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LETTER TO THE EDITOR

Pediatric Reference Intervals for Total Antioxidant Capacity, Oxidant Status, and Ferric Reducing Antioxidant Power

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The assessment of oxidative stress biomarkers becomes important, whether for evaluation during a pathological process or for a better understanding of the antioxidant effects in the body [1]. The antioxidant status can be assessed through biomarkers such as total antioxidant capacity (TAC) and ferric reducing antioxidant power (FRAP), whereas the oxidative status can be estimated, for instance, using total oxidant Status (TOS) [2,3].

Interpreting oxidative stress markers requires defining reference values suited to the studied population, since normal ranges may vary with age, gender, geographic region, and ethnicity [4]. The absence of well-established reference intervals for specific groups, especially in pediatrics, restricts both clinical application and research comparability. Therefore, this study aims to establish reference intervals for TAC, TOS, and FRAP in healthy children from southern Brazil, thereby contributing to the expansion of pediatric reference data.

A pediatric population of 134 public school students from Santa Maria, Brazil, was enrolled. Venous blood samples were collected after 8 hours of fasting into tubes without anticoagulant. Serum was obtained by centrifugation at 2,800 rpm for 15 minutes, aliquoted, and stored at -80°C until analysis. Inclusion criteria were children whose parents/guardians signed informed consent and who assented at collection. Exclusion criteria were chronic or acute renal failure, infectious, in-

Table 1. Laboratory profile of the pediatric population evaluated.

Variable	Median (Q1 - Q3)
Age (years)	7.0 (6.0 - 7.0)
BMI (kg/m^2)	15.8 (14.7 - 18.1)
Urea (mg/dL)	23.0 (17.0 - 27.0
Creatinine (mg/dL)	0.51 (0.44 - 0.68)
AST (U/L)	28.0 (25.0 - 55.0)
ALT (U/L)	19.0 (14.0 - 25.0)
WBC (x 10^3/mm^3)	7.7 (6.3 - 9.3)
HBG (g/dL)	12.6 (12.1 - 13.1)
CRP (mg/L)	0.18 (0.08 - 0.42)
TAC (µmol Trolox Equiv/L)	540.5 (509. 3 - 585.0)
TOS (µmol H_2O_2 Equiv/L)	22.0 (19.0 - 28.0)
FRAP (µmol/L)	338.5 (297.3 - 654.0)

BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, WBC: white blood cells, HGB: hemoglobin, CRP: C-reactive protein, TAC: total antioxidant capacity, TOS: total oxidant capacity, FRAP: ferric reducing antioxidant power.

flammatory, or malignant diseases. The study was approved by the Research Ethics Committee of the Federal University of Santa Maria (protocol 3.972.052, CA EE 29792120.9.0000.5346).

The biomarkers TAC and TOS were quantified using Erel's method, with modifications in the TAC assay. TAC was measured by automated colorimetric assay at 660 nm, calibrated with Trolox [5]. TOS was measured by automated colorimetric method based on ferrous oxidation to ferric ions, calibrated with hydrogen peroxide [6]. FRAP levels were determined following Benzie and Strain's protocol, using an automated analyzer at 593 nm [7]. All analyses were performed using the BS 380 automated chemistry analyzer (Mindray, China). Reference intervals (RI) were established according to the Approved Recommendations on the Theory of Reference Values issued by the International Federation of Clinical Chemistry (IFCC) [8].

Data were analyzed using Statistica v14 and GraphPad Prism v8.2.1. Normality was assessed with the Shapiro-Wilk test. As variables were non-normal, results were expressed as medians and interquartile range, with intergroup comparisons by Mann-Whitney U test. Reference intervals for TAC, TOS, and FRAP were estimated from the 2.5th - 97.5th percentiles and $p < 0.05$ was adopted.

The study population consisted of 134 children, including 66 boys and 68 girls, aged between 5 and 11 years. The hematological parameters and biochemical markers were within the normal reference ranges for this age group, as shown in Table 1, indicating the absence of clinically significant alterations.

The reference intervals were established based on the percentages of 2.5% and 97.5% of the evaluated values. For TAC, the reference intervals derived for the pediatric population was 451.63 and 668.40 µmol Trolox Equiv/L, while for TOS the values were between 14.00 and 39.68 µmol H_2O_2 Equiv/L and finally the range for FRAP was between 239.30 and 590.83 µmol/L.

The reference intervals were estimated for TAC, TOS, and FRAP in children of both genders. For females, the observed values were TAC between 463 and 649.00 µmol Trolox Equiv/L, TOS between 14 and 40 µmol H_2O_2 Equiv/L, and FRAP between 213 and 604 µmol/L. In male participants, the reference intervals ranged from 449 to 703 µmol Trolox Equiv/L for TAC, 12 to 39 µmol H_2O_2 Equiv/L for TOS, and 238 to 597 µmol/L for FRAP. The values between sexes for each variable, however no statistically significant differences were observed in the reference intervals between males and females.

The present study established, for the first time, reference intervals for the oxidative stress biomarkers TAC, TOS, and FRAP in children, representing an important advance in the field. Defining these values is essential, as such biomarkers have been shown to contribute to the assessment of pathological processes [1]. Therefore, the availability of reference intervals specific to children provides support for both future research and clinical practice, as these biomarkers have been widely investigated in studies addressing various pathologies. In this context, the study conducted by Yilmaz et al. reported a statistically significant difference in TAC levels between children with cancer and the control group, with increased values observed in the cancer group [9]. These biomarkers have also been employed in the assessment of childhood obesity. In this context, Rowicka et al. reported that obese children exhibited higher TOS levels, whereas TAC values were significantly lower when compared to children with adequate weight [2]. Furthermore, a Brazilian study conducted by Novaes et al. identified a positive association between body fat percentage and FRAP levels, demonstrating a dose-response relationship [3].

The derivation of region-specific reference intervals enhances diagnostic accuracy, as biomarker levels are influenced by genetic, environmental, and nutritional factors [4]. Despite some limitations, including the relatively small sample size, which was insufficient to establish gender-specific reference intervals, the findings of this study offer valuable contributions to the field. Given the limited data available in existing reference databases, further research is warranted to refine and expand the established reference intervals for TAC, TOS and FRAP in children.

This study established, for the first time, pediatric reference intervals for TAC, TOS, and FRAP in healthy Brazilian children aged 5 - 11 years. These values improve the interpretation of oxidative stress biomarkers in clinical and research contexts, especially in childhood

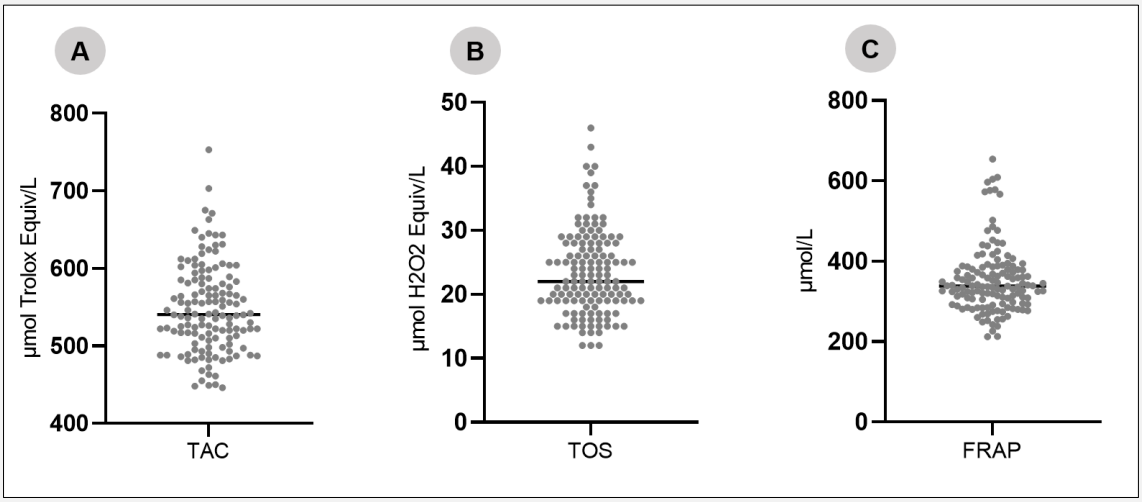

Figure 1. Distribution of oxidative stress biomarkers in the studied pediatric population.

Distribution of oxidative stress biomarkers in the pediatric population studied. A: Total Antioxidant Capacity (TAC) expressed in µmol Trolox Equiv/L, B: Total Oxidant Status (TOS) expressed in µmol H_2O_2 Equiv/L, and C: Ferric Reducing Ability of Plasma (FRAP) expressed in µmol/L. Each dot represents an individual value, and the horizontal line indicates the median.

diseases influenced by oxidative balance. The findings highlight the need for region- and age-specific reference intervals to enhance diagnostic accuracy and study comparability. Future research should validate these intervals in diverse populations to support their standardization in diagnostic protocols.

Declaration of Interest:

None.

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