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Hematological and biochemical reference ranges for population with sickle cell disease at steady-state in Tanzania

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Abstract: Hematological and biochemical reference values in sickle cell disease (SCD) are crucial for patient management and evaluation of interventions. This study was conducted at Muhimbili National Hospital (MNH) in Dar es Salaam, Tanzania, to establish laboratory reference ranges among children and adults with SCD at steady-state. Patients were grouped into five age groups and with respect to their sex. Aggregate functions were used to handle repeated measures within the individual level in each age group. A nonparametric approach was used to smooth the curves and a parametric approach was used to determine SCD normal ranges. Comparison between males and females and against the general population was documented. Data from 4,422 patients collected from 2004-2015 were analyzed. The majority of the patients (35.41%) were children aged between 5-11 years. There were no significant differences (p≥0.05) in mean corpuscular hemoglobin concentration (MCHC), lymphocytes, basophils and direct bilirubin observed between males and females. Significant differences (p < 0.05) were observed in all selected parameters across age groups except neutrophils and MCHC in adults, as well as platelets and alkaline phosphatase in infants when SCD estimates were compared to the general population. Laboratory reference ranges in SCD at steady-state were different from those of the general population and varied with sex and age. The established reference ranges for SCD at steady-state will be helpful in the management and monitoring of the progress of SCD.

Keywords: Hematological parameters; biochemical parameters; reference ranges; sickle cell disease; Tanzania; steady-state.

1. Introduction

Sickle cell disease (SCD) is the most common genetic disorder globally, with an estimated 330,000 babies born with the condition annually [1]. Sub-Saharan Africa is disproportionately overburdened with SCD, where currently 75% of all patients reside in the region, which is estimated to increase

to 85% by the year 2050 [2]. In the absence of optimal care, up to 80% of children with SCD may die before reaching adulthood [3–5]. In high-income countries, effective management of SCD has been achieved through public health education, early diagnosis (including newborn screening), and therapeutic intervention to prevent or treat infections, anemia and other sickling crisis [5]. Curative options such as bone marrow transplantation are becoming increasingly available although challenged by the high risk of complications of the procedure and, in low-income countries, high cost, lack of expertise and lack of facilities [6, 7].

SCD primarily affects red blood cells but also causes changes in other hematological components such as the white blood cells and platelets [8, 9]. Individuals with SCD go through alternating periods of stable health (steady-state) and crises associated with complications such as anemia and pain [10]. These are generally associated with alteration in the quantity and function of the cellular elements in the blood. Significant alterations in hematological parameters occur in severe health conditions such as chronic pains and may predict prognosis [11]. While the low neutrophil count has been associated with a good prognosis in SCD patients, this is generally rare among patients with SCD as steady-state [8]. Furthermore, research has shown that patients with high leukocyte count, low steady-state hemoglobin and lower fetal hemoglobin level (HbF) have increased risks for sickle cell crises, including stroke [12].

Overall, most of the hematological and biochemical parameters in individuals with SCD are different from those of the general population [8, 9, 13, 14]. Thus, the significance of laboratory reference values for SCD has received considerable attention in recent years [8, 9, 13–15]. Unfortunately, there is a scarcity of reliable data to serve as reference values for hematological and biochemical parameters among patients with SCD in most African countries [16–18]. Only a few laboratories have their reference interval established from their population. Most other laboratories rely on reference values created many years ago and those generated from a different population whose characteristics are likely to be very different [19]. Since laboratory reference values are critical in medical decision-making, it is imperative to understand their reference values for individuals with SCD at steady-state in Sub-Saharan Africa. In this study, we aimed to characterize the hematological and biochemical parameters among children and adult patients with SCD at steady-state, which are essential in monitoring the progress of patients under different interventions.

2. Materials and Methods

2.1. Study design and setting

This was a prospective cohort study to establish the hematological and biochemical reference values of individuals with SCD at steady-state. The study utilized the Muhimbili Sickle Cohort data from the Muhimbili National Hospital, Tanzania [20]. The Muhimbili Sickle Cohort, established in 2004,

had enrolled over 5,000 patients with SCD. The patients enrolled in the cohort came from different geographical regions of the country, with varying prevalences of SCD [20]. Grounded on ethnic descent, almost all patients are homozygous for the Bantu or Central African Republic haplotype [20, 21]. Common outpatient services offered to patients at SCD clinics included routine physical and hematological monitoring, folic acid supplementation, penicillin prophylaxis for children below six years and health education. The use of hydroxyurea was not common and none of the patients in this study was using this medication. Patients requiring further care were channeled to appropriate inpatient departments. Other services such as pneumococcal vaccines (since 2013) were provided to patients free of charge through appropriate national programmes.

2.2. Patients

Hematological and biochemical data were collected longitudinally from 2004 to 2015. Data on 4,422 patients with confirmed homozygous SCD (HbSS, via hemoglobin electrophoresis, isoelectric focusing or high-performance liquid chromatography) and 15,461 visits were available for analysis. Individuals with all the other hemoglobin genotypes were excluded. Patients were categorized into pediatrics (infants, children, and adolescents) and adult groups. The age groups included <1 year (infants); 1-4 years, and 5-11 years (children); 12-17 years (adolescents), and those aged from 18 years and above (adults). Only records of patients at steady-state visits were included in the current study. A steady-state was defined as the point in time where SCD patient was in good health (absence of clinical events) and had not received treatments other than routine penicillin prophylaxis and folate supplements. Furthermore, a patient was not on hydroxyurea, had no history of blood transfusion during the last four months and had no history of acute pain that required treatment in the emergency department within one month before the observation [10, 22].

2.3. Hematological and biochemical parameters

Twenty-two laboratory parameters were used in this study. Among those, sixteen were hematological parameters, including hemoglobin (Hb) count, fetal hemoglobin (HbF), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell (RBC) count, mean corpuscular volume (MCV), red cell distribution width (RDW), reticulocyte count, platelet count, and mean platelet volume (MPV), as well as white blood cells (WBC), neutrophils, lymphocytes, eosinophils, monocytes, and basophils. The other six were biochemical parameters, which included bilirubin total, direct bilirubin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and creatinine. Hematological parameters were assessed using an automated hematology analyzer (Sysmex XT 2000i Kobe, Japan). The biochemical parameters were assessed using Roche Cobas integral 400plus. All laboratory procedures were done using standard protocols, including appropriate controls.

2.4. Statistical analysis

We assessed data for outliers using box-and-whisker plots. Values below the lowest and above the highest observations were considered outliers and excluded from the analysis. Repeated measurements within the individual were collapsed by calculating the mean value for everyone within the age range using an aggregate function in R. Variables were tested for normality of distribution using diagnostic plots (i.e., Q-Q plots, boxplots and histograms), Shapiro-Wilk test and Anderson-Darling test. Some variables were transformed using log and square root for those that didn't pass normality tests. Upper and lower normal values were determined using the mean plus or minus 1.96 standard deviations [23]. The sample population was grouped by sex for each age category. Mean differences between males and females and against the general population were evaluated using the t-test and Mann Whitney U test, respectively. Loess regression was used to smooth curves for all parameters, which were identified to be significantly different. All ages above 35 were rounded to 35 years to restrict fewer observations (only 1.46% of the study population) in higher ages, thus removing potential bias in the approximation of the means due to wide bandwidths above 35 years of age. A p-value of less than 0.05 was considered significant.

3. Results

3.1. Demographic characteristics

A total of 4,422 patients with SCD were included in this study. The male-to-female ratio was 1:1. The majority of patients (1566, 35.41%) were children aged between 5-11 years, while a minority (135, 3.05%) were infants. Overall, the females were slightly older than males (11.59 vs. 10.30 years; $p < 0.05$). The mean difference across the age groups when compared by sex was not significant except in the age group of 1-4 years, as presented in Table 1.

Table 1. Demographic parameters of SCD Patients. Means values are obtained after finding the mean ages within the individual level for each group.

Parameters	Overall	Females	Males	P-values*
Infants (<1 year)				
Frequency (%)	135 (3.05)	59 (1.33)	76 (1.72)	
Mean age ±SD	0.71 ± 0.23	0.69 ± 0.26	0.72 ± 0.21	0.426
Children (1-4 years)				
Frequency (%)	998 (22.6)	478 (10.80)	520 (11.75)	
Mean age ± SD	3.3 ± 1.02	3.39 ± 1.03	3.25 ± 1.00	0.023
Children (5-11 years)				
Frequency (%)	1566 (35.41)	766 (17.32)	800 (18.09)	
Mean age ± SD	8.36 ± 1.90	8.44 ± 1.90	8.29 ± 1.89	0.144

Adolescent (12-17 years)			
Frequency (%)	956 (21.61)	476 (10.76)	480 (10.85)	
Mean age ± SD	14.66 ± 1.57	14.74 ± 1.59	14.59 ± 1.55	0.125
Adults (≥ 18 years)			
Frequency	767 (17.34)	456 (10.31)	311 (7.03)	
Mean age ± SD	23.36 ± 5.22	23.61 ± 5.28	23.0 ± 5.11	0.112
Total			
Frequency (%)	4422 (100.0)	2235 (50.54)	2187 (49.46)	
Mean age ± SD	10.95 ± 7.42	11.59 ± 7.75	10.30 ± 7.01	<0.05

[*] Welch Two Sample t-test for the mean difference.

3.2. Comparison of haematological parameters

The mean values of hematological indices are shown in Table 2. The levels of hemoglobin and MCH were significantly different in all age groups except in infants, where the results were analyzed by gender. The mean HbF was higher in females, but the difference was statistically significant in children aged 5-11 years, adolescence and adults (p-values of 0.0006, 0.0341, and 0.0005, respectively). While MCHC slightly increased with age, the mean levels were comparable between females and males. RBCs and reticulocyte count were significantly higher in males than females among patients aged ≥18 years, with p-values of <0.0001 and 0.0411 or 0.0276, respectively. The mean MCV and RDW in the infant groups were alike when the results were examined by gender. Mean platelet counts were lower in females, but the difference was only evident in children aged 1-4 years (p=0.0183). MPV was significantly higher in female children aged 5-11 years compared to males (p = 0.0002). Furthermore, the mean WBC was higher in males. However, the observed difference was not significant among infants (<1 year) and adults (≥18 years). WBC components, i.e., neutrophils and eosinophils, were evidently different in adolescents and adults when their mean levels were analyzed by sex. The mean lymphocytes (percentage) were higher in females, although significantly so only in the age groups of <1 and 5-11 years. On the other hand, the absolute lymphocytes were significantly higher in male children aged between 5-11 years and adolescence (12-17 years), with the p = 0.0128 and 0.0468, respectively. Monocytes were more elevated in males, but the difference was not statistically significant in infants and children aged 5-11years. The mean basophil counts were comparable between males and females.

Table 2 Steady-state hematological reference values calculated as mean (mean ± 1.96SD) in SCD categorized by age groups and sex.

Age group (years)	Females		Males		Combined		P-values*
	n	Mean (Mean ±1.96SD)	N	Mean (Mean ±1.96SD)	n	Mean (Mean ±1.96SD)	
Hemoglobin (g/dL)							
<1	59	7.3(4.8-9.8)	76	7.2(4.5-9.8)	135	7.2(4.6-9.8)	0.4574
1-4	477	7.4(5.0-9.7)	518	7.2(4.7-9.6)	995	7.3(4.9-9.7)	0.0128
5-11	766	7.4(5.1-9.6)	799	7.2(5.1-9.3)	1565	7.3(5.1-9.5)	0.0018
12-17	476	7.6(5.4-9.7)	480	7.4(5.1-9.7)	956	7.5(5.3-9.7)	0.0171
≥18	456	7.6(5.1-10.0)	310	8.2(5.0-11.3)	766	7.8(5.0-10.6)	<0.0001
HbF (%)							
<1	29	16.5(4.0-67.7)	42	16.0(3.1-81.2)	71	16.2(3.5-75.2)	0.7882
1-4	278	9.8(2.7-35.4)	272	9.3(2.9-29.5)	550	9.6(2.8-32.4)	0.1696
5-11	379	6.6(1.9-23.1)	377	5.5(1.5-19.9)	756	6.0(1.6-21.8)	0.0006
12-17	150	5.8(1.3-24.4)	135	4.9(1.3-18.0)	285	5.3(1.3-21.4)	0.0341
≥18	162	5.9(1.5-22.5)	103	3.9(0.9-16.6)	265	5.0(1.2-21.1)	0.0005
MCH (pg)							
<1	58	23.4(17.4-29.4)	75	22.4(14.9-30.0)	133	22.9(15.9-29.8)	0.1109
1-4	476	25.0(18.7-31.3)	514	24.5(18.2-30.9)	990	24.8(18.4-31.1)	0.0357
5-11	762	25.8(19.0-32.6)	798	25.4(18.8-32.1)	1560	25.6(18.9-32.4)	0.0392
12-17	476	26.8(20.2-33.4)	478	25.9(19.2-32.7)	954	26.4(19.6-33.1)	<0.0001
≥18	453	27.6(20.3-34.9)	311	26.5(19.6-33.5)	764	27.2(20.0-34.4)	<0.0001
MCHC (g/dL)							
<1	58	31.0(28.6-33.5)	76	30.9(27.4-34.4)	134	31.0(27.9-34.0)	0.5994
1-4	477	31.4(28.1-34.7)	518	31.4(28.0-34.9)	995	31.4(28.0-34.8)	0.9934
5-11	765	32.1(28.9-35.4)	799	32.1(28.6-35.6)	1564	32.1(28.7-35.5)	0.8559
12-17	476	32.8(29.5-36.1)	478	32.9(29.6-36.1)	954	32.8(29.6-36.1)	0.5454
≥18	456	32.9(29.8-35.9)	311	32.9(29.7-36.1)	767	32.9(29.8-36.0)	0.9422
RBC Count (×10¹²/l)							
<1	59	3.2(1.7-4.6)	76	3.3(1.4-5.2)	135	3.2(1.5-4.9)	0.4144
1-4	478	3.0(1.6-4.4)	518	3.0(1.5-4.4)	996	3.0(1.6-4.4)	0.3762
5-11	766	2.9(1.6-4.2)	800	2.8(1.5-4.2)	1566	2.9(1.6-4.2)	0.2956
12-17	476	2.8(1.7-4.0)	478	2.9(1.5-4.3)	954	2.9(1.6-4.2)	0.2519
≥18	456	2.8(1.5-4.1)	310	3.1(1.4-4.9)	766	2.9(1.4-4.5)	<0.0001
MCV (fL)							
<1	58	75.4(57.8-92.9)	76	72.7(50.9-94.4)	134	73.8(53.7-94.0)	0.2167
1-4	477	79.4(61.2-97.7)	517	78.1(59.9-96.2)	994	78.7(60.5-97.0)	0.0106
5-11	765	80.3(60.7-99.9)	799	79.2(60.7-97.7)	1564	79.7(60.7-98.8)	0.0140
12-17	476	81.8(64.0-99.5	478	78.9(60.2-97.5)	954	80.3(61.9-98.8)	<0.0001
≥18	455	83.9(64.1-103.7)	311	80.7(61.5-99.9)	766	82.6(62.8-102.4)	<0.0001
RDW (%)							
<1	59	23.7(16.9-30.5)	75	24.3(17.4-31.3)	134	24.1(17.2-30.9)	0.1860

| Age group (years) | Females | | Males | | Combined | | P-values* |
	n	Mean (Mean ±1.96SD)	N	Mean (Mean ±1.96SD)	n	Mean (Mean ±1.96SD)	
1-4	476	22.6(16.2-28.9)	518	23.3(16.9-29.7)	994	22.9(16.5-29.4)	<0.0001
5-11	764	21.6(15.4-27.8)	800	22.3(16.3-28.4)	1564	22.0(15.8-28.1)	<0.0001
12-17	476	21.2(15.0-27.5)	478	21.9(16.1-27.6)	956	21.6(15.5-27.6)	<0.0001
≥18	456	20.3(14.5-26.1)	310	21.6(15.6-27.6)	766	20.8(14.8-26.8)	<0.0001
Reticulocyte (%)							
<1	20	12.1(3.0-27.2)	17	13.0(3.2-29.4)	37	12.5(3.2-28.0)	0.7032
1-4	201	13.4(3.6-29.3)	221	12.5(2.0-31.9)	422	12.9(2.7-30.7)	0.4093
5-11	336	12.4(3.8-26.0)	365	12.1(3.6-25.6)	701	12.3(3.7-25.8)	0.9524
12-17	235	12.0(3.8-24.7)	198	11.1(3.4-23.3)	433	11.6(3.6-24.1)	0.0894
≥18	202	11.9(3.9-24.2)	122	11.0(3.9-21.8)	324	11.6(3.9-23.4)	0.0411
Reticulocyte Count (×10¹²/l)							
<1	20	0.38(0.07-0.70)	17	0.42(0.12-0.72)	37	0.40(0.09-0.71)	0.4462
1-4	201	0.42(0.04-0.79)	221	0.39(0.01-0.78)	422	0.40(0.02-0.79)	0.1977
5-11	336	0.37(0.04-0.70)	365	0.37(0.06-0.68)	701	0.37(0.05-0.69)	0.7346
12-17	235	0.36(0.07-0.66)	198	0.33(0.66-0.61)	433	0.35(0.06-0.64)	0.0371
≥18	202	0.34(0.07-0.61)	122	0.38(0.09-0.66)	324	0.36(0.08-0.63)	0.0276
Platelet Count (×10⁹/l)							
<1	59	397.9(73.3-722.4)	76	410.8(56.8-764.7)	135	405.1(64.8-745.5)	0.7935
1-4	476	375.1(55.8-694.4)	517	399.2(67.0-731.4)	993	387.7(60.9-714.4)	0.0183
5-11	766	422.3(110.9-733.6)	798	440.2(103.8-776.6)	1564	431.4(106.7-756.2)	0.1210
12-17	476	447.0(130.6-763.4)	477	453.3(142.5-764.1)	953	450.2(136.7-763.7)	0.9320
≥18	456	442.9(149.5-736.3)	309	447.0(149.2-744.9)	765	444.6(149.6-739.6)	0.7580
MPV (fL)							
<1	53	8.5(6.8-9.9)	68	8.3(6.4-10.5)	121	8.4(6.5-10.2)	0.9604
1-4	458	8.7(6.9-10.3)	498	8.6(6.8-10.1)	956	8.6(6.9-10.2)	0.2977
5-11	741	8.6(7.0-10.2)	781	8.4(6.8-10.1)	1522	8.6(6.9-10.2)	0.0002
12-17	467	8.5(6.9-10.4)	470	8.5(7.0-10.3)	937	8.5(7.0-10.4)	0.7275
≥18	443	8.5(7.1-10.5)	304	8.6(7.1-10.6)	747	8.6(7.1-10.5)	0.3750
WBC (×10⁹/l)							
<1	57	16.4(3.8-29.1)	72	16.6(1.3-31.3)	129	16.4(2.4-30.3)	0.4381
1-4	473	17.1(6.5-27.6)	518	18.2(7.3-29.2)	991	17.7(6.9-28.5)	0.0006
5-11	798	14.7(5.6-23.8)	766	15.7(7.1-24.4)	1564	15.2(6.3-24.1)	<0.0001
12-17	476	12.5(5.8-19.2)	477	13.3(6.0-20.7)	953	12.9(5.9-20.0)	0.0003
≥18	455	11.6(4.7-18.4)	311	11.9(4.3-19.6)	766	11.7(4.5-18.9)	0.4009
Neutrophils (%)							
<1	56	27.2(7.0-47.3)	69	29.3(8.2-50.4)	125	28.3(7.6-49.1)	0.1985
1-4	447	36.5(18.1-55.0)	482	36.7(18.1-55.2)	929	36.6(18.1-55.1)	0.9277
5-11	724	41.6(25.2-58.0)	783	42.2(25.4-58.9)	1507	41.9(25.3-58.5)	0.2629

Age group (years)	Females		Males		Combined		P-values*
	n	Mean (Mean ±1.96SD)	N	Mean (Mean ±1.96SD)	n	Mean (Mean ±1.96SD)	
12-17	465	45.5(29.1-62.0)	460	44.0(27.6-60.5)	925	44.8(28.3-61.3)	0.0091
≥18	449	48.5(28.9-68.0)	305	46.9(25.5-68.1)	754	47.8(27.6-68.1)	0.0132
Neutrophils Count (×10⁹/l)							
<1	56	4.3(0.02-8.6)	69	4.7(-0.9-10.4)	125	4.5(-0.5-9.6)	0.3250
1-4	447	6.1(1.6-10.6)	482	6.5(2.1-10.8)	929	6.3(1.8-10.7)	0.0087
5-11	724	6.0(1.8-10.2)	783	6.6(2.2-10.9	1507	6.3(2.0-10.6)	<0.0001
12-17	465	5.7(1.9-9.5)	460	5.8(1.7-9.9)	925	5.8(1.8-9.7)	0.1927
≥18	449	5.6(1.1-10.2)	305	5.5(1.0-10.1)	754	5.6(1.1-10.2)	0.6492
Lymphocytes (%)							
<1	59	56.6(29.7-83.5)	75	53.3(29.3-77.4)	134	54.8(29.3-80.2)	0.0740
1-4	472	49.4(29.4-69.5)	516	48.6(28.2-69.1)	988	49.0(28.8-69.3)	0.2216
5-11	763	43.9(26.7-61.1)	796	43.0(26.2-59.8)	1559	43.5(26.4-60.5)	0.0612
12-17	474	40.1(24.1-56.1)	476	39.6(23.6-55.7)	950	39.9(23.9-55.9)	0.3484
≥18	454	37.0(20.5-53.5)	310	36.5(17.5-55.5)	764	36.8(19.2-54.3)	0.4455
Lymphocytes Count (×10⁹/l)							
<1	59	9.3(0.04-18.7)	75	8.7(0.0-18.2)	134	9.0(0.0-18.4)	0.4594
1-4	472	8.5(1.7-15.2)	516	8.9(1.9-15.8)	988	8.7(1.8-15.5)	0.0758
5-11	763	6.4(1.3-11.6)	796	6.8(1.8-11.7)	1559	6.6(1.5-11.7)	0.0128
12-17	474	5.0(1.6-8.3)	476	5.2(2.1-8.3)	950	5.1(1.8-8.3)	0.0468
≥18	454	4.2(1.5-6.9)	310	4.2(1.1-7.3)	764	4.2(1.3-7.1)	0.6696
Monocytes (%)							
<1	57	8.8(3.3-23.4)	74	9.7(3.7-25.7)	131	9.3(3.5-24.7)	0.1492
1-4	466	8.3(3.7-18.6)	507	8.8(3.7-20.5)	973	8.6(3.7-19.6)	0.0252
5-11	756	8.7(4.2-18.1)	793	8.9(4.1-19.0)	1549	8.8(4.1-18.5)	0.3807
12-17	473	9.4(4.6-19.2)	472	9.9(4.8-20.4)	945	9.7(4.7-19.8)	0.0121
≥18	449	9.5(4.7-19.0)	306	10.5(5.0-22.2)	755	9.9(4.8-20.4)	<0.0001
Monocytes Count (×10⁹/l)							
<1	57	1.4(0.0-7.5)	74	1.6(0.4-15.4)	131	2.2(0.1-12.0)	0.2325
1-4	466	1.4(0.0-8.3)	507	1.6(0.0-10.4)	973	2.1(0.0-9.4)	0.0002
5-11	756	1.2(0.0-5.9)	793	1.3(0.2-7.4)	1549	1.6(0.0-6.7)	0.0001
12-17	473	1.1(0.0-5.0)	472	1.2(0.03-4.2)	945	1.4(0.01-5.3)	0.0002
≥18	449	1.0(0.0-3.8)	306	1.2(0.0-4.3)	755	1.2(0.0-4.6)	<0.0001
Eosinophils (%)							
<1	59	3.7(1.2-11.5)	72	3.4(1.1-10.3)	131	3.5(1.1-10.8)	0.4710
1-4	462	3.5(1.2-9.8)	501	3.5(1.2-9.7)	963	3.5(1.2-9.8)	0.8414
5-11	743	3.8(1.3-11.4)	790	4.0(1.4-11.5)	1533	3.9(1.3-11.5)	0.1706
12-17	470	3.6(1.3-9.9)	463	4.5(1.5-12.8)	933	4.0(1.4-11.5)	<0.0001
≥18	454	3.2(1.1-8.9)	307	3.8(1.2-12.2)	761	3.5(1.1-10.3)	<0.0001

Age group (years)	Females		Males		Combined		P-values*
	n	Mean (Mean ±1.96SD)	N	Mean (Mean ±1.96SD)	n	Mean (Mean ±1.96SD)	
Eosinophils Count (×10⁹/l)							
<1	59	0.53(0.0-1.44)	72	0.45(0.0-1.20)	131	0.48(0.0-1.31)	0.3034
1-4	462	0.51(0.0-1.34)	501	0.54(0.0-1.39)	963	0.53(0.0-1.37)	0.2644
5-11	743	0.51(0.0-1.53)	790	0.58(0.0-1.65)	1533	0.55(0.0-1.59)	0.0078
12-17	470	0.40(0.0-1.17)	463	0.57(0.0-1.69)	933	0.48(0.0-1.46)	<0.0001
≥18	454	0.32(0.0-0.93)	307	0.44(0.0-1.40)	761	0.37(0.0-1.15)	<0.0001
Basophils (%)							
<1	58	2.5(1.3-5.0)	72	2.3(0.9-5.5)	130	2.4(1.1-5.3)	0.1711
1-4	471	2.1(1.2-3.8)	510	2.1(1.2-3.8)	`981	2.1(1.2-3.8)	0.6519
5-11	754	2.0(1.1-3.4)	795	2.0(1.2-3.4)	1549	2.0(1.1-3.4)	0.9251
12-17	473	1.9(1.1-3.4)	474	1.9(1.1-3.5)	947	1.9(1.1-3.5)	0.9398
≥18	456	1.9(1.2-3.0)	310	1.9(1.1-3.4)	766	1.9(1.1-3.1)	0.2761

*Welch Two Sample t-test. SD; Standard deviation

Figure 1 represents smoothed means curves of hematological parameters, which were statistically different between males and females. In the figure, the bandwidth represents a 95% confidence interval. Therefore, there is a 0.95 probability that the true regression line for the population lies within the confidence interval.
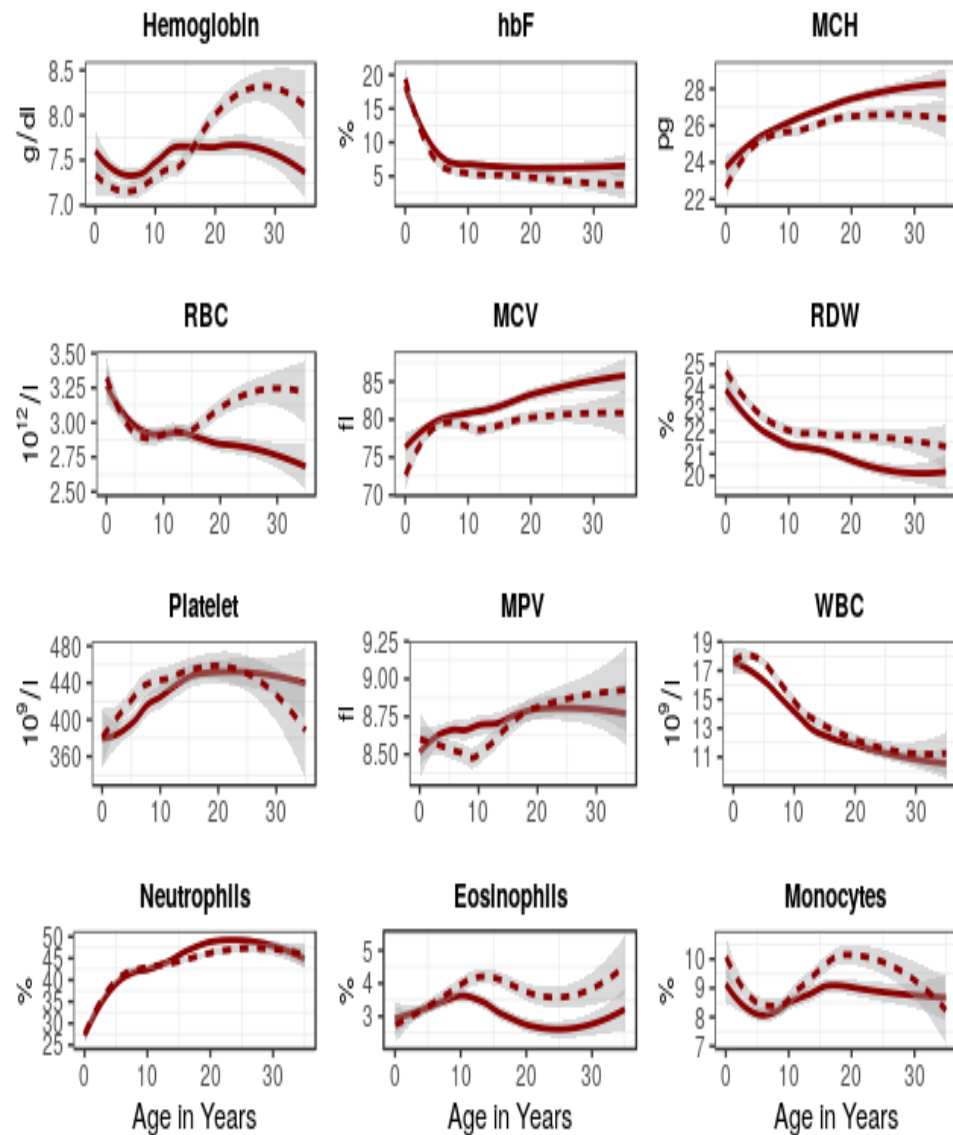
Figure 1. Smoothed mean curves for selected hematological parameters by sex. Solid lines denote the mean values for females and dashed lines denote the mean values for males.

3.3. Comparison of biochemical parameters

The variations of the mean values of biochemical parameters between males and females cases are shown in Table 3. While the mean direct bilirubin was comparable between males and females across age groups, the total bilirubin was significantly different only in adults (p=0.0243). The mean AST was significantly higher in females among infants and significantly higher in males among children aged 5-11 years and adolescents. The mean ALP and creatinine were higher in adult males compared to adult females. Females were aged 12-17 years had significantly lower levels of LDH (by 75.5U/L) compared to males.

Table 3 Steady-state biochemical reference values calculated as mean (mean ± 1.96SD) in SCD categorized by age groups and sex.

Age group (years)	Females		Males		Combined		P-values*
	n	Mean (Mean ±1.96SD)	n	Mean (Mean ±1.96SD)	n	Mean (Mean ±1.96SD)	
Bilirubin direct (μmol/L)							
<1	13	9.9(0.8-119.4)	17	8.6(1.2-59.6)	30	9.2(1.0-80.2)	0.6752
1-4	154	7.4(1.0-54.8)	169	6.7(0.7-64.1)	323	7.0(0.8-59.6)	0.5992
5-11	308	7.8(1.0-56.6)	353	7.6(1.1-53.3)	661	7.7(1.1-54.8)	0.5685
12-17	198	6.0(0.5-62.0)	184	6.0(0.6-55.5)	382	6.0(0.6-58.7)	0.8477
≥18	166	6.7(0.9-48.4)	106	6.1(0.6-61.2)	272	6.5(0.7-53.3)	0.7343
Bilirubin total (μmol/L)							
<1	17	35.4(10.7-117.3)	18	30.2(8.2-110.8)	35	32.6(9.4-113.1)	0.7165
1-4	153	39.5(11.4-136.8)	158	36.4(11.4-116.4)	311	37.9(11.4-126.2)	0.2966
5-11	280	50.1(14.6-170.9)	318	50.5(15.4-165.9)	598	50.3(15.0-168.1)	0.8201
12-17	162	59.4(15.6-225.7)	160	58.0(11.5-291.3)	322	58.7(13.4-257.4)	0.8052
≥18	152	48.9(15.1-158.5)	85	59.9(13.7-161.7)	237	52.6(14.3-193.4)	0.0243
AST (U/L)							
<1	18	51.6(13.3-114.9)	16	39.4(18.3-88.5)	34	45.7(14.0-95.4)	0.0180
1-4	156	44.8(20.1-79.4)	162	46.2(21.2-80.7)	318	45.5(20.7-80.0)	0.2745
5-11	283	43.1(20.9-73.4)	321	46.4(22.8-78.3)	604	44.9(21.8-76.2)	0.0029
12-17	165	40.7(16.2-76.4)	161	45.9(19.8-82.8)	326	43.2(17.7-80.0)	0.0009
≥18	155	33.1(12.2-64.0)	87	34.7(12.7-67.5)	242	33.6(12.4-65.3)	0.5586
ALP (IU/L)							
<1	18	305.4(89.4-650.0)	16	335.5(90.8-734.5)	34	319.4(91.6-685.0)	0.3427
1-4	158	290.2(119.0-563.7)	163	300.1(106.5-577.8)	321	297.6(112.7-570.6)	0.8089
5-11	285	235.7(86.1-458.9)	322	240.2(107.0-426.6)	607	238.1(96.6-442.3)	0.4232
12-17	167	222.6(61.9-482.6)	162	240.3(89.3-464.4)	329	231.2(74.2-475.1)	0.1035
≥18	154	125.1(30.0-285.3)	89	158.3(35.8-367.9)	243	136.8(30.5-319.5)	0.0005
LDH (U/L)							
<1	18	794.1(123.4-2047.8)	16	829.4(234.0-1789.4)	34	810.6(174.1-1913.9)	0.9313
1-4	151	903.0(314.8-1794.2)	159	946.9(352.7-1828.6)	310	925.4(333.9-1812.0)	0.2646
5-11	279	840.1(316.5-1614.4)	316	819.2(236.5-1752.7)	595	829.0(271.3-1690.2)	0.5434
12-17	166	784.7(208.7-1728.8)	159	860.2(282.7-1750.8)	325	821.2(241.8-1744.1)	0.0207
≥18	153	637.9(156.4-1444.6)	87	714.9(131.8-1763.4)	240	665.3(145.0-1563.8)	0.2412
Creatinine (μmol/L)							
<1	18	31.7(6.7-75.3)	18	32.8(17.4-52.9)	36	32.2(10.8-65.0)	0.9620
1-4	158	32.6(18.9-50.0)	162	33.3(18.1-53.0)	320	32.9(18.5-51.5)	0.4853
5-11	284	31.7(16.8-51.4)	320	33.4(17.1-55.2)	604	32.6(16.9-53.5)	0.0547
12-17	167	35.1(18.4-62.3)	168	37.8(16.1-63.1)	335	36.4(17.2-62.7)	0.2763
≥18	151	48.8(20.4-75.9)	87	51.3(23.8-89.0)	238	46.4(21.1-81.6)	0.0002

*Welch Two Sample t-test. SD; Standard deviation

Similarly, we represent smoothed means curves of biochemical parameters statistically different between males and females. As shown in Figure 2, the bandwidth represents a 95% confidence interval. Therefore, there is a 0.95 probability that the true regression line for the population lies within the confidence interval.
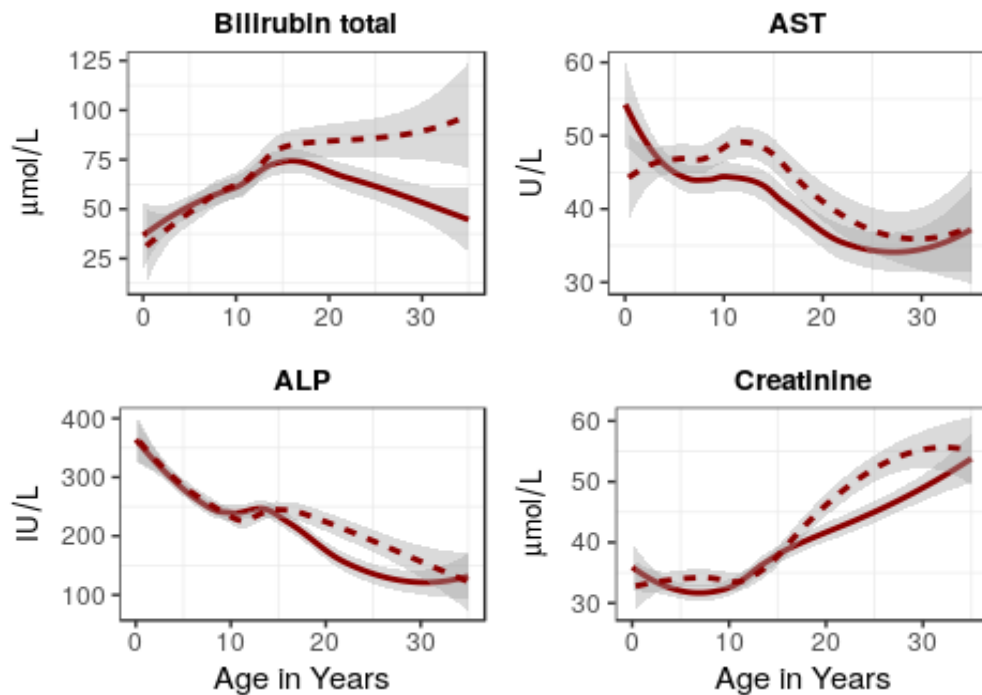
Figure 2. Smoothed mean curves for selected biochemical parameters by sex. Solid lines denote the mean values for females and dashed lines denote the mean values for males.

3.4. Laboratory parameters in SCD against the general population

Table A1 compares values in SCD and that of the general Tanzanian population published elsewhere [16, 24, 25]. Individuals with SCD had significantly lower reference values for hemoglobin, MCH, and lymphocytes. In comparison, values for platelets (except infants' group), WBC, bilirubin direct, bilirubin total, and AST were significantly higher when compared to that of the general population (almost p<0.0001 in each case). MCHC, Neutrophils, platelets, and ALP in infants had estimated ranges comparable to the general population. Children with SCD below five years had significantly higher MCV and creatinine reference values than those of the general population, but this changed for the older age groups where reference values from the general population were higher compared to those of individuals with SCD.

4. Discussion

Understanding reference ranges for laboratory parameters is critical to the focused care of SCD patients. This study is the first to report reference values for hematological and biochemical parameters for patients with SCD at steady-state by age groups and by sex in Tanzania. Most laboratory parameters (platelets, white blood cells, bilirubin direct, bilirubin total, aspartate aminotransferase, and alkaline phosphatase) were found to have higher values when compared to that of the general population, which was also different across the age groups. The reference ranges in the SCD were more or less similar between males and females.

The observed steady-state hemoglobin concentration of 7.2-7.3g/dL in children with SCD under-five years of age was higher than 6.3-6.9g/dL reported elsewhere [9, 14, 26]; but lower than 8.1-9.3g/dL, which was reported in the Cooperative Study involving patients with SCD conducted in Western countries [27]. These observations of differences in hemoglobin may be due to different factors such as quality of care in children with SCD malaria infection, nutritional deficiency [28] and parasitic infections [29], of which the latter three are more common in the least developed countries [30].

Moreover, the lower steady-state hemoglobin concentration in SCD patients when compared to the general population can be explained by continual haemolysis of the red cells with their consequent shortened survival in individuals with SCD [15]. The mean hemoglobin, HbF, MCH, RBC, MCV, RDW, eosinophils, bilirubin total, ALP, and creatinine were different between females and males, with most deviations happening after the age of 15 years. This is likely due to the start of the menstruation period, which has also been associated with changes in levels of these parameters in female macaques [31].

We found significantly higher MCV values in children with SCD less than five years of age compared to that of the general population. These higher values are in agreement with findings from another study [13]. It was reported that higher MCV in children is largely due to folate deficiency [32]. Lacking vitamin B9 (resulting from not eating enough foods like leafy green vegetables, beans, and grains) may be worse, especially in under-fives who are more dependent and may have lower compliance with supplementary folate [32].

Our study reported higher platelet counts among individuals with SCD compared to the values reported by the previous study [8]. Furthermore, there was a significant difference in platelet levels in all age groups except among infants when the values were compared between individuals with SCD and the general population. After four years of life, platelet counts were gradually elevated in individuals with SCD but declined in the general population. The advancement of platelet count in patients with SCD could be due to deficient splenic pooling of young active platelets due to hyposplenism [33].

The mean WBC counts were higher among patients with SCD compared to the general population. A previous study suggested that reaction to commonly used medication and recurrent infections are factors that could increase the number of white cells in individuals with SCD [34]. Additionally, elevated WBC

counts were shown to occur in children with SCD children during bacterial infections and vaso-occlusive crises [35]. Based on these findings, we believe that periodic evaluation of SCD patients' thorough physical examination will help identify those with high WBC so that appropriate measures can be taken to moderate causes related to high WBC.

We reported normal alkaline phosphatase levels in infants while other patients with SCD had shown a significant increase compared to the general population. As documented elsewhere, raised plasma levels of alkaline phosphatase in patients with SCD could result from persistent vaso-occlusive crises involving the bones. The observed elevation of alkaline phosphatase was above the expected rise in children who are still growing [36].

Creatinine levels were considerably lower in patients with SCD after four years of age. Low creatinine values in patients with SCD are in agreement with those reported in other studies [37, 38]. It was reported that the decrease of creatinine in patients with SCD was caused by smaller physical stature [39]. This finding is consistent with that of another study which investigated uric acid, urea, and creatinine levels in patients with SCD, which reported that the decline of creatinine resulted from the reduced muscle mass [38].

In conclusion, our study is the first to report steady-state hematological and biochemical reference values for SCD for different age groups (infants, children, adolescents, and adults) by sex. We reported an increase in the means of MCH, MCV, and neutrophils with significantly higher values in females. Other parameters such as HbF, RDW, WBC, and ALP significantly decreased with age in males and females. Moreover, we observed the means of some parameters such as hemoglobin, RBC, platelet, MPV, AST, eosinophils, total bilirubin, and creatinine fluctuate over time between males and females. These data provide useful reference ranges that can be used in monitoring the status and management of patients with SCD in Tanzania and other populations sharing similar environments, particularly in Sub-Saharan Africa.

4.1. Limitations

Although the sample size was large in our study, the number of males and female patients in the infants' group was less than 120, as recommended elsewhere [40, 41]. This may be the reason for the failure of a considerable number of parameters in this age group to attain the significance level. Furthermore, the interpretation of laboratory results may have been limited when the variation of a specific group is minimal from that of the population. Therefore, the criterion of partitioning the population reference ranges into subgroups (males and females in our case) using a significant difference criterion may seem insufficient than utilizing the clinical and physiological approach [40, 41].

Author Contributions: B.P.M., R.Z.S., and Jmakani designed the research study; B.P.M., R.Z.S., and A.D.F. designed the methodology section; R.Z.S.,

Jmgaya, D.S., F.T., U.M., E.B., S.N., Jmakani and B.P.M. collected and curated the data; A.D.F. and B.P.M. performed data analysis; A.D.F., R.Z.S., B.P.M., E.B. and Jmakani interpreted the results; A.D.F. wrote the original draft of the manuscript; A.D.F., R.Z.S., Jmgaya, D.S., F.T., U.M., E.B., S.N., Jmakani and B.P.M. reviewed the manuscript; B.P.M., R.Z.S., and Jmakani provided project administration and supervision. All authors have read and agreed to the final draft.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Muhimbili National Hospital (MNH) and approved by Muhimbili University of Health and Allied Science (MUHAS) ethical committee (MU/RP/AEC/VOL XI/33).

Informed Consent Statement: During data collection, informed consent was obtained from all SCD patients or parents/guardians in the case of children and adolescents (below 18 years).

Data Availability Statement: The Muhimbili data sharing policy is not finalized. The data used to support the findings of this study are available from the institute upon reasonable request.

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Competing interests: The authors declare that they have no competing interests.

Appendix A:

Table A1. Medians (95% reference ranges) in selected laboratory parameters for SCD at steady-states compared to population published data in Tanzania.

Analytes (Unit)	Age group in years (sex)	SCD Patients Median (2.5-97.5%)	General Population Median (2.5-97.5%)	P-values*
Hemoglobin (g/dL)	<1	7.3(4.6-9.5)	10.7(8.1-13.2) [‡]	<0.0001
	1-4	7.3(4.5-9.7)	11.3(8.1-13.9) [‡]	<0.0001
	5-11	7.3(4.9-9.4)	12.6(10.3-14.7) [‡]	<0.0001
	12 -17(F)	7.6(5.3-9.7)	13.0(10.0-14.9) [‡]	<0.0001
	12 -17(M)	7.4(5.0-9.8)	13.7(10.8-17.0) [‡]	<0.0001

Analytes (Unit)	Age group in years (sex)	SCD Patients Median (2.5-97.5%)	General Population Median (2.5-97.5%)	P-values*
	≥18 (F)	7.6(5.1-10.0)	13.5(11.1-15.7) ‡	<0.0001
	≥18 (M)	8.3(4.7-11.0)	15.4(13.7-17.7) ‡	<0.0001
	≥18 (M&F)	7.9(4.8-10.6)	14.7(11.7-17.2) ‡	<0.0001
MCH (pg)	-	-	-	-
	≥18(F)	28.0(19.9-34.2)	30.0(23.1-33.2) ‡	<0.0001
	≥18(M)	26.6(19.9-33.1)	29.3(24.2-33.1) ‡	<0.0001
	≥18(M&F)	27.5(19.9-34.0)	29.7(23.6-33.1) ‡	<0.0001
MCHC (g/dL)	-	-	-	-
	≥18(F)	32.8(29.9-36.1)	33.3(30.6-35.1) ‡	<0.0001
	≥18(M)	32.6(30.0-36.5)	32.7(30.4-34.8) ‡	0.9239
	≥18(M&F)	32.8(30.0-36.3)	33.0(30.6-34.9) ‡	0.9856
MCV (fL)	<1	74.0(53.4-91.7)	71.0(53.3-96.6) †	<0.0001
	1-4	79.0(59.7-95.3)	75.0(54.7-91.6) †	<0.0001
	5-11	80.0(60.5-98.0)	82.5(66.0-90.0) †	<0.0001
	12 -17(F)	82.0(64.3-99.1)	84.9(62.2-94.5) †	<0.0001
	12 -17(M)	79.0(63.4-93.5)	82.0(63.2-91.0) †	<0.0001
	≥18 (F)	84.4((64.0-102.8)	89.5(77.7-97.9) ‡	<0.0001
	≥18 (M)	81.7(62.8-99.2)	89.3(76.4-98.8) ‡	<0.0001
	≥18 (M&F)	83.3(63.0-101.4)	89.4(77.6-98.1) ‡	<0.0001
Platelet Count (×10⁹/l)	<1	399(135-724)	384(25-708) †	0.2923
	1-4	376(104-758)	359(79-650) †	0.0087
	5-11	432(126-762)	320(94-530) †	<0.0001
	12 -17(F)	450(132-771)	282(107-482) †	<0.0001
	12 -17(M)	445(179-808)	271(119-458) †	<0.0001
	≥18(F)	448(143-738)	271(151-425) ‡	<0.0001
	≥18(M)	435(179-777)	224(147-356) ‡	<0.0001
	≥18(M&F)	439(148-765)	244(150-359) ‡	<0.0001
WBC count (×10⁹/l)	<1	13.8(7.8-33.7)	9.2(5.0-17.3) †	<0.0001
	1-4	17.2(8.2-30.1)	7.7(3.7-13.2) †	<0.0001
	5-11	14.7(7.7-25.3)	5.6(3.7-9.1) †	<0.0001
	12 -17	12.6(6.8-21.1)	5.3(3.2-10.3) †	<0.0001
	≥18	-	-	-
Lymphocytes (%)	-	-	-	-
	≥18 (F)	37.3(19.7-53.1)	40.4(20.9-56.7) ‡	<0.0001
	≥18 (M)	36.8(16.9-54.2)	40.8(20.8-57.3) ‡	<0.0001
	≥18(M&F)	37.1(18.2-54.6)	40.4(20.8-56.7) ‡	<0.0001
Neutrophils (%)	-	-	-	-
	≥18 (F)	48.6(30.4-67.3)	48.4(32.5-71.3) ‡	0.7035
	≥18 (M)	46.8(26.8-69.7)	47.3(31.7-69.3) ‡	0.4763
	≥18(M&F)	47.6(28.5-69.6)	48.1(32.0-69.1) ‡	0.6826
	-	-	-	-

Analytes (Unit)	Age group in years (sex)	SCD Patients Median (2.5-97.5%)	General Population Median (2.5-97.5%)	P-values*
Bilirubin direct (µmol/L)	≥18 (F)	8.1(0.0-23.4)	1.81(0.70-5.83) ‡	<0.0001
	≥18 (M)	7.5(0.0-32.9)	2.53(0.93-8.43) ‡	<0.0001
	≥18(M&F)	7.8(0.0-29.3)	2.21(0.72-8.22) ‡	<0.0001
Bilirubin total (µmol/L)	<1	32.2(10.4-90.5)	4(2-19) ⌐	<0.0001
	1-4	36.9(7.8-132.3)	4(2-9) ⌐	<0.0001
	5-11	46.4(1.9-184.0)	4(2-11) ⌐	<0.0001
	12 -17(F)	55.8(16.7-237.9)	6(3-22) ⌐	<0.0001
	12 -17(M)	57.8(10.5-295.2)	6(2-15) ⌐	<0.0001
	≥18 (F)	45.5(10.2-170.9)	13.9(6.0-42.0) ‡	<0.0001
	≥18 (M)	52.6(12.9-251.8)	10.0(4.5-31.3) ‡	<0.0001
	≥18(M&F)	48.6(10.9-216.0)	11.5(5.2-41.0) ‡	<0.0001
AST (U/L)	<1	46.0(11.8-101.2)	38(26-65) ⌐	0.0057
	1-4	43.0(24.4-89.3)	36(27-55) ⌐	<0.0001
	5-11	43.5(24.0-86.8)	31(21-51) ⌐	<0.0001
	12 -17(F)	38.0(20.0-86.2)	23(17-36) ⌐	<0.0001
	12 -17(M)	44.0(22.5-90.0)	27(19-42) ⌐	<0.0001
	≥18 (F)	32.0(16.7-66.0)	20.1(13.5-35.2) ‡	<0.0001
	≥18 (M)	31.0(18.0-78.9)	28.2(15.2-53.4) ‡	<0.0001
	≥18(M&F)	31.0(18.0-61.8)	22.8(14.3-48.1) ‡	<0.0001
ALP (IU/L)	<1	322(53-586)	307(164-589) ⌐	0.1998
	1-4	288(120-600)	253(153-410) ⌐	<0.0001
	5-11	237(110-468)	278(174-460) ⌐	<0.0001
	12 -17(F)	216(71-556)	191(68-498) ⌐	<0.0001
	12 -17(M)	229(122-488)	302(124-537) ⌐	<0.0001
	≥18 (F)	115(46-310)	85.0(45.4-170.4) ‡	<0.0001
	≥18 (M)	153(59-408)	75.4(45.3-155.0) ‡	<0.0001
	≥18(M&F)	123(47-396)	80.0(45.6-158.4) ‡	<0.0001
Creatinine (µmol/L)	<1	32.5(13.2-57.1)	18(12-28) ⌐	<0.0001
	1-4	32.0(20.0-56.0)	24(15-50) ⌐	<0.0001
	5-11	32.0(18.0-56.0)	36(24-49) ⌐	<0.0001
	12 -17(F)	35.0(19.1-68.0)	45(32-64) ⌐	<0.0001
	12 -17(M)	34.0(18.0-69.1)	48(36-80) ⌐	<0.0001
	≥18 (F)	42.5(25.0-86.0)	56(40-81) ‡	<0.0001
	≥8 (M)	51.0(26.3-83.4)	69(48-96) ‡	<0.0001
	≥18 (M&F)	44.7(25.0-85.3)	62(42-90) ‡	<0.0001

*Mann Whitney U test for significance difference. ‡Obtained from [16]; ⌐from [24] and ‡from [25]. In [16] and [24] age ranges were listed as; <12 months, 1 to <5 years, 5 to <13 years, 13 to <18 years (M), 13 to <18 years (F). In [25] age range is 19 to 48 years.

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