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Pediatric reference intervals for transferrin saturation in the CALIPER cohort of healthy children and adolescents

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ABSTRACT

Background

Transferrin saturation reference intervals specific for age and sex have not been previously reported for the pediatric population. The reference values for transferrin saturation have been previously reported to be lower in children compared to adults, caused by a combination of low serum iron and high serum transferrin levels in children, warranting specific reference intervals.

Here we use the original iron and transferrin data from the CALIPER cohort to establish age- and sex-specific pediatric reference intervals for transferrin saturation.

Methods

Iron and transferrin concentrations were measured in serum samples from the CALIPER cohort of healthy children and adolescents on the Abbott Architect c8000. Transferrin saturation was subsequently calculated and statistically relevant age- and sex-partitions were determined.

After removing outliers, age- and sex-specific reference intervals with corresponding 90% confidence intervals were calculated using CLSI C28-A3 guidelines.

Results

Transferrin saturation required 3 separate age partitions, with an additional sex partition for 14-<19 year olds. Transferrin saturation was more variable during the first year of life, evident by a wider reference interval, which subsequently narrowed at one year until adolescence. Upon adolescence, a sex difference was apparent with females having lower percent transferrin saturation than males.

Conclusions

Age- and sex-specific pediatric reference intervals for transferrin saturation were established based on a large cohort of healthy pediatric subjects. Transference studies suggest that these intervals established using Abbott assays are comparable to those on Beckman, Ortho, Roche, and Siemens assays. Individual laboratories should however verify these reference intervals for their individual instrument and local population as per CLSI guidelines.

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INTRODUCTION

Transferrin is a plasma glycoprotein synthesized by the liver, that controls the level of free iron in the circulation. Transferrin has several functions related to iron activity and transport including rendering iron soluble, preventing iron-mediated free-radical toxicity, and facilitating iron transport into cells. One transferrin molecule can bind two Fe^{3+} ions and, together with ferritin, binds essentially all circulating plasma iron (1). The sum of these iron binding sites on transferrin, defined as the total iron binding capacity (TIBC), can be easily assessed by measuring serum transferrin concentration. The percentage of these iron binding sites occupied by iron is defined as the transferrin saturation. Determining levels of iron and TIBC, and calculating transferrin saturation are useful parameters in distinguishing between several conditions including iron deficiency, hemochromatosis, chronic illness, hemolytic anemia, and iron poisoning.

Reference intervals for these parameters are essential to correctly interpret patient laboratory test results. Traditionally, the normative range for transferrin saturation in adults has been reported as between 20-50%, with less than 20% indicating iron deficiency. However, this adult reference interval cannot be generalized to the pediatric population, as normal transferrin saturation levels have been shown to be lower in children than adults, caused by a combination of low serum iron and high serum transferrin levels in children (2). Unfortunately, in many cases pediatric laboratory test results are interpreted based on reference intervals established from an adult reference population. This is due to several challenges that are encountered in establishing pediatric reference intervals, including small sample volumes and collecting blood from a sufficient number of healthy children and adolescents to cover the extensive periods of growth and development (3).

To address these gaps, the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) program has established reference intervals for several analytes including common biochemical markers, protein markers, lipids and enzymes (4), specialty endocrine markers (5), fertility hormones (6), cancer biomarkers (7), vitamins (8), metabolic disease biomarkers (9), testosterone indices (10), and specialized biochemical markers (11). In our first publication of 40 routine biochemical markers we established age- and sex-specific reference intervals for serum iron and transferrin (4). Here we use the original iron and transferrin data from healthy children and adolescents to calculate transferrin saturation and establish age- and sex-specific pediatric reference intervals for this parameter.

METHODS

This study was approved by the Institutional Review Board at the Hospital for Sick Children, Toronto, Canada.

Participant recruitment and sample acquisition

Healthy children and adolescents (age 1 to < 19 years) were recruited from schools and community centres in the Greater Toronto Area. Samples from subjects <1 year were collected by heel stick from apparently healthy and metabolically stable subjects from outpatient clinics at the Hospital for Sick Children and Mount Sinai Hospital.

Participation in the study consisted of written informed consent, completion of a health questionnaire, anthropometric measurements (height, weight, and waist circumference) and donation of a blood sample. Prior to statistical analysis, participants were excluded if they were pregnant, had a history of chronic illness (including chronic low iron status) or metabolic disease, an acute illness within the previous month, or use of prescribed medication within the previous two weeks.

Blood samples were drawn into serum separator tubes (SST™; Becton, Dickinson and Company, Franklin Lakes, NJ, USA), centrifuged, separated and aliquoted within 4 hours of collection, and stored at –80 °C until testing.

Sample analysis

Serum samples were analyzed for iron, transferrin, and C-reactive protein (CRP) on the Abbott Architect c8000 analyzer.

Analytical methods were controlled according to the manufacturer's instructions using preventative maintenance, function checks, calibration, and quality control.

Statistical analysis and reference interval determination

Data were analyzed in accordance with Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines on defining, establishing, and verifying reference intervals in the clinical laboratory (12). Statistical analysis was performed using Microsoft Excel and R software. Transferrin saturation (%) was calculated by ((Iron (µmol/L)/Transferrin (g/L) x 25.1) x 100) (13). To determine if subjects with an elevated CRP value should be excluded from analysis, the Spearman's rank correlation coefficient between transferrin saturation and CRP concentration was calculated. Transferrin saturation and CRP were significantly negatively correlated, and therefore all subjects with an elevated CRP value (≥ 10 mg/L) were removed from the reference population. To determine if subjects taking oral contraceptives should be excluded from analysis, the Harris and Boyd method (14) was used to determine if transferrin saturation was significantly different between female adolescents using and not using oral contraceptives. If transferrin saturation was significantly different between these groups, those using oral contraceptives would be removed from the reference population. Subjects were chosen to ensure the ethnic composition of study participants was in accordance with the 2006 Canadian census data for the province of Ontario (15). The statistical approach for calculating reference intervals has been described previously (4). Briefly, scatter plots were generated to visually inspect the data and manually exclude extreme outliers. If data were not skewed, outliers were removed using the Tukey test (16). Conversely, if data were skewed, outliers were removed using the adjusted Tukey test by multiplying the interquartile range by a factor using the medcouple measure of skewness (17). The Harris and Boyd method was used to determine statistically relevant age and sex partitions (14).

Reference intervals for partitions with a sample size ≥ 120 participants were calculated using the nonparametric rank method. For partitions containing < 120 and > 40 participants, the robust statistical method of Horn and Pesce (18) was used to calculate the reference interval. For each reference limit, corresponding 90% confidence intervals were calculated.

RESULTS

Prior to calculating transferrin saturation pediatric reference intervals, the effects of CRP and oral contraceptive use on transferrin saturation values were determined. The Spearman's rank correlation coefficient between transferrin saturation and CRP concentration was -0.280 ($p < 0.0001$). As transferrin saturation and CRP were shown to be significantly (negatively) correlated, participants with an elevated CRP value (≥ 10 mg/L) were excluded. Using the Harris and Boyd method, transferrin saturation was not significantly different between female adolescents using oral contraceptives and those not using oral contraceptives. Therefore, females using oral contraceptives were not excluded from the reference population.

A total of 852 subjects had available data on both serum iron and transferrin concentrations, after excluding subjects based on the above stated criteria. Figure 1 shows a scatterplot of percent transferrin saturation plotted against age, with males shown in blue and females shown in pink. Age- and sex-specific pediatric reference intervals for percent transferrin saturation are shown in Table 1.

Transferrin saturation required 3 separate age partitions (0-<1 year, 1-<14 years, and 14-<19

Figure 1 Age- and sex-specific scatter plot of percent transferrin saturation in the CALIPER pediatric population

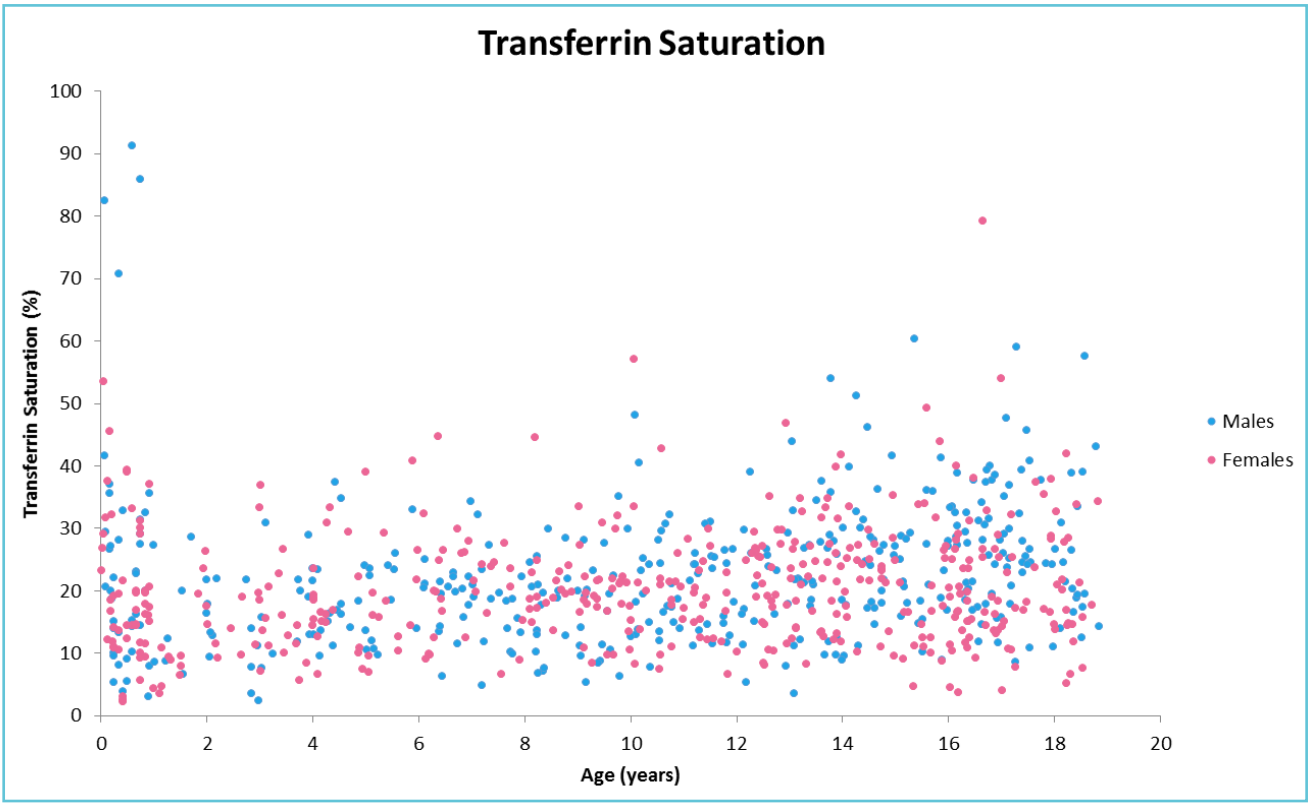

Table 1 Pediatric age- and sex-specific reference intervals for transferrin saturation

Transferrin saturation (%)	Males					
	Age range	n	Lower limit	Upper limit	Lower Confidence Interval	Upper confidence interval
	0-<1 year	92	4.1	59	(3.0, 5.3)	(51, 67)
	1-<14 years	473	6.5	39	(5.3, 7.1)	(35, 42)
	14-<19 years	136	9.6	58	(8.6, 11.7)	(43, 60)
Transferrin saturation (%)	**Females**					
	Age range	n	Lower limit	Upper limit	Lower confidence interval	Upper confidence interval
	0-<1 year	92	4.1	59	(3.0, 5.3)	(51, 67)
	1-<14 years	473	6.5	39	(5.3, 7.1)	(35, 42)
	14-<19 years	135	5.2	44	(4.6, 9.1)	(37, 54)

Underlined partition indicates sex-specific reference intervals.

years), with an additional sex partition for 14-<19 year olds. This parameter was shown to be more variable during the first year of life, evident by a wider reference interval, which subsequently narrowed at one year until adolescence. Upon adolescence, a sex difference was apparent with females having lower transferrin saturation than males.

DISCUSSION

Reference intervals for serum iron and transferrin were previously established by the CALIPER program (4). The present study is an extension to these previously determined reference intervals, by establishing age- and sex-specific pediatric reference intervals for transferrin saturation using Abbott assays.

Our transferrin saturation reference intervals demonstrated a slight sex difference, with males having higher transferrin saturation than females after 14 years of age. Previous studies also showed a higher transferrin saturation levels in male adolescents (19,20), while others showed no difference (2,21). As iron has been shown to also be higher in males, but transferrin has no apparent sex difference, the transferrin saturation sex difference is most likely a result of higher iron levels in males (4). Although transferrin saturation is statistically different between sexes when comparing the mean and standard deviation of transferrin saturation between sexes, further examination is needed to determine if this difference is clinically significant. The upper limits reported for males and females differ by 27% and the lower limits differ

by 59%. To determine if these differences are clinically significant, they must result in different clinical decisions. Unlike statistical significance, wide-spread accepted criteria to judge clinical significance is lacking and is most often based on the judgement of the clinician (22). Additionally, similar to our study, others have demonstrated a small increase in transferrin saturation with age (2,19–21). CALIPER reference values were also similar to those published most recently by Aldrimer, et al, who defined reference intervals for 6 months – 11 years and 12 – 18 years as 6.2%-41% and 6.2%-48%, respectively (21). These values aligned closely with our reference intervals, which span from 4.1%-59% transferrin saturation.

To ensure that the established transferrin saturation reference intervals sufficiently represented the healthy pediatric population, those with a CRP concentration ≥ 10 mg/L were excluded. The acute phase response down-regulates the hepatic synthesis of transferrin, therefore decreasing the TIBC, meaning there are less available transferrin binding sites for iron (19). Additionally, inflammation is accompanied by lower concentrations of iron in serum (23). As a result, transferrin saturation may also be affected by inflammation. The calculation Spearman's rank correlation coefficient between transferrin saturation and CRP concentration was -0.280 ($p < 0.0001$). Although not very strong correlation, transferrin saturation and CRP were shown to be significantly (negatively) correlated. Therefore participants with a CRP concentration ≥ 10 mg/L were excluded to ensure falsely low transferrin saturation values were not included.

Estrogen greatly affects both iron and transferrin concentrations (24). Contraceptive medication can increase transferrin levels by as much as 15% (25). Additionally, contraceptives raise serum iron concentration and after cessation of contraceptive intake, serum iron concentrations decrease by as much as 30% concurrently with uterine bleeding (26). Another study showed serum iron, TIBC, and serum transferrin levels were significantly greater in users of oral contraceptives, while transferrin saturation was not different from the control group's level (27). The stimulatory effect of estrogen on the liver's biosynthesis of protein may explain the increase in serum transferrin and TIBC levels with oral contraceptive use (28). Additionally, increased serum iron concentration as a result of contraceptive use may be due to a decrease in menstrual blood loss. Oral contraceptive use did not have a significant impact on percent transferrin saturation in our pediatric reference population. Therefore, female adolescents using oral contraceptives were not excluded from the reference population prior to calculating reference intervals for transferrin saturation.

In this study, we establish pediatric reference intervals for transferrin saturation using an Abbott platform. However, transferrin saturation values obtained using Abbott assays should also be applicable to values obtained using Beckman, Ortho, Roche, and Siemens assays. Iron and transferrin, the two values used to compute transferrin saturation, were previously successfully transferred and verified from Abbott assays to Beckman, Ortho, Roche, and Siemens assay through a series of CALIPER transference studies (29–32). To perform transference, a method comparison was performed using 200 patient samples spanning the analytical measuring range. If there was a sufficient correlation between the methods and the residuals were normally distributed, the regression line equation was used to transfer the reference interval established using the Abbott assay to each of the other manufacturer assays. The transferred reference intervals were then validated if ≥ 90% of 100 healthy pediatric samples from the CALIPER cohort fell within the transferred reference interval. Therefore, these studies suggest that these intervals established

using Abbott assays are comparable to those on Beckman, Ortho, Roche, and Siemens assays.

This study provides age- and sex-specific reference intervals for percent transferrin saturation based on a multi-ethnic Canadian population aged 0-<19 years of age. These data will improve test result interpretation for pediatric patients. Prior to implementing these reference intervals into clinical practice, they should be validated by individual laboratories for their individual instrument and local population.

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