Original Articles

NORMAL REFERENCE VALUES FOR HEMATOLOGICAL PARAMETERS, RED CELL INDICES, HB A2 AND HB F FROM EARLY CHILDHOOD THROUGH ADOLESCENCE IN SAUDIS

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Background: Values of hematological parameters are affected by factors such as age, sex, ethnic background and social, nutritional and environmental factors. The objective of this study was to determine the values of hematological parameters, red cell indices and Hb A2 and Hb F levels in normal Saudi children, living in the Central Province of Saudi Arabia.

Materials and Methods: The study was carried out on 1526 apparently healthy children, with ages ranging from 1-15 years, and selected during a household screening program. Hematological parameters, red cell indices, and hemoglobin types (Hb A2 and Hb F) were estimated, and the children were divided into 15 groups depending on the age. Male and female children were separated, and the mean and standard deviation of each parameter was calculated for each age group.

Results: No significant differences were observed in the red cell count in the male and female children. White blood cells gradually decreased from 2 years onwards, while hemoglobin and hematocrit levels increased significantly from 2 to 15 years. Mean cell volume and mean cell hemoglobin also showed slight increases, while mean cell hemoglobin concentration remained more or less constant. Hemoglobin A2 and Hb F showed slight but nonsignificant fluctuations. Comparison of the results with those reported in the literature shows that Saudi children have some values similar to Caucasians, while others have values which are intermediate between Caucasians and African children.

Conclusion: The values reported in this study can be used as normal reference values for Saudi children and adolescents.

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Key Words: Hemoglobin, red blood cells, hematocrit, red cell indices, Hb A2 and Hb F.

The values of hematological parameters are affected by a number of factors even in apparently healthy populations. These factors include age, sex, ethnic background, body build and social, nutritional and environmental factors, especially altitude.[1-5] It has been shown in several studies that some of the hematological parameters exhibit considerable variations at different periods of life. At birth, the total hemoglobin (Hb) level, red blood cell (RBC) count and packed cell volume (PCV) are shown to be higher than at any other period of life.[6,7] The levels of these parameters then decrease during the next few months after birth, some more steeply than others, with the cells becoming hypochromic with the development of "physiologic" iron-deficiency anemia.[8] The hemoglobin content and the red cells then gradually rise to adult levels by the age of puberty. In general, the female levels are lower than the male levels.[9-10]

Several studies have been carried out in children at various ages and in adolescents, and significant differences have been reported in different populations, seasons, racial and ethnic groups and gender subgroups.[10-24] Genetic factors are shown to significantly contribute to all blood cell measures, accounting for between 61%-96% of variance.[11,25] In almost all studies, the ethnic and sex differences are significant, with Caucasian values being higher than those of Africans and Afro-Caribbeans.[15,17,18,24,26] It has, therefore, been stressed that each population must establish its own "normal reference values" for use in clinical assessments.[27]

In an attempt to establish the normal reference values for hematological parameters in Saudi children and adolescents with ages ranging from 1-15 years, we conducted this investigation and determined the values in apparently healthy children screened during a household screening program. The children were grouped according to age and sex, and the ranges for the commonly used hematological parameters were determined. This paper reports our findings, and compares the results with those reported in the literature.

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TABLE 1. *The total number of children, and each age group investigated during this study.*

Age (years)	No. investigated Male	Female
Up to 1	5	5
>1-2	21	25
>2-3	39	37
>3-4	39	42
>4-5	40	50
>5-6	79	53
>6-7	60	52
>7-8	81	73
>8-9	50	68
>9-10	69	67
>10-11	66	45
>11-12	74	56
>12-13	53	50
>13-14	67	63
>14-15	43	54
Total	786	740

TABLE 2. *Hematological parameters in 1-15-year-old Saudi children.*

Age (years)	Sex	RBC $x10^{12}$/L	WBC $x10^{9}$/L	Hb g/dL	PCV (%)
Up to 1	M	4.8±0.52	7.2±1.2	12.3±1.2	36.0±2.3
	F	4.8±0.41	6.7±1.1	13.1±1.0	38.0±2.6
>1-2	M	4.8±0.39	8.7±2.2	11.3±1.0	34.0±2.8
	F	4.9±0.45	8.5±1.9	11.7±1.0	36.0±2.4
>2-3	M	4.8±0.32	8.6±2.3	12.2±1.0	36.0±3.1
	F	4.9±0.62	8.0±2.4	12.2±1.3	37.0±3.4
>3-4	M	4.8±0.36	7.4±1.6	12.0±1.1	36.0±2.5
	F	4.8±0.43	7.6±1.8	12.7±0.9	38.0±2.5
>4-5	M	4.7±0.30	7.5±2.2	12.5±1.1	37.0±2.7
	F	4.69±0.3	7.5±2.2	12.6±1.1	37.0±2.8
>5-6	M	4.9±0.47	7.7±2.2	12.9±1.1	38.0±2.9
	F	4.7±0.55	7.5±2.2	12.9±1.17	38.0±3.7
>6-7	M	4.8±0.49	7.8±2.2	12.9±1.2	38.0±2.9
	F	4.7±0.4	7.6±2.2	13.1±0.98	38.0±3.3
>7-8	M	4.9±0.28	7.0±2.0	13.5±0.90	39.0±3.4
	F	4.8±0.5	7.5±2.2	13.1±1.8	38.0±3.5
>8-9	M	4.9±0.28	7.0±2.0	13.5±0.90	39.0±2.6
	F	4.8±0.5	7.5±2.8	13.1±1.8	39.0±3.8
>9-10	M	4.9±0.37	6.7±2.2	13.6±1.9	40.0±3.7
	F	4.8±0.39	7.0±1.9	13.0±1.7	38.3±3.5
>10-11	M	4.8±0.44	6.5±2.3	13.3±1.9	39.5±3.5
	F	4.8±0.44	6.5±2.2	13.5±0.79	39.7±2.2
>11-12	M	4.9±0.43	6.5±2.1	13.5±1.3	39.0±3.1
	F	4.9±0.38	6.7±1.75	13.4±1.28	39.0±3.3
>12-13	M	4.86±0.45	6.8±1.9	13.5±0.84	39.8±2.6
	F	4.8±0.46	6.9±2.8	13.7±1.25	40.3±3.3
>13-14	M	5.1±0.52	6.5±1.7	14.1±1.4	41.4±3.0
	F	4.9±0.41	7.0±2.0	13.8±1.3	40.7±3.3
>14-15	M	5.1±0.26	7.9±2.1	14.2±1.1	41.8±2.8
	F	4.9±0.59	7.14±2.1	13.8±1.2	39.4±3.0

Materials and Methods

The study group included 1526 apparently healthy Saudi children with ages ranging from 1-15 years (Table 1), who had been selected during a household screening program of the Central Province of Saudi Arabia to screen for diabetes mellitus. The screening was conducted according to a statistically designed household screening program, the details of which have been published elsewhere.[28-29] The Central Province was divided into sectors and individual sectors were randomly selected for inclusion in the study. In each sector, every 10th street was selected and on every street, every 10th house was selected for screening. The family was contacted by phone and invited to join in the study. Only those who volunteered were included. An early morning visit was made on a mutually agreed date by a doctor, nurse and a laboratory technician. Physical details were recorded and blood samples were drawn by venipuncture from each member of the family over 2 years of age, and placed in a heparinized tube. Only 10 children with ages up to one year were included. The whole blood was transported at 4°C to King Khalid University Hospital, Riyadh, where all the analyses were conducted at the Medical Biochemistry Department on the day of blood extraction. The hematological parameters were estimated on a Coulter Counter ZF6 with a hemoglobinometer attachment. Blood smears were prepared from fresh blood and air dried. The red cell morphology was assessed after appropriate staining. Red cell indices as mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were determined using the Coulter Counter. The red cells were separated from the plasma by centrifugation and washed twice with cold physiological saline. Fresh hemolysate was used to determine the hemoglobin phenotypes by electrophoresis at alkaline[30] and acid pH.[31] Hb A2[30] and Hb F[32] were estimated for each sample using freshly hemolysed cells.

The data were fed into the mainframe computers at the Computer Center, King Saud University, Riyadh, and analyzed after grouping the children according to sex and age. According to age, there were 15 groups as follows: up to 1 year, >1-2, >2-3, >3-4, >4-5, >5-6, >6-7, >7-8, >8-9, >9-10, >10-11, >11-12, >12-13, >13-14 and >14-15 years. The data analysis was conducted separately for each group using Statistical Analysis System (SAS). Student's *t*-test was used to make comparisons between any two groups. $P<0.05$ was considered statistically significant.

Results

On the basis of the results of electrophoresis, none of the children was suffering from sickle cell disease, and were apparently healthy. The values of RBC count, WBC, total hemoglobin (Hb) and packed cell volume (PCV) in the different age groups are presented in Table 2. The RBC count did not show a significant change in the 1 to 13-year-olds but rose slightly beyond this age. Also, the difference between the values of the girls and the boys was not significant ($P>0.05$).

White blood cells increased from the level in the one-year-olds to the two-year-old children. The highest level in

TABLE 3. *Red cell indices in 1-15- year-old Saudi children.*

Age (years)	Sex	MCV (fl)	MCH (pg)	MCHC (g/dL)
Up to 1	M	75.0±4.9	26.0±3.0	34.0±1.5
	F	78.0±7.3	26.9±2.7	34.0±0.7
>1-2	M	72.0±6.8	24.0±2.9	33.0±1.3
	F	72.0±7.8	24.0±3.1	33.0±1.2
>2-3	M	75.0±5.0	26.0±3.2	33.0±2.2
	F	75.0±7.7	25.0±3.0	33.0±1.2
>3-4	M	74.0±6.1	25.0±2.5	34.0±1.4
	F	78.0±4.9	26.0±1.8	34.0±1.0
>4-5	M	78.0±4.6	27.0±2.5	33.0±1.2
	F	79.0±5.7	27.0±2.8	34.0±1.0
>5-6	M	79.0±4.9	27.0±2.1	34.0±1.2
	F	80.0±4.9	27.0±2.0	34.0±1.3
>6-7	M	78.0±9.0	27.0±2.8	34.0±1.3
	F	81.0±4.0	27.0±2.1	34.0±2.1
>7-8	M	79.0±7.7	27.0±1.8	33.9±1.8
	F	79.0±6.5	27.0±2.7	34.0±1.2
>8-9	M	80.0±5.1	27.0±1.8	34.0±1.3
	F	80.0±5.2	27.0±3.1	34.0±1.3
>9-10	M	81.0±5.1	27.6±2.3	34.0±1.2
	F	79.6±6.1	27.0±2.3	34.0±1.3
>10-11	M	80.4±8.1	27.6±2.1	34.1±1.3
	F	80.8±4.4	27.5±1.8	34.0±1.0
>11-12	M	80.3±5.1	27.3±2.2	34.0±1.6
	F	80.0±6.3	27.0±2.5	34.0±1.5
>12-13	M	81.8±5.6	27.6±2.1	33.9±1.3
	F	81.0±5.4	27.6±2.0	33.9±1.1
>13-14	M	80.0±5.9	27.0±2.3	34.0±1.3
	F	83.6±4.7	28.6±2.3	34.0±1.2
>14-15	M	81.0±6.4	27.8±1.8	34.0±1.12
	F	81.0±6.4	27.1±2.9	33.6±1.8

TABLE 4. *Hemoglobins A2 and F in 1-15- year-old Saudi children.*

Age (years)	Sex	Hb A2 (%)	Hb F (%)
Up to 1	M	3.2±0.2	1.0±0.2
	F	3.2±0.2	4.0±0.2
>1-2	M	3.0±0.13	0.9±0.57
	F	3.1±0.65	1.2±0.39
>2-3	M	2.4±0.5	0.7±0.2
	F	3.0±0.17	1.0±0.96
>3-4	M	2.7±0.49	0.73±0.2
	F	2.8±0.5	0.70±0.3
>4-5	M	2.8±1.6	0.7±0.3
	F	2.8±1.0	0.7±0.3
>5-6	M	2.8±0.41	0.73±0.4
	F	3.1±0.4	0.73±0.4
>6-7	M	3.0±0.5	0.9±0.5
	F	2.5±0.5	1.0±0.5
>7-8	M	2.9±0.23	0.7±0.18
	F	2.9±0.3	1.0±1.1
>8-9	M	3.0±0.14	0.7±0.2
	F	3.6±1.0	0.8±0.6
>9-10	M	2.8±0.3	1.0±0.2
	F	2.9±0.07	1.0±0.1
>10-11	M	2.8±0.29	0.75±0.21
	F	2.9±0.5	0.85±0.34
>11-12	M	2.6±0.49	0.8±0.2
	F	3.1±0.07	0.85±0.2
>12-13	M	2.6±0.5	0.6±0.3
	F	2.7±0.3	0.6±0.3
>13-14	M	2.9±0.11	0.74±0.76
	F	3.15±0.35	0.8±0.14
>14-15	M	3.0±0.29	0.75±0.33
	F	2.7±0.13	0.8±0.7

both boys and girls were in the two-year-olds. From four years onwards, the level remained more or less similar and the difference between girls and boys was not significant ($P>0.05$).

Total hemoglobin level was lowest in the two-year-olds, both in the boys and girls, and then gradually increased up to 15 years of age. The difference in hemoglobin level between boys and girls up to 15 years of age was not significant. In this age group, the hemoglobin level of the boys was almost 3 g/dL more than in the two-year-olds, while in the girls, the difference was almost 2 g/dL. Hematocrit also decreased in the two-year-olds, but rose gradually from three years onwards in both boys and girls, and in the 14-year-olds onwards, the boys had a higher level than girls.

The red cell indices in the different age groups are presented in Table 3. The MCV and MCH were slightly lower in those aged less than 5 years of age, but from then on increased and reached the adult level by six years of age. MCHC did not vary much in the different age groups.

Hb A2 level showed some fluctuations but remained less than 3.5% in the majority of children. Hb F level was highest in the one-year-olds, reached around 1% in the two-year-olds, and remained around 1%-1.5% in all other age groups.

Discussion

Extensive studies during the last three to four decades conducted in different populations have worked out normal reference ranges for hematological parameters in adults and children,[10-24] and have reported significant differences in different ethnic groups.[3,5,12,14,18,24,26] In African-American, African and Afro-Caribbean children, the levels of these parameters are generally lower than in Caucasians.[5,12,19,24,26] Environmental factors and altitude also influence the value of hematological parameters.[10]

Saudi Arabia forms the largest part of the Arabian Peninsula and occupies over 2.2 million square kilometers.[33] It is probably among the driest countries in the world, with long, hot and almost totally dry summers, and a short very cool winter season during which rain occurs. In the East and the West, the weather is humid, and due to the large numbers of oases, these areas have been malarial endemic in the past.[34] The frequency of genetic anemias due to the presence of sickle cell, α- and β-thalassemias and glucose-6-phosphate dehydrogenase deficiency is high, and this affects the overall health status of the population.[35-36] A high prevalence of anemia has been reported in these malaria endemic areas.[37-38]

For this study, we investigated children in the Central

Province of Saudi Arabia, where the frequency of genetic disorders is low (<1%), and the population is generally healthy.[35-38] The results of this study can be regarded as normal reference values in Saudi children. This study has covered the entire age range from 1-15 years, separately in the males and females, to determine the value of hematological parameters, red cell indices and Hb A2 and F. The number of infants under two years was small in this study. The results obtained follow more or less an expected pattern, though a few interesting exceptions are worth discussing.

The mean red cell count was more or less constant in the 1-13-year-olds, but rose slightly beyond this age. The difference in the male and female results was similar to the RBC values reported by Ghafouri et al.[39] in children from Jeddah, and by Serjeant et al.[5] in children from Jamaica, but were higher than the values reported for some Caucasian groups.[40,41] The total hemoglobin and hematocrit levels were lowest in the >1-2 years group, but from then onwards increased up to the 15 years age group. Up to the age of 13 years, the male and female values were similar, but were lower in female adolescents (14-15 years of age). Ghafouri et al.[39] reported total hemoglobin level of 13.7±1.0 g/dL and 13.5±1.0 g/dL, respectively, in the male and female children, with ages ranging from 12-15 years. No significant differences were seen in the male and female results, though beyond 14 years of age, the male values were higher than the female values. The values are similar to those reported from Jamaica,[6] but lower than the Caucasian values.[40-41]

Ghafouri et al. reported a significantly higher WBC count (10.9±3.8 x 10^{12}/L) in one-year-olds, which decreased when they were in the 9-11-year-old age group (6.9±3.1x10^9/L), but then increased to 7.3±2.0x10^9/L in the 12-15-years age group.[39] These values are significantly higher than those reported by Gilles[42] from seven countries in the tropics, where the WBC count ranged from 5.0-5.94x10^9/L, but lower than the studies reported in Caucasians. Dallman[40] reported a mean of 7.8x10^9/L and a range of 4.5-13.0 x10^9/L in adults, while our range in the 15-year-old children was around 3.0-11.2x10^9/L in females and 3.8-12.1x10^9/L in males. These values in females are similar to the values reported from Jeddah,[39] but higher in males compared to the results from Jeddah.

The red cell indices did not show any significant difference between the male and female results in all age groups. The mean cell volume decreased in the >1-2-year-olds, but then increased to reach the adult value. However, the MCV was significantly lower in all age groups in our study population. In a study reported earlier on 544 children from the Central Province, 13.6% had hypochronic-microcytic anemia.[37,38] This may be due to the presence of subclinical iron deficiency or α- or β-thalassemia trait.

Both clinical and subclinical iron deficiencies constitute one of the most frequently encountered nutritional anemias, and the prevalence of iron-responsive microcytic hypochromic anemia varies from 9%-70% in different population groups,[43] the incidence being higher in the less developed countries compared to the developed nations of the world. In the United States and Canada, using transferrin saturation, free erythrocyte protoporphyrin and serum ferritin as diagnostic criteria for clinical and subclinical iron deficiency, the prevalence was reported to range from 2.3%-27%. There are several causes of iron deficiency in children, including many parasitic diseases and other infections, consumption of highly refined foods, use of aluminum and steel cookware rather than iron utensils, lower intake of iron-rich foods, particularly vegetables, and gastrointestinal disorders, leading to impaired iron absorption. Subclinical (mild) iron deficiency, due to diminished iron storage, occurs long before anemia develops, and requires early diagnosis and intervention to prevent the development of full-blown symptoms of iron deficiency.

Among Saudi children, detailed investigations are necessary to achieve a differential diagnosis of the hypochromic-microcytic state. Since the diagnosis of the carrier states of α- or β-thalassemia requires molecular analysis, we suggest that in this apparently healthy age groups, there were some children suffering from sub-clinical iron deficiency and others who were carriers of α- or β-thalassemias. Thus our results are closely comparable to the report from Jeddah,[39] but the values are lower than those reported in the literature.[40,41] Mean cell hemoglobin concentration did not differ significantly.

The levels of Hb A2 and Hb F also showed variations. The mean Hb A2 was lower than 3.2% in most of the age groups. Hb A2 may be regarded as an indicator of the presence of α- and β-thalassemia, but is not always confirmatory. In the α-thalassemia patients, the Hb A2 levels may be normal or decreased (<2.5%), while in the β-thalassemias, the values may be normal or higher (>3.5%). Furthermore, Hb A2 levels may be decreased in patients with iron deficiency. Hence, the variability and heterogeneity in the level of Hb A2 in different conditions makes it an unreliable test for differential diagnosis of thalassemia. On the other hand, the Hb F level, a useful parameter for diagnosis of thalassemia, depends on the rate of expression of Gγ and Aγ genes 3' to the ε-gene on chromosome 11, in the β-globin gene cluster. These genes are actively expressed during the fetal life and in young children, but are switched off by the age of two years and then remains more or less constant at a value of <1%. In some of our age groups, the Hb F was slightly higher but in others it was the same as the adult value.

This study has attempted to establish the normal reference range for hematological parameters and red cell indices in a group of healthy children. We suggest that these values should be regarded as the normal reference range for hematological parameters, red cell indices, Hb F and Hb A2 levels in Saudi children and adolescents.

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