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# Use of Gentamicin for Childhood Brucellosis: Effect on Calcium Homeostasis and Relapse

## Çocukluk Çağı Brusellozunda Gentamisin Kullanımı: Kalsiyum Homeostazı ve Relaps Üzerine Etkileri

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**Abstract**

**Objective:** Brucellosis is a permanent health problem in many developing countries globally, and the search for simple and effective treatments is of high importance. Gentamicin, used in the treatment of brucellosis, has effects on calcium homeostasis as an extracellular calcium-sensing receptor (CaSR) agonist. In this study, it was aimed to compare post-treatment relapse rates between patients who were administered with intravenous gentamicin for 14 days and patients who were administered oral antibiotics in order to investigate the effects of gentamicin on calcium homeostasis.

**Material and Methods:** Retrospectively, a positive Rose Bengal test result in patients younger than 18 years, Wright agglutination titer ≥1/160 or *Brucella* spp. patients diagnosed with brucellosis in any of the culture samples were scanned through their files. Patients receiving oral therapy (Group 1/Regimen 1) and patients receiving intravenous therapy (Group 2/Regimen 2), complete blood count (hemogram); erythrocyte sedimentation rate; spot urinary electrolyte levels; and serum urea, creatinine, aspartate aminotransferase, alanine aminotransferase, calcium, phosphorus, magnesium, alkaline phosphatase, PTH, 25 hydroxy vitamin D3, albumin, total protein and electrolyte levels were evaluated.

**Results:** There was a statistically significance variability in serum potassium, calcium, and phosphorus levels, parathyroid hormone, 25-dihydroxyvitamin D3, and alkaline phosphatase in the patients who were administered with gentamicin. In seven of the 21 patients (33.3%) who were administered with Regimen 1, relapse/treatment failure was observed within the first six months after the treatment. There was no relapse observed in any of the 61 patients who were administered with Regimen 2.

**Öz**

**Giriş:** Bruselloz, dünya çapında birçok gelişmekte olan ülkede kalıcı bir sağlık sorunudur ve basit ve etkili tedavi arayışları büyük önem taşımaktadır. Bruselloz tedavisinde kullanılan gentamisin, hücre dışı kalsiyum algılayıcı reseptör (CaSR) agonisti olarak kalsiyum homeostazına etki eder. Gentamisinin kalsiyum homeostazı üzerindeki etkilerini araştırmak için 14 gün süreyle intravenöz (IV) gentamisin ve oral antibiyotik uygulanan hastalar arasında tedavi sonrası nüks oranlarını karşılaştırmayı amaçladık.

**Gereç ve Yöntemler:** Retrospektif olarak, 18 yaşından küçük hastalarda, Wright aglütinasyon titresi ≥1/160 veya *Brucella* spp. Kültür örneklerinden herhangi birinde bruselloz tanısı alan hastalar dosyaları üzerinden tarandı. Oral tedavi alan hastalar (Grup 1/Rejim 1) ve intravenöz tedavi alan hastalarda (Grup 2/Rejim 2) tam kan sayımı (hemogram); eritrosit sedimantasyon hızı; nokta idrar elektrolit seviyeleri; serum üre, kreatinin, aspartat aminotransferaz, alanin aminotransferaz, kalsiyum, fosfor, magnezyum, alkalin fosfataz, PTH, 25-OH D3, albümin, toplam protein ve elektrolit düzeyleri değerlendirildi.

**Bulgular:** Gentamisin verilen hastalarda serum potasyum, kalsiyum, fosfor, paratiroid hormon, 25-OH D3 ve alkalen fosfataz düzeylerinde istatistiksel olarak anlamlı değişkenlik vardı. Rejim 1 uygulanan 21 hastanın yedisinde (%33.3) tedaviden sonraki ilk altı ay içinde nüks/tedavi başarısızlığı gözlendi. Rejim 2 uygulanan 61 hastanın hiçbirinde nüks gözlenmedi.

**Sonuç:** Çocukluk çağı brusellozu tedavisinde nüks riskini ortadan kaldırmak için 14 gün intravenöz gentamisin ile 45 gün doksisiklin/trimetoprim-sülfametoksazol tedavisi uygulanabilir. CaSR agonisti olarak kul-

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**Conclusion:** Fourteen days of intravenous gentamicin with 45 days of doxycycline/trimethoprim-sulphamethoxazole treatment can be administered in order to eliminate relapse risk in the treatment of childhood brucellosis. Gentamicin, used as a CaSR agonist, may cause temporary changes in calcium homeostasis.

**Keywords:** Brucellosis, calcium-sensing receptor, child, gentamicin, relapse

**Amaç:** Çocukluk çağı brusellozunun tedavisinde nüks riskini ortadan kaldırmak için 45 günlük doksisiklin/trimetoprim-sulfametoksazol tedavisi ile birlikte 14 günlük intravenöz gentamisin uygulanabilir. CaSR agonisti olarak kullanılan gentamisin, kalsiyum homeostazında geçici değişikliklere neden olabilir.

**Anahtar Kelimeler:** Bruselloz, kalsiyum duyarlı reseptör, çocuk, gentamisin, relaps

## Introduction

Brucellosis is one of the most prevalent zoonotic bacterial infections. Human brucellosis is among the most prevalent zoonotic diseases in the world with more than 500.000 new cases per year. Brucellosis is an important public health issue in developing regions, especially in the Mediterranean region, North and East Africa, Middle East, Arabic Peninsula, Indian Peninsula, South America, and Central Asia regions. In some endemic countries, its prevalence is >10/100.000 people per year (1). It affects people from all age groups, including the pediatric population. In endemic regions, childhood brucellosis constitute approximately one-third of all cases of human brucellosis (2). There are many differences in epidemiological and clinical characteristics of brucellosis in pediatric patients (3). In newborns and pediatric patients <8 years of age, optimal treatment for brucellosis has not been clearly described. Tetracyclines are contraindicative because they permanently stain teeth and prevent bone growth, and aminoglycosides, co-trimoxazole, and rifampicin are usually the recommended medications (4).

Relapse rate is seen as an important predictor of treatment success in brucellosis. Although many studies indicate the factors increasing the probability of relapse, the best method to control relapse and the disease is still unknown. Relapse rate is dependent on the type of antibiotics used, their combinations, and treatment durations (5).

Gentamicin is more active in vitro against *Brucella* spp. compared with streptomycin. Although gentamicin (5 mg/kg/day for 7-14 days) has been administered in combination with doxycycline, the experience regarding this regimen is very limited to confirm its superiority over the use of streptomycin plus doxycycline. No study has been published comparing the results of doxycycline plus streptomycin and doxycycline plus gentamicin. Unless there is further experience regarding the use of gentamicin instead of streptomycin, optimal dose and treatment duration will remain unknown (4). Additionally, regarding duration selection for brucellosis treatment, it has been reported that gentamicin can be used for 5-14 days based on the presence of a focal disease (spondylitis or endocarditis) and underlying conditions, such as kidney failure, which may contraindicate certain antibiotic treatments (6,7). In a study conducted on childhood brucellosis, 3% of the patients administered with gentamicin for 7-10 days has experienced a relapse, whereas in another study, no patients administered with gentamicin for 5-14 days have experienced a relapse (8). Therefore, a longer duration of gentamicin use for brucellosis treatment may eliminate the relapse risk. However, there are some side effects related to gentamicin use.

Gentamicin, known as a nephrotoxic drug, affects calcium and magnesium homeostasis. Calcium-sensing receptor (CaSR) plays an important role in the protection of calcium homeostasis by regulating parathyroid hormone (PTH) secretion (9). CaSR does not only respond to calcium but also to bivalent, trivalent, and polyvalent cations (10). Thus, gentamicin is also known as a type I CaSR agonist that activates CaSR without calcium. It directly causes loss of calcium and magnesium in urine and may rarely cause clinically severe hypocalcemia or hypomagnesemia (9).

We were unable to find any reports regarding the use of gentamicin for 14 days to eliminate relapse/treatment failure in childhood brucellosis and its effects on nephrotoxicity and calcium homeostasis in the literature. Therefore, we aimed to compare the rates of relapse following treatment between 61 pediatric patients with brucellosis <18 years of age, without a focal disease or kidney function problems, who were administered with intravenous gentamicin for 14 days and 21 pediatric patients with brucellosis without focal diseases or kidney function disorders <18 years of age who were administered with oral antibiotics to investigate the effects of gentamicin.

## Materials and Methods

### Patients

This study was conducted on 82 pediatric patients who were admitted to our tertiary university hospital clinic between January 2012 and December 2019 with the diagnosis of brucellosis and administered with oral and intravenous antibiotics.

The following were used as definitive diagnosis criteria for brucellosis: being under the age of 18 and showing compatible clinical findings (fever, perspiration, arthralgia, hepatomegaly, splenomegaly, or focal disease symptoms) as well as Rose Bengal test positivity (Seromed, Türkiye) and detection of Wright agglutination titer ≥1/160 or growth of *Brucella* spp. in blood, bone marrow or tissue culture samples." Exclusion criteria were the presence of known or suspected hypersensitivity

or other contraindications against tetracyclines or aminoglycosides and severe comorbid diseases. The patients were enrolled in the study only once. The study was approved by the institutional review board of our center (2018-80576354-050-99/103), and informed consent was obtained from the legal representatives of all patients.

### Study Design

In this cross-sectional, retrospective designed study, the treatment of 21 out of the 82 patients diagnosed with brucellosis was initiated with oral antibiotics (Group 1/ Regimen 1), whereas the treatment of 61 patients was initiated with intravenous antibiotics (Group 2/ Regimen 2) according to their clinical signs an need to hospitalization based on guidelines (4).

Thus, out of the 21 patients who were administered with Regimen 1, the combination of trimethoprim-sulphamethoxazole (10 mg/kg/day) and rifampicin (20 mg/kg/day) was administered for 45 days to patients <8 years of age. Patients >8 years of age were administered a combination of doxycycline (4 mg/kg/day) and rifampicin (20 mg/kg/day) for 45 days. First of all, the patients were started on Regime 1 treatment according to guidelines. However, in the clinical follow-up, difficulty in compliance with oral treatment and frequent relapses were observed, and rifampicin suspension for patients under eight years of age was not available in pharmacies, which subsequently led clinicians to apply Regime 2 therapy to selected patients as mandatory for patient benefit.

Out of the 21 patients included in this study, seven (33.3%) patients experienced relapse/treatment failure within the first six months after treatment. Relapse diagnosis was established based on patient complaints, clinical findings, and increase in Brucella titers. Seventeen of 61 patients who were administered with Regimen 2, a combination of trimethoprim-sulphamethoxazole (10 gm/kg/day for 45 days) and gentamicin (5 mg/kg/day for 14 days) was administered due to the their age being >8. A combination of doxycycline (4 mg/kg/day for 45 days) and gentamicin (5 mg/kg/day for 14 days) was administered to pediatric patients >8 years of age.

### Relapse and Treatment Failure

Treatment failure caused by lack of efficacy was described as the continuing of any symptoms of the disease (arthritis/arthralgia, hepatosplenomegaly, fatigue, vomiting, stomach,..) and increasing the Brucella titers in the serum at the end of treatment. Relapse of brucellosis was defined as the reoccurrence of new positive blood cultures or increase in antibody titers within six months after treatment (10).

### Clinical and Laboratory Evaluation

Complete blood count (hemogram); erythrocyte sedimentation rate, spot urine electrolyte levels, and serum urea, creatinine, aspartate aminotransferase, alanine aminotransferase, calcium, phosphorus, magnesium, alkaline phosphatase, PTH, 25 hydroxy vitamin D3, albumin, total protein, and electrolyte levels were measured. Estimated glomerular filtration rate based on the Schwartz formula {eGFR= 0.413 × (height (cm)/serum creatinine (mg/dL) = mL/min/1.73 $m^2$)} was calculated at the beginning and on day 14 of the treatment and after the treatment in months one, two, three, six, nine, and 12 and when clinical symptoms reoccurred (11). Cochlear and vestibular toxicities were evaluated clinically. Our youngest patient's age was 24 months and generally was accepted that anemia is Hb <11.5 g/dL, leukopenia is leukocyte <4000/$mm^3$, leukocytosis is leukocyte >11.000/$mm^3$, thrombocytopenia is thrombocyte <150.000/$mm^3$, thrombocytosis is thrombocyte >450.000/$mm^3$, high sedimentation rate >20 mm/h, C-reactive protein positivity >0.5 mg/dL.

### Microbiological Analyses

Rose Bengal slide agglutination test (Seromed, Türkiye) was used as the screening test. Rose Bengal test antigen (*B. abortus* S99 strain) was mixed with the patient serum in this test.

Brucella IgM and IgG were measured by ELISA in patients with a negative Rose Bengal test and clinical suspicion of brucellosis (12). Blood cultures were incubated for one week using the BD BACTEC 9120 system (Becton Dickinson-Spain, Madrid, Spain). Culture positive samples were sent to the reference laboratory in our country. All isolated Brucella strains were defined as *B. melitensis*.

### Statistical Analysis

The analyses of the study were performed using SPSS 22 (IBM, Chicago, USA) package software. Frequencies, percentages and measures of central tendency (mean and median), and measures of central prevalence (standard deviation and minimum-maximum values) were used in the study. Paired samples t-test was used for statistical comparisons in the analyses, and p-values <0.05 were accepted as statistically significant.

## Results

### Analysis of the Patients

There were 74 patients with a positive Wright agglutination test above 1/160. A diagnosis of active brucellosis in the family history and brucellosis was considered clinically, but the Wright agglutination test was 1/40 in one patient and 1/80 in four patients. There were three patients with a family history and clinically suspected Brucella, with a negative Wright agglutination test but positive for Brucella IgM. *Brucella melitensis* growth was observed in the blood cultures of 15 patients (three in Group 1 and 12 in Group 2).

**Table 1.** Demographic, clinical, and laboratory characteristics of the patients with brucellosis at the beginning of the study

| | Group 1 | Group 2 | Total |
|---|---|---|---|
| Number of patients | 21 (25.6) | 61 (74.4) | 82 (100) |
| Age, mean ± SD (months) | 130.1 ± 50.8 | 132.4 ± 54.3 | 131.8 ± 53.1 |
| Age, median (minimum-maximum) (months) | 146 (22-215) | 146 (24-216) | 146 (22-216) |
| Male sex | 13 (61.9) | 42 (68.9) | 55 (67.1) |
| **Risk factor for brucellosis** | | | |
| Occupational exposure (livestock breeding) | 11 (52.4) | 41 (67.2) | 52 (63.4) |
| Use of unpasteurized milk products | 11 (52.4) | 40 (65.6) | 51 (62.2) |
| Family history | 8 (38.1) | 26 (42.6) | 34 (41.5) |
| **Clinical findings** | | | |
| Arthralgia | 12 (57.1) | 41 (67.2) | 53 (64.6) |
| High fever | 9 (42.9) | 21 (34.4) | 30 (36.6) |
| Night sweats | 2 (9.5) | 19 (31.1) | 21 (25.6) |
| Splenomegaly | 4 (19.0) | 9 (14.8) | 13 (15.9) |
| Hepatomegaly | 0 (0) | 2 (3.3) | 2 (2.4) |
| **Laboratory findings** | | | |
| Leucocyte ($mm^3$) | 6.130 ± 1.549 | 7.402 ± 2.363 | 7.076 ± 2.245 |
| Hemoglobin (g/dL) | 12.8 ± 1.8 (8.3-15.6) | 13.2 ± 1.5 (9.3-16.7) | 13.1 ± 1.6 (8.30-16.7) |
| Thrombocyte ($mm^3$) | 297 ± 70 (178-473.000) | 313 ± 100 (92-668.000) | 309 ± 94 (92-668.000) |
| CRP[1] (mg/dL) | 0.7 ± 1.2 (0.01-4.10) | 0.9 ± 1.4 (0.01-7.54) | 0.8 ± 1.3 (0.01-7.54) |
| ESR[2] (mm/hour) | 18 ± 15 (1-48) | 18 ± 14 (3-68) | 18 ± 14 (1-68) |
| AST[3] (U/L) | 54 ± 80 (16-388) | 36 ± 25 (10-191) | 40 ± 45 (10-388) |
| ALT[4] (U/L) | 47 ± 82 (10-382) | 28 ± 27 (7-190) | 33 ± 47 (7-382) |
| Median agglutination titer (range) | 1/40-1/1280 | 1/80-1/5120 | 1/40-1/5120 |

CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, AST: Aspartate aminotranspherase, ALT: Alanine aminotranspherase.

In Group 1, 13 (61.9%) patients were males, and eight (38.1%) were females, and their median age was 130.1 ± 50.8 (24-215) months. In Group 1, 17 (81%) patients were >8 years of age, and four (19%) were <8 years of age. In Group 2, 42 (68.9%) patients were males, and 19 (31.1%) were females; their mean age was 132.4 ± 54.3 (24-216) months. In Group 2, 44 (72.1%) patients were >8 years of age, and 17 (27.9%) were <8 years of age.

Demographic, clinical, and laboratory characteristics of the patients with brucellosis at the beginning of the study in both groups are shown in Table 1. It was detected that all brucellosis cases were most common during summer with a rate of 50% (n= 41), followed by spring (19.5%) (n= 16), fall (17.0%) (n= 14), and winter (13.5%) (n= 11) (Figure 1). While C-reactive protein positivity (>0.5 mg/dL) was 37.8% and aspartate aminotransferase >40 U/L was 30.5%, thrombocytopenia was only 1.2%. The number (%) of abnormal laboratory results found in all patients (Group 1 and Group 2) is shown in Table 2. Changes in urea, creatinine, sodium, chlorine, potassium and GFR levels in patients who were administered with Regimen 2 at the beginning and on the day of 14 are shown in Table 3. Serum potassium levels were higher on the day of 14 but there was no significant other parameters (Table 3). The change in biochemical and spot urine parameters playing a role in the calcium metabolism levels in patients who were administered with Regimen 2 are shown in Table 4. While serum calcium and phosphorus levels were higher, PTH and 25-OH VitD3 levels were lower on the day 14 of the treatment (Table 4). Relapse rates of the patients who were administered with Regimen 1 and Regimen 2 are shown in Table 5. Relapse/

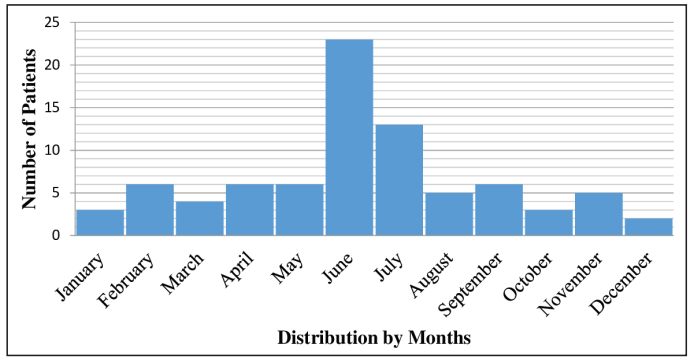


**Figure 1.** Distribution of the incidence of brucellosis by months.

**Table 2.** Abnormal laboratory results of the 82 patients from both groups

| Parameter | Number of cases (%) |
|---|---|
| Anemia (<11.5 g/dL) | 13 (15.9) |
| Leukopenia (<4000) | 6 (7.3) |
| Leukocytosis (>11.000) | 5 (6.1) |
| Thrombocytopenia (<150.000) | 1 (1.2) |
| Thrombocytosis (>450.000) | 4 (4.9) |
| High sedimentation (>20 mm/h) | 24 (29.3) |
| C-reactive protein positivity (>0.5 mg/dL) | 31 (37.8) |
| AST (>40 U/L) | 25 (30.5) |
| ALT (>40 U/L) | 17 (20.7) |
| AST: Aspartate aminotranspherase, ALT: Alanine aminotranspherase. | |

treatment failure was observed within the first six months after the treatment in seven (33.3%) out of 21 patients included in this study. There was no relapse/treatment failure in the 61 patients who received Regimen 2 (Table 5).

## Discussion

In this study, we showed that intravenous gentamicin could be administered for 14 days to inpatients to decrease or eliminate the relapse risk in the treatment of childhood brucellosis. In patients who were administered with gentamicin treatment, there was a significant increase in mean serum potassium, serum phosphorus, serum/urine calcium, and urine magnesium levels and a significant decrease in mean serum alkaline phosphatase and PTH levels on day 14 of treatment compared with the levels at the beginning of treatment.

The ability of Brucella to live inside macrophages may cause relapse or chronic disease. Relapse is not rare despite treatment with a few antibiotic regimens. It is estimated that 5-40% of the patients with acute brucellosis experience relapse till one year depending on the duration and combination of antibiotic treatment (13). Oral regimens, such as eight weeks of co-trimoxazole/doxycycline and co-trimoxazole/rifampicin, have been previously investigated. However, it has been reported that with these regimens, treatment failure and relapse occurred in 15.7-26.4% of the cases.

**Table 3.** Changes in urea, creatinine, sodium, chlorine, potassium and GFR levels in patients who were administered Regimen 2

| | Beginning, mean ± SD | On day 14 of the treatment, mean ± SD | p |
|---|---|---|---|
| Urea (mg/dL) | 25.7 ± 6.7 | 25.4 ± 6.7 | 0.741 |
| Creatinine (mg/dL) | 0.59 ± 0.15 | 0.61 ± 0.15 | 0.097 |
| Sodium (mEq/L) | 136.7 ± 3.5 | 137.1 ± 3.4 | 0.373 |
| Chlorine (mEq/L) | 101.4 ± 2.2 | 102.2 ± 3.8 | 0.412 |
| Potassium (mEq/L) | 4.2 ± 0.4 | 4.5 ± 0.4 | <0.001 |
| GFR (mL/min/1.73 $m^2$) | 105.7 ± 27 | 102.4 ± 25 | 0.115 |
| GFR: Glomerular filtration rate. | | | |

**Table 4.** The change in biochemical and spot urine parameters playing a role in the calcium metabolism levels in patients who were administered Regimen 2

| | Beginning, mean ± SD | On day 14 of the treatment, mean ± SD | p |
|---|---|---|---|
| Calcium (mg/dL) | 9.4 ± 0.8 | 9.8 ± 0.4 | **0.002** |
| Phosphorus (mg/dL) | 4.3 ± 0.9 | 5.3 ± 0.8 | **0.000** |
| ALP (U/L) | 206.9 ± 88 | 183.1 ± 73 | **0.000** |
| Magnesium (mmol/L) | 2.09 ± 0.2 | 2.03 ± 0.2 | 0.165 |
| PTH (pg/mL) | 37.02 ± 11.4 | 20.4 ± 8.2 | **0.003** |
| 25-OH $VitD_3$ (ng/mL) | 19.14 ± 8.2 | 15.3 ± 5.6 | **0.021** |
| Total Protein (g/L) | 7.5 ± 0.7 | 7.1 ± 0.6 | **0.000** |
| Albumin (g/L) | 4.2 ± 0.5 | 4.1 ± 0.3 | **0.031** |
| u.Ca (mg/dL) | 9.2 ± 7.6 | 10.7 ± 4.9 | 0.551 |
| u.Mg (mg/dL) | 4.9 ± 5.1 | 6.8 ± 9.1 | 0.327 |
| u.Ca/Cr | 0.13 ± 0.10 | 0.22 ± 0.13 | 0.063 |
| ALP: Alkalenephosphatase, PTH: Parathormone, u.Ca: Urine calcium, u.Mg: Urine Magnessium, u.Ca/Cr: Urine calsium creatinine rate. | | | |

**Table 5.** Relapse rates, blood culture growths and organ involvements in patients who were administered Regimen 1 and Regimen 2

| | Group 1/Regimen 1 | Group 1/Regimen 1 (Relapse) | Group 2/Regimen 2 | Group 2/Regimen 2 (Relapse) |
|---|---|---|---|---|
| >8 years of age (%) | 17 (81) | 6 (28.6) | 51 (73.1) | 0 (0) |
| <8 years of age (%) | 4 (19) | 1 (4.7) | 17 (27.9) | 0 (0) |
| Organ involvements | | | | |
| Hepatomegaly | 0 | 0 | 2 (3.3) | - |
| Splenomegaly | 4 (19) | 1 (14.2) | 9 (14.7) | - |
| Arthritis | 12 (57.1) | 7 (100) | 41 (67.2) | - |
| Osteomyelitis | 1 | 0 | 0 | - |
| Neurobrucellosis | 0 | 0 | 0 | - |
| Genitourinary | 0 | 0 | 0 | - |
| Other (endocarditis, colitis, etc) | 0 | 0 | 0 | |
| Blood culture growth | 3 (14.3) | 0 | 12 (19.6) | 0 (0) |
| Total number of patients (%) | 21 (100) | 7 (33.3) | 61 (100) | 0 (0) |

Shorter treatment durations with different treatment regimens have been associated with 30-40% relapse rates (14). In our study, relapse/treatment failure occurred in seven (33.3%) out of the 21 patients who were administered with oral antibiotics (Group 1), which is compatible with the findings reported in the literature. In a study comparing the use of gentamicin and streptomycin in adults, the relapse rate of the group receiving 45 days of doxycycline with seven days of gentamicin has been found as 5.2%, whereas the relapse rate of the group receiving 45 days of doxycycline with 14 days of streptomycin has been concluded as 7.4% (15). In two other studies testing the duration of use of doxycycline with gentamicin, it has been reported that gentamicin is more effective in decreasing relapse compared with streptomycin and that shortening the duration of doxycycline increases the relapse rate (16,17).

It has been reported that the relapse rate is 4.5-9% in patients administered with various antibiotic combinations for brucellosis in many countries, including Türkiye (3,19,20). Relapse has been observed in 3% of the patients with osteoarticular uptake who used gentamicin for 7-10 days for childhood brucellosis (8). In Israel, in a study conducted on a total number of 105 patients, including 58 (55.2%) pediatric patients and 47 (44.8%) adults, 90% of the pediatric patients and 64% of the adult patients have been treated by admitting to the hospital as inpatients. It has been reported that as treatment, gentamicin was administered for 5-14 days and doxycycline + rifampin or TMP-SMX was administered for six weeks and no relapses were observed (7). Based on the last two aforementioned studies, it can be stated that extending the duration of treatment in patients treated with gentamicin may eliminate relapse (7,8). Thus, to eliminate the risk of relapse, gentamicin was used for 14 days in our patients with no aminoglycoside nephrotoxicity risk factors (decrease of volume, renal failure, concomitant use of other nephrotoxic agents, long-term aminoglycoside use, and potassium and magnesium loss) (9). Although relapse occurred in seven (33.3%) of the 21 patients who were administered with Regimen 1, no relapse occurred in any of the 61 patients who were administered with Regimen 2. We believe that our study is the first study that reported such numbers with no relapse in any of the patients who were administered with Regimen 2 besides a few unorganized studies.

While there was no statistically significant difference between eGFR at the beginning of the treatment and eGFR at the end of the treatment, there was a statistically significant increase in the calcium and phosphorus levels at the end of the treatment, and there was a statistically significant decrease in PTH and alkaline phosphatase levels. In large cross-sectional population studies, average serum phosphorus levels in subjects with normal kidney functions remain quite stable at approximately 3.8 mg/dL. When the glomerular filtration rate (GFR) drops below 30 mL/min/1.73 $m^2$, serum phosphorus levels increase (20). Thus, the increase in serum phosphorus levels may be a result of the decrease in GFR (below 30 mL/min/1.73 $m^2$). However, eGFR was found to be within normal limits in our study. Thus, the increase in phosphorus levels may have been the result of a decrease in PTH levels. As in hypoparathyroidism, when there is a decrease in PTH levels, there must be a decrease in calcium levels. However, a statistically significant increase was detected in calcium levels. The increase in both calcium and phosphorus levels despite the statistically significant decrease in serum PTH levels on day 14 of the treatment compared with the levels at the beginning of the treatment can be explained with CaSR activation.

CaSR, secreted by parathyroid cells, regulates PTH secretion and controls blood calcium concentration (21). It has been reported that CaSR can specifically mimic the effect of bivalent ($Mg^{+2}$, $Cd^{+2}$), trivalent ($Gd^{+3}$, $La^{+3}$), or polyvalent (neomycin) cations and free calcium on parathyroid gland cells (22). Aminoglycosides, including gentamicin, which are polyvalent cations, are well-known strong agonists of CaSR. A decrease in serum PTH level as well as Bartter-like syndrome develops in patients treated with gentamicin (9). Therefore, gentamicin

use may have resulted in the activation of CaSR in parathyroid gland cells and decreased the levels of serum PTH in our study. Because the phosphaturic effect decreases due to the decrease of PTH levels, serum phosphorus levels may have increased. CaSR is expressed in various organs besides parathyroid glands, especially in kidneys. The ascending thick limb of the loop of Henle (LOH) plays a very important role in the renal retention of $Na^+$, $K^+$, $Cl^-$, $Ca^{++2}$ and $Mg^{++2}$, whereas the distal curved tubule (DCT) plays a part in urinary electrolyte excretion and especially in renal magnesium excretion (9). CaSR expression has been reported in DCT and connected tubule cells (22). Fast adaptation for hypercalciuria and hypomagnesuria related to the use of gentamicin has been reported. The upregulation of distal tubule transport molecules TRPV5, TRPV6, TRPM6, and calbindin-D28k occurs six hours after treatment with gentamicin. This kidney adaptation has also prevented further mineral loss related to gentamicin treatment (10). Serum PTH, 25-hydroxyvitamin D3, and albumin levels were low in our patients. It is expected/required that total serum calcium levels are lower with these low levels. Thus, higher serum calcium level on day 14 of treatment compared with the levels at the beginning of treatment despite the low PTH, 25-hydroxyvitamin D3, and albumin levels may be related to the increased activity of gentamicin with DCT and the transport molecules (TRPV5, TRPV6, TRPM6, and calbindin-D28k) in the connecting tubule cells.

It has been reported that the use of gentamicin increases CaSR expression and increases urinary fractional calcium and magnesium excretion (11,23). Gentamicin may inhibit apical NKCC2 (Na/K/Cl cotransporter) channels by activating CaSR in the basolateral membrane (blood side) of the tubular cell of the ascending thick limb of LOH. The inhibition of apical NKCC2 channels may cause an impairment in the luminal reabsorption of $Na^+$, $K^+$, $Cl^-$, $Ca^{++2}$, and $Mg^{++2}$. The exceeding concentration of these ions beyond the re-absorptive capacity of the distal tubule may cause the disruption of distal transmission and loss via kidney (9). Hence, higher urine calcium, magnesium, and calcium/creatinine levels on day 14 of treatment compared with the levels at the beginning of treatment in our study may be associated with the activation of CaSR in the parathyroid gland by gentamicin and decreasing PTH secretion and inhibition of apical NKCC2 channels.

Normally almost all potassium is reabsorbed from the proximal tubule and secreted from the distal tubule at various rates (24). The use of gentamicin may cause the production of arachidonic acid metabolites by activating CaSR, which might be the cause of statistically significant increase in the serum potassium level. Arachidonic acid metabolites may have also inhibited the apical potassium channels in the distal tubule and increased the serum potassium levels (21).

Renal tubular function impairment associated with aminoglycosides can be divided into two types. One of these is Fanconi-like syndrome of the proximal tubule damage defined by frequent increases in the serum creatinine as well as metabolic acidosis, hypophosphatemia, and glucosuria. The other is Bartter-like syndrome of the distal tubule function disorder defined by hypokalemic metabolic alkalosis, hypocalcemia, and hypomagnesemia without a significant increase in serum creatinine (9). Because the aforementioned biochemical abnormalities were not observed in our patients, Fanconi-like syndrome and Bartter-like syndrome were not considered. Because all biochemical abnormalities normalized after gentamicin was discontinued, temporary distal tubular function disorder linked to the use of gentamicin was considered in our patients.

Relapse rate after treatment depends on the types of the antibiotics used, their combination, and treatment duration (5,23). Gentamicin is more active in vitro against *Brucella* spp. compared with streptomycin. Because optimal dose and treatment duration is unknown unless further experience is gained by using gentamicin instead of streptomycin, gentamicin (5 mg/kg/day) was used for 14 days in our patients without aminoglycoside nephrotoxicity risk to eliminate the relapse risk. Although relapse occurred in seven (33.3%) of the 21 patients who were administered with Regimen 1, relapse was not observed in any of the 61 patients who were administered with Regimen 2. We believe that our study is the first study in childhood brucellosis to report the absence of relapse in any of the patients who were administered with Regimen 2.

The most important limitation of the study is the small number of patients. In addition, the study is retrospective, open-label and not controlled. However, considering the limited data and high relapse rates in childhood brucellosis, our study made significant contributions to the literature.

## Conclusion

In conclusion, intravenous gentamicin can be administered for 14 days to inpatients to decrease or eliminate the relapse risk in the treatment of childhood brucellosis. In patients administered with gentamicin treatment, there was a significant increase in the mean serum potassium, serum phosphorus, serum/urine calcium, and urine magnesium levels and a significant decrease in the mean serum alkaline phosphatase and PTH levels on day 14 of treatment compared with the levels at the beginning of treatment. The temporary increase in the mean levels of serum potassium, serum phosphorus, serum/urine calcium, and urine magnesium despite the decrease in PTH levels may be related to CaSR activation. Serum potassium, phosphorus, calcium, and PTH levels in patients must be examined due to temporary distal tubular function disorder related to the use of gentamicin. Gentamicin as a

CaSR agonist may cause temporary changes in calcium homeostasis, but studies with large population are needed.

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**Informed Consent:** Patient consent was obtained.

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