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Normal Hematologic Values and Prevalence of Anemia in Children Living on Selected Pacific Atolls

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Abstract. The hematologic status of infants and children living on the small islands of the Pacific basin has been poorly documented. This report determines the normal ranges for hemoglobin (Hb) and mean corpuscular volume (MCV) for children residing on four of the small atolls of the Republic of the Marshall Islands in the archipelago of Micronesia. The difficulty in establishing normal hematologic values in pediatric populations is discussed and a methodology suggested that does not exclude any Hb value above the mean in determining the normal range for Hb. The study population was comprised of 563 Marshallese children representing approximately 3.4% of all children less than 16 years of age living in the Marshall Islands. The local prevalence of anemia and iron deficiency was also established.

Introduction

Anemia is the most commonly described hematologic problem of infants and children. However, the prevalence of anemia may vary depending on the method used to determine normal hematologic values. Published information regarding the hematologic status of the pediatric populations of the Pacific Islands is limited [1] and specific reference to the archipelago of Micronesia is virtually unavailable. Micronesia is comprised of the Republic of the Marshall Islands and the Caroline, Kiribati and Mariana Islands. This article reports the hematologic status of children residing on the small atolls of the Republic of the Marshall Islands where the median age is 14.8 years and 52.2% of the inhabitants are less than 16 years of age [2]. Normal ranges of hemoglobin (Hb) and mean corpuscular volume (MCV) were established for ages 1–11 years, and the prevalence of iron deficiency anemia determined. The statistical methodology used to determine the normal range for Hb did not exclude any Hb value above the mean. The difficulties in determining normal values for Hb and MCV in large populations of infants and children are discussed and the rationale supporting the proposed methodology presented.

The Population of the Marshall Islands

The Republic of the Marshall Islands is located north of the equator in the central Pacific, approximately 6,700 km (4,200 miles) southwest of San Francisco, California. The 30,000 inhabitants are distributed throughout 30 atolls and 5 mountain-top islands with a land mass totalling 110 km^2 (69 square miles) scattered across an ocean area of 480,000 km^2 (300,000 square miles). Almost half of the population lives in isolated island communities which typically contain fewer than 1,000 persons. The remainder of the inhabitants live on one of the two major population centers, Majuro atoll or Kwajalein atoll, the former being the capital of the Republic. The ethnic origin of the Marshallese, as inferred from archeological studies, is eastern Melanesian, with the original inhabitation of the Marshall Islands occurring perhaps 2,000 years ago [3]. It is clear that the remote island populations enjoyed extreme isolation for many cen-

turies, although interisland travel by large outrigger sailing vessels permitted the evolution of a relatively homogeneous society.

The Marshall Islands were made a United Nations Trust Territory under United States trusteeship following World War II. In 1954, during a nuclear weapons test series, the populations of Rongelap and Utirik atolls, numbering 253 including those in utero, were exposed to radioactive fallout from a United States thermonuclear test on Bikini atoll [4]. Since that time these exposed populations have received annual medical examinations and treatment carried out by the Medical Department of Brookhaven National laboratory under contract to the Atomic Energy Commission (now the US Department of Energy) [5]. Over the years, the Brookhaven medical team has, by virtue of its periodic field visits to the exposed populations, come into contact with a much larger general population which is resident in these islands. To the extent that its schedule and resources permit, the team has volunteered assistance to the local health authorities and a measure of direct patient care to the larger group. Approximately 1,500 Marshallese have been seen in this way, including a large number of children. They are the subject of this report.

The annual medical examinations of exposed and unexposed adults have included evaluation for anemia. Hb electrophoresis has been performed on blood specimens from approximately 200 Marshallese. No variant Hbs have been detected. Splenomegaly is distinctly rare and when present has been associated with nonhematologic disorders. Malaria is not present on the atolls. Evaluation of selected individuals with microcytosis has yet to lead to a confirmed diagnosis of either α or β thalassemia. Glucose-6-phosphate dehydrogenase deficiency has not been identified among several hundred persons tested. It therefore appears probable, from the results of routine evaluations of the adult population, that genetically determined anemias have, at best, a low prevalence among the Marshallese.

Subjects and Methods

Venous or finger-stick blood specimens were obtained from 563 Marshallese children ranging from 6 months to 15 years of age as part of their medical examination. The recorded age was that of the most recent birthday. The study population resided on 4 atolls; Rongelap (n = 110), Utrik (n = 158), Kwajalein (n = 169) and Majuro (n = 126). Two hundred and thirteen were children of radiation-exposed parents. In the aggregate the study population represented nearly 3.4% of all Marshallese under 16 years of age. Parents or guardians of all children were aware of the testing and consented to their child's participation. Copies of blood test results were placed on individual health records and given to the parent/guardian. Venous blood was collected in EDTA-anticoagulated vacutainers (Becton Dickinson, No. 6453) and skin puncture specimens were collected in 80-μl finger-stick diluting pipettes and diluted in Haema-Line 2 (Baker Instruments, Allentown, Pa.) before analysis. Hb and MCV values were determined by direct measurement on a Baker 500 electronic cell sizer and counter; calibration of the cell counter was done with control cells supplied by the manufacturer. Free erythrocyte protoporphyrin (FEP) determinations were performed on light-protected EDTA-anticoagulated specimens after they had been transported at 4 °C to the Hematopathology Laboratory, University of California, Irvine Medical Center. Zinc protoporphyrin levels were assayed with an internally calibrated (hematocrit 35%) commercial hematofluorometer employing front surface illumination and digital computer output (Environmental Science Associates, Bedford, Mass.). One hundred μl of whole blood were placed on a glass coverslip and introduced into the instrument. All determinations were performed in triplicate. The laboratory's normal range (100 subjects) is 13.8 ± 10.9 μg/dl and values exceeding 60 μg/dl are considered definitely abnormal. Five whole blood controls were drawn to indicate possible changes associated with storage. The coefficient of variation for these controls was 0.3%.

Statistical analysis was performed utilizing BMDP software [6] on a DEC VAX-11 computer. Among the types of analyses performed were simple and detailed data descriptions including analysis of variance and covariance, frequencies and correlations.

All Hb and MCV values were used in analysis for differences among groups (i.e., island of residence, history of parental radiation exposure). Because of insufficient numbers, values of persons < 1 and > 13 years of age were excluded in evaluation of normal ranges. To derive approximate age-specific normal ranges for Hb and MCV for Marshallese children the method of Dallman and Siimes [7] was used. This procedure derives limits by excluding outlying values prior to analysis. The exclusion limit for Hb is an MCV $> 5\%$ from the mean; for MCV it is an Hb > 1 SD from the mean. It is acknowledged that a considerable number of normal values will be among the excluded, but the strength of the method is that most common abnormalities will be removed. In following this approach in analyzing the Marshallese data, the mean rather than median values were used because the small number of determinations in each age group made percentile analyses statistically weak.

The appropriateness of excluding any Hb greater than the mean was questioned in view of the rarity of pathologically high Hb levels in children. For this reason exclusion of Hb values on the basis of MCV values $> 5\%$ from the mean was recalculated to encompass only the Hb levels *less* than the mean.

Results

Age-specific mean values for Hb and MCV in the 1- to 13-year age groups (table I) show the previously reported increase with age [8, 9]. The FEP, in contrast,

Table I. Age-specific hematologic parameters in Marshallese children

Age years	Hb		MCV		FEP	
	Mean ± SD g/dl	n	Mean ± SD fl	n	Mean ± SD mg/dl	n
1	11.5 ± 0.7	29	77 ± 7	29	30 ± 11	9
2	12.1 ± 0.9	62	78 ± 4	62	30 ± 24	25
3	12.3 ± 0.9	46	80 ± 5	45	17 ± 12	27
4	12.3 ± 1.1	49	80 ± 5	49	22 ± 23	33
5	12.3 ± 0.9	57	82 ± 5	56	18 ± 21	50
6	12.6 ± 0.6	38	83 ± 5	38	10 ± 10	32
7	12.7 ± 0.7	42	84 ± 5	42	10 ± 8	39
8	12.8 ± 0.9	35	84 ± 4	35	14 ± 18	28
9	12.9 ± 0.9	35	85 ± 4	35	10 ± 11	30
10	12.9 ± 0.7	35	86 ± 5	35	10 ± 11	31
11	13.0 ± 0.9	29	86 ± 4	29	6 ± 8	24
12	13.3 ± 0.6	34	86 ± 5	34	9 ± 9	32
13	13.2 ± 0.9	27	85 ± 5	27	9 ± 10	24

n = Number of children tested.

Table II. Comparison of Hb and MCV values (mean ± SD) obtained by finger puncture or venipuncture techniques in 2- and 3-year-old Marshallese children

	n tested	Hb, g/dl	MCV, fl
Finger puncture	70	12.1 ± 1.0	78.2 ± 4.7
Venipuncture	35	12.2 ± 0.7	78.8 ± 4.2

Table III. Prevalence (%) of anemia in Marshallese children, as defined by the World Health Organization (WHO)[a] and the 2nd National Health and Nutrition Examination Survey (NHANES II)[b]

Age, years	n tested	WHO	NHANES II
1–2	91	14.3	8.8 (5.7)[c]
3–5	152	5.3	5.7 (3.5)
6–8	116	13.8	2.6 (2.3)
9–11	99	9.1	3.0 (2.8)
12–14			
Males	44	2.3	2.3 (2.9)
Females	36	2.8	2.8 (3.6)
Total	538	8.9	4.5

[a] The WHO [10] recommended a lower limit for a normal Hb in children aged 6 months to 6 years of 11.0 g/dl and for ages 6–14 years of 12.0 g/dl.

[b] Based on 95% ranges after exclusion of subjects with abnormal FEP, MCV or tranferrin saturation, the lower limits of normal Hb determined from NHANES II [11] are: < 10.7 g/dl for ages 6 months to 2 years; < 10.9 g/dl for ages 3–5 years; < 11.0 g/dl for ages 6–8 years; < 11.4 g/dl for ages 9–11 years, and < 12.0 and < 11.5 g/dl for males and females, respectively, of ages 12–14 years.

[c] The percentages in parentheses are the prevalences of anemia found in the US by NHANES II [11].

was higher in the younger groups. By one-way analysis of variance there was no significant difference in age-specific Hb and MCV levels when analyzed by history of parental radiation exposure ($p = 0.24$, $F = 1.40$ for Hb; $p = 0.32$, $F = 1.19$ for MCV). Distinct differences for Hb and MCV were detected, however, when age-specific values were evaluated according to atoll of residence. The probability of significant difference among atolls was $p < 0.001$, $F = 8.77$ for Hb, and $p < 0.002$, $F = 3.52$ for MCV. The highest levels for Hb and the lowest values for MCV were found in children living on Kwajalein atoll.

To determine if significant systematic error might have been introduced by using the finger puncture technique in young children, the Hb and MCV of 2- and 3-year-olds who had either venipuncture or finger puncture were compared. The mean values were similar (table II). An attempt to exclude variant hemoglobins, especially Hb E, was only partially successful. Of 25 specimens obtained from children with lower MCV values, only 6 were satisfactory for Hb electrophoresis. All 6, with MCV values ranging from 73–78 fl, were Hb AA.

The age-specific mean and median values for the Hb and MCV were found to be in close agreement. They are displayed, along with age-specific mean values after exclusion of the outlying values as described by Dallman and Siimes [7], in figures 1 and 2. A fourth age-specific curve graphed in figure 1 is higher for Hb at all points than the Hb curve derived by the exclusion limits as defined by Dallman and Siimes [7]. The difference, while small at each point (mean difference = 0.12 ± 0.06 g/dl), is statistically significant by sign test analysis when all points are analyzed together ($p < 0.001$). Using the same approach for the MCV (i.e., no exclusion of an MCV in a child with a Hb higher than mean) no such difference was detected (fig. 2).

In determining the prevalence of anemia two lower limits were compared; those recommended by the World Health Organization [10] and those derived by Dallman et al. [11] (table III). The overall prevalences

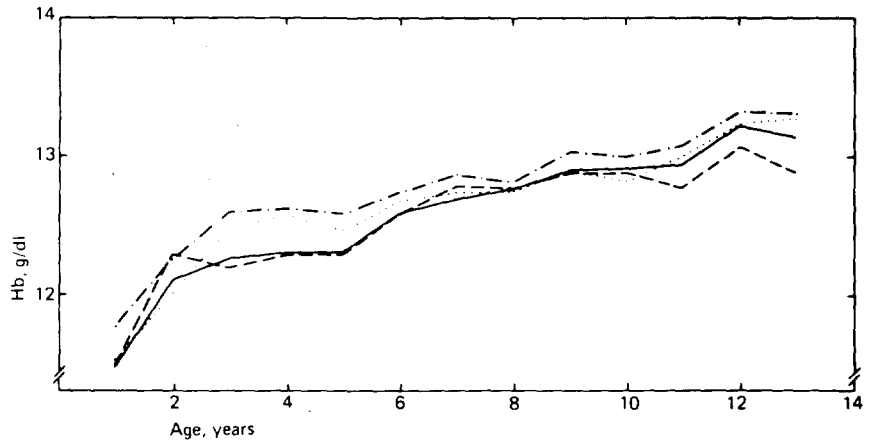

Fig. 1. Age-specific values of Hb in Marshallese children. —— = Mean; --- = median; ..... = mean, with Hb exclusions defined by Dallman and Siimes [7]; ·-·-· = same as ....., but without exclusion of any Hb values from subjects with Hb higher than mean.

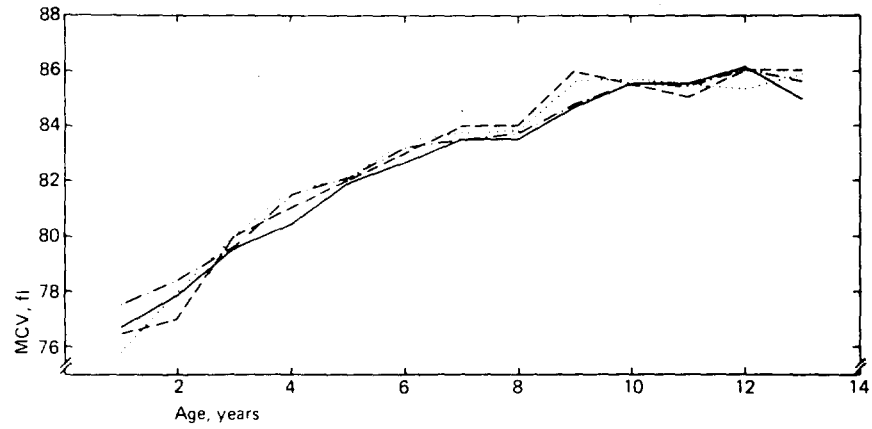

Fig. 2. Age-specific values for mean corpuscular volume in Marshallese children. —— = Mean; --- = median; ..... = mean, with MCV exclusions defined by Dallman and Siimes [7]; ·-·-· = same as ....., but without exclusion of MCV values from subjects with Hb higher than mean.

were found to be 8.9 and 4.5%, respectively (table III). In estimating the prevalence of iron deficiency it was found that 12 of 412 children tested (2.9%) had an FEP which, by laboratory criteria, was 'definitely' elevated (> 60 μg/dl). The MCV was below the limits for normal set by Dallman and Siimes [7] for 21 of the 538 children tested (3.9%).

Discussion

What is the best way to determine the normal range of Hb in a population of infants and children? Which values should be included in the analysis and which values excluded? The reported normal ranges for Hb tend to be similar even though the exclusion limits may be derived differently. The lower limit for normal Hb in children aged 3–5 years has been placed at 11.0, 11.0, 10.9 or 11.2 g/dl using 4 different approaches [7, 9–11]. Nevertheless, it is important to minimize systematic bias [12]. The distribution of Hb values in a normal population is known to be Gaussian, or nearly so [13], but abnormal values need not be so distributed. In populations where diseases associated with anemia are prominent, skewing of the total population's Hb distribution will be toward lower levels. Smoking will skew values upward. Mean values will be shifted similarly. Therefore, it is an oversimplification to exclude high and low values of equivalent deviation from the mean (or median) if clinical experience suggests one pattern of deviation greatly predominates. Hb levels above the mean (or median) should be included in derivation of the normal range of Hb in children since a pathologically elevated Hb level in childhood is an infrequent occurrence and iron deficiency is common. Using this approach, the present analysis detected a small but systematic bias in the method used by Dallman and Siimes [7] which is apparent at every year for ages 1–13 (fig. 1). Because of the evidence of systematic

Table IV. Comparison of normal ranges of Hb (gm/dl) for children in the US [NHANES II; 11] with the suggested normal ranges for the Republic of the Marshall Islands

Age, years	95% range[a] (US, NHANES II)	± 2 SD of mean[b] (Marshall Islands)
1-2	10.7-13.8	10.7-13.5
3-5	10.9-14.5	11.0-14.2
6-8	11.0-14.3	11.4-14.2
9-11	11.6-14.8	11.5-14.7

The US range for ages 1-2 years is for all races; for other age groups the values for white children are shown.

[a] After exclusion of persons with abnormal values for transferrin saturation, high FEP or low MCV.

[b] After exclusion of persons with *both* an MCV > 5% from the mean and an Hb > 5% *below* the mean.

bias resulting from the exclusion of some Hb levels above the mean in determining the normal range of Hb in children, the Marshallese data were analyzed with their inclusion. The analysis indicated the normal range (± 2 SD from the mean) for Marshallese children was similar to the ranges established by Dallman et al. [11] for children in the US (table IV). There are no previous reports on the hematologic status of Marshallese children.

As indicated by a low MCV or high FEP, the prevalence of iron deficiency (approximately 3%) in the Marshallese children surveyed is similar to that derived from analysis of data collected on American children in the National Health and Nutrition Examination Survey II [NHANES II; 11]. The prevalence of a low MCV or anemia and a higher age-specific mean FEP were greatest for ages 1-5 years, indicating that children in this age bracket are more likely to be iron-deficient. Although the study population represented nearly 90% of children residing on Rongelap and Utirik atolls and more than 3% of all Marshallese children, it is not known if the same prevalence of iron deficiency applies throughout the Republic of the Marshall Islands or Micronesia.

The similarity of the Marshallese and US values (table IV) indicates that racial differences in norms for Hb for Marshallese and American children are probably not significant. These results differ from the small but well-documented differences in normal Hb levels of Blacks and Whites in all childhood age groups in the United States [12, 14, 15]. Although the prevalence of anemia in Marshallese children in this study was not much different from that found in children living in the United States (table III), the distribution of age-specific Hb and MCV by island was not random. The children tested on Kwajalein atoll had both the highest mean Hb and lowest MCV. The significance of this finding is unknown. However, it could be explained if dietary folic acid deficiency were more common on the other three atolls.

Only 5 of the 563 children (0.8%) had moderate to severe anemia (Hb < 10 g/dl). The values ranged from 8.8 to 9.8 g/dl. Four had a low MCV and of 3 children tested all had an elevated FEP. Iron deficiency appears, therefore, to be the principal cause of the most severe as well as the most frequent anemias in Marshallese children.

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