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Routine Hematological Reference Values in 7 to 17 Years-Old Children Living at An Intermediate Altitude (1869 meter, Erzurum, Turkey)

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ÖZET:

Orta Derecede Yüksek Bir Rakımda (1869 metre Erzurum, Türkiye) 7-14 Yaş Arası Çocuklarda Rutin Hematolojik Referans Değerleri

Referans değerler elde etmek üzere, 1869 metre yükseklikte yaşayan 718 sağlıklı çocuktan alınan venöz kan örneklerinde bir otomatik hematoloji analizeri (Cell-Dyn 1500) ile tam kan sayımları yapıldı. Öncelikle referans değerleri elde etmeye uygun popülasyonları elde etmek için, fert başına düşen aylık gelir, anababanın eğitim durumu ve antesedan infeksiyonlar herbir parametre üzerindeki tesirleri yönünden değerlendirildi. Bu faktörlere göre bölünmüş subgruplardan hematolojik değerler açısından etkilenmiş olanları ekarte edildi. Böylece oluşturulan modifiye popülasyonlardan elde edilen ölçümlerin histogramları normal dağılım gösterdi. İlgili parametrelerde yaş ve cinsiyetin etkileri değerlendirildi ve referans değerler kilinik kullanımı kolaylaştırmak üzere gruplandı. Bizim yaklaşık 2000 metre yükseklikte yaşayan çocuklar için teklif ettiğimiz değerlerden hemoglobin, hematokrit, eritrosit ve ortalama eritrosit volümüne ait olanlar deniz seviyesindeki değerlere göre yüksek bulundu. Ayrıca sonuçlarımız orta derecede yüksek rakımın diğer rutin hematolojik değerler üzerinde herhangi bir etkisi olmadığını işaret etti.

Anahtar kelimeler: Referans değerler, yüksek rakım, kan sayımı.

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Shortened title: Hematological references at moderate altitude.

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Abstract

In order to obtain reference values, complete blood count was performed on venous blood samples from 718 healthy children living at 1869 meter altitude by an automated hematology analyzer (Cell-Dyn 1500). At first, to form the populations proper to obtaining reference valuses, some factos, "per capita income" "parent's educational status", and "antecedent infection (s)" were assessed in respect with their effects on each parameter. Of subgroups divided according to these, factors, those which were affected in terms of hematological values were excluded. The histograms of measurements from the modified population were obtained, and they showed "Gaussian" distribution. Effects of age and sex on the parameters were evaluated, and reference values were arranged according to grouped ages to facilitate clinical use. Of the reference values which we suggest for children living at about 2000 m altitude, those of hemoglobin, hematocrit, red blood cell count and mean cell volume are significantly higher than sea-level values. In adddition, our results indicate that intermediate altitude has no effect on the other routine hematological values.

Indexing words: reference values, high altitude, blood count.

Introduction

There are satisfactory studies on reference values of anemia-related routine hematologicl parameters in childhood period (1,2). However , almost all of them have been reported as values of those living at sea level, and it is well known that high altitude causes some hematological adaptations (3). Knowledge of erythrocytosis due to living at high altitude has a history of about a hundred-years. Nevertheless, hematological reference values of high altitude residents, especially those of children, have not been obtained adequately. It has been suggested that a correction for hemoglobin could be done by increasing the sea level values 4% for each 1000 m (4). this suggestion is based on a study which was performed on adults in the early 1940 s (5). Therefore, it seems to be necessary to obtain reliable hematological reference values for intermediate and high altitude residents. This is particularly important for screening of minor changes such as mild iron deficiency anemia which is still often seen in childhood in developing countries.

Even in some recently published hematological textbooks, the suggested reference values for white blood cell count and differential cell count in childhood have been based on a study carried out approximately 30 years ago (6,7). in addition, although reference values of platelet count have been recorded the same as those of adults (8), reference values of platelet volume and related parameters for children have not been available, as desired, until now.

Materials and Methods

The survey population consisted of 785 children aged 7-14 years, living at Erzurum city center (1869 m above sea level) in Turkey for at least one year. Parents informed, written consents were obtained for all the children included in the study. Detailed medical history was taken and physical examination was carried out carefully for each child. Children with symptoms suggesting any illness, even the ones with mild infections (23 children) were excluded. For each child a single blood sample was collected at 10.00-11.00 am, while the children were at rest following about 2-hours fasting period (5ml of blood sample was taken from antecubital vein by a sterile, disposable syringe (21G) and put into a vacutainer tube containing 10mg disodium EDTA according to standart protocols (9)). Thirty-one collected samples were excluded because of partial coagulation.

The measurements were performed by an automated hematology analyzer (Cell-Dyn 1500, Sequoia Turner Corporation), which is an impedance type analyzer (10), between two to three hours after collection.

The calibration status of the analyzer was checked and proper calibration was provided if necessary according to the manufacturer's recommendations every time before samples were run.The parameters measured were: white blood cell count (WBC); lymphocyte count (LYM); granulocyte count (GRAN); monocyte, eosionophil, and basophil counts all together (MID); red blood cell count (RBC); hemoglobin (Hg); hematocrit (Htc); mean cell volume (MCV); mean cell hemglobin (MCH); mean cell hemoglobin concentration (MCHC); red cell distribution width (RDW); platelet count (PLT); mean platelet volume (MPV); platecrit (PCT); platelet distribution width (PDW), These fifteen parameters for each sample were measured, except for MPV, PCT, PDW mesurements of 88 samples.

After obtaining all the measurements, the children were evaluated in terms of possible mild iron deficiency anemia by using RDW values. We had 31 cases with slightly higher RDW values (between 14.5-16.0 %). In 7 out of remaining 24 cases did not show any difference from those of the whole group, and they were not excluded.

The final population for statistical analysis was 718 children considered as healthy. At this point, the possible effects of three variables on reference values were evaluated. These were parent's educational status, per capita income, and antecedent infection (s). The first two, parent's educational status and per capita income, were considered together in terms of three categories, lower, middle and higher. The third, that of antecedent infections (s), was considered in terms of those with or without such infections in last month. For these evalulations, the method "Least-Squares Analysis of Data With Unequal Subclass" was used by means of the computer program created by Harvey (11). Duncen's LSD test (12) was also used to be able to select the values of biased subgroup from the overall group result.

After the exclusion of biased subgroups, the reference values were calculated from the constituted reference populations. Firlty, the the mean values and ± 2SD of each age and set were obtained and the "Gaussian" characteristic of histogram distributions were observed. Secondly, in order to simplify the clinical approach, by using the Harvey's computer program (11), Duncen's LSD test (12), and Minitab II computer program (13), the values of grouped ages and sex were obtained.

Results

Of the factors evaluated to form reference populations, parent's educational status was found to be very significant on Hb and Htc values ($p< 0.01$) and signficant on MCV values ($p<0.05$).

The mean value of these parameters from the children whose parents were in the lowest education level was lower than that of the other two groups (data not shown). For this reason, the results from this group of 191 children were not included in the subsequent calculations of RBC and related parameters (Hg,Hct,MCV,MCH,MCHC, and RDW) for reference values. Effects with regard to per capita income was not significant enough to require any exclusions. Results of the group with antecedent infections (s) were not included in the subsequent calculations of PLT and related parametres since antecedent infections showed significant effect on mean PLT ($p<0.05$), increasing mean PLT (data not shown).

Finally, The reference populations consisted of 718 children for WBC, 527 for RBC, and 535 for PLT (also for related parameters).

The values separately obtained for each age and sex are not given in this article. Sex as a determinant was significant on RDW, MCV and MCHC (p values, respectively, <0.01, <0.01 and <0.05). Age as a determinant was significant on WBC, RBC and eryhtrocyte related parameters (p values, for RDW <0.05 and $p<0.001$ for the others). Reference values according to the final groupings were shown in tables, I, II and III.

Discussion

The use of the correct populations from which the biased subgroups have been excluded is recommended in order to obtain reference values (1,2). For that reason, in any study aimed for reference values, the first step should be to form a reference population. There are various reports about the effects of socio-economical status on hematological values, (2, 14-16). We evaluated the effects of parent's education status and per capita income, which have been thought to be important especially for anemia related parameters. After these evaluation, we excluded the biased subgroup to be able to avoid possible abnormal values. In this article we have not mentioned the details of this socio-economical assessment. The relevant details and discussion will be presented elsewhere.

On the other hand, one should be aware of the abnormalities which may cause slight changes in the hematological values. The main reasons for these changes are nutritional iron deficiency, ß-thalassemia trait, acute infections, including mild cases, and antecedent infections (1,4,17,18). The children with one or more of these abnormalities should be excluded from the reference population. Distinguishing mild iron deficiency from normal cases in particularly difficult, (4,17). We excluded children with mild iron deficiency anemia using RDW, which has been proposed to detect iron deficiency in its earlier stage with high sensitivity (19,20). and response to iron treatment.

We did not attempt to detect ß-thalassemia trait cases because the previous studies showed its much lower prevalance in our region (21). In accordance with previous reports (18), we found that antecedent infection(s) might cause increased PLT, and did necessary exclusions. However we did not see any effect of antecedent infectioin(s) on anemia related parameters.

On the subject of routine hematological values in children residing at sea level, three satisfactory studies on properly selected populations have given values similar to one-another (1,2,14). The report by Yip and colleagues and our results are compared on table IV. (Different age groups in their study have been put together and the mean value of 6-14 yaars old have been recalculated by us in order to be able to compare the rusults). As shown on table IV, the mean values of Hg, Htc, RBC, and MCV at 1869 m altitude have been found higher than the sea level values (respectively, 1.3 g/dl for Hb, 4% for Hct, $0.45x10^9/L$, 2fL for MCV).

It was reported that up to moderately high altitude, an adaptation mechanism causes increasingly higher number of red blood cells which are relatively microcytic (22). We however found increased MCV as well as RBC. The difference between these two results is likely to be related to some additional factors as mentioned before (23), and needs further investigations.

The reason for the suggestion that estimation-based corrections of Hb values shauld be done at different altitudes (4) is likely to be scarcity of the reference values on this subject. Estrella at al evaluated iron deficiency in children living at 2700 m altitude using therapeutic iron trial and found 9 iron-deficient children out of 38 children. At the beginning, with the estimation-based approach, they had predicted that only one child could have had iron deficiency anemia. Therefore they addressed the necessity of using proper reference values for high altitude residents (24). Our results indicate higher Hb values compared to the estimation-based values according to Hurtado and colleagues' study, and suggest that if Estrella and colleagus could have used proper reference values for at their altitude, they would have predicted larger number of anemic children in their study.

Our reference values for WBC and differential leucocyt count are very close to the values given in recent textbooks, which were obtained by manual methods at sea level about thirty years ago (25). Also PLT did not show any difference from the previous manual values. Our results also confirm that a moderate altitude has no effect on WBC, differential leucocyte count and PLT.

In this study, we also present reference values for RDW, MPV,PCT and PDW for school-aged children. The mean and upper limit of RDW values presented by our study are very close to the values given for adults (26). Our MPV reference values for children are also similar to adult values (27). The reference values of PCT and PDW will become more meaningful with the accumulation of their data.

Conclusions

Our results strongly suggest that different altitudes need different reference values in terms of anemia related parameters. Since some regional variations can occur, it appears necessary to perform multi-center studies capable of evaluating additional factors as well as different altitudes. As data collected in this way accumulates, it will facilitate assessment of the abnormalities such as iron deficiency anemia, acute infection anemias, and ß-thalassemia trait in intermediate and high altitude residents.

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Table I- Reference values of eryhtrocyte count and eryhtrocyte related parameters.

Parameters	Mean (±2SD) values according to grouped ages 7-10 years(n=262)		11-14 years (n=265)	
For both sexes				
RBC (X10/L)	4.90 (4.30-5.50)		5.07 (4.42-5.71)	
Hg (g/dL)	14.3 (12.8-15.7)		15.0 (13.4-16.5)	
Htc (%)	41.4 (36.8-46.1)		44.0 (39.2-48.7)	
MCH (pg/red cell)	29.1 (26.1-32.2)		29.6 (26.8-32.4)	
For different sexes	Boys	Girls	Boys	Girls
MCV (fL/red cell)	84(76-92)	85 (76-94)	86 (80-93)	87 (80-95)
MCHC (g/dL)	34.5 (31.7-37.3)	34.4 (33.1-37.1)	34.3 (32.4-36.2)	33.8 (31.9-35.6)
RDW (%)	13.2 (11.6-14.8)	12.8 (11.2-14.3)	13.0 (11.5-14.5)	12.7 (11.3-14.1)

Table II- Reference values of leucocyte and differential count according to grouped ages.

Grouped ages*	Parameters		Mean (±2SD) values
7-10 (n=394)	Leucocyte count ($10^3/\mu L$)		8.5 (4.0- 13.0)
11-14 (n=324)	Leucocyte count ($10^3/\mu L$)		7.8 (4.1- 11.4)
7-14 (n=718)	Granulocyte count	%	53.7 (34.0-73.4)
		($10^3/\mu L$)	4.4 (2.6-5.9)
	Lymphocyte count	%	37.7 (21.5-53.9)
		($10^3/\mu L$)	3.0 (1.7 - 4.4)
	Other cells count	%	8.6 (2.2-15.0)
		($10^3/\mu L$)	0.7 (0.2-1.2)

* both sex together

Table III- Reference values of platelet count and related parametres.

Grouped ages*	Mean (±2SD) values			
	PLT ($10^3/L$)	MPV (fL)	PCT (%)	PDW (10GSD)
7-14 (n=535)	276 (155-397)	9.7 (7.2-12.3)	0.267 (0.205-0.330)	18.6 (16.0-21.3)

*both sex together

Table IV- The comparison of our reference values for anemia related parameters at 1869 meter with those of Yip's study at sea level.

Studies	gruped ages	HGB (g/dL)	HCT (%)	RBC ($10^6/\mu L$)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)
Yip et al (1984) at sea level	6-14 (n=1322)	13.3	38.7	4.52	84	28.9	34.4	—
Our results (1991), at 1869 meter	7-14 (n=527)	14.6	42.7	4.99	86	29.3	34.2	13.0

References

1- Yip R, Johnson C, Dallman PR: Age Related Changes In Laboratory Values Used In The Diagnosis of Anemia and Iron Deficiency. Am J Clin Nutr 39: 427, 1984.

2- Garn GM, Ryan AS, Abraham S, et al: Suggested Sex and Age Appropriate Values for "Low" and "Deficient" Hemoglobiln Levels. Am J Clin Nutr 34: 1648, 1981.

3- Tufts DA, Haas JD, Beard JL, et al: Distribution of Hemoglobin and Functional Consequences of Anemia in Adult Males at High Altitude. Am J Clin Nutr 42: 1, 1985.

4- Dallman PR, Siimes MA, Stekel A: Iron Deficiency in Infancy and Childhood.Am J Clin Nutr 33: 86, 1980

5- Hurtado A, Merino C, Delgrado A: Influence of Anoxemia on Hematopoietic Activity. Arc Intern Med 75; 284, 1945

6- Lubin BH. Reference Values in Infancy and Childhood. In Nathan DG, Oski FA, eds. Hematology of Infancy and Childhood. 3rd ed. Philadelphia: Saunders, 1987

7-Williams WJ, Nelson DA, Morris MW. Examination of the Blood. In Williams WJ,Beutler E, Erslev AJ, Lichtman MA, eds. Hematology. 4th ed. Internationa edition: McGraw Hill, 1991.

8- Wintrobe MM, ed. Clinical Hematology. 8th ed. Philadelphia: Lea and Febiger, 1981.

9- Raphael SS, ed. Lync'h Medical Laboratory Technology. 4th ed. Philadelphia: Saunders, 1983.

10- Cell-Dyne 1500 Operator's Reference Manual. SuquoiaTurner Corporation, 850 Maude Venue, Muntain View, CA 9443, USA, 1986.

11- Harvey WR. Instructions for Use of Least Sequares and Maximum Likelihood General Purpose Program, Ohio State University. Columbus, USA, 1972.

12- Wine R L. Statistics for Scientists and Engineers, New Delhi: Prentice Hall of India Ltd. 1966.

13- Minitab Project Statistic Department. Minitab 2, 215 Pond Laboratory The Pennsylvania StateUniversity,University Park, USA, 1982.

14- Dallman PR, Siimes MA: Percentile Curves for Hemoglobin and Red Cell Volume in Infancy and Childhood. J Pediatr 94: 26, 1979.

15-Owen GM, Nelsen CE, Garry PI: Nutritional Status of Preschool Children. Hemoglobin, Hematocrit and Plasma Iron Values. J Pediatr 76: 762, 1970.

16- Lovric JA: Normal Hematological Values in Children Aged 6 to 36 Months and Socio-Medical Implications. Med J Aust 2: 366, 1970.

17- Reeves JD, Yip R, Kiley V, et al: Iron Deficiency in Infants: The Influence of Antecedent Infection. J Pediatr 105: 874, 1984.

18- Heath HW, Pearson HA: Reply to "Thrombocytosis in Hospitalized Children" (letter). J Pediatr 116: 835, 1990.

19- Oski FA Sadowitz PD, Helu B: The Red Cell Volume Distribution Width (RDW) and The Diagnosis of Iron Deficiency. Pediatr Res 19: 265A, 1985.

20- Vvan Zeben D, Bieger R, van Wermeskerken RKA, et al: Evaluation of Microcytosis, Serum Ferritin and Red Blood Cell Distribution Width. Eur J Haematol 44: 105, 1990.

21- Gençelli Y: Doğu Anadolu Bölgesinde Hemoglobin Elektroforezi ile Thalassemia Trait ve Anormal Hemoglobinler Taraması. Uzmanlık Tezi.Atatürk Üniversitesi Tıp Fakültesi,Erzurum,Türkiye, 1983.

22- Ruiz-Arguelles GJ, Sanchez-Medal L, Loria Piedras J, et al: Red Cell Indices in Normal Adults Residing at Altitudes from Sea Level to 2670 Meters. Am J Hematol 8: 265, 1980

23- Beal CM, Goldstein MC: Hemoglobin Concentration of Pastoral Numads Permanently Resident at 4850-5450 Meters in Tibet. Am J Physic Anthrop 73; 433, 1987.

24- Estrella R, Hercberg, S,Maggy G, et al: Evaluating Of Iron-Deficiency Anemia an Iron Supplemantation ,Trial in Childlren Living at A 2800-m Altitude. Clinica Chimica Acta 164: 1,1987.

25-Altman PL, Dittmer DS: Blood and Other Body Fluids. Federation of American Societies for Experimental Biology, Washington, USA, 1961.

26- Bessman JD, Williams LJ, Gardner FH: Improved Classification of Anemias by MCV and RDW. Am J Clin Pathol 80: 322, 1983.

27- Bessman JD, Williams LJ, Gilmer P, et al : Mean Platelet Volume: The Inverse Relation of Platelet Size and Count in Normal Subjects and an Artifact of Other Particles. Am J Clin Pathol 76: 289, 1981.