length of stay, presence of fever, and dehydration.

Within the framework of the Central Laboratory surveillance program; The rotavirus genotypes were determined by sending stool samples of rotavirus positive patients. The frequency distribution of genotyped patients was made according to months and seasons. Statistical analysis of the data obtained as a result of the study was performed using the SPSS 24 (Statistical Package for the Social Sciences, Chicago, IL, USA) program. Chi-square test, Mann Whitney-U test, and median tests were used for demographic data. The Kruskal Wallis test (ANOVA) was used to evaluate whether there was any difference between the determined rotavirus genotypes in terms of clinical score, length of stay, season distribution, and duration of diarrhea. Average ages were given in months. Results are given as n (%) or mean±standard deviation (SD) (lower-upper value). p<0,05 was considered statistically significant.

Table 1. The rotavirus diarrhea clinical severity score.

	Vesikari Score		
Parameters	**1**	**2**	**3**
Maximum Number Stools per Day	1-3	4-5	≥6
Diarrhea Duration (Days)	1-4	5	≥6
Maximum Number Vomiting Episodes per Day	1-2	2-4	≥5
Vomiting Duration (Days)	1	2	≥3
Temperature (C^0)	37.1-38.4	38.5-38.9	≥39
Dehydration	Normal or light	1-5%	≥6%
Treatment	Rehydration	Hospitalization	Dehydration despite treatment

RESULTS

A total of 100 patients were evaluated in the study. Of these, 54 (54%) were male and 46 (46%) were female. The median age of the patients was 13 months (IQR 5-45 months). When the distribution of patients hospitalized due to rotavirus gastroenteritis by months was examined, it was observed that the most frequent hospitalizations were made in

December (n=17, 17%), followed by November

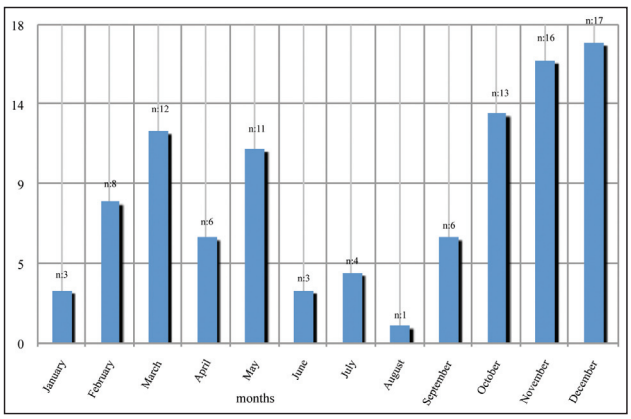

Figure 1. Distribution of patients hospitalized due to rotavirus gastroenteritis by months.

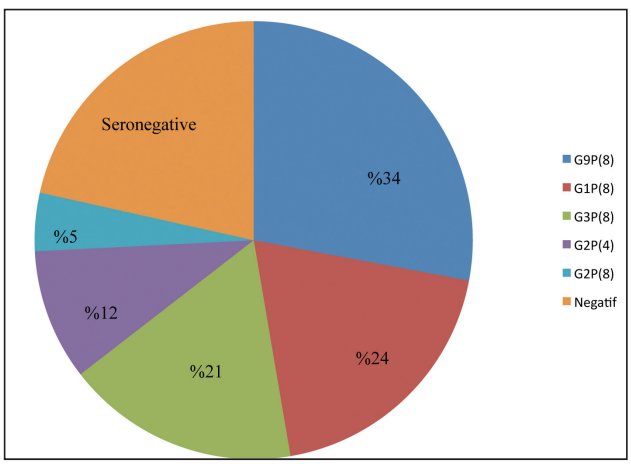

Figure 2. Distribution of PCR-detected serotypes in patients with positive rotavirus antigen .

(n=16, 16%) and October (n=13, 13%). Distrubition of the patients were shown in Figure 1.

Most frequently detected serotypes were G9P (n=26, 34%), G1P (n=18, 30%), and G3P (8) (n=16, 24%). In 20 (20%) patients, although rotavirus antigen was found to be positive in the stool, no serotype was detected by RT-PCR. Serotypes detected by PCR are shown in Figure 2. There was no statistically differences between the detected serotypes and clinical findings, duration of symptoms, and severity of the course (p>0.05).

When the patients who took breast milk and those who did not were compared, no statistically significant difference was found in terms of the presence of vomiting (p=0.069), duration of diarrhea (p=0.143), or length of stay (p=0.417). However, it was found that the frequency of fever was higher and the degree of dehydration was more severe in those who did not breastfeed (p=0.038 and p=0.025, respectively). It was found that among inpatients with positive rotavirus, Vesikari scores at the time of admission were higher than those who received breast milk, and there was a statistically significant difference (p=0.001).

It was found that among inpatients with positive rotavirus, Vesikari scores at the time of admission were higher than those who received breast milk, and there was a statistically significant difference (p=0.001).

Vesikari scores of patients admitted to the intensive care unit; It was statistically higher than patients who were not hospitalized in intensive care (p=0.001). There was no statistically significant difference in patients in the mild and moderate disease groups according to the vesikari classification.

Table 2. The degree of dehydration and Vesikari skore according to their breast milk intake.

Dehydration	Breastfeeding (n=41)	Not Breastfeeding (n=59)	P-value
Mild dehydration n (%)	27 (65.8%)	29 (49.2%)	0.025
Moderate dehydration n (%)	14 (34.2%)	22 (37.2%)	
Severe dehydration n (%)	0 (0.0%)	8 (13.6%)	
Vesikari skore mean (±sd)	8.4±2.0	9.9±2.1	0.001

DISCUSSION

Acute gastroenteritis is one of the most important causes of mortality and morbidity in the world, especially in developing countries. In prevalence studies conducted in different parts of the world, the frequency of rotavirus varies and it is reported that rotavirus is the agent in 10-70% of viral gastroenteritis cases [1,2,9,15,19]. In previous studies, it has been reported that rotavirus diarrhea is most common in children between the ages of 6 and 24 months, and it peaks at 9-12 months [20]. The median age of the patients in our study was 13 months. Although these values are similar to the literature, 69% of the patients were children older than 24 months. These results can only be due to the evaluation of inpatients. Indeed, in a large-scale study in which rotavirus surveillance in our country was tried to be determined, it was shown that rotavirus gastroenteritis was more common in children aged 13 to 24 months, and this age group was followed by children between 25 and 36 months [21]. It has been reported that rotavirus infection shifts to older ages, especially in developed countries [20,21].

It is thought to be due to the increase in the average age, virus genotype changes, and the provision of hygiene conditions. Rotavirus is more common in cold seasons. The frequency of rotavirus in America and Europe increases in the period of December-March, while in Africa, the frequency of rotavirus was higher in dry seasons [22]. In our country, it has been reported that rotavirus gastroenteritis cases are frequently seen between September and May [23,24]. In our study, it was observed that hospitalizations due to rotavirus gastroenteritis were more frequent in November and December, and seasonally, most frequently in autumn.

Rotavirus can cause serious and life-threatening dehydration accompanied by vomiting [25]. Fluid electrolyte loss, metabolic acidosis, nutritional deficiency, malnutrition, and dermatitis seen in acute gastroenteritis are also common in rotavirus infection. It may cause severe dehydration, especially in children younger than 2 years old [25,26]. In a study

conducted in Africa, dehydration was reported to be more severe in the rotavirus positive group [27]. In our study, mild dehydration was found in 28%, moderate dehydration in 64%, and severe dehydration in 8% of the patients hospitalized due to rotavirus gastroenteritis. It was noted that 19 (19%) of the patients in our study received antibiotic treatment during their hospitalization, and 17 of these patients started treatment with a pre-diagnosis of respiratory tract infection. Similarly, in a study conducted in Mexico, it was reported that antibiotics were used at a rate of 17.6% in patients with rotavirus positive gastroenteritis [28]. Apart from fever and symptoms related to the gastrointestinal system, it can also lead to respiratory tract-related symptoms associated with rotavirus infection. Studies are reporting that 30-50% of the cases have respiratory tract symptoms, cough and nasal discharge are common [29,30].

It is a matter of debate whether this is due to the factor or to the viruses that affect the accompanying respiratory tract since the time of its occurrence is mostly in the winter months. Diagnosis of rotavirus gastroenteritis is generally indistinguishable from other gastroenteritis by physical examination and clinical findings. Since the treatment of rotavirus gastroenteritis is hydration and supportive therapy like other viral gastroenteritis, specific microbiological diagnosis is not necessary in most cases. However, in the case of prolonged diarrhea, patients with the suppressed immune system, severe chronic diseases, and in surveillance studies, rotavirus can be examined as an etiological agent. Definitive diagnosis may also prevent the use of unnecessary and potentially harmful antibiotics in rotavirus gastroenteritis [31]. It is known that most physicians cannot determine whether there is rotavirus infection at the beginning of the clinic, and therefore, antibiotic treatment is started considering other diagnoses [32]. Many scoring systems have been established to determine the course and treatment approach of acute gastroenteritis. The most used scoring is Vesikari scoring [7]. In a cohort study, Vesikari score was reported to be related in terms of dehydration and treatment duration [33]. In our study, the median value of the Vesikari score of the patients was determined to be 9,2. According to the degree of Vesikari, 35% of the cases were evaluated as mild, 57% as moderate, and 8% as a serious disease. Within the framework of different studies, it was observed that Vesikari scoring was more significant in outpatients [34]. In our study, the median value of Vesikari score of 7 (7%) patients hospitalized in the intensive care unit was 14 points, and we found statistically significantly higher. Therefore, it has been observed that the Vesikari score can also be used in severely ill patients.

Monitoring of serotypes is important in terms of the development of rotavirus infection and vaccine studies. Rotavirus serotypes differ by region. The most common serotypes in acute gastroenteritis caused by group A rotaviruses in the world are G1P(8), G2P(4), G3P(8), G4P(8), G9P(8) [35]. In a study conducted in our country in 2014, the most common G9 genotype (48%) was found, followed by G1 (25.9%), G2 (16.2%) and G3 (4.3%) [21]. In another study conducted between 2014 and 2016, 1396 RT-PCR detected the most common G1 (28.3%), followed by G3 (21%), G9 (18.8%), G2 (16.3%) [36]. While the prevalence of G9P(8) serotype was 2-10% in prevalence studies conducted in India between 2003 and 2007, it reached 40% in the prevalence studies conducted in 2013 [37]. In our study, we found G9P(8) with a frequency of 32,5% [26] as the most common serotype in patients. Then, 22,5% [18] G1P(8), 20% [16] G3P(8) followed. The types of virus serotypes in terms of frequency vary from country to country and from year to year. The most common serotype G9P(8) in our study is increasing in epidemiological studies conducted worldwide. It is the most common serotype in many studies. These findings support our study. It is necessary to determine virus serotypes and to monitor genotypic changes. Besides, it is important for which serotypes in the country for the use of the vaccine to be developed. The relationship between rotavirus serotypes in terms of the severity of the infection has not been established. In a study conducted in India, Vesikari scoring of G9 serotypes was found higher than G1 serotypes [38]. In another study, it was reported that the G2P(4) serotype caused a more severe clinical picture [39]. In our study,

we performed Vesikari scoring on our patients to investigate whether there is a difference in clinical severity of rotavirus serotypes. We found no significant difference in terms of the clinical severity of the disease.

Rotavirus, outside the intestine; has been detected in extraintestinal tissues such as the liver, heart, lung, and central nervous system. In our study, hypovolemic shock in 2 (2%) patients, acute renal failure secondary to dehydration in 3 (3%) patients, septic shock in 2 (2%), metabolic acidosis in 6 (6%) patients, increased liver function test in 5 (5%) patients, 4 (4%) patients had hyponatremia, 6 (6%) patients had hypernatremia, and 19 (19%) patients had respiratory tract infection. 7 (7%) patients were followed up in the intensive care unit.

Vesikari score was higher in patients with severe disease. Therefore, we determined that the vesikari score could be used in severe patients in terms of predicting the severity of the disease.The main principle in treatment is to replace lost fluid and electrolytes. There is no specific antiviral therapy. Rotavirus infections in infants younger than 4-6 months are often asymptomatic due to the protective effect of maternal antibodies. It is symptomatic in only 10-20% of cases and is usually mild. However, serious infections may be seen in premature babies. It has been reported that breastfeeding reduces the risk of rotavirus gastroenteritis [44]. We found that the frequency of fever and dehydration was more severe in patients with rotavirus gastroenteritis compared to patients who did not take breast milk. Besides, we found higher Vesikari scores in patients who did not receive breast milk. It has been observed that breastfeeding is protective in rotavirus gastroenteritis and the clinical course is milder, and breast milk intake should be encouraged in patients who can be fed orally in rotavirus diarrhea. The major limitation of the study was that it has been planned retrospectively. Since the data in our study were analyzed retrospectively, instant clinical data and outcomes were not included.

CONCLUSION

Our study shows the 5-year surveillance of rotavirus genotypes and is also important in terms of including the broad clinical features of rotavirus diarrhea.

Ethics Committee Approval: S.B.U. Izmir Dr. Behçet Uz Pediatrics and Surgery Training and Research Hospital Clinical Research Ethics Committee approval was obtained (2017/17-06).
Conflict of Interest: The authors declared that there were no conflicts of interest.
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Informed Consent: Since our study was retrospective, consent was not obtained from the patients.

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Research Article

Does Block Anesthesia Used in Hypospadias Surgery Increase Complication Rates?

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Hipospadias Ameliyatında Kullanılan Blok Anestezisi Komplikasyon Oranlarını Arttırıyor mu?

ABSTRACT

Objective: *Caudal and penile block are two commonly used methods for providing analgesia during and after hypospadias surgery. Although caudal block is a more popular method than penile block, it is thought to contribute to venous congestion and thus to poor healing and fistula formation at the suture line. In our study, we compared postoperative complication rates in patients operated for distal hypospadias with penile or caudal block.*
Methods: *The records of 44 patients who underwent distal hypospadias surgery at pediatric surgery clinic of Okmeydani Trainig and Research Hospital between 2013 and 2018 were retrospectively reviewed. Patients were divided into two groups as caudal block and penile block groups. Patients were evaluated for meatal stenosis and urethral fistula development.*
Results: *There were 26 patients in the penile block gruop and 18 patients in caudal block grop. Mean age was 2.3 in the penile block group and 3.5 in the caudal block group. In penile block grop, fistula was observed in 2 patients (7.6%) and meatal stenosis in 4 patients (15.3%). In caudal block group fistula was observed in 1 patient (5.5%) and meatal stenosis was observed in 2 patients (11.1%). There was no statistically significant difference between the two groups.*
Conclusion: *Both penile and caudal block are commonly used in hypospadias surgery for providing analgesia. In the literature, there are studies showing that the caudal block contributes to urethral fistula formation. In our study, there was no significant difference in the complication rates between the two groups.*

Keywords: *Hypospadias, regional anesthesia, complications*

ÖZ

Amaç: *Kaudal ve penil blok hipospadias cerrahisi sırasında ve sonrasında analjezi sağlanması için sık kullanılan iki yöntemdir. Kaudal blok penil bloğa göre daha popüler bir yöntem olmasına rağmen venöz konjesyona ve bu sebeple dikiş hattında kötü iyileşme ve fistül oluşumuna katkısı olduğu düşünülmektedir. Çalışmamızda penil ve kaudal blok uygulanan distal hipospadiaslı hastalarda postoperatif komplikasyon oranlarını karşılaştırdık.*
Yöntem: *2013-2018 yılları arasında Okmeydanı Eğitim Araştırma Hastanesi Çocuk Cerrahisi kliniğinde distal hipospadias tanılı 44 hastanın dosyaları geriye dönük olarak incelendi. Hastalar kaudal blok ve penil blok uygulananlar olarak iki gruba ayrıldı. Hastalar meatal stenoz ve üretrakutanöz fistül gelişimi açısından değerlendirildi.*
Bulgular: *Penil blok uygulanan 26, kaudal blok uygulanan 18 hasta mevcuttu. Penil blok grubunda yaş ortalaması 2.3, kaudal blok grubunda 3.5 olarak saptandı. Hastaların ameliyat sonrası 7. gününde üretral kateteri çekildi. Penil blok uygulanan hastaların 2' sinde fistül (%7,6), 4'ünde meatal stenoz (%15,3) gözlendi. Kaudal blok uygulanan hastaların 1'inde fistül (%5,5), 2'sinde meatal stenoz (%11,1) gözlendi. İki grup arasında istatiksel olarak anlamlı bir fark saptanmadı.*
Sonuç: *Kaudal ve penil blok hipospadias cerrahisinde analjezi sağlanması için sık kullan iki yöntemdir. Literatürde kaudal bloğun üretrakutanöz fistül oluşumunda katkısı olduğunu gösteren çaışmalar mevcut olsada, bizim çalışmamızda kaudal ve penil blok uygulanan hastalarda komplikasyon oranları açısından anlamlı fark saptanmamıştır.*

Anahtar kelimeler: *Hipospadias, rejyonel anestezi, komplikasyonlar*

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INTRODUCTION

Caudal-epidural and penile block are the most commonly methods used for intraoperative and postoperative pain control in hypospadias surgery. Recent studies reported that penile block has similar efficacy to that of caudal-epidural block. Dorsal penile block is associated with relatively rare occurrence of serious adverse effects in penile surgery [1,2]. The landmark-based technique was first described by Bateman in 1972 [3]. Its most common adverse effect was reported as block failure and minimal redness and bleeding at the injection site [4]. Penile block not only provides faster and long-lasting pain control but is also associated with lower complication rates, as reported by larger scale studies [5].

The other reliable and efficacious anesthetic method for penile surgery, the caudal-epidural block, is comparably less frequently used due to its lower efficacy in older children. Vasodilation and venous congestion induced by sympathetic blockade secondary to caudal anesthesia in penile region poses a limitation for bleeding control during the surgery. In addition, Saavedra-Belaunde et al. [6] recently reported that caudal anesthesia was associated with a higher risk of fistula formation after distal hypospadias repair.

In this study, we aimed to compare complication rates in patients who underwent caudal-epidural block and penile block for hypospadias surgery.

MATERIAL and METHODS

After approval by local ethics committee, we retrospectively reviewed medical records of 81 patients with a diagnosis of distal hypospadias followed in pediatric surgery department of Okmeydanı Training and Research Hospital between October 2013 and April 2021. Patients were divided into two groups as those who were applied penile or caudal-epidural block.

All patients were to be operated by the pediatric surgeons and pediatric urologist of the same department using the same techniques and materials. No patients had received premedication except prophylactic antibiotherapy with ampicillin at a single dose of 50 mg/kg one hour before the surgery. All cases were monitored with electrocardiogram, noninvasive blood pressure measurement, peripheral oxygen pressure, and end-tidal carbon dioxide pressure. Laryngeal mask airway was placed after anesthesia induction with propofol 3-5 mg/kg or sevoflurane 8%. Once the position of the mask was confirmed, dorsal penile block and caudal block were applied with bupivacaine 0.25% at a dose of 3-5 ml (at a maximal dose of 0.5 mg/kg) and at a dose of 0.5 ml/kg in penile and caudal block groups, respectively. Anesthesia was maintained with 66% nitrous oxide, 1 MAC isoflurane, and 33% oxygen under spontaneous respiration. The cases where mean arterial pressure was increased or a >15% increment in heart rate were considered as block failure, which prompted bolus administration of fentanyl 1 mcg/kg. Pre- and post-block heart rate, mean blood pressure, and end-tidal carbon dioxide pressure were continuously monitored and recorded. This monitoring was maintained till 30 minutes after transfer to the recovery room.

Postoperative analgesia was maintained with 10 mg/kg intravenous paracetamol administered at every eight hours. The patients were discharged on postoperative day 0 and 1. On the 7th postoperative day, the dressing was opened and the catheter was withdrawn. The patients were followed up for 6-18 months postoperatively. Those who developed meatal stenosis and fistulae were recorded.

Statistical Analysis

Statistical analysis was performed via IBM SPSS 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). Descriptive data were expressed as numbers and percentages for categorical variables and as the mean, standard deviation, minimum, and maximum values for continuous variables. The comparison of continuous variables between the two groups was made using Mann- Whitney U test as the normality test showed non-normal distribution. Categorical variables were compared with chi-square test and Fisher's exact test. Statistical significance was set as "$p<0.05$"

RESULTS

The mean age of study population was 3.4±3.5 years (range: 6 months-16 years). Forty-six patients had received caudal block (56.79%), and the remaining 35 patients (43.20%) penile block. The mean age of patients in caudal block group (3.9±3.8 years, range: 6 months- 8 years) was higher than that in penile block group (2.7±3.1 years, range: 1-16 years), (p=0.045).

The patients who developed urethral fistulae had undergone fistula repair at month 6 of follow-up. Most of the cases (n=72, 88.9%) had undergone TIPU (Tubularized Incised Plate Urethroplasty) The remaining nine cases (11.1%) underwent MAGPI (Urethral (meatal) Advancement and Glanuloplasty). No local adrenaline was administered intraoperatively. Chordee assessment was performed via artificial erection for each patient. Chordee was detected in eight patients with five cases in caudal block and three cases in penile block of the study groups , and chordee correction was performed in these patients. While urethral repair was performed through meatal advancement and glanuloplasty (MAGPI) in fourteen patients who had glanular meatus, those with coronal, subcoronal or midpenile meatus or megameatus had undergone tubularized incised plate urethroplasty (TIPU). During postoperative follow-up, ten patients (12.3%) developed meatal stenosis and two cases (2.5%) had fistula. Dorsal meatotomy was performed after six months if routine dilation proved no benefit in patients with meatal stenosis. Those developing fistula underwent fistula repair with meatotomy provided that meatal stenosis

Table 1. Postoperative clinical characteristics of the study groups.

	Anesthesia	Anesthesia		
	Mean±SD Median (IQR)	Caudal-epidural Block (n=46) Mean±SD Median (IQR)	Penile Block (n=35) Mean±SD Median (IQR)	
Age	3.4±3.5 2 (1-5)	3.9±3.8 2 (1-6)	2.7±3.1 2 (1-3)	p=0.045[a]
	n (%)[b]	**n (%)[c]**	**n (%)[c]**	
Mea position				
Coronal	39 (48.1)	24 (52.2)	15 (42.9)	
Glanular	13 (16.0)	8 (17.4)	5 (14.3)	
Megameatus	1 (1.2)	-	1 (2.9)	x^2=2.566
Midpenile	5 (6.2)	2 (4.3)	3 (8.6)	p=0.633
Subcoronal	23 (28.4)	12 (26.1)	11 (31.4)	
Chordee				
Yes	8 (9.9)	41 (89.1)	31 (91.4)	p=0.519[c]
No	73 (90.1)	5 (10.9)	3 (8.6)	
Operation technique				
MAGPI	9 (11,1)	4 (8.7)	5 (14.3)	x^2=1.534
TIPU	72 (88.9)	42 (91.3)	30 (85.7)	p=0.464
Postoperative Fistula				
Yes	2 (2.5)	-		
No	79 (97.5)	46 (100.0)	2 (5.7)	p=0.184
Postoperative meatal stenosis			33 (94.3)	
Yes	10 (12.3)	4 (8.7)	6 (17.1)	p=0.316[c]
No	71 (87.7)	42 (91.3)	29 (82.9)	

[a]Mann-Whitney u test; [b]Column percentage; [c]Fisher's exact test
IQR, Inter Qartile Range; SD, standard deviation; MAGPI, meatal advancement and glanuloplasty; TIPU, tubularized incised plate urethroplasty.

was accompanied at month six postoperatively. Different types of anesthesia techniques did not differ in meatal position, presence of chordee, and surgical technique, or Dartos flap (p=0.633, p=0.519, p=0.464, and p=1.145, respectively). Complication rates were not also statistically significantly different between the study groups (Table 1).

Complication rates between the surgical techniques were not different for fistula or meatal stenosis (p=0.184 and p=0.316, respectively) (Table 2).

No complications occurred in patients undergoing MAGPI whereas two patients (2.5%) developed urethral fistula and ten patients (12.3%) had meatal stenosis among those undergoing TIPU.

Table 2. Comparison of surgical techniques in terms of postoperative fistula and meatal stenosis.

	Postoperative Fistula n (%)	Postoperative meatal stenosis n (%)
MAGPI (n=9)	-	1 (12.5)
TIPU (n=72)	2 (2.8)	9 (12.5)
	χ^2=1.534 p=0.464	χ^2=0.256 p=0.880

MAGPI, meatal advancement and glanuloplasty; TIPU, tubularized incised plate urethroplasty.

DISCUSSION

Penile block and caudal-epidural block are the two most commonly used peripheral block methods in distal hypospadias surgery. Recent studies suggested superiority of the penile block in perioperative and postoperative analgesia [7]. Major contributor of the innervation of the penis is dorsal penile nerve. The proximal part is innervated by the posterior branch of the penile nerve. The branches of genitofemoral and ilioinguinal nerves also contribute to the innervation. Therefore, penile nerve cannot be blocked with a single lateral injection. The caudal-epidural block is another popular block method that can be used as an alternative to penile block. The sympathetic block induced in this method could lead to vasodilation and penile congestion in penile venous sinuses [8], an unwanted adverse effect during and after surgery. On the other hand, formation of hematoma and necrosis could be listed among the threatening complications of the penile block, though a study reported that these could be reduced when the method was performed under the guidance of ultrasonography [9].

The failure at hypospadias surgery is a consequence of many factors including surgeon's skill, defect severity, competence of the technique used, patient-related factors, and presence of adequate urinary drainage [10,11]. The most common complication of the hypospadias surgery is urethra-cutaneous fistula. In fact, the recent advances in surgical techniques have dramatically reduced the rates of such complications [12,13]. Zaidi et al. [14] in their retrospective study involving patients who underwent primary hypospadias repair, reported association of fistula formation to the more proximal positioning of urethral meatus and use of subcutaneous epinephrine consistent with the literature and unlike the findings of the study by Kundra et al . any association to the caudal anesthesia could not be found [7]. This finding seems consistent with that of our study. In the study by Kundra et al. surgical techniques and surgical services in terms of complication rates of caudal-epidural and penile block methods were not mentioned [7]. In our study, we compared complication rates of these block methods where the surgery was performed by the same surgical services.

Whereas, Zaidi et al. reported its negative impact on wound healing as shown through the rabbit model by Kajbafzadeh et al. In fact, the authors reported markedly higher rates of cellular damage and higher number of apoptotic myocytes after bleeding control with epinephrine in the hemostatic technique with application of tourniquet compared to the non-hemostatic technique [14,15]. Tourniquet was not applied to our study patients who received local block with epinephrine administration

In our case series of distal primary hypospadias repair, we compared complication rates in patients who underwent caudal-epidural block or penile block for hypospadias surgery. We found that there was no significant difference in the complication rates between the two groups.

CONCLUSION

Our study eliminated such factors that limits the assessment of the association between peripheral block and the complications of hypospadias surgery; including use of multiple techniques, local application of epinephrine and tourniquet for hemostasis, presence of patients with proximal hypospadias, or performing hypospadias surgery by different departments. Nevertheless, the surgery is a very sophisticated procedure, requiring a high level of skill and experience. In fact, the number of procedures performed by surgeons was reported to be associated with complication rates [16]. Those with lower number of procedures were reported to have higher incidence of complications such as meatal stenosis, urethral fistula, or stricture [17]. Accordingly, the smaller number of the procedures performed by the surgeon appears to be a limiting factor in our study.

Ethics Committee Approval: Okmeydanı Training and Research Hospital Ethics Committee approval was obtained (17.04.2018/889).

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Diurnal Enuresis Secondary to Aripiprazole in a Child with Autistic Disorder: A Case Report

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Otizm Tanılı Bir Çocukta Aripiprazole İkincil Gelişen Diurnal Enürezis: Vaka Bildirimi

To the Editor;

Aripiprazole is a commonly used second-generation antipsychotic agent for the treatment of schizophrenia, bipolar disorder, and other mood disorders. The Food and Drug Administration (FDA) approved aripiprazole for autism spectrum disorder (ASD)-related irritability such as aggressive symptoms, temper tantrums, and deliberate self-injuriousness in children and adolescents aged 6-17 years. Frequently reported adverse effects of aripiprazole are extrapyramidal symptoms, akathisia and tremor. Diurnal enuresis exists in approximately 10% of children [1,2]. Although drug-induced urinary incontinence is noted as one of the substantial side effects of selective serotonin reuptake inhibitors (SSRIs); reports on antipsychotic-induced enuresis are available [2,3]. However, side effects of aripiprazole in children as urinary retention and enuresis has been rarely reported. In particular, there are very little information regarding aripiprazole-induced enuresis in ASD [4-6]. In this case presentation, we aimed to report diurnal enuresis developed secondary to aripiprazole treatment in a six-year-old boy with ASD.

CASE

A 6-year-old boy was referred to our outpatient clinic with complaints of aggressiveness, hyperactivity, repetitive and self-injurious behaviors. According to his developmental history, he was diagnosed with ASD because of verbal developmental delay, lack of eye contact, social-emotional reciprocity and stereotyped behaviors when he was four years old. The case was diagnosed with ASD according to DSM-5 criteria. Risperidone was prescribed at a dose of 0.25 mg/day; but discontinued because of the drug-related severe sedation. Thus, aripiprazole was started at the dose of 1 mg/day. On the fifth day of aripiprazole treatment, he developed new-onset diurnal enuresis recurring 5-6 times a day for 3 weeks until the medication was stopped.

His medical history, results of physical and neurological examinations and urinalysis were not remarkable. He had urinary bladder control at the age of 3 and had no history of urinary incontinence. Following discontinuation of aripiprazole, his enuresis ceased. After four weeks, due to re-exacerbation of behavioral symptoms, aripiprazole was restarted at a dose of 1 mg/day and then enuresis reappeared and recurred on the fifth day of treatment 5-6 times a day.

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Drug-induced adverse effects were measured with the Naranjo probability scale [7], which indicated a probable adverse effect associated with aripiprazole. Consequently, we could not maintain the treatment with aripiprazole due to the severity of enuresis. This case study demonstrates the development of diurnal enuresis in a child with ASD when aripiprazole was added to the pharmacological treatment. We also experienced that enuresis disappeared when aripiprazole was discontinued. Moreover, the reoccurrence of enuresis when aripiprazole was used for the second time indicates that the relationship between aripiprazole and enuresis may not be coincidental.

Consistent to our clinical experience, though rarely seen,there are some reports of aripiprazole-induced urinary retention and enuresis in children [2,4,5,8]. Although SSRIs cause enuresis in most of the cases [9,10] and aripiprazole is a treatment option for enuresis [3,11], sometimes aripiprazole might be the reason of nocturnal enuresis [2,5,6]. In this case, 5-HT2A antagonism of 5-HT2A receptors on detrusor muscle and the antagonism of alpha-1 receptors on internal sphincter might constitute the mechanisms of enuresis triggered by aripiprazole. In addition, aripiprazole with its serotonin reuptake effect might have another mediating role on enuresis due to cholinergic neuromuscular impact of serotonin on isolated detrusor muscle [10]. This is also an important reason of enuresis developing after SSRI use [12]. Another enuresis-enhancing effect of aripiprazole may occur through 5-HT1A system [4]. Antagonism of 5-HT1A inhibits bladder contractions [13]. Partial agonism of aripiprazole on 5-HT1A receptors may facilitate enuresis by increasing bladder contractions.

Herein, we are reporting a rarely seen case of aripiprazole-induced diurnal enuresis in autistic children. It is noteworthy that most of the case reports of aripiprazole-induced enuresis including the current study have come from Turkey. The etiopathogenesis of this condition is not clear . Frequent use of aripiprazole in ASD in Turkey or an unknown genetic background across Turkish population might be responsible for its more frequent occurrence in our country. Although aripiprazole has become widely common in treating behavioral problems associated with autistic disorder, this case report also highlights that development of enuresis with low doses of aripiprazole should be considered when using it in children with neurodevelopmental disorders such as ASD.

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