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Hematological Parameters and Indices Reference Interval in Pediatric Egyptian Population; A Single Institution Experience

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Abstract

OBJECTIVE: Differentiating a health from a non-health state requires meticulous knowledge of the reference interval (RI). Over the past years, numerous working groups generated ethnic, age, and gender tailored RI in all laboratory tests. The paucity of regional studies covering the normal hemogram values resulted in the adoption of the western population RI which is influenced by ethnic and geographical differences.

AIM: The current study, aimed at generating the Egyptian pediatric group hemogram RI.

SUBJECTS AND METHODS: At Abu-Rish Japanese hospital, Cairo University Specialized Pediatric Hospital, the study included 1099 Egyptian surgical patients, of both sexes, with an age range between 1-month and 144 months. The electronic peripheral blood count test results archived in the laboratory information system were collected from January 2006 to December 2017 followed by a non-parametrical comparative study to compute the consecutive age groups for generation of continuous indirect RI (2.5th and 97.5th percentiles).

RESULTS: The age clusters, 2–5 months, 6–11 months 12–23 months, 24–59 months, and 60–144 months, were created based on hemoglobin (Hb), Red blood cells count, and platelet count data.

CONCLUSION: Previously published pediatric hemogram, Western and African-based, RIs show great variation. The comparative study of Hb between each month of life created different consecutive age groups than those adopted by Western countries and other African countries. The Hb results within each group showed substantial differences, between the RI of the western countries and those of Egyptian subjects specifically a lower limit of anemia diagnosis as well as a lower limit of leucopenia diagnosis. The age-specific RI we propose would change the threshold for anemia, and leucopenia diagnosis. The results might justify the use of national RI, after its validation, instead of the international ones.

Introduction

The pediatric group lags behind the adult group in regards to the established norms of the laboratory tests. This is partially caused by difficulties associated with this age group. For the pediatric population, the challenges of establishing benchmarks are related mostly to child growth and development. Another major obstacle in establishing normal intervals is the lack of healthy volunteers with infants being the least likely group to be sampled. The proposition on which the reference interval (RI) is fashioned is that subjects' values, with a non-clinically relevant disorder to the analyte in question, approximate normal values, although they were obtained for a clinical indication, not from healthy volunteers [1], [2].

The lack of Egyptian RI for a long period forced physicians to adopt those conducted through studies of other nationalities and races living in different geographical areas. However, through the work of different investigators [3], [4], [5], [6], a variation of RI across the geographical regions and ethnic groups was noticeable. The Mediterranean population is not a phenotypically homogenous group. Through different investigation modalities, e.g., Human leukocyte antigen genotyping and 3D imaging techniques, Egyptians were proved to be different, from a racial perspective, than those of white descent [7], [8].

Regional studies involving oriental, in general, and Egyptian population, in specific, are non-existent, to the best of our knowledge. The current work addressed the imperative need for generating Egyptian-oriented RI which makes it a pilot study with a secondary objective of evaluating the currently employed RI and its comparability to the collected one from an economic perspective.

Subjects and Methods

The current work was a single institution-based study of a tertiary hospital, where attendees of the outpatient pediatric surgical clinic of Cairo University Specialized Pediatric Hospital were screened during the day working hours. Protocols for sample acquisition

and processing including sampling time, sampling procedure, anticoagulant, and the instrument used were the same the whole time in which samples were recorded.

Age was defined as age in days at the date of venipuncture, for example, an age group referred to as being 2 years old included individuals from the age of 366 to 729 days old. All subjects had an International Federation of Clinical Chemistry (IFCC) defined normal routine chemical analysis (e.g., kidney function test, liver function tests, and iron profile) as well as a coagulation profile. Participants were considered consistent with the indirect Hoffmann method, that is, participants had minimal pathology relevant to the studied parameters [9].

Hematology parameters were measured with either the Abbots Cell-Dyn Ruby or Cell-Dyn 3700 analyzers, which were maintained per the manufacturer's user manual. The laboratory service employed a successful external quality control program as well as daily quality control provided by the manufacturer throughout the study period. The patient's test results archived in the laboratory information system were collected for a given time frame (from January 2006 to December 2017) without any filtering. The Kasr Al-Aini institutional review board approved the study.

Eight hematological parameters were studied; hemoglobin (Hb), red blood cells count (RBCs), hematocrit (Htc), mean corpuscular volume (MCV), mean corpuscular volume hemoglobin (MCH), MCH concentration (MCHC), white blood cells (WBCs), and platelets.

The whole study population both males and females included 1099 subjects with the age range of 1-month to 144 months with a minimum of 120 participants in each preliminary age group/subclass, to achieve the 95% confidence limits stated by the Clinical and Laboratory Standards Institute (CLSI) guidelines [10].

Statistical analysis

All calculations for determining RI were per CLSI guideline document C28-A3. SPSS(Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA), overall data were examined for normality using the Shapiro–Wilk's test and the Kolmogorov–Smirnov test with Lilliefors correction. Values were examined for outliers. The one-third rule for the D/R ratio (where the D is the absolute difference between an extreme observation and the next, while R is the range of all observations) was applied for outlier identification. However, outliers were not excluded from the data set following CLSI guidelines CA28-A3. Statistical significance was set at $P < 0.05$. Multiple linear regressions with a stepwise model were performed to explore age and sex as potentially affecting the results and so should be considered as a partitioning factor. According to a normality study conducted, most data required a non-parametrical comparative study with *post hoc* analysis between different ages in a 1-month interval was used to construct age intervals instead of the fixed age group or a qualified guessing approach. Descriptive statistics were calculated. The 2.5^{th} and 97.5^{th} percentiles were used as the lower and upper reference limits to express the data. For the probability testing, the probability density function was calculated after data transformation.

Results

Creation of continuous age groups

In the current study, 800 males and 299 females (with a frequency of 72.8 % and 27.2 % respectively) were included in the study. Regression analysis revealed that no effect of gender was found ($p > 0.05$) and therefore it was not considered during the process of group creation. In contrast, age had an impact on all parameters and thus the need for it to be a partitioning factor was crucial ($p < 0.001$). With both graphical inspection and normality statistical testing, most of the data set proved to be skewed.

In the age group 2–2 years, computation created Hb-based clustering as follows 24–59 months and 60–144 months. RBCs and platelets did not create any cluster in this subgroup. In the 1^{st} year of life age group, computation created RBCs based clustering as follows 2–5 months, 6–11 months, and 12–23 months. Hb and platelets did not create any cluster in this subgroup. The 95% confidence interval (CI) results of different age groups regarding all parameters are displayed in Table 1.

Discussion

Dynamic changes in the hematological parameters are evidently observed in pediatric subjects who reflect the need for age-specific RI [1]. Complicated by the small sample volume and scarcity of healthy volunteers, the generation of young children RI in Egypt was not addressed before. With the advancing statistical methods and data mining techniques, namely, the indirect techniques, the construction of pediatric RI was made possible [9].

Originally, the textbook adopted RIs are usually derived from a large-scale single institution recruitment study which makes geographical limitation a major

obstacle to the generalization of the data acquired [3]. This was later addressed through national programs such as Unites States National Health and Nutrition Examination Survey, the Canadian Laboratory Initiative on Pediatric RI Database and the Canadian Health Measures Survey, and the German Health Interview and Examination Survey for Children and Adolescents. However, they still reflected the Western industrialized population. Appling such recommendations to the pediatric population living in developing countries such as Egypt would pose a potential misguided medical decision. The African-based RI study conducted by Lugada *et al.* [11] would not be discussed as they had a caveat in their subject selection, namely, based their selection on HIV negativity status rather than overall health status.

Setting a country-based RI was </AQ9>among the recommendation of IFCC to address both the ethnic and the geographical effect on the different parameters which later proved to be extremely effective for the population of Nordic countries [12].

In concordance with other published studies concerning children younger than 12 years old, the current study considered age and not gender to be a major determinant of pediatric hemogram RI and accordingly was used as partitioning factors [3], [11], [13], [14], [15], [16], [17].

Age clusters from the 1st month to the 23 months did not fulfill the 120-subject criterion to be considered a reliable indicator of the RI. Each cluster included less than 120 subjects in the 6–11 months age group and 12–23 months age group. The first 24 months of life are a pilot study. The number of participants in the 1st month of life (neonates) (26 subjects) did not fulfill the CLSI criteria for the RI subgrouping limit of 120 subjects nor the 60-participant limit recommended in pilot studies, to be included in the main analysis. The neonatal period, therefore, was excluded from the analysis. Results of 1-month-old subjects are only included for demonstration. The RI utilized included 95% CI in contrast to the recommended 90% CI of CLSI to allow the comparison between the RI adopted by Egyptian clinical practice.

In the age group 2–23 months, a tendency for higher RBCs counts with aging was detected. In the age group 24 months to144 months, RBCs count and MCHC were stable while Hb, Htc, MCV, and MCH showed a significant rise with age.

In the age group 2 month-144 months, WBCs count demonstrated a significant change with age ($p < 0.001$). Applying a *post hoc* test revealed that the age group of 2–23 months of life compared to the 2–12 years age group, had a significantly higher WBCs count range ($p < 0.05$). Age did not affect platelet count throughout the childhood period from 2 months to 144 months ($p > 0.05$).

On comparing clusters created by our statistical analysis to clusters by others, namely Aldrimer *et al.* [2], Greer *et al.* [18], Adeli *et al.* [13], and Bain *et al.* [14], an exact match was not detected in most ages. Consequently, the closest cluster found was compared in the different age groups.

The 2– 5 months age group of the current study was closest to the 3–6 months age group of Greer *et al.* [18] and the 3–6 months age group of Bain *et al.* [14]. The current study agreed with both of them regarding Hb, RBCs, MCV, and MCH-specific RIs. In the same age group, considering WBCs RI, the 2–5 months age group current study had a higher 97.5th percentile (23.5×10^3/cmm) in comparison to the 3–6 months age group of Greer *et al.* [18] (15×10^3/cmm) as well as 3–6 months age group of Bain *et al.* [14] (18×10^3/cmm) in the. In the current study, only four subjects (2.8% of the group) had WBCs count greater than 17×10^3/cmm, with a probability of 0.5%, and were clinically asymptomatic. Concerning the platelet count RI in the age group 2–5 months, the current study reported a lower 2.5th percentile (140×10^3/cmm) compared to the 3–6 months age group of Bain *et al.* [14] (200×10^3/cmm). In the current study, only nine subjects (6.3 % of the group) had Platelet count lower than 200×10^3/cmm, with a probability of 0.1%, and were clinically asymptomatic.

The current study age group cluster from 6 to 11 months was most related to the 0.5–2 years group of Greer *et al*. [18]. Current work 97.5th percentile was in agreement with Greer *et al.* [18], however, showed a lower 2.5th percentile of Hb and RBCs count (10 g/dl in contrast to 10.5 g/dl). In the current study, only 12 subjects (13% of the group) had Hb lower than 10.5g/dl, with a probability of 0.5%. This age group in the current study had a wider WBCs range ($4–22.8\times 10^3$/cmm)

Table 1: The calculated hemogram reference intervals in the normal Egyptian age group of 1 month–12 years expressed as the median and the 95% range

Analyte	Median (95% range)					
	First month of life (*n*=26)	2–5 months (*n*=138)	6–11 months (*n*=92)	12–23 months (*n*=110)	2–4 years (24–59 months) (*n*=369)	5–12 years (60–144 months) (*n*=364)
Hb (g/dl)	12 (10–16)	11 (10–13.47)	11 (10–13.6)	11 (10–13.2)	12 (10–14)	12 (11–14)
Htc (%)	32.5 (27–47)	32 (28–39.5)	34 (27.8–39)	34 (29.8–39)	36 (31–42)	37 (33–43)
RBCs ($\times 10^6$/cmm)	4 (3–7)	4 (3–5)	4 (3.9–5.6)	5 (4–5.5)	5 (4–5.5)	5 (4–5.3)
MCV (fl)	89.5 (67–102)	80 (71–91.5)	75 (70.4–87.3)	76 (70–88.5)	77 (71–88.7)	79 (72–88)
MCH (pg)	32 (23–40)	27 (23–33)	25 (23–31.3)	25 (23–32)	26 (23–31.7)	26.5 (24–29)
MCHC (%)	34 (24–43)	34 (27.63–38)	33 (29.8–37)	33 (30–38.05)	34 (30–36)	33 (30–36)
WBC ($\times 10^3$/cmm)	10 (3–24)	10 (5.55–23.5)	10 (4–22.8)	10 (4–20.6)	8 (5–17)	7 (4–14)
Platelets ($\times 10^3$/cmm)	372 (122–635)	373 (140.73–588)	347 (190–629)	337 (168–558)	312 (197–550)	301 (177–500)

Hb: Hemoglobin, Htc: Hematocrit, RBCs: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cells.

compared to 0.5–2 years group of Greer *et al.* [18] (6–17.5× 10^3/cmm). Five subjects had WBCs count equal to or greater than 17.5 × 10^3/cmm (frequency of 5.4% and probability 0.05%. Nine subjects had WBCs count equal to or lower than 6 × 10^3/cmm (frequency of 9.8 %, with a probability of 0.03%).

The present work's 12–23 months age group was closest to the 1–2 years age group of Bain *et al.* [14]. Bain *et al.* [14] reported a higher Hb and RBCs 2.5th percentile (11.1 g/dl) compared to the current study (10g/dl).

In the current study, the 2.5th percentile limit for leucopenia diagnosis (4 × 10^3/cmm) was lower than that adopted by Greer *et al.* [18] (6 × 10^3/cmm) and Bain *et al.* [14] (6 × 10^3/cmm) in the age group of 12–23 months. In that age group, the present work included nine subjects with WBCs range < 6 × 10^3/cmm, frequency of 7.9 %, and were clinically asymptomatic. For the platelet count RI in the age group 12–23 months, the current study reported a lower 2.5th percentile (167 × 10^3/cmm) compared to Bain *et al.* [14] 1–2 years age group (200 x10^3/cmm).

For comparison of the current study age group 24–59 months (2–4 year of life), no exact match of the cluster was detected. The 2–6 years age group of Greer *et al.* [18] and 2–6 years age group of Bain *et al.* [14] were possible similar age groups. In the critical values of erythrocytes parameters for anemia determination (Hb and RBCs), the current study showed a lower Hb threshold for anemia diagnosis (i.e., a higher 2.5th percentile), in contrast to RI adopted by Greer *et al.* [18] and Bain *et al.* [14], 10 g/dl in contrast to 11.5, 11, and 11.1 g/dl, respectively. Twenty-three subjects with a frequency of 6.2% had Hb lower than 11g/dl with a probability of 19%. With respect to MCV, current study revealed a lower 2.5th percentile (71 fl) compared to the 2–6 years age group of Greer *et al.* [18] (75 fl) and the 2–6 years age group of Bain *et al.* [14] (75 fl). In the current study, the 2–4 years age group had a WBCs RI of 5–17× 10^3/cmm with a lower 97.5th percentile compared to both Greer *et al.* [18] (range of 5–15.5× 10^3/cmm) and Bain *et al.* [14] (range of 5.5–15.5× 10^3/cmm). Fifteen subjects with a frequency of 4% had WBC higher than 15.5 × 10^3/cmm with a probability of 19% and were clinically asymptomatic. The current study and Bain *et al.* [14] were in agreement over the platelet RI in the 24–59 months (2–4 years) age group.

The age group from 60 to 144 months (5–12 years of life) in the current study was related to the 6–12 years age group of Greer *et al.* [18] and the 6–12 years age group of Bain *et al*. [14]. In the critical values of erythrocytes parameters for anemia determination (Hb and RBCs), the current study showed a lower Hb threshold for anemia diagnosis (median Hb 12 and range 11–14 g/dl)), in contrast to RI adopted by Greer *et al.* [18] (median Hb 13.5 and range 11.5–15.5 g/dl) and Bain *et al.* [14] (median Hb 13.5 and range 11.5–15.5 g/dl).

Conclusion

In all age clusters studied, a lower 2.5th percentile value for the Hb and RBCs parameters was found in the current study compared to similar clusters in Western-based RIs. Thus, adopting any of the previous RIs would result in overdiagnosis of anemia in the relevant age group. A higher 97.5th percentile value of WBCs count as an indicator of infection in the current study compared to similar age groups in Western-based RIs. Thus, adopting any of the previous RIs would result in overdiagnosis of infection in the relevant age group. The difference between the current study and the Western-based RIs could be attributed to different ethnic origin, habits, and environmental conditions.

Being a retrograde study, a major limitation faced by the authors was the low number of subjects in the neonatal age as well as the lack of birth weight knowledge and whether a full term or a preterm neonate.

As a pilot study for the first 23 months of life age group in Egyptians, a large-scale disease determinant comprehensive study is mandatory such as the study of the factors affecting the erythropoiesis to determine which parameters are mostly affected by geographical area is pivotal for the proper generation of population-based RI.

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