



Establishment of Age-specific Reference Intervals for Serum Magnesium Concentrations in Healthy Children Aged 0-18 Years: A Comparative Analysis of Non-parametric, Bootstrap, and Gaussian Mixture Model Approaches

0-18 Yaş Arası Sağlıklı Çocuklarda Serum Magnezyum Düzeylerinin Yaşa Özgü Referans Aralıklarının Belirlenmesi: Non-parametrik, Bootstrap ve Gaussian Mixture Model Yaklaşımlarının Karşılaştırmalı Analizi

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ABSTRACT

Objective: This study aimed to establish age-specific reference intervals (RIs) for serum magnesium (Mg) concentrations in healthy children aged 0-18 years and to compare the derived intervals with manufacturer-provided reference ranges.

Methods: This retrospective, cross-sectional study included 1,725 healthy children who presented to the Medical Biochemistry Laboratory of University of Health Sciences Türkiye, Haydarpaşa Numune SUAM between November 1, 2022, and February 18, 2023. Serum Mg was measured using the colorimetric end-point method on the Roche Diagnostics COBAS 8000 (c702 module). RIs were calculated using the non-parametric 2.5th-97.5th percentile method according to CLSI EP28-A3c and IFCC guidelines, and validated with bootstrap resampling (95% confidence interval) and Gaussian mixture model (GMM) analysis.

ÖZ

Amaç: Bu çalışma, 0-18 yaş arası sağlıklı pediatrik bireylerde serum magnezyum (Mg) düzeyleri için yaşa özgü referans aralıklarını belirlemeyi ve elde edilen sonuçları üretici firma referanslarıyla karşılaştırmayı amaçlamıştır.

Yöntemler: Retrospektif ve kesitsel olarak tasarlanan bu çalışmada, 1 Kasım 2022 ve 18 Şubat 2023 tarihleri arasında Sağlık Bilimleri Üniversitesi, Haydarpaşa Numune SUAM Tıbbi Biyokimya Laboratuvarı'na başvuran 1,725 sağlıklı çocuk değerlendirilmiştir. Serum Mg düzeyleri Roche Diagnostics COBAS 8000 (c702) analizöründe kolorimetrik end-point yöntemle ölçülmüştür. Referans aralıkları, CLSI EP28-A3c ve IFCC önerileri doğrultusunda non-parametrik yüzde 2,5.-97,5. yöntemiyle hesaplanmış; bootstrap (%95 güven aralığı) ve Gaussian mixture model (GMM) analizleriyle doğrulanmıştır.

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ABSTRACT

Results: Serum Mg concentrations differed significantly among age groups (Kruskal-Wallis, $p<0.05$). The highest median level was found in the 7-12-year group (2.21 mg/dL), and the lowest in the 13-18-year group (2.06 mg/dL). Serum magnesium concentrations increased from early to middle childhood, reaching their highest median levels at 7-12 years, followed by a decline during adolescence (13-18 years). The GMM-derived reference limits were generally comparable to those obtained using the non-parametric method. The derived age-specific reference intervals generally exhibited higher upper reference limits than the age-dependent expected values reported in the manufacturer's documentation.

Conclusion: This study provides locally derived, age-specific serum magnesium reference intervals for a Turkish pediatric population, contributing to the available evidence on population-specific reference intervals in pediatric laboratory medicine. The combined application of non-parametric, bootstrap, and GMM methods enhances methodological robustness in RI establishment. These findings emphasize the necessity of population-specific RIs and offer a foundation for developing a national pediatric RI database in Türkiye.

Keywords: Magnesium, pediatric reference intervals, non-parametric method, Gaussian mixture model, bootstrap analysis, clinical biochemistry

Öz

Bulgular: Serum Mg düzeyleri yaş grupları arasında anlamlı farklılık göstermiştir (Kruskal-Wallis, $p<0,05$). En yüksek ortalama düzey 7-12 yaş grubunda (2,21 mg/dL), en düşük ortalama düzey ise 13-18 yaş grubunda (2,06 mg/dL) saptanmıştır. Serum magnezyum düzeyleri erken çocukluktan orta çocukluk dönemine kadar artış göstererek 7-12 yaş grubunda en yüksek ortalama düzeye ulaşmış, ergenlik döneminde (13-18 yaş) ise azalmıştır. GMM ile elde edilen referans sınırları, non-parametrik yöntemle hesaplanan sınırlarla genel olarak benzerlik göstermiştir. Elde edilen yaşa özgü referans aralıklarının üst sınırları, üretici dokümanında bildirilen yaşa bağlı beklenen değerlerin üst sınırlarından genel olarak daha yüksek bulunmuştur.

Sonuç: Bu çalışma, Türkiye'deki tek merkezli bir pediatrik popülasyonda 0-18 yaş grubuna özgü serum magnezyum referans aralıklarını belirleyerek pediatrik laboratuvar tıbbında popülasyona özgü referans aralıklarına ilişkin mevcut bilimsel verilere katkı sağlamıştır. Non-parametrik, bootstrap ve GMM yaklaşımlarının birlikte kullanımı, referans aralığı belirlemede metodolojik güvenilirliği artırmaktadır. Bulgular, ulusal düzeyde pediatrik referans aralık veri tabanı oluşturulması için önemli bir temel sunmaktadır.

Anahtar Kelimeler: Magnezyum, pediatrik referans aralıkları, non-parametrik yöntem, Gaussian mixture model, bootstrap analizi, klinik biyokimya

Introduction

Magnesium (Mg) is an essential macromineral required for the maintenance of vital physiological functions and represents the second most abundant intracellular cation after potassium. Approximately 60% of the body's total Mg content is stored within bone tissue, 20% within skeletal muscle, and the remaining fraction in soft tissues such as the liver, heart, and kidneys (1). Only a small fraction of total body magnesium is present in the circulation, with approximately 55-70% of serum magnesium existing in the free ionized form, 20-30% bound to proteins (predominantly albumin), and the remainder complexed with various anions (2).

Mg acts as a mandatory cofactor for more than 300 enzymatic reactions and is intimately involved in virtually all major metabolic pathways. It plays a central role in adenosine triphosphate (ATP) synthesis and energy metabolism, nucleic acid synthesis, protein translation, glucose homeostasis, muscle contraction, cardiac excitability, neuromuscular transmission, and regulation of vascular tone (3,4). Consequently, disturbances in Mg homeostasis have been associated with a wide range of clinical conditions, including cardiovascular diseases, metabolic syndrome, type 2 diabetes, neuromuscular disorders, arrhythmias, and hypertension (1,5).

In routine laboratory practice, serum total Mg concentration remains the most commonly used biomarker. Although

serum Mg represents only a small fraction of total body Mg stores, it is widely utilized because of its practicality and applicability in clinical decision-making (6). However, serum Mg levels are influenced by numerous biological and analytical variables—including age, sex, dietary intake, hormonal status, pregnancy, comorbid conditions, medication use, and methodological differences—resulting in significant inter-laboratory variability. This heterogeneity underscores the necessity of establishing locally validated reference intervals (RIs) (7,8).

Historically, adult RIs for serum Mg have been reported as 0.75-0.95 mmol/L (1.82-2.31 mg/dL), largely based on NHANES-I data obtained in the 1970s (6). Emerging evidence has suggested that serum Mg concentrations below approximately 0.85 mmol/L may be associated with increased clinical risk or suboptimal Mg status (2,9). However, this proposed value should be interpreted as a clinical risk or adequacy threshold rather than as a statistically derived RI lower limit. In contrast, RI boundaries are established from the distribution of measurements in a defined healthy population, typically using the 2.5th and 97.5th percentiles according to Clinical and Laboratory Standards Institute (CLSI)/International Federation of Clinical Chemistry (IFCC) recommendations. In pediatric populations, Mg levels differ substantially from those in adults due to factors such as rapid somatic growth, endocrinological variation, and ongoing organ maturation. As a result, age-appropriate and population-specific RIs are essential for accurate clinical interpretation (10,11).

To address this need, the IFCC and the CLSI recommend that each laboratory establish its own RIs specific to its population, analytical platforms, and measurement procedures, as outlined in the EP28-A3c guideline (12). This recommendation is particularly critical for pediatric RI studies, where age, sex, biological variation, and analytical uncertainty must be carefully considered. Supporting this approach, the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) project has demonstrated significant age-dependent variability in numerous biochemical parameters, including Mg (10). However, despite the growing recognition of population-specific pediatric RIs, nationally validated serum Mg RI studies in the Turkish pediatric population remain limited. Most laboratories in Türkiye continue to rely on manufacturer-provided intervals or internationally derived datasets, which may not adequately reflect local demographic, ethnic, nutritional, and analytical characteristics. Therefore, establishing locally derived pediatric serum Mg RIs may contribute to improving laboratory interpretation and clinical decision-making in Turkish pediatric populations.

Given the methodological heterogeneity across laboratories and the physiological variability in pediatric age groups, the establishment of locally derived, method-specific RIs is of paramount importance. In this context, the present study aims to define age-specific RIs for serum Mg concentrations in healthy children aged 0-18 years who presented to the Medical Biochemistry Clinic of University of Health Sciences Türkiye, Haydarpaşa Numune SUAM, thereby contributing novel and clinically meaningful data to pediatric laboratory medicine.

Methods

Study Design and Ethical Approval

This study was designed as a retrospective and cross-sectional descriptive analysis. The objective was to establish age-specific RIs for serum Mg concentrations in pediatric individuals admitted to the Medical Biochemistry Clinic of University of Health Sciences Türkiye, Haydarpaşa Numune SUAM. All procedures were performed in accordance with the CLSI EP28-A3c guideline and the recommendations of the IFCC.

Ethical approval was obtained from the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences Türkiye (decision no: 2025-4/12, date: 27.02.2025). Because the study was retrospective and based exclusively on anonymized laboratory and hospital information system data, the requirement for individual informed consent was waived by the relevant ethics committee.

The study adhered to the ethical principles of the Declaration of Helsinki (2013 revision), and confidentiality of personal data was strictly maintained.

Study Population

The dataset consisted of routine biochemical test results from pediatric patients aged 0-18 years (inclusive) who

presented to the laboratory between November 1, 2022, and February 18, 2023. Age was calculated in completed years, and all participants aged 0 through 18 years were eligible for inclusion. Data were retrieved from the Laboratory Information System (LIS) using Structured Query Language queries, including age, sex, admission date, and serum Mg results. Only the first measurement per individual was included.

Clinically healthy individuals were included. Subjects with acute infection, chronic disease, metabolic or hormonal disorders, systemic illness, intensive care follow-up, or chronic medication use were excluded. Samples showing hemolysis, lipemia, or icterus interference were rejected. Individuals with pathological albumin, calcium, or creatinine levels were also excluded. Records that were incomplete, erroneous, or duplicated were removed.

Clinical health status was assessed using available hospital information system records, International Classification of Diseases (ICD)-coded diagnoses, clinical admission data, and laboratory-based exclusion criteria. Although the retrospective design precluded direct clinical examination, individuals with documented acute or chronic systemic diseases, renal, hepatic, endocrine, metabolic, or inflammatory disorders were excluded from the reference population.

A total of 1,725 pediatric samples met the inclusion criteria. Each age-specific subgroup included more than 120 individuals (0-6 years: n=512; 7-12 years: n=641; 13-18 years: n=572), satisfying the minimum sample size recommendation for the non-parametric estimation of reference intervals according to CLSI EP28-A3c guidelines.

Sample Collection and Biochemical Measurements

Blood samples were collected from the antecubital vein between 08:00 and 10:00 into serum separator tubes containing clot activator. After clot formation, samples were centrifuged at 3000×g for 10 minutes. Serum magnesium concentrations were measured using a colorimetric end-point assay based on the formation of a magnesium-xylidyl blue complex on the cobas 8000 c702 analyzer (Roche Diagnostics, Mannheim, Germany). Measurements were performed according to the manufacturer's reagent instructions, and results were expressed in mg/dL (13). Internal quality control was performed using two levels of PreciControl ClinChem Multi control materials (Roche Diagnostics GmbH, Mannheim, Germany). External quality assessment was conducted through the Randox International Quality Assessment Scheme (RIQAS; Randox Laboratories Ltd., Crumlin, United Kingdom) (14,15). The analytical coefficient of variation (CVa) for serum magnesium measurements was 1.38%, indicating low analytical imprecision under the laboratory's routine quality control conditions.

Data Processing and Quality Control

During preprocessing, incomplete or inconsistent records were excluded. Potential outliers were identified using the interquartile range (IQR) method, with lower and upper fences defined as $Q1 - 1.5 \times IQR$ and $Q3 + 1.5 \times IQR$, respectively. The application of these criteria and the number of observations excluded from each age group should be reported to clarify their potential impact on the estimated reference limits. Distribution characteristics were evaluated using histograms and boxplots, and normality was assessed via the Kolmogorov-Smirnov test.

Age Group Stratification

Because pediatric Mg levels vary with growth, hormonal maturation, and developmental physiology, the population was divided into three age groups:

- 0-6 years: early childhood,
- 7-12 years: middle childhood,
- 13-18 years: adolescence.

Age-specific partitioning was evaluated using the Harris-Boyd and Lahti approaches. Sex-specific partitioning within the predefined age groups was assessed according to CLSI/IFCC recommendations using the Harris-Boyd criteria. Although minor differences in serum magnesium concentrations were observed between males and females during adolescence, separate sex-specific reference intervals were not established because the available findings did not provide sufficient evidence to support further partitioning.

Determination of RIs

RIs were calculated using non-parametric methods according to CLSI EP28-A3c and IFCC guidelines. The 2.5th and 97.5th percentiles were estimated for each age group. Bootstrap resampling was used to estimate 95% confidence intervals (CIs) for the lower and upper non-parametric reference limits within each age group. Gaussian mixture model (GMM) analysis was performed to evaluate potential multimodality in serum Mg distributions. The optimal number of mixture components for each age group was determined using the Bayesian information criterion (BIC), and the model with the lowest BIC value was selected as the best-fitting model. GMM analysis was performed to explore the distributional characteristics of serum magnesium concentrations within each age group. The number of mixture components was selected using the BIC. The resulting model-based reference limits were descriptively compared with the corresponding non-parametric reference limits. When required, Box-Cox transformation improved distribution symmetry.

Quantile regression analysis was performed to evaluate continuous age-related changes in serum Mg concentrations across the 2.5th, 50th, and 97.5th percentiles without assuming Gaussian distribution. Quantile regression coefficients and

pseudo-R² values were estimated to characterize age-related changes in serum magnesium concentrations at the 2.5th, 50th, and 97.5th percentiles. The estimated coefficients and model fit statistics are presented in Supplementary Table 1 (16,17).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA), Python version 3.11 (Python Software Foundation, Wilmington, DE, USA), and R version 4.4.2 (R Foundation for Statistical Computing, Vienna, Austria). One-way analysis of variance (ANOVA) with Tukey post-hoc testing was used for normally distributed variables; Kruskal-Wallis and Dunn tests were applied otherwise. Bonferroni correction was applied to account for multiple pairwise comparisons. Effect sizes were assessed using eta-squared (η^2) for analysis of variance and the rank-based effect size (r) for non-parametric pairwise comparisons. A p-value <0.05 indicated statistical significance. RIs obtained in this study were compared with manufacturer-provided pediatric values (Roche Diagnostics).

Ethical Considerations

All analyses were performed retrospectively using anonymized data. The study fully complied with the ethical standards of the Declaration of Helsinki (2013 revision).

Results

A total of 1,725 healthy pediatric individuals who met the inclusion criteria and presented to the Medical Biochemistry Clinic of University of Health Sciences Türkiye, Haydarpaşa Numune SUAM between November 1, 2022, and February 18, 2023, were included in the analysis. The mean age of participants was 9.8 ± 4.6 years, with a sex distribution of 52.4% male and 47.6% female.

Serum Mg concentrations exhibited a right-skewed distribution, and the Kolmogorov-Smirnov test indicated a statistically significant departure from normality ($p < 0.05$). Given the distributional characteristics of the data, age-specific reference intervals were estimated using the non-parametric 2.5th and 97.5th percentile method in accordance with CLSI EP28-A3c recommendations.

Serum Mg Levels by Age Group

Participants were stratified into three age groups based on developmental physiology:

- Group 1: 0-6 years ($n=512$),
- Group 2: 7-12 years ($n=641$),
- Group 3: 13-18 years ($n=572$).

The Kruskal-Wallis test indicated an overall statistically significant difference in serum magnesium concentrations among the three age groups ($p < 0.05$). Descriptively, the highest median serum magnesium concentration was

Table 1. Age-specific pediatric serum magnesium reference intervals determined using non-parametric percentiles with 95% bootstrap confidence intervals (CIs) for the lower and upper reference limits

Age group	n	Lower limit (2.5 th) (CI)	Upper limit (97.5 th) (CI)	Median
0-6	512	1.79 (1.76-1.82)	2.42 (2.40-2.47)	2.15
7-12	641	1.82 (1.81-1.84)	2.48 (2.45-2.51)	2.21
13-18	572	1.74 (1.71-1.76)	2.37 (2.35-2.39)	2.06

Reference intervals were calculated using the non-parametric 2.5th-97.5th percentile method. Values in brackets indicate 95% bootstrap CIs for the lower and upper reference limits

observed in the 7-12-year age group (2.21 mg/dL), followed by the 0-6-year group (2.15 mg/dL), whereas the lowest median was observed in the 13-18-year group (2.06 mg/dL). These findings indicate an age-related variation in serum magnesium concentrations, although the overall Kruskal-Wallis result does not establish which individual age groups differ significantly.

RI Determination

Following CLSI EP28-A3c and IFCC recommendations, RIs were established using the non-parametric 2.5th-97.5th percentile method. The lower and upper reference limits, their corresponding 95% bootstrap CIs, and median values are presented in Table 1.

GMM and Bootstrap Analysis

GMM analysis was performed to examine the distributional characteristics of serum magnesium concentrations within each age group. The resulting GMM-derived reference limits are presented alongside the non-parametric reference limits in Table 2. GMM-derived reference limits were generally comparable to those obtained using the non-parametric method, with relatively small differences in the estimated lower and upper reference limits across age groups.

Quantile regression analysis was used to evaluate age-related changes in serum magnesium concentrations across the 2.5th, 50th, and 97.5th percentiles. The estimated trajectories suggested relatively stable magnesium concentrations during early childhood, followed by lower concentrations during adolescence. These patterns should be interpreted in conjunction with the estimated regression coefficients and their associated statistical uncertainty.

Figure 1 presents the quantile regression curves for serum Mg concentrations according to age. The 2.5th, 50th, and 97.5th percentile trajectories demonstrate relatively stable Mg levels during early childhood, followed by lower serum Mg concentrations during adolescence.

Comparison with Manufacturer RIs

The Roche Diagnostics Magnesium Gen.2 reagent documentation provides age-dependent expected values for serum magnesium, including 1.7-2.3 mg/dL for children aged 5 months to 6 years, 1.7-2.1 mg/dL for those aged 6-12 years, and 1.7-2.2 mg/dL for those aged 12-20 years

(13). In the present study, the upper reference limits were 2.42 mg/dL, 2.48 mg/dL, and 2.37 mg/dL for the 0-6, 7-12, and 13-18-year age groups, respectively. These limits were higher than the upper expected values reported in the manufacturer's documentation for the corresponding pediatric age ranges. However, because the manufacturer's age categories do not fully align with those used in the present study, direct age-matched comparisons were not possible. The observed differences may reflect population-specific biological variation, differences in reference population selection, and preanalytical factors. Therefore, these findings should be interpreted descriptively, and the relative contributions of these factors cannot be determined from the present data.

Overall Assessment

Serum Mg levels showed marked variability across pediatric developmental stages. The decline during adolescence may reflect increased growth velocity, hormonal changes, and dietary variations. The observed differences between manufacturer-provided and locally derived reference intervals highlight the importance of evaluating population-specific reference intervals in clinical laboratories. This study provides age-specific local RIs for serum Mg in healthy children aged 0-18 years and demonstrates the complementary value of non-parametric, bootstrap, and GMM methods in RI studies.

Discussion

In this study, age-specific RIs for serum Mg concentrations in healthy children aged 0-18 years were established using non-parametric, bootstrap, and GMM approaches in accordance with CLSI EP28-A3c and IFCC recommendations and were subsequently compared with manufacturer-provided intervals (8). Our findings demonstrate significant age-related differences in serum Mg concentrations, with the highest median concentrations observed during middle childhood and relatively lower values during adolescence. Given the central role of Mg in ATP-dependent reactions, nucleic acid and protein synthesis, muscle contraction, neuromuscular transmission, and intracellular signaling, reliable pediatric RIs are essential for the accurate identification and clinical interpretation of hypo- and hypermagnesemia (1,3,4).

The observed age-dependent differences are biologically plausible. Relatively higher serum Mg concentrations

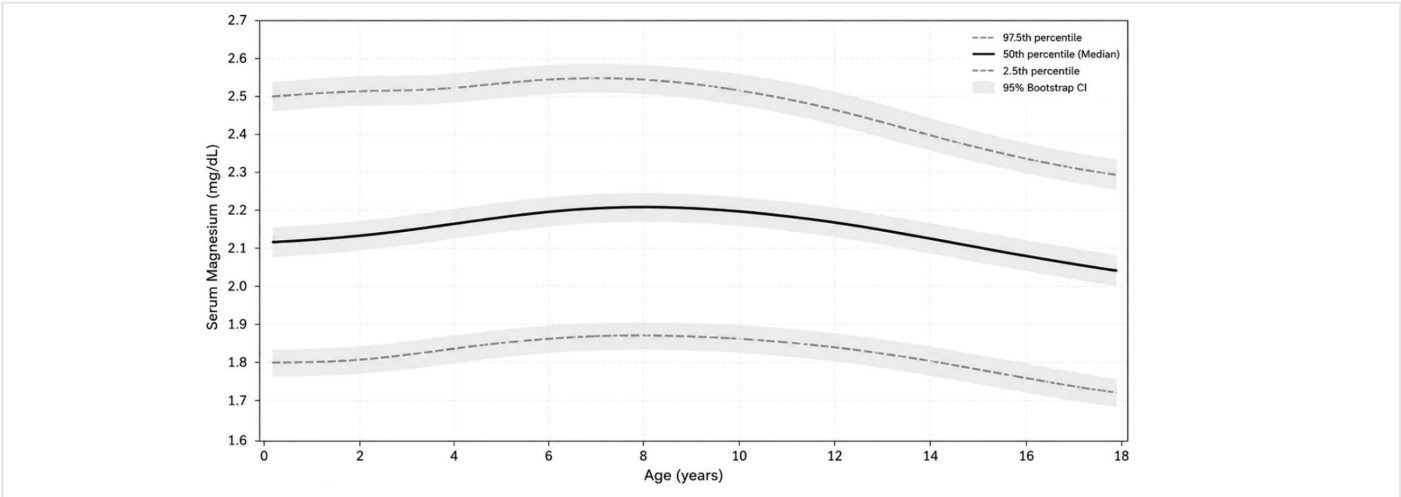


Figure 1. Estimated age-related trajectories of serum magnesium concentrations at the 2.5th, 50th (median), and 97.5th percentiles. The curves suggest an increase in median magnesium concentrations during childhood, followed by a decline during adolescence. Shaded regions represent the reported 95% bootstrap confidence bands around the estimated quantile trajectories
CI: Confidence interval

Table 2. Descriptive comparison of non-parametric and Gaussian mixture model-derived serum magnesium reference limits across pediatric age groups

Age group	Method	Lower limit	Upper limit	Mean ± SD	Correlation (r)
0-6	Non-parametric	1.79	2.42	2.15±0.18	-
	GMM	1.78	2.44	2.16±0.17	0.95
7-12	Non-parametric	1.82	2.48	2.21±0.19	-
	GMM	1.80	2.47	2.22±0.18	0.94
13-18	Non-parametric	1.74	2.37	2.06±0.20	-
	GMM	1.72	2.36	2.05±0.19	0.93

Differences between non-parametric and GMM-derived reference limits are presented descriptively. Formal equivalence or agreement between the two estimation approaches was not established
SD: Standard deviation.

during early and middle childhood may be associated with active bone mineralization, protein synthesis, and increased energy requirements. In contrast, the relatively lower concentrations observed during adolescence may reflect pubertal hormonal changes, rapid somatic growth, sex-related physiological differences, changing dietary patterns, and increased metabolic utilization. Consistent with our findings, data from the CALIPER initiative have demonstrated substantial age- and sex-dependent variability across numerous pediatric biochemical analytes, including electrolytes and minerals (10,11).

From a methodological perspective, the non-parametric 2.5th-97.5th percentile approach was appropriate because serum Mg concentrations deviated significantly from a Gaussian distribution. Bootstrap resampling provided 95% confidence intervals for the estimated lower and upper reference limits, allowing an assessment of their sampling uncertainty. However, these confidence intervals should be interpreted as measures of statistical precision rather than as evidence of the clinical validity or external generalizability of the derived reference intervals (18). Furthermore, the

GMM-derived reference limits were generally comparable to those obtained using the non-parametric approach. However, agreement between reference limits does not independently establish unimodality, which should be evaluated based on the optimal number of mixture components selected by the BIC. The parallel application of non-parametric, bootstrap, and GMM approaches therefore strengthened the methodological reliability of the derived RIs.

Compared with the age-dependent expected values reported in the Roche Diagnostics Magnesium Gen.2 documentation, the reference intervals derived in the present study generally exhibited higher upper reference limits. Nevertheless, differences in age-group definitions and reference population characteristics should be considered when interpreting these findings. These differences may be attributable to population-specific biological characteristics, nutritional and demographic factors, preanalytical conditions, and analytical differences related to the measurement system, reagents, and calibration procedures (7). The need for age-based

partitioning, as recommended by the Harris-Boyd and Lahti approaches, was further supported by both the visual and statistical findings of the quantile regression analysis (19,20).

The principal strengths of the present study include its large sample size ($n=1,725$), low analytical imprecision ($CVa=1.38\%$), verification of analytical performance through two-level internal quality control and external quality assessment, and the simultaneous use of complementary statistical methods. Direct comparison with manufacturer-provided intervals also enhances the clinical relevance of the findings. In addition, the use of LIS-based real-world data reflects routine clinical laboratory practice and increases the practical applicability of the proposed RIs.

Collectively, these findings support the use of locally derived, age-specific, population-specific, and method-specific RIs to improve the interpretation of serum Mg measurements and clinical decision-making in pediatric laboratory medicine.

Study Limitations

The present study has several limitations. First, its retrospective design precluded the direct clinical examination of participants and limited the availability of detailed information regarding several preanalytical and biological variables that may influence serum Mg concentrations, including fasting status, dietary Mg intake, hydration status, and physical activity. Although individuals with abnormal serum albumin concentrations were excluded, variations in albumin levels within the reference range may still have influenced total serum magnesium concentrations, as approximately 20-30% of circulating magnesium is protein-bound, predominantly to albumin.

Second, the study was conducted at a single tertiary-care center, which may limit the generalizability of the findings to the broader pediatric population. Referral-based hospital populations may include a greater proportion of individuals with subclinical or early-stage conditions than community-based cohorts, potentially introducing selection bias into the derived RIs. Nevertheless, extensive laboratory-based exclusion criteria, ICD-coded diagnoses, clinical admission records, and available laboratory findings were reviewed to minimize this potential effect.

Third, dietary, socioeconomic, regional, and ethnic factors could not be comprehensively evaluated. Moreover, although sex-specific partitioning was assessed, the number of participants within narrower age- and sex-specific subgroups may not have been sufficient to identify subtle sex-related differences, particularly during puberty. Therefore, prospective multicenter studies involving geographically, socioeconomically, and demographically diverse pediatric populations are required to externally validate the proposed intervals and support the

development of nationally representative pediatric serum Mg RIs for Türkiye.

Conclusion

This study establishes age-specific serum Mg RIs for healthy children aged 0-18 years using complementary non-parametric, bootstrap, and GMM approaches. The observed age-dependent pattern, characterized by the highest median concentrations during middle childhood and relatively lower concentrations during adolescence, highlights the importance of developmental stage-specific interpretation. The differences between the locally derived RIs and the manufacturer-provided values further demonstrate the clinical importance of population- and method-specific RIs. These findings provide locally derived RI data for a Turkish pediatric population and may support future multicenter studies and the development of broader pediatric RI datasets in Türkiye.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences Türkiye (decision 2025-4/12, date: 27.02.2025).

Informed Consent: Given the retrospective design of the study and the use of anonymized data obtained from laboratory and hospital information systems, the requirement for individual informed consent was waived by the Hamidiye Scientific Research Ethics Committee of the University of Health Sciences Türkiye.

Acknowledgements

Confidentiality of Data

All patient-related data were anonymized before analysis, and the confidentiality of personal information was maintained throughout the study.

Data Availability

The datasets generated and analyzed during the present study are available from the corresponding author upon reasonable request and subject to institutional and ethical restrictions concerning patient confidentiality.

Footnotes

Authorship Contributions

Concept: A.Ö., F.K., M.M.Y., E.M.G., Design: A.Ö., F.K., M.M.Y., E.M.G., Data Collection or Processing: Ö.P., Analysis or Interpretation: Ö.P., Literature Search: A.Ö., F.K., M.M.Y., E.M.G., Writing: Ö.P., E.M.G.

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