

# Determine Reference Intervals for Selected Clinical Chemistry Parameters Using Selected Healthy Adult Population in Sri Lanka

DRM Panagoda<sup>1#</sup>, GHRE Karunaratne<sup>1</sup>, V Abeysuriya<sup>2</sup> and L Chandrasena<sup>2</sup>

<sup>1</sup> Faculty of Science, Horizon Campus Malabe, Sri Lanka

<sup>2</sup> Nawaloka Hospital Research and Education Foundation, Sri Lanka

#panagoda.rishandi97@gmail.com

**Abstract** - This study was aimed to determine the reference interval values for selected clinical chemistry parameters: Full Blood Count, ESR, TSH, blood sugars, liver function test, lipid profile test, and renal profile test using selected healthy adult population of Colombo city, Sri Lanka. Data was collected from March 2019 - October 2019 using a selected total of 991 healthy adults (656 males and 335 females) population in the Nawaloka Hospital laboratory database. Descriptive statistics were used to calculate mean, median, 2.5<sup>th</sup> - 97.5<sup>th</sup> percentiles range, 95% CI, maximum, and minimum using Minitab 17.3.1 software. Data were statistically analyzed using the paired sample T-test and one-way ANOVA to see the association between age and gender. There was a significant difference ( $p < 0.05$ ) between gender and the levels of eosinophil, basophils, haemoglobin, PCV, MCHC, RBC, MCH, MCV, platelet count, specific gravity, TSH, ESR, Albumin, Globulin, Bilirubin, ALT, GGT, AST, Triglyceride, HDL, Total HDL ratio, VLDL, Urea, Creatine, Uric acid Calcium and Phosphorus. Further, there was a significant difference in the values of WBC, Hemoglobin, PCV, RBC, MCH, MCV, platelet count, TSH and ESR, ALT, total cholesterol, triglycerides, HDL, LDL, Total HDL ratio, VLDL, Chloride, Creatine, Phosphorus, FBS and PPBS ( $p < 0.05$ ) according to the age groups.

**Keywords:** *reference interval, biochemical investigation, liver function test*

## I. INTRODUCTION

Reference interval is crucial for disease screening, diagnosis, monitoring, progression and treatment efficacy. Due to lack of locally derived reference values for the parameters, RI derived from western population is widely used in most of the laboratories (Edvardsson et al., 2017) (Longmore et al., 2014). However studies conducted in different countries have shown differences between locally and western derived RI values (Concordet et al.,

2009). Therefore, strict adherence to the reference values generated in developed countries could lead to inappropriate diagnosis and treatment of patients.

Different studies also indicated considerable variation in clinical chemistry RIs by several variables such as age, sex, geographical location, environment, lifestyle and genetic variation. (Rustad et al., 2004) (Ozarda et al., 2013). RI values for clinical chemistry parameters are vital for assessment of the health status of human population as they are used as baseline data in clinical trials. In Sri Lanka, American Standards are used as the RI values but the values have not been checked for the healthy Sri Lankan individuals. These clinical parameters may vary with the other factors like age and sex. Therefore, this study is aimed to determine the RI values for selected clinical chemistry parameters and determine the variation with age and gender using selected healthy adult population. This may be the first private sector study to establish RIs using Sri Lankan population considering age and gender.

## II. METHODOLOGY

### A. Reference Population

In this cross-sectional study, conveniently sampled, clinical records of healthy adults living in Colombo, the main city of Sri Lanka, were selected from existing database of Nawaloka Hospital Laboratory for 8 months period starting from March 2019 through October 2019. Based on inclusion exclusion criteria 1054 individual records were ultimately selected in the study.

Briefly, the inclusion criteria included: male and female elders between 18 and 80 years old. Conversely, individuals with the following conditions were excluded: Age  $\leq 18$  years, adults with common intestinal parasitic infections, hemoparasite, HIV, HCV, HBV positives and HCG

positive (for females), hospitalized persons, chronic diseases, cardiovascular diseases, kidney diseases, medication and acutely ill as per the recommendations were excluded from this study.

### B. Sample Size

Sample size calculation based on WINPEPI (Version 11.48) comparison of proportions of two groups, ( $p=0.05$ , Power 80%). Assumed proportional difference of two groups is 10%. Ratio of sample size B: A=1. Therefore, need minimum of 336 patients for the study (Tang, 2014). In this study, population stratification was based on sex and age.

### C. Variables

Reference intervals of common clinical chemistry parameters were the dependent variable with receptively sex and age.

### D. Operational Definitions

**Healthy Adults:** Individuals (adults) with age  $\geq 18$  years and without disease or disabilities based on clinical sign and symptom plus laboratory investigations.

**Reference Intervals:** the range between, and including two reference values defined by a specific percentage (usually 95%) for common clinical chemistry parameters of healthy individuals.

**95<sup>th</sup> percentile ranges:** It is the range between, and including the 2.5<sup>th</sup> percentile and the 97.5<sup>th</sup> percentile.

### E. Method of Data Collection

Bio-chemical investigations data from existing data base in the Nawaloka Hospital Laboratory in Sri Lanka was collected.

### F. Data Management and Statistical Analysis

All statistical calculations were performed on the Minitab version 17.3.1 software. Descriptive statistics was used to determine the mean, median, 95%CI, minimum, maximum and 2.5<sup>th</sup> -97.5<sup>th</sup> percentile range of each parameter. Independent sample T-test and one-way ANOVA were employed to see the association between variables. All statistical tests were two tailed, and p-value  $< 0.05$  considered as statistically significant.

### G. Ethical clearance

Ethical clearance was obtained from the Nawaloka Hospital Research and Education Foundation.

## III. RESULTS AND DISCUSSION

### A. Screening Results

Table 1 describes the characteristics of the study participants. After carefully screening of 1621 records, complete data records of 1054 (670 males and 384 females) were included for analysis.

Table 1 - Demographic Characteristics of Study Population

Variable	Frequency (number)	Percent
<b>Gender</b>		
Male	670	63.56
Female	384	36.43
<b>Age (in years)</b>		
18 – 24	163	16.66
25 – 34	210	21.51
35 – 44	210	21.51
45 – 54	205	21.00
55 – 64	130	13.31
$\geq 65$	60	6.14
<b>Biochemical Investigations</b>		
Full Blood Count (FBC)	790	74.95
Urine Full Report (UFR)	921	87.38
Erythrocyte	581	55.12
Sedimentation		
Rate (ESR)		
Thyroid Stimulating Hormone (TSH)	353	33.49
Liver Function Test (LFT)	371	35.19
Lipid Profile Test (LPT)	607	57.59
Renal Profile Test (RPT)	316	29.18
Hemoglobin A1C (HbA1C)	241	22.86
Glucose Post Prandial Blood Sugar	240	22.77

### B. Demographic characteristics

In table 1 the mean age of both the study participants at the study entry period was 40.921 years. The mean age of the females and males were 42.727 and 39.924 years respectively.

### C. Clinical Chemistry Reference Intervals

The calculated mean, median, 95% CI for mean, 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile range values of clinical

chemistry parameters based on gender and age were summarized in tables 2 and 3 respectively. The overall mean value of WBC, Neutrophil, Lymphocytes, Eosinophil, Monocytes and Basophil of participants were 7161.8 (\*10<sup>9</sup>/l), 51.1 (\*10<sup>9</sup>/l), 37.2 (\*10<sup>9</sup>/l), 4.0 (\*10<sup>9</sup>/l), 6.7 (\*10<sup>9</sup>/l), and 0.4 (\*10<sup>9</sup>/l), respectively. Males had reference intervals of WBC of 3806 – 10407 (\*10<sup>9</sup>/l) against females of 3877 – 10663 (\*10<sup>9</sup>/l), Neutrophil values of 35.14 – 67.25 (\*10<sup>9</sup>/l) against female of 36.11 – 68.37 (\*10<sup>9</sup>/l), Lymphocytes of 21.66 – 52.94 (\*10<sup>9</sup>/l) against females of 21.31 – 52.72 (\*10<sup>9</sup>/l), Eosinophil of (-1.19) – 9.68 (\*10<sup>9</sup>/l) against females of (-1.53) – 9.00 (\*10<sup>9</sup>/l), Monocytes of 2.30 – 11.31 (\*10<sup>9</sup>/l) against females of 2.75 – 10.49 (\*10<sup>9</sup>/l) and Basophile of (-0.14) – 1.05 (\*10<sup>9</sup>/l) against females of (- 0.24) – 1.05 (\*10<sup>9</sup>/l). Males had significantly different (p<0.05) higher 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile ranges of Eosinophil and Basophiles than females. The mean values of Eosinophil and Basophils were significantly different (p<0.05) between males and females. However, significant difference in gender was not observed in the mean values of WBC, Neutrophils, Lymphocytes and Monocytes.

As shown in table 2 participants had overall mean value of Hemoglobin of 14.19 (g/dl), PCV of 42.44 (\*10<sup>9</sup>/l), MCHC of 33.42 (g/dl), RBC of 4.97 (\*10<sup>9</sup>/l), MCH of 28.35 (pg) and MCV of 84.77 (fl). Males had reference intervals of Hemoglobin 12.48 – 17.44 (g/dl) against females of 10.45 – 14.94 (g/dl), PCV of 37.65 – 51.25 (\*10<sup>9</sup>/l) against females of 32.39 – 44.67 (\*10<sup>9</sup>/l), MCHC

of 30.23 – 37.13 (g/dl) against females of 30.67 – 35.19 (g/dl), RBC of 4.25 – 6.17 (\*10<sup>9</sup>/l) against females of 3.68 – 5.30 (\*10<sup>9</sup>/l), MCH of 23.75 – 33.28 (pg) against females of 23.83 – 32.26 (pg) and MCV of 73.05 – 96.14 (fl) against females of 74.62 – 95.60 (fl). Males had significant (p<0.05) higher 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile ranges of Hemoglobin, PCV, MCHC, RBC, MCH and MCV.

The overall mean values of platelet count, specific gravity, pH, TSH and ESR of participants were 286675 (\*10<sup>9</sup>/l), 1.02, 5.86, 2.38 (micro Iu/ml) and 12.65 (mm) respectively. Males had reference intervals of platelet count of 162189 – 393073 (\*10<sup>9</sup>/l) against females of 179960 – 428684 (\*10<sup>9</sup>/l), specific gravity of 1.01 – 1.03 against females of 1.01 – 1.03, pH of 4.75 – 6.94 against females of 4.73 – 7.08, TSH of (-1.32) – 5.39 (micro Iu/ml) against females of (-1.46) – 6.96 (micro Iu/ml) and ESR of (-11.68) – 28.27 (mm) against females of (-4.50) – 44.44 (mm). Males had significantly (p<0.05) higher 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile ranges of platelet count, specific gravity, TSH and ESR.

As shown in above table the difference in the 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile range of pH were not statistically significant between males and females (p>0.05). The means values of platelet count, specific gravity, TSH and ESR were significantly different between males and females (p<0.05), whereas significant gender difference was not observed in the mean value of pH (p>0.05)

Table 2 - Biochemical Parameters of Full Blood Count, ESR and TSH according to the Gender

Parameters	Male		Female		Combined		p- Value
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	95% CI for mean	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	95% CI for mean	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	95% CI for mean	
WBC (*10 <sup>9</sup> /l)	3806-10407	6961.70-7251.00	3877- 10663	7062-7479	3828- 10495	7043.00-7280.60	0.204
Neutrophils(*10 <sup>9</sup> /l)	35.10-67.20	50.40-51.80	36.10- 68.30	36.60-37.70	35.40- 67.60	50.90-52.10	0.091
Lymphocytes(*10 <sup>9</sup> /l)	21.60- 52.90	36.60-37.90	21.30- 52.70	36.00-37.90	21.50- 52.80	36.60-37.70	0.635
Eosinophil(*10 <sup>9</sup> /l)	(-1.10)-9.60	4.00- 4.40	(-1.50)-9.00	3.40- 4.00	(-1.30)-9.40	3.80- 4.20	0.014
Monocytes(*10 <sup>9</sup> /l)	2.30- 11.30	6.60- 7.00	2.70- 10.40	6.30- 6.80	2.40- 11.00	6.50- 6.80	0.245
Basophils(*10 <sup>9</sup> /l)	(-0.10)-1.00	0.40- 0.40	(-0.20)- 1.00	0.30- 0.40	(-0.10)- 1.00	0.40- 0.40	0.026
Hemoglobin(g/dl)	12.40-17.40	14.80-15.00	10.40- 14.90	12.50-12.80	11.00- 17.30	14.00-14.30	0.000

PCV(*10 <sup>9</sup> /l)	37.60-51.20	44.1- 44.70	32.30- 44.60	38.10-38.90	33.80- 51.00	42.10-42.70	0.000
MCHC(g/dl)	30.20-37.10	33.50-33.80	30.60- 35.10	32.70-33.00	30.20- 36.60	33.30-33.50	0.000
RBC(*10 <sup>9</sup> /l)	4.20- 6.10	5.10- 5.20	3.60- 5.30	4.40- 4.50	3.80- 6.10	4.90- 5.00	0.000
MCH(pg)	23.70-33.20	28.30-28.70	23.80- 32.20	27.70-28.30	23.70- 32.90	28.10-28.50	0.000
MCV(fl)	73.00-96.10	84.00-85.10	74.60- 95.60	84.40-85.70	73.50- 95.90	84.30-85.10	0.000
Platelet Count (*10 <sup>9</sup> /l)	162189-393073	272562-282701	179960-428684	296677-311968	165651-407699	282357-290993	0.000
Specific Gravity	1.00- 1.00	1.00- 1.00	1.00- 1.00	1.00- 1.00	1.00- 1.00	1.00- 1.00	0.005
PH	4.70- 6.90	5.80- 5.80	4.70- 7.00	5.80- 5.90	4.70- 6.90	5.80- 5.90	0.139
TSH(micro lu/ml)	(-1.30)-5.30	1.70- 2.20	(-1.40)- 6.90	2.40- 3.00	(-1.40)- 6.20	2.10- 2.50	0.001
ESR(mm)	(-11.60)-28.20	7.20- 9.30	(-4.50)- 44.40	18.20-21.60	(-11.70)-37.00	11.60-13.60	0.000

As shown in table 3, the values of Neutrophils, Lymphocytes, Eosinophils, Monocytes, Basophiles, MCHC, specific gravity and pH across all age groups of participants were similar (p>0.05).

However, there was significant difference in the values of WBC, Hemoglobin, PCV, RBC, MCH, MCV, platelet count, TSH and ESR (p<0.05).

Table 3 - Biochemical Parameters of Full Blood Count, ESR and TSH according to the Age

Parameters		Age group						p-Value
		18-24	25-34	35-44	45-54	55-64	>= 65	
WBC (*10 <sup>9</sup> /l)	Mean	7530	7294	7177	6938	6872	7069	0.038
	Median	7105	7240	6900	6770	6850	7060	
	2.5th- 97.5th percentile range	3424-11636	43.97-10191	3871-10483	3473-10403	4057-9687	3457-10681	
	95% CI for mean	7130-4930	7063-7526	6916-7437	6667- 7210	6590- 7154	6464-7675	
Neutrophils (*10 <sup>9</sup> /l)	Mean	52.71	50.84	50.73	51.12	52.15	53.80	0.111
	Median	52.10	50.90	50.70	50.80	50.75	54.60	
	2.5th- 97.5th percentile range	34.56-70.87	34.68-67.00	35.46-66.01	33.65- 68.59	37.57-66.74	42.41-64.20	
	95% CI for mean	50.94-54.48	49.54-52.13	49.53-52.49	49.75-52.49	50.69- 53.61	52.06-55.55	
Lymphocytes (*10 <sup>9</sup> /l)	Mean	36.32	38.00	37.88	37.40	36.89	35.17	0.255
	Median	36.35	37.70	37.20	37.50	37.20	34.95	
	2.5th- 97.5th percentile range	19.83-52.82	21.66-54.35	23.35-52.41	20.15-54.66	22.10- 51.68	23.02- 47.32	
	95% CI for mean	34.71-37.93	36.69-39.31	36.73-39.02	36.05-38.75	35.41- 38.37	33.13- 37.21	
Eosinophil (*10 <sup>9</sup> /l)	Mean	3.77	3.98	4.21	4.17	4.10	3.36	0.483
	Median	3.15	3.40	3.40	3.70	3.60	2.30	
	2.5th- 97.5th percentile range	(-1.2)- 8.77	(-1.10)-9.06	(-2.05)-10.47	(-1.26)-9.61	(-0.25)- 8.45	(-2.19)- 8.91	
	95% CI for mean	3.29- 4.26	3.57- 4.38	3.71- 4.70	3.74- 4.60	3.66- 4.53	2.43- 4.29	
Monocytes (*10 <sup>9</sup> /l)	Mean	6.76	6.74	6.74	6.82	6.43	7.28	0.494
	Median	7.35	7.00	7.10	7.00	6.90	8.00	
	2.5th- 97.5th percentile range	1.96- 11.56	2.69- 10.79	2.49- 10.99	2.80- 10.84	1.65- 11.21	2.11- 12.45	
	95% CI for mean	6.29- 7.23	6.41- 7.06	6.40- 7.08	6.50- 7.13	5.95- 6.91	6.41- 8.15	
Basophils (*10 <sup>9</sup> /l)	Mean	0.41	0.45	0.42	0.47	0.41	0.39	0.394
	Median	0.40	0.40	0.40	0.40	0.40	0.30	
	2.5th- 97.5th percentile range	(-0.18)-1.01	(-0.35)-1.25	(-0.09) -0.93	(-0.08) -1.04	(-0.17) - 1.00	(-0.22) - 1.01	
	95% CI for mean	0.35- 0.47	0.38- 0.51	0.38- 0.46	0.43- 0.52	0.35- 0.47	0.29- 0.49	
Hemoglobin (g/dl)	Mean	14.69	14.43	14.42	13.99	13.87	12.92	0.000
	Median	15.00	14.75	14.60	14.20	13.70	12.80	
	2.5th- 97.5th percentile range	11.93-17.46	11.35-17.52	11.27-17.58	10.73-17.25	10.71- 17.03	10.32- 15.54	

	95% CI for mean	14.426-14.965	14.185-14.680	14.172-14.671	13.737-14.249	13.554-14.187	12.491-13.367	
PCV (*10 <sup>9</sup> /l)	Mean	43.97	43.36	42.92	41.79	41.56	39.17	0.000
	Median	44.40	43.70	43.35	42.00	41.30	39.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	36.66-51.29	34.74-51.99	34.53-51.32	33.55-50.03	33.05- 50.08	31.67- 46.69	
	95% CI for mean	43.26-44.68	42.67-44.05	42.26-43.59	41.14-42.43	40.71- 42.41	37.92-40.43	
MCHC (g/dl)	Mean	33.40	33.35	33.57	33.43	33.34	32.96	0.459
	Median	33.40	33.60	33.40	33.50	33.30	33.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	31.22-35.59	28.02-36.69	31.35-35.80	30.92-35.96	30.88- 35.81	30.58- 35.35	
	95% CI for mean	33.18-33.61	32.92-33.78	33.39-33.74	33.24-33.63	33.09- 33.59	32.56-33.36	
RBC (*10 <sup>9</sup> /l)	Mean	5.21	5.10	5.03	4.90	4.79	4.39	0.000
	Median	5.30	5.10	5.10	4.90	4.70	4.40	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	4.13- 6.29	3.98- 6.22	3.92- 6.15	3.95- 5.85	3.71- 5.87	3.39- 5.38	
	95% CI for mean	5.11- 5.32	5.01- 5.19	4.95- 5.12	4.82- 4.97	4.68- 4.90	4.22- 4.55	
MCH (pg)	Mean	28.01	28.19	28.43	28.17	28.72	29.20	0.049
	Median	28.30	28.60	28.65	28.70	28.90	29.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	24.36-31.68	23.51-32.88	24.67-32.19	21.85-34.51	24.26- 33.20	26.59- 31.82	
	95% CI for mean	27.66-28.37	27.82-28.57	28.13-28.72	27.68-28.67	28.28- 29.17	28.76-29.64	
MCV (fl)	Mean	83.95	84.18	84.66	84.70	86.10	88.04	0.000
	Median	84.50	84.20	85.20	85.40	85.90	88.40	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	74.36-93.54	73.57-94.80	75.17-94.17	72.36-97.01	75.43- 96.79	78.52- 97.57	
	95% CI for mean	83.01-84.88	83.33-85.03	83.91-85.41	83.73-85.66	85.03- 87.17	86.44-89.64	
Platelet count (*10 <sup>9</sup> /l)	Mean	296778	299127	291852	274964	277167	251579	0.000
	Median	289500	293000	290000	278000	268500	250500	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	171613-421943	182495-415759	179948-403756	156810-393118	135767-418567	161593-341565	
	95% CI for mean	284596-308960	289776-308477	282993-300710	265697-284230	262996-291337	236488-266670	
Specific Gravity	Mean	1.02	1.02	1.02	1.02	1.02	1.02	0.336
	Median	1.02	1.02	1.02	1.02	1.02	1.02	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	1.01- 1.03	1.01- 1.03	1.01- 1.03	1.01- 1.03	1.01- 1.03	1.01- 1.03	
	95% CI for mean	1.02- 1.02	1.02- 1.02	1.02- 1.02	1.02- 1.02	1.02- 1.02	1.02- 1.02	
pH	Mean	5.88	5.91	5.85	5.84	5.77	5.96	0.329
	Median	5.50	6.00	5.50	5.50	5.50	5.50	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	4.87- 6.88	4.56- 7.26	4.80- 6.90	4.78- 6.90	4.79- 6.75	4.60- 7.31	
	95% CI for mean	5.79- 5.96	5.81- 6.01	5.77- 5.93	5.76- 5.91	5.67- 5.87	5.76- 6.15	
TSH (micro Iu/ml)	Mean	1.41	2.20	2.05	2.39	2.64	3.61	0.004
	Median	1.05	1.87	1.52	1.67	2.14	2.54	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	(-0.29)-3.11	(-0.19)-4.60	(-1.34)-5.46	(-1.63)-6.42	(-1.57)- 6.87	(-2.07)- 9.29	
	95% CI for mean	0.60- 2.21	1.88- 2.51	1.69- 2.42	1.94- 2.83	2.08- 3.21	2.52- 4.69	
ESR (mm)	Mean	6.29	9.78	9.74	12.95	19.00	26.23	0.000
	Median	4.00	7.00	6.00	11.00	12.50	26.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	(-6.36)-18.96	(-6.89)-26.45	(-5.84)-25.34	(-7.02)-32.93	(-14.71)-52.71	(-18.16)-70.62	
	95% CI for mean	4.53- 8.05	8.16-11.39	8.35-11.14	11.18-14.72	15.36- 22.64	17.92-34.53	

Table 5 Biochemical Investigations of Blood Sugar Level according to Age group

Parameters		Age group						p-Value
		18-24	25-34	35-44	45-54	55-64	>= 65	
Fasting Blood Sugar (FBS)(mg/dl)	Mean	93.47	95.75	109.70	122.66	133.09	121.71	0.000
	Median	93.50	92.00	95.00	104.00	107.00	106.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	69.05-117.90	53.31-133.20	19.86-199.50	33.66-211.70	6.90-259.30	41.55-201.90	
	95% CI for mean	90.54-96.40	92.06-99.45	101.84-117.56	114.78-130.54	120.57-145.61	109.42-134.00	
Hemoglobin A1C (HbA1C)	Mean	8.95	7.55	6.89	7.30	7.53	6.88	0.272
	Median	8.95	7.20	5.90	6.50	6.80	6.70	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	8.25-9.64	3.27-11.84	2.93-10.85	3.43-11.18	3.72-11.36	4.61-9.16	
	95% CI for mean	5.77-12.12	6.17-8.94	6.35-7.43	6.83-7.78	7.00-8.07	6.46-7.30	
Glucose post prandial Blood Sugar (PPBS)(mg/dl)	Mean	112.00	108.70	134.64	164.40	219.60	160.60	0.000
	Median	99.00	92.00	105.00	151.00	205.50	153.50	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	(-2.46)-226.50	31.38-186.0	(-11.20)-280.50	11.13-317.70	78.47-360.70	39.08-282.10	
	95% CI for mean	58.00-166.00	96.56-120.84	118.19-151.00	143.50-185.40	187.60-251.50	127.60-193.70	

Table 4 Biochemical Investigations of Blood Sugar, according to gender

Parameters	Male		Female		Combined		p-Value
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	95% CI for mean	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	95% CI for mean	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	95% CI for mean	
Fasting Blood Sugar (FBS) (mg/dl)	25.27-203.3	109.56-118.98	24.17-197.5	105.56-116.12	24.79-200.8	109.29-116.30	0.340
Hemoglobin A1C (HbA1C)	(-10.20)-26.54	6.70-9.63	3.24-11.29	6.81-7.72	(-7.28)-23.02	6.88-8.84	0.244
Glucose post prandial Blood Sugar (PPBS) (mg/dl)	5.32-305.4	142.97-167.76	(-5.32)-276.7	120.71-150.68	0.31-295.5	138.33-157.48	0.299

The calculated mean, median, 95% CI for mean, 2.5<sup>th</sup> - 97.5<sup>th</sup> percentile range values of Blood sugar parameters based on sex and age are shown in table 4 and 5 respectively. As shown in table 4, participants had overall mean value of FBS of 112.79 (mg/dl), HbA1C of 7.867, PPBS of 147.90 (mg/dl). Males had reference intervals of FBS 25.27 - 203.3 (mg/dl) against females of 24.17 - 197.5 (mg/dl), HbA1C of (-7.289) -

26.54 against females of 3.244 - 11.29, PPBS of 5.325 - 305.4 (mg/dl) against females of (-5.329) - 276.7 (mg/dl). The values of FBS, HbA1C and PPBS were not significantly difference between males and females. As shown in table 5, the values of FBS and PPBS across all age groups of participants were significantly different (P<0.05)

Table 6 Biochemical investigations of lipid, liver, renal according to the Gender

Parameter s	Male				Female				Combined				p- Value
	Mean	Media n	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile	95% CI for mean	Mean	Medi an	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile	95% CI for mean	Mean	Medi an	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	95% CI for mean	

			range				range						
Total Protein (g/dl)	7.50	7.50	0.60	7.44-7.55	7.38	7.40	0.60	7.28-7.49	7.45	7.50	0.50	7.39-7.50	0.066
Albumin (g/dl)	4.38	4.40	0.50	4.33-4.43	4.11	4.20	0.60	4.06-4.17	4.27	4.30	0.50	4.23-4.31	0.000
Globulin (g/dl)	3.11	3.00	0.80	3.04-3.18	3.31	3.20	0.80	3.22-3.39	3.19	3.10	0.80	3.14-3.25	0.000
Albumin/Globulin Ratio (g/dl)	1.45	1.40	0.50	1.41-1.50	1.34	1.30	0.40	1.23-1.45	1.40	1.40	0.50	1.35-1.46	0.059
Bilirubin (mg/dl)	0.76	0.66	0.43	0.70-0.82	0.56	0.51	0.30	0.52-0.60	0.67	0.57	0.41	0.63-0.71	0.000
Alanine aminotransferase (ALT) (u/l)	45.54	40.50	30.00	42.77-48.30	32.61	28.00	20.00	29.85-35.36	39.96	33.00	26.75	37.93-42.00	0.000
Gamma-glutamyltransferase (GGT) (u/l)	52.39	38.00	29.50	46.74-58.04	35.02	26.00	20.25	30.68-39.36	45.35	34.00	26.00	41.48-49.23	0.000
Alkaline phosphatase (ALP) (u/l)	76.01	75.00	27.00	73.18-78.85	75.85	73.00	28.00	71.99-79.70	75.94	74.00	25.25	73.65-78.24	0.945
Aspartate aminotransferase (AST) (u/l)	28.15	25.00	13.00	26.34-29.96	24.12	21.00	10.00	22.51-25.73	26.54	23.00	13.00	25.27-27.81	0.001
Cholesterol, Total (mg/dl)	194.07	193.00	57.00	189.37-198.76	195.94	193.50	56.50	190.42-201.46	194.81	193.00	56.00	191.23-198.38	0.612
Triglyceride (mg/dl)	146.84	125.00	95.00	137.85-155.83	109.52	103.00	64.75	102.75-116.30	131.91	115.00	82.50	125.69-138.14	0.000
High Density Lipoprotein (HDL)(mg/dl)	45.75	43.00	14.00	44.23-47.28	52.90	51.00	13.25	50.90-54.90	48.62	46.00	16.00	47.37-49.86	0.000
Low Density Lipoprotein (LDL)(mg/dl)	119.18	119.00	55.00	114.42-123.95	119.10	115.00	55.50	113.38-124.81	119.15	117.00	56.00	115.50-122.80	0.981
Total/HDL Ratio	4.49	4.30	1.80	4.32-4.67	3.87	3.70	1.30	3.70-4.05	4.25	4.00	1.80	4.12-4.37	0.000
Very Low Density Lipoprotein (VLDL) (mg/dl)	29.59	25.00	18.50	27.42-31.77	22.64	20.00	11.00	20.68-24.59	26.86	23.00	16.00	25.40-28.57	0.000
Non-HDL (mg/dl)	143.94	147.00	63.00	137.03-150.84	137.79	127.00	45.00	124.06-151.53	141.65	139.00	55.25	134.98-148.31	0.430



Sodium (mmol/l)	138.99	139.00	133.10-144.90	138.53-139.44	143.49	140.00	133.70-153.30	135.30-151.68	141.05	140.00	134.40-147.70	137.30-144.81	0.280
Potassium (mmol/l)	4.39	4.40	3.61-5.17	4.33-4.45	4.35	4.40	3.52-5.18	4.28-4.42	4.37	4.40	3.57-5.17	4.33-4.42	0.440
Chloride (mmol/l)	99.32	99.00	94.72-103.90	98.97-99.68	99.08	100.00	82.00-116.20	97.65-100.51	99.21	100.00	87.17-111.30	98.53-99.89	0.743
Urea (mg/dl)	25.49	25.00	8.71-42.28	24.20-26.78	21.91	21.00	8.44-35.39	20.78-23.04	23.85	23.00	8.13-39.58	22.97-24.74	0.000
Creatinine (mg/dl)	1.01	0.93	(-0.38)-2.41	0.92-1.09	0.76	0.69	(-0.48)-2.01	0.68-0.85	0.90	0.81	(-0.45)-2.26	0.84-0.96	0.000
Uric Acid (mg/dl)	5.51	5.70	3.01-8.00	5.32-5.70	4.35	4.40	2.30-6.40	4.18-4.52	4.98	5.00	2.42-7.54	4.84-5.12	0.000
Calcium (mg/dl)	9.16	9.20	8.43-9.90	9.10-9.22	9.02	9.00	8.35-9.69	8.97-9.08	9.10	9.10	8.38-9.81	9.06-9.14	0.001
Phosphorus (mg/dl)	3.36	3.30	2.67-4.65	3.29-3.43	3.66	3.70	-	3.57-3.74	3.49	3.50	2.48-4.29	3.44-3.55	0.000

As shown in above table 6, there were significant difference ( $P < 0.05$ ) between males and females in the levels of Albumin, Globulin, Bilirubin, ALT, GGT, AST, Triglyceride, HDL, Total HDL ratio, VLDL, Urea, Creatinine, Uric acid Calcium and Phosphorus. The reference intervals of Total protein, Albumin/Globulin

ratio, ALP, Total cholesterol, LDL, Non- HDL, Sodium, Potassium and Chloride were not significantly different between the two sex groups ( $P > 0.05$ ).

Table 7 Biochemical investigation of Lipid, liver, Renal According to Age group

Parameters		Age group						P-Value
		18-24	25-34	35-44	45-54	55-64	$\geq 65$	
Total Protein (g/dl)	Mean	7.82	7.49	7.41	7.4790	7.4219	7.393	0.257
	Median	8.00	7.50	7.50	7.5000	7.4000	7.300	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	7.06-8.59	6.67-8.31	5.87-8.96	6.768-8.190	6.605-8.239	6.239-8.547	
	95% CI for mean	7.56-8.08	7.38-7.60	7.25-7.57	6.76- 8.19	7.32-7.51	7.16-7.61	
Albumin (g/dl)	Mean	4.31	4.32	4.29	4.28	4.25	4.10	0.200
	Median	4.30	4.40	4.40	4.30	4.30	4.30	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	3.62-5.01	3.58-5.06	3.52-5.06	6.60- 4.96	3.45-5.06	3.24-4.96	
	95% CI for mean	4.08-4.55	4.22-4.42	4.21-4.37	4.20- 4.35	4.16-4.35	3.93-4.27	
Globulin (g/dl)	Mean	3.50	3.17	3.18	3.19	3.16	3.29	0.417
	Median	3.70	3.10	3.10	3.20	3.00	3.30	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	2.47-4.54	2.12-4.22	2.17-4.20	2.20- 4.18	2.01-4.31	2.11-4.47	
	95% CI for mean	3.15-3.86	3.03-3.31	3.08-3.29	3.08- 3.30	3.02-3.29	3.06-3.52	
Albumin/Globulin Ratio (g/dl)	Mean	1.28	1.41	1.47	1.38	1.38	1.30	0.591
	Median	1.10	1.40	1.40	1.30	1.40	1.20	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	0.56-1.99	0.76-2.06	(-0.76)-3.12	0.78- 1.99	0.67-2.10	0.64-1.95	
	95% CI for mean	1.03-1.52	1.32-1.50	1.30-1.64	1.31- 1.45	1.31-1.46	1.17-1.42	
Bilirubin	Mean	0.64	0.71	0.72	0.66	0.64	0.64	0.767



(mg/dl)	Median	0.62	0.68	0.59	0.56	0.54	0.57	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	0.30-0.98	0.49-1.38	(-0.07)-1.52	0.02- 1.31	(-0.22)-1.52	(-0.03)-1.31	
	95% CI for mean	0.50-0.79	0.61-0.81	0.63-0.80	0.5965-0.7425	0.543-0.7538	0.50-0.77	
Alanine aminotransferase (ALT) (u/l)	Mean	34.63	42.44	44.25	44.43	34.48	30.29	0.000
	Median	27.00	33.00	37.50	41.00	33.00	30.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	(-18.45)-87.71	(-7.48)-92.36	(-11.28)-99.78	(-4.922)-93.78	4.453-64.51	4.281-56.30	
Gamma-glutamyltransferase (GGT) (u/l)	Mean	35.17	45.07	41.59	49.40	51.04	38.55	0.284
	Median	31.00	29.00	35.00	38.50	35.00	27.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	1.57-66.76	(-47.32)-137.50	(-8.36)-91.55	(-21.71)-120.50	(-50.62)-152.70	(-40.20)-117.30	
Alkaline phosphatase (ALP) (u/l)	Mean	28.77-41.57	32.89-57.24	36.36-46.81	41.43-57.37	38.85-36.23	23.27-53.84	0.730
	Median	78.50	73.05	74.67	74.90	78.62	77.55	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	36.69-120.30	32.54-113.60	26.93-122.40	24.23-125.60	43.32-113.90	38.86-116.20	
Aspartate aminotransferase (AST) (u/l)	Mean	64.94-92.06	67.80-78.30	69.73-79.60	69.19-80.62	74.45-82.80	70.04-85.06	0.911
	Median	26.67	25.68	26.19	27.64	27.32	25.38	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	2.05-51.29	(-0.17)-51.53	4.787-47.59	0.4945-54.79	(-10.47)-65.11	3.918-46.84	
Cholesterol, Total (mg/dl)	Mean	18.68-34.65	22.48-28.87	24.15-28.22	25.06-30.22	23.32-31.31	21.87-28.88	0.000
	Median	187.28	202.86	203.26	197.56	186.63	173.67	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	128.00-246.60	114.20-291.50	113.70-292.90	112.00-283.10	95.20-278.10	83.22-264.10	
Triglyceride (mg/dl)	Mean	178.68-195.88	193.74-211.97	195.62-210.90	190.13-204.99	177.28-195.98	159.80-187.53	0.013
	Median	95.50	146.20	130.37	130.37	141.57	104.98	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	(-5.43)-196.40	(-39.80)-332.20	(-14.98)-275.70	(-14.98)-275.70	(-6.388)-289.50	15.66-194.30	
High Density Lipoprotein (HDL) (mg/dl)	Mean	70.60-120.30	124.30-168.00	117.30-143.45	117.30-143.45	126.44-156.71	91.29-118.67	0.007
	Median	42.42	48.46	47.78	48.25	48.05	56.18	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	-	13.25	17.00	16.00	18.00	27.00	
Low Density Lipoprotein (LDL) (mg/dl)	Mean	37.93-46.91	44.27-52.65	45.14-50.42	45.92-50.57	45.77-50.33	50.64-61.72	0.000
	Median	131.16	125.84	127.56	119.66	111.64	96.67	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	47.00	53.00	50.50	49.50	61.00	58.50	
Total/ HDL Ratio	Mean	115.48-146.84	115.38-136.30	119.96-135.15	112.51-126.80	103.16-120.12	85.72-107.61	0.000
	Median	4.76	4.55	4.56	4.28	3.99	3.27	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	4.80	4.40	4.15	3.90	4.10	3.00	
Very Low Density Lipoprotein (VLDL) (mg/dl)	Mean	2.10	1.30	2.10	1.80	1.75	1.05	0.005
	Median	4.06-5.47	4.18-4.92	4.26-4.85	4.02- 4.53	3.76-4.22	2.96-3.57	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	21.33	29.37	25.76	26.94	31.18	19.41	
(mg/dl)	Mean	18.00	25.00	22.00	23.00	25.00	17.00	0.005
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	24.00	19.00	12.00	13.00	18.00	12.00	

	percentile range							
	95% CI for mean	9.45-33.22	25.03-33.70	22.41-29.11	24.17-29.72	26.95-35.41	16.58-22.24	
Non-HDL (mg/dl)	Mean	134.00	153.90	142.64	140.86	137.15	141.00	0.814
	Median	130.00	153.00	139.00	140.50	141.00	113.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	13.00	53.50	62.25	46.25	68.00	66.00	
	95% CI for mean	99.10-168.90	139.24-168.57	131.37-153.91	132.71-149.01	125.38-148.91	86.60-195.30	
Sodium (mmol/l)	Mean	139.86	152.70	139.39	139.39	138.88	138.29	0.344
	Median	140.00	139.00	140.00	140.00	140.00	139.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	5.00	3.00	3.00	3.50	4.00	7.00	
	95% CI for mean	137.69-142.02	125.20-180.20	138.82-139.96	138.65-140.12	137.88-139.88	136.51-140.05	
Potassium (mmol/l)	Mean	4.22	4.29	4.37	4.31	4.47	4.47	0.060
	Median	4.20	4.30	4.40	4.30	4.40	4.45	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	0.80	0.50	0.50	0.40	0.50	0.40	
	95% CI for mean	3.81-4.63	4.17-4.41	4.28-4.45	4.22-4.40	4.37-4.58	4.31-4.64	
Chloride (mmol/l)	Mean	99.28	99.25	99.79	99.61	99.62	95.11	0.022
	Median	100.00	99.00	100.00	100.00	100.00	99.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	2.00	2.50	2.00	2.00	4.00	4.75	
	95% CI for mean	97.70-100.86	98.75-99.74	99.34-100.25	99.08-100.14	98.80-100.45	87.76-102.46	
Urea (mg/dl)	Mean	26.38	22.59	22.53	24.81	24.55	25.54	0.243
	Median	26.00	22.00	22.00	25.00	24.00	25.00	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	7.75	10.00	10.00	12.25	11.00	16.50	
	95% CI for mean	22.53-30.22	20.39-24.79	21.02-24.03	23.10-26.25	22.67-26.42	20.11-30.96	
Creatinine (mg/dl)	Mean	1.41	0.78	0.95	0.83	0.96	0.93	0.040
	Median	0.86	0.75	0.87	0.80	0.80	0.79	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	0.18	0.26	0.29	0.28	0.26	0.41	
	95% CI for mean	0.20-2.62	0.74-0.82	0.79-1.10	0.80-0.86	0.77-1.14	0.82-1.05	
Uric Acid (mg/dl)	Mean	4.83	5.17	5.12	5.01	4.93	4.54	0.413
	Median	5.30	4.90	5.30	5.00	4.90	4.40	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	2.15	2.10	1.80	1.85	1.72	1.47	
	95% CI for mean	3.40-6.26	4.73-5.60	4.84-5.40	4.68-5.33	4.63-5.22	4.06-5.02	
Calcium (mg/dl)	Mean	9.04	9.10	9.05	9.10	9.18	9.014	0.267
	Median	9.20	9.10	9.10	9.10	9.10	9.05	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	0.40	0.45	0.50	0.40	0.40	0.70	
	95% CI for mean	8.71-9.37	8.98-9.22	8.96-9.14	9.02-9.18	9.10-9.26	8.86-9.16	
Phosphorus (mg/dl)	Mean	3.68	3.45	3.37	3.44	3.61	3.67	0.020
	Median	3.55	3.50	3.40	3.40	3.70	3.40	
	2.5 <sup>th</sup> - 97.5 <sup>th</sup> percentile range	0.70	0.67	0.40	0.70	0.80	0.90	
	95% CI for mean	3.37-4.00	3.29-3.60	3.28-3.45	3.30-3.57	3.48-3.74	3.40-3.93	

As shown in table 7, the analyzed data of total protein, Albumin, Globulin, Albumin/Globulin

ratio, Bilirubin, GGT, ALP, AST, Non-HDL, Sodium, Potassium, Urea, Uric acid and Calcium

across all age groups of participants were similar ( $p>0.05$ ), but, there was a significant difference in the values of ALT, total cholesterol, Triglycerides, HDL, LDL, Total HDL ratio, VLDL, Chloride, Creatine and Phosphorus ( $p<0.05$ )

#### IV. CONCLUSION

In the current study, some of the calculated reference intervals of clinical chemistry parameters were significantly different according to the age and gender. Therefore, there is a possibility of inappropriate diagnosis of some clinical conditions in healthy adults. However, further studies are required to establish unique reference intervals for Sri Lankan population.

#### REFERENCES

- Concordet, D., Geffré, A., Braun, J. and Trumel, C., (2009). A new approach for the determination of reference intervals from hospital-based data. *Clinica Chimica Acta*, 405(1-2), pp.43-48.
- Edvardsson, M., Sund-Levander, M., Milberg, A., Wressle, E., Marcusson, J. and Grodzinsky, E., (2017). Differences in levels of albumin, ALT, AST,  $\gamma$ -GT and creatinine in frail, moderately healthy and healthy elderly individuals. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 56(3), pp.471-478.
- Horowitz, G. L. (2008). *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory: Approved Guideline*.
- Katayev, A., Balciza, C. and Seccombe, D., (2010). Establishing Reference Intervals for Clinical Laboratory Test Results. *American Journal of Clinical Pathology*, 133(2), pp.180-186.
- Longmore, M., Wilkinson, I., Baldwin, A. and Wallin, E., (2014). Reference intervals, etc. *Oxford Medicine Online*,
- Mekonnen Z, Amuamuta A, Mulu W, Yimer M, Zenebe Y, Adem Y, et al. (2017) Clinical chemistry reference intervals of healthy adult populations in Gojjam Zones of Amhara National Regional State, Northwest Ethiopia. *PLoS ONE*12 (9): e0184665.
- Ozarda, Y., Ichihara, K., Barth, J. and Klee, G., (2013). Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. *Clinical Chemistry and Laboratory Medicine*, 51(5).
- Rustad, P., Felding, P., Franzson, L., Kairisto, V., Lahti, A., Mårtensson, A., Petersen, P., Simonsson, P., Steensland, H. and Uldall, A., (2004). The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. *Scandinavian Journal of Clinical and Laboratory Investigation*, 64(4), pp.271-284.
- Tang, M., (2014). Sample Size Determination for Clinical Trials. *Wiley StatsRef: Statistics Reference Online*.

#### ACKNOWLEDGEMENT

Nawaloka Research and Education Foundation and the laboratory staff of Nawaloka Hospital PLC Colombo

#### AUTHOR BIOGRAPHY



Rishandi Panagoda is received BSc (Hons) Biotechnology at the Horizon Campus, Malabe affiliated with Nilai University, Malaysia.