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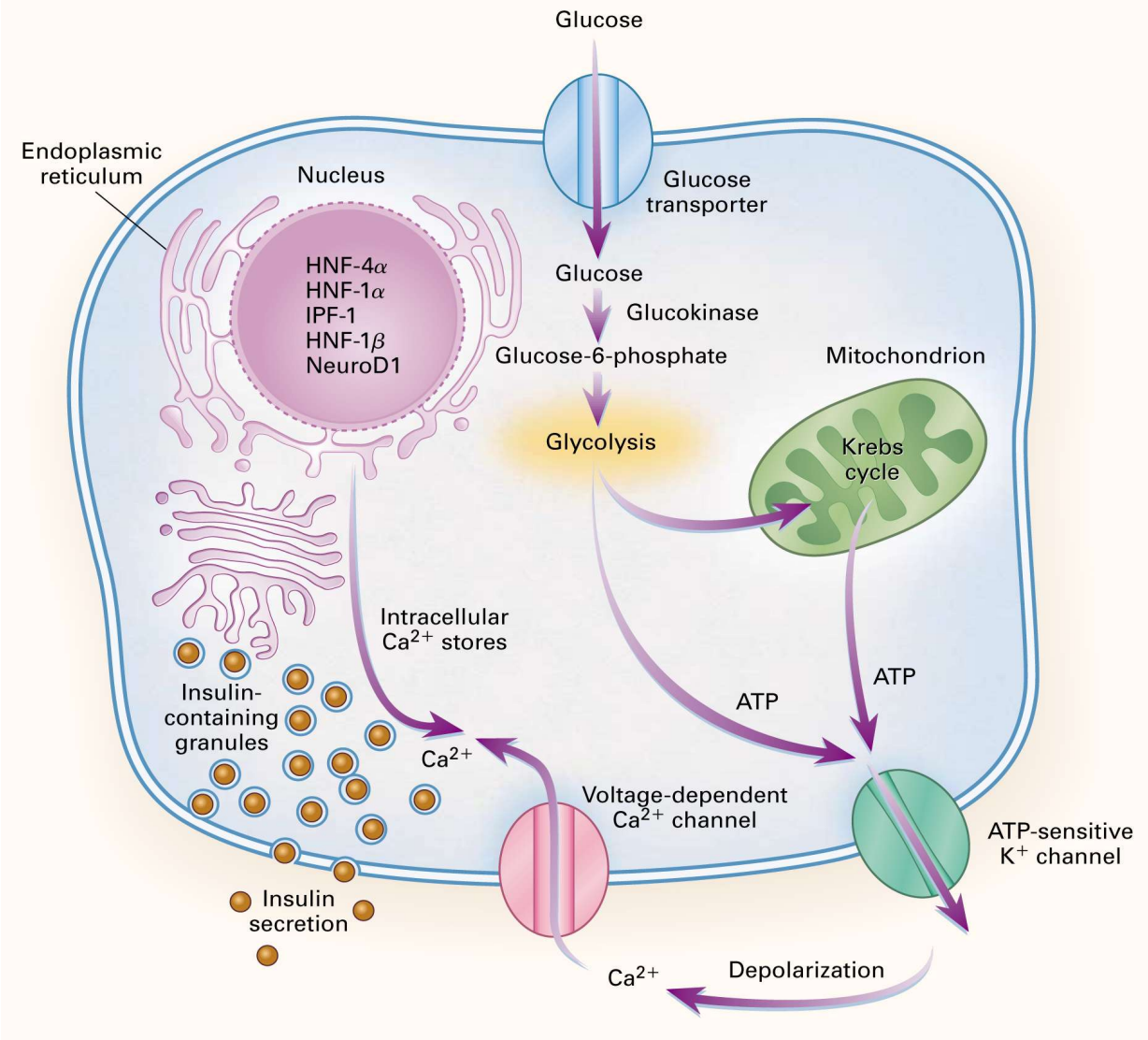

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# Contents

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# Editorial

Dear readers,

On behalf of the Editorial Board, I am delighted to announce the publication of the first issue of the Trends in Pediatrics. Trends in Pediatrics is a high quality, peer reviewed journal which focus on childhood growth and diseases and publishing articles of scientific excellence in pediatrics. Abstracting in an international index is a paramount objective of our journal.

In this issue, we present you with 6 articles including 4 original articles, 1 review article, and 1 case report. The original articles describe; (1) GCK-MODY in children with 4 novel variants, (2) increased B-cell activating factor of the tumour necrosis factor family (BAFF) levels in childhood brucellosis, (3) the relation between vitamin D levels and migraine, and (4) the risk factors of acute kidney injury in neonates. The topic of the review article is food allergy among children. Kohler's disease is the case report of this issue. I would like to thank to the editorial team, the reviewers, all authors, and the team of Logos Publishing House for their magnificent support in preparing the first issue of this journal.

It is my pleasure to invite all researchers interesting in pediatrics and pediatric surgery to submit their scientific articles to our journal. We would highly appreciate receiving readers' feedback, so please feel free to share your ideas with us, provide your thoughts and comments through our web site www.trendspediatrics.com.

We look forward to your scientific contributions in the future issues of the journal.

Sincerely yours,

**Assoc. Prof. Ahmet Anık, MD**
**Editor-in-Chief**

# Food Allergy in Childhood

**Sibel Sonmez-Ajtai**

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**ABSTRACT**

*Food allergy (FA) is a common and increasing problem globally and often co-exist with other atopic diseases. Trigger foods differ according to country and ethnic origin. Some FAs are more likely to resolve but some continue lifelong. Teenagers carry the highest risk of fatal anaphylaxis. The main diagnostic tool is allergy focused clinical and diet history, and further supportive allergy tests must be guided by clinical history. Current management consists of strict avoidance of trigger food(s). Management of allergic reactions with regular clinical re-assessment is essential to educate, support, and determine if the condition is resolving by time. Research on FA treatments and prevention is ongoing.*

## PREVALENCE and CAUSES

The incidence of food allergy (FA) is increasing worldwide and has reached an alarming 3-6% prevalence amongst children in developed countries [1]. FA is said to be the second wave of the allergy epidemic [2], following the first wave of atopic diseases (asthma, allergic rhinitis, and eczema) which emerged in the first half of the 20th-century hand in hand with the adoption of the so-called "Western lifestyle". Though the puzzle is a complex one and beyond the scope of this review, it is important to recognize that there are both genetic and environmental factors at play in the development of FA. Many environmental influences culminate in reduced biodiversity and change in the composition of the human microbiota which has crucial effects on our immune system [3].

## CLASSIFICATION and DIAGNOSIS

Food can cause a host of adverse reactions, which are not always allergic to their pathophysiology. Allergy is an immunologically mediated adverse reaction. It is essential to understand the distinction between allergic and non-allergic hypersensitivity reactions to food (e.g. cow's milk protein allergy vs lactose intolerance) as well as different types of FA, as the diagnostic and management approach differs, as does the prognosis. There are two distinct types of FA: IgE-mediated and non-IgE mediated. They present with different clinical signs and symptoms, and the main diagnostic tool for FAs is the allergy focused clinical history [4,5] (Table 1).

There are several supportive diagnostic tests including skin prick tests, specific IgE tests to whole food allergens or single food proteins (called components), and food challenge (or provocation) tests which can be utilized to confirm or rule out IgE-mediated FA. These tests must be carefully selected and interpreted in the light of the clinical history and the importance of this point cannot be overemphasized. These tests are not appropriate for the investigation of non-IgE mediated FA, where the only investigative tool beyond clinical history is an exclusion diet followed by re-introduction to prove causality between suspect food and clinical symptoms.

There are also many unvalidated alternative medicine tests such as kinesiology, Vega testing, hair testing, and IgG which patients must be strongly warned against [6].

**Table 1. Characteristic features of food allergy related disorders**

| Disorder | Age Group | Clinic | Diagnostic Tools | Prognosis |
|---|---|---|---|---|
| **IgE-mediated Disorders** | | | | |
| Acute Allergic Hypersensitivity | Any age; mostly early childhood period | Onset between minutes to 2 hours; immediate cutaneous (pruritus, erythema, angioedema, urticaria, etc.), respiratory (cough, respiratory distress, wheeze, etc.), cardiovascular (hypotension, tachycardia, shock, etc.), gastrointestinal (nausea, diarrhea, abdominal pain, etc.), and neurologic symptoms (loss of consciousness, seizure, etc.) | History<br>Positive skin prick test (SPT) and or allergen specific immunoglobulin (sIgE),<br>Confirmation of the diagnosis with oral food challenge test (OFC) | Depends on the type of food: milk, soy, egg, and wheat may outgrown in childhood; but peanut, tree nuts, seeds and fish persist lifelong |
| Oral Allergy Syndrome (Pollen-Food Allergy Syndrome) | Any age; mostly in teenagers or young adults | Immediate symptoms on contact to raw fruit with oral mucosa; pruritus, tingling, angioedema of the lips or tongue | History<br>Positive SPT with raw fruits or vegetables and/or allergen sIgE, confirmation of the diagnosis with OFC | Depends on the type of pollen allergy; symptoms increase with the pollen season, and symptoms may improve with pollen immunotherapy |
| **Ig-E and Non-IgE-mediated Disorders** | | | | |
| Eosinophilic Esophagitis | Any age; particularly in childhood | Intermittant or chronic symptoms of dysphagia, abdominal pain, heartburn, emesis, gastroesophageal reflux symptoms unresponsive to conventional treatment | History<br>Positive SPT and/or allergen sIgE with a poor concordance with the diagnosis (50%), atopy patch test may be of valuable, elimination diet and OFC, endoscopy and biopsy for confirmation of the diagnosis and follow-up the treatment response | Varies, but not well established. Elimination diet improves the symptoms within 6-8 weeks, in case of unresponsive to elimination diet topical or systemic corticosteroids, and/or elemental diet may be required |
| Allergic Eosinophilic Gastroenteritis | Any age | Intermittent or chronic symptoms of abdominal pain, emesis, irritability, weight loss, failure to thrive, anemia, and protein-losing enteropathy | History<br>Positive SPT or allergen sIgE with a poor concordance with the diagnosis (50%), elimination diet and OFC, endoscopy and biopsy for confirmation of the diagnosis and follow-up the treatment response | Varies, but not well established. Elimination diet improves the symptoms within 6-8 weeks. In case the child is unresponsive to elimination diet elemental diet may be required |
| **Non-IgE-mediated Disorders** | | | | |
| Food Protein-Induced Allergic Proctocolitis (Allergic Proctocolitis) | Young infants (≤6 months), mostly breast-fed healthy appearing infants | Blood-streaked and/or mucuous stool | History<br>Response to elimination diet with suspected food (mostly cow's milk and egg) within 48-72 hours, rarely diagnosis is confirmed by rectal biopsy | Mostly tolerance is achieved by 1-2 years of age |
| Food Protein-Induced Allergic Enterocolitis Syndrome (FPIES) | Young infants | Acute-subacute: Repetitive or intractable emesis, dehydration, shock in 15% of the cases<br>Chronic: emesis, diarrhea, failure to thrive, anemia | History<br>Response to elimination diet with suspected food (milk, soy, rice, etc.) rarely diagnosis is confirmed by OFC | Mostly resoltion is achieved by 1-3 years of age, rarely persists into late adolescence |
| Food Protein-Induced Enteropathy | Young infants | Protracted diarrhea (steatorrhea), emesis, anemia in 40%, failure to thrieve | History<br>Endoscopy and biopsy<br>Elimination diet | Mostly resoltion is achieved by 1-2 years of age |

**Table 2. Most common foods implicated in food allergy**

| *EU label requirement |
|---|
| 1. Milk* |
| 2. Egg* |
| 3. Gluten containing grains (wheat, barley, rye, spelt, etc.)* |
| 4. Fish* |
| 5. Shellfish (mollusks and crustaceans)* |
| 6. Peanut (a legume)* |
| 7. Soya (a legume)* |
| 8. Tree nuts (hazelnut, almond, cashew, pistachio, walnut, pecan, Brazil nut, Macadamia nut)* |
| 9. Mustard (a seed)* |
| 10. Celery* |
| 11. Sesame (a seed)* |
| 12. Lupin (a legume)* |
| 13. Other legumes (peas, beans, chickpeas, lentils, fenugreek, etc.) |
| 14. Other seeds (sunflower seed, linseed, pumpkin seed, etc.) |
| 15. Fruits (peach, kiwi, melon, apple, banana, etc.) |
| 16. Vegetables (potato, cucumber, etc.) |
| 17. Other grains (rice, oat, etc.) |

## RISK FACTORS

Eczema and family history of atopy are the two major risk factors for the development of FA in childhood [4]. The earlier the onset (i.e. the longer the duration) and the higher the severity of eczema, the higher the risk of FA. Accordingly, the children at the highest risk are infants with severe eczema onset in the first year of life. Males are affected more than females in childhood, whereas in adults FA is more common in females.

## FOODS CAUSING FOOD ALLERGY

The most common foods causing FA vary according to country. For example, milk, egg, and peanut allergies are the top 3 in the UK and Australia, whereas milk, egg, and wheat top the charts in Japan and sesame in Saudi Arabia. Besides, differences are observed amongst different ethnic groups living in the same country in terms of the type and the number of trigger foods [4]. In the European Union (EU), it is a legal requirement for some top allergens to be distinctly labeled and declared on all foods for sale (Table 2). Most FAs develop without a history of prior regular consumption, but nut, fish, and shellfish allergies can also develop after prior tolerance. Some food allergies (e.g. fruits, vegetables, and nuts) develop secondary to pollen sensitization, causing localized oral mucosa symptoms only in the majority of patients.

## PROGNOSIS

Many FAs with childhood-onset will resolve in time, but the pace and rate of resolution are declining [2]. In general, non-IgE mediated FAs are more likely to resolve faster than IgE-mediated FAs. Also, the prognosis depends on the specific food. For instance, nut and fish/shellfish allergies are much more persistent, whereas egg and milk allergy are more likely to resolve by time.

## MORBIDITY and MORTALITY

Fatal anaphylaxis due to FA is very rare [7], though anaphylaxis from all causes including food has increased up to 6-fold in the last two decades in some counties [8]. Anaphylaxis is only possible if one has IgE-mediated FA and there are currently no tests that can estimate the probability or even the threshold for anaphylaxis in a given individual. Patients with a previous history of anaphylaxis and those with poorly controlled asthma are at higher risk of anaphylaxis. Young people in their mid and late-teens have the highest risk of fatal anaphylaxis [8]. Foods responsible for most cases of anaphylaxis will vary according to country, but nuts and milk appear to be the most common ones in the UK [8].

The main morbidities resulting from FAs are (i) Allergic reactions due to accidental exposure, (ii) Avoidance diet which impacts on nutrition and growth unless carefully managed by the dietician and substituted with suitable alternatives to the foods avoided, (iii) Psychosocial impact due to having to follow a food avoidance diet and fear of allergic reactions, (iv) Co-existent atopic diseases/co-morbidities including asthma, eczema, and allergic rhinoconjunctivitis.

## PREVENTION and MANAGEMENT

Studies have shown that it is possible to reduce the risk of peanut and egg allergy in children if these foods are introduced into the weaning diet of infants early and eaten regularly [9,10]. The studies aiming to prevent infant eczema (and later FA) by application of moisturizers have not shown benefit so far, but more are ongoing [11]. It is also not possible to prevent milk allergy by feeding high-risk non-breastfed infants hypoallergenic formula [1].

There is no cure for FA, through induction of a degree of tolerance is possible with desensitization (immunotherapy) and such interventions have been the subject of intensive research for the past decade. So far, there is no licensed food immunotherapy product for routine clinical use but several are in the development and approval application stage, particularly for peanut.

The current standard treatment for FA consists of (i) Avoidance with suitable diet substitution, guided by a dietician, (ii) Treating the symptoms of allergic reactions due to accidental exposure (e.g. oral antihistamine +/- adrenaline for IgE-mediated reactions, fluid resuscitation for food protein-induced enterocolitis syndrome (FPIES), treatment of eczema flares, or simply time and patience for gut symptoms caused by non-IgE mediated allergy), (iii) Provision of written allergy management plans and emergency medications is essential for children with IgE-mediated FAs, though not all require Adrenaline autoinjector (AAI) provision [12]. Education of patient and family/school staff in self-management, (iv) Treatment of other atopic co-morbidities, (v) Regular review and re-assessment to determine if the FA is resolving.

It is increasingly recognized that many FA patients and their families need professional psychology support as well as extra social support, as FA is proven to adversely and significantly affect patients' health-related quality of life (HRQL).

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# Clinical, Laboratory and Genetic Characteristics of Children with GCK-MODY (MODY2): Report of Four Novel Variants in GCK Gene

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## ABSTRACT

***Objective:*** *Maturity-Onset Diabetes of the Young (MODY) is the most common type of monogenic diabetes. Heterozygous inactivating variants in glucokinase (GCK) gene are related to MODY2 (GCK-MODY). In this study, we aimed to investigate the phenotype and genotype characteristics of patients with GCK-MODY.*
***Methods:*** *Anthropometric and clinical characteristics (age, gender, weight, height and body mass index, complaints, family history), laboratory data (glucose, insulin, glycated hemoglobin, lipid levels) and molecular analysis of the GCK gene were collected from the hospital records.*
***Results:*** *The median age was 9.50 y (1.0-16.0), weight SDS -0.27 (-1.50-2.50), height SDS -0.09 (-2.20-1.60), and body mass index SDS -0.10 (-1.30-2.40). The median level of fasting blood glucose was 121 mg/dL (101-143), insulin was 9.50 mIU/mL (1.80-21.0), and HbA1c was 6.35% (6.20-6.60) at the time of diagnosis. Fourteen patients (78%) were diagnosed incidentally with asymptomatic hyperglycemia, while 4 patients (22%) had symptoms of polyuria and polydipsia. Ten different variants were detected in the GCK gene of 18 cases; one variant was nonsense, one variant was deletion, and the rest of the variants were missense mutations. Exon 7 was the most common location in coding regions and missense was the primary mutation type. The most common variant was c.802G>T (p.Glu268Ter) and detected in 5 (28%) patients. Four (22%) of the variants were novel; seven missense (p.Asp132Gly, p.Arg191Gln, p.Met238Thr, p.Met238Ile, p.Leu243Pro, p.Arg250Cys, p.Arg275Cys), one deletion (p.Pro153del) and one splice site mutation (c.863+3A>G).*
***Conclusion:*** *Since there is no specific treatment for GCK–MODY, GCK gene mutation screening should be considered in cases with early onset mild hyperglycemia, family history of impaired fasting glycemia and negative beta-cell antibodies to avoid unnecessary use of insulin or oral antidiabetic drugs. In this study, ten different variants were detected in the GCK gene of the 18 cases, four of which were novel.*

## INTRODUCTION

Maturity-Onset Diabetes of the Young (MODY), is a group of monogenic defects in β cell functions characterized by autosomal dominant inheritance and non-insulin dependent form of diabetes. Classically, patients with MODY have a family history of diabetes at two or three generations and the diagnosis is made before the age of 25 years [(1)].

MODY is the most common form of monogenic diabetes in Europe and accounts for 1-2% of all cases of diabetes. The prevalence of MODY is 21-45/1,000,000 in children and 100/1.000.000 in adults [(2–4)]. The most common causes of MODY are MODY2 and MODY3, due to variants in glucokinase (GCK) and hepatocyte nuclear factor 1-alpha (HNF1A) genes, respectively [(1)]. The prevalence of GCK-MODY is higher in countries such as Germany, France, Spain and Italy, where glucose screening in asymptomatic children is a routine procedure [(1)]. Similar to these countries, GCK-MODY is the most common form of MODY in Turkey [(5)].

GCK-MODY is characterized by stable, mild and non-progressive hyperglycemia, low glycosylated hemoglobin (HbA1c) levels with good prognosis that does not require specific treatment [1]. Considering that MODY patients often misdiagnosed with type 1 or type 2 diabetes mellitus (DM), correct molecular diagnosis of MODY is crucial for choosing the optimal treatment and identifying at-risk family members [6].

In this study, we aimed to describe (i) the molecular spectrum of GCK gene, (ii) clinical and laboratory characteristics of these children, and (iii) to present 4 novel variants.

## MATERIALS and METHODS

### Patient Data

Children with a clinical diagnosis of GCK-MODY and available molecular results for GCK gene were enrolled in the study. Demographic, clinical and laboratory characteristics of the subjects were collected from the hospital records. Inclusion criteria were: (i) stable and mild hyperglycemia (fasting plasma glucose 100-144 mg/dL), (ii) family history of diabetes with autosomal dominant inheritance, and (iii) absence of beta-cell autoantibodies [6]. Patients with type 1 or type 2 DM, and HbA1c level greater than 7.5% were excluded from the study. Patients were classified and compared in two groups: Subjects with documented variant in GCK gene were classified as GCK-MODY (+) and subjects that exhibit typical characteristics of GCK-MODY but without any variant in GCK gene were classified as GCK-MODY (-).

### Genetic Analysis

Genomic DNA was extracted from peripheral blood using standard techniques (QIAGEN® (Hilden, Germany) were used following the manufacturer's instructions. All coding GCK (NM_000162) exons and their flanking regions were amplified by PCR. The amplicons were cleaned up with Sephadex (GE Healthcare). ABI PRISM 3100 DNA analyzer (Applied Biosystems) and Big Dye Terminator Cycle Sequencing V3.1 Ready Reaction Kit (Life Technologies) were used to elucidate the DNA sequence. Variants were evaluated according to the reference genome of GRCh37(h19) [RefSeqIDs: GCK (NM_000162.5)]. Novel variants in the GCK gene were analyzed in three different bioinformatics tools that examine functional effects of variants: PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/), Mutation Taster (http://www.mutationtaster.org) and SIFT (Sorting Intolerant From Tolerant) (http://sift.jcvi.org/www/SIFT_enst_submit.html) [7-9]. The CADD and DANN scores of the single nucleotide variants as well as the insertion/deletion variants detected in the analysis were calculated by reference to PubMed publications [10,11].

### Statistical Analysis

Statistical Package for Social Sciences (SPSS) software (version 25.0, SPSS Inc., Chicago, IL, USA) was used for the statistical. Data from groups with and without variants were compared using Mann-Whitney U-test. The results of non-parametric tests were given as median (minimum-maximum). A p value less than 0.05 were defined as statistically significant.

## RESULTS

**Table 1. Demographic, clinical and laboratory characteristics of children with GCK–MODY***

| Characteristics | n=18 |
|---|---|
| Age (year) | 9.50 (1.0-16.0) |
| Female/Male | 8/10 |
| Weight SDS | -0.27 (-1.50-2.50) |
| Height SDS | -0.09 (-2.20-1.60) |
| BMI SDS | -0.10 (-1.30-2.40) |
| Glucose 0 min (mg/dL) | 121 (101-143) |
| Glucose 120 min (mg/dL) | 152 (99-249) |
| Insulin 0 min (mIU/mL) | 9.50 (1.80-21.0) |
| Insulin 120 min (mIU/mL) | 29.30 (2.20-109.30) |
| HbA1c (%) | 6.35 (6.20-6.60) |
| C peptide (mg/dL) | 0.69 (0.40-4.30) |
| Total Cholesterol (mg/dL) | 190 (101-232) |
| LDL- Cholesterol (mg/dL) | 106 (26-153) |
| HDL- Cholesterol (mg/dL) | 53 (46-90) |
| Triglyceride (mg/dL) | 70 (33-180) |

**Data were given as median (min-max)*
*BMI, body mass index; SDS, standard deviation score; LDL, low-density lipoprotein; HDL, high-density lipoprotein*

Molecular analysis of the GCK gene was performed in 29 patients and variant was detected in 18 cases [10 male, 56%] from 15 different families [GCK-MODY (+)], and no variants were detected in 11 cases [6 male, 55%] [GCK-MODY (-)].

The median age was 9.50 y (1.0-16.0), weight SDS -0.27 (-1.50–2.50), height SDS -0.09 (-2.20-1.60), and body mass index SDS -0.10 (-1.30-2.40) in children with GCK-MODY (+). The median level of fasting blood glucose was 121 mg/dL (101-143), insulin was 9.50 mIU/mL (1.80-21.0), and HbA1c was 6.35% (6.20-6.60) at the time of diagnosis (Table 1). Fourteen patients (78%) were diagnosed incidentally with asymptomatic hyperglycemia, while 4 patients (22%) were symptomatic (polyuria and polydipsia). According to the oral glucose tolerance test (OGTT) the increase in blood glucose level was <83 mg/dL except for one case who had high postprandial glucose level (249 mg/dL).

There was no significant difference between the GCK-MODY (+) and GCK-MODY (-) groups with respect to age, gender, anthropometric measurements, and laboratory tests (Table 2).

Ten different variants were detected in the GCK gene of 18 cases; one variant was nonsense, one variant was deletion, and the rest of the variants were missense mutations. The variants were evaluated according to American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines recommendations [12]. The most common variant was 802G>T (p.Glu268Ter) and detected in 5 (28%) patients. Four (22%) of the variants were novel; 7 missense (p.Asp132Gly, p.Arg191Gln, p.Met238Thr, p.Met238Ile, p.Leu243Pro, p.Arg250Cys, p. Arg275Cys), one deletion (p.Pro153del) and one splice site mutation (c.863+3A>G) (Table 3). In addition, targeted next-generation sequencing analysis of other genes in the MODY etiology was planned in cases with no mutation.

**Table 2. Comparison of GCK–MODY (+) and GCK–MODY (-) groups***

| Characteristics | GCK–MODY (+) n= 18 | GCK–MODY (-) n= 11 | p** |
|---|---|---|---|
| Age (year) | 9.5 (1.0-16.0) | 10.0 (1.0-17.0) | 0.63 |
| Female/Male | 8/10 | 5/6 | 1.000 |
| Weight SDS | -0.27 (-1.50–2.50) | 0.65 (-3.90–1.40) | 0.46 |
| Height SDS | -0.09 (-2.20–1.60) | 0.60 (-2.40–1.50) | 0.61 |
| BMI SDS | -0.10 (-1.30–2.40) | 0.05 (-3.30–1.20) | 0.92 |
| Glucose 0 min (mg/dL) | 121 (101–143) | 121 (104–142) | 0.88 |
| Glucose 120 min (mg/dL) | 152 (99–249) | 146 (99–218) | 0.64 |
| Insulin 0 min (mIU/mL) | 9.5 (1.8–21.0) | 6.4 (3.3–21.6) | 0.52 |
| Insulin 120 min (mIU/mL) | 29.30 (2.20–109.30) | 35.1 (8.8–137.0) | 0.82 |
| HbA1c (%) | 6.35 (6.20–6.60) | 6.05 (5.00–6.40) | 0.26 |
| C peptide (mg/dL) | 0.69 (0.40–4.30) | 1.20 (1.0–1.50) | 0.43 |
| Total Cholesterol (mg/dL) | 190 (101–232) | 142 (126–178) | 0.06 |
| LDL-Cholesterol (mg/dL) | 106 (26–153) | 78 (60–105) | 0.23 |
| HDL-Cholesterol (mg/dL) | 53 (46–90) | 49 (31–55) | 0.07 |
| Triglyceride (mg/dL) | 70 (33–180) | 87 (41–129) | 0.49 |

**Data were given as median (min-max), **Mann-Whitney U test*
*BMI, body mass index; SDS, standard deviation score; LDL, low-density lipoprotein; HDL, high-density lipoprotein*

**Table 3. The characteristics of the variants in GCK gene and prediction tools scores**

| Nucleotide changes | Location | Aminoacid changes | dbSNP number | Mutation Taster score | Polyphen-2 score | SIFT score | DANN score | CADD score | ACMG 2015 Criteria | Reported |
|---|---|---|---|---|---|---|---|---|---|---|
| c.395A>G | Exon 4 | p.Asp132Gly | - | 0.9999 | 0.53 possibly damaging | 0.006 damaging | 0.9964 | 24.6 | PM1, PM2, PP2, PP3 | Novel |
| c.457_459 delCCT | Exon 4 | p.Pro153del | - | 1 | N/A | 0.894 damaging | N/A | N/A | PM1, PM2, PM4, PP3 | Novel |
| c.572G>A | Exon 5 | p.Arg191Gln | rs886042610 | 1 | 1 probably damaging | 0.001 damaging | 0.9995 | 32 | PM1, PM2, PM5, PP2, PP3 | Massa 2001 (22) |
| c.713T>C | Exon 7 | p.Met238Thr | - | 1 | 0.1 benign | 0.276 tolerated | 0.9906 | 24.2 | PM1, PM2, PP2, PP3 | Novel |
| c.714G>A | Exon 7 | p.Met238Ile | - | 1 | 0.48 possibly damaging | 0.053 tolerated | 0.9941 | 23.6 | PM1, PM2, PP2, PP3 | Novel |
| c.728T>C | Exon 7 | p.Leu243Pro | rs1470562535 | 1 | 1 probably damaging | 0.028 damaging | 0.9989 | 31 | PM1, PM2, PP2, PP3 | Borowiec, 2012 (23) |
| c.748C>T | Exon 7 | p.Arg250Cys | rs1057524904 | 1 | 1 probably damaging | 0.001 damaging | 0.9994 | 32 | PM1, PM2, PP2, PP3 | Pinterova, 2006 (24) |
| c.802G>T | Exon 7 | p.Glu268Ter | - | 1 | N/A | N/A | 0.9977 | 42 | PVS1, PM2, PP3 | Pruhova, 2003 (25) |
| c.823C>T | Exon 7 | p.Arg275Cys | rs556436603 | 1 | 0.99 probably damaging | 0.002 damaging | 0.9991 | 26.4 | PM1, PM2, PM5, PP2, PP3 | Gloyn, 2003 (26) |
| c.863+3A>G | Intron 7 | Non coding variant | rs193922334 | 1 | - | - | 0.9178 | 22.5 | PM2, BP4 | No publication |

*N/A: Not available. PM1: Mutation hotspot. PM2: Absent from controls. PM4: Change in protein length. PM5: Other aminoacid change in same codon. PP2: Rare benign missense. PP3: In silico pathogenic evidence. PVS1: Loss of function. BP4: In silico benign evidence.*

## DISCUSSION

GCK-MODY is the most common form of MODY in some countries with the highest prevalence in southern European countries [13-15]. According to the three recent studies from Turkey, the frequency of GCK-MODY in Turkish children with MODY was 24-64% [5,16,17].

GCK-MODY is an autosomal dominant disease characterized by mild hyperglycemia and mild elevation of HbA1c [1]. The glucose threshold for insulin secretion has increased in GCK–MODY and mild fasting hyperglycemia is present from birth [6]. The characteristic biochemical features of GCK-MODY are, (i) mild fasting hyperglycemia (100-145 mg/dL), (ii) HbA1c lower than 7.5%, and (iii) increment of glucose at 2h in OGTT lower than 83 mg/dL [1]. In the present study, the inheritance pattern was compatible with autosomal dominant, fasting glucose levels of the patients were between 100-145 mg/dL, HbA1c levels ≤7.5% and increment of glucose level in OGTT <83 mg/dL in accordance with the literature data. Classical symptoms of diabetes are rare in patients with GCK-MODY. Patients with GCK-MODY are usually asymptomatic and the majority of the patients are discovered during routine screening or accidental discovery of elevated blood glucose [18]. In our study, most of the patients were referred for accidental discovery of elevated blood glucose compatible with the literature data.

Glucokinase which encoded by the 50Kb ten-exon

GCK gene, phosphorylates glucose [19]. More than 700 GCK variants have been reported in different populations with no common variants or hotspots [20]. In the present study, the missense variants (n=1/10: 1 known), nonsense variants (n=7/10, 3 novel and 4 known), deletions (n=1/10, 1 novel) and splice site variants (n:1/10, 1 known) were distributed throughout the protein: 3/10 (30%) in the small domain and 7/10 (70%) in the large domain of the protein.

Missense variants of GCK gene are the most frequent causes of GCK-MODY [20]. Exon 7 was the most common location in coding regions and missense was the primary mutation type.

Clinical and laboratory characteristics of patients with molecularly proven GCK-MODY were similar with the patients with suspicion of GCK-MODY but without mutation. This suggested that there may be variants in the GCK gene that we could not detect with existing methods, or unknown genes that cause the GCK-MODY phenotype.

There are some limitations in our study. Firstly, functional studies could not be performed for novel variants. Secondly, we may not be able to detect all variants, such as large deletions and duplications that account for up to 3.5% of disease-causing variants in the GCK gene [21]. In addition to other limitations, other genes in MODY etiology were not studied in cases with no mutation in the GCK gene.

Since there is no specific treatment in GCK-MODY, the correct diagnosis is essential for optimal management of patients to prevent unnecessary use of insulin or oral antidiabetic medications. This study revealed 4 novel heterozygous variants in the GCK gene that caused GCK-MODY disease and showed that the phenotypic properties of the novel variants are similar.

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# Increased B-cell Activating Factor Levels in Children with Acute Brucellosis

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## ABSTRACT

***Objective:*** *Brucellosis is a chronic infectious disease. We aimed to show the association of serum 'A PRoliferation-Inducing Ligand (APRIL) (TNFSF13A)' and 'B-cell Activating Factor (BAFF)' levels belonging to the tumor necrosis factor family in children with acute brucellosis.*

***Methods:*** *Fifty children with acute brucellosis and 35 healthy controls who were admitted during a two-year period between 2018 and 2020 were prospectively included in the study. All patients and healthy children were tested for complete blood counts, C-reactive protein, BAFF, and APRIL levels, and erythrocyte sedimentation rate.*

***Results:*** *The mean age of 50 patients with acute brucellosis was 10.2±3.6 years, and 37 (74%) of them was male. BAFF levels were significantly higher in children with acute brucellosis than the control group (548.6±253.4 vs. 280.5±83.6, p<0.001). In addition to APRIL levels, other laboratory tests were not statistically significantly different between the patients and control group. Neither BAFF and APRIL, nor age, gender, Rose-Bengal test results, transaminase levels, and other laboratory parameters were significantly different between the patients with and without any complication(s). ROC analysis showed that BAFF values above 330 pg/mL were 78% sensitive and 72% specific for the detection of acute brucellosis in children (area under curve:0.864, p<0.001, CI:0.787-0.941).*

***Conclusion:*** *BAFF levels were significantly elevated in children with acute brucellosis. BAFF levels above 330 pg/mL were 78% sensitive and 72% specific for the detection of the disease, but BAFF could not discriminate between patients with and without complications. APRIL, which has relatively similar effects, is not associated with acute pediatric brucellosis.*

## INTRODUCTION

Brucellosis, which is also called as Malta fever, Bang disease, and Mediterranean fever, is a chronic infectious disease. The disease is frequently seen in a wide geography such as the Mediterranean countries, Asia, Africa, Central and South America and the Near East, including our country. While the disease can be asymptomatic or present with mild symptoms, it can also manifest itself with morbidities such as central nervous system, heart and musculoskeletal involvement. The definitive diagnosis of the disease is made by the recovery of *Brucella* spp. from blood, bone marrow, or other tissue samples, but serological tests are used in the absence of bacteriological confirmation. Although many serological methods have been used, the standard method is serum agglutination test [(1)]. These cheap and rapidly performed tests sometimes ensue in cross-reactivity with other Gram-negative infections, incompatibility with the clinical situation, inability to detect *Brucella canis* infections, false negative/positive results due to inhibition of antibodies or prozone phenomenon [(2,3)]. As with other infectious diseases, nonspecific routine laboratory tests, such as white blood cell count, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) help in the diagnosis of brucellosis. However, previous studies have shown that variabilities can be seen even in children with definite diagnosis of brucellosis [(4,5)]. In addition, these parameters are not specific and may vary depending on the presence of many other diseases that develop simulta-

neously. For this reason, specific and sensitive tests, which can be used in the diagnosis and monitorization of pediatric brucellosis, are necessary.

Molecules such as 'A Proliferation-Inducing Ligand (APRIL) (TNFSF13A)' and 'B-cell Activating Factor belonging to the tumor necrosis factor family (BAFF)' could be promising. APRIL and BAFF are members of TNF superfamily and play a critical role in activation, proliferation, homeostasis, and existence of B cells [6]. These cytokines are also present in astrocytes, dendritic cells, macrophages, and monocytes [7]. Changes in the levels of BAFF and APRIL occurred in various diseases, including autoimmune diseases, immunodeficiencies, and cancers [8-10]. BAFF/APRIL system and infections are also associated. BAFF levels increase in infections due to hepatitis C, human immune deficiency, respiratory syncytial, H1N1, Dengue, Sendai, Reovirus-1, and Epstein-Barr viruses, [11]. In addition, an association between a mutation in any of BAFF/APRIL receptors in common variable immunodeficiency patients has been reported to result in increased frequency of sinopulmonary infections [12]. BAFF/APRIL system was investigated in children with Kawasaki disease. It was reported that BAFF and APRIL were inversely correlated with each other during the course of the disease [13]. BAFF levels significantly decreased with IVIG treatment, while APRIL levels increased to higher levels in response to the treatment. A clinical study investigating the relationship between brucellosis and BAFF/APRIL system has not been conducted to date.

In this study, we aimed to show the significance of serum BAFF and APRIL levels in children with acute brucellosis.

## MATERIALS and METHODS

Fifty children with acute brucellosis and 35 healthy controls, who were admitted to Department of Pediatric Infectious Diseases during a two-year period between 2018 and 2020, were prospectively included in the study. First episode of brucellosis with specific symptoms and clinical and laboratory findings persisting for up to two months, was defined as acute brucellosis. Patients with chronic diseases or any clinical or laboratory evidence of any disease or users of any medication that might interfere with inflammation and response of acute phase reactants were not included in the study. Brucella tube (Wright) and Coombs agglutination tests with titers ≥ 1:160 or blood cultures where *Brucella* spp. proliferated yielded laboratory diagnosis of brucellosis. The children in the control group consisted of age- and sex-matched healthy children with negative brucellar serology. All patients and healthy children were tested using complete blood cell counts, CRP, BAFF, APRIL levels, and ESR.

An enzyme linked immunosorbent assay (ELISA) was used to detect BAFF and APRIL levels in the collected blood plasma samples. After centrifugation at 2000xg for 15 min at room temperature, the plasma samples were stored at -80˚C until the tests were performed. All samples were thawed only once prior to use and diluted to 1:10 concentration with buffer solutions in triplicates. Serum TNFSF13/APRIL levels were analyzed by means of a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Tumor Necrosis Factor Ligand Superfamily member13 (TNFSF13/APRIL) Elisa Kit-Fine Test Catalogue Number: EH00315) with inter-assay CV: <8% and intra-assay CV: <10%, respectively. The mean minimum detectable dose (MDD) of human TNFSF13/APRIL was 0.78 ng/mL. Serum BAFF levels were measured using an ELISA kit (Human B-Cell Activating Factor (BAFF) Elisa Kit-Fine Test Catalogue Number: EH0042). Inter-, and intra-assay CVs of the kit were <8%, and <10%, respectively. The MDD for human BAFF was 62.5 pg/mL.

Informed consent was taken from each one of the legal guardians of all children for their inclusion in the study. Local institutional ethics committee approved the study (2018/04-26).

Data were analyzed with the SPSS software version 16.0 for Windows (SPSS Inc, Chicago, IL, USA). Kolmogorov-Smirnov test was used to verify the normality of the distribution of continuous variables. Continuous variables were presented as mean±standard deviation or median (interquartile range) and categorical variables were expressed as numbers and percentages. The independent samples t test or Mann-Whitney U test was used for continuous variables and the chi-square test for categorical variables. Receiver operating characteristic

(ROC) curve analysis was used to determine the optimum cut-off level of BAFF in association with *Brucella* infection. Statistical significance was defined as $p<0.05$

## RESULTS

The mean age of 50 patients with acute brucellosis was 10.2±3.6 years, and thirty-seven (74%) of them was male, which were not statistically different from the healthy children (p=0.69 and p=0.52, respectively). BAFF levels were significantly higher in children with acute brucellosis than the control group (548.6±253.4 vs. 280.5±83.6, p<0.001). Other laboratory tests, in addition to APRIL levels were not statistically different between the patient and control groups.

Twenty (40%) patients had complications. Majority (n=15, 75%) of them had arthritis, and 2 (10%) patients had osteomyelitis, each one (5%) of the remaining three patients had thrombocytopenia, hepatitis, and lymphadenopathy. Age, gender, Rose- Bengal test results, transaminase levels, and other laboratory parameters were not significantly different between the patients with and without complication(s) (Table 2). The symptomatic periods were not different between the patients with and without complications [30(20-60) vs. 25(11-30), p=0.07]. BAFF and APRIL levels were also indiscriminative between the patients, as shown in Table 2.

In ROC analysis, it was calculated that BAFF values >330 pg/mL had 78% sensitivity and 72% specificity for the detection of acute brucellosis in children

**Table 1. The comparison of laboratory parameters of the study group with control group**

| Parameter | Study group (n=50) | Control group (n=35) | p value |
|---|---|---|---|
| Hemoglobin, gr/dL, mean±SD | 12.7±1.3 | 13.1±1.3 | 0.21 |
| Platelet, /mm$^3$, mean±SD | 326,136±105,558 | 327,214±71,449 | 0.95 |
| Leukocytes, /mm$^3$, median (IQR) | 8330 (6684-9943) | 7096 (6080-9400) | 0.13 |
| Neutrophils, /mm$^3$, median (IQR) | 3363 (2426-5285) | 3400 (2415-4869) | 0.33 |
| Eosinophil, /mm$^3$, median (IQR) | 64 (16-203) | 73 (30-230) | 0.45 |
| ESR, mm/h, median (IQR) | 11 (5-19) | 10 (7-14) | 0.62 |
| CRP, mg/dL, median (IQR) | 0.45 (0.30-1.03) | 0.40 (0.30-0.70) | 0.52 |
| BAFF, pg/mL, mean±SD | 548.6±253.4 | 280.5±83.6 | <0.001* |
| APRIL, pg/µL, median (IQR) | 13.1 (8.4-20.5) | 10.3 (5.9-17.1) | 0.17 |

*SD, standard deviation; IQR, interquartile range; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; BAFF, B-cell Activating Factor; APRIL, A PRoliferation-Inducing Ligand*

**Table 2. The comparison of laboratory parameters of the patients with and without complication**

| Parameter | Patients with complication(s) (n=30) | Patients without complication(s) (n=20) | p value |
|---|---|---|---|
| Hemoglobin, gr/dL, mean±SD | 12.9±1.3 | 12.4±1.1 | 0.20 |
| Platelet, /mm$^3$, mean±SD | 324,017±99,790 | 329,315±116,273 | 0.86 |
| Leukocytes, /mm$^3$, median (IQR) | 7505 (6080-9420) | 8800 (7590-12600) | 0.11 |
| Neutrophils, /mm$^3$, median (IQR) | 3463 (2313-5236) | 3830 (3140-5906) | 0.40 |
| Eosinophil, /mm$^3$, median (IQR) | 62 (15-185) | 73 (9-210) | 0.85 |
| ESR, mm/h, median (IQR) | 10 (3.5-16) | 11 (7-14) | 0.07 |
| CRP, mg/dL, median (IQR) | 0.3 (0.3-0.9) | 0.6 (0.3-1.2) | 0.29 |
| BAFF, pg/mL, mean±SD | 560.0±271.9 | 531.5±228.7 | 0.70 |
| APRIL, pg/µL, median (IQR) | 13.1 (9.9-20.8) | 10.5 (7.2-20.3) | 0.14 |
| Brucella tube agglutination test, median (IQR) | 320 (320-1280) | 320 (0-640) | 0.30 |
| Brucella Coomb's agglutination test, median (IQR) | 640 (320-640) | 640 (320-640) | 0.26 |
| Serum free iron, (65-175 ug/dL), median (IQR) | 37 (30-59) | 28 (22-53) | 0.18 |
| Iron binding capacity, (120-370 ng/dL), median (IQR) | 256 (228-322) | 301 (257-328) | 0.19 |
| Serum ferritin, (21.8-274.6 ng/mL), median (IQR) | 62 (21-186) | 77 (47-115) | 0.52 |
| Serum vitamin D, (20.0-60.3 ng/mL), median (IQR) | 22.3 (13.9-27.5) | 22 (16-27) | 0.57 |

*SD, standard deviation; IQR, interquartile range; ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; BAFF, B-cell Activating Factor; APRIL, A PRoliferation-Inducing Ligand*

(area under curve:0.864, p<0.001, CI:0.787-0.941) (Figure 1).

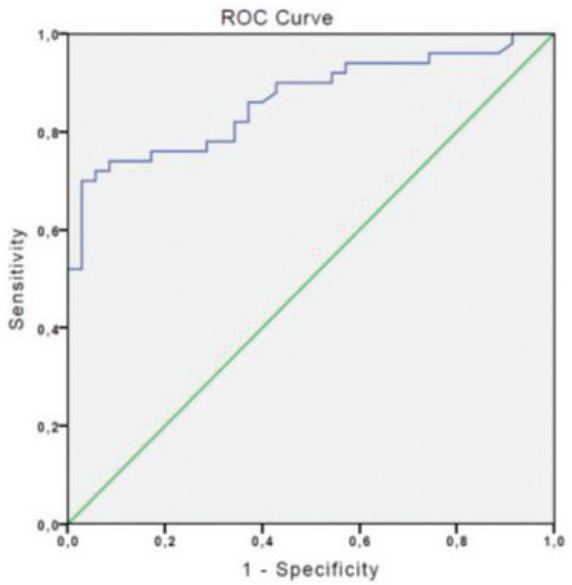


**Figure 1.** ROC analysis presenting the sensitivity and specificity of BAFF levels in children with acute brucellosis

## DISCUSSION

Brucellosis is a multisystemic infection without a predilection to any specific organ or body system. Definitive diagnosis by means of microbiological and molecular tests are challenging, so easier and faster methods such as use of biomarkers can aid in diagnosis. BAFF can be a promising diagnostic marker as this study showed that children with acute brucellosis has higher BAFF levels. Additionally, BAFF values above 330 pg/mL had 78% sensitivity and 72% specificity for the detection of acute brucellosis in children. Other traditional markers, such as white blood cell counts and CRP levels are not ensuring. Previous studies have shown that both leukocytosis and leukopenia, just as increased and normal acute phase reactants levels can be seen even in children with brucellosis [4,5].

BAFF, discovered in 1999 by Schneider et al. [14], is mainly expressed as a mRNA by naive and activated T cells, and monocyte-derived dendritic cells. BAFF exerts its effects by its three different receptors: B-cell maturation, transmembrane activator and CAML interactor, and B-cell-activating factor receptor/BLys receptor 3 (BR3). Its main receptor BAFF-R (BR3) has been shown to be almost exclusively expressed by B cells. BAFF and its receptors are essential for survival and activation of plasmablasts derived from memory B cells and inhibition of apoptosis of immunoglobulin secreting cells with resultant increase in immunoglobulin synthesis [15]. Any pathology in this mechanism is assumed to be responsible for the development of possible autoimmune diseases and malignities [16]. In animal studies, it was seen that when BAFF was overexpressed, B-cells secreting malaria-specific antibodies increased, hence mice were protected from lethal malaria infection [17]. Also, BAFF deficiency was shown to result in increased mortality rates related to West Nile virus infection and decreased virus-specific IgG and neutralizing antibody responses [18]. However overexpression of BAFF has been associated with progression of infectious diseases as HIV/SIV [19]. Excessive overexpression of BAFF may contribute to the inhibition of self-reactive B-cells during the B-cell development and impair memory B-cell generation and functions. Anyway, neutralization of BAFF may speed up the SIV-induced death [20].

The effects of APRIL and BAFF are relatively similar. As BAFF, and APRIL have been shown to enhance Ig production [21]. APRIL acts by enhancing Ig production in response to antigens in the serum. Adults with sepsis hospitalized in intensive care unit were compared with healthy controls and their serum APRIL levels were found to be higher than controls which were related to poor prognosis [22]. Similarly, higher APRIL levels are associated with Th1 immune response in pulmonary tuberculosis in adults [17]. Tuberculosis is another infectious disease caused by an intracellular microorganism just like brucellosis. Conversely, an association was not found between APRIL levels and brucellosis in this study.

Brucellosis can result in significant changes, such as increased AST, ALT, and GGT levels, [23]. Similarly, white blood cell, and neutrophil counts, and CRP levels can be significantly higher in the brucellosis patients [24]. The most common complication in brucellosis is osteoarticular pathologies [25]. Yılmaz Çiftdoğan and Aslan [26] reported that ESR and CRP values are more prominently elevated if osteoarticular involvement is present. In our study, neither the acute phase reactants and other laboratory parameters, nor BAFF and APRIL levels were different bet-

ween children with and without complications. In childhood brucellosis, lower hemoglobin, iron, and vitamin D and higher leukocyte counts, CRP, and ferritin levels have been suggested to be predictive for definitive diagnosis [27]. In this study none of these laboratory parameters was significantly different between children with, and without acute brucellosis.

This study has also some limitations. Due to the small size of the study population, statistically significant results could not be achieved in some parameters. Additionally, it would be better if we could analyze main BAFF receptors to interpret their relationship in conjunction with BAFF itself. Anyway, to the best of authors' knowledge, this is the first trial to date to show an association between BAFF and APRIL levels and childhood brucellosis. Animal studies modelling brucellosis would be beneficial to clearly define the molecular interactions between the biomarkers and the disease.

As a conclusion, BAFF levels are significantly elevated in children with acute brucellosis. BAFF levels above 330 pg/mL had 78% sensitivity and 72% specificity for the detection of the disease and can be used as a novel biomarker to diagnose brucellosis in these children. However, BAFF cannot discriminate between patients with and without complications and APRIL, which has relatively similar effects, is not associated with acute pediatric brucellosis.

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# Frequency, Risk Factors, Clinical Course, and Effect on Mortality of Acute Kidney Injury in Newborns

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## ABSTRACT

***Objective:*** *Acute kidney injury (AKI) is still an important cause of morbidity and mortality in the neonatal period, despite recent improvements on perinatal care. In this study, the frequency, etiological causes, clinical characteristics, and mortality of newborns with AKI in a tertiary neonatal intensive unit (NICU) were investigated.*
***Methods:*** *Medical records of newborns admitted between 2007 and 2011 were evaluated and patients who developed AKI in the first 28 days of life were determined. Clinical characteristics, primary cause, mortality, and highest creatinine of newborns with AKI were recorded.*
***Results:*** *It was determined that 94 of 677 (13.9%) patients (80% of them in the first seven days of life) developed AKI. Hypovolemia, birth asphyxia, congenital heart disease, sepsis, and genitourinary system (GUS) anomalies were found to be the most frequent causes of AKI. The incidence of AKI and mortality rates were higher in patients with gestational age under 28 week and birth weight under 1000 g. Mortality tends to rise in the presence of AKI regardless of the underlying disease, but this was statistically significant only for sepsis and cardiac disease. The presence of AKI increases length of stay and, the creatinine level was found to be lower in those who survived.*
***Conclusions:*** *AKI is still an important morbidity in patients treated in NICU despite improvement on perinatal care. Low birth weight, prematurity, birth asphyxia, sepsis, hypovolemia, cardiac diseases, and GUS anomalies were common causes and mortality increases in patients with AKI regardless of gestational age, birth weight, and underlying etiology.*

## INTRODUCTION

Diagnosis of acute kidney injury (AKI) in newborns is challenging due to difficulties in monitoring urine output, serum creatinine levels reflecting maternal serum creatinine level in the first days of life, and the frequent occurrence of non-oliguric renal failure. The incidence of AKI in the neonatal period varies according to the birth weight, gestational age, the severity and number of concomitant diseases. With the development of neonatal and perinatal care, the survival rates of critically ill newborns have increased. However, this also increases the incidence of AKI [(1)]. There are few studies showing the relationship between etiological causes and mortality of AKI in newborns admitted to the neonatal intensive care unit (NICU). In these studies, most causes of AKI in the neonatal period were sepsis, hypovolemia, birth asphyxia, congenital heart disease (CHD), genitourinary system (GUS) anomalies, and drugs. However, their relationship with AKI and their effects on mortality are not well established [(1-4)]. Although AKI in the neonatal period may cause chronic kidney disease [(5-8)], but there are few studies of AKI and long-term effects in this patient group. The aim of this study is to determine the relationship, incidence, etiological causes, clinical course, mortality rates and mortality risk factors of AKI in newborns.

AKI is the accumulation of harmful wastes in the body and reduced kidney function resulting in fluid retention. Clinical manifestations can range from mild dysfunction to severe anuric renal failure. A decrease in urine output or an abnormal elevation in

serum creatinine supports the diagnosis [2]. In order to diagnose and stage AKI in other pediatric age groups, Akcan-Arıkan et al. modified the RIFLE (risk, injury, failure, loss, end-stage disease) criteria and pediatric RIFLE (pRIFLE) criteria were developed [9]. However, there is not enough data about using those criteria's in the newborn age group.

## MATERIAL and METHODS

This study was conducted in accordance with the amended Declaration of Helsinki, approved by the Local Ethical Committee. Medical records of all newborns admitted to the NICU between 2007-2011 were evaluated and those having AKI within 0-28 days of life were determined. The referral status, birth weight, gestational age, mode of delivery, clinical characteristics, presence of maternal morbidity, length of stay, and mortality status were recorded. In addition to other data, main disease-causing AKI was determined, the highest creatinine level and prognosis were recorded. AKI was defined as a two-fold increase between two measurements or a serum creatinine level of >1.5 mg/dL. The etiology of AKI was determined by the underlying disease. In the presence of more than one etiological factor, the primary etiological factor was determined by clinical decision.

Categorical and continuous variable were reported as frequencies and percentiles and means with standard deviations (SD) or medians with interquartile ranges (IQRs). The Mann-Whitney U test was used to compare non-parametric variables and Student's t test was performed for parametric data. Multivariate regression analysis was used to determine risk factors for mortality. All data obtained were analyzed using an IBM SPSS V15.0 program and P-values ≤0.05 were considered significant for all comparisons.

## RESULTS

Total of 677 patients were included to the study during the study period. High creatinine value for age was found in 94 (13.9%) patients. Table 1 shows the risk factors related to AKI for newborns admitted to the NICU. AKI incidence was higher in patients with birth weight under 1000 g weight and gestational age under 28 weeks.

The time of the occurrence of AKI according to the underlying etiology and the mortality rates in those patients are shown in Table 2.

Mortality increased in patients with AKI regardless of birth weight and gestational age. Mortality tends to increase in the presence of AKI regardless of the underlying disease, but this was statistically significant only for sepsis and cardiac disease (Table 3).

**Table 1. Risk factors related to AKI**

| | Acute Kidney Injury | | |
|---|---|---|---|
| **Characteristics** | **No** | **Yes** | **P** |
| Gender | | | |
| Male - no. (%) | 338 (86.2%) | 54 (13.8%) | 0.923 |
| Female - no. (%) | 245 (86%) | 40 (86%) | |
| Birth Weight | | | |
| <1000 gr - no. (%) | 66 (63.5%) | 38 (36.5%) | <0.001 |
| 1000-1500 gr - no. (%) | 133 (88.7%) | 17 (11.3%) | |
| 1500-2000 gr - no. (%) | 124 (89.9%) | 14 (11.1%) | |
| 2000-2500 gr - no. (%) | 81 (91%) | 8 (9%) | |
| >2500 gr - no. (%) | 179 (91.3%) | 17 (8.7%) | |
| Gestational Age | | | |
| <28 week - no. (%) | 54 (61.4%) | 34 (38.6%) | <0.001 |
| 28-32 week - no. (%) | 203 (87.1%) | 30 (12.9%) | |
| 33-37 week - no. (%) | 202 (92.2%) | 17 (7.8%) | |
| >37 week - no. (%) | 81 (90.5%) | 8 (9.5%) | |
| Route of Delivery | | | |
| C/S - no. (%) | 470 (86.7%) | 72 (13.3%) | 0.365 |
| NSPD - no. (%) | 113 (83.7%) | 22 (16.3%) | |
| Maternal Morbidity | | | |
| No - no. (%) | 347 (87%) | 52 (13%) | 0.442 |
| Yes - no. (%) | 236 (84.9%) | 42 (15.1%) | |

**Table 2. AKI etiology and mortality rates by age groups**

| | Age (days) | | | | |
|---|---|---|---|---|---|
| **Characteristics (n)** | **0-2** | **3-7** | **8-15** | **16-30** | **Mortality n (%)** |
| Sepsis (27) | 1 | 12 | 8 | 6 | 9 (33.3) |
| Birth Asphyxia (23) | 20 | 3 | - | - | 8 (34.8) |
| Hypovolemia (19) | 6 | 9 | 2 | 2 | 5 (26.3) |
| Congenital Heart Disease (14) | 7 | 6 | - | 1 | 7 (50) |
| Genitourinary Anomalies (5) | 4 | 1 | - | - | 2 (40) |
| Other (6) | 2 | 3 | 1 | - | 3 (50) |

**Table 3. Primary etiological and risk factors and mortality rates leading to AKI**

| Characteristics | Acute Kidney Injury | n | Mortality n (%) | P | OR |
|---|---|---|---|---|---|
| Sepsis | Yes | 27 | 9 (33.3) | <0.001 | 7.9 |
| | No | 203 | 12 (5.9) | | |
| Birth Asphyxia | Yes | 23 | 8 (34.7) | 0.619 | - |
| | No | 55 | 16 (29) | | |
| Hypovolemia | Yes | 19 | 5 (26.3) | 0.223 | - |
| | No | 25 | 3 (12) | | |
| Congenital Heart Disease | Yes | 14 | 7 | <0.001 | 5.6 |
| | No | 265 | 40 | | |
| Genitourinary Anomalies | Yes | 5 | 2 | 0.140 | - |
| | No | 15 | 1 | | |
| Birth Weight | | | | | |
| <1000 gr | Yes | 38 | 16 (42.1) | <0.324 | - |
| >1000 gr | Yes | 56 | 18 (32.1) | | |

## DISCUSSION

Acute kidney injury in newborns is generally defined by determining the urine output and serum creatinine level. However, there is no single serum creatinine value agreed upon. Different definitions are used in the literature. Mostly, increase in serum creatinine level was defined as AKI, while in some studies only the renal replacement therapy requirement was defined as AKI [10]. Recently, studies on new biomarkers have been intensified due to the disadvantages of serum creatinine level in the diagnosis of AKI. These include serum and urine neutrophil gelatinase-associated lipocalin, urine liver fatty acid binding protein, serum and urine cystatin-C, urine interleukin-18, urine aprotinin, urine netrin-1, kidney damage molecule-1, beta-2 microglobulin and osteopontin [5]. In 2013, a working group concluded Kidney Disease Improving Global Outcomes (KDIGO) criteria for the definition of AKI. According to KDIGO criteria, AKI was defined as ≥ 0.3 rise or ≥ 1.5-1.9 × rise from baseline serum creatinine level (defined as previous lowest/trough value) [27]. Due to the retrospective nature of our study, it is not possible to use the above-mentioned new biomarkers and KDIGO criteria for the diagnosis of AKI. Therefore, AKI was defined as a serum creatinine level >1.5 mg/dl or a 2-fold increase between any 2 measurements despite a serum creatinine level of <1.5 mg/dl [4]. The incidence of AKI in our study was found to be 13.9%. Moghal et al. found that the incidence of AKI was higher in critically ill patients in newborns than in other age groups [11]. However, in several studies, the incidence of AKI in critically ill newborns has been found in a wide range of 1-31%. This was interpreted as there was no consensus on the diagnosis of AKI.

When the development time of AKI were examined, it was found that AKI developed with a rate of 78.8% in the first week of life, while the most common etiological reasons were birth asphyxia, hypovolemia, CHD, GUS anomalies and drugs (aminoglycosides, indomethacin, ibuprofen), but the relationship between AKI and their effects on mortality are not well known [13-19]. In a study conducted in India, the incidence of AKI was found to be 26% in 200 newborns with sepsis and it was observed that mortality was higher in patients with AKI [20]. Birth asphyxia has been defined as the most common cause of AKI in many studies. While Aggarwal et al. found the inci-

dence of AKI to be 54% in patients with 5th minute Apgar scores of 6 and below [15], Korlowicz et al. [16] found the incidence of AKI to be 66% in term newborns exposed to severe birth asphyxia. In patients with hypoxic ischemic encephalopathy, a significant relationship was found between AKI and the severity of the neurological disease and serum creatinine level and the neurological outcome of the patients [21]. Studies investigating the incidence of AKI in newborns with CHD, Mortazavi et al. [22] found the incidence of AKI to be 21% in newborns with heart disease, whereas in another study, the incidence of AKI in congenital heart diseases was reported to vary between 5-20% [23]. The incidence of AKI was found to be 24% in premature babies of <30 weeks of age who were given indomethacin treatment due to patent ductus arteriosus [24]. It is controversial whether AKI in these patients is caused by patent ductus arteriosus induced circulatory disorder or indomethacin treatment.

There is no specific treatment for AKI that occurs in the neonatal period, underlying factors need to be treated. In postrenal AKI, the treatment is usually surgical intervention. Renal replacement therapy is the most effective modality in AKI [25].

It is known that mortality increases with acute kidney damage in neonatal period. Studies examining the relationship between mortality and AKI, the mortality rate was found between 6% and 58% depending on the underlying diseases [26]. AKI is not usually directly causing mortality, and the disease that causes death and the etiology of AKI may not be the same. Therefore, mortality rates in AKI are mostly associated with the underlying disease [10].

The limitation of the study is the AKI criteria we used. We defined AKI as serum creatinine level > 1.5 mg/dl or a 2-fold increase between any 2 measurements despite a serum creatinine level of < 1.5 mg/dl. However, KDIGO criteria defined in 2013 for the diagnose of AKI, this may cause underdiagnose and lower incidence of AKI.

## CONCLUSION

AKI is more common in critically ill newborns than in other age groups. The development of perinatal care has increased the survival rates and the incidence of AKI in critically ill newborns. However, diagnosing AKI in newborns is more difficult than in other age groups. The frequency of AKI in the neonatal period varies according to the gestational age, birth weight, severity of comorbid diseases and NICU possibilities. Most common causes of AKI in the neonatal intensive care unit are sepsis, hypovolemia, birth asphyxia, CHD, GUS anomalies and nephrotoxic drugs. It is known that mortality rates increase in newborns with acute kidney damage. On the other hand, AKI in the neonatal period can cause chronic kidney disease that requires lifelong follow-up and treatment.

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# The Relationship Between Serum Vitamin D Levels and the Severity of Headache in Children with Migraine

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## ABSTRACT

***Objective:*** *Migraine is a primary episodic headache disorder accompanied by neurological, gastrointestinal, and autonomic changes. The aim of this study is to compare vitamin D levels with the severity of headache in patients with migraine.*
***Methods:*** *A total of 108 children diagnosed with migraine were evaluated. We measured vitamin D levels and 25-hydroxy vitamin D3 with the enzyme-linked immunosorbent assay (ELISA) method. Serum vitamin D was defined as <12 ng/ml insufficiency. Serum vitamin D was defined as deficiency in those with 12-30 ng/ml. Serum vitamin D was defined as normal in those with >30 ng/ml. The severity of the headache was assessed according to the Migraine Disability Assessment Score (MIDAS).*
***Results:*** *The mean serum 25-hydroxy vitamin D3 levels of migraine patients was 16.6±5.9 ng/ml. As the level of vitamin D decreased, so the severity of the headache increased, with a higher MIDAS grade ($p<0.05$). No relationship was determined between the MIDAS grade and levels of calcium, phosphorus, and alkaline phosphatase ($p>0.05$).*
***Conclusion:*** *The severity of headache is associated with reduced serum vitamin D levels in children with migraine.*

## INTRODUCTION

Migraine is a common disease characterized by progressive and recurrent headaches in children and adolescents. Its prevalence is about 7.7% [(1)]. Its characteristic features are (i) attacks last more than 4 hours and less than 3 days without medication, (ii) headache is one-sided and throbbing, (iii) patients experience discomfort from light and sound and attacks often accompanied by nausea or vomiting, (iv) pain is moderate or severe [(2-6)].

Vitamin D deficiency is a global public health problem. The incidence of vitamin D deficiency is estimated to be 30-80% in children and adults worldwide [(2,3,7)]. Vitamin D deficiency leads to many health problems including cardiovascular diseases, autoimmune diseases, infectious diseases, diabetes mellitus, osteoarthritis, inflammatory diseases, mental and skin disorders [(8-11)]. Additionally, vitamin D deficiency has been suggested to play an important role in pathogenesis of a number of neurological diseases particularly in migraine [(12,13)].

## MATERIAL and METHODS

In this prospective cross-sectional study, 108 children with recently physician-diagnosed migraine between the ages of 3 and 15 yr were recruited from a tertiary referral pediatric neurology center between December 2017 and December 2019. The demographic data, symptoms and findings at the time of

diagnosis, frequency and duration of the headache, and the diagnostic methods of cranial magnetic resonance imaging (MRI) and electroencephalography (EEG) were recorded.

The diagnosis of migrane was based on diagnostic criteria of the International Classification of Headache Disorders-III (ICHD-III) published by the International Headache Society (IHS) in 2013 [14]. The severity of the headache was determined according to the MIDAS scale as; 0-5: Grade I, 6-10: Grade II, 11-20: Grade III, and ≥21: Grade IV [15]. The participants were assigned to two groups as those with aura and those without aura.

Subjects with any previous neurological diseases (mental retardation, seizure, epilepsy, etc.), any systemic or autoimmune diseases, any drug use (steroids, immunosuppressants, vitamin D supplements, etc.), acute/chronic infection, or hospitalization within the previous four weeks were excluded from the study.

After eight hours of fasting, blood samples were taken from all subjects. The samples were processed immediately by centrifugation at 4000 rpm at room temperature. Laboratory tests including complete blood count, serum calcium, phosphorus, alkaline phosphatase, albumin, vitamin B12 as well as liver, thyroid, and kidney function tests and vitamin D [25-hydroxy vitamin D3, 25(OH)D3] were obtained and measured by routine methods. Study groups were assigned according to serum vitamin D levels as deficiency (<12 ng/ml), insufficiency (12-30 ng/ml), and sufficiency (>30 ng/ml).

The study was approved by the Ethics Committee. Informed consent was obtained from all parents.

**Statistical Analysis**

For statistical analysis, Social Sciences Statistics Package for Windows (SPSS Inc., Chicago) 21 package program was used. Variables were expressed as mean±standard deviation, number (n), and percentage (%). Kolmogorov Smirnov test was used to determine the normal distribution of numerical variables. Student's t test or one-way analysis of variance (ANOVA) was used for the comparison of the parameters with normal distribution. Mann Whitney U-test or Kruskal Wallis test was used for the comparison of non-normally distributed parameters. Chi-square test was used for comparison of categorical variables. One-way ANOVA test was used to determine the arithmetic mean of a dependent variable between two independent groups and whether there was a significant difference. A P value of <0.05 was considered statistically significant.

## RESULTS

There were 108 patients in the study, consisting of 70 female and 38 male with an age of 11.72±3.21 years (range, 6-17 years). A family history of migraine was determined in 42 (38.9%) patients and there was a triggering factor for the pain in 86 (79.6%) patients. According to the history and neurological examination, cranial imaging was applied to 78 (63%) patients (CT to 11 patients, MRI to 67 patients) and the results of those were determined as normal. EEG was determined as normal in all patients. The frequency of headache was reported as every day in 15, 2-6 times per week in 37, once a week in 33, and 1-3 times in a month in 25 patients, respectively. According to the MIDAS grade evaluations, 31 (28.7%) patients were grouped as MIDAS grade 1, 41 (37.9%) were MIDAS grade 2, 21 (19.5%) were MIDAS grade 3, and 15 (13.9%) were MIDAS grade 4, respectively (Table 1).

The mean (±SD) level of vitamin D was 13.37±8.43 ng/ml (range, 1.34 ng/ml-35 ng/ml). The mean level of vitamin D in males was significantly higher than that of females (15.6±8.52 ng/ml vs. 12.1±8.18 ng/ml) (p=0.042). According to vitamin D status, sixty (55.6%) patients were vitamin D deficient, 38 (35.2%) were insufficient, and 10 (9.3%) were sufficient. There was a significant difference among patients with MIDAS Grade 1 and Grade 2 and those with MIDAS Grade 3 and Grade 4 in terms of vitamin D levels (p< 0.001). Also, 95.2% of the subjects with MIDAS Grade 3 and in all subjects with MIDAS Grade 4 were vitamin D deficient (Table 2). Migraine with aura was determined in 19 patients and migraine without aura in 89 patients. There was no difference between patients with aura and patients without aura in terms of the mean vitamin D levels (p=0.121) (Table 2). The rate of vitamin D deficiency in subjects with aura was 47.4% and 57.3% in subjects without

**Table 1. Comparison of vitamin D status among MIDAS grades**

| | MIDAS Grade 1 (n=31) n (%) | MIDAS Grade 2 (n=41) n (%) | MIDAS Grade 3 (n=21) n (%) | MIDAS Grade 4 (n=15) n (%) | p |
|---|---|---|---|---|---|
| Vitamin D deficiency (<12 ng/ml) | 11 (35.5) | 14 (34.1) | 20 (95.2) | 15 (100) | <0.0001 |
| Vitamin D Insufficiency(12-30 ng/ml) | 13 (41.9) | 24 (58.5) | 1 (4.8) | 0 | |
| Vitamin D Sufficiency (>30 ng/ml) | 7 (22.6) | 3 (7.3) | 0 | 0 | |

*Statistics: *Crosstabs-Chi-square. Statistically significant value: (p<0.05)*
***Abbreviations:*** *MIDAS: Migraine Disability Assesment Score*

**Table 2. Comparison of vitamin D levels among study groups**

| | Serum Vitamin D level Mean ± SD | P |
|---|---|---|
| MIDAS Grade 1 | 18.2±6.4 | 0.0001* |
| MIDAS Grade 2 | 16.0±5.7 | |
| MIDAS Grade 3 | 7.1±4.3 | |
| MIDAS Grade 4 | 4.9±1.4 | |
| Migraine with aura | 16.0±11.0 | 0.121** |
| Migraine without aura | 12.8±7.7 | |

*Statistics: *One Way ANOVA, Posthoc=Scheffe Alpha Test, **Student t test, values are given in mean (standard deviation). Statistically significant value: (p<0.05)*
***Abbreviations:*** *MIDAS: Migraine Disability Assessment Score*

**Table 3. Comparison of vitamin D status in patients with or without aura**

| | Migraine with aura n (%) | Migraine without aura n (%) | p |
|---|---|---|---|
| Vitamin D deficiency (<12 ng/ml) | 9 (47.4) | 51 (57.3) | 0.148 |
| Vitamin D Insufficiency (12-30 ng/ml) | 6 (31.6) | 32 (36) | |
| Vitamin D Sufficiency (>30 ng/ml) | 4 (21.1) | 6 (6.7) | |

*Statistics: Crosstabs-Chi-square. Statistically significant value p<0.05*

aura (Table 3). There was no correlation between the scores of MIDAS and serum levels of calcium, phosphorus, and alkaline phosphatase (p=0.574, p=0.148, p=0.893, respectively).

## DISCUSSION

In present study, we found a significant negative relation between the serum levels of vitamin D and severity of migraine. The role of vitamin D in bone mineralization diseases is well defined, also low vitamin D levels may be related to non-specific pain and non-inflammatory skeletal myopathy [16-19]. Many studies reported that low vitamin D levels may be associated with headache [20-22]. In a cross-sectional study of 11.614 subjects, Kjærgaard et al. demonstrated a significantly low level of serum 25(OH)D3 in non-migraine headaches [23]. In another cross-sectional study, lower levels of serum 25(OH)D3 were measured in patients with musculoskeletal pain, fatigue, and headache [24,25]. Prakash et al. found that chronic tension headaches improved with vitamin D and calcium supplements in patients with vitamin D deficiency and osteomalacia [20].

Several studies reported vitamin D deficiency or insufficiency in migraine subjects, while many other studies showed normal levels of vitamin D [8,26]. In a study, authors found a higher serum vitamin D level (50-100 ng/mL) was associated with a lower odds ratio of migraine headaches than those with low serum vitamin D levels (<20 ng/mL) [27]. In another study, it has been reported that serum levels of both vitamin D and vitamin D receptors were lower in subjects with migraine than that of controls [8]. In addition, some studies reported no correlations between the levels of serum vitamin D and some parameters of headache such as aura, severity, and duration [28].

In a few studies, the authors found an increase in migraine attacks in the autumn and winter months and a decrease in vitamin D levels in the same period. The incidence of both migraine and vitamin D deficiency has been reported to increase at higher altitudes far from the equator [29-31]. The prevalence of childhood migraine in Turkey, height above sea level increases the frequency is higher than 2 times [32,33]. The patients in the current study lived in a region of the lowest altitude in Turkey. Prakash et al. showed that there is a relationship between altitude and headache [34]. A similar study found an association between vitamin D deficiency and headache [26].

In a study by Stewart et al, the relationship between severity and characteristics of headache and the MIDAS grade was examined [15]. The MIDAS grades of patients aged <25 years were found to be significantly higher and the MIDAS scale was reported to be reliable. In the current study, it was determined that as the vitamin D level decreased, so there was a significant increase in the severity of the headache. Bıçakçı et al studied university students with migraine and determined severity of MIDAS grade 1 in 49%, MIDAS grade 2 in 19.3%, MIDAS grade 3 in 29.1% and MIDAS grade 4 in 9.7%. In the same study, the rate of migraine without aura was determined as 6%. The low rates were attributed to denial of headaches by university students [35]. In the current study, these rates were higher. On the contrary, when MIDAS grades were evaluated, there was no statistically significant difference between those with migraine with aura and those with migraine without aura.

## CONCLUSION

In the present study we showed that the severity of headache is associated with reduced serum vitamin D levels in children with migraine. The strongest connection reported to date is between serum vitamin D levels and migraine headaches; but our results need to be confirmed by large-scale population based studies.

**Ethics Committee Approval:** Approval was obtained from Kahramanmaras Sutcu Imam University Faculty of Medicine Clinical Research Ethics Committee (22/11/2017/198).

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# Kohler's Disease: Acute Foot Pain and Limping in Pediatric Emergency Department

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## ABSTRACT

*Kohler's disease is a rare cause of limping in children which is hypothetically related with impaired blood supply of navicular bone by the compression of other tarsal bones. The clinical manifestations are pain and swelling in the midfoot and limping with typical steps on the lateral aspect of affected foot. As it is an elusive entity for physicians, the lack of clinical suspicion leads to utilization of advanced diagnostic tools and delayed diagnosis. Here we present a case of Kohler's disease as a 5 years-old girl with acute foot pain and limping.*

## INTRODUCTION

The limping is a frequent symptom in children who admitted to pediatric emergency department (PED) [1-3]. Due to broad spectrum of underlying causes, facing a limping child can be a challenge for physicians. Although the vast majority of final diagnoses were transient synovitis, by the concern about missing out more serious disorders such as septic arthritis and malignancies physicians usually appeal to laboratory tests or imaging methods despite their mostly worthless effects [1,4-6]. But the key part of diagnosis is detailed history combined with a systematic examination, even for uncommon conditions. Kohler's disease (KD) is one of the rare cause of gait abnormality in children which can be easily distinguished by clinical suspicion [1,3,7-9]. Thus we present a case of KD with an aim of pointing out the clinical and radiological clues for diagnosis.

## CASE REPORT

A 5-year-old girl admitted to PED with limping and right foot pain for two days. Pain was unresponsive

to appropriate doses of ibuprofen and she refused to walk on last day due to the exacerbation of pain by weight-bearing. Her parents denied any fever, recent or chronic illness, overuse or trauma, even trivial foot injuries. The vital signs were appropriate for her age. Even though she had taken 10 mg/kg of ibuprofen 2 hours before admission, physical examination revealed tender to palpation and swelling at the dorsomedial side of her right foot and antalgic gait with steps on lateral side of the right foot. But, there was no warmth, redness, abrasion or ecchymosis and no abnormal neurological finding. The mobility of her hips and ankles was normal and painless. The radiographs indicated a flattened, fragmented and sclerotic navicular bone of right foot which suggested KD (Fig 1). The orthopedic consultant gave information about KD to her parents and recommended avoidance of weight-bearing and short course administration of ibuprofen for pain relief. Short leg walking cast was applied for immobilization to decrease the duration of symptoms. At the follow-up visits the patient's pain and limping regressed after four weeks.

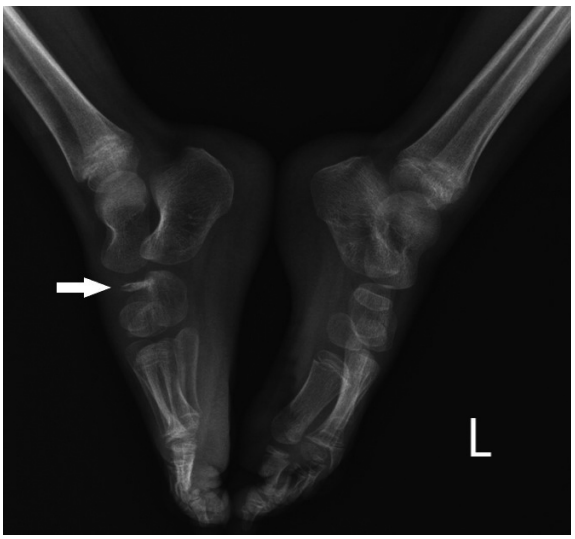


**Figure 1.** X-ray image of the flattened, fragmented and sclerotic navicular bone (white arrow)

## DISCUSSION

Osteochondrosis is the degeneration of growing bones related with the interruption of the blood supply by unknown etiology. The clinical manifestations are pain and disability on the affected part of extremity which are usually hip, knee or foot [8]. Kohler's disease is a scarce one involved navicular in the foot [7,8,10,11]. As reported in our 5 years-old patient, typically it appears between 2-10 years old and boys are affected more and later than girls [7,10,12,13]. As these epidemiological data supported, KD is likely related with the ossification of tarsal navicular that is terminated 18-24 months of age in girls and 30-36 months of age in boys [7,10,12,14]. Although pathogenesis is not clearly understood, navicular bone is the last bone to ossify so it is hypothesized that navicular bone compressed by other earlier ossified tarsal bones while the body weight of child instantly increases. Additionally, the central one third of navicular's blood supply is a watershed zone that increases the incidence of avascular necrosis. Concurrently, the interruption of the blood supply via dorsalis pedis and medial plantar arteries which maintain the perfusion of navicular bone, leads to avascular necrosis [7,14].

The characteristic features of KD are pain and swelling in the midfoot with or without redness, point tenderness localized to navicular and limping. Patients typically step on the lateral aspect of effected foot to avoid exacerbation of pain with weight-bearing on medial aspect [3,7,11]. Accordingly, our patient had tenderness on right midfoot and antalgic gait with steps on lateral side of the right foot. Although KD is usually idiopathic, can also reveal after overuse or trauma [3,7]. The plain radiograph of foot is enough for diagnosis due to its characteristic radiological findings of the navicular in KD. These are flattening called "waferlike or wafer-thin", increased radiodensity related to patchy or uniform sclerosis, fragmentation due to loss of trabecular pattern [3,7,11,14]. Thus, we could easily make diagnosis of our patient due to flattened, fragmented sclerotic right navicular bone on X-ray. But it should be kept in mind that the radiological findings without relevant symptom don't indicate KD [7,14].

As the perichondrial vascular ring of navicular maintains the rearrangement of blood supply, it is a spontaneously resolving condition [14,15]. The treatment of KD is conservative with nonsteroidal anti-inflammatory drugs, rest and avoidance of weight-bearing provided by short leg walking cast, crutches, arch supports, controlled ankle motion (CAM) boots [3,7,11,15]. The symptoms completely resolve within six

weeks to 18 months, but our patient completely recovered within the shorter time period [7,12,13]. Although the duration of symptoms is related with the type and length of therapy, the long-term prognosis is independently favorable [10,12,13].

In conclusion, Kohler's disease is a rare cause of limping in children that is simply diagnosed by radiographs of the foot in case of clinical suspicion. However, the lack of awareness of the physicians physicians may result with underdiagnosis and unnecessarily utilized time-wasting diagnostic tools.

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