Reference Intervals for Common Laboratory Tests in Melanesian Children

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Abstract. Pediatric reference intervals for biochemical tests are often derived from studies in Western countries and may not be applicable to the developing world. No such intervals exist for Melanesian populations. The aim of this study was to provide specific reference intervals for children from Papua New Guinea (PNG). We assayed plasma from 327 healthy Melanesian children living in Madang Province for common biochemical and hematological analytes. We used well-validated commercially available assay methodology. Compared with reference intervals from children from Western countries and/or African children, there were substantial differences in hemoglobin, soluble transferrin receptor, ferritin, calcium, phosphate, and C-reactive protein. Differences in the upper limits of reference intervals for bilirubin and alanine aminotransferase were also observed. Available reference intervals from Western and African countries may be inappropriate in PNG and other Melanesian countries. This has implications for clinical care and safety monitoring in pharmaceutical intervention trials and vaccine studies.

INTRODUCTION

Reference intervals for common laboratory tests have been derived traditionally from samples of adults living in industrialized countries.¹ However, these intervals can differ substantially from those in children from developing countries, such as those in Africa.^{1–4} Optimal cost-effective detection and management of common conditions such as malaria, anemia, and malnutrition depends on the availability of appropriate local age-specific reference ranges.⁵ Although Melanesian children face many of the health issues prevalent in Africa, marked genetic and environmental differences mean that African pediatric reference intervals may not be applicable in countries such as Papua New Guinea (PNG). In this study, we have derived normal reference intervals for a number of laboratory tests for apparently healthy Melanesian children and compared these to published reference intervals reported for children from Western countries and Africa.

SUBJECTS

This study was performed in Madang Province on the north coast of mainland PNG. The majority of the population (450,000 people) is subsistence farmers living on lowland coastal plains,⁶ and there is hyperendemic transmission of *Plasmodium falciparum* and *Plasmodium vivax* with an estimated 50 infective bites per child per year.⁷ Approximately 90% of children have alpha thalassemia trait.⁸

As part of a case-control study of the genetic determinants of severe malaria, we recruited 327 healthy PNG children 12 months to 10 years of age. These children had been matched by age, sex and, where possible, village of residence, to children with severe malaria presenting to Modilon Hospital, the provincial referral hospital, and were therefore representative of the local population most vulnerable to malaria. Written informed consent was obtained from the parents or

guardians before each child's participation in the study that was approved by the PNG Institute of Medical Research's Institutional Review Board and the PNG Medical Research Advisory Committee. The study was conducted according to the Helsinki Declaration.

The concept of normality in developing countries has been considered philosophical,⁹ but can be defined as “not suffering significant illness” or being in “reasonable health.”¹⁰ The present children conformed to this definition in that they did not have 1) a history of malaria within the previous fortnight, 2) current fever (axillary temperature > 37.5°C), 3) respiratory distress (respiratory rate > 40/minute plus in-drawing of chest wall or dyspnea), 4) impaired consciousness (Blantyre Coma Score ≤ 4),¹¹ or 5) a hemoglobin (Hb) concentration < 50 g/L. In this part of PNG, malarial parasitemia without acute illness is common⁷ and therefore children with asymptomatic parasitemia were included. Similarly, although children with mild to moderate anemia are not considered to be in optimal health, anemia represents part of the spectrum of normality in this epidemiologic situation. Nevertheless, to minimize the effects of low Hb on hematinic indices, only children with Hb concentrations > 100 g/L were tested for plasma ferritin, soluble transferrin receptor (sTFR), and vitamin B₁₂ (see Methods).

METHODS

Demographic data including the provincial origin of both parents were recorded. All children underwent a standardized clinical examination that was performed by trained research nursing officers. This examination included weight, height, axillary temperature, pulse rate, respiratory rate, and spleen size. Anthropometric Z-scores for weight-for-age, height-for-age, and weight-for-height were obtained for children < 5 years of age using World Health Organization reference data.¹²

Hemoglobin concentrations were measured using the Hb201 + system (Hemocue, Ängelholm, Sweden). Venous blood (5 mL) was collected into lithium heparin tubes (BD Vacutainer, Beckton Dickinson, Franklin Lakes, NJ). After gentle mixing and prompt centrifugation, separated plasma was stored at –70°C until analyzed. A Giemsa-stained thick blood film was prepared for all subjects regardless of disease

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status and examined for malaria parasites by at least two experienced microscopists.

Plasma was assayed for concentrations of electrolytes, urea and creatinine, albumin and total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total bilirubin, calcium, phosphate, C-reactive protein (CRP), total cholesterol, triglycerides, creatine kinase (CK), ferritin, sTFR, vitamin B₁₂, vitamin D, and cystatin C. Other than vitamin B₁₂ (Elecsys 2010, Roche Diagnostics, Mannheim, Germany), vitamin D (Liaison 25-OH Vitamin D Total Assay, Diasorin Inc., Stillwater, MN), and cystatin C (N Latex cystatin C kit, Siemens Healthcare Diagnostics, Newark, NJ), all assays were performed on the Cobas Integra 800 platform (Roche Diagnostics) using reagents supplied by the manufacturer. Compensated plasma creatinine values are reported and were automatically calculated in accordance with the manufacturer's instructions. The lower limit of detection for plasma creatinine was 18 µmol/L. Corrected plasma calcium concentrations were calculated using the formula $Ca_{corrected} = Ca_{measured} + ([40 - \text{Albumin}] \times 0.02)$.¹³ Assays were monitored for accuracy and imprecision using appropriate internal quality control procedures and, with the exception of cystatin C, through proficiency testing as part of the Quality Assurance Program of the Royal College of Pathologists of Australasia. The laboratories were accredited by the National Association of Testing Authorities as satisfying the requirements of ISO15089:2003.

Statistical methodologies from published guidelines were applied to development of PNG-specific reference intervals, which were defined as from the 2.5th to 97.5th centile.^{14,15} Outlying values were removed using Dixon's Outlier Statistic or the one-third rule. For each analyte, we applied the D'Agostino and Pearson method to test for a normal distribution for both raw data values and, when appropriate, log-transformed values¹⁶; however, because only four of 24 analytes tested were normally distributed after log-transformation, we used non-parametric methods to determine reference intervals for all parameters.¹⁵

Reference intervals for biochemical analytes change with age, particularly in the early years.^{1,2,17} Because of the present sample size, we were unable to test for more than two age partitions that included more than the recommended 120 children in each group. As an alternative approach, we derived a number of clinically relevant partitions for age-specific reference intervals for our setting. The youngest 123 children (aged 12–35 months) were partitioned from the remaining children aged 36–120 months. A second partition was applied to include children 1–5 years of age, primarily to enable an appropriate comparison with studies in African children but also because the majority of antimalarial efficacy and pharmacokinetic studies and associated safety monitoring performed in PNG involve this age group.

Reference intervals were determined separately by sex and the presence or absence of malaria parasites on microscopy. Partitioning tests to define a separate reference interval for each group were applied if the difference between the means or medians for each partition was > 25% of the calculated reference interval of the whole sample,^{15,18} and if the difference between medians was < 25%, a single reference interval was reported. Sample sizes for reference intervals were above the recommended numerical threshold of 120 for each individual test and within those intervals selected for partitioning^{15,19}

apart from vitamin D, ferritin, sTFR, vitamin B₁₂, and cystatin C (*N* = 97, 115, 116, 100, and 98, respectively).

Pediatric reference intervals for industrialized countries^{17,20–22} and, in the case of ALT, bilirubin, creatinine, sTFR, and CRP for African children,^{1,4,23} were obtained from published data. For the data that contained different age-specific reference intervals,¹⁷ we selected comparable age ranges from the children in this study to allow direct comparison. This included those 1–5 years of age, an age range used commonly in African studies. The level of agreement between the calculated reference interval and African and Western pediatric reference intervals was evaluated with the Kappa statistic and interpreted using criteria proposed by Landis and Koch.²⁴

RESULTS

Details of the children recruited to this study are summarized in Table 1; only one child was screened for recruitment but subsequently excluded because of severe anemia. The majority (79%) had parents who were both from Madang Province. Most of the remainder had either both parents from neighboring Sepik Province or one parent from Madang and one from Sepik Province. Eight values were considered outliers and were removed from further analysis: two plasma bicarbonate concentrations and one plasma urea, ALP, CK, ferritin, CRP, and vitamin D concentration.

Reference intervals for PNG children 12–35 months of age (the youngest 123 children), children 3–10 years of age, children < 5 years of age, and all children in the present cohort are shown in Table 2 together with comparative reference interval data and kappa statistics for Western and African children. Compared with reference intervals from African children and children from Western countries, there were substantial differences in Hb, sTFR, ferritin, calcium, phosphate, and CRP. Differences in the upper limits of reference intervals for bilirubin and ALT were also observed. For cystatin C, the kappa statistic was low despite close agreement between reference intervals because of the low prevalence of discordant results.²⁵ After the partition testing, there were no significant differences between children with or without parasites detectable by thick film microscopy, nor was partitioning required by sex.

DISCUSSION

The present laboratory reference intervals are the first for healthy PNG children and for pediatric Melanesian populations in general. Because there were a number of substantial

TABLE 1
Characteristics of the 327 children studied*

Age (months)	43 (25–50)
Males (%)	57
Anthropometric measures (Z score):	
Weight-for-age	–1.28 (–2.05 to –0.72)
Height-for-age	–2.24 (–3.3 to –1.24)
Weight-for-height	–0.03 (–1.12 to +0.98)
Palpable spleen (%)	13
Malarial parasitemia (%):	
<i>Plasmodium vivax</i>	14.1
<i>Plasmodium falciparum</i>	8.2
<i>Plasmodium malariae</i>	1.4
Hemoglobin (g/dL)	106 (96–119)

*Data are percentages or median and (interquartile range).

TABLE 2
Reference intervals for the current cohort of PNG children, and for Western and African children*

Analyte (units)	PNG reference interval 2.5th-97.5th centile				Western reference interval			African reference interval		
	12-35 mths (N = 123)	3-10 yrs (N = 204)	< 5 yrs (N = 246)	1-10 yrs (N = 327)	2.5th-97.5th centile	Kappa statistic	Landis and Koch	2.5th-97.5th centile	Kappa statistic	Landis and Koch
Hematology										
Haemoglobin (g/L)	65-133	72-130	67-131	71-131	107-138	0.01	Slight	1-2 y 68-124 2-3 y 7.2-12.5 3-4 y 79-132 4-5 y 80-135 (1)	0.65	Substantial
Ferritin (mg/L)				12-166†	36-84	0.08	Slight			
sTFR (mg/L)				3.6-10.9‡	1.1-3.1 (20)	0.00	None	3.9-9.5 (4)	0.73	Substantial
Vitamin B12 (pmol/L)				160-830§	197-897	0.65	Substantial			
Electrolytes										
Sodium (mmol/L)	124-141	129-141	127-141	127.5-141	134-143	0.23	Fair			
Potassium (mmol/L)	3.2-5.2	3.1-4.8	3.2-5.0	3.2-4.9	3.7-5.0	0.14	Slight			
Bicarbonate (mmol/L)	11.5-19.1	13.0-20.5	12.4-19.8	12.2-20.7	13-29	0.70	Substantial			
Calcium (mmol/L)	2.00-2.71	1.95-2.51	1.99-2.67	1.97-2.65	1-3 y 2.17-2.44 4-6 y 2.19-2.51 7-9 y 2.19-2.51	0.20	Fair			
Corrected Calcium (mmol/L)	2.08-2.64	2.05-2.46	2.07-2.61	2.06-2.58	2.19-2.64	0.06	Slight			
Phosphate (mmol/L)	1.32-2.48	1.24-2.22	1.28-2.35	1.27-2.29	1-3 y 1.25-2.10 4-6 y 1.30-1.75 7-9 y 1.20-1.80	0.16	Slight			
Kidney function										
Creatinine	18-38	18-44	18-37	18-45	18-62	0.54	Moderate	1-2 y 25-43 2-3 y 25-46 3-4 y 27-49 4-5 y 27-49 (1)	0.00	None
Urea (mmol/L)	0.9-4.5	1.3-5.5	1.0-5.2	1.0-5.1	1-3 y 1.8-6.0 4-6 y 2.5-6.0 7-9 y 2.5-6.0	0.18	Slight			
Cystatin C (mg/L)				0.56-0.90 (21)¶	0.51-0.95	0.00	None⊥			
Liver Function										
ALT (IU/L)	5-43	5-52	5-43	5-45	1-3 y 5-45 4-6 y 10-25 7-9 y 10-35	0.30	Fair	1-2 y 11-68 2-3 y 11.9-66 3-4 y 11.9-56.3 4-5 y 12.9-56.3 (1)	0.05	Slight
ALP (IU/L)	104-307	103-246	106-267	104-259	1-3 y 104-345 4-6 y 93-309 7-9 y 86-315	0.56	Moderate			
GGT (IU/L)	6-32	6-18	6-23	6-19	1-3 y 5-16 4-6 y 8-18 7-9 y 11-21	0.19	Slight			
Bilirubin (μmol/L)	1.7-8.3	1.4-7.8	1.6-7.9	1.5-7.8	0-17	0.20	Fair	1.7-18.9 (1)	0.61	Substantial
Plasma proteins										
Total Protein (g/L)	58-88	60-91	60-89	60-89	1-3 y 59-70 4-6 y 59-78 7-9 y 62-81	0.05	Slight			
Albumin (g/L)	32-47	33-46	32-46	32.5-46	1-3 y 34-42 4-6 y 35-52 7-9 y 37-56	0.26	Fair			
CRP (mg/L)	0-48	0-35	0-50	0-32	0-8.2	0.32	Fair	0-122 (23)	0.21	Fair
Other										
Cholesterol (mmol/L)	1.8-5.4	1.7-4.5	1.8-5.0	2.2-4.7	1-3 y 1.15-4.7 4-6 y 2.8-4.8 7-9 y 2.9-6.4	0.25	Fair			
Triglycerides (mmol/L)	0.42-2.7	0.36-2.7	0.37-2.7	0.37-2.7	1-3 y 0.31-1.41 4-6 y 0.36-1.31 7-9 y 0.32-6.4	0.21	Fair			
Vitamin D (nmol/L)				44-138**	30-150	0.66	Substantial			
Creatine kinase (IU/L)	11-234	15-210	14-233	15-215	1-3 y 2-163 4-6 y 18-158 7-9 y 2-177	0.63	Substantial			

* Unless indicated in parentheses, comparator reference intervals are from Reference 17. All children in the cohort are included when comparing Western PNG reference intervals, whereas only children < 5 years of age are included when comparing reference intervals between African and PNG children.

† Plasma ferritin measured in 115 children.

‡ Plasma soluble transferrin receptor measured in 116 children.

§ Plasma vitamin B12 measured in 97 children.

¶ Plasma cystatin measured in 98 children.

⊥ The kappa statistic was low despite tight agreement between reference intervals caused by the low prevalence of discordant results. See Reference 25 for detailed explanation.

** Plasma vitamin D measured in 98 children.

differences between these intervals and those published for children from Western and African countries, we recommend that they be adopted in clinical practice and epidemiologic and intervention studies in PNG. In the absence of reliable local data, we also recommend that they be used in other Melanesian countries. The present data also add to the growing literature that underscores the need for local reference ranges for common laboratory tests.⁵

The reference intervals for many of the variables in this study imply that not all the children selected were in optimal health despite appearing healthy and being drawn from the community. Plasma CRP and variables such as creatinine, Hb, sTFR, and ferritin may be related to undernutrition,²⁶ malaria,¹ and other subclinical infections.²⁷ Approximately 20% of enrolled children had asymptomatic malarial parasitemia by microscopy, 13% had enlarged spleens, and many were anemic. However, the most recent Clinical and Laboratory Standards Institute guidelines for establishing reference intervals note that "health is a relative condition lacking a universal definition."¹⁵ The guidelines also recommend that the "designation for good health for a candidate reference individual may involve a variety of examinations such as history and physical and/or certain clinical laboratory tests."¹⁵ The present children fulfilled the principles and definitions outlined in this document and were selected as controls in the parent study of the genetic determinants of severe malaria. Similar principles have been applied to other studies of reference intervals in developing countries.¹⁻⁴

We measured all analytes in an Australian nationally accredited laboratory under optimal conditions with strict calibration and quality control measures. Although the assays are all in common contemporary use, methodological differences might contribute to the differences between our reference intervals and those of others. For example, in the case of plasma creatinine, the Cobas Integra 800 platform (Roche Diagnostics) uses a compensated picric acid assay, whereas samples in the study from Africa were assayed on the Vitros DT60 II analyzer (Orthoclinical Diagnostics, Rochester, NY) that uses an enzymatic reaction. Without assaying the same samples on both systems, methodology-dependent differences are difficult to assess, but it is very likely that reference interval differences between those for PNG children and their African or Western counterparts are real.

Children in PNG have high rates of anemia due mainly to recurrent malaria, intestinal helminthic infection, and red cell polymorphisms including alpha thalassemia trait.⁸ This was reflected in the lower Hb and ferritin, and increased sTFR ranges relative to those in Western children. African children are also exposed to malaria and helminths,¹ and there was good agreement between African and PNG reference intervals for both Hb and sTFR.

Among the electrolytes measured in our pediatric sample, serum calcium, corrected calcium, and phosphate reference intervals were different from published Western ranges. The relatively low serum calcium concentrations in PNG children were not caused by a lower serum vitamin D as the reference interval for this analyte was similar to that in Western children, whereas none of our subjects had severe deficiency (< 27.5 nmol/L). This probably reflects frequent sun exposure which, by contrast, may not be adequate in urbanized Pacific Island children living in New Zealand, 24% of whom are vitamin D deficient.²⁸ A lower set point for parathyroid hormone

regulation of calcium homeostasis may be a characteristic of Melanesians, consistent with ethnic differences in European studies.²⁹ The serum phosphate reference range was higher for PNG compared with Western children, perhaps reflecting a high phosphate diet including seafood.

Although renal function reference intervals were similar, plasma creatinine levels in PNG children appeared lower than those in children from Western countries. This reflects the present anthropometric data showing that although they have a median weight to height Z-score close to zero, PNG children have lower body weights, are shorter, and have less muscle mass than children from Western countries,¹² a situation that also applies to African children.²⁶ Serum cystatin C is another analyte used to assess glomerular filtration rate (GFR) in adults and children.²¹ The cystatin C reference intervals for PNG and children from Western countries showed tight agreement, suggesting similar GFR despite variability in serum creatinine caused by differences in muscle mass.

Plasma CRP is an acute phase reactant that reflects tissue inflammation.³⁰ Although the level of agreement between PNG, Western, and African pediatric reference ranges was fair, the upper limit was 8.2 mg/L in Western children in comparison with 32 and 122 mg/L, respectively, in PNG and African children. This difference is likely to be caused by the presence of malaria parasites,³¹ intestinal helminthes, and skin infections²⁷ that frequently occur in apparently healthy children from Africa and Melanesia.

The PNG reference intervals for serum bilirubin showed some agreement with children from Western countries, and substantial agreement with those from African children. However, the upper limits were much lower in PNG children (7.8 versus 17 μ mol/L and 18.9 μ mol/L, respectively). For ALT, the upper limit of reference intervals for PNG children was similar to Western children in the younger age groups (43 versus 45 IU/L), but higher than that for Western children > 3 years of age (52 versus 25–35 IU/L). The PNG children also had upper limits of reference intervals for ALT across all age groups that were lower than in their African counterparts. In addition to clinical practice, these data have potential implications for trials of drugs and vaccines conducted in countries such as PNG. Entry into such trials is usually restricted to subjects with a serum bilirubin less than twice the upper limit of normal (ULN) because of the risk of liver injury with potentially hepatotoxic interventions,³² whereas the intervention should be discontinued if the ALT is > 5 times the ULN for more than 2 weeks or > 3 times the ULN in combination with a serum bilirubin > 2 times the ULN.³² Therefore, the availability of a locally derived ULN is an important component of safety and monitoring.

The present reference intervals are likely to be valid for Melanesian children living in coastal provinces of PNG and perhaps other ethno-geographically similar regions of Oceania, but they may not be applicable to children from highland areas of the country where there is no malaria transmission. In addition, poor health infrastructure and limited accessibility to laboratory investigations outside major centers may hinder their broad clinical application. Nevertheless, their availability should facilitate ongoing and future trials of new drugs and vaccines, and should assist in the implementation of improved standards of laboratory diagnosis should these be provided by government or other sources.

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