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Alkaline phosphatase: reference interval transference from CALIPER to a pediatric Brazilian population

Fosfatase alcalina: transferência de intervalos de referência do CALIPER para uma população pediátrica brasileira

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Introduction: Interpreting laboratory tests requires reference intervals (RI) that may vary between different populations. For the diagnosis of hypophosphatasia (HPP), lower limits of alkaline phosphatase (ALP) levels must be determined. **Objective:** To transfer the RI findings for ALP obtained by the Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) in children and adolescents, adjusted for the Brazilian population. **Methods:** The ALP measures from 1690 subjects (aging from 1-18 years) were analyzed. The CALIPER subgroups and the Clinical and Laboratory Standards Institute (CLSI) guideline were used for validation. Inclusion criteria were patients with normal range of hepatic and renal function, bone metabolism, and blood counts. Exclusion criteria were hospitalization, low weight, and use of drugs that could interfere in the ALP measurement and patients in with more than three orders for ALP measuring test. The RI obtained were considered valid if more than 90% of patients were whitin of the CALIPER RI. **Results:** The ALP RI results (IU/l) obtained were: 149-301 for both sexes aged 1-9 years; 127-326 for both sexes aged 10-12 years; 62-212 for girls and 129-437 for boys aged 13-14 years; 52-120 for girls and 78-268 for boys aged 15-16 years; 45-97 for girls and 40-129 for boys aged 17-18 years. In 92.4% of the patients, the results were comparable with those of the CALIPER study. **Conclusion:** The results demonstrated that the ALP RI for Brazilian children and adolescents are comparable to the CALIPER study in 92.4% of the patients and can be used for this population.

Key words: alkaline phosphatase; child; reference intervals.

INTRODUCTION

For a clinical decision regarding the establishment of a diagnosis and treatment implementation, additional tests often follow the history and physical examination of a certain patient. Clinical laboratories usually use the reference intervals (RI) provided by the manufacturers' lab kits for the interpretation of laboratory tests. However, it is necessary to keep in mind that there are several influences that can change the test results. Therefore, the RIs may not be the same when considering different sites.

RI is usually the central range of values limited by the reference limit values at certain designated percentiles, so it refers to a range of values observed in a control group defined by a specific percentage – usually the 95% central[1]. The definitions, principles, and procedures for the determination of RI were provided by the expert panel of theory of reference values (EPTRV) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the Standing Committee on Reference Values (SCRV) of the International Council for Standardization in Hematology (ICSH)[1, 2]. The settings arising from these documents were the basis of a guideline published by the Clinical and Laboratory Standards Institute (CLSI), "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory" in its 2008 review[1]. This document recommends the rules to determine, validate, or transfer RIs from other laboratories. According to the CLSI, when a valid RI study has already been conducted, it is possible to transfer it if the population and laboratory methods are comparable. This procedure is especially recommended for populations in which there are restrictions or difficulties for sample collection, such as in children.

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Alkaline phosphatase (ALP) or orthophosphoric-monoester phosphohydrolase is a membrane-bound enzyme expressed by embryonic, primordial, and neural stem cells[3]. It is found in bone tissue, vascular endothelium, kidney tubules, apical brush border of enterocytes, biliary epithelium, airways mucosal surface, brain, and in hair follicles[4]. Four isoenzymes can be found, depending upon the site of tissue expression; different classifications include intestinal ALP (IALP), placental ALP (PALP), germ cell ALP (GCALP), and tissue-nonspecific ALP (TNSALP)[5, 6]. The last one is especially abundant and presents three isoforms relative to their presence, these are: liver, bone, and kidney-type (also called L/B/KALP)[7, 8].

The physiological function of each ALP isoform is not well defined in all tissues. The one that has a more well-defined function is the bone TNSALP isoform, which has a role in normal skeletal mineralization and is an early marker of collagen matrix synthesis[7, 9]. The other functions remain obscure, both in physiological and pathological conditions. The natural substrates for TNSALP appear to include at least three phosphocompounds: phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP)[9, 10].

The Roche platform provides assays that are widely used in many laboratories. For children and adolescents, the kit provides higher ALP reference values but not the lower ones. For the suspicion and diagnosis of diseases, if the result present low ALP (e.g., hypophosphatasia)[10, 11], laboratories must provide the acceptable lower limits as reference.

In 2013, Estey *et al*.[12] published the Canadian Laboratory Initiative in the Pediatric Reference Intervals (CALIPER) study, in which RIs for various analytes, including total ALP, were determined in Canadian children using the CLSI standards. The results were obtained by several laboratory platforms, and the results of ALP were comparable between them.

In this study, we aim to determine the ALP RI, specially the lower limits, for Brazilian children and adolescents, by transference from the previous RI obtained by the CALIPER.

METHODS

Patients

A retrospective survey of ALP carried out in a private laboratory was conducted. Patient serum specimens of 1950 children and adolescents from 1 to 18 years old, for which the ALP measurement was ordered, were selected between April 2015 and March 2016. The CLSI guideline was used to confirm whether the results obtained could be transfered according to the original study[1].

Inclusion criteria were children and adolescents between one and 18 years of age, residents in the city of Rio de Janeiro, Brazil, whose results for analytes other than ALP including liver function, bone metabolism, kidney function, and blood count tests were within the RI. No stratification by race or ethnicity was carried out due to racial miscegenation that composes the Brazilian population. Exclusion criteria were hospitalization, lack of full registration data from patients, abnormal results of other tests of liver or bone metabolism assessment, anemia (hemoglobin below the reference value for the age range), low weight, which could suggest malnutrition, use of drugs that could interfere physiologically or analytically in ALP measurement (ibuprofen, theophylline, cefoxitin, doxycycline, amphotericin B, tetracycline, antiepileptics, anticoagulants, lipid-lowering drugs, and inhibitors of bone matrix formation and resorption), and patients in which this test was ordered more than 3 times because of a greater chance of having the disease. The criteria used for low weight was that of the World Health Organization (WHO) (available at http://www.who.int/childgrowth/standards/Technical_report.pdf). Those who were ≤ 2 standard deviation (SD) in relation to the standard curve were excluded.

The percentage of children and adolescents excluded with these criteria were 22.8% of both sexes from 1 to 12 years old; older than this age, girls and boys were excluded with 13% and 6% from ages 13-14 years old, 13.3% and 15.6% from ages 15-16 years old, and 17% and 17.2% for ages 17-18 years old, respectively.

As the ALP requires several age- and sex-stratified RI, the same subgroups proposed by the CALIPER study have been used, since we intended to compare both results. The same RIs were also applied for both sexes for the ranges 1 to 9 and 10 to12 years old, and different values were established for girls and boys for the ranges 13 and 14, 15 and 16, and 17and 18 years old. Once these data were extracted from the study population by applying the proposed exclusion and partitioning criteria from the initial group of 1950 patients, 1690 were selected for the statistical study to estimate if both RIs were comparable. The groups' data are shown in **Table**.

Data collection and sampling

Blood was collected under identical specimen standard tubes that contain separator gel. The measurements were performed on the same day in serum of primary tubes after blood centrifugation at 3200 rpm for 15 min.

TABLE – General data of patients selected for the study, RI, CI of ALP in serum, and results of this Brazilian study compared to the CALIPER study

Partition of the groups		Total number of patients (*n*)	Patients excluded (*n*/%)	Patients selected (*n*)	ALP (IU/l)		CALIPER RI (IU/l)	Outsiders (*n*)	Agreement with CALIPER results (%)
Age (years)	Gender (*n*)				RI obtained	95% CI			
1-9	Both	322	32/11.3	290	149-301	(134-319)	135-320	24	91.7
10-12	Both	232	24/11.5	208	127-326	(122-401)	122-400	19	90.9
13-14	Girls	199	26/13	173	62-212	(51-246)	52-243	4	97.7
	Boys	197	12/6	185	129-437	(101-454)	109-449	18	90.3
15-16	Girls	248	33/13.3	215	52-120	(45-122)	46-110	21	90.2
	Boys	250	39/15.6	211	78-268	(62-334)	77-317		95.7
17-18	Girls	252	43/17	209	45-97	(40-99)	41-82	9	90.4
	Boys	250	43/17.2	207	40-129	(38-141)	50-142		92.3

RI: reference interval; CI: confidence interval; ALP: alkaline phosphatase; CALIPER: Canadian Laboratory Initiative in Pediatric Reference Intervals.
Patients selected: total number of patients minus patients excluded. Outsiders: number and percentage of subjects tested in the present study that fell outside 10% above or below the original reported limits.
Obs: percent difference to plus (+) or minus (-) of the results obtained in this study compared to the CALIPER study; the first percentage refers to the inferior reference value difference, and the second percentage refers to the superior reference value difference.

Biochemical data

ALP was measured by the enzymatic colorimetric method on Roche/Hitachi Cobas platform (Roche Diagnostics Australia Pty Ltd, Castle Hill, NSW, Australia), the same Roche platform and analyzer used in the definition of reference intervals by the refence study. The measuring range was 5-1200 U/l. The intra-assay percentage coefficient of variation (intra %CV) was 0.7% at concentrations of 84.3 ± 0.6 U/l, 0.5% at concentrations of 222 ± 1 U/l, and 0.3% at concentrations of 996 ± 3 U/l. The inter-assay percentage coefficient of variation (inter %CV) was 2.4% at concentrations of 92.8 ± 2.2 U/l, 1.7% at concentrations of 224 ± 4 U/l, and 0.9% at concentrations of 1025 ± 9 U/l.

Statistical analysis

The GraphPad Prism® software version 6.0 was used for the statistical analysis (GraphPad Software, Inc, California). In order to assess if ALP exhibited a Gaussian distribution, the Kolmogorov-Smirnov test was performed. Since the analyte did not exhibit a Gaussian distribution, a logarithmic transformation was used. Outlying observations were calculated using the test proposed by Dixon[13] using the ratio D/R, where D is the absolute difference between an extreme observation (large or small) and the next largest or smallest observation, and R is the range of all observations, including extremes. The method of Harris and Boyd (1990)[14] could be used to decide if it was necessary to separate the RI for gender; this method was not applied because the purpose was to reproduce the pre-established criteria of the original study. The RIs were taken as the central 95%. The RIs obtained in this study were considered valid if less than 10% of patients studied were out of the RIs of the original study[1].

RESULTS

The RIs results and 95% confidence intervals (CIs) obtained of ALP are shown in Table. The CI provides a quantitative measure of the variability of the results. As the number of patients was large enough, we achieved a good precision for the estimated RI. The comparison of this study results with the CALIPER's and the percentage difference between both RI are shown in Table.

In 92.4% of the patients, the results were comparable to those of the CALIPER.

DISCUSSION

This study aimed to transfer the RIs obtained from a Brazilian population of children and adolescents by comparing them with the previously conducted CALIPER study; this methodology was suggested by the CLSI guideline, which establishes rules to validate or transfer RIs for the same or acceptable comparable analytical system. This is a common procedure in clinical laboratories, and its application is extremely useful in populations such as those studied here because of the difficulty of selecting and obtaining blood samples in minors. We assessed this query by examining the subject population of a large laboratory and comparing the RI obtained with those of the CALIPER study.

RIs obtained by a Roche platform assay employed in the present study were age- and sex-stratified for children over 1 year of age and adolescents until 18 years old. In the original CALIPER study, ALP measured with several assays presented good RI correspondence. That included the Roche Cobas platform[12].

Under normal conditions, ALP levels are significantly higher in childhood and adolescence[15]. In childhood, the enzyme levels increase gradually and reach high values in prepuberty and puberty, because ALP is a product of osteoblast activity, and during growth it is required for bone formation[16]. At the end of puberty, such levels tend to decrease and reach lower levels in adulthood[17]. In various conditions, the serum total ALP measurement has been used in children for the routine investigation of diseases because it has a long half-life and is a marker of chronic changes[18]. Elevation of this enzyme is often associated with the presence of bone and liver diseases[19]. Damaged liver cells release increased amounts of ALP into the blood as in choledocholithiasis, biliary atresia, and viral hepatitis[5]. In rickets, increased ALP is associated with typical radiological changes[20]; it can also be increased in other diseases that affect bone metabolism but also in consolidation of fractures[21]. Some other conditions or diseases may lead to reduced levels of ALP, as in children with hypophosphatasia, malnutrition, magnesium deficiency, and severe anemia, including pernicious and aplastic anemia[5,6].

Subjects with genetic generalized deficiency of TNSALP activity present hypophosphatasia and decreased PLP metabolism – the predominant form of vitamin B6[22]. The biochemical characteristics are an increased vitamin B6 in serum and PEA in urine.The hypomineralization of the skeleton and teeth due to defective bone mineralization is the differential diagnosis of rickets. The most severe cases are lethal, and death occurs during intrauterine life or during infancy with virtually complete absence of TNSALP in all tissues[10]. Severe forms of the disease are transmitted as an autosomal-recessive trait. A mutation in the TNSALP gene confirms the diagnosis[11].

For the ALP RI, we excluded those subjects with high probability of being sick, so that a healthy population was likely to be selected. Furthermore, outliers were excluded to obtain a more homogeneous population. As the two sets of RIs may be treated in the same manner for population subclasses, we used the same partitioning criteria as the reference study. Then, we determined whether there was a significant difference in each subclass between the original and the present study. In all age groups, both for girls and for boys, the percentage of patients outside the RI was less than 10%. Therefore, it was verified that the results of the present study are suitable for acceptance.

CONCLUSION

The results of this study demonstrate that ALP RIs for Brazilian children and adolescents are comparable to the CALIPER study. Moreover, the establishment of ALP RIs can improve the health care of the pediatric population and promote comparable clinical studies among these patients.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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RESUMO

Introdução: *A interpretação de exames laboratoriais necessita de intervalos de referência (IR) que podem variar entre diferentes populações. Para o diagnóstico de hipofosfatasia, deve-se determinar limites inferiores do IR da fosfatase alcalina (FA).* ***Objetivo:*** *Transferir os resultados de IR da FA obtidos pela Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) em crianças e adolescentes, ajustados para a população brasileira.* ***Métodos:*** *Analisaram-se as dosagens de FA de 1690 indivíduos (1 a 18 anos). Subgrupos do CALIPER e diretrizes do Clinical and Laboratory Standards Institute (CLSI) foram utilizados. Os critérios de inclusão foram pacientes com função hepática, renal, exames do metabolismo ósseo e hemograma normais; já os de exclusão, hospitalização, baixo peso, uso de drogas interferentes na dosagem de FA e pacientes com mais de três solicitações de FA. Os IR seriam considerados válidos se mais de 90% dos pacientes se encontrassem dentro dos IR do CALIPER.* ***Resultado:*** *Os resultados dos IR de FA (UI/l) obtidos foram: 149-301 para ambos os sexos entre 1-9 anos; 127-326 para ambos os sexos entre 10-12 anos; 62-212 para meninas e 129-437 para meninos entre 13-14 anos; 52-120 para meninas e 78-268 para meninos entre 15-16 anos; 45-97 para meninas e 40-129 para meninos entre 17-18 anos de idade. Em 92,4% dos pacientes os resultados eram comparáveis com os do CALIPER.* ***Conclusão:*** *Os resultados demonstraram que os IR de FA para crianças e adolescentes brasileiras são comparáveis com o estudo CALIPER em 92,4% dos pacientes e podem ser utilizados para essa população.*

Unitermos: *fosfatase alcalina; criança; valores de referência.*

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