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Department of Biochemistry and Molecular Biology MPhil Thesis

2016

A Study of Reference Ranges for Biochemical and Hematological Parameters in the Healthy Population of Bangladesh

Ali, Md. Akshad

University of Rajshahi

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M.Phil Thesis

A STUDY OF REFERENCE RANGES FOR BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS IN THE HEALTHY POPULATION OF BANGLADESH

Md. Akshad Ali

M.Phil Thesis

Thesis submitted to the University of Rajshahi, Bangladesh in the fulfillment of the requirements for the degree of Master of Philosophy in Biochemistry and Molecular Biology

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June 2016

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June, 2016

Declaration

I do here by declare that the materials embodied in this entitle “A study of reference ranges for biochemical and hematological parameters in the healthy population of Bangladesh” Prepared for submission in the Department of Biochemistry and Molecular biology, University of Rajshahi, Rajshahi Bangladesh for the degree of Master of Philosophy, are original research work of mine and have not been previously submitted for the awards of any degree anywhere.

Md. Akshad Ali

Certificate from Supervisor

This is to certify that the thesis entitled "A study of reference ranges for biochemical and hematological parameters in the healthy population of Bangladesh" submitted by Md. Akshad Ali enrolled with Roll No.11308,Session:2011-2012 to University of Rajshahi Rajshahi, Bangladesh, for the partial fulfillment of the Degree of 'Masters of Philosophy in Biochemistry and Molecular biology, is a bona-fide record of his research work accomplished under signed supervision at the Department, of Biochemistry and Molecular biology University of Rajshahi, Rajshahi Bangladesh. The thesis contains no materials previously published or written by another person except when due reference in made in the next of the thesis. The author and the supervisor give permission to use this thesis for academic purpose only. No part of the thesis may be reproduced or transmitted in any form or by any means, electronically or mechanically without prior permission of the author and the supervisors.

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DEDICATED

TO

PARENTS

LIST OF CONTENTS

Contents	Page
List of Contents	i-iv
List of Table	v-vi
List of figure	viii-ix
List of Abbreviation and Symbols	x
List of Annexes	xi
Abstract	xii-viii
	xiv-xvi
Chapter 1: Introduction	**1-36**
1.1 Reference Intervals	1
1.2 History and Concept of Reference Intervals	2
1.3 Role in national development, planning and to increase human knowledge	4
1.4 The Current Guidelines and Limitations of Reference Interval Determination	5
1.5Use of reference range	6
1.6 Concept of health and normality	7
1.7 Interpretation of the reference limits	8
1.8 Analyte description	9
1.8.1Biochemical tests parameters	9
1.8.1.1 Glucose	10
1.8.1.2 Creatinine	10
1.8.1.3 Urea	11
1.8.1.4 Uric acid	11
1.8.1.5 Sodium	12
1.8.1.6 Potassium	12
1.8.1.7 Chloride	12
1.8.1.8 Calcium	13
1.8.1.9 Magnesium	13
1.8.1.10 Phosphorus	14
1.8.1.11 Cholesterol	15
1.8.1.12 HDL.Cholesterol	15
1.8.1.13 LDL.Cholesterol	16
1.8.1.14 Triglyceride	16
1.8.1.15 Total protein	17
1.8.1.16 Albumin	17
1.8.1.17 Bilirubin-total	18
1.8.1.18 Alanine Aminotransferase (ALT)	19
1.8.1.19 Aspartate Aminotransferase (AST)	20
1.8.1.20 Alkaline Phosphatase (ALP)	21

Contents	Page
1.8.2 Hematological tests parameters	22
1.8.2.1 (RBCs)	23
1.8.2.2 Hemoglobin	23
1.8.2.3 Hematocrit	24
1.8.2.4 Mean corpuscular volume (MCV)	24
1.8.2.5Mean corpuscular hemoglobin (MCH)	25
1.8.2.6 Mean corpuscular hemoglobin Concentration (MCHC)	25
1.8.2.7 Red cell distribution width (RDW)	26
1.8.2.8 Platelets (Thrombocytes)	26
1.8.2.9 Mean platelet volume (MPV)	27
1.8.2.10White blood cells, (WBC)	27
1.8.2.11Neutrophils	28
1.8.2.12Lymphocytes	29
1.8.2.13 Monocytes	29
1.8.2.14 Eosinophils	30
1.8.2.15 Basophils	31
1.8.3 Literature Review	31
1.8.4 Aim and objectives	34
1.8.4.1 General Objective	35
1.8.4.2 Specific objectives	36
Chapter 2: Materials and methods	**37-88**
2.1 Study area and Reference population / Participants:	37
2.2 Ethical consideration and Consent:	37
2.3 Selection criteria:	37
2.3.1 Inclusion Criteria	38
2.3.2 Exclusion Criteria	38
2.4 Specimen Collection, transportation, rejection, processing and storage	38
2.4.1 Specimen Collection and Handling Techniques	38
2.4.2 Specimen transportation	39

Contents	Page
2.4.3 Specimen Rejection	39
2.4.4 Specimen processing and storage	39
2.5 Analytes and Instrumentation	40
2.6 Analytical methods and Reaction Principles	40
2.7 Calibration procedure and calibrator traceability	44
2.8 Quality Assurance (QA)/ Quality Control (QC)	44
2.9 Data Management and Statistical Analysis	48
2.9.1 Data Management	48
2.9.2 Frequency distributions	49
2.9.3 Normality test	66
2.9.3.1Normal Q-Q Plot	67
2.9.4 Non Parametric method	86
2.9.5 Partitioning of Values	87
Chapter 3: Results	**89-155**
3.1 Sample Volume with age and sex distribution	89
3.2 Reference Intervals for Biochemical Analytes	94
3.1.1 Reference Intervals for Glucose	95
3.1.2 Reference Intervals for Creatinine	96
3.1.3 Reference Intervals for Urea	99
3.1.4 Reference Intervals for Uric acid	101
3.1.5 Reference Intervals for Sodium	102
3.1.6 Reference Intervals for Potassium	104
3.1.7 Reference Intervals for Chloride	106
3.1.8 Reference Intervals for Calcium	107
3.1.9 Reference Intervals for Magnesium	109
3.1.10 Reference Intervals for Phosphorus	111
3.1.11 Reference Intervals for Cholesterol	112
3.1.12 Reference Intervals for HDL.Cholesterol	114
3.1.13 Reference Intervals for LDL.Cholesterol	116
3.1.14 Reference Intervals for Triglyceride	118
3.1.15 Reference Intervals for Total protein	120
3.1.16 Reference Intervals for Albumin	122
3.1.17 Reference Intervals for Bilirubin-total	123
3.1.18 Reference Intervals for Alanine Aminotransferase (ALT)	125
3.1.19 Reference Intervals for Aspartate Aminotransferase (AST)	127
3.1.20 Reference Intervals for Alkaline Phosphatase (ALP)	129
3.2 Reference intervals of Hematological Analytes	131
3.2.1 Reference Intervals for Red Blood Cell (BRC)	131
3.2.2 Reference Intervals for Hemoglobin	133
3.2.3 Reference Intervals for Hematocrit	135

Contents	Page
3.2.4 Reference Intervals for Mean corpuscular volume (MCV)	136
3.2.5 Reference Intervals for Mean corpuscular hemoglobin (MCH)	138
3.2.6 Reference Intervals for Mean corpuscular hemoglobin Concentration (MCHC)	140
3.2.7 Reference Intervals for Red cell distribution width (RDW)	141
3.2.8 Reference Intervals for Platelets (Thrombocytes)	143
3.2.9 Reference Intervals for Mean Platelet volume (MPV)	145
3.2.10 Reference Intervals for White blood cells, (WBC)	146
3.2.11 Reference Intervals for Neutrophils	148
3.2.12 Reference Intervals for Lymphocytes	149
3.2.13 Reference Intervals for Monocytes	151
3.2.14 Reference Intervals for Eosinophils	152
3.2.15 Reference Intervals for Basophils	154
Chapter 4: Discussion and Conclusion	**156-171**
4.1 Discussion	156
4.2 Conclusion	171
Chapter 5: References	**172-179**
Annexes	**180-182**

LIST OF TABLES

Tables	Page
Table-2.1: Analytes included in the study. All tests were performed using reagents specific for the Beckman Coulter AU640 andSysmex XE 2100 analyzer.	41
Table -2.2: The analytical methods and reaction principles used for the analysis of biochemistry tests.	42
Table -2.3: The analytical methods used for the analysis of hematology on Sysmex XE2100.	43
Table-2.4: Total allowable error, analytical imprecision and Measurement Uncertainty.	47
Table-2.5: Significance of the normality for data distribution, p value as per Shapiro-Wilk test is given.(P<0.05 is considered significant).	68
Table- 2.6 Nonparametric Confidence Intervals of Reference Limits	88
Table 3.1Sex and age distribution of recruited participants in this study.	89
Table 3.2Sex and age distribution of selected participants in this study.	90
Table 3.3 Sex and age distribution of participants to construct reference intervals in this Study.	91
Table 3.4 Significance of the difference between samples values for the sex male and females subjects, p value as per Mann-Whitney test.	92
Table 3.5 Significance of the difference between samples values for the age group less than 40 and more than 40 years, p value as per Mann-Whitney test.	93
Table 3.6 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for fasting glucose.	95
Table 3.7 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervalscreatinine.	97
Table 3.8 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervalsfor urea.	99
Table 3.9 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervalsfor uric acid.	101
Table 3.10 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for sodium.	103
Table 3.11 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Potassium.	104
Table 3.12 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for chloride.	106
Table 3.13 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for calcium.	108

LIST OF TABLES

Tables	Page
Table 3.14 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for magnesium.	109
Table 3.15 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for inorganic phosphate.	111
Table 3.16 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for cholesterol.	113
Table 3.17 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for HDL. Cholesterol	115
Table 3.18 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for LDL. Cholesterol	116
Table 3.19 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum triglyceride.	118
Table 3.20 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for total protein.	120
Table 3.21 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for albumin.	122
Table 3.22 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum bilirubin total.	124
Table 3.23 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum ALT.	126
Table 3.24 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum AST.	127
Table 3.25 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum ALP.	129
Table 3.26 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Red Blood Cell (BRC).	132
Table 3.2795% reference values (2.5th and 97.5th percentiles values) w their 90% confidence intervals for hemoglobin	134
Table 3.28 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals forhematocrite.	135
Table 3.29 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals forMCV.	137
Table 3.30 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for MCH.	139
Table 3.31 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for MCHC.	140
Table 3.32 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for RDW-CV	142

LIST OF TABLES

Tables	Page
Table 3.33 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Platelets	143
Table 3.34 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for MPV	145
Table 3.35 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for WBC	147
Table 3.36 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for neutrophil	148
Table 3.37 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Lymphocyte	150
Table 3.38 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Monocyte	151
Table 3.39 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Eosinophil	153
Table 3.40 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for basophil	154
Table-4.1Compare the observed reference values of fasting glucose, creatinine, urea and uric acid for Bangladeshi population and other populations.	162
Table-4.2 Compare the observed reference values for lipid profiles for Bangladeshi population and other populations.	163
Table-4.3 Compare the observed reference values for Na, K, Cl, Ca, Mg and $PO_4$for Bangladeshi population and other populations.	166
Table-4.4 Compare the observed reference values for TP, Alb, Bil-T ALT, AST and ALP for Bangladeshi population and other populations.	167
Table 4.5 Compare the observed reference values for hematology parameters RBC, Hb, HCT, MCV, MCH, MCHC, RDW-CV, PLT for Bangladeshi population and other populations.	169
Table 4.6 Compare the observed reference values for hematology parameters white bloodcell and it's differential counts for Bangladeshi and other populations.	170

LIST OF FIGURES

Figure	Page
Figure 1.1: Definitional Schematic used in reference Interval Determination	6
Figure 2.1:Frequency histograms of Blood Glucose and serum Creatinine.	49
Figure 2.2:Frequency histograms of Blood Urea and serum Uric acid.	50
Figure 2.3:Frequency histograms of serum Sodium and Potassium	51
Figure 2.4:Frequency histograms of serum Chloride and total Calcium	52
Figure 2.5:Frequency histograms of serum Magnesium and Inorganic Phosphate	53
Figure 2.6:Frequency histograms of total Cholesterol and HDL. Cholesterol	54
Figure 2.7:Frequency histograms of HDL. Cholesterol and Triglyceride	55
Figure 2.8:Frequency histograms of total Protein and Albumin	56
Figure 2.9:Frequency histograms of Bilirubin-Total and Alanine amino transferase	57
Figure 2.10:Frequency histograms of Aspartate amino transferase and alkaline phosphatas	58
Figure 2.11:Frequency histograms of Red Blood Cell (RBC) and Hemoglobin	59
Figure 2.12 :Frequency histograms of Hematocrite and MCV	60
Figure 2.13 :Frequency histograms of MCH and MCHC	61
Figure 2.14:Frequency histograms of RDW-CV and Platelets (PLT)	62
Figure 2.15:Frequency histograms of MPV and White blood cell (WBC)	63
Figure 2.16:Frequency histograms of Neutrophil and Lymphocyte	64
Figure 2.17:Frequency histograms of Monocyte and Eosinophil	65
Figure 2.18:Frequency histograms of Basophile	66
Figure 2.19 :Normal Q-Q plot of Glucose and Creatinine	69
Figure 2.20:Normal Q-Q plot of Urea and Uric acid	70
Figure 2.21:Normal Q-Q plot of Sodium and Potassium	71

Figure	Page
Figure 2.22:Normal Q-Q plot of Chloride and Calcium	72
Figure 2.23:Normal Q-Q plot of Magnesium and Inorganic Phosphorous	73
Figure 2.24:Normal Q-Q plot of total Cholesterol and HDL. Cholesterol	74
Figure 2.25:Normal Q-Q plot of LDL. Cholesterol and Triglyceride	75
Figure 2.26:Normal Q-Q plot of total Protein and Albumin	76
Figure 2.27:Normal Q-Q plot of Bilirubin Total and Alanine amino transferase (ALT)	77
Figure 2.28:Normal Q-Q plot of Aspartate amino transferase (AST) and Alkaline phosphatas (ALP)	78
Figure 2.29:Normal Q-Q plot of Red blood cell (RBC) and Hemoglobin	79
Figure 2.30:Normal Q-Q plot of Hematocrite (HCT) and MCV	80
Figure 2.31:Normal Q-Q plot of MCH and MCHC	81
Figure 2.32:Normal Q-Q plot of RDW-CV and Platelets	82
Figure 2.33:Normal Q-Q plot of MPV and White Blood Cell (WBC)	83
Figure 2.34:Normal Q-Q plot of Neutrophil and Lymphocyte	84
Figure 2.35 :Normal Q-Q plot of Monocyte and Eosinophil	85
Figure 2.36 :Normal Q-Q plot of Basophile	86
Figure 3.1Sex and age distribution of recruited participants	89
Figure 3.2 Sex and age distribution of selected participants	90
Figure 3.3 Sex and age distribution of participantto construct reference intervals	91

LIST OF ANNEXES

List of Annexes	Page
1. Annex I: Donor Consent Form (English and Bangla)	180
2. Annex II: Donor Questionnaire Form	182

ABBREVIATIONS AND SYMBOLS

Alb	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMP	adenosine monophosphate
Apo B	Apo lipo protein
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
ATPase	Adenosine Triphosphatase
Baso	Basophil
Bil-T	Bilirubin-Total
Ca	Total Calcium
CBC	Complete Blood Count
CHD	Coronary Heart Disease
Cl	Chloride
CCLM	Clinical Chemistry and Laboratory Medicine
Chol	Cholesterol
chem	Chemistry
CLSI	Clinical and Laboratory Standards Institute
CMIA	chemiluminescentmicroparticle immunoassay
COP	Colloidal Osmotic Pressure
CSF	Cerebrospinal fluid
CV	Coefficient of Variation
Da	Dalton
ECF	Extracellular Fluid Compartment
EDTA	Ethylenediaminetetraacetic acid
Eosino	Eosinophil
EQA	External Quality Assessment
EQC	External Quality Control
FBS	Fasting blood glucose
GFR	Glomerular filtration rate
Hb	Hemoglobin
HCT	Hematocrite
HDL.C	High density lipoprotein cholesterol
IFCC	International Federation of Clinical Chemistry

ABBREVIATIONS AND SYMBOLS

IBM	International Business Machines
ICSH	International Council for Standardization in Hematology
ITP	Immune Thrombocytopenic Purpura
IQC	Internal Quality Control
IQR	Inter quartile range
K	Potassium
KDa	Kilo Dalton
LDL.C	Low density lipoprotein cholesterol
L-J chart	Levey-Jennings control chart
LL	Lower limit
Lympho	Lymphocyte
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
Med	Median
Mg	Magnesium
mg	Milligram
mg/dL	Milligram per deciliter
ml	Milliliter
mm	Millimeter
mmol/L	Millimol per liter
Mono	Monocyte
MPV	Mean Platelets Volume
MW	Molecular weight
Na	Sodium
NCEP	National Cholesterol Education Program
NADP	Nicotinamide adenine dinucleotide phosphate
Neutro	Neutrophil
NIST	National institute of standards and technology
PO_4	Inorganic Phosphorous
PLT	Platelets
PMN	Polymorphonuclear
PTH	Parathyroid hormone
Q1	First quartile
Q2	Second quartile
Q3	Third quartile

ABBREVIATION AND SYMBOLS

QA	Quality Assurance
QC	Quality Control
RI	Reference interval
RBC	Red Blood Cell
RDW-CV	Red cell Distribution- Coefficient of Variation
RIQAS	Randox International Quality Assessment Scheme
RIs	Reference intervals
SD	Standard deviation
SGPT	Serum glutamic-pyruvic transaminase
SPSS	Statistical Package for Social Sciences
SRM	Standard Reference Material
SST	serum separator tube
TG	Triglyceride
TP	Total Protein
UA	Uric Acid
UL	Upper limit
WBC	White Blood cell
%	Percentage
<	Less than
>	More than
”	Less than and equal
•	More than and equal
µg	Microgram
µl	Microlitre

sensitivity of 96%, with a diagnostic sensitivity of 86% at 12 hours after onset of chest pain. AST levels may be increased in viral hepatitis and liver disease associated with hepatic necrosis, with 20 to 50 fold elevations frequently encountered. The evaluation of AST activity in relation to ALT (De Ritis ratio; AST/ALT) is a useful indicator of liver damage. Ratios <1.0 are indicative of mild liver damage, and are particularly associated with diseases of an inflammatory nature. Ratios >1.0 are indicative of severe liver disease, usually involving necrosis.

Increased AST levels may be detected in cirrhosis, extrahepatic cholestasis, progressive muscular dystrophy, dermatomyositis, acute pancreatitis, haemolytic disease, gangrene, crushed muscle injuries and pulmonary emboli. Slight or moderate increases in AST levels may also be observed after ingestion of alcohol, or administration of drugs including penicillin, salicylates or opiates.

1.8.1.20 Alkaline Phosphatase (ALP)[41]

Alkaline phosphatase (ALP) belongs to a group of enzymes that catalyze the hydrolysis of various phosphomonoesters at an alkaline pH. Consequently, ALP is a nonspecific enzyme capable of reacting with many different substrates.

ALP activity is present on cell surfaces in most human tissue. The highest concentrations are found in the intestine, liver, bone, spleen, placenta, and kidney. In the liver, the enzyme is located on both sinusoidal and bile canalicular membranes; activity in bone is confined to the osteoblasts, those cells involved in the production of bone matrix. The specific location of the enzyme within this tissue accounts for the more predominant elevations in certain disorders.

Elevations of ALP are of most diagnostic significance in the evaluation of hepatobiliary and bone disorders. In hepatobiliary disorders, elevations are more predominant in obstructive conditions than in hepatocellular disorders; in bone disorders, elevations are observed when there is involvement of osteoblasts.

In biliary tract obstruction, ALP levels range from 3 to 10 times ULN. Increases are primarily a result of increased synthesis of the enzyme induced by cholestasis. In contrast, hepatocellular disorders, such as hepatitis and cirrhosis, show only slight increases, usually less than three times ULN. Because of the degree of overlap of ALP elevations that occurs in the various liver disorders, a single elevated ALP level is difficult to interpret. It assumes more diagnostic significance when evaluated along with other tests of hepatic function.

Elevated ALP levels may be observed in various bone disorders. Perhaps the highest elevations of ALP activity occur in Paget's disease (osteitisdeformans). Other bone disorders include osteomalacia, rickets, hyperparathyroidism, and osteogenic sarcoma.

In normal pregnancy, increased ALP activity, averaging approximately 1.5 times ULN, can be detected between weeks 16 and 20. ALP activity increases and persists until the onset of labor. Activity then returns to normal within 3 to 6 days. ALP levels are significantly decreased in the inherited condition of hypophosphatasia. Subnormal activity is a result of the absence of the bone isoenzyme and results in inadequate bone calcification.

1.8.2Hematological tests parameters

In this study reference intervals or range have been identified for twenty biochemical test parameters. These are

1.Red Blood Cell (RBC)
2.Hemoglobin (Hb)
3.Hematocrit (HCT)
4.Mean Corpuscular Volume(MCV)
5.Mean Corpuscular Hemoglobin(MCH)
6.Mean Corpuscular Hemoglobin Concentration (MCHC)
7.Red cell Distribution- Coefficient of Variation (RDW-CV)
8.Platelets (PLT)
9.Mean Platelets Volume(MPV)
10.White Blood cell(WBC)
11.Neutrophil
12.Lymphocyte
13.Monocyte
14.Eosinophil
15.Basophil

1.8.2.1 Red blood cells (RBCs),[42]

Red blood cells (RBCs), also called erythrocytes, are cells that circulate in the blood and carry oxygen throughout the body. The RBC count totals the number of red blood cells that are present in a person's sample of blood. The typical lifespan of an RBC is 120 days; thus the bone marrow must continually produce new RBCs to replace those that age and degrade or are lost through bleeding.

Changes in the RBC count usually mirror changes in the hematocrit and hemoglobin level. When the values of the RBC count, hematocrit, and hemoglobin decrease below the established reference interval, the person is said to be anemic. When the RBC and hemoglobin values increase above the normal range, the person is said to be polycythemic. Too few RBCs can affect the amount of oxygen reaching the tissues, while too many RBCs can cause decreased blood flow and related problems.

1.8.2.2 Hemoglobin[43]

Hemoglobin is the iron-containing protein found in all red blood cells (RBCs) that gives the cells their characteristic red color. Hemoglobin enables RBCs to bind to oxygen in the lungs and carry it to tissues and organs throughout the body. It also helps transport a small portion of carbon dioxide, a product of cell metabolism, from tissues and organs to the lungs, where it is exhaled.

Clinical significance: Several diseases and conditions can affect RBCs and consequently the level of hemoglobin in the blood. In general, the hemoglobin level and hematocrite rise when the number of red blood cells increases. The hemoglobin level and hematocrite fall to less than normal when there is a drop in production of RBCs by the bone marrow, an increase in the destruction of RBCs, or if blood is lost due to bleeding. A drop in the RBC count, hemoglobin and hematocrite can result in anemia, a condition in which tissues and organs in the body do not get enough oxygen, causing fatigue and weakness.

If too many RBCs are produced, polycythemia results and the blood can become thickened, causing sluggish blood flow and related problems.

1.8.2.3 Hematocrit[44]

A hematocrit is a test that measures the proportion of a person's blood that is made up of red blood cells (RBCs). Blood consists of RBCs, white blood cells (WBCs), and platelets suspended in a fluid portion called plasma. The hematocrit is a ratio of the volume of red blood cells to the volume of all these components together, called whole blood. The value is expressed as a percentage or fraction. For example, a hematocrit value of 40% means that there are 40 milliliters of red blood cells in 100 milliliters of blood.

The hematocrit is a fairly quick and simple way of evaluating a person's red blood cells and checking for conditions such as anemia. The hematocrit reflects both the number of red blood cells and their volume (mean corpuscular volume or MCV). If the size of the RBCs decreases, so will the hematocrit and vice versa. In general, the hematocrit will rise when the number of red blood cells increases and the hematocrit will fall to less than normal when there is a drop in production of RBCs by the bone marrow, an increase in the destruction of RBCs, or if blood is lost due to bleeding. If the bone marrow is not able to produce new RBCs fast enough, then the overall number of RBCs and hematocrit will drop, resulting in anemia and in this condition, the body does not have the capacity to deliver enough oxygen to tissues and organs, causing fatigue and weakness.

1.8.2.4 Mean corpuscular volume (MCV)[45]

Mean corpuscular volume (MCV) is the average volume of red cells in a specimen. MCV is elevated or decreased in accordance with average red cell size, i.e., low MCV indicates microcytic (small average RBC size), normal MCV indicates normocytic (normal average RBC size), and high MCV indicates macrocytic (large average RBC size).

Indicates RBCs are smaller than normal (microcytic); caused by iron deficiency anemia or thalassemia. Indicates RBCs are larger than normal (macrocytic), for example in anemia caused by vitamin B12 or folate deficiency, myelodysplasia, liver disease, hypothyroidism.

1.8.2.5 Mean corpuscular hemoglobin (MCH)[46]

Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin per red blood cell in a blood sample. MCH is used to help diagnose the type, cause and severity of anemia. It is one of the RBC indices. The MCH is usually either high or low if the size of red blood cells (measured by the MCV) is high or low. Bigger red blood cells usually have more hemoglobin and smaller red blood cells usually have less hemoglobin simply due to their size.

A low MCH is known as hypochromic, because there is less hemoglobin in the red cells, which give them their red color. A high MCH with anemia is known as hyperchromic anemia.

1.8.2. 6 Mean corpuscular hemoglobin Concentration (MCHC)[47]

The mean corpuscular hemoglobin concentration, a measure of the concentration of hemoglobin in a given volume of packed red blood cells. It is reported as part of a standard complete blood count. It is calculated by dividing the hemoglobin by the hematocrite. It is thus a mass or molar concentration.

Decreased MCHC values (hypochromia) are seen in conditions such as iron deficiency anemia and thalassemia. Increased MCHC values (hyperchromia) are seen in conditions where the hemoglobin is more concentrated inside the red cells, such as autoimmune hemolytic anemia, in burn patients, and hereditary spherocytosis, a rare congenital disorder.

1.8.2.7 Red cell distribution width (RDW)[48]

Red cell distribution width (RDW) is a parameter that measures variation in red blood cell size or red blood cell volume. RDW is elevated in accordance with variation in red cell size (anisocytosis), i.e., when elevated RDW is reported on complete blood count, marked anisocytosis (increased variation in red cell size) is expected on peripheral blood smear review.

Mathematically, the RDW-CV is calculated with the following formula:

RDW-CV = (Standard deviation of MCV ÷ mean MCV) × 100**.**

Usually presents with high RDW with low MCV in iron Deficiency Anemia and presents with high RDW and high MCV in Folate and vitamin B12 deficiency anemia. Also presents with high RDW with MCV being high, low or often normal range in mixed Deficiency (Iron + B12 or folate) anemia.

1.8.2.8 Platelets (Thrombocytes)[49]

Platelets, also called thrombocytes, are tiny fragments of cells that are essential for normal blood clotting. They are formed from very large cells called megakaryocytes in the bone marrow and are released into the blood to circulate. The platelet count is a test that determines the number of platelets in a person's sample of blood. When there is an injury to a blood vessel or tissue and bleeding begins, platelets help stop bleeding in three ways.

They (i). Adhere to the injury site (ii).Clump together (aggregate) with other platelets (iii). Release chemical compounds that stimulate further aggregation of other platelets.

High count of platelet in Blood are known as thrombocytosis. Causes of thrombocytosis are acute bleeding and blood loss, allergic reactions, cancer chronic kidney failure or another kidney disorder, heart attack, coronary artery bypass, infections, including tuberculosis, iron and vitamin deficiency, removal

of spleen, hemolytic anemia, inflammation, major surgery, pancreatitis, trauma burns and exercise. Medications that can cause reactive thrombocytosis include: epinephrine, tretinoin and heparin sodium.

Low count of platelet in Blood are known as thrombocytopenia. Causes of thrombocytopenia are aplastic anemia, a vitamin B-12 deficiency, a folate deficiency, an iron deficiency, viral infections, including HIV, Epstein-Barr virus, and chickenpox, exposure to chemotherapy, radiation, or toxic chemicals ,consuming too much alcohol cirrhosis and leukemia.

1.8.2.9 Mean platelet volume (MPV)[50]

Mean platelet volume (MPV) is a machine-calculated measurement of the average size of platelets found in blood and is typically included in blood tests as part of the CBC. Since the average platelet size is larger when the body is producing increased numbers of platelets, the MPV test results can be used to make inferences about platelet production in bone marrow or platelet destruction problems.

MPV is higher when there is destruction of platelets. This may be seen in inflammatory bowel disease, immune thrombocytopenic purpura (ITP), myeloproliferative diseases and Bernard-Soulier syndrome. It may also be related to pre-eclampsia, and recovery from, transient hypoplasia.

Abnormally low MPV values correlate with thrombocytopenia when it is due to impaired production as in aplastic anemia. In addition, low MPV, can correlate with abnormally small platelet size, sometimes a symptom of a spectrum referred to as Wiskott-Aldrich Syndrome, caused by a genetic mutation of the WAS gene.

1.8.2.10 White blood cells, (WBC)[54]

White blood cells, also called leukocytes, are cells that exist in the blood, the lymphatic system, and tissues and are an important part of the body's defense

system. They help protect against infections and also have a role in inflammation, allergic responses, and protecting against cancer. The white blood cell (WBC) count totals the number of white blood cells in a person's sample of blood. It is one test among several that is included in a complete blood count (CBC), which is often used in the general evaluation of a person's health.

Low count of WBC in Blood are known as leukopenia. Causes of Leukopenia are Bone marrow disorders or damage, conditions, Severe infections (sepsis)Lymphoma or other cancer that spread to the bone marrow, Dietary deficiencies and Diseases of immune system (e.g. HIV/AIDS).

High count of WBC in Blood are known as leukocytosis. Causes of leukocytosis are Infection, most commonly bacterial or viral, Inflammation, Leukemia, myeloproliferative disorders, Allergies, asthma and tissue death (trauma, burns, heart attack) Intense exercise or severe stress.

1.8.2.11 Neutrophil [55]

Neutrophils are the most abundant white blood cell, constituting 60-70% of the circulating leukocytes. They defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers forms pus. Neutrophils are active in phagocytosing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes (used in digesting microbes) and die after having phagocytosed a few pathogens. Neutrophils are the most common cell type seen in the early stages of acute inflammation. The life span of a circulating human neutrophil is about 5.4 days.

Low count of neutrophil in blood are known as neutropenia. Causes of neutropenia are Severe, overwhelming infection (sepsis), autoimmune disorders, dietary deficiencies, reaction to drugs, chemotherapy, immunodeficiency, myelodysplasia and bone marrow damage (e.g. chemotherapy, radiation therapy)

High count of neutrophil in blood are known as neutrophilia. Causes of neutrophilia are acute bacterial infections, inflammation, trauma, heart attack, or burns, Stress, rigorous exercise and cushing syndrome.

1.8.2.12 Lymphocytes[56]

Lymphocytes are much more common in the lymphatic system than in blood. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. Lymphocytes include: natural killer cells(NK cells) (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). They are the main type of cell found in lymph, which prompted the name lymphocyte.

Low count of lymphocytes in blood are known as lymphocytopenia. Causes of lymphocytopenia are autoimmune disorders (e.g. lupus, rheumatoid arthritis),infections (e.g. HIV, viral hepatitis, typhoid fever, influenza),bone marrow damage (e.g. chemotherapy, radiation therapy), corticosteroids.

High count of lymphocytes in blood are known as lymphocytosis. Causes of lymphocytosis are Acute viral infections (e.g., chicken pox, cytomegalovirus (CMV),Epstein-Barr virus (EBV), herpes, rubella),Certain bacterial infections (e.g. whooping cough and tuberculosis), toxoplasmosis, chronic inflammatory disorder (e.g. ulcerative colitis), lymphocytic leukemia, lymphoma and stress (acute).

1.8.2.13 Monocytes[57]

Monocytes, the largest type of WBCs, share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an extra role: they present pieces of pathogens to T cells so that the pathogens may be recognized

again and killed. This causes an antibody response to be mounted. Monocytes eventually leave the bloodstream and become tissue macrophages, which remove dead cell debris as well as attack microorganisms. Neither dead cell debris nor attacking microorganisms can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney shaped nucleus and are typically agranulated.

Causes of low counts ofmonocytes are bone marrow damage or failure, hairy cell leukemia and aplastic anemia.

Causes of high counts of monocytes are Chronic infections (e.g., tuberculosis, fungal infection), infection within the heart (bacterial endocarditis), collagen vascular diseases

(e.g., lupus, scleroderma, rheumatoid arthritis, vasculitis), Monocytic or myelomonocytic and leukemia (acute or chronic)

1.8.2.14 Eosinophils[58]

Eosinophil compose about 2-4% of the WBC total. This count fluctuates throughout the day, seasonally, and during menstruation. It rises in response to allergies, parasitic infections, collagen diseases, and disease of the spleen and central nervous system. They are rare in the blood, but numerous in the mucous membranes of the respiratory, digestive, and lower urinary tracts.

One or an occasional low number is usually not medically significant.High counts of eosinophil can indicate the following such as asthma, allergies such as hay fever, drug reactions, parasitic infections, inflammatory disorders (celiac disease, inflammatory bowel disease), some cancers, leukemias or lymphomas and addison disease.

1.8.2.15 Basophils[59]

A basophil is a type of white blood cell and a type of granulocyte. They are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing the dilation of blood vessels. Because they are the rarest of the white blood cells (less than 0.5% of the total count) and share physicochemical properties with other blood cells, they are difficult to study. They can be recognized by several coarse, dark violet granules, giving them a blue hue.

As with eosinophil count are normally low in the blood; usually not medically significant. High counts of basophils can indicate the following such as rare allergic reactions (hives, food allergy), inflammation (rheumatoid arthritis, ulcerative colitis), some leukemia's and uremia

1.8.3 Literature Review

Health and disease can only be distinguished by accurate and reliable reference values of a particular laboratory test. It is now a proven fact that there is considerable variation in biochemistry/hematology reference intervals depending on the demographic and preanalytical variables. There are evidences that values provided by manufacturers do not have appropriate application for all populations. Moreover, reference ranges provided by different laboratory manuals and books also do not solve this problem.

Roshan*et al.*[22].represented normal reference ranges of Malaysian population. These values were determined by using Sysmex XE-2100 and ACL 9000 hematology and coagulation analyzers. Their study showed that there were considerable differences in the reference values from manufacturers, western population or laboratory manuals compared with those from the local population.

The hematological profile of the population in central province of Iran were studied by Amitis Ramezani[23](The median and 95% reference values (2.5^{th}-

97.5th) for Hb and platelet counts were 15.5 g/dl (14.1-17.7) and 209 $\times 10^9$ cells/L (151-322) respectively. The median for total WBC count, neutrophil, lymphocyte, monocyte and eosinophil were 6.7 $\times 10^9$ cells/L (4.3-11.2), %58 (%50-%70), 40% (30-49%), 0% (0-2) and %1 (0-3%), respectively) was different from the reports of other countries and also the standards reference ranges described in textbook.

Clement Leh *et al* (2011) showed locally-derived age-specific clinical laboratory reference ranges of healthy Africans in sub-Saharan Africa. Reference values from North American and European populations are being used for African subjects despite showing significant differences. Clinical laboratory reference values for African adolescents and young adults that can be used in clinical trials and for patient management[25].

Reference ranges for haematological and other laboratory tests in most African countries are based on populations in Europe and America and, because of environmental and genetic factors, these may not accurately reflect the normal reference ranges in African populations. The distribution of haematological parameters in healthy individuals residing in Blantyre, Malawi and also examined the effect of socio demographic and nutritional factors on the haematological variables (Chisale *et al*,2015)

Clinical reference intervals among Indian population are poorly defined. Therefore, there is an urgent need to establish local clinical laboratory reference intervals for healthy Indian population. 95 % reference interval for hematological and biochemical parameters in apparently healthy Indian population. 10,665 reference individuals identified as healthy by physicians. The 95 % of the reference distribution was estimated using 2.5th and 97.5th percentile reference limits. The 95 % reference intervals for hemoglobin (Males: 12.3–17 g/dL; Females: 9.9–14.3 g/dL), platelet count (Males: 1.3–3.8;Females: 1.3–4.2 Lakhs/µL), erythrocyte sedimentation rate (Males: 2–22; Females: 4–55 mm/h),

serum uric acid in males: 3.5–8.2 mg/dL, gamma glutamyltransferase (Males: 13–61 U/L), fasting blood glucose (Males: 78–110 mg/dL), total cholesterol (Males: 115–254 mg/dL), low density lipoprotein (Males: 60–176 mg/dL) and triglycerides (Males: 55–267 mg/dL, Females: 52–207 mg/dL) were different from currently used reference values. Additionally need for gender based partitioning were observed for triglycerides and gamma glutamyltransferase. The observed findings are of clinical significance and it needs to be validated with additional community based studies (Shrilekha Saira*et al*,2014)[24].

YC LO, *et al.* analytes reference intervals of common clinical chemistry for adults in Hong Kong"[63].The samples were tested for twenty-five routine analytes named Albumin(BCG), ALT ALP, Amylase, AST, Bil-T, Calcium, Chloride, Creatinine,

Cholesterol , D-HDL, Glucose, GGT, Phosphate, Potassium, Sodium, Total Protein, Urea, Uric acid , TIBC, Iron, CPK, CKMB, LDH and Triglyceride on the Abbott ARCHITECT clinical chemistry system. Results from this study obtained serum uric acid (males:3.57–9.32mg/d, females 2.71-6.27 mg/d)[72],chloride(102–109mmol/L)[9]. Calcium(2.19-2-2.50mmol/L)[35], Magnesium (males: 0.821.15mmol/L, females 0.77-1.13mmol/L)[98], Cholesterol(115.7 -254.2 mg/dl)[75], Triglyceride (15.45 -390 mg/dl)[75], ALT(males: 8.0–57 U/L, females 7-39 U/L)[74], (males: 8.0–57 U/L, females 7-39 U/L)[75],ALP (39-142 U/L)[73] was different from the reports of other countries and also the standards reference ranges described in textbook [35,36,72,73,74,75].

K.S. Chua*et al.* established reference ranges reference ranges of seventeen serum biochemical constituents in a Singapore population. Seventeen biochemical constituents were named Albumin, ALP, Bil-T, Bicarbonate, Calcium, Chloride, Creatinine, Glucose, Phosphate, Potassium, Sodium, Total Protein, Urea, Uric acid Protein bound iodine, Cholesterol and Triglyceride. The 95 % reference intervals for ALP (32-105 U/L)[73],Bil-T(0.175- 1.40 mg/dl)[72],Creatinine (0.5-1.60

mg/dl)[72], Glucose (3.10 -6.6 mmol/L)[35],Urea (17- 46 mg/dl)[35], Uric acid(3.90-8.30 mg/dl)[72] were vary from the reports of other countries and also the standards reference ranges described in textbook [35.72.73].

Reference values for 26 elements, namely Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg,Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn and Zr are proposed in serum and blood of 110 healthy adults of the urban area of Rome . They were included in the study on the basis of strict criteria of eligibility and exclusion. With the exception of Ba, Bi, Co, Cr, Ni, Sb, SnTl in serum, and Bi, Hg, Si, V and W in whole blood, experimental data for each all the other analytes were found to approach a normal distribution. The estimated 5 - 95% references ranges (in ng/ ml) were reported. For several elements the reference ranges observed overlapped information available in the literature. Gender, age, body mass index,

smoking habits and alcohol consume were used as grouping variables. Mutual associations were observed for several elements, as follows: Be, Ca, Co, Cr, Cu, Li, Mo, Pb and Zn with sex; Ca, Pb and Si with age (< and > 45 years); Co, Cr, Mo, Sb and Tl with body mass index; Cd and Pb with smoking habit; Cr and Pb with alcohol consume(Alessandro ALIMONTI *et al*,2005)[26].

1.8.4 Aim and objectives

Reference ranges are sets of values used by the health professionals to interpret the results and are considered the most authoritative tools in laboratory science to assist in the decision making phase, hence useful for patient care.

The more traditional, widespread and practiced method for interpreting the laboratory results is based on the comparison made with reference range or reference intervals. As defined by the international federation of clinical chemistry (IFCC) and Clinical and Laboratory Standards Institute (CLSI) the terms reference range or reference interval mean a range of values obtained from

individuals randomly chosen but appropriately selected in order to satisfy suitably defined criteria.

There are no published reference ranges for biochemical and hematological parameters for Bangladeshi population. All clinical laboratory of Bangladesh relies on reference values provided by the manufacturer'sof reagents as well as those in literature in medical books to interpret patient results. This can be of a grave mistake having in mind that parameters vary from region (Waithaka*et al.*, 2009). The patient's results can also vary due to the methods used in analyzing, that is either manual or by automation. Reagent manufactures and medical text books reference ranges which may not adequately represent Bangladeshi population, when it is used to aid decision-making regarding clinical diagnosis and treatment that may not precisely applicable.Europe and America , are the source of reagents used in analyzing different biochemical and hematological parameters, and their reference values are established with healthy population from these regions. Since reference ranges may vary with age, sex, life style, diet and geographical location. Life style, diet and geographical location of Bangladeshi population are differ from American and European population.

So it should remain own reference range based upon Bangladeshi population characteristics. The objectives of this study were therefore to establish reference ranges for biochemical and hematological parameters for the population of Bangladesh for the fish time.

1.8.4 .1 General Objective

To establish reference ranges for biochemical and hematological parameters for the population of Bangladesh**.**

1.8.4 .2 Specific objectives

a) To identify 95% reference ranges or intervals of biochemical and hematological parameters for healthy Bangladeshi population.

b) Partitioning of the data for age, sex and recommend separate reference interval if significantly different.

MATERIALS AND METHODS

2.1 Study area and Reference population / Participants:

In this study, the apparently healthy members of the Bangladeshi population included Dhaka, Chittagong, Rajshahi and Khulna were selected as reference individuals who fulfill the CLSI selection criteria. Reference population consisted of healthy volunteer blood donors aged between 18 to 65 years old who had lived in different division of Bangladesh and those who gave written informed consent for this study were interviewed through a questionnaire. Those who did not meet the inclusion criteria were excluded from the study.

2.2 Ethical consideration and Consent:

Ethical approval was obtained from institute of Biological Sciences ethical committee, Rajshahi University. Written informed consent was obtained from each participant and questionnaire were completed using specific form as per interview prior to blood collection.

2.3Selection criteria:

The reference individuals are those persons identified as healthy from this reference population. Defining healthy and normal has been a challenge. Drawing from the guidance published by Clinical laboratory standards institute (document EP-28-A3c) strict inclusion, exclusion criteria were used to identify reference individuals.

2.3.1Inclusion Criteria

- Citizen of Bangladesh with apparently healthy.
- Age range 18 - 65 years.
- Body mass index(BMI) of 18 to 35 Kg/m2

2.3.2Exclusion Criteria

- Blood donor with known pathophysiologic states: diabetes mellitus, chronic renal insufficiency, hypertension, ischemic heart disease, anaemia, thyroid and liver diseases, HbsAg, HCV and HIV positive were excluded.
- volunteer with complaints that may reflect biochemical and hematological abnormality such as weight loss, fever, chest pain, giddiness, polyarthralgia and loss of appetite were excluded.
- Donor with past illness of typhoid, tuberculosis, malaria, dengue within 6 months of the collection date and jaundice or major surgery within 1 year were excluded.
- Pregnant and lactating women were excluded.
- Donor's whose physical examination revealed abnormalities or whose blood pressure was more than 140/90 were excluded.

2.4 Specimen Collection, transportation, rejection, processing and storage:

Some pre-analytical factors affect results so these are considered when performing the study. Examples include sample matrix such as serum, plasma and whole blood, Patient condition such as fasting , Proper sample identification, common interferences such as hemolysis, icteric and lipemic, temperature during specimen storage. Sample were collected from 2013 to 2015.

2.4.1 Specimen Collection and Handling Techniques:

Specimens were collected from all the reference individuals between 7.30 and 9.30 am in the morning after an overnight fast. All participants were advised to take rest at least for 15 min before blood collection. All participants were seated

and cubital vein sampling was most often done. Evacuated blood collection tubes were used. Blood was collected in

plain or SST gel tubes for analysis of liver function tests, renal function tests, lipid profile, electrolyte, protein ,enzyme or Sodium Fluoride/EDTA tubes for glucose analysis and EDTA tubes for hematological parameters. Once the specimens were acquired, they were labeled with the donor and study numbers.

2.4.2 Specimen transportation:

Specimens were transported from the collection centre to the processing laboratory in ice packed cool boxes within 30 minute.

2.4.3 Specimen Rejection:

Specimens were rejected if any one or more of the following rejection criteria are met

1.The Questionnaire and consent form are not completed.

2.The specimen is collected in incorrect collection tube.

3.Specimens lacking proper identification or not labeled.

4.Specimen containers are broken or leaking.

5.Specimen is less than the minimal volume needed for testing.

6.Haemolysed blood sample.

7.Clotted blood in EDTA tube.

2.4.4 Specimen processing and storage:

Hematological specimen were rolled prior to analysis. For biochemical analysis once clotted, the blood specimens were centrifuged at 4000 revolutions per

minute for five minutes at room temperature and serum separated immediately and were analyzed the sample within 10 minutes. If analysis is not possible serum specimens were then stored at -70oC awaiting laboratory analysis.

2.5 Analytes and Instrumentation:

Table-2.1 lists the assays included in the study. Twenty biochemical and fifteen hematological total thirty five tests were performed following the manufacturer's package inserts and the standard operating procedures for Beckman Coulter reagents on the Beckman Coulter AU640 Chemistry analyzer (Beckman Coulter Inc Japan). andSysmex reagents on the Sysmex XE 2100 hematology analyzer (Sysmex Corporation Kobe, Japan). Serological testing for HBsAg , an anti-HCV and HIV antibodies were done by Architect ci2000 system using chemiluminescent microparticle immunoassay (CMIA) technology.

2.6 Analytical methods and Reaction Principles:

The method and reaction principles used in the laboratory to produce patient results is an important factor in setting reference intervals. Same analyte value may obtained different based on different analytical method and reaction principles. Table -2.2 and Table -2..3 summarized analytical methods and reaction principles used on Beckman Coulter AU640 and Sysmex XE 2100.\

Table-2.1:Analytes included in the study. All tests were performed using reagents specific for the Beckman Coulter AU640 andSysmex XE 2100 analyzer.

Biochemistry Tests	**Hematology Tests**
1. Glucose	1.Red Blood Cell (RBC)
2.Creatinine	2.Hemoglobin (Hb)
3.Urea	3.Hematocrite (HCT)
4.Uric Acid(UA)	4.Mean Corpuscular Volume(MCV)
5.Sodium(Na)	5.Mean Corpuscular Hemoglobin(MCH)
6.Potassium(K)	6.Mean Corpuscular Hemoglobin Concentration (MCHC)
7.Chloride(Cl)	7.Red cell Distribution- Coefficient of Variation (RDW-CV)
8.Total Calcium(Ca)	8.Platelets (PLT)
9.Magnesium(Mg)	9.Mean Platelets Volume(MPV)
10.Inorganic Phosphorous(PO4)	10.White Blood cell(WBC)
11.Cholesterol	11.Neutrophil (Neutro)
12.HDL.Cholesterol(HDL.C)	12.Lymphocyte (Lympho)
13.LDL.Cholesterol (LDL.C)	13.Monocyte (Mono)
14.Triglyceride(TG)	14.Eosinophil (Eosino)
15.Total Protein (TP)	15.Basophil(Baso)
16.Albumin (Alb)	
17.Bilirubin-Total (Bil-T)	
18.Alanine amino Transferase (ALT)	
19.Aspartate amino Transferase (AST)	
20.Alkaline phosphates (ALP)	

Table -2.2: The analytical methods and reaction principles used for the analysis of biochemistry tests

Test	Analytical Method	Reaction Principle
Glucose	Spectrophotometry (End Point Assay)	Hexokinase /G-6-PDH
Creatinine	Photometry (Fixed Point Assay)	Modified Jaffe Method
Urea	Spectrophotometry(Rate Assay)	Urease
Uric Acid	Photometry (End Point Assay)	Uricas
Sodium	Potentiometric	Not applicable
Potassium	Potentiometric	Not applicable
Chloride	Potentiometric	Not applicable
Calcium	Photometry (End Point Assay)	Arsenazo
Magnesium	Spectrophotometry (End Point Assay)	Xylidyl Blue
Inorganic Phosphate	Spectrophotometry (End Point Assay)	Phosphomolybdate
Cholesterol	Photometry(End Point Assay)	Enzymatic
HDL.Cholesterol	Photometry(End Point Assay)	Enzymatic Immuno inhibition
LDL.Cholesterol	Photometry(End Point Assay)	Enzymatic Selective Protection
Triglyceride	Photometry(End Point Assay)	Glycerol Phosphate Oxidase
Total Protein	Photometry(End Point Assay)	Biuret
Albumin	Photometry(End Point Assay)	Bromcresol Green
Bilirubin-Total	Photometry(End Point Assay)	DPD (Dichloro phenyl diazonium tetra fluoroborate)
Alanine amino Transferase	Spectrophotometry(Rate Assay)	NADH (without pyridoxal-5-phosphate).
Aspartate amino Transferase	Spectrophotometry(Rate Assay)	NADH ((without pyridoxal-5-phosphate).
Alkaline phosphates	Photometry (Rate Assay)	Para-nitro phenyl Phosphate

Table -2.3: The analytical methods used for the analysis of hematology on Sysmex XE2100

Test	Analytical Method
Red Blood Cell (RBC)	Optical Impedance
Hemoglobin	Photometric
Hematocrite	Calculated
Mean Corpuscular Volume	Calculated
Mean Corpuscular Hemoglobin	Calculated
Mean Corpuscular Hemoglobin Concentration	Calculated
Red cell Distribution-Coefficient of Variation	Calculated
Red Blood Cell (RBC)	Optical Impedance
Hemoglobin	Photometric
Hematocrite	Calculated
Mean Corpuscular Volume	Calculated
Mean Corpuscular Hemoglobin	Calculated
Mean Corpuscular Hemoglobin Concentration	Calculated
Red cell Distribution-Coefficient of Variation (RDW-CV)	Calculated

2.7 Calibration procedure and calibrator traceability:

All the analytical instruments are calibrated as per manufacturers' recommendations. Biochemistry automated system, calibration done when new reagent lot are used, internal quality control are outlines, reagents calibration stability over, after severe breakdown and preventive maintenance. On the other hand Haematology automated system calibration done when the analyzer is first installed and put into operation, if a daily quality control shows a drift, if any component of the analyzer is replaced or repaired and as a routine once in a year.

Calibrator traceability and measurement uncertainty information for all assays were provided by the manufacturer. In Biochemistry automated analyzer, Beckman coulter system calibrator were used as calibration materials which are traceable to national institute of standards and technology (NIST) SRM 965,SRM 967 L1,SRM 909b L1,SRM 909b L1,SRM 2201,SRM 2202,SRM 2201,NIST SRM 909b L1,SRM 909 b L2,SRM 909b,SRM 927c,CRM 470 and SRM 916a for Glucose, Creatinine, Urea, Uric Acid, Sodium, Potassium, Chloride, Calcium, Magnesium Cholesterol, Total Protein, Albumin and Total Bilirubin respectively.

In hematology automated analyzer, SCS-1000 hematology calibrators were used as calibration materials which are traceable to ICSH (International Council for Standardization in Hematology)and Expert Panel on Cytometry.

2.8 Quality Assurance (QA)/ Quality Control (QC)

The key factors are the accuracy and assay precisionare important factor in setting reference intervals. In general, assay precision has a smaller effect than bias on setting reference intervals, especially when the precision is small compared to the combined within and between-person biological variation. To determine the precision of analysis

for the serum constituents studied, pooled sera were included in every batch of analysis of each constituent. At the end of the study period between batches values obtained for the pooled sera were used for the calculation of mean, standard deviation and coefficient of variation values.Table-2.4shown analytical imprecision and Measurement uncertainty with total allowable error during the time of this study.

To ensure accuracy and precision of the test results, all pre analytical, analytical and post analytical precautions were taken into consideration. Two type of control were performed for biochemistry and hematology.

- Internal Quality Control(IQC)
- External Quality Control(EQC)

Internal Quality control manufacture by BIORAD which were run daily and External Quality Control materials from Randox Laboratory Limited USA, named RIQAS (Randox International Quality Assessment Scheme) monthly Programme were run once in a month which monitors the performance test results. RIQAS is the largest global External Quality Assessment (EQA) scheme, used by more than 35,000 participants in 123 countries. This large number of participants makes RIQAS the best quality EQA scheme in the world and ensures an extensive database of results for many analytical methods, directly increasing statistical validity as a result.

The laboratory has satisfactory performance in RIQAS clinical Chemistry and RIQAS Hematology over the past three years

Step involved in Internal Quality Control for Biochemistry and Hematology as follow

- Run two levels(biochemistry) and three level (hematology) of QC materials daily
- If the QC is not within the control range (+/-3sd), stop analysis and take corrective action.
- Calculate Mean, Standard Deviation, and Coefficient of Variation
- Plot L-J Charts
- Apply Westgard rules for evaluation and passing the run
- Make a chart of monthly CV for the year.
- Review monthly QC performed.
- Maintain records of the above and review them.

Table-2.4:Total allowable error, analytical imprecision and Measurement Uncertainty

Test	**Total allowable Error**	**Mean Coefficient of variation(CV)**	**Measurement Uncertainty**
Glucose	5.5	1.9993	3.9986
Creatinine	8.87	2.2342	4.4684
Urea	15.55	3.5415	7.0831
Uric Acid	11.97	1.8736	3.7472
Sodium	0.73	1.26623	2.5324
Potassium	5.61	1.83572	3.6714
Chloride	1.5	1.4355	2.8711
Total Calcium	2.55	2.4514	4.90297
Magnesium	4.8	2.8388	5.67775
Inorganic Phosphorous	10.11	3.2371	6.47426
Cholesterol	9.01	2.48589	4.9717
HDL.Cholesterol	11.63	4.78538	9.5707
LDL.Cholesterol	11.9	5.13879	10.277
Triglyceride	25.99	2.41656	4.8331
Total Protein	3.63	3.859	7.7181
Albumin	4.07	2.0277	4.0555
Alanine amino Transferase	27.48	2.5805	5.1611
Aspartate amino Transferase	16.69	1.8816	3.7632
Alkaline phosphates	12.04	4.22478	8.4495
Red Blood Cell	4.4	0.9408	1.8816
Hemoglobin	4.19	0.85613	1.71226
Hematocrite	3.97	0.963803	1.9276
Mean Corpuscular Volume	2.5	0.9857	1.9714
MCH	2.42	0.97856	1.95712
MCHC	1.27	0.96897	1.93794
RDW-CV	4.6	0.99589	1.99178
Platelets	13.4	2.49881	4.99763
Mean Platelets Volume	5.84	1.9985	3.9971
White Blood cell	15.49	1.88649	3.77298
Neutrophil	23.35	1.76569	3.531396
Lymphocyte	17.9	1.802365	3.60473
Monocyte	27.9	1.985674	3.971349
Eosinophil	37.1	1.896562	3.793124
Basophil	38.5	2.012564	4.025128

2.9 Data Management and Statistical Analysis

2.9.1 Data Management

730 persons met the inclusion criteria and were included in the study. Data on biochemical parameters, fasting blood glucose(FBS), Creatinine, Urea, Uric Acid(UA), Sodium(Na), Potassium(K), Chloride (Cl), Total Calcium(Ca), Magnesium(Mg), Inorganic phosphorous(PO4),total Cholesterol, high density lipoprotein (HDL) (HDL.C), low density lipoprotein (LDL) cholesterol (LDL.C), Triglyceride(TG),Total Protein (TP),Albumin (Alb),Bilirubin-Total(Bil-T),Alanine amino Transferase (ALT) , Aspartate amino Transferase (AST), Alkaline phosphates (ALP) and hematology parameters Red Blood Cell (RBC), Hemoglobin (Hb), Hematocrite (HCT),Mean Corpuscular Volume(MCV) Mean Corpuscular Hemoglobin(MCH),Mean Corpuscular Hemoglobin Concentration(MCHC),Red cell Distribution- Coefficient of Variation(RDW-CV).Platelets (PLT),Mean Platelets Volume(MPV),White Blood cell (WBC), Neutrophil (Neutro), Lymphocyte (Lympho), Monocyte (Mono), Eosinophil(Eosino), and Basophil(Baso) count , were recorded. All Data were entered into a Microsoft Excel database, compared, and corrected for data entry errors then imported into Statistical Package for Social Sciences (IBM SPSS -20).

The data was visually inspected for extreme values and the values for single parameters that appeared physiologically impossible were removed. Outliers in the remaining data were identified using 3 or 4 standard deviations and 1.5 inter quartile range (IQR) criteria. The first quartile (Q1), the median (Q2) and third quartile (Q3) were calculated for a given analyte that is, the interquartile range (IQR) by subtracting the first quartile from the third quartile (Q3 - Q1). When a value was lower than Q1 -1.5(Q3-Q1) or higher than Q3+1.5(Q3-Q1) it was considered as an outlier and removed prior to analysis.

2.9.2 Frequency distributions

Figure 2.1 to 2.18show the frequency distributions obtained for the twenty biochemical and fifteen hematological analytes studied where the X-axis shows the concentration of the analyte and the Y-axis shows the number of individuals.

Figure 2.1 : Frequency histograms of Blood Glucose and serum Creatinine.

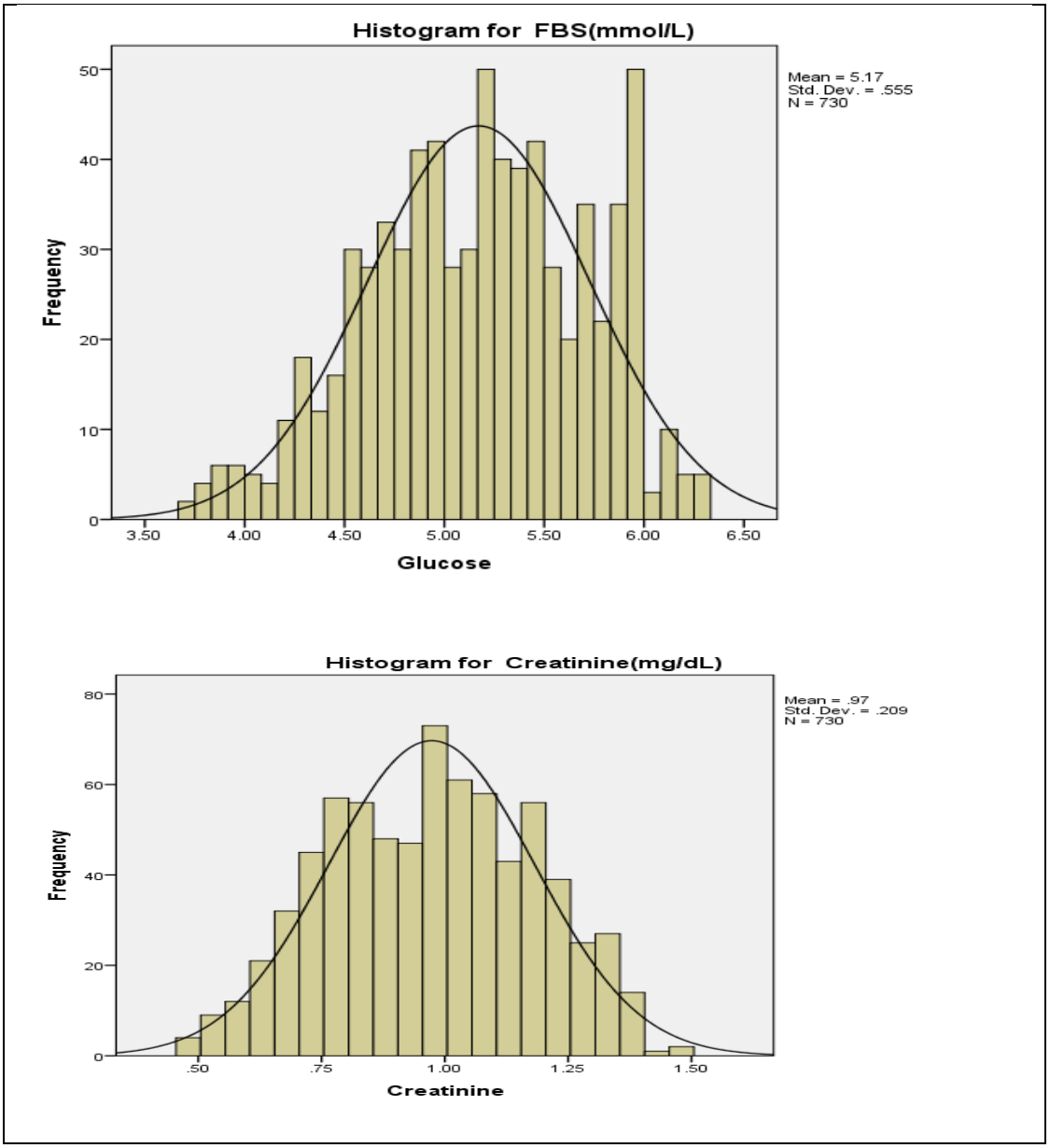


Figure 2.2: Frequency histograms of Blood Urea and serum Uric acid.

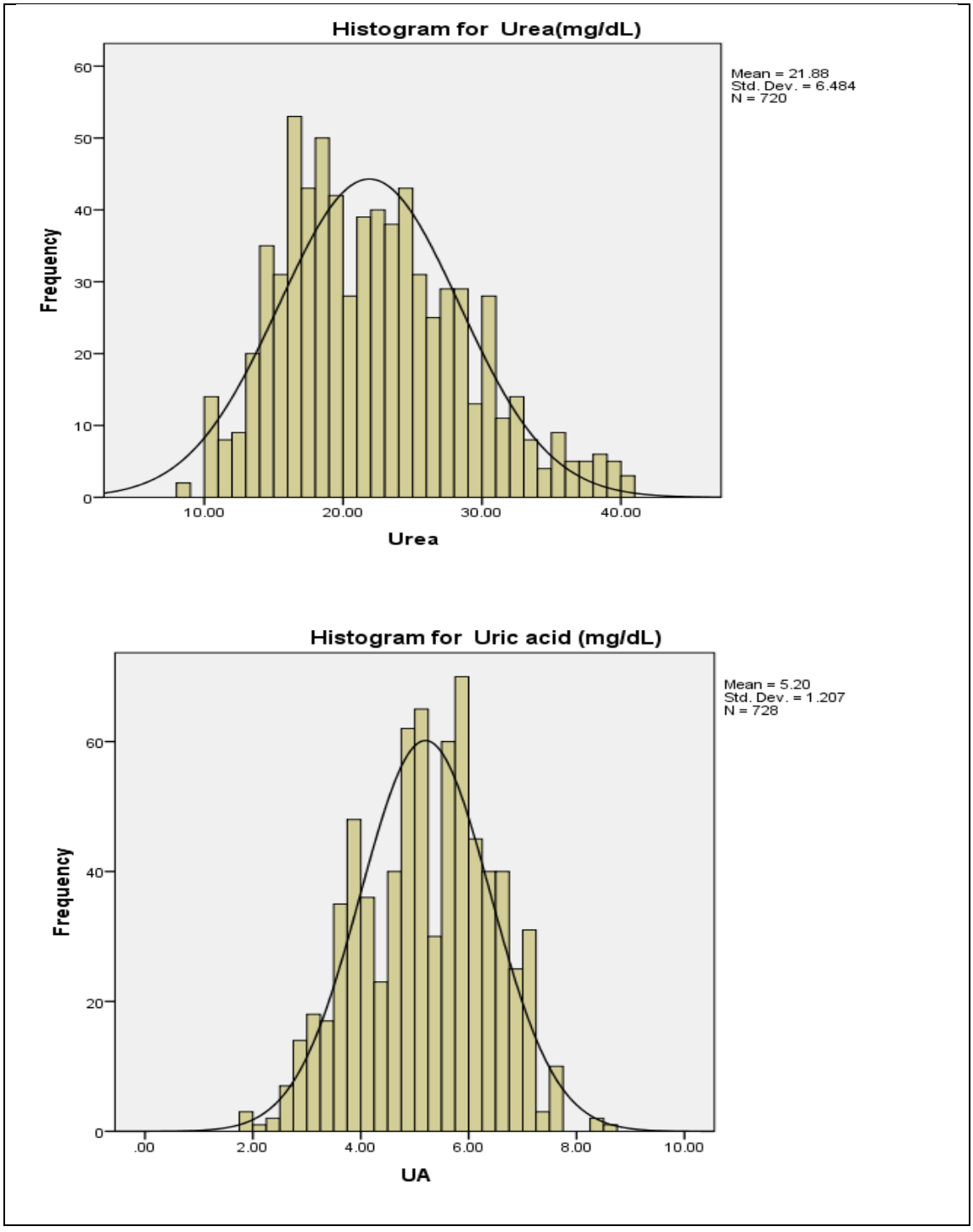


Figure 2.3: Frequency histograms of serum Sodium and Potassium

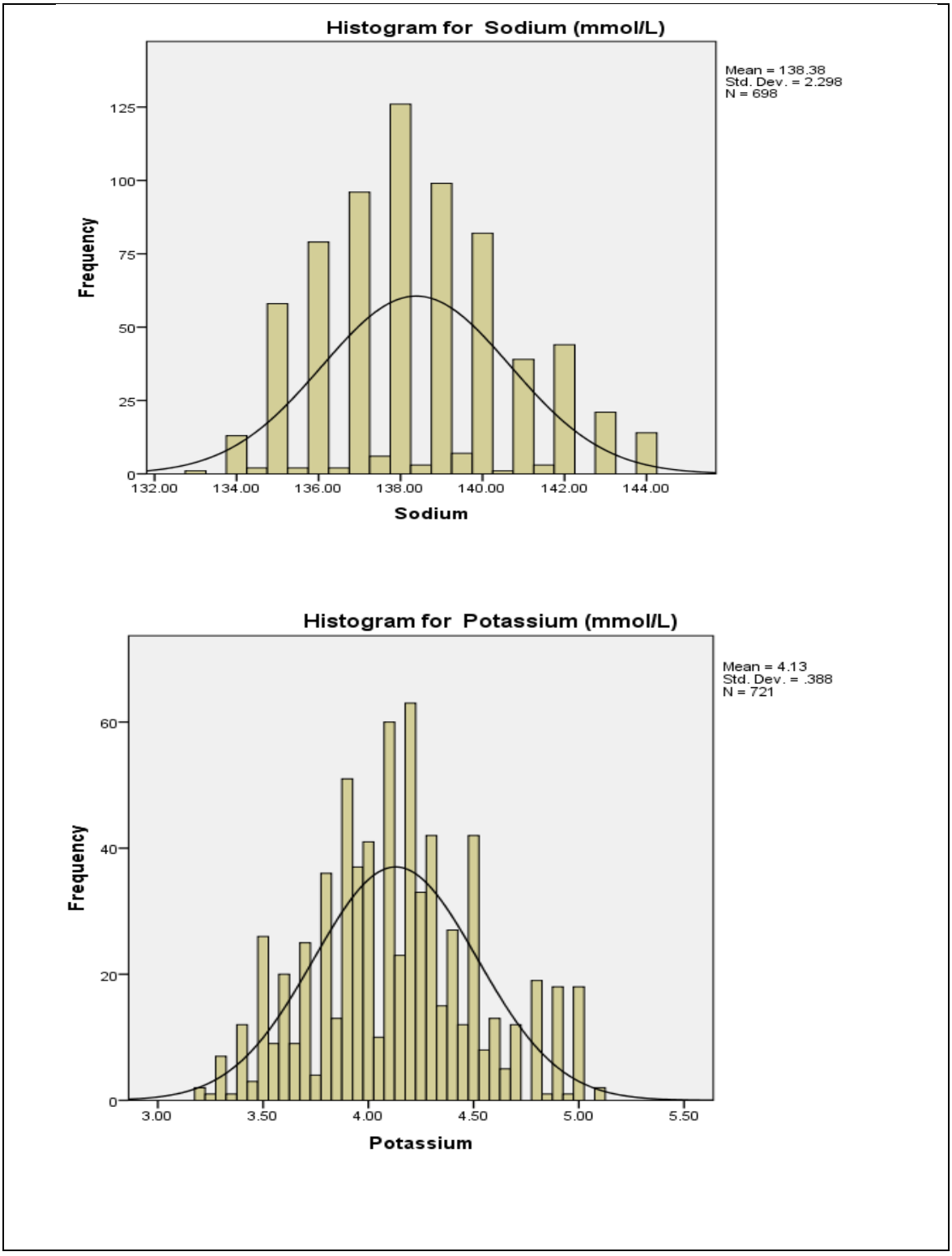


Figure 2.4: Frequency histograms of serum Chloride and total Calcium

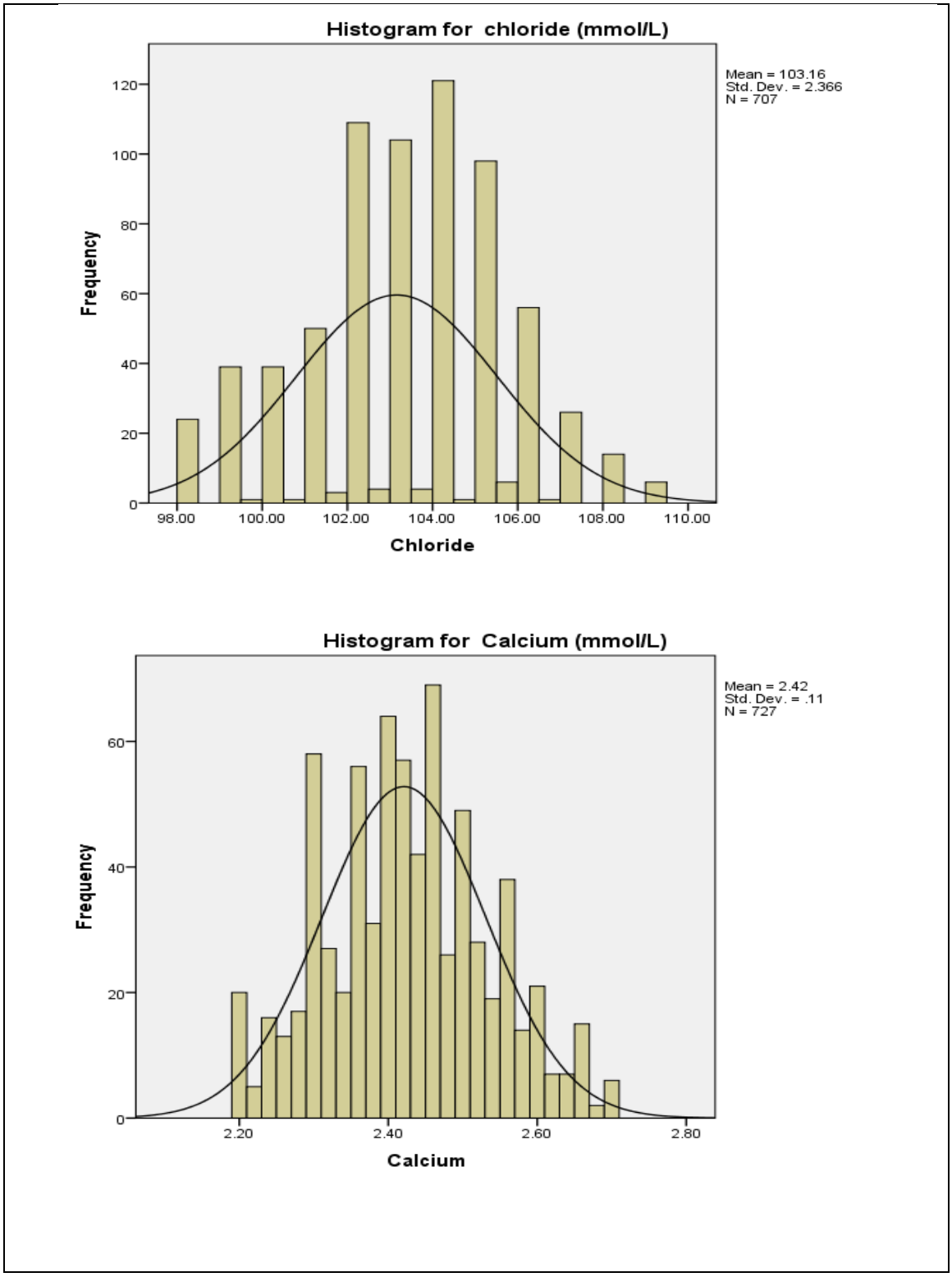


Figure 2.5: Frequency histograms of serum Magnesium and Inorganic Phosphate

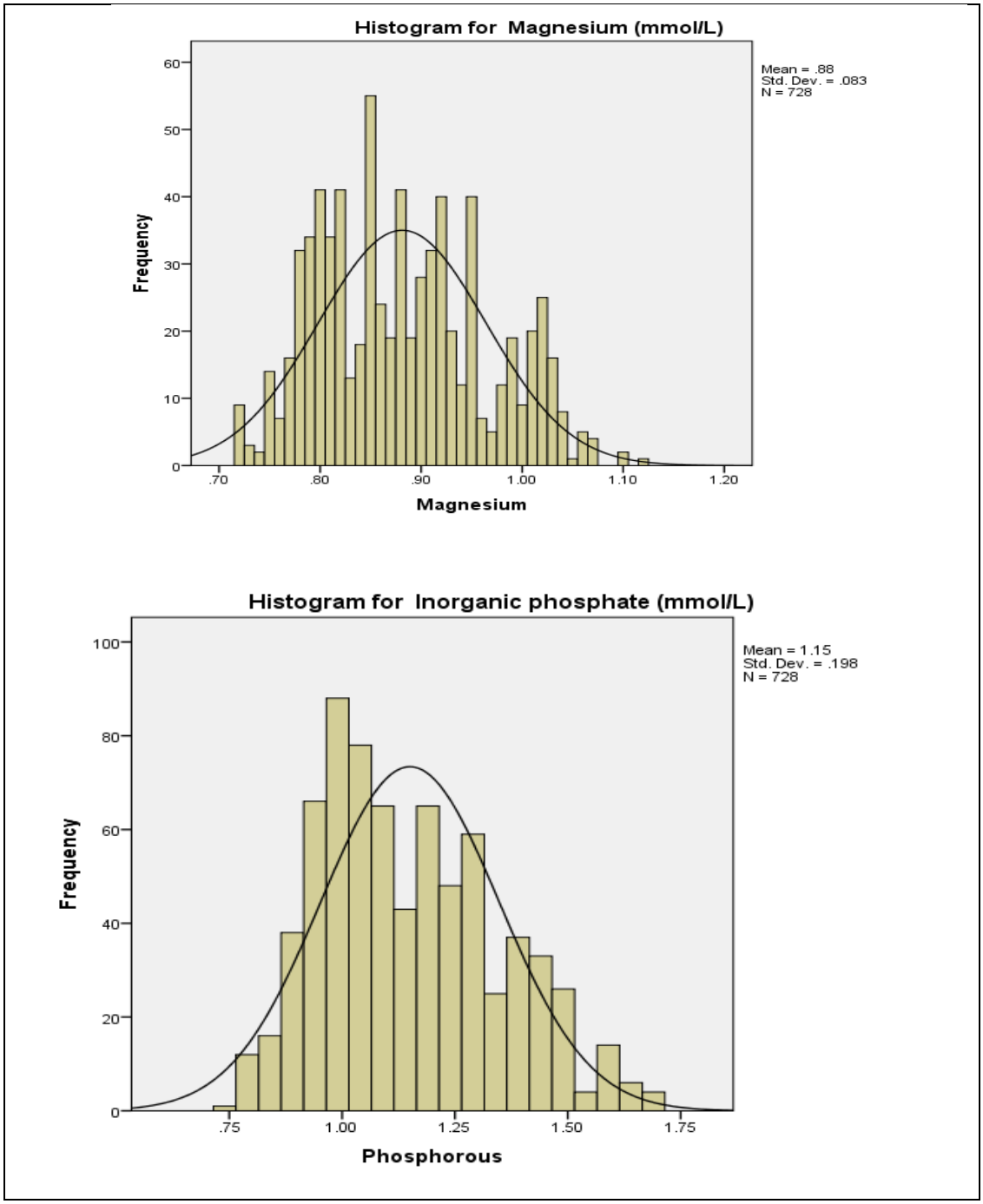


Figure 2.6: Frequency histograms of total Cholesterol and HDL.Cholesterol

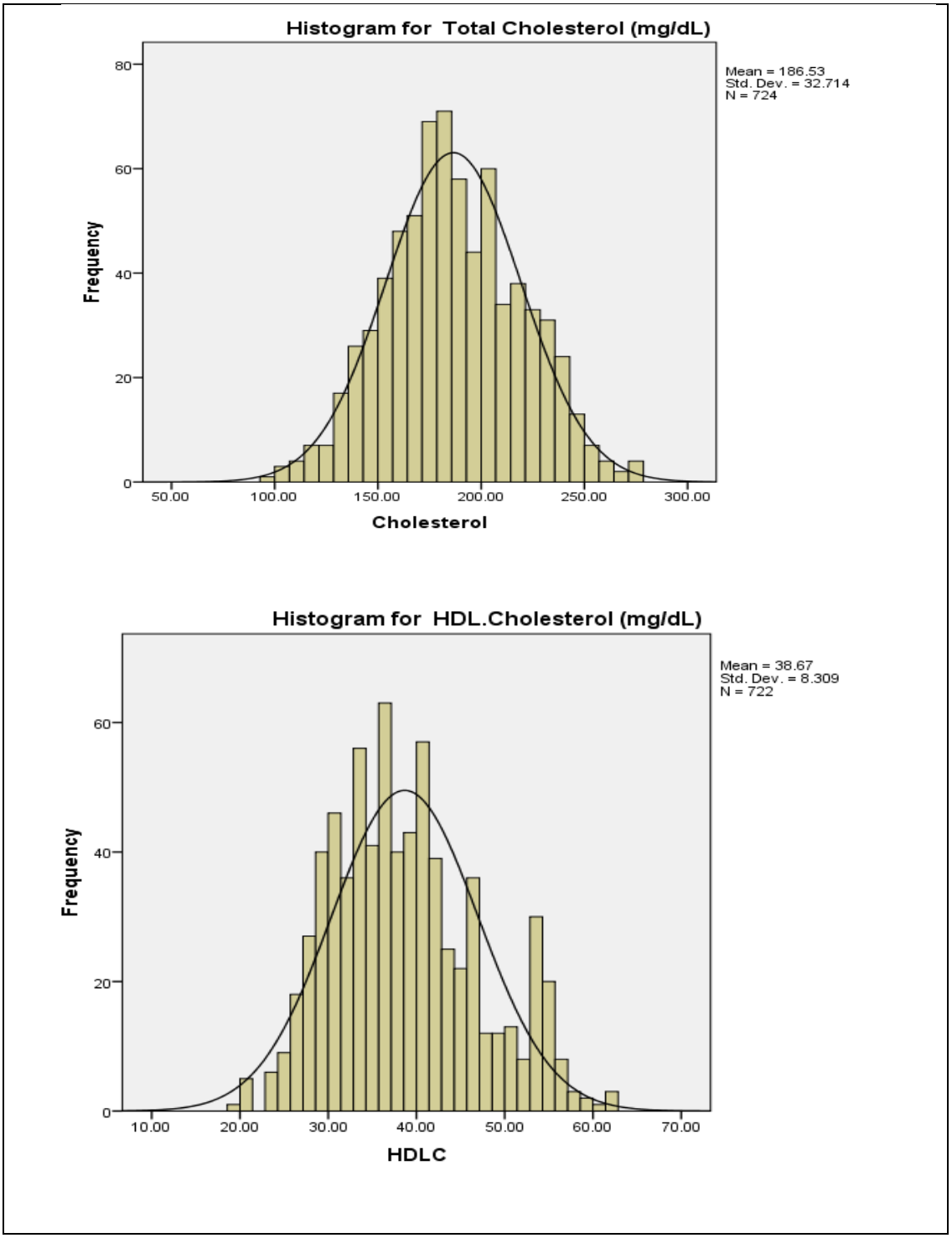


Figure 2.7: Frequency histograms of LDL.Cholesterol and Triglyceride

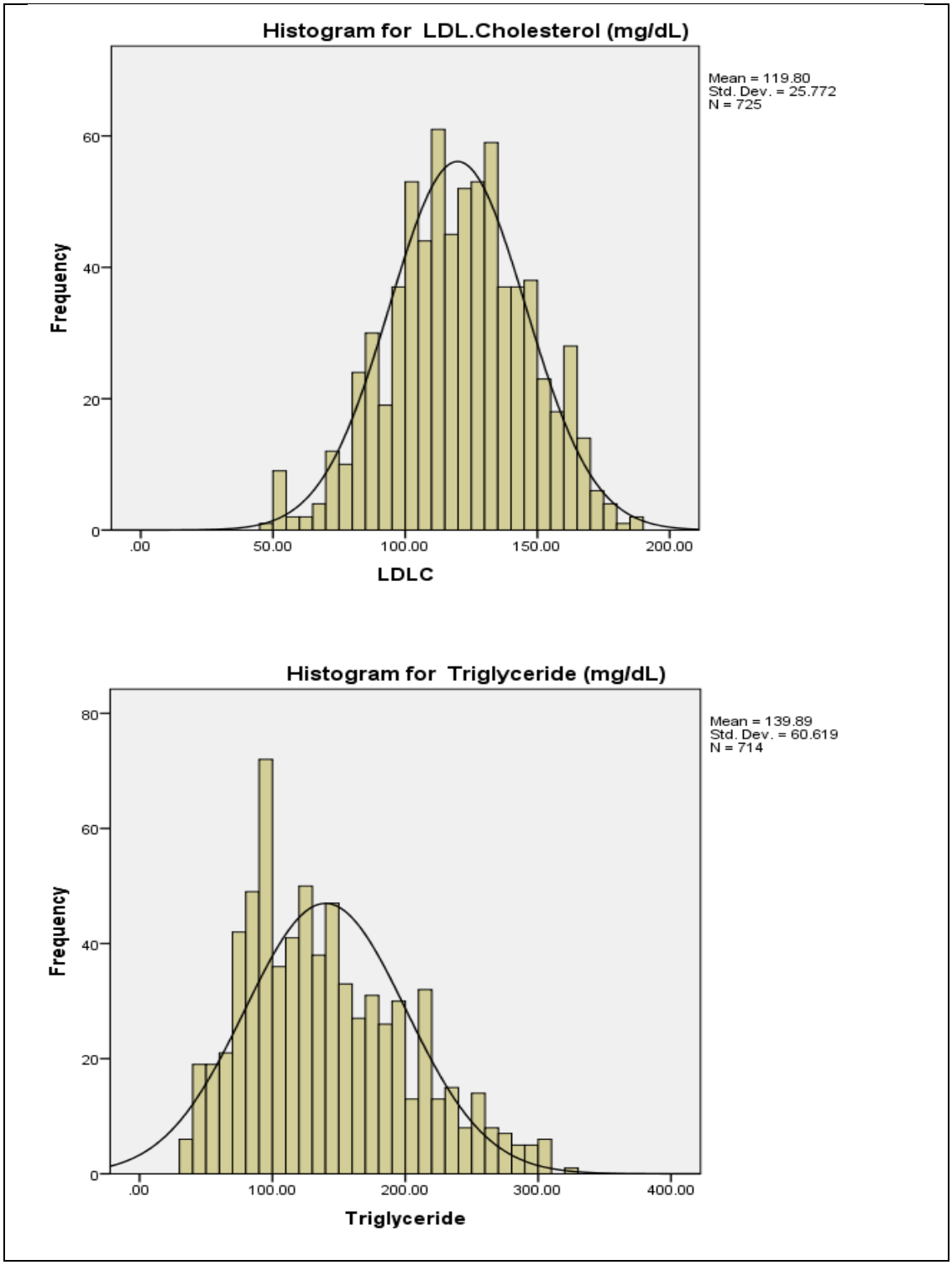


Figure 2.8: Frequency histograms of total Protein and Albumin

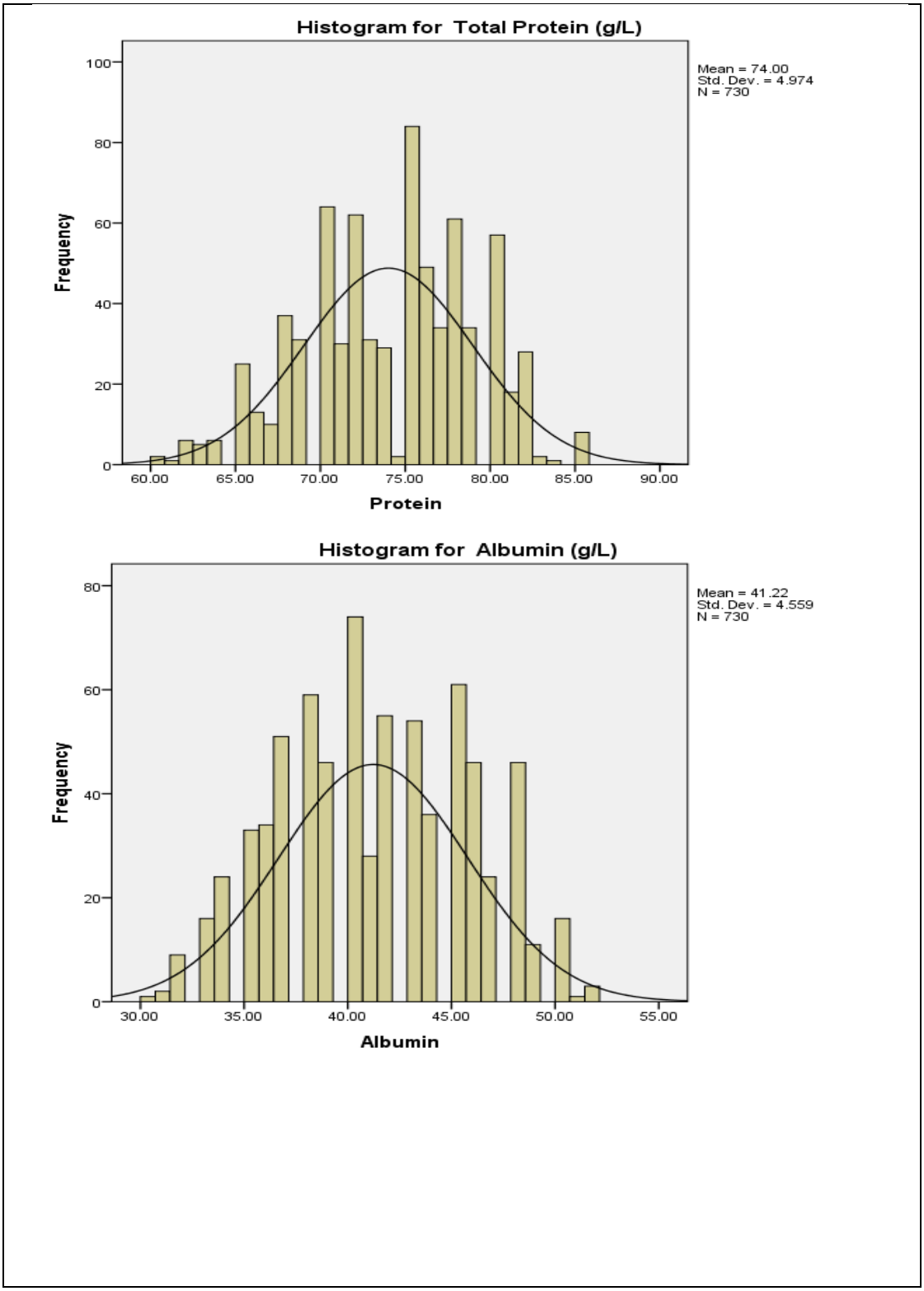


Figure 2.9: Frequency histograms of Bilirubin-Total and Alanine amino transferase

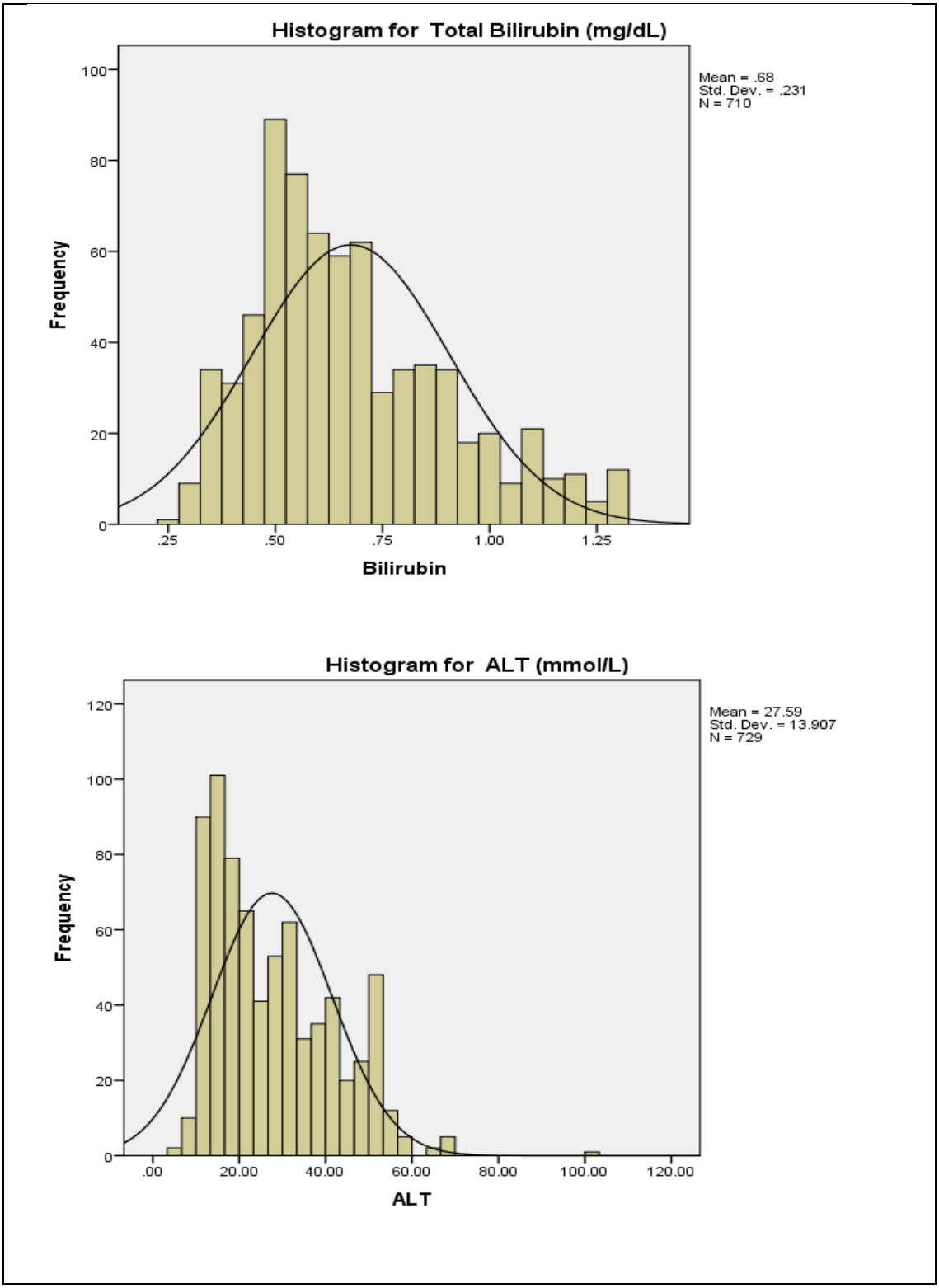


Figure 2.10: Frequency histograms of Aspartate amino transferaseand alkaline phosphatase

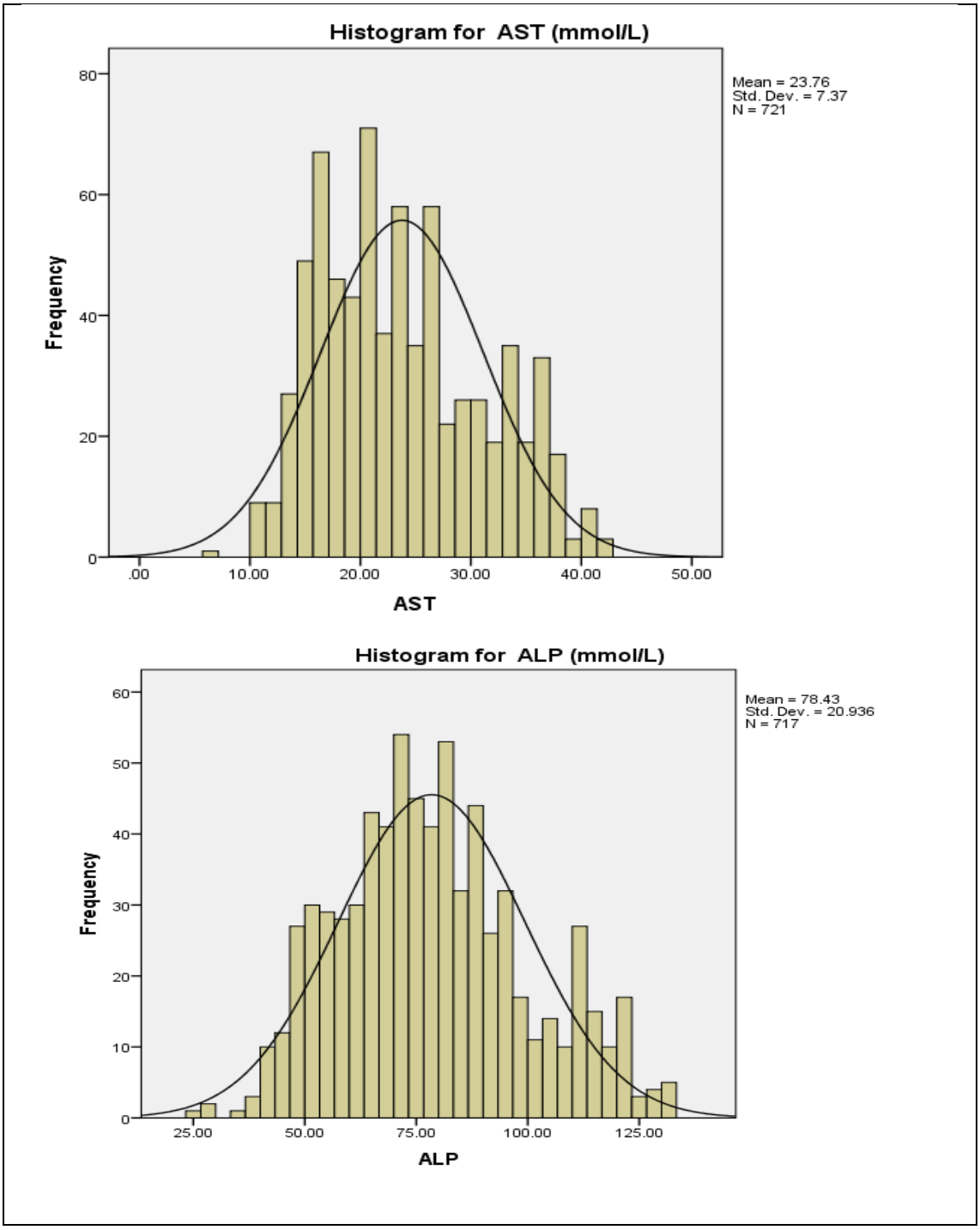


Figure 2.11: Frequency histograms of Red Blood Cell (RBC) and Hemoglobin

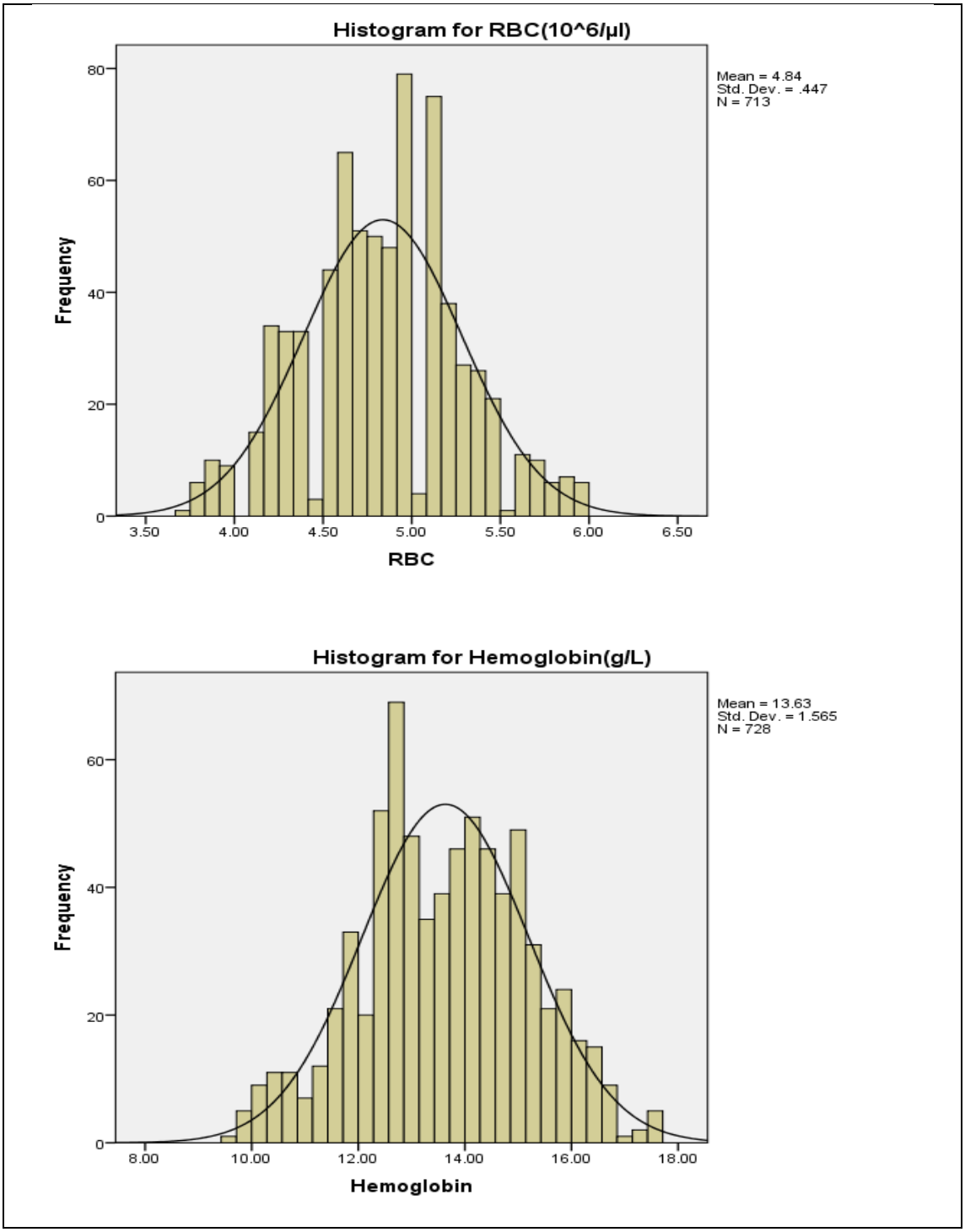


Figure 2.12 : Frequency histograms of Hematocrite and MCV

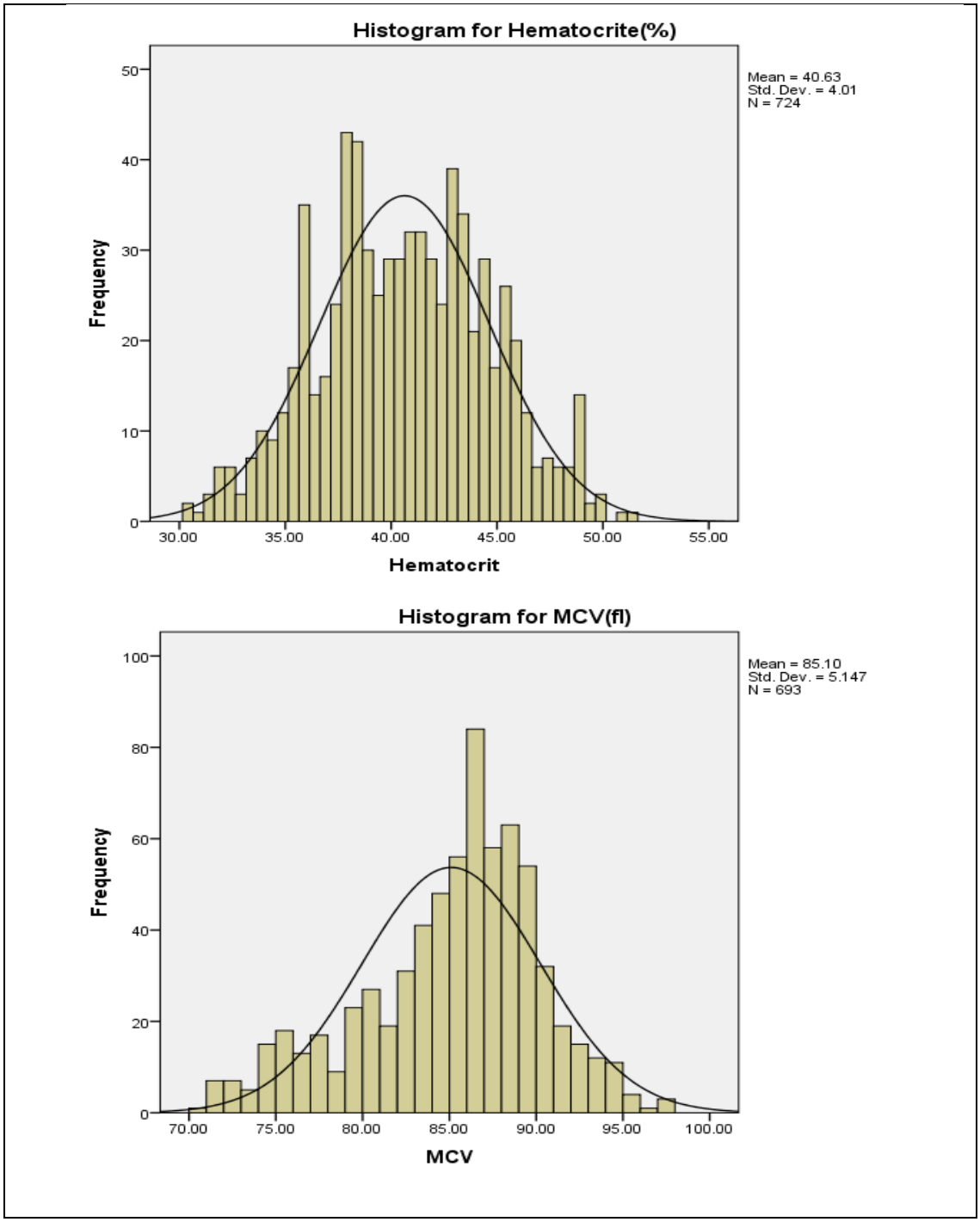


Figure2.13: Frequency histograms of MCH and MCHC

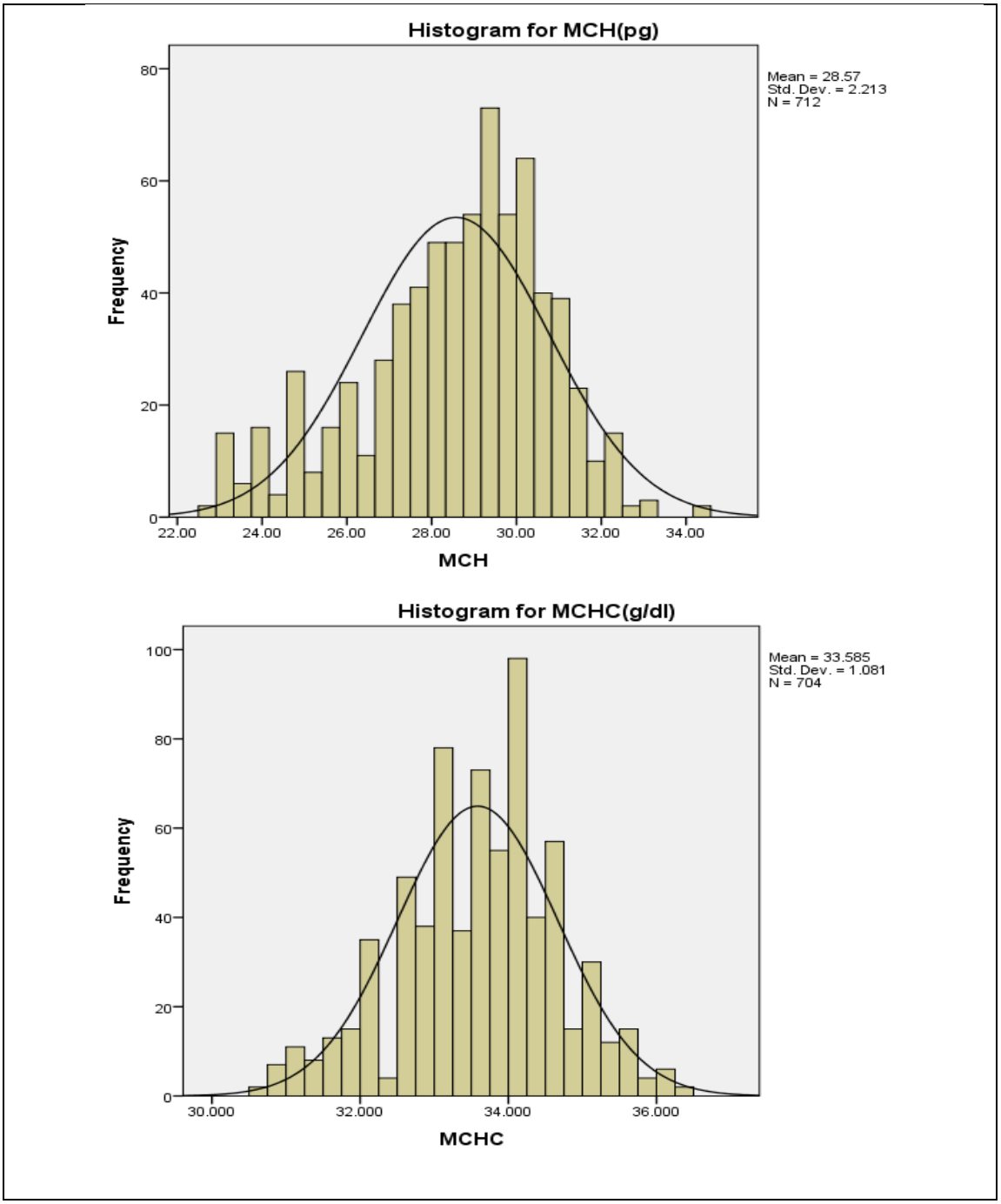


Figure 2.14: Frequency histograms of RDW-CV and Platelets (PLT)

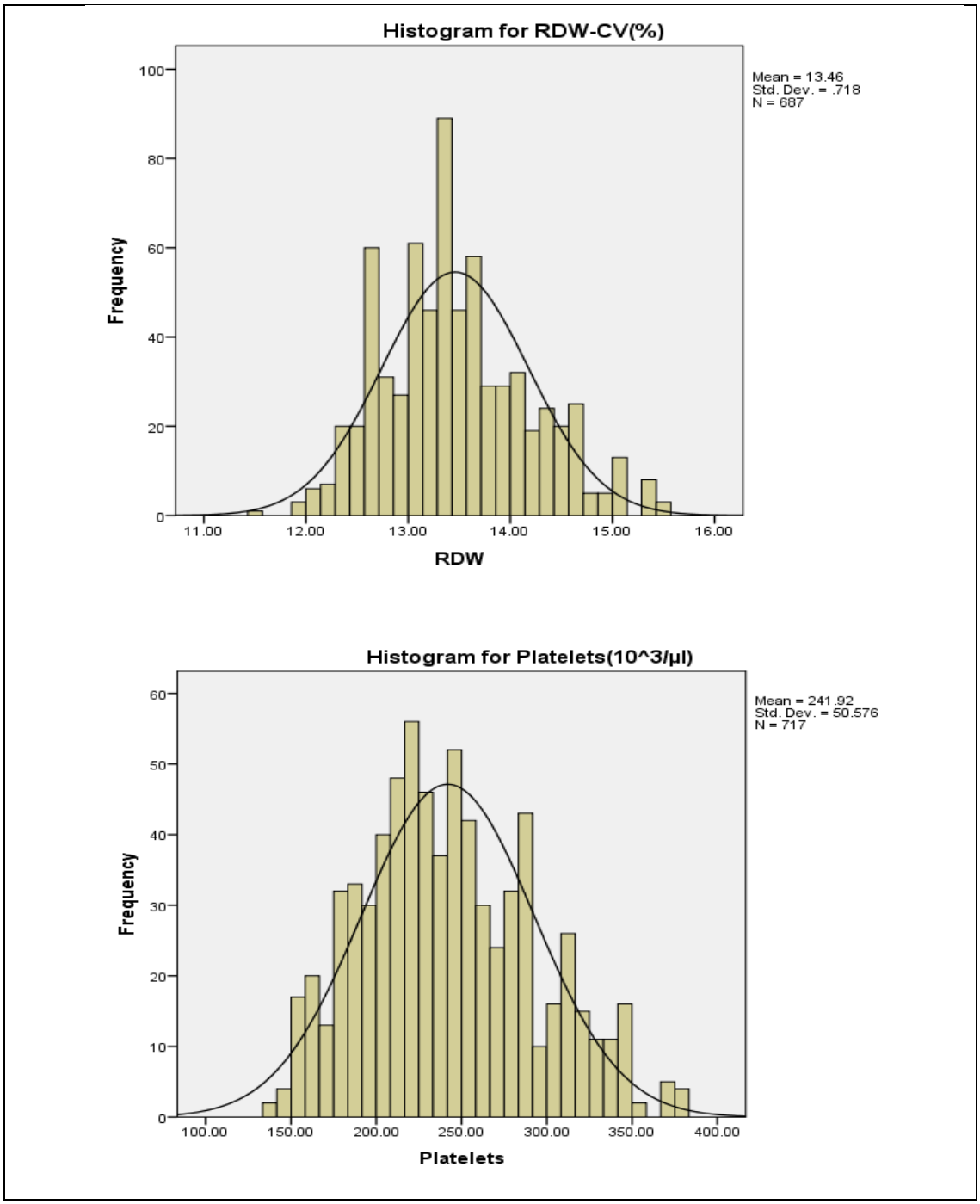


Figure 2.15: Frequency histograms of MPV and White blood cell (WBC)

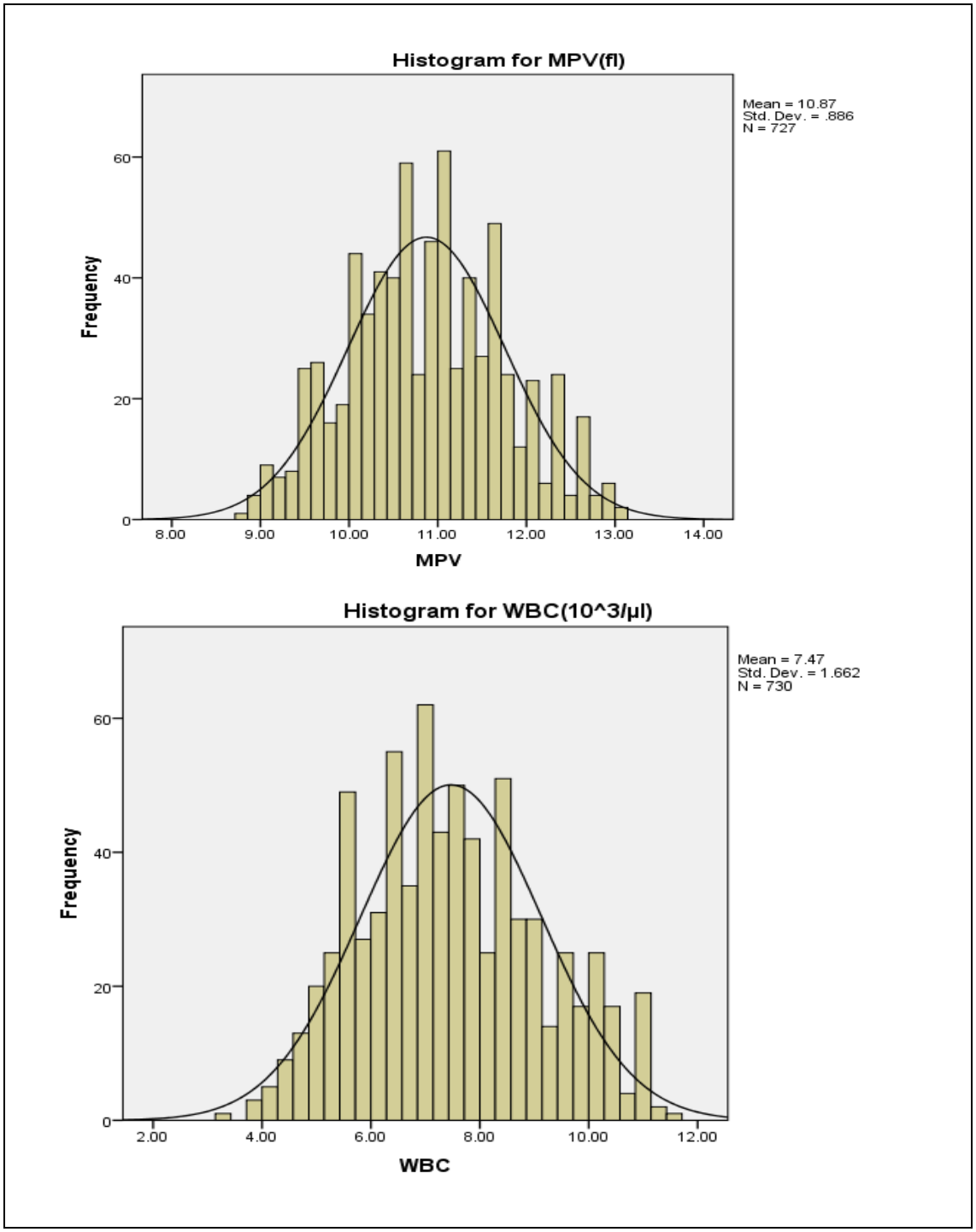


Figure 2.16: Frequency histograms of Neutrophil and Lymphocyte

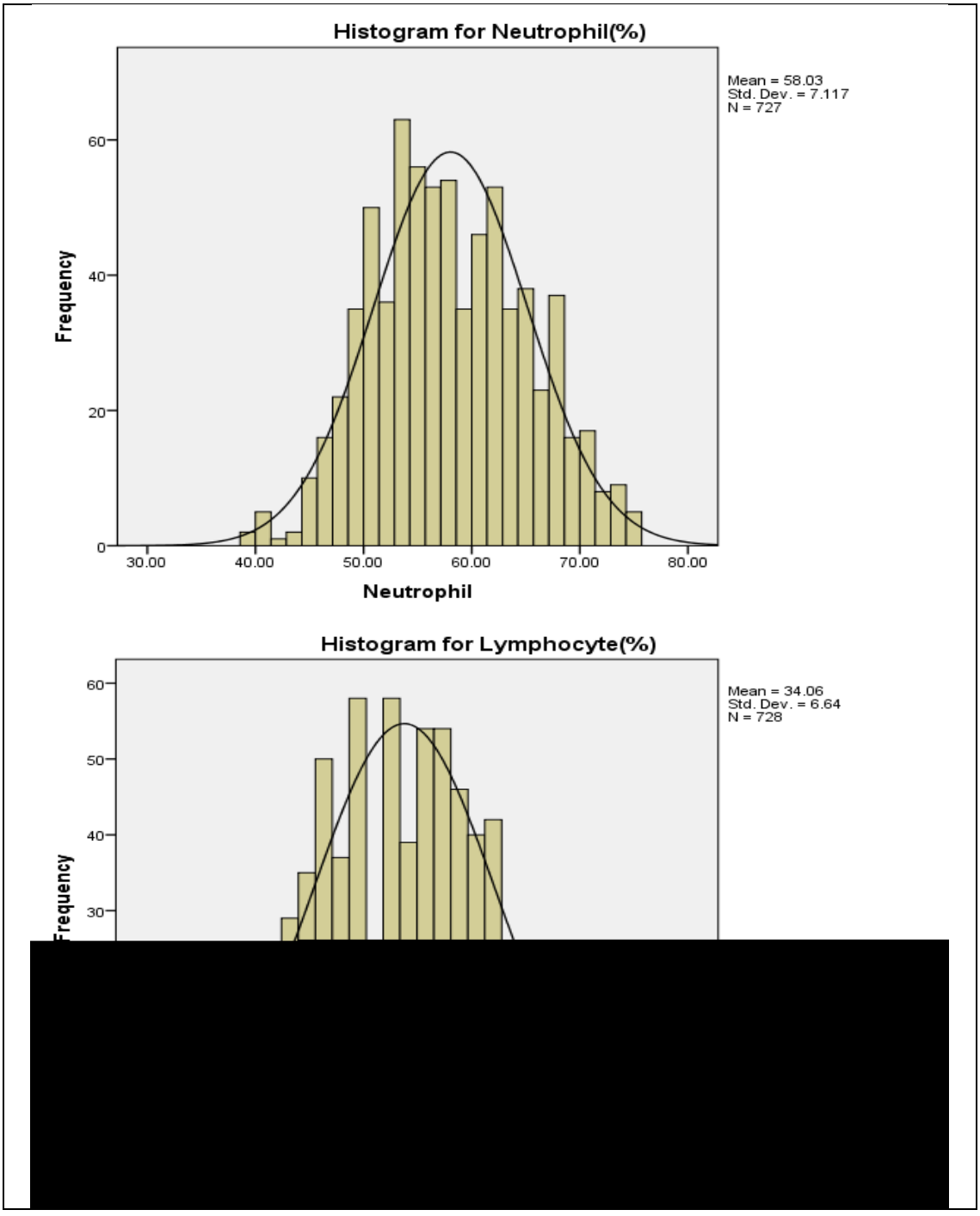


Figure 2.27: Normal Q-Q plot of Bilirubin Total and Alanine amino transferase (ALT)

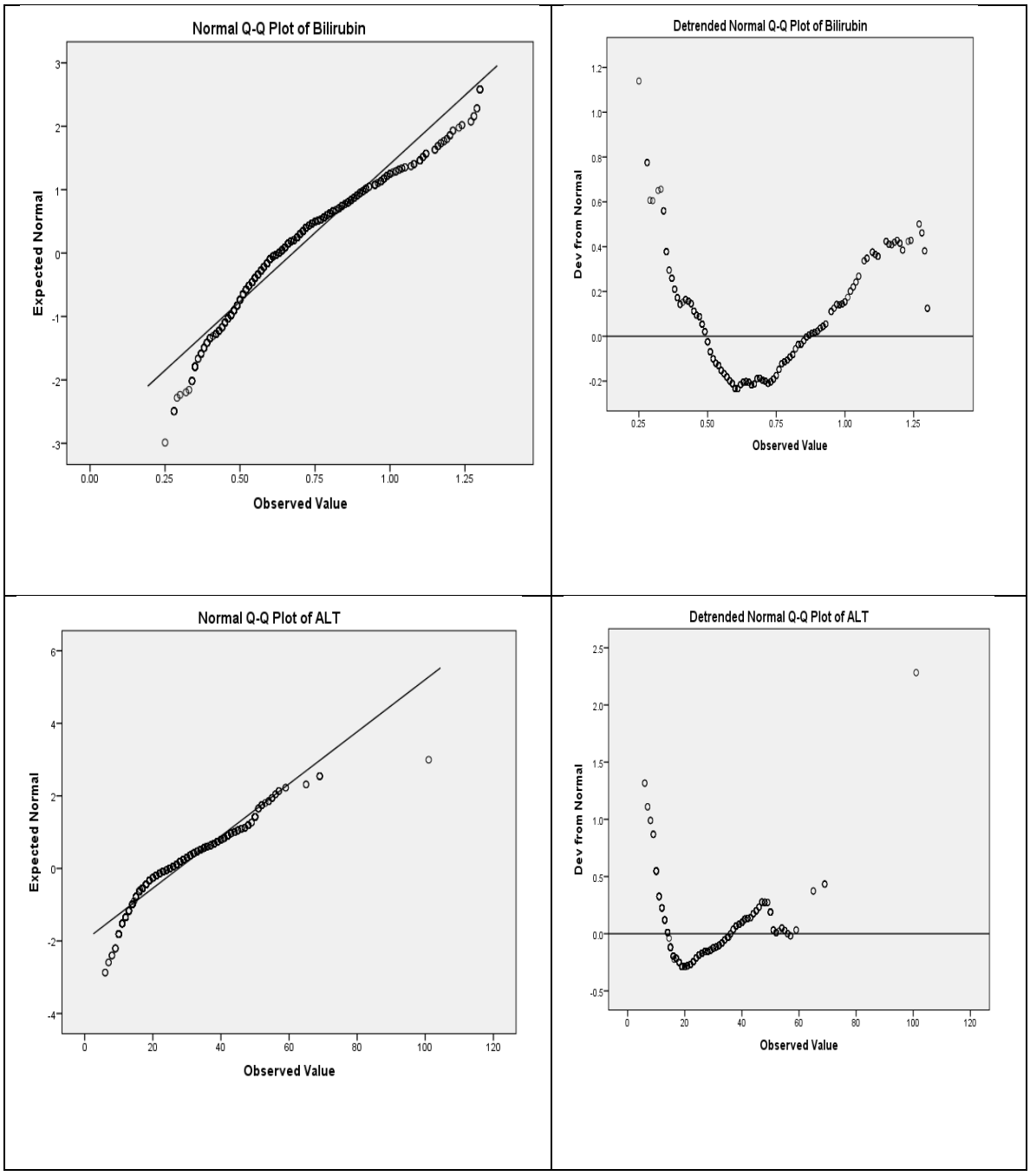


Figure 2.28: Normal Q-Q plot of Aspartate aminotransferase (AST) and Alkaline phosphatas (ALP)

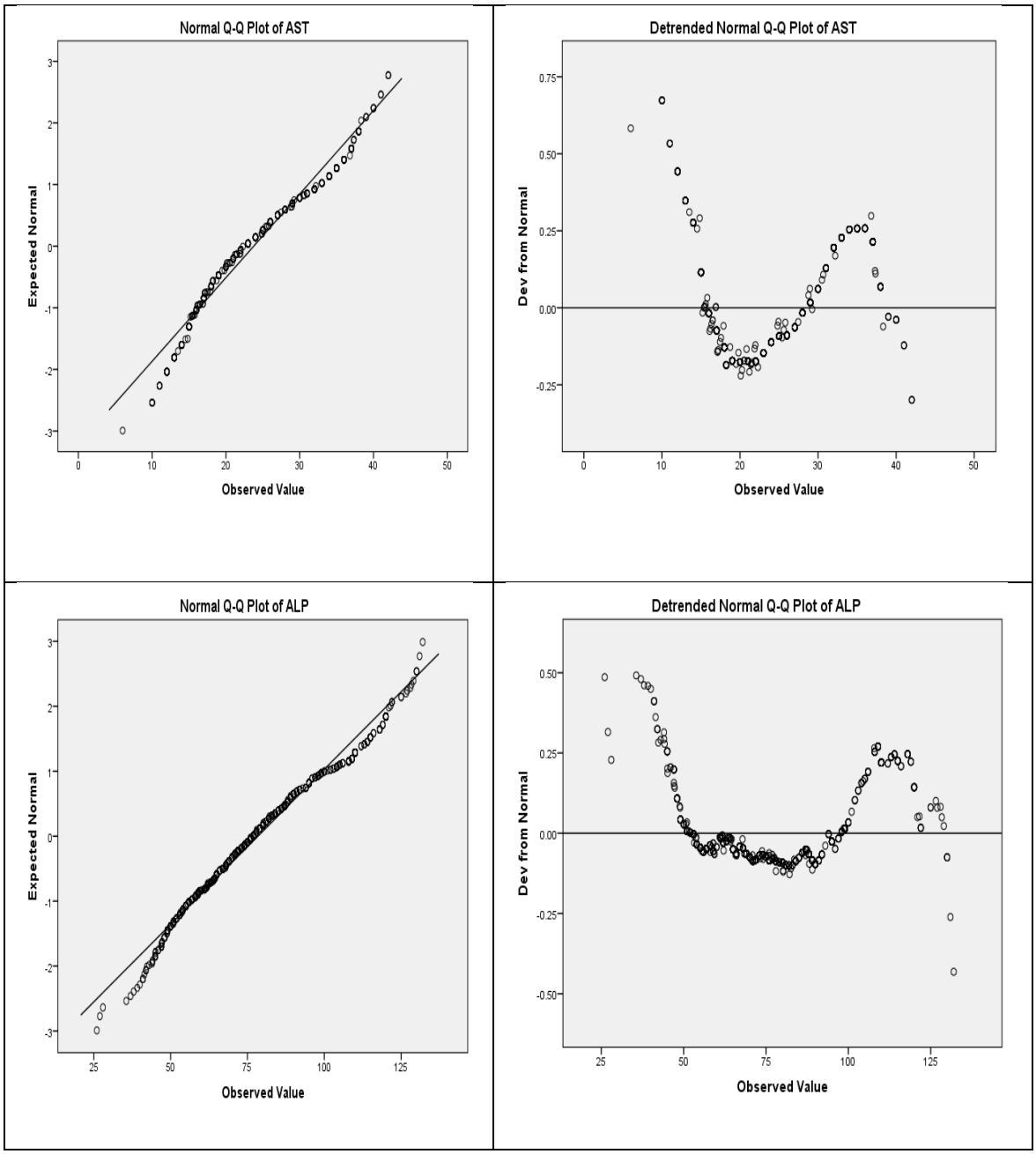


Figure 2.29: Normal Q-Q plot of Red blood cell (RBC) and Hemoglobin

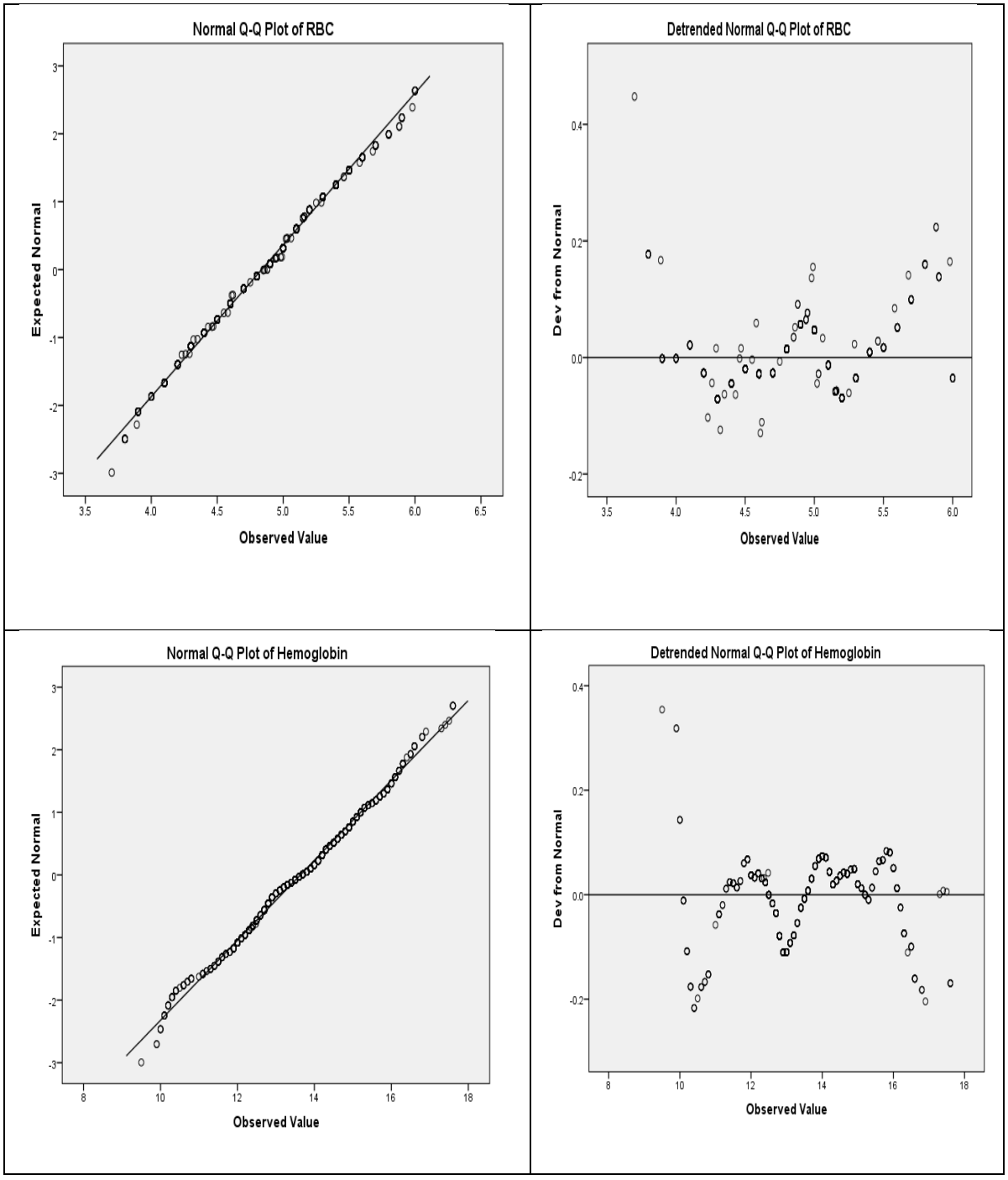


Figure 2.30: Normal Q-Q plot of Hematocrite (HCT) and MCV

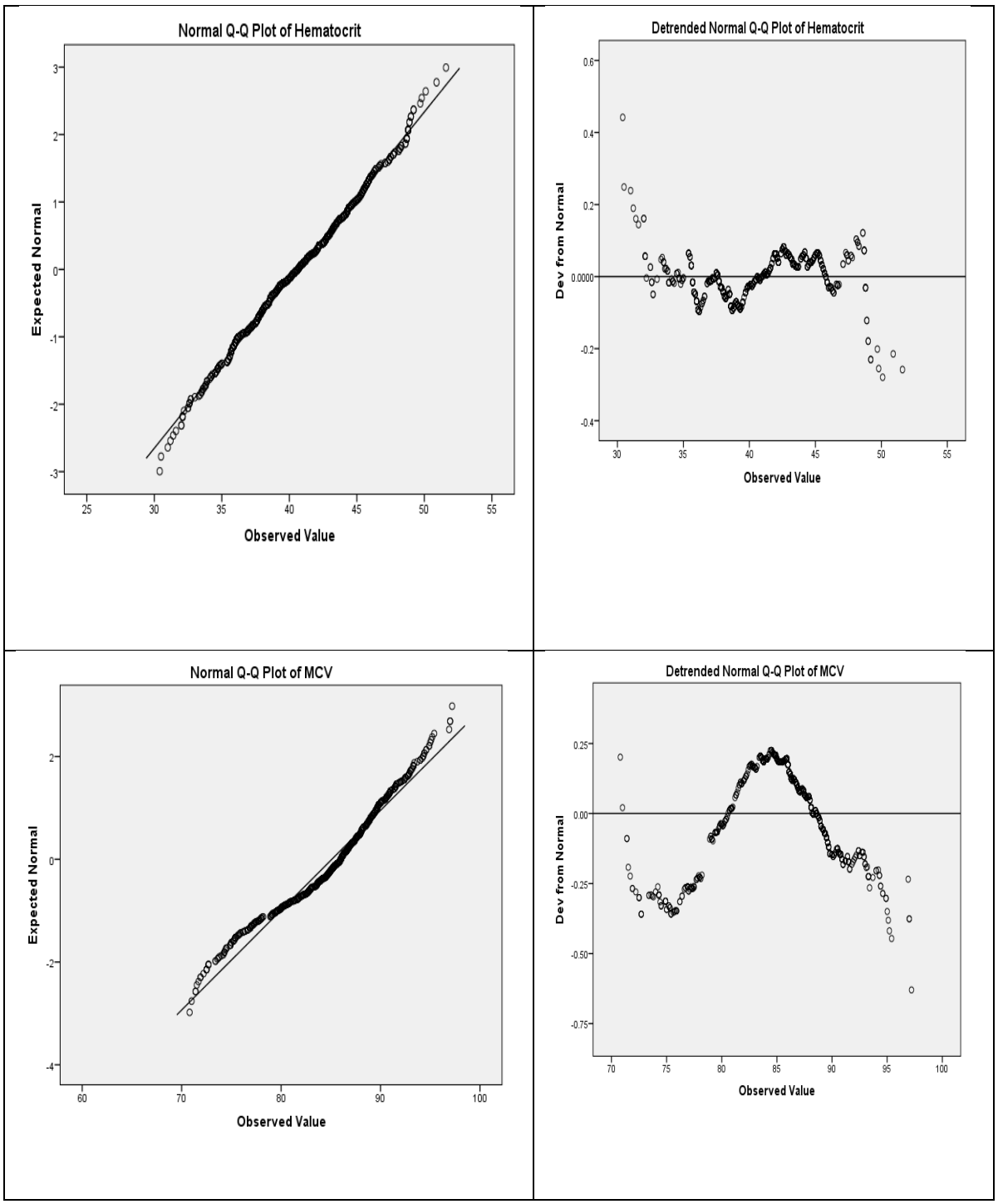


Figure2.31: Normal Q-Q plot of MCH and MCHC

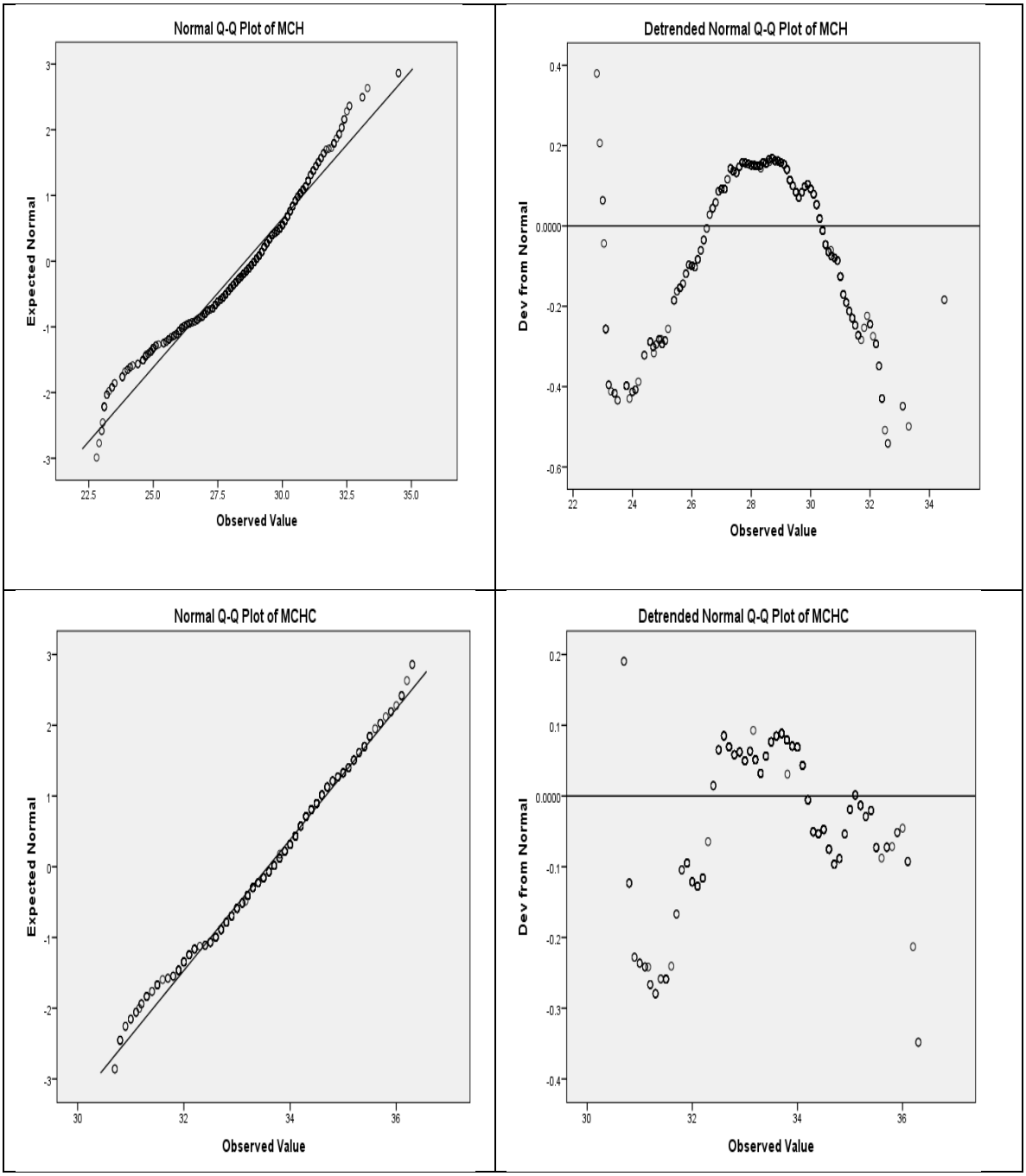


Figure2.32: Normal Q-Q plot of RDW-CV and Platelets

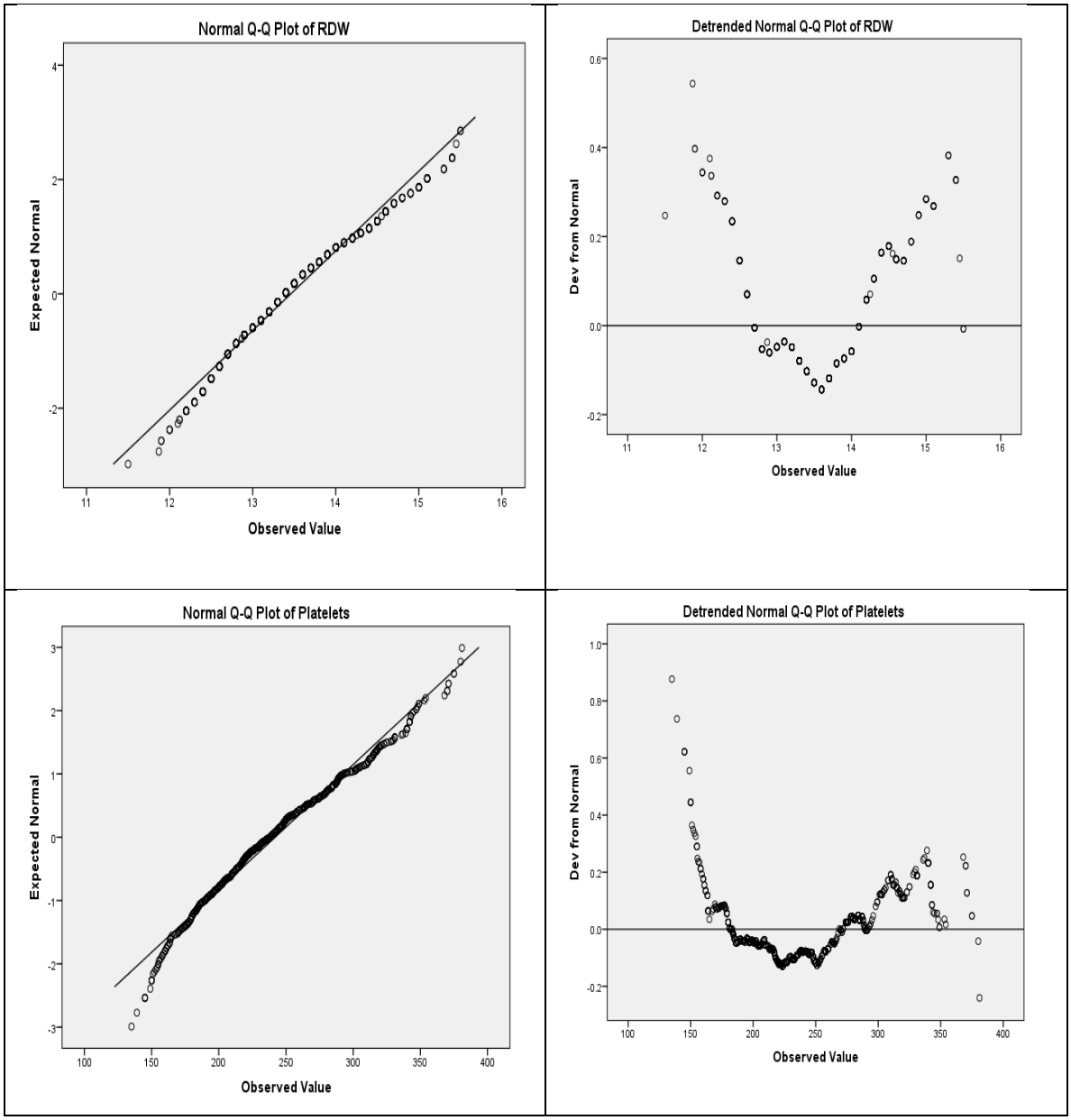


Figure2.33:Normal Q-Q plot of MPV and White Blood Cell (WBC)

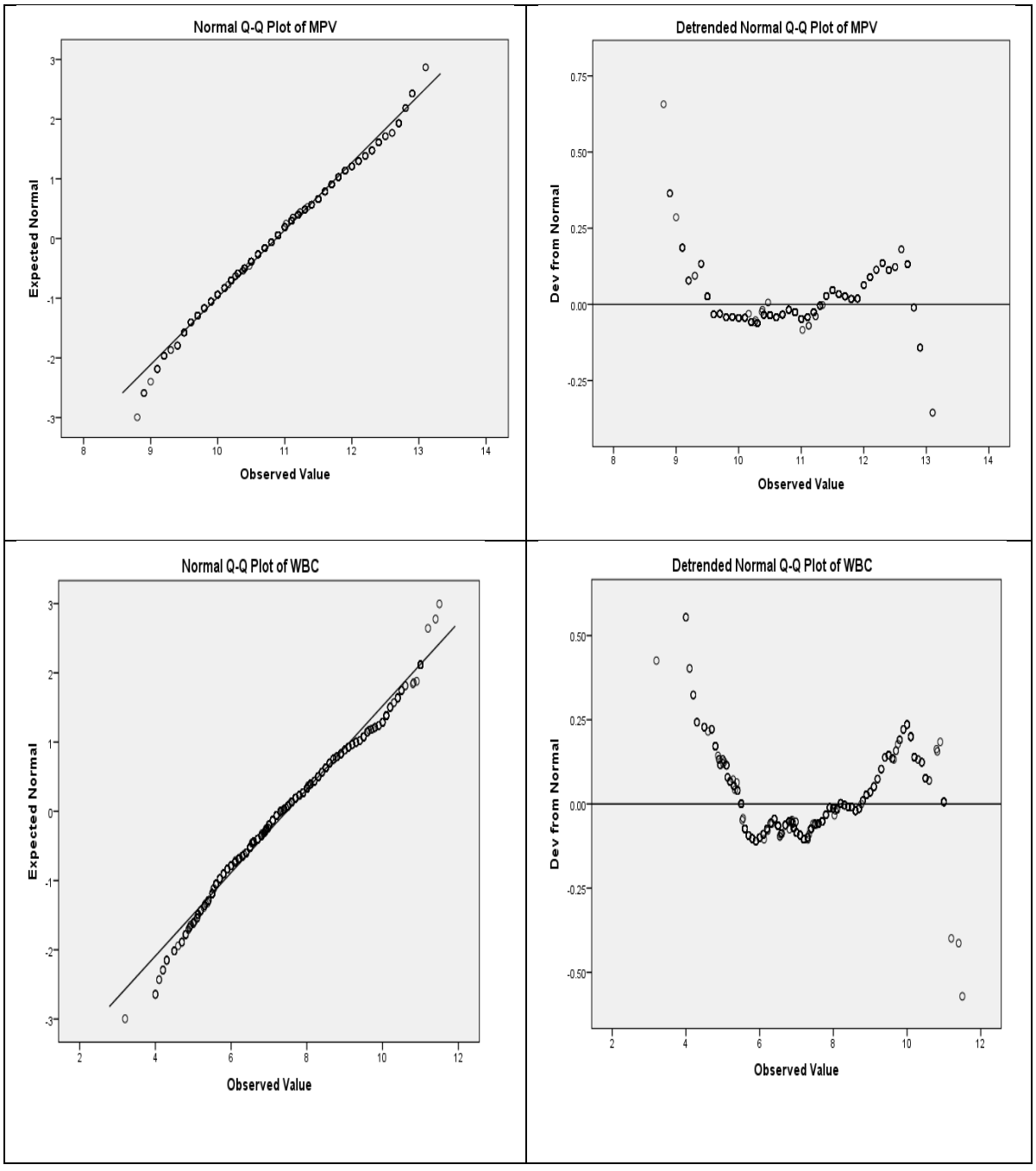


Figure2.34:Normal Q-Q plot of Neutrophil and Lymphocyte

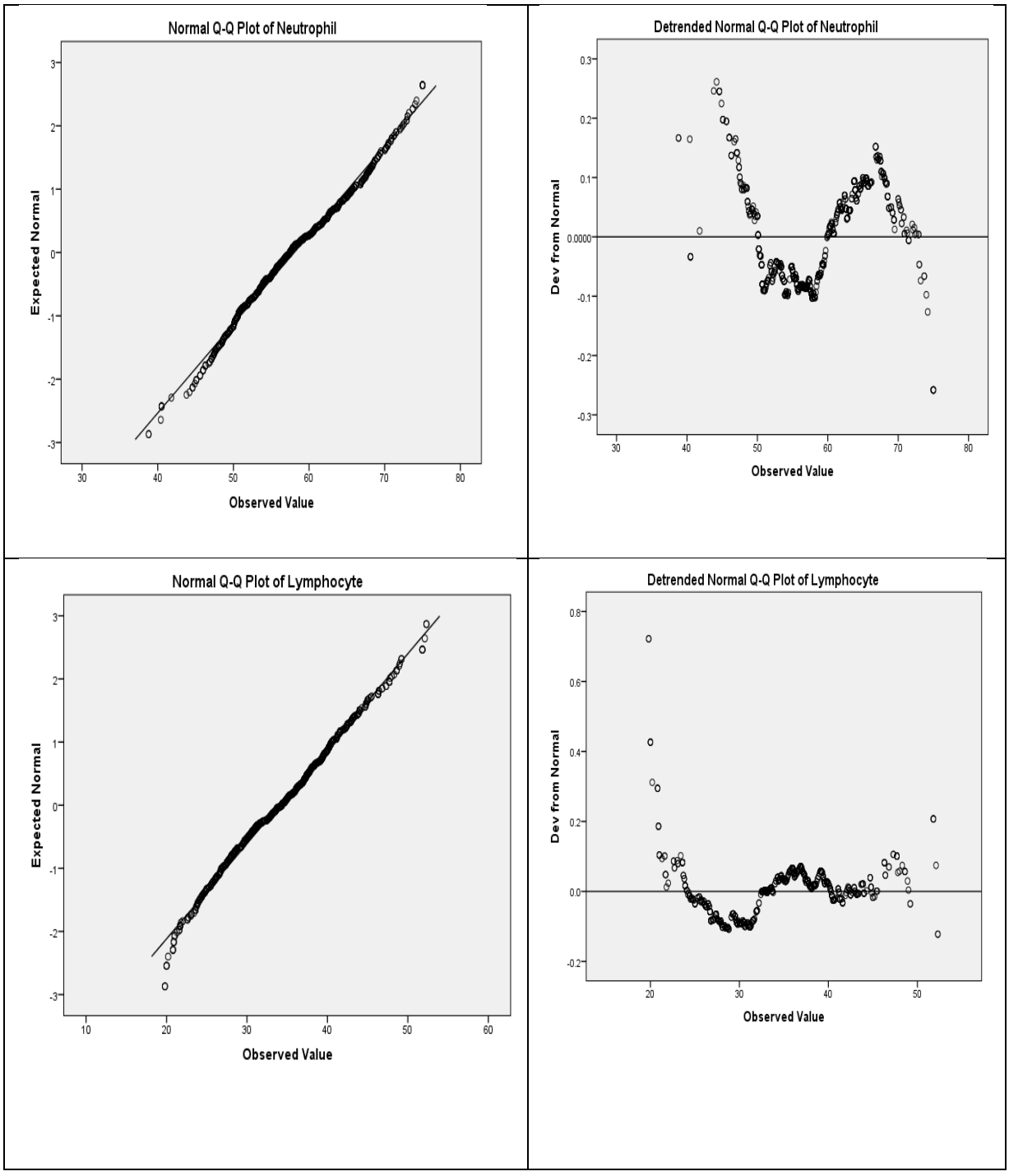


Figure2.35:Normal Q-Q plot of Monocyte and Eosinophil

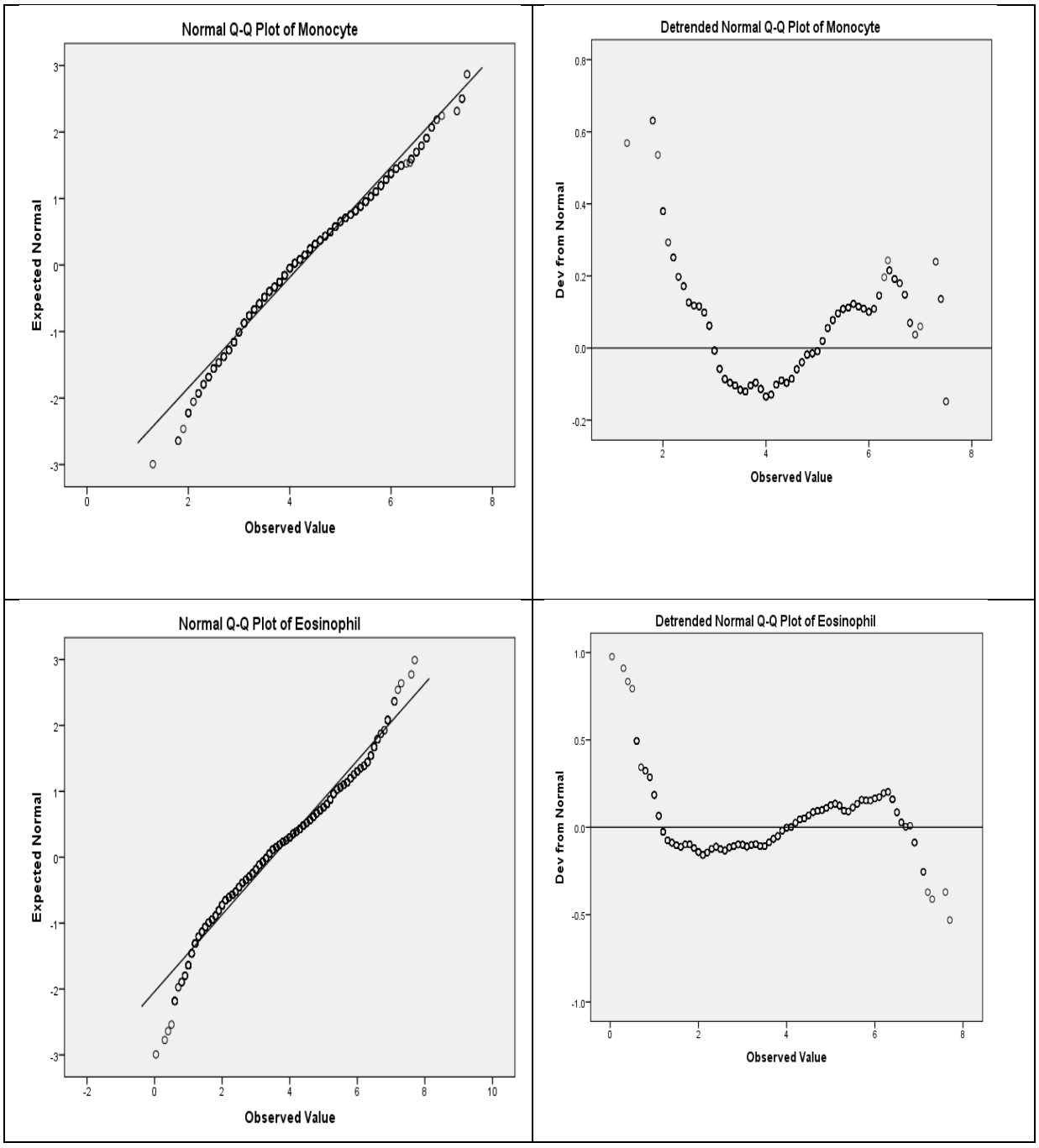
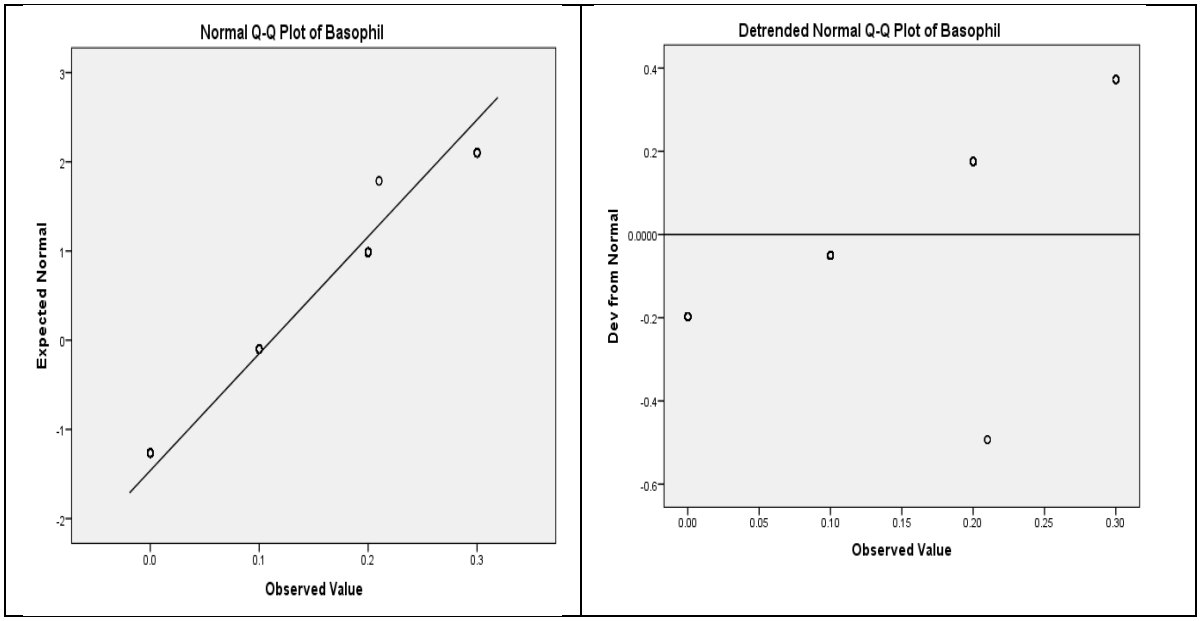


Figure2.36:Normal Q-Q plot of Basophile



2.9.4 Non Parametric method

The steps in a nonparametric procedure as follows

1. The n reference values were sorted in ascending order of magnitude and rank the values. The minimum value has rank number 1, the next value number 2, and so on until the maximum value, rank n, is reached. Consecutive rank numbers were given to two or more values that are equal ("ties"). The sorting and ranking were done with spreadsheet software such as EXCEL.

2. The rank numbers of the 2.5 and 97.5 percentiles were compute as 0.025(n + 1) and 0.975(n +1), respectively3. The percentiles by finding the original reference values were determined that correspond to the computed rank numbers, provided that the rank numbers are integers. Otherwise, interpolation between the two limiting values is necessary.

4. Finally, the confidence intervals of each percentile were determined through use of the binomial distribution. Nonparametric confidence intervals of reference

limits table 4.6 facilitate this step for the 0.90 confidence interval of 2.5 and 97.5 percentiles. The bounding rank numbers for each percentile located in the table.

2.9.5 Partitioning of reference ranges/intervals

The Mann-Whitney U test (also called the Wilcoxon-Mann-Whitney test) is a rank-based nonparametric test that can be used to determine if there are differences between two groups on a continuous or ordinal dependent variable. This test is often presented as the nonparametric alternative to the independent-samples t-test, which can be used when your data fail the assumptions of the independent-samples t-test.P-values for the difference between two sex groups male and female participants as well as between two age groups less than 40 and more than 40 years old subjects were estimated using the Mann-Whitney test where $p<0.05$ were considered significantly different.

Separate reference intervals were calculated for subclass age and sex. Sex based partitioning suggested statistically significant difference in reference values between males and females for most analytes. In order to make the partitioning clinically meaningful, we decided to report separate reference intervals for males and females only if the lower and upper reference limits fell outside the 90 % confidence intervals of the two groups. Otherwise single reference interval for both males and females was calculated.

For age based partitioning, the data was broadly divided into two age groups (based on less than forty years and more than 40 years). Significance between two groups were carried out by p value with 90% confidence intervals values and clinical consideration.

Table- 2.6 Nonparametric Confidence Intervals of Reference Limits

Sample Size	Rank Number		Sample Size	Rank Number	
	Lower	Upper		Lower	Upper
119-132	1	7	566-574	8	22
133- 160	1	8	575-598	9	22
161-187	1	9	599-624	9	23
188-189	2	9	625-631	10	23
190-218	2	10	632-665	10	24
219-248	2	11	666-674	10	25
249-249	2	12	675-698	11	25
250-279	3	12	699-724	11	26
280-307	3	13	725-732	12	26
308-309	4	13	733-765	12	27
310-340	4	14	766-773	12	28
341-363	4	15	774-799	13	28
364-372	5	15	800-822	13	29
373-403	5	16	823-833	14	29
404-417	5	17	834-867	14	30
418-435	6	17	868-871	14	31
436-468	6	18	872-901	15	31
469-470	6	19	902-919	15	32
471-500	7	19	920-935	16	32
501-522	7	20	936-967	16	33
523-533	8	20	968-970	17	33
534-565	8	21	971-1000	17	34

RESULTS

3.1 Age and sex distribution

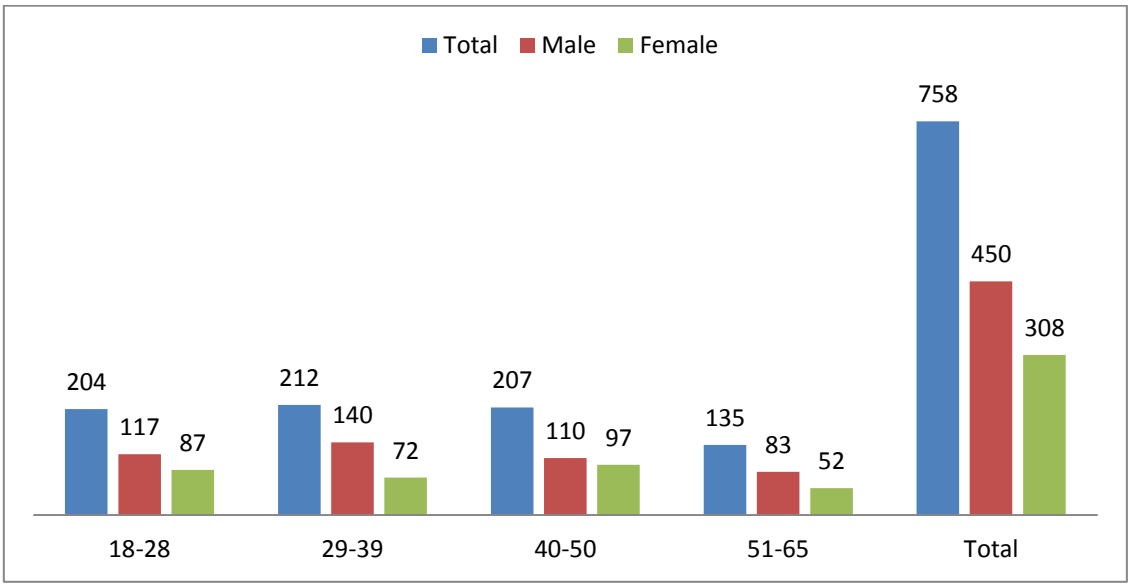
758 participant recruited for the study, among them 730 subjects were involved in the study, 436(59.726%) males and 294(40.274%) females were included .Twenty eight participant were excluded, among them six HbsAg positive, one HCV positive, eight lipeamic, four icteric and nine were hemolyzed.

Table showed the distribution of age and sex of the studied population

Table 3.1Sex and age distribution of recruited participants

SN	Age group	Total	Male	%	Female	%
1	18 to 28	204	117	15.435	87	11.477
2	29 to 39	212	140	18.469	72	9.498
3	40 to 50	207	110	14.511	97	12.796
4	50 to 65	135	83	10.949	52	6.860
5	Total	758	450	59.366	308	40.633

Figure 3.1Sex and age distribution of recruited participants

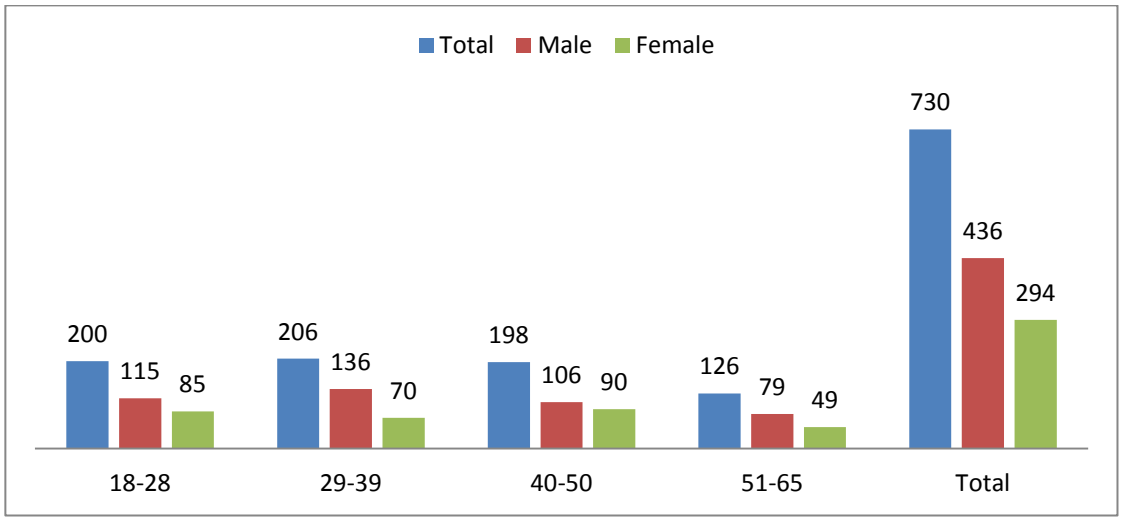


After selection procedure the following distribution was done according to age and sex in this study

Table 3.2Sex and age distribution of selected participants

SN	Age group	Total	Male	%	Female	%
1	18 to 28	200	115	15.753	85	11.643
2	29 to 39	206	136	18.630	70	9.589
3	40 to 50	198	106	14.520	90	12.328
4	50 to 65	126	79	10.821	49	6.712
5	Total	730	436	59.726	294	40.274

Figure 3.2 Sex and age distribution of selected participants

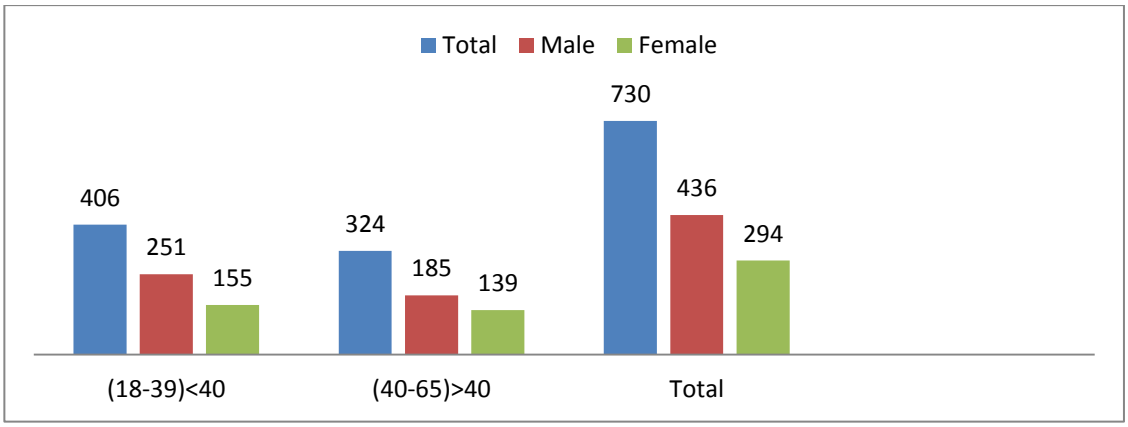


To select participants for establishing reference intervals in between18-28 age group.200 participants were in 8-28age group, where as155 (15.75) subjects were males and 85(11.64%) females. In 29-39 age group total participants were 206. 136(18.63%) subjects were males and 70(9.59%) females. In 40-50 age group total participants were 198. 106(14.52%) subjects were male and 90(12.33%) female. As well as in 50-65 age group total participants were 126, however 79(10.82%) participants were males and 49(6.71%) females.

Table 3.3Sex and age distribution of participants to construct reference intervals

SN	Age group	Total	Male	%	Female	%
1	(18-39)<40	406	251	61.822	155	38.177
2	(40-65)>40	324	185	57.098	139	42.901
3	Total	730	436	59.726	294	40.274

Figure 3.3 Sex and age distribution of participant to construct reference intervals



To construct reference intervals total no of males 436(59.726%) and females 294(40) participants were involved in the study. To set up age specific reference intervals of analytes, the data was broadly divided into two age groups (based on less than forty years and more than forty years) where as406 (55.616%) data for less than 40 and 325(44.52%) data for more than 40 years old participant.

For less than forty years age group 251(61.822%) subjects were males and 155(38.177%) females but more than forty years age group 185(57.098%) subjects were males and 139(42.901%) females.

Tables 3.4 and 3.5 showed the significance of the differences in the samples values between males and females as well as between age groups of less than 40 and more than 40 years respectively where p-values less than 0.05 were considered statistically significant.

Table 3.4 Significance of the difference between samples values for the sex males and females subjects, p value as per Mann-Whitney test .

Tests	P- value (Mann-Whitney)
Glucose	0.040
Creatinine	0.000
Urea	0.000
Uric Acid	0.000
Sodium	0.001
Potassium	0.532
Chloride	0.000
Total Calcium	0.001
Magnesium	0.828
Inorganic Phosphorous	0.823
Cholesterol	0.044
HDL Cholesterol	0.000
LDL.Cholesterol	0.000
Triglyceride	0.000
Total Protein	0.673
Albumin	0.005
Bilirubin-Total	0.000
Alanine amino Transferase	0.000
Aspartate amino Transferase	0.000
Alkaline phosphates	0.000
Red Blood Cell (RBC)	0.000
Hemoglobin	0.000
Hematocrit (HCT)	0.000
Mean Corpuscular Volume(MCV)	0.015
Mean Corpuscular Hemoglobin(MCH)	0.000
Mean Corpuscular Hemoglobin Concentration(MCHC)	0.000
RDW-CV	0.001
Platelets (PLT)	0.000
Mean Platelets Volume(MPV)	0.620
White Blood cell(WBC)	0.819
Neutrophil	0.000
Lymphocyte	0.003
Monocyte	0.000
Eosinophil	0.000
Basophil	0.002

Table3.5 Significance of the difference between samples values for the age group less than 40 and more than 40 years, p value as per Mann-Whitney test

Tests	P- value (Mann-Whitney)
Glucose	0.000
Creatinine	0.000
Urea	0.000
Uric Acid	0.000
Sodium	0.052
Potassium	0.000
Chloride	0.005
Total Calcium	0.013
Magnesium	0.000
Inorganic Phosphorous	0.001
Cholesterol	0.000
HDL Cholesterol	0.000
LDL.Cholesterol	0.001
Triglyceride	0.000
Total Protein	0.401
Albumin	0.000
Bilirubin-Total	0.000
Alanine amino Transferase	0.001
Aspartate amino Transferase	0.000
Alkaline phosphates	0.000
Red Blood Cell (RBC)	0.000
Hemoglobin	0.000
Hematocrit (HCT)	0.000
Mean Corpuscular Volume(MCV)	0.982
Mean Corpuscular Hemoglobin(MCH)	0.264
Mean Corpuscular Hemoglobin Concentration(MCHC)	0.000
RDW-CV	0.247
Platelets (PLT)	0.377
Mean Platelets Volume(MPV)	0.001
White Blood cell(WBC)	0.078
Neutrophil	0.000
Lymphocyte	0.000
Monocyte	0.835
Eosinophil	0.942
Basophil	0.749

3.2 Reference Intervals for Biochemical Analytes

The reference values were constructed using 2.5th and 97.5th percentiles as lower and upper limits at 95%confidence interval in accordance with CLSI (NCCLS, 2000) guideline for determining reference intervals. The medians for males and females were statistically compared using Mann-Whitney test. $p<0.05$ was considered statistically different. Difference is not significant if $p<0.05$ but reference value fell inside their 90% confidence interval of lower and upper limits.

The following tables (from 3.6 to 3.26) showed mean, median, standard deviation and 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals, p value of the biochemical analytes for combined males and females), age less than 40 and age more than 40 years respectively .Participants used for determining the reference values for each biochemistry parameter which were all above the minimum sample size (N = 120) suggested by CLSI.

The biochemical tests results for Glucose Creatinine, Urea, Uric acid, Sodium, chloride, calcium, Cholesterol, HDL Cholesterol, LDL. Cholesterol, Triglyceride, Albumin, Total Bilirubin, Alanine amino Transferase, Aspartate amino Transferase and Alkaline phosphatase showed statistically significant sex differences ($p<0.05$) as well as potassium, magnesium, inorganic phosphorus and total protein statistically not significant sex differences($P>0.05$) in Tables 3.4 and Table 3.5.

But Considering both statistical ($P<0.05$) and 90% Confidence interval limits are shown in Table 3.7, table 3.8 , table 3.10table 3.17, table 3.19 , table 3.22table 3.23, table 3.24 and table 3.25.Creatinine, Urea, Uric acid, Sodium HDL. Cholesterol, Triglyceride, Total Bilirubin, Alanine amino Transferase, Aspartate

amino Transferase and Alkaline phosphatase levels were significant for sex difference.

From biochemical tests Glucose, chloride, calcium Cholesterol, LDL. Cholesterol and albumin level were statistically significant difference between the males and females intervals. Considering 90% Confidence interval limits are summarized in table 3.6, table 3.12, table 3.13,table 3.16, table 3.18 and table 3.21,reference intervals of these parameters were not significant for sex difference.

3.1.1Reference Intervals for Glucose

Table 3.6 showed presented SD were 0.555, 0.56, 0.54 and mean were 5.17, 5.20, 5.12 as well as median were 5.2, 5.26, 5.1 for combined(M&F), males and females subject respectively.

Table 3.6: 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for fasting glucose.

Analyte: Glucose, Unit: mmol/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	730	5.2	5.17	0.555	4.0 to 6.1	3.87- 4.12	6.0 -6.12	
Males	436	5.26	5.20	0.56	4.01 to 6.1	3.87 - 4.24	6.0- 6.2	0.040
Females	294	5.1	5.12	0.54	3.99 to 6.1	3.79 - 4.20	6.0- 6.2	
< 40 Y old	406	4.91	4.96	0.53	3.99 to 6.0	3.86 - 4.08	5.97 - 6.12	0.000
> 40 Y old	324	5.45	5.42	0.47	4.24 to 6.11	3.86 - 4.49	6.0 - 6.2	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year,>40=More than forty year and P<0.05 is consider significant.

The reference intervals for fasting glucose for males were 4.01 -6.1 mmol/L with their 90% confidence intervals 3.87-4.24mmol/L and 6.0- 6.2 mmol/L. Where as reference intervals for fasting glucose for females 3.99 - 6.1mmol/L with their 90% confidence intervals 3.79 - 4.20 mmol/L and 6.0- 6.2mmol/L as

well as combined (M&F) 4.0 - 6.1 mmol/L with their 90% confidence intervals3.79 - 4.20mmol/L and6.0 -6.12 mmol/L for lower and upper limits respectively as shown in table3.6.

Difference between two sex groups was statistically significant (P=0.04), but the lower and upper reference limits fell inside the 90% confidence interval of the two sex groups. So glucose was not significant for sex difference.

It was observed that, 406 subjects were less than 40 years and 325 subjects were more than 40 years, age specific reference intervals for fasting glucose level were 3.99 - 6.0 under 40 years subjects, with their 90% confidence intervals 3.86 - 4.08mmol/L and 5.97 - 6.12 mmol/L and above 40 years old subjects intervals is 4.24 to 6.11 mmol/L with their 90% confidence intervals 3.86 - 4.49mmol/L and 6.0 - 6.2 mmol/L for lower and upper limits respectively, presented in table 3.6.

It was statistically significant difference (P=0.000), and also lower and upper reference limits fell inside the 90% confidence interval of two age groups. As results glucose was not significant for age difference.

Reference values for glucose obtained from the present study was similar to North Indian study[70][4.04-6.27 mmol/L for males and 4.0-6.24 mmol/L for females and 4.0-6.27mmol/L for combined]

3.1.2 Reference Intervals for Creatinine

To establish reference Intervals for Creatinine, total 724 data were included where 436 males and 288 females. Table 3.7 showed in SD were 0.208, 0.15, 0.13 and mean were 0.973, 1.09, 0.78 as well as median were 0.98, 1.08, 0.79 for combined(M&F), males and females subject respectively.

Table 3.7 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for creatinine.

Analyte: Creatinine, Unit: mg/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	724	0.98	0.973	0.208	0.57 to 1.35	0.53- 0.61	1.33 -1.37	
Males	436	1.08	1.09	0.15	0.76 to 1.37	0.75 – 0.79	1.35- 1.40	0.000
Females	288	0.79	0.78	0.13	0.52 to 1.08	0.48 – 0.53	1.04- 1.13	
< 40 Y old	400	0.99	0.93	0.20	0.53 to 1.31	0.52 – 0.57	1.28 – 1.34	0.000
> 40 Y old	324	1.02	1.01	0.20	0.65 to 1.39	0.63 – 0.69	1.36 – 1.40	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for creatinine presented in table 3.7 formales0.76 to 1.37 mg/dl with their 90%confidence intervals 0.75-0.79mg/dl and 1.35-1.40 mg/dl ,for females 0.52 - 1.08mg/dl with their 90%confidence intervals 0.48-0.53mg/dl and 1.04-1.13 mg/dl and the combined(M&F) 0.57 - 1.35 mg/dl with their 90%confidence intervals 0.53-0.61mg/dl and1.33-1.37mg/dl for lower and upper limits respectively.

Statistically significant sex difference were observed (P=0.000) between males and females reference interval. Also the lower and upper reference limits fell outside the 90% confidence interval of the two groups. So creatinine are significant for sex difference where males had significantly higher creatinine values of 0.76 - 1.37 mg/dl against 0.52 - 1.08 mg/dl for females. Sex differences in Creatinine have been known to exist due to differences in muscle mass.

To construct age specific reference intervals for creatinine, 400 data for below 40 and 324 data for above 40 years subjects were involved in this study. Table 3.7 presented SD were 0.20, 0.20 and mean were 0.93, 1.01 as well as median were 0.91, 1.02, for the less than 40 years and more than 40 years subjects respectively.

The study obtained the age specific reference intervals for creatinine for below 40 years old subjects 0.53 - 1.31 mg/dl with their 90%confidence intervals ,0.52-0.57 mg/dl and 1.28-1.34 mg/dl and above 40 years old subjects interval 0.65 - 1.39 mg/dl with their 90%confidence intervals 0.63-0.69 mg/dl and 1.36-1.40 mg/dl for lower and upper limits respectively. Table 3.7 showed above 40 years old subjects had significantly higher creatinine of 0.65 - 1.39 mg/dl against 0.53 - 1.31 for below 40 years old subjects.

Difference between two age group reference interval was statistically significant (P=0.000) and also the lower and upper reference limits fell outside 90% confidence interval of two age groups. So creatinine is significant for age difference.

The increased in serum reference range for creatinine in males and females with progression of age could be due to the decrease of renal integrity with advancing age; similar results have been reported by Verma et al. (1992).[66]

Obtained reference values for creatinine separately in males and females have the same finding with Hinduja National Hospitalstudy[71][0.7-1.3 mg/dl for males and 0.6-1.0 mg/dl for female] and standard Text Book [72][0.8-1.3 mg/dl for males and 0.6- 1.1mg/dl for females] .

3.1.3 Reference Intervals for Urea

Table 3.8 showed SD were 6.48, 6.38, 5.53 and mean were 21.88, 23.57, 19.15 as well as median were 21, 23, 18 for combined (M&F), males and females subject respectively.

Table 3.8 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for urea.

Analyte: Urea, Unit: mg/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	716	21	21.88	6.48	11 to 37	10.5-12	35-38.3	
Males	430	23	23.57	6.38	13 to 38.3	10.86 –13.58	37- 39	0.000
Females	286	18	19.15	5.53	10.5 to 34	10 - 11	30- 35	
< 40 Y old	400	19.05	20.07	5.36	10.86 to30.8	10 - 12	30.5–33.8	0.000
> 40 Y old	316	24	24.16	7.14	11 to 40	10.13 -13.58	38.3 - 41	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year,>40=More than forty year and P<0.05 is consider significant.

Reference intervals for urea for the male 13 - 38.3 mg/dl with their 90% confidence intervals 10.86-13.58 mg/dl and 37-39 mg/dl, the females intervals 10.5 to 34 mg/dl with their 90% confidence intervals10-11 mg/dl and 30-35 mg/dl and the combined(M&F) intervals 11 - 37 mg/dl with 90% confidence intervals 10.5-12 mg/dl to 35-38.3 mg/dl for lower and upper limits respectively as shown in table 3.8.

Statistically significant sex difference was observed (P=0.000) between males and females reference interval. Also the lower and upper reference limits fell outside 90% confidence interval of two groups. So urea was significant for sex difference where males had significantly higher urea values of 13.0 - 38.3 mg/dl against 10.5 - 34.0 mg/dl for females.

Urea a waste product of protein metabolism, in the blood. Urea is formed by the liver and carried by the blood to the kidneys for excretion. Because urea is synthesized by the liver cells where male liver cells usually higher than females, as a results higher level of urea are synthesized than females.

Table 3.8 showed SD were 5.36, 7.14 and mean were 20.07, 24.16 as well as median were 19.05, 24 for less than 40 years and more than 40 years subjects respectively.

Age specific reference intervals for urea for below 40 years old subjects 10.86 - 30.8 mg/dl with their 90% confidence intervals 10-12 mg/dl and30.5-33.8 mg/dl and above 40 years old subjects interval 11 - 40 with 90% confidence intervals 10.13-13.58 mg/dl and 38.3-41.0 mg/dl for lower and upper limits respectively. In this study 40 years old subjects had significantly higher urea of 11.0 - 40.0 mg/dl against 10.86 - 30.8 mg/dl for less than 40 years old subjects as shown in table 3.8.

Statistically significant age difference was found (P=0.000) between two age group reference interval and also the lower and upper reference limits fell outside the 90% confidence interval of the two age groups. So urea are significant for age difference. The increased in serum urea in males and females with progression of age could be due to the decrease of renal integrity with advancing age; similar results have been reported by Verma et al. (1992).[66]

Similar reference values for urea were found in Hinduja National Hospital study[71][10.7-36.38 mg/dl for males and 10.7 -34.4 mg/dl.]

3.1.4 Reference Intervals for Uric acid

To establish reference intervals for uric acid, total 728 data were included where 434 males and 294 females. Table 3.9 presented SD 1.20, 1.02, 1.04 and mean 5.19, 5.70, 4.44 as well as median 5.23, 5.8, 4.33 for combined(M&F), males and females subject respectively.

Table 3.9 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for uric acid**.**

Analyte: Uric acid, Unit : mg/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	728	5.23	5.19	1.20	2.85 to 7.22	2.7- 2.97	7.14 -7.5	
Males	434	5.8	5.70	1.02	3.48 to 7.5	2.98–3.87	7.22 - 7.60	0.000
Females	294	4.33	4.44	1.04	2.5 to 6.41	1.98–2.80	6.2- 6.7	
< 40 Y old	404	5.01	4.98	1.24	2.7 to 7.2	2.4–3.5	7.0-7.6	0.000
> 40 Y old	324	5.59	5.46	1.10	3.5 to 7.3	2.70–3.67	7.0 –7.5	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for uric acid presented in table 3.9 for male 3.48 - 7.5 mg/dl with their 90% confidence interval 2.98-3.87 mg/dl and 7.22-7.6 mg/dl, for females interval 2.5 to 6.41 mg/dl with their 90% confidence intervals1.98-2.8 mg/dl and 6.2-6.7 mg/dl and the combined (M&F) interval 2.85 - 7.22 mg/dl with their 90% confidence interval 2.7-2.97 mg/dl and 7.14-7.5 mg/dl for lower and upper limits respectively.

Statistically significant sex difference was observed (P=0.000) between males and females reference interval and also the lower and upper reference limits fell outside the 90% confidence interval of the two groups. So uric acid was significant for sex difference where males had significantly higher uric acid values of 3.48- 7.5 mg/dl against 2.5 - 6.41 mg/dl for females. The serum uric acid concentration of females, which is lower than in

males, is thought to be related to a higher renal clearance of uric acid in female, possibly due to their higher plasma estrogen levels.

Table 3.9 showed the number of data 404 and 324,SD 1.24 and 1.10 , mean 4.98 and5.46 as well as median 5.01 and 5.59for below 40 years and above 40 years subjects respectively.

Table 3.9 demonstrated age specific reference intervals for uric acid for below 40 years old subjects2.7 - 7.2 mg/dl with their 90% confidence interval 2.4-3.5 mg/dl and7.0-7.6 mg/dl and above 40 years old subjects interval 3.5- 7.3 with their 90% confidence interval 2.70-3.67 mg/dl 7.0 - 7.5 mg/dl for lower and upper limits respectively.

Difference between two age groups was statistically significant (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. So uric acid was not significant for age difference.

Reference values for uric acid in Hinduja National Hospital study[71][3.8-7.8 mg/dl for males and 2.6 -6.3 mg/dl] and North Indian study[70][3.44 - 7.81 mg/dl for males, 2.40-6.84 mg/dl for females and 2.50-7.70 mg/dl for combined] as well as standard Text Book[72][3.5-7.2 mg/dl for males and 2.3 -6.6 for females]were found similar values. It is also observed that for females reference interval of YC LO, David A. Armbruster *et al*[63] were almost similar to present study.

3.1.5 Reference Intervals for Sodium

To set up reference intervals for sodium, total 710data were included where 435 males and 275 females. Table 3.10 showed SD were 2.29, 2.76, 1.82 and mean were 138.4, 138.8, 138.03 as well as median were 138, 139, 138 for combined (M&F), males and females subject respectively.

Table 3.10 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for sodium.

Analyte: Sodium, Unit: mmol/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	710	138	138.4	2.29	134.8 to 144	134- 136	143 -145	
Males	435	139	138.8	2.76	134.8 to 145	134 - 136	145- 146	0.001
Females	275	138	138.03	1.82	134.8 to 142	134 - 136	141- 142	
< 40 Y old	390	138	138.21	2.21	134.8 to 143	134 - 135	142 - 144	0.052
> 40 Y old	320	138	138.58	2.38	134 to 144	134 - 135	143 - 144	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for sodium for male 134.8 - 145 with their 90% confidence interval 134-136 mmol/L and 145-146mmol/L, for females interval 134.8 to142 mmol/L with their 90% confidence intervals 134-136 mmol/L and 141-142 mmol/L and combined interval 134.8 - 144 with their 90% confidence intervals 134-136 mmol/L and 143-144 mmol/L for lower and upper limits respectively as shown in table 3.10.

Statistically significant sex difference was found (P=0.001) between males and females reference interval and the lower reference limits of males and females were similar but the upper reference limits fell outside the 90% confidence interval of the two sex groups. So sodium are significant for sex difference where males had significantly higher upper reference limits of sodium of 134.8-145 mmol/L against 134.8 - 142 mmol/L for females. Sodium is the tight physiological control key analyte where usually predicts that minimal gender difference would be observed.

To establish age specific reference intervals for sodium, 390 data for less than 40 years and 320 data for more than 40 years subjects were involved. Table 3.10 showed SD were 2.21 and 2.38, mean were 138.21 and 138.58 as well as

median were 138 and 138 for the less than 40 years and more than 40 years subjects respectively.

Age specific reference intervals for sodium for less than 40 years old subjects 134.8- 143 with their 90% confidence intervals134-135mmol/L and 142-144 mmol/Land more than 40 years old subjects interval 134 to 144 mmol/L with their 90% confidence intervals 134-135 mmol/Land 143 –144 mmol/L for lower and upper limits respectively Presented in table 3.10.

It was no statistically significant difference (P=0.052) and the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. So sodium are not significant for age difference.

Values of sodium in combined with males and females in comparable with USA population[77] [136-145 mmol/L] and the standard Text Book[9] [136-145 mmol/L].

3.1.6 Reference Intervals for Potassium

To construct reference intervals for potassium, total 716 data were included in this study where 431 males and 285 females. Data presented SD were 0.38, 0.41, 0.32 and mean were 4.12, 4.13, 4.10 as well as median were 4.12, 4.11, 4.12 for combined (M&F), males and females subjects respectively as shown table 3.11.

Table 3.11 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Potassium.

Analyte: Potassium , Unit: mmol/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	716	4.12	4.12	0.38	3.4 to 5.0	3.36- 3.45	4.9 -5.0	
Males	431	4.11	4.13	0.41	3.4 to 5.0	3.3 – 3.48	4.9- 5.0	0.532
Females	285	4.12	4.10	0.32	3.48 to 4.8	3.4 – 3.5	4.7- 5.0	
< 40 Y old	392	4.10	4.05	0.36	3.4 to 4.9	3.3 – 3.48	4.8 – 4.9	0.000
> 40 Y old	324	4.19	4.17	0.37	3.4 to 4.9	3.3 – 3.5	4.89 – 5.0	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Table 3.11 presented reference intervals for serum potassium for the male 3.4 - 5.0 mmol/L with their 90% confidence interval 3.3-3.48 mmol/L and4.90-5.1 mmol/L, for female interval 3.48- 4.8 mmol/L with their 90% confidence intervals 3.4-3.5 mmol/L and 4.7-5.0mmol/L and the combined interval 3.4 to 5.0 mmol/L with their 90% confidence intervals3.36-3.45mmol/L and 4.9-5.0mmol/L for lower and upper limits respectively.

It was no statistically significant difference (P=0.532) and the lower and upper reference limits fell inside the 90% confidence interval of the two sex groups. So potassium was not significant for sex difference.

The number of data 392 and 324for below 40 years and above 40 years subjects respectively were included to set up age specific reference interval for serum potassium. Table 3.11 showed SD were 0.36 and 0.37, mean were 4.05 and 4.17 as well as median were 4.10 and 4.19 for below 40 years and above 40 years subjects respectively.

It was observed age specific reference interval for serum potassium for below 40 years old subjects 3.4- 4.9 mmol/L with their 90% confidence intervals3.3-3.48 mmol/L and 4.8-4.9mmol/L and above 40 years old subjects interval 3.4 - 4.9 mmol/L with 90% confidence intervals 3.3-3.5 mmol/L and 4.89-5.0 mmol/L for lower and upper limits respectively presented in table 3.11.

It was statistically significant difference (P=0.000) between two age groups but the lower and upper reference limits fell inside 90% confidence interval of two age groups. As results serum potassium was not significant for age difference.

Observed values for serum potassium separately male and female in similar with YC LO, David A. Armbruster *et al*[63][3.5- 5.2 mmol/L for males , 3.6- 5.2 mmol/L for females and 3.6-5.2 mg/dl for combined] and USA population[77][3.5-5.0 mmol/L for combined] as well as standard Text Boo[36][3.5-5.1mmol/L for combined].

3.1.7 Reference Intervals for Chloride

To establish reference intervals for chloride, total 722 data were included, where 436 males and 286 females. Data presented SD were 2.36, 2.58, 2.39 and mean were 103.15, 102.69, 103.15 as well as median were 103, 103, 103.9 for combined(M&F), males and females subject respectively as shown in table 3.12.

Table 3.12 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for chloride.

Analyte: Chloride, Unit: mmol/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	722	103	103.15	2.36	99 to 108	98- 99	107 -108	
Males	436	103	102.69	2.58	97 to 107	96 - 99	107- 108	0.000
Females	286	103.9	103.15	2.39	99 to 108	97 - 99	107- 109	
< 40 Y old	406	103	102.72	2.56	97 to 107	96 - 98	106 - 107	0.005
> 40 Y old	316	103	103.41	2.41	98 to 108	97 - 99	107 - 109	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for chloride for male 97- 107mmol/L with their 90% confidence intervals 96-99 mmol/L and 107-108 mmol/L, for female interval 99- 108mmol/L with their 90% confidence intervals97-99mmol/L and 107-109mmol/L and combined(M&F) intervals 99- 108mmol/L with their 90% confidence intervals98-99 mmol/L and 107-108 mmol/L for lower and upper limits respectively presented in table 3.12.

Statistically significant difference was observed between two sex groups (P=0.000). But the lower and upper reference limits fell inside the 90% confidence interval of two sex groups. So chloride was not significant for sex difference.

Data 406for less than 40 years and 316 for more than 40 years subjects were comprised to set up age specific reference interval for serum chloride. Table 3.12 showed SD were2.56 and 2.41, mean were 102.72 and103.41for less than 40 years and more than 40 years subjects respectively as well as median were 103 for both groups.

It was found in table 3.12,age specific reference interval for serum chloride for below 40 years old subjects 97- 107mmol/L with their 90% confidence intervals96-98mmol/L and 106-108 mmol/L and above 40 years old subjects intervals 98to 108 mmol/L with 90% confidence interval 97-99 mmol/L and 107-109mmol/L for lower and upper limits respectively.

While it was statistically significant difference (P=0.005), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result serum chloride was not significant for age difference.

Obtained values for serum chloride in combined male and female in similar with USA population[77][98-106 mmol/L] and the standard Text Book[36] [98-107 mmol/L].

3.1.8 Reference Intervals for Calcium

To set up reference intervals for calcium, total 724 data were included where 435 males and 289 females. Table 3.13 showed SD were 0.10, 0.11, 0.10 and mean were 2.42, 2.43, 2.40 as well as median were 2.42, 2.43, 2.40 for combined (M&F), males and females subject respectively.

Table 3.13 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for calcium.

Analyte: Calcium, Unit: mmol/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	724	2.42	2.42	0.10	2.2 to 2.65	2.2- 2.23	2.64 -2.65	
Males	435	2.43	2.43	0.11	2.2 to 2.65	2.2 – 2.24	2.65-2.65	0.001
Females	289	2.40	2.40	0.10	2.2 to 2.63	2.2 – 2.23	2.59- 2.65	
< 40 Y old	403	2.40	2.41	0.11	2.2 to 2.65	2.2 – 2.24	2.62 – 2.68	0.013
> 40 Y old	321	2.43	2.43	0.10	2.23 to 2.65	2.2 – 2.25	2.63 – 2.66	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for calcium for males 2.5- 2.65mmol/L with their 90% confidence intervals 2.2-2.24 mmol/L and 2.65-2.65mmol/L, for female interval 2.2- 2.63mmol/L with their 90% confidence intervals 2.2-2.23mmol/L and 2.59-2.65mmol/L and the combined interval 2.2- 2.65with their 90% confidence intervals 2.2-2.23 mmol/L and 2.64-2.65 mmol/L for lower and upper limits respectively present in table 3.13.

While it was statistically significant sex difference (P=0.001), the male and female are both so close to the combined interval that is deemed not to be a clinically significant difference. Also the lower and upper reference limits fell inside the 90% confidence interval of two sex groups. So serum calcium was not significant for sex difference.

Data 403for less than 40 years and 321 for more than 40 years subjects were involved to construct the age specific reference interval for serum calcium. Table 3.13 showed SD were 0.11 and 0.10, mean were 2.41 and2.43 as well as median were 2.40 and 2.43for less than 40 years and more than 40 years subjects respectively.

It is found the age specific reference interval for serum calcium for less than 40 years old subjects 2.2 - 2.65 mmol/L with their 90% confidence interval 2.2-2.24 mmol/L and 2.62-2.68mmol/L and more than 40 years old subjects interval 2.23 - 2.65 mmol/L with their 90% confidence intervals 2.2-2.25 mmol/L and 2.63-2.66 mmol/L for lower and upper limits respectively in table 3.13.

Although it was statistically significant difference (P=0.013), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result serum calciumwas not significant for age difference.

Values for serum calcium in combined male and female are comparable with the standard Text Book[35] [2.20-2.65 mmol/L].

3.1.9 Reference Intervals for Magnesium

To construct reference intervals for magnesium, total 728 data were included where 435 males and 293 females. Data presented SD were 0.08, 0.08 and 0.07for combined (M&F), males and females subjects respectively. Mean and median were equal (o.88) for the three groups.

Table 3.14 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for magnesium.

Analyte: Magnesium, Unit: mmol/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	728	0.88	0.88	0.08	0.75 to 1.05	0.73- 0.75	1.03 -1.06	
Males	435	0.88	0.88	0.08	0.75 to 1.06	0.73 – 0.75	1.04- 1.07	0.823
Females	293	0.88	0.88	0.07	0.75 to 1.04	0.72 – 0.77	1.02- 1.06	
< 40 Y old	405	0.88	0.89	0.07	0.77 to 1.03	0.74 – 0.78	1.03 – 1.06	0.000
> 40 Y old	323	0.85	0.86	0.08	0.74 to 1.06	0.72 – 0.78	1.03 – 1.07	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for magnesium presented in table 3.14 for males 0.75-1.06mmol/L with their 90% confidence intervals0.73-0.75mmol/L and 1.04-1.07 mmol/L, for females 0.75- 1.04mmol/L with their 90% confidence intervals 0.72-0.77mmol/L and 1.02-1.06mmol/L and combined (M&F) 0.75-1.05mmol/L with their 90% confidence intervals 0.73-0.75mmol/L and 1.03-1.06mmol/L for lower and upper limits respectively.

It was not statistically significant difference (P=0.828) and also the lower and upper reference limits fell inside the 90% confidence interval of two sex groups. So serum magnesium was not significant for sex difference.

To establish age specific reference intervals for magnesium, 405 data for less than 40 years and 323 data for more than 40 years subjects were involved. Table 3.14 showed SD were0.07 and 0.08, mean were 0.89 and0.86 as well as median were 0.88 and 0.85for less than 40 years and more than 40 years subjects respectively.

It was found age specific reference interval for serum magnesium for less than 40 years old subjects 0.77 - 1.03 mmol/L with their 90% confidence intervals 0.74-0.78 mmol/L and 1.03-1.06mmol/L and more than 40 years old subjects interval 0.74 - 1.06 mmol/L with their 90% confidence interval 0.72-0.78 mmol/L and 1.03-1.07 for lower and upper limits respectively in table 3.14.

While it was statistically significant difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result serum magnesium was not significant for age difference.

Reference values for serum magnesium in combined male and female are similar with the standard Text Book[35] [0.73-1.06mmol/L for males and 0.77–1.03mmol/L for females].

3.1.10 Reference Intervals for Inorganic phosphate

To establish reference intervals for inorganic phosphate, total 728 data were included where 434 males and 294 females. Table 3.15 presented SD were 0.19, 0.20, 0.18 and mean were 1.15, 1.15, 1.10 as well as median were 1.11, 1.13, 1.10 for combined(M&F), males and females subject respectively.

Table 3.15 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for inorganic phosphate.

Analyte: Inorganic Phosphate, Unit: mmol/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	728	1.11	1.15	0.19	0.85 to 1.59	0.81- 0.86	1.46 -1.60	
Males	434	1.13	1.15	0.20	0.83 to 1.58	0.80- 0.87	1.52 -1.64	0.823
Females	294	1.10	1.14	0.18	0.87 to 1.59	0.83- 0.88	1.51 -1.62	
< 40 Y old	404	1.09	1.13	0.20	0.85 to 1.58	0.80- 0.87	1.51 -1.62	0.001
> 40 Y old	324	1.16	1.17	0.19	0.82 to 1.62	0.80- 0.85	1.51 -1.67	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for Inorganic phosphate presented in table 3.15 for the males 0.83- 1.58 mmol/L with their 90% confidence interval 0.80-0.87mmol/L and 1.52-1.64 mmol/L, for female interval 0.87to 1.59mmol/L with their 90% confidence interval 0.83-0.88 mmol/L and 1.51-1.62mmol/L and combined(M&F) interval 0.85- 1.59mmol/L with their 90% confidence interval 0.81-0.86 mmol/L and 1.53-1.60 mmol/L for lower and upper limits respectively.

It was not statistically significant difference (P=0.823) and also the lower and upper reference limits fell inside the 90% confidence interval of two sex groups. So Inorganic phosphate was not significant for sex difference.

To set up age specific reference interval for Inorganic phosphate,404 data for less than 40 years and 324 data for more than 40 years subjects were involved.

Table 3.15 showed SD were 0.20 and 0.19, mean were 1.13 and1.17 as well as median were1.09 and 1.16for less than 40 years and more than 40 years subjects respectively.

Table 3.15 presented age specific reference interval for Inorganic phosphate for below 40 years old subjects 0.85 to 1.58mmol/L with their 90% confidence intervals0.80-0.87mmol/L and 1.51-1.62 mmol/L and above 40 years old subjects interval 0.82to1.62mmol/L with their 90% confidence intervals 0.80-0.85mmol/L and 1.51-1.67mmol/L for lower and upper limits respectively.

While it was statistically significant difference (P=0.001), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result Inorganic phosphate was not finally significant for age difference.

Observed values for serum Inorganic phosphate in combined males and females are comparable with the standard Text Book[35] [0.81-1.45].

3.1.11 Reference Intervals for Cholesterol

To set up reference intervals for cholesterol, total 722 data were included where 431 males and 291 females. Table 3.16 showed SD were 32.71, 32.73, 31.91 and mean were 186.5, 187.7, 184.2 as well as median were 185, 187.7, 184.2 for combined (M&F), males and females subject respectively.

The reference intervals for cholesterol showed in table 3.16 for the males 118-244 mg/dl with their 90% confidence intervals 113.5-122.8 mg/dl and 241-251 mg/dl, the female interval 122- 250mg/dl with their 90% confidence intervals 117-125mg/dl and 242-260mg/dl and the combined(M&F) interval 122-249mg/dl with their 90% confidence intervals 117-131.4mg/dl and 244-257 mg/dl for lower and upper limits respectively.

Table 3.16 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for cholesterol.

Analyte: Cholesterol, Unit: mg/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	722	185	186.5	32.71	122 to 249	117- 131.4	244 -257	
Males	431	187.1	187.7	32.73	118 to 244	113.5- 122.8	241 -251	0.044
Females	291	180	184.2	31.91	122 to 250	117- 125	242 -260	
< 40 Y old	402	180.6	181.9	27.53	131.4 to 240	128- 132.2	231 -241.5	0.000
> 40 Y old	320	195	193.6	37.74	117 to 270	112.3- 122.8	257 -275	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

While it was statistically significant sex difference (P=0.044), but the lower and upper reference limits fell inside the 90% confidence interval of two sex groups. So serum Cholesterol was not significant for sex difference.

To construct age specific reference interval forcholesterol,402 data for less than 40 years and 320 data for more than 40 years subjects were involved. Table 3.16 showed SD were 27.53 and 37.74, mean were 181.9 and193.6 as well as median were180.6 and 195for the less than 40 years and more than 40 years subjects respectively .

It was found in table 3.16 age specific reference interval for serum cholesterol for less than 40 years old subjects 131.4- 240mg/dl with their 90% confidence intervals 128-132.2mg/dl and 231.0-241.5 mg/dl and more than 40 years old subjects interval 117- 270mg/dl with their 90% confidence intervals 112.32-122.85mg/dl and 257-275mg/dl for lower and upper limits respectively.

It was statistically significant age difference (P=0.000) and also the lower and upper reference limits fell outside the 90% confidence interval of two age groups. As a result serum cholesterol was significant for age difference.

It was observed the upper reference limit is higher in aged subjects than young subjects that means the reference intervals are higher in above 40 years old subjects below 40 years old subjects.

There are the possible cause diet and physical activity and weight for higher cholesterol in aged than young. As usual diet and physical activity are change with increasing aged when they take rich food and decrease physical activity. Lack of physical activity can lead to weight gain and increase total cholesterol level as well as rich food contain high cholesterol .

The study obtained reference values for serum cholesterol separately males and females are generally supported by other studies such as YC LO, David A. Armbruster *et al*[63][115.0-257.3 mg/dl for males and 116.9-247.7 for females] and Hinduja National Hospital study[71][126-267 mg/dl for males and 123 -263 mg/dl for females]as well as North Indian study[70][115.47-255.75 mg/dl for males, 118.6-244.0 mmol/L for females]

3.1.12 Reference Intervals for HDL. Cholesterol

To establish reference intervals for HDL. Cholesterol, total 722 data were included in this study where 427 males and 295 females. Table 3.17 presented SD were 8.30, 6.80, 8.52 and mean were 38.66, 35.74, 42.74 as well as median were 38, 35, 41 for combined(M&F), males and females subject respectively.

Reference intervals for serum HDL. Cholesterol presented in table 3.17. for the males 24- 52 mg/dl with their 90% confidence interval 21.0-25.1mg/dl and 49.5-53 mg/dl, the female interval 27to 59 mg/dl with their 90% confidence interval 25.2-30mg/dl and 56-65mg/dl and the combined interval 25to 55.51mg/dl with their 90% confidence interval 24-26mg/dl and 55-57 mg/dl for lower and upper limits respectively.

Table 3.17 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for HDL. Cholesterol.

Analyte: HDL.Cholesterol, Unit: mg/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	722	38	38.66	8.30	25 to 55.51	24- 26	55 -57	
Males	427	35	35.74	6.80	24 to 52	21 – 25.1	49.5- 53	0.000
Females	295	41	42.74	8.52	27 to 59	25.2 - 30	56- 65	
< 40 Y old	405	37	37.55	7.85	24.5 to 55	20 - 26	54 - 58	0.000
> 40 Y old	317	39	40.09	8.66	25 to 57.7	24 - 27	55 – 60.3	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Statistically significant sex difference was observed (P=0.000), and also the lower and upper reference limits fell outside the 90% confidence interval of the two sex groups. So serum HDL. Cholesterol was significant for sex difference where females had higher HDL. Cholesterol concentration in blood than males. The estrogen produced by female has been found to raise HDL cholesterol, generally giving females higher HDL levels than males.

To set up age specific reference interval forHDL.Cholesterol,405 data for less than 40 years and 317 data for more than 40 years subjects were involved. Table 3.17 showed SD were 7.85 and 8.66, mean were 37.55 and40.09 as well as median were 37 and 39for the less than 40 years and more than 40 years subjects respectively.

It was found in the table 3.17 age specific reference interval for serum HDL. Cholesterol for the less than 40 years old subjects is 24.5- 55mg/dl with their 90% confidence interval 20.0-26.0mg/dl and 54.0-58.0 mg/dl and the more than 40 years old subjects interval 25- 57.7mg/dl with their 90% confidence intervals 24-27mg/dl and 55-60.3 mg/dl for lower and upper limits respectively.

While it was statistically significant difference between two age groups (P=0.000) but the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a results serum HDL.Cholesterol was not significant for age difference.

3.1.13 Reference Intervals for LDL. Cholesterol

To construct reference intervals for LDL. Cholesterol, total 725 data were included in this study where 432 males and 293 females. Data presented SD 25.77, 26.19 and 24.45 and mean 119.8, 122.9, 115.2 as well as median 120,125,110 for combined (M&F), males and females subjects respectively in table 3.18.

Table 3.18 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for LDL. Cholesterol.

Analyte: LDL.Cholesterol, Unit:mg/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	725	120	119.8	25.77	69 to 167	55.65- 72	165 -170	
Males	432	125	122.9	26.19	62 to 168	53 - 74	166- 176	0.000
Females	293	110	115.2	24.45	71.5 to 167	62 - 76	162- 170	
< 40 Y old	404	116.8	117.6	23.18	71.5 to 162	70 – 76.5	160 - 167	0.001
> 40 Y old	321	125	123.22	29.19	54.59 to 176	51.41 - 67	168 - 186	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for serum LDL. Cholesterol for the males 62- 168mg/dl with their 90% confidence intervals 53-74mg/dl, and 166-176 mg/dl, for female interval 71.5- 167mg/dl with their 90% confidence intervals 62-76mg/dl and 162-170mg/dl and the combined(M&F) interval 69- 167mg/dl with their 90% confidence intervals 55.65-72.0 mg/dl and 165-170 mg/dl for lower and upper limits respectively as shown in table 3.18.

While it was a statistically significant sex difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of the two sex groups. So serum LDL. Cholesterol was not finally significant for sex difference.

To construct age specific reference interval forLDL.Cholesterol,404 data for less than 40 years and 321 data for more than 40 years subjects were involved. Table 3.18 showed SD 23.18 and 29.19, mean 117.6 and123.22 as well as median 116.8 and 125for the less than 40 years and more than 40 years subjects respectively.

It was found in table 3.18 age specific reference interval for serum LDL. Cholesterol for less than 40 years old subjects 71.5- 162mg/dl with their 90% confidence interval 70-76.5 mg/dl and 160.0-167.0 mg/dl and the more than 40 years old subjects interval 54.59- 176mg/dl with their 90% confidence intervals 51.41-67.0mg/dl and168-186mg/dl for lower and upper limits respectively.

Statistically significant difference was observed between two age groups (P=0.001) and also the lower and upper reference limits fell outside the 90% confidence interval of the two age groups. As a results serum LDL. Cholesterol was significant for age difference where the reference intervals for LDL. Cholesterol are higher in more than 40 years old subjects than less than 40 years old subjects. There are the possible cause diet and physical activity for higher LDL .Cholesterol in aged than young. As usual diet and physical activity are change with increasing aged in subjects when they take rich food and decrease physical activity. Lack of physical activity can lead to weight gain and increase LDL. Cholesterol along with total cholesterol level as well as rich food contain high LDL. Cholesterol level.

In the present study values of serum LDL. Cholesterol in combined and separately male and female are generally supported by the study North Indian study[70][50-170.55 mg/dl for males, 42.87-170.0 mg/dl for females and 47.22-

170.0] where upper limit were similar to this study and lower limit were lower than this study and another study Hinduja National Hospital study[71][67-192 mg/dl for males and 65-185 mg/dl for females] where lower limit were similar to this study and upper limit were higher than this study in both males and females.

3.1.14 Reference Intervals for Triglyceride

To set up reference intervals for triglyceride, total 715 data were included where 427 males and 288 females. Table 3.19 showed SD were 60.61, 62.05, 52.06 and mean were 139.88, 155.8, 117.12 as well as median were 130, 145, 102 for combined (M&F), males and females subject respectively.

Table 3.19 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum triglyceride.

Analyte: Triglyceride, Unit: mg/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	715	130	139.88	60.61	47 to 277	45.0- 50	269 -290	
Males	427	145	155.8	62.05	62.5 to 297	55-65	281 - 306	0.000
Females	288	102	117.12	52.06	45 to 237	34 - 46	210- 248	
< 40 Y old	393	124.9	133.9	54.29	45 to 253	35-47	250 - 285	0.000
> 40 Y old	322	142	147.16	64.81	60 to 282	59-67	274 - 304	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

It was found in table 3.19 reference intervals for serum triglyceride for the males 62.5- 297mg/dl with their 90% confidence intervals 55-65 mg/dl and 281-306 mg/dl, the females interval 45- 237mg/dl with their 90% confidence intervals 34 - 46 mg/dl and 210-248 mg/dl and the combined(M&F) interval 47- 277mg/dl with their 90% confidence intervals 45.0-50.0mg/dl and 269 -290 mg/dl for lower and upper limits respectively.

Difference between males and females reference interval was statistically significant (P=0.000).Also the lower and upper reference limits fell outside the 90% confidence interval of the two groups. So triglyceride was significant for sex difference where males had significantly higher triglyceride values of 62.5-297.0 mg/dl against 45.0 – 237.0 mg/dl for females. The serum triglyceride concentration of female, which is lower than in male due to hormonal effect, higher plasma estrogen levels are responsible for higher HDL. Cholesterol and lower triglyceride.

To construct age specific reference intervals for serum triglyceride 393 data for less than 40 years old subjects and 322 data for more than 40 years old subjects were included. Table 3.19 summarized SD were 54.29 and 64.81,Mean were133.9 and 147.16 as well as median were 124.9and 142for the less than 40 years and more than 40 years subjects respectively.

It was observed in table 3.19 age specific reference intervals for triglyceride for less than 40 years old subjects 45 - 253 mg/dl with their 90% confidence intervals 35-47 mg/dl and 250-285 mg/dl and more than 40 years old subjects interval 60 - 282 with their 90% confidence intervals 59-67 mg/dl274- 304 mg/dl for lower and upper limits respectively .

Statistically significant difference was observed (P=0.000), and also the lower and upper reference limits fell outside the 90% confidence interval of the two age groups. So triglyceride was significant for age difference where more than 40 years old subjects had significantly higher reference interval of triglyceride than less than 40 years old subjects due to Participants whose post menopausal(estrogen effect) were included in more than 40 years old subjects group. Plasma estrogen levels are low in post menopausal female, this lower concentration of estrogen are responsible for higher level of triglycerides.

Observed reference values of triglyceride separately males and females are generally supported by the study Hinduja National Hospital study[71][41-256 mg/dl for males and 35 -218 mg/dl for female] and YC LO, David A. Armbruster*et al*[63][47.27- 450.0 mg/dl for males 43.0- 285.4 mg/dl for females and 45.45-390.0 mg/dl for combined] where female reference range were similar to present study but male reference range of YC LO, David A. Armbruster*et al*[63] were in higher than our study and male reference range of Hinduja National Hospital study[71] were in lower than present study.

3.1.15 Reference Intervals for Total Protein

To set up reference intervals for total protein, total 730 data were included in this study where 436 males and 294 females. Table 3.20 showed SD were 4.97, 5.08, 4.80 and mean were74, 74.07, 73.90for combined (M&F), males and females subject respectively. Median 75 that were equal for the three groups.

Table 3.20 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for total protein.

Analyte: Total Protein, Unit:g/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	730	75	74	4.97	64 to 82	63- 65	82 -84	
Males	436	75	74.07	5.08	64 to 82	63 - 65	82- 84	0.673
Females	294	75	73.90	4.80	64 to 82	62- 65	81- 85	
< 40 Y old	406	75	74.18	4.75	65 to 82	63 - 66	82 - 83	0.401
> 40 Y old	324	74	73.78	5.23	63 to 82	62 - 65	82 - 85	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for total protein presented in table 3.20 for males 64- 82g/L with their 90% confidence interval 63-65g/L and 82-84 g/L, for female interval 64- 82g/L with their 90% confidence interval 62-65g/L and 81-85 g/L and the combined(M&F) interval 64to 82g/L with their 90% confidence interval 63-65 g/L and 82-84 g/L for lower and upper limits respectively.

It was not statistically significant sex difference (P=0.673), and also the males and females both were similar to the combined interval. So serum total protein was not significant for sex difference.

To establish age specific reference interval for serum total protein 406 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included. Table 3.20 summarized SD were 4.75 and 5.23 Mean were 74.18 and 73.78 as well as median were75 and74 for the less than 40 years and more than 40 years subjects respectively.

It was found in table 3.20 age specific reference interval for serum total protein for less than 40 years old subjects is 65 - 82 g/L with their 90% confidence intervals 63-66 g/L and 82-83 g/L and more than 40 years old subjects interval 63 - 82 g/L with their 90% confidence intervals 62-65 g/L and 82-85 g/L for lower and upper limits respectively.

While it was statistically significant difference (P=0.401), but the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result serum total protein was not significant for age difference.

Obtained reference values of serum total protein in combined and separately male and female are supported by the standard Text Book[35][64-83g/L] and Hinduja National Hospital study[71][64-80g/L for male and 63-80 g/L for female] .

3.1.16 Reference Intervals for Albumin

To construct reference intervals for serum albumin, total 730 data were included in this study where 436 males and 294 females.Table 3.21presented SD were 4.55, 4.69, 4.30 and mean were 41.22, 41.60, 40.64 as well as median were 41, 42, 40for combined (M&F), males and females subjects respectively.

Table 3.21 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for albumin.

Analyte: Albumin, Unit: g/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	730	41	41.22	4.55	33 to 50	32- 33	49 -50	
Males	436	42	41.60	4.69	33 to 50	32 - 34	49- 50	0.005
Females	294	40	40.64	4.30	33 to 49	32 - 34	48- 50	
< 40 Y old	406	42	42.15	4.40	34 to 50	32 - 34	48 - 50	0.000
> 40 Y old	324	40	40.05	4.49	32 to 48.2	32 - 34	48 - 50	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for albumin for the males33- 50g/L with their 90% confidence intervals 32-34g/L and 49-50 g/L, for female interval 33- 49 g/L with their 90% confidence interval 32-34 g/L and 48-50 g/L and combined(M&F) interval 33- 50with their 90% confidence interval 32-33g/L and 49-50 g/L for lower and upper limits respectively as shown in table 3.21.

While it was a statistically significant sex difference (P=0.005), but the lower and upper reference limits fell inside the 90% confidence interval of the two sex groups. So serum albumin was not significant for sex difference.

To set up study age specific reference interval for serum albumin 406 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included. Table 3.21 summarized SD were 4.40 and 4.49 Mean were42.15 and 40.05 as well as median were42 and 40for the less than 40 years and more than 40 years subjects respectively.

It is found in table 3.21 age specific reference interval for serum albumin for the less than 40 years old subjects 34- 50g/L with their 90% confidence intervals32-34g/L and 48-50 g/L and more than 40 years old subjects interval 32g/L-48.2g/L with their 90% confidence intervals 32-34g/L and 48-50 g/L for lower and upper limits respectively.

Statistically significant difference was observed (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result serum albumin was not significant for age difference.

Observed values of serum albumin in combined and separately male and female are supported by the standard Text Book[35][33-50g/L]and Hinduja National Hospital study[71][35-49g/L for male and 34-48 g/L for female].

3.1.17 Reference Intervals for Bilirubin-total

To construct reference intervals for serum bilirubin-total, total 710 data were included where 429 males and 281 females.Table 3.22summarized SD were 0.23, 0.25, 0.17 and mean were 0.67 ,0.74, 0.57 as well as median were 0.63, 0.69, 0.54 for combined (M&F), males and females subjects respectively.

It was found in table 3.22 reference intervals for serum bilirubin-total for males 0.35 - 1.35 mg/dl with their 90% confidence intervals 0.34 - 0.42 mg/dl and 1.3- 1.37 mg/dl, for females interval 0.33 to 0.97 mg/dl with their 90% confidence intervals 0.28 - 0.34 mg/dl and 0.92- 1.07 mg/dl and combined(M&F) interval 0.34 to 1.21 mg/dl with their 90% confidence

intervals 0.33- 0.35 mg/dl and 1.19 -1.28 mg/dl for lower and upper limits respectively.

Table 3.22 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum bilirubin total.

Analyte: Albumin, Unit: g/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	710	0.63	0.67	0.23	0.34 to 1.21	0.33- 0.35	1.19 -1.28	
Males	429	0.69	0.74	0.25	0.35 to 1.35	0.34 - 0.42	1.3- 1.37	0.000
Females	281	0.54	0.57	0.17	0.33 to 0.97	0.28 - 0.34	0.92- 1.07	
< 40 Y old	400	0.66	0.71	0.24	0.35 to 1.3	0.33 - 0.37	1.20 - 1.37	0.000
> 40 Y old	310	0.58	0.62	0.20	0.33 to 1.20	0.28 - 0.36	1.19 - 1.30	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

It was statistically significant sex difference (P=0.000) between males and females reference intervals and also the lower and upper reference limits fell outside 90% confidence interval of the two groups. So bilirubin total was significant for sex difference where males had significantly higher bilirubin-total reference values of 0.35 to 1.35 mg/dl against 0.33 to 0.97 mg/dl for females. The serum bilirubin total concentration for females which is lower than in male due to influence of sex hormones.

To establish age specific reference interval for serum bilirubin-total 400 data for less than 40 years old subjects and 310 data for more than 40 years old subjects were included. Table3.22 summarized SD were 0.24 and 0.20 Mean were 0.71 and 0.62 as well as median were 0.66 and 0.58 for the less than 40 years and more than 40 years subjects respectively.

It was found in table 3.22 age specific reference interval for serum bilirubin-total for the less than 40 years old subjects 0.35- 1.3 gm/dl with their 90% confidence intervals 0.33 - 0.37gm/dl and 1.20 - 1.37gm/dl and more than 40 years old subjects interval

0.33 - 1.20gm/dl with their 90% confidence intervals 0.28 - 0.36gm/dl and 1.19 - 1.30gm/dl for lower and upper limits respectively.

While it was a statistically significant difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result serum bilirubin-total was not significant for age difference.

Values of serum bilirubin -total in combined and separately males and females supported by other similar studies "Reference intervals of common clinical chemistry analytes for adults in Hong Kong" done in Hong Kong (YC LO, David A. Armbruster *et al*.)[63] published by the journal of the International Federation of clinical chemistry and laboratory medicine. Manolio et al. (1992)

3.1.18 Reference Intervals for Alanine Aminotransferase (ALT)

To set up reference intervals for serum ALT, total 725 data were included where 434 males and 291 females. Table 3.23 summarized SD 13.90, 13.47, 10.59 and mean 27.58, 31.79, 20.74 as well as median 25, 29, 16for combined (M&F), males and females subjects respectively.

Reference intervals for serum ALT for males 10 - 57 U/L with their 90% confidence intervals 10- 11U/L and 55-58 U/L , for females interval 9 - 48 U/L with their 90% confidence intervals 7 - 10 U/L and 42- 50 U/L and combined(M&F) interval 10 - 55 U/L with their 90% confidence intervals 9.0-10.0 U/L and 52 -57 U/L for lower and upper limits respectively presented in table 3.23.

Table 3.23 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum ALT.

Analyte:Alanine Aminotransferase (ALT) , Unit: U/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	725	25	27.58	13.90	10 to 55	9.0- 10.0	52 -57	
Males	434	29	31.79	13.47	10 to 57	10- 11	55- 58	0.000
Females	291	16	20.74	10.59	9 to 48	7 - 10	42- 50	
< 40 Y old	404	21	26.34	14.12	9 to 54	8 - 10	52 - 56	0.001
> 40 Y old	321	27	28.66	12.55	10 to 55	9 - 12	51 - 57	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Statistically significant sex difference was observed (P=0.000) between males and females reference intervals and also the lower and upper reference limits fell outside 90% confidence interval of the two groups. So ALT was significant for sex difference where males had significantly higher ALT reference values of 10 - 57U/L against 9 - 48U/L for females. The serum ALT concentration of males which is higher than in females due to differences in muscle mass, usually male muscle mass is higher than female.

To establish age specific reference interval for serum ALT 404 data for less than 40 years old subjects and 321 data for more than 40 years old subjects were included. Table 3.23 summarized SD were 14.12 and 12.55 Mean were 26.34 and 28.66 as well as median were 21 and 27 for less than 40 years and more than 40 years subjects respectively

It was found from in table 3.23 age specific reference interval for serum ALT for less than 40 years old subjects 9 - 54U/L with their 90% confidence intervals8 - 10U/L and 52 - 56U/L and the more than 40 years old subjects

interval 10 - 55U/L with their 90% confidence intervals 9 - 12U/L and 51-57U/L for lower and upper limits respectively.

Statistically significant difference was observed (P=0.001), but the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result serum ALT was not significant for age difference.

Observed values for serum ALT in combined and separately male and female are supported by is supported by other similar studies "Reference intervals of common clinical chemistry analytes for adults in Hong Kong" done in Hong Kong (YC LO, David A. Armbruster *et al*.)[63]

3.1.19 Reference Intervals for Aspartate Aminotransferase (AST)

To construct reference intervals for serum AST, total 723 data were included in this study where 430 males and 293 females.Table 3.24summarized SD were 7.37, 7.43, 5.47 and mean were 23.75, 25.89, 20.09 as well as were median 23, 25, 19for combined (M&F), males and females subjects respectively.

Table 3.24 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum AST.

Analyte: Aspartate Aminotransferase (AST) , Unit: U/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	723	23	23.75	7.37	12 to 38	12-13	38 -40	
Males	430	25	25.89	7.43	12 to 39	11 - 13	38- 41	0.000
Females	293	19	20.09	5.47	12 to 34	11 - 13	32- 35	
< 40 Y old	399	21	22.66	7.24	12 to 38	10 - 13	37 - 40	0.000
> 40 Y old	324	24	25.35	7.97	13 to 40	12 - 15	38 - 42	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

It was found in table 3.24 reference intervals for serum AST for the males 12 - 39 U/L with their 90% confidence intervals 11-13 U/L and 38-41 U/L, for females interval 12 - 34 U/L with their 90% confidence intervals 11-13 U/L and 32- 35 U/L and combined(M&F) interval 12 - 38 U/L with their 90% confidence intervals 12-13 U/L and 38 -40 U/L for lower and upper limits respectively.

Statistically significant sex difference was observed (P=0.000) between males and females reference intervals and also the lower and upper reference limits fell outside the 90% confidence interval of the two groups. So AST was significant for sex difference where males had significantly higher AST reference values of 12 - 39 U/L against 12 - 34 U/L for females. The serum AST concentration of males which is higher than in females due to differences in muscle mass, usually muscle mass of males is higher than females.

Observed reference values of serum AST are supported by other similar studies (YC LO, David A. Armbruster *et al*.)[63]

To establish the age specific reference interval for serum AST 399 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included.

Table 3.24 summarized SD were 7.24 and 7.97 Mean were 22.66 and 25.35 as well as median were 21 and 24for the less than 40 years and more than 40 years subjects respectively.

It was presented in table 3.24 the age specific reference interval for serum AST for less than 40 years old subjects 12 - 38 U/L with their 90% confidence intervals10 - 13U/L and 37 - 40U/L and more than 40 years old subjects interval 13 - 40U/L with their 90% confidence intervals 12 - 15U/L and 38 - 42U/L for lower and upper limits respectively.

Statistically significant difference was observed (P=0.000), but lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result serum AST was not significant for age difference.

Values of serum AST in combined and separately males and females are supported by is supported by other similar studies "Reference intervals of common clinical chemistry analytes for adults in Hong Kong" done in Hong Kong (YC LO, David A. Armbruster *et al.*)[63] published by the journal of the International Federation of clinical chemistry and laboratory medicine. Manolio*et al*. (1992)

3.1.20 Reference Intervals for Alkaline Phosphatase (ALP)

To set up reference intervals for serum ALP, total 718 data were included where 433 males and 285 females.Table 3.25showedSD were 20.93, 21.18, 20.08 and mean were 78.42, 81.47, 74.02 as well as median were 77, 79, 74for combined (M&F), males and females subjects respectively.

Table 3.25 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for serum ALP.

Analyte: Alkaline Phosphatase(ALP) , Unit: U/L								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	718	77	78.42	20.93	44 to 120	41-45.0	120- 125	
Males	433	79	81.47	21.18	45 to 125	42 - 47.1	122- 130	0.000
Females	285	71	74.02	20.08	41.5 to 118	28 - 45	115- 120	
< 40 Y old	396	74	75.51	19.56	44.2 to120	40 - 45	115 - 123	0.000
> 40 Y old	322	80	81.44	21.62	41 to 122	37 - 48	120 - 130	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

It was found in table 3.25 the reference intervals for serum ALP for the males 45 -125 U/L with their 90% confidence intervals 42 - 47.1U/L and122- 130U/L,

for females interval 41.5 - 118U/L with their 90% confidence intervals 28 - 45U/L and 115- 120 U/L and the combined(M&F) interval 44 - 120 U/L with their 90% confidence intervals 41-45.0U/L and 120- 125 U/L for lower and upper limits respectively.

It was observed statistically significant sex difference (P=0.000) between males and females reference intervals and also the lower and upper reference limits fell outside the 90% confidence interval of two sex groups. So ALP was significant for sex difference where males had significantly higher ALP reference values of 45 -125U/L against 41.5 - 118U/L for females.

The serum ALP concentration of males which is higher than in females due to differences bone mass, usually bone mass of male is higher than female.

To construct age specific reference interval for serum ALP 396 data for less than 40 years old subjects and 322 data for more than 40 years old subjects were included. Table 3.25 summarized SD were 19.56 and 21.62 Mean were 75.51 and 81.44 as well as median were 74 and 80for the less than 40 years and more than 40 years subjects respectively.

It was found in table 3.25 age specific reference interval for serum ALP for the less than 40 years old subjects 44.2 -120 U/L with their 90% confidence intervals40 - 45U/L and 115 - 123U/L and more than 40 years old subjects interval 41 - 122 U/L with their 90% confidence intervals 37 - 48 U/L and 120 - 130 U/L for lower and upper limits respectively .

While it was a statistically significant difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result serum ALP was not significant for age difference.

Obtained values for serum ALP in separately males and females are supported by the Hinduja National Hospital study[91][38-123U/L for male and 35-120 U/L for female].

3.2 Reference intervals of Hematological Analytes

The hematological tests results for RBC, Hemoglobin, HCT, MCV , MCH, MCHC, RDW-CV platelets, neutrophil,lymphocyte, monocyte, eosinophil and basophile showed statistically significant sex differences ($p<0.05$) as well as MPV and WBC statistically not significant sex differences ($P>0.05$) presented in table 3.4.

But Considering both statistical ($P<0.05$) and 90% Confidence interval limits shown in table 3.26, table3.27, table 3.28,table 3.30, table 3.31, table 3.33the tests results for RBC, hemoglobin, hematocrite, MCV, MCH, MCHC and platelets are significant for sex difference.

The hematological tests RDW-CV, neutrophil, lymphocyte, monocyte, eosinophil and basophile had statistically significant ($P<0.05$) difference between the males and females intervals. When Considering 90% Confidence interval limits shown in table 3.32, and from table 3.36 to table 3.40 reference intervals of these tests were not significant sex difference . Here the study were chosen to use combined sex intervals shown in table 3.32 and from table3.36 to table 3.40.

3.2.1 Reference Intervals for Red Blood Cell (BRC)

To set up reference intervals for RBC, total 713 data were included, where 420 males and 293 females.Table 3.26 showed SD were 0.429, 0.35, 0.35 and mean were 4.83, 5.03, 4.54 as well as median were 4.86, 5.1 and 4.6for combined (M&F), males and females subject respectively.

The reference intervals for RBC showed in table 3.26 for males 4.4 - 5.6x10^6/µl with their 90% confidence intervals 4.3-4.4x10^6/µl and 5.59-5.8x10^6/µl, for female interval 3.9 - 5.2x10^6/µl with their 90% confidence intervals3.8-3.9x10^6/µl and 5.1- 5.4x10^6/µl and the combined(M&F) interval 4.0 - 5.59x10^6/µl with their 90% confidence intervals 3.9- 4.4x10^6/µl and 5.58 - 5.6x10^6/µl for lower and upper limits respectively

Table 3.26 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Red Blood Cell (BRC).

Analyte:Red Blood Cell (BRC), Unit: 10^6/µl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	713	4.86	4.83	0.429	4.0 to 5.59	3.9- 4.4	5.58-5.6	
Males	420	5.1	5.03	0.35	4.4 to 5.6	4.3 - 4.4	5.59- 5.8	0.000
Females	293	4.6	4.54	0.35	3.9 to 5.2	3.8- 3.9	5.1- 5.4	
< 40 Y old	390	5.0	4.92	0.43	4.1 to 5.2	4 - 4.2	5.1 - 5.6	0.000
> 40 Y old	323	4.7	4.57	0.48	3.89 to 5.5	3.8 - 4.1	5.2 - 5.7	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

It was statistically significant sex difference (P=0.000) between males and females reference interval and also lower and upper reference limits fell outside the 90% confidence interval of two groups. So RBCs were significant for sex difference where males had significantly higher RBC values of 4.4 - 5.6x10^6/µl against3.9 - 5.2x10^6/µl for females. The reasons for these differences have been attributed to factors such as the influence of the androgen hormone on erythropoiesis and menstrual blood loss in females [61,69].

Reference values for serum RBC in separately males and females are supported by Massachusetts General Hospital study [77] [4.5 – 5.9x10^6/µl for male and 4.0 – 5.2x10^6/µl for female].Diagnostic Perspectives V-1 [78][4.44-5.63x10^6/µl for male and 3.92- 5.08x10^6/µl for female].

To establish age specific reference interval for RBC 390 data for less than 40 years old subjects and 323 data for more than 40 years old subjects were involved. Table 3.26 summarized SD were 0.43 and 0.48 Mean were 4.92 and 4.57 as well as were median 5.0 and 4.7 for less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for RBC for less than 40 years old subjects 4.1 -5.2x10^6/µl with their 90% confidence intervals 4 - 4.2x10^6/µl and 5.1 - 5.6x10^6/µland more than 40 years old subjects interval 3.89 - 5.5x10^6/µl with their 90% confidence intervals 3.8 - 4.1x10^6/µl and 5.2 - 5.7x10^6/µlfor lower and upper limits respectively as shown in table 3.26.

While it was statistically significant difference (P=0.000), but lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result RBC was not significant for age difference

3.2.2 Reference Intervals for Hemoglobin

To construct reference intervals for hemoglobin, total 710 data were included where 421 males and 289 females.Table 3.27 showed SD were 1.56, 1.03, 0.83 and mean were 13.63, 14.55, 12.32 and median were 13.7, 14.5, 12.5 for combined (M&F), males and females subject respectively.

The reference intervals for hemoglobin for males 12.4 - 16.55gm/dl with their 90% confidence intervals12.3 - 12.6gm/dl and 16.3- 16.6gm/dl , for female interval 10.3 - 13.6gm/dl with their 90% confidence intervals 10.2- 10.4gm/dl and 13.5- 13.8 gm/dl and the combined(M&F) interval 10.3 - 16.5gm/dl with their 90% confidence intervals10.2- 10.5gm/dl and 16.3 -16.6 gm/dl for lower and upper limits respectively as shown in table 3.27.

Table 3.27 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for hemoglobin.

Analyte:Hemoglobin , Unit: gm/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	710	13.7	13.63	1.56	10.3 to 16.5	10.2- 10.5	16.3 -16.6	
Males	421	14.5	14.55	1.03	12.4 to 16.55	12.3 - 12.6	16.3- 16.6	0.000
Females	289	12.5	12.32	0.83	10.3 to 13.6	10.2- 10.4	13.5- 13.8	
< 40 Y old	404	14.0	13.88	1.59	10.3 to 15	10.1 - 10.7	14.9 - 17.5	0.000
> 40 Y old	306	13.3	13.32	1.47	10.2 to 16.2	10.0 - 10.6	15 - 16.5	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

It was observed in table 3.27 statistically significant sex difference (P=0.000) between males and females reference interval and also the lower and upper reference limits fell outside the 90% confidence interval of two groups. So hemoglobin was significant for sex difference where males had significantly higher hemoglobin values of 12.4 - 16.55gm/dlagainst10.3 - 13.6gm/dl for females. The reasons for these differences have been attributed to factors such as the influence of the androgen hormone on erythropoiesis and menstrual blood loss in females [61,69].In this study, reference values of hemoglobin in male are supported by Diagnostic Perspectives V-1 [78][13.5- 16.9gm/dl for male]

To set up age specific reference interval for hemoglobin 404 data for less than 40 years old subjects and 306 data for more than 40 years old subjects were included. Table 3.27 summarized SD were 1.59 and 1.47 Mean were 13.88 and 13.32 as well as median were14.0 and 13.3 for less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for hemoglobin for less than 40 years old subjects 10.3 - 15gm/dl with their 90% confidence intervals 10.1 - 10.7gm/dl and 14.9 - 17.5gm/dl and more than 40 years old subjects interval 10.2 -

16.2gm/dl with their 90% confidence intervals 10.0 - 10.6gm/dl and 15 - 16.5gm/dl for lower and upper limits respectively as shown in table 3.27.

It was statistically significant difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result hemoglobin was not significant for age difference

3.2.3 Reference Intervals for Hematocrit

To establish reference intervals for hematocrit, total 713 data were involved, where 431 males and 282 females.Table 3.28demonstratedSD were 4.01, 2.97, 2.29 and mean were 40.63, 42.95, 37.21 as well as median were 40.6, 43, 37.6for combined (M&F), males and females subject respectively.

Table 3.28 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for hematocrit.

Analyte:Hematocrite , Unit: %								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	713	40.6	40.63	4.01	32.6 to 48.7	32.1- 33.5	48.2 -48.9	
Males	431	43	42.95	2.97	36.5 to 48.8	35.6 - 37.7	48.7- 49.2	0.000
Females	282	37.6	37.21	2.29	32.1 to 41.7	32- 32.6	40.8- 42.7	
< 40 Y old	403	41.3	41.04	4.16	33.5 to 48.8	32.1 - 34.1	48.3 - 49	0.000
> 40 Y old	310	39.9	39.99	3.87	32.2 to 48.3	31.4 - 33.7	46.6 - 48.9	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference interval for hematocrit for the males 36.5 - 48.8%with their 90% confidence intervals35.6 - 37.7% and48.7- 49.2% , for female interval 32.1 - 41.7 %with their 90% confidence intervals32- 32.6 % and 40.8- 42.7 % and the combined(M&F) interval 32.6 - 48.7%with their 90% confidence intervals32.1- 33.5% and 48.2 -48.9 %for lower and upper limits respectively as shown in table 3.28.

It was statistically significant sex difference (P=0.000) between males and females reference interval and also the lower and upper reference limits fell outside the 90% confidence interval of two groups. So hematocrit was significant for sex difference where males had significantly higher hematocrit values of 35.6 - 37.7% against32.1- 41.7%for females.

The hematocrit of males which is higher than in females due to higher count of RBC in male than female .

To construct age specific reference interval for hematocrit 403 data for below 40 years old subjects and 310 data for above 40 years old subjects were included. Table 3.28 summarized SD were 4.16 and 3.87 Mean were 41.04 and 39.99 as well as median were 41.3 and 39.9 for less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for hematocrit for below 40 years old subjects 33.5 - 48.8% with their 90% confidence intervals 32.1 - 34.1% and 48.3 - 49% and above 40 years old subjects interval 32.2 - 48.3% with their 90% confidence intervals 31.4 - 33.7% and 46.6 - 48.9% for lower and upper limits respectively as shown in table 3.28.

It was statistically significant difference (P=0.000), but lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result hematocrit was not significant for age difference.

3.2.4 Reference Intervals for Mean Corpuscular Volume (MCV)

To establish reference intervals for MCV, total 699 data were included in this study where 411 males and 288 females.Table 3.29demonstratedSDwere 5.15, 4.86, 5.86 and mean 85.10, 85.44, 84.28 as well as median 86, 86.2, 85.2for combined (M&F), males and females subject respectively.

Table 3.29 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Mean Corpuscular volume (MCV)

Analyte:Mean Corpuscular volume, Unit: fl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	699	86	85.10	5.15	73.4 to 94.2	72.5- 74.3	93.3 -94.6	
Males	411	86.2	85.44	4.86	74.5 to 94	72.4 - 75.4	93.2- 94.9	0.015
Females	288	85.2	84.28	5.86	70.6 to 94.3	69.5- 72.5	92.8- 95.1	
< 40 Y old	378	86	85.17	4.65	74.3 to 93.4	72.3 - 74.9	91.5 - 94.9	0.982
> 40 Y old	321	86	84.90	5.84	72.4 to 94.3	70 - 74.6	93.3 - 95.1	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference interval for MCV showed in table 3.29 for males 74.5 - 94fl with their 90% confidence interval 72.4-75.4 fl and 72.4 -75.4fl, for females interval 70.6 - 94.3 fl with their 90% confidence interval 69.5-72.5fl and 92.8-95.1 fl and the combined(M&F) interval 73.4 - 94.2 fl with their 90% confidence interval 72.5- 74.3fl and 93.3 -94.6 fl for lower and upper limits respectively .

It was statistically significant sex difference (P=0.015) between males and females reference interval and also lower and upper reference limits fell outside their 90% confidence interval of two groups. So MCV was f significant for sex difference where males had significantly higher MCV values of 74.5 to 94flagainst70.6 to 94.3flfor females. Table 3.29demonstrated lower limit of MCV in female had lower than male that was clinically significance.

To construct age specific reference interval forMCV378 data for less than 40 years old subjects and 321 data for more than 40 years old subjects were included. Table 3.29 summarized SD 4.65 and 5.84 Mean 85.17 and 84.90for

less than 40 years and more than 40 years subjects respectively. Median was equal for both groups that was 86.

Age specific reference intervals of presented in table 3.29 MCV for below 40 years old subjects were 74.3 - 93.4fl with their 90% confidence intervals 72.3 - 74.9fl and 91.5 - 94.9fl and above 40 years old subjects interval 72.4 to 94.3 g/L with their 90% confidence intervals 70 - 74.6 g/L and 93.3 - 95.1 g/L for lower and upper limits respectively .

It was not statistically significant difference (P=0.982) and also lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result MCV was not significant for age difference.

Reference values for MCV in combined male and female are supported by the standard Text Book[76]De Gruchy's clinical Hematology in medical Practice 5th edition 1993[combined(M&F) 76 - 94 fl].

3.2.5 Reference Intervals for Mean Corpuscular Hemoglobin (MCH)

To construct reference intervals for MCH, total 706 data were included where 419 males and 287 females.Table 3.30demonstrated SD 2.21, 1.95, 2.33 and mean28.57, 29.02, 27.92 and median 28.9, 29.3, 28.2for combined (M&F), males and females subject respectively.

The reference intervals for MCH showed in table 3.30 for males 24.6 - 32.3Pgwith their 90% confidence interval 24 -24.9Pg and 32-32.4Pg , for female interval 23 - 31.6Pg with their 90% confidence interval 22.1- 23.1Pg and 31.6 - 32.3 Pg and combined(M&F) interval 23.4 - 32.2 Pg with their 90% confidence intervals23.1- 23.8Pg and 32 -32.4 Pg for lower and upper limits respectively.

Table 3.30 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for MCH.

Analyte:Mean Corpuscular Hemoglobin , Unit: Pg								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	706	28.9	28.57	2.21	23.4 to 32.2	23.1- 23.8	32 -32.4	
Males	419	29.3	29.02	1.95	24.6 to 32.3	24 - 24.9	32- 32.4	0.000
Females	287	28.2	27.92	2.33	23 to 31.6	22.1- 23.1	31.3- 32.5	
< 40 Y old	385	29.1	28.72	2.00	23.8 to 32	23.8 - 24.6	31.6 - 32.3	0.264
> 40 Y old	321	28.9	28.5	2.28	23.2 to 32.3	23 - 23.8	32 - 32.6	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Difference between males and females reference interval was statistically significant (P=0.000) and also lower and upper reference limits fell outside the 90% confidence interval of two groups. So MCH was significant for sex difference where males had significantly higher MCH values of 24.6 to 32.3Pgagainst23 to 31.6Pgfor females .

To establish age specific reference interval for MCH 385 data for less than 40 years old subjects and 321 data for more than 40 years old subjects were included. Table 3.30 demonstrated SD 2.00 and 2.28 Mean 28.72 and 28.5 as well as median 29.1 and 28.9for less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for MCH presented in table 3.30 for less than 40 years old subjects were 23.8 - 32Pg with their 90% confidence intervals 23.8 - 24.6Pg and 31.6 - 32.3Pg and more than 40 years old subjects interval 23.2 to 32.3Pg with their 90% confidence intervals 23 - 23.8Pg and 32 - 32.6Pg for lower and upper limits respectively.

It was statistically significant age difference (P=.264), but lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result MCH was not significant for age difference.

3.2.6 Reference Intervals for MCHC

To set up reference intervals for MCHC, total 714 data were involved where 420 males and 294 females.Table 3.31showed SD were 1.08, 1.00, 1.21 and mean were 33.58, 33.89, 32.96 and median were 33.7, 34.0, 33.2for combined (M&F), males and females subject respectively.

Table 3.31 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals MCHC,

Analyte:MCMC , Unit:gm/dl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	714	33.7	33.58	1.08	31.2 to 35.6	31.1- 31.4	35.5 -35.9	
Males	420	34	33.89	1.00	31.9 to 35.9	31.3 - 32	35.7- 36.1	0.000
Females	294	33.2	32.96	1.21	30.2 to 35.2	30.1- 30.4	34.8- 35.3	
< 40 Y old	390	33.8	33.77	0.99	31.9 to 35.7	31.2 - 32	35.3 - 35.9	0.000
> 40 Y old	324	33.5	33.39	1.08	31.1 to 35.3	30.9 - 31.2	35.1 - 35.8	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for MCHC for the males 31.9 - 35.9gm/dl with their 90% confidence interval 31.3 - 32gm/dl and 35.7- 36.1gm/dl , for female interval 30.2 - 35.2gm/dl with their 90% confidence interval 30.1- 30.4gm/dl and 34.8- 35.3gm/dl and combined(M&F) interval 31.2 - 35.6gm/dl with their 90% confidence interval 31.1- 31.4gm/dl and 35.5 -35.9gm/dl for lower and upper limits respectively as shown in table 3.31.

It was statistically significant sex difference (P=0.000) between males and females reference interval and also the lower and upper reference limits fell outside the 90% confidence interval of two groups. So MCHC was significant

for sex difference where males had significantly higher MCHC values of 31.9 - 35.9gm/dlagainst30.2 - 35.2gm/dl for females .

To establish age specific reference interval forMCHC390 data for below 40 years old subjects and 324 data for above 40 years old subjects were included. Table 3.31 summarized SD were 0.99 and 1.08 Mean were 33.77 and 33.39 as well as median were 33.8 and 33.5for the below 40 years and above 40 years subjects respectively .

Age specific reference interval for MCHC for below 40 years old subjects were 31.9 - 35.7gm/dl with their 90% confidence intervals 31 - 32gm/dl and 35.3 - 35.9gm/dl and above 40 years old subjects interval 31.1 - 35.3gm/dl with their 90% confidence intervals 30.9 - 32gm/dl and 35.1 - 35.8gm/dl for lower and upper limits respectively as shown in table 3.31.

It was statistically significant difference (P=0.000), but lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result MCHC was not significant for age difference.

Obtained reference values of MCHC in combined males and females are supported by the standard Text Book[76][31.0 - 35.0 gm/dl]

3.2.7 Reference Intervals for Red cell Distribution- Coefficient of Variation (RDW-CV)

To construct reference intervals for RDW-CV, total 685 data were included where 416 males and 269 females.Table 5.32demonstratedSD 0.717, 0.65, 0.79 and mean 13.46, 13.37, 13.59 and median 13.4, 13.3, 13.5for combined (M&F), males and females subject respectively.

Table 3.32 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for RDW-CV.

Analyte:RDW-CV , Unit: %								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	685	13.4	13.46	0.717	12.2to 15.1	12.2- 12.4	14.9 -15.3	
Males	416	13.3	13.37	0.65	12.3 to 14.8	12.12 - 12.4	14.7- 15.4	0.001
Females	269	13.5	13.59	0.79	12.2 to 15.4	12- 12.4	15.1- 16	
< 40 Y old	370	13.4	13.41	0.60	12.4 to 14.8	12.4 - 12.5	14.6 - 15.7	0.247
> 40 Y old	315	13.4	13.53	0.86	12.1 to 15.6	11.9 - 12.4	14.8 - 15.8	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for RDW-CV presented in table 5.32 for males 12.3 - 14.8%with their 90% confidence interval 12.12 - 12.4 % and 14.7- 15.4% , for female interval 12.2 to 15.4 % with their 90% confidence interval 12- 12.4% and 14.6 - 15.7 % and combined(M&F) interval 12.1 - 15.6%with their 90% confidence interval 11.9 - 12.4% and 14.8 - 15.8 % for lower and upper limits respectively

Statistically significant sex difference (P=0.001), but lower and upper reference limits fell inside the 90% confidence interval of the two sex groups. So RDW-CV was not significant for sex difference.

To establish age specific reference interval for RDW-CV 370 data for less than 40 years old subjects and 315 data for more than 40 years old subjects were included. Table 3.32 summarized SD were 0.60 and 0.86 Mean were 13.41 and 13.53for less than 40 years and more than 40 years subjects respectively as well as median were 13.4 that was equal for both group.

Age specific reference interval for RDW-CV showed in table 5.32 for less than 40 years old subjects were 12.4 - 14.8% with their 90% confidence intervals 12.1 - 12.5% and 14.6 - 15.7% and more than 40 years old subjects interval 12.1 - 15.6 %

with their 90% confidence intervals 11.9 - 12.4 % and 14.8 - 15.8% for lower and upper limits respectively.

Difference of reference intervals between two age groups were statistically significant (P=0.401),but lower and upper reference limits fell inside the 90% confidence interval of the two age groups. As a result RDW-CV was not significant for age difference.

3.2.8 Reference Intervals for Platelets (PLT)

To establish reference intervals for platelets, total 719 data were included where 428 males and 291 females.Table 3.33demonstrated SD50.57, 46.23, 56.37 and mean 241.9, 228.9, 265.5 and median 238, 224, 258for combined (M&F), males and females subject respectively.

Table 3.33 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Platelets.

Analyte: Platelets , Unit: 10^3/µl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	719	238	241.9	50.57	155.4 to 344	151- 160	342 -353	
Males	428	224	228.9	46.23	151 to 331	149 - 155	321- 344	0.000
Females	291	258	265.5	56.37	175 to 420	158- 180	375- 425	
< 40 Y old	394	235	240.2	48.47	160 to 342	152 - 163.4	340 - 352	0.377
> 40 Y old	325	241	243.9	53.03	152 to 350	145 - 160	340 - 375	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for platelets showed in table 3.33 for males 151 - 331x10^3/µl with their 90% confidence intervals149 - 155x10^3/µl and 321-344x10^3/µl, for female interval 175 - 420x10^3/µl with their 90% confidence interval 158- 180x10^3/µl and 375- 425x10^3/µl and combined(M&F) interval 155.4 - 344x10^3/µl

with their 90% confidence interval 151- 160 $x10^3$/µl and 342 -353$x10^3$/µl for lower and upper limits respectively.

It was statistically significant sex difference (P=0.000) between males and females reference interval and also lower and upper reference limits fell outside the 90% confidence interval of two groups. So platelets were significant for sex difference where females had significantly higher platelets values of 175 to 420$x10^3$/µl against151 to 331$x10^3$/µl for males.

To set up age specific reference interval forplatelets394 data for below 40 years old subjects and 325 data for above 40 years old subjects were included. Table 5.33showed SD were 48.47 and 53.03 Mean were 240.2 and 243.9 as well as median were235 and 241for less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for platelets for below 40 years old subjects 160 - 342$x10^3$/µl with their 90% confidence intervals 152 - 163.4$x10^3$/µl and 340 - 352$x10^3$/µland above 40 years old subjects interval 152 - 350$x10^3$/µl with their 90% confidence intervals 145 - 160$x10^3$/µl and 340 - 375$x10^3$/µl for lower and upper limits respectively a shown in table 3.33.

It was a statistically significant difference (P=0.377), but lower and upper reference limits fell inside the 90% confidence intervals of two age groups. As a result platelets were not significant for age difference.

Observed reference values for serum platelets in combined males and females are supported by Massachusetts General Hospital study [77] [150 – 350$x10^3$/µl for combined (M&F)].

3.2.9 Reference Intervals for Mean Platelets Volume (MPV)

To construct reference intervals for MPV, total 727 data were included in this study where 435 males and 292 females.Table 3.34 showed SD were 0.886, 0.90, 0.87 and mean were 10.87, 10.90, 10.83and median were 10.9, 10.8, 10.9for combined (M&F), males and females subject respectively.

Table 3.34 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for MPV.

Analyte:Mean Platelets Volume , Unit:fl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	727	10.9	10.87	0.886	9.2 to 12.7	9.1- 9.4	12.6 -12.8	
Males	435	10.8	10.90	0.90	9.4 to 12.8	9.1 - 9.5	12.7- 12.9	0.620
Females	292	10.9	10.83	0.87	9.10 to 12.7	8.9- 9.4	12.4- 12.8	
< 40 Y old	404	10.9	10.93	0.88	9.4 to 12.7	9.2 - 9.5	12.6 - 12.9	0.001
> 40 Y old	323	10.7	10.75	0.86	9.2 to 12.7	8.9 - 9.4	12.4 - 12.8	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

The reference intervals for MPV presented in table 3.34 for males 9.4 - 12.8flwith their 90% confidence intervals9.1 - 9.5fl and 12.7- 12.9 , for female interval 9.10 - 12.7flwith their 90% confidence interval 8.9- 9.4fl and 12.4- 12.8fl and combined(M&F) interval 9.2 - 12.7flwith their 90% confidence interval 9.1- 9.4 fl and 12.6 -12.8flfor lower and upper limits respectively.

Difference between males and females reference interval was statistically significant (P=0.620) and also lower and upper reference limits fell inside the 90% confidence interval of two sex groups. So MPV was not significant for sex difference.

To establish age specific reference interval forMPV,404 data for less than 40 years old subjects and 323 data for more than 40 years old subjects were included.

Table 3.34 summarized SD were 0.88 and 0.86 Mean were 10.93 and 10.75 as well as median were 10.9 and 10.7 for less than 40 years and more than 40 years subjects respectively.

Reference intervals for MPV showed in table 3.34 for less than 40 years old subjects were 9.4 - 12.7 fl with their 90% confidence intervals 9.2 - 9.5 fl and 12.6 - 12.9fl and more than 40 years old subjects interval 9.2 - 12.7fl with their 90% confidence intervals 8.9 - 9.4fl and 12.4 - 12.8fl for lower and upper limits respectively . It was statistically significant difference (P=0.001), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result MPV was not significant for age difference.

3.2.10 Reference Intervals for White Blood Cell (WBC)

To establish reference intervals for WBC, total 730 data were included in this study where 436 males and 294 females.Table 3.35demonstrated SD 1.66, 1.58, 1.77 and mean 7.47, 7.46, 7.48 and median 7.3, 7.4, 7.2for combined (M&F), males and females subject respectively.

The reference intervals for WBC for males 4.6 - 10.82x10^3/µl with their 90% confidence interval 4.2 - 4.8x10^3/µl and 10.4- 11x10^3/µl, for female interval 4.3 - 11x10^3/µl with their 90% confidence interval 4.1- 4.9x10^3/µl and 10.8- 11x10^3/µl and combined(M&F) interval 4.5 - 11x10^3/µl with their 90% confidence interval 4.3- 4.8 x10^3/µl and 10.6 -11x10^3/µl for lower and upper limits respectively as shown in table 3.35.

Table 3.35 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for WBC.

Analyte:White Blood Cell , Unit: 10^3/µl								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	730	7.3	7.47	1.66	4.5 to 11	4.3- 4.8	10.6 -11	
Males	436	7.4	7.46	1.58	4.6 to 10.82	4.2 - 4.8	10.4- 11	0.819
Females	294	7.2	7.48	1.77	4.3 to 11	4.1- 4.9	10.8- 11	
< 40 Y old	406	7.2	7.36	1.6	4.5 to 11	4.3 - 4.8	10.4 - 11	0.078
> 40 Y old	324	7.5	7.59	1.72	4.7 to 11	4.1 - 4.8	10.5 - 11	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Difference between two age groups were statistically significant (P=0.819) and also the lower and upper reference limits fell inside 90% confidence interval of two sex groups. So WBC counts were not significant for sex difference.

To construct age specific reference interval for WBC 406 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included. Table 3.35 summarized SD were 1.60 and 1.72 Mean were 7.36 and 7.59 as well as median were 7.2 and 7.5for the less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for WBC for less than 40 years old subjects were 4.5 - 11x10^3/µl with their 90% confidence intervals4.3 - 4.8x10^3/µl and 10.4 - 11x10^3/µland more than 40 years old subjects interval 4.7 - 11x10^3/µl with their 90% confidence intervals 4.1 - 4.8x10^3/µl and 10.5-11x10^3/µl for lower and upper limits respectively as shown in table 3.35.

It was statistically significant difference (P=0.078), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result WBC was not significant for age difference.

Reference values of WBC in combined male and female are supported by Massachusetts General Hospital study [77] [4.5 to 44x10^3/µl for combined (M&F)].

3.2.11 Reference Intervals for Neutrophil

To construct reference intervals for neutrophil, total 728 data were included 436 males and 292 females.Table 3.36demonstrated SD 7.12, 7.12, 7.0 and mean 58.03, 57.13, 59.30 and median 57.5, 56.6, 58.5for combined (M&F), males and females subject respectively

Table 3.36 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for neutrophil

Analyte:Neutrophil , Unit: %								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	728	57.5	58.03	7.12	45.6 to 72.1	44.6- 46.3	70.9 -73.0	
Males	436	56.6	57.13	7.12	44.9 to 70.8	43.8 - 46	69.2- 73.4	0.000
Females	292	58.5	59.30	7.00	45.1 to 73.2	40.5- 47.5	70.5- 74.2	
< 40 Y old	404	56.4	56.96	7.05	44.6 to 70.8	40.5 - 46.6	69.4 - 75.0	0.000
> 40 Y old	324	58.85	59.30	7.07	46.3 to 72.6	44.5 - 47.5	70.1 - 73.2	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for neutrophil presented in table 3.36 for males 44.9 - 70.8 %with their 90% confidence interval 43.8 - 46% and 69.2- 73.4 %, for female interval 45.1 - 73.2 %with their 90% confidence interval 40.5- 47.5 % and 70.5- 74.2 % and combined(M&F) interval 45.6 - 72.1 % with their 90% confidence interval 44.6- 46.3 % and 70.9 -73.0 % for lower and upper limits respectively.

Difference between two sex groups was statistically significant (P=0.000), but the lower and upper reference limits fell inside 90% confidence interval of two sex groups. As a result neutrophil was not significant for sex difference.

To set up age specific reference interval for neutrophil404 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included. Table 3.36showed SD were 7.05 and 7.07 Mean 56.96 and 59.30 as well as median 56.4 and 58.85 for less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for neutrophil for below 40 years old subjects were 44.6 - 70.8 % with their 90% confidence intervals 40.5 - 46.6 % and 69.4 -75.0 % and above 40 years old subjects interval 46.3 - 72.6 % with their 90% confidence intervals 44.5 - 47.5 % and 70.1 - 73.2 % for lower and upper limits respectively as shown in table 3.36.

It was statistically significant difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result neutrophil was not significant for age difference.

Reference values of neutrophil in separately male and female are supported by Diagnostic Perspectives V-1[78][41.0 to 70.7 % for males and 42.9 to 74.3 % for females]

3.2.12 Reference Intervals for Lymphocyte

To construct reference intervals for lymphocyte, total 728 data were included where 436 males and 292 females.Table 3.37showedSD were6.64, 6.82, 6.01 and mean were 34.05, 34.60, 33.05 and median were34.3, 35.05, 33.1for combined (M&F), males and females subject respectively.

Table 3.37 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Lymphocyte.

Analyte:Lymphocyte , Unit: %								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	728	34.3	34.05	6.64	21.6 to 47.7	20.9- 22.6	46.4-48.6	
Males	436	35.05	34.60	6.82	21.7 to 48	20.9 - 22.7	46.3- 49	0.003
Females	292	33.1	33.05	6.01	21 to 46.3	20.2- 23.6	43- 48	
< 40 Y old	404	34.8	35.01	6.51	22.6 to 48.9	20 - 24.1	46.0 - 51.8	0.000
> 40 Y old	324	32.85	32.86	6.60	21 to 46.3	20.9 - 22.7	44.8 - 49.0	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for lymphocyte presented in table 3.37 for males 21.7 - 48%with their 90% confidence interval 20.9 - 22.7% and 46.3- 49 %, for female interval 21 to 46.3%with their 90% confidence interval 20.2- 23.6% and 43- 48 % and combined(M&F) interval 21.6 - 47.7%with their 90% confidence interval 20.9- 22.6% and 46.4-48.6 %for lower and upper limits respectively.

Difference was statistically significant (P=0.003), but the lower and upper reference limits fell inside the 90% confidence interval of two sex groups. As a result lymphocyte was not significant for sex difference.

To establish age specific reference interval forlymphocyte404 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included. Table 3.37 demonstrated SD 6.51 and 6.60Mean 35.01 and 32.86 as well as median 34.8 and 32.85for less than 40 years and more than 40 years subjects respectively.

Age specific reference interval for lymphocyte for less than 40 years old subjects were 22.6 to 48.9 % with their 90% confidence intervals 20 - 24.1 % and 46.0 - 51.8 % and more than 40 years old subjects interval 21 to 46.3%with their 90% confidence intervals 20.9 - 22.7 % and 44.8 - 49.0 % for lower and upper limits respectively as shown in table 3.37.

It was statistically significant difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of two age groups. As a result lymphocyte was not significant for age difference.

Observed reference values of lymphocyte in separately males and females are supported by Diagnostic Perspectives V-1[78][19.1 - 47.9 % for males and 18.3 - 45.7 % for females]

3.2.13 Reference Intervals for Monocyte

To establish reference intervals for Monocyte, total 726 data were included where 436 males and 290 females.Table 3.38showed SD were 1.20, 1.17, 1.22 and mean were4.22, 4.35, 4.02 and median were4.0, 4.20, 3.9for combined (M&F), males and females subject respectively

Table 3.38 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Monocyte.

Analyte: Monocyte , Unit: %								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	726	4.0	4.22	1.20	2.2 to 6.7	2.0- 2.3	6.6 -6.8	
Males	436	4.2	4.35	1.17	2.3 to 6.7	2.2 - 2.5	6.6- 6.9	0.000
Females	290	3.9	4.02	1.22	2.2 to 6.8	2.0- 2.4	6.2- 7.3	
< 40 Y old	402	4.0	4.21	1.15	2.3 to 6.7	2.2 - 2.5	6.4 - 6.9	0.835
> 40 Y old	324	4.2	4.22	1.26	2.2 to 6.9	1.9 - 2.4	6.6 - 7.4	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for monocytes showed in table 3.38 for the males 2.3 - 6.7 %with their 90% confidence interval 2.2 - 2.5% and 6.6- 6.9 %, for female interval 2.2 - 6.8%with their 90% confidence interval 2.0- 2.4% and 6.2- 7.3% and combined(M&F) interval 2.2 - 6.7%with their 90% confidence interval 2.0- 2.3% and 6.6 -6.8 %for lower and upper limits respectively.

It was statistically significant difference (P=0.000), but the lower and upper reference limits fell inside 90% confidence interval of two sex groups. As a result monocytes counts were not significant for sex difference.

To set up age specific reference interval for monocytes counts 402 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included. Table 3.38 summarized SD were 1.15 and 1.26 Mean were 4.21 and 4.22 as well as median were 4.0 and 4.2for less than 40 years and more than 40 years subjects respectively.

As shown in table 3.38, age specific reference interval for monocytes counts for the less than 40 years old subjects were 2.3 - 6.7 % with their 90% confidence intervals 2.2 - 2.5 % and 6.4 - 6.9 % and the more than 40 years old subjects interval 2.2 - 6.9 % with their 90% confidence intervals 1.9 - 2.4 % and 6.6 - 7.4 % for lower and upper limits respectively.

Difference was not statistically significant (P=0.835) and also the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. So monocytes counts were not significant for age difference.

3.2.14 Reference Intervals for Eosinophil

To construct reference intervals for eosinophil, total 721 data were included where 430 males and 291 females.Table 3.39demonstrated SD 1.71, 1.65, 1.73 and mean 3.48, 3.71, 3.14 and median 3.3, 3.70, 3.0for combined (M&F), males and females subject respectively.

Table 3.39 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for Eosinophil.

Analyte:Eosinophil , Unit: %								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	721	3.3	3.48	1.71	0.7 to 6.9	0.6- 0.9	6.6 -6.9	
Males	430	3.7	3.71	1.65	0.8 to 6.8	0.6 - 1.0	6.5- 6.9	0.000
Females	291	3.0	3.14	1.73	0.6 to 6.9	0.6- 1.0	6.6- 7.3	
< 40 Y old	397	3.3	3.49	1.69	0.8 to 6.8	0.6 - 1.0	6.5 - 6.9	0.942
> 40 Y old	324	3.3	3.48	1.72	0.6 to 6.9	0.6- 1.0	6.6 - 7.1	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for eosinophil for males 0.8 - 6.8%with their 90% confidence intervals0.6 - 1.0% and 6.5- 6.9 %, for female interval 0.6 - 6.9%with their 90% confidence intervals0.6- 1.0% and 6.6- 7.3 % and combined(M&F) interval 0.7 - 6.9%with their 90% confidence intervals0.6- 0.9% and 6.6 -6.9 %for lower and upper limits respectively as shown table 3.39.

It was statistically significant difference (P=0.000), but the lower and upper reference limits fell inside the 90% confidence interval of two sex groups. As a result Eosinophil counts were not significant for sex difference.

To establish age specific reference interval for Eosinophil counts 397 data for less than 40 years old subjects and 324 data for more than 40 years old subjects were included. Table 3.39 summarized SD 1.69 and 1.72 Mean 3.49and 3.48for the less than 40 years and more than 40 years subjects respectively. Median 3.3 that was equal for both age groups.

It was found from table 3.39 age specific reference interval for eosinophil counts for less than 40 years old subjects were 0.8 - 6.8 % with their 90% confidence intervals 0.6 -1.0 % and 6.5 - 6.9 % and more than 40 years old subjects interval 0.6 - 6.9 %

with their 90% confidence intervals 0.6- 1.0 % and 6.6 -7.1 % for lower and upper limits respectively.

Difference between two age groups was not statistically significant (P=0.942) and also the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. So eosinophil counts were not significant for age difference.

3.2.15 Reference Intervals for Basophil

To construct reference intervals for basophils count, total 727 data were included where 435 males and 292 females.Table 3.40showedSD were0.076, 0.07, and 0.06 and mean were 0.111, 0.11, and 0.09for combined (M&F), males and females subject respectively and median 0.1 that was equal for three groups.

Table 3.40 95% reference values (2.5th and 97.5th percentiles values) with their 90% confidence intervals for basophil.

Analyte:Basophil , Unit: %								
Subjects	**N**	**Med**	**Mean**	**SD**	**95% Reference Intervals**	**90% Confidence Intervals for Lower Limit**	**90% Confidence Intervals for Upper Limit**	**P Value**
M & F	727	0.1	0.111	0.076	0.0 to 0.3	0.0- 0.0	0.21 -0.3	
Males	435	0.1	0.11	0.07	0.0 to 0.3	0.0 - 0.0	0.2- 0.3	0.002
Females	292	0.1	0.09	0.06	0.0 to0.2	0.0- 0.0	0.2- 0.3	
< 40 Y old	406	0.1	0.1	0.07	0.0 to 0.2	0.0 - 0.0	0.2 - 0.3	0.749
> 40 Y old	321	0.1	0.11	0.08	0.0 to 0.3	0.0 - 0.0	0.2 - 0.3	

N= number, Med= Median and SD= standard deviation <40 Y =less than forty year, >40=More than forty year and P<0.05 is consider significant.

Reference intervals for basophils count for the males 0.0 to 0.3 %with their 90% confidence interval 0.0- 0.0% and 0.2- 0.3 %, female interval 0.0 to 0.2%with their 90% confidence interval 0.0- 0.0% and 0.2- 0.3% the combined (M&F) interval

0.0 - 0.3%with their 90% confidence interval 0.0- 0.0% and 0.21 -0.3%for lower and upper limits respectively as shown in table 3.40.

It was statistically significant difference (P=0.002), but lower and upper reference limits fell inside the 90% confidence interval of two sex groups. As a result basophils counts were not significant for sex difference.

To establish age specific reference interval for basophils counts406 data for less than 40 years old subjects and 321 data for more than 40 years old subjects were included. Table 3.40 summarized SD were 0.07 and 0.08 Mean were 0.10 and 0.11for less than 40 years and more than 40 years subjects respectively. Median 0.1 that was equal for three groups.

It was found from table 3.40 age specific reference interval for basophils counts for less than 40 years old subjects were 0.0 - 0.2 % with their 90% confidence intervals 0.0 - 0.0 % and 0.2 - 0.3% and more than 40 years old subjects interval 0.0 to 0.3% with their 90% confidence intervals 0.0 - 0.0 % and 0.2 - 0.3 % for lower and upper limits respectively.

Difference between two age groups was not statistically significant (P=0.749) and also the lower and upper reference limits fell inside the 90% confidence interval of the two age groups. So basophils counts were not significant for age difference.

DISCUSSION AND CONCLUSION

4.1 Discussion

To identify patients whose laboratory value may indicate illness and assist the clinicians make meaningful decisions in front of this aim Saris and Graesbeck introduced the reference values conception in 1969. The 95% reference interval established from healthy individuals was used as the reference value for most biochemical and hematological parameters. From then as per the recommendation of IFCC and NCCLS (new name CLSI) several international laboratories have adopted 95% reference interval to identify the reference value for their laboratory parameters.

After twenty years, several prospective studies came out showing association of various biochemical and hematological parameters to clinical events. The availability of several new drugs and development of pharmaceutical industries that effectively reduce clinical events have resulted in moving from reference values to decision limits.

Now as per guidelines for various associations such as American Diabetes Association, National Cholesterol Education Program, and Joint National Commission are used to arrive at recommended values and decision limits. Recommended values for biochemical parameters blood glucose, total Cholesterol, HDL Cholesterol, LDL cholesterol, triglycerides has been revised in the last ten years as our understanding of risk factors and opportunity and options for their modifications are available. Therefore using 95% reference interval for these parameters are useful both the laboratory physicians and clinicians.

Good understanding of diseases condition and their pathophysiologic has resulted in recommended values for optimal functioning. A Person needs hemoglobin of 12 gm/dl for optimal functioning and creatinine of less than 1.4 mg/dl indicate normal kidney function. Knowledge such as these has also resulted in recommended values making 95% reference interval measure irrelevant for the clinician.

Reference values or normal values for biochemical and hematological parameters are used to aid physicians to interpret results of clinical measurements. Reference values for Bangladeshi populations are not readily available and the values used in all over the country are usually based on results of measurements in advanced countries, taken from the literature or from package inserts that accompany reagent kits. However, these parameters even in the healthy state are affected by several factors including age, ethnicity, gender and environment. As more healthy individuals now undergo routine health check-up, it is important to know the distribution of the laboratory parameters to make meaningful clinical decisions. So it is very necessary to select reference ranges for biochemical and hematological parameters for the population of Bangladesh.

The present study carried out to established biochemical and hematological reference values to serve as standards for the interpretation of laboratory results during screening and follow-ups in clinical trials and routine healthcare in Bangladesh.

As clinical laboratory reference ranges of biochemistry and hematology for adult males and females have not previously been established in Bangladesh. Although the number of males (436) was more than those of females (294), each group exceeded the minimum of 120 participants per subgroup for nonparametric estimates required for 95% reference interval determination as recommended by CLSI(NCCLS 2000).

The lower proportion of females is likely due to physiological factors such as pregnancy, lactation and menstruation, therefore less frequent participation in blood donation.

The rigorous screening process employed by the laboratory presumably resulted in blood collection from overall healthy adults. The results for reference values for each parameter under study were obtained using similar analytical methods and unit of measure as those in applicable. Emphasis was laid on external and internal quality control methods which ensured accuracy and precision.
To consider statistical significance with 90% confidence interval and Clinical consideration the values for Glucose, Potassium, Chloride, Calcium, Magnesium, Inorganic phosphate, Cholesterol, LDL. Cholesterol, Total Protein, Albumin in biochemistry and values of MPV and RDW-CV total white blood cell count, percentage count of Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophile in hematology did not vary between male and female subjects. It could be recommend that combined reference values for the above mentioned test parameters.

Findings of significant higher values in males than females for the following biochemistry parameters are: Creatinine, Urea, Uric acid, Sodium, Total Bilirubin, ALT, AST ALP Triglyceride (shown in table from 3.7 to 3.10 and from 3.22to 3.25 as well as table 3.19)are supported by Koram K et al. (2007)[60;]Kibaya et al. (2008)[61] and 3. Saathoff E, et al. (2008)[62]

Significant higher values of reference ranges for Creatinine, Urea, Uric Acid, BIL-T, ALT, AST, ALP and Triglycerides in males compared to females shown in table from 3.7 to 3.9 and from 3.22to 3.25 and table 3.19 supported YC LO, David A. Armbruster *et al.*[63]andManolio*et al.*(1992)

Sex differences in Creatinine, Urea, Uric Acid, AST, ALT and ALP have been known to exist due to differences in muscle mass which affects AST and ALT and bone mass which influences ALP.

Sex differences in the BIL-T values could be partly due to influence of sex hormones. These findings are in agreement with those of similar studies done in Uganda (Eller *et al*. 2008) for adults. Sex differences in Uric acid have been previously documented by Roche Diagnostics (2005), Eller *et al*. (2008) and Saathoff *et al*. (2008) which could be due to the influence of sex hormones and differences in body mass between genders.

Sex differences observed for Sodium and Uric could be attributed to the differences in response to dietary salts due to effects of sex hormone patterns and sex-related genetic factors. Similar findings were noted in Rwanda by Gahutu & Wane (2006) for adult humans.

There were no significance difference in lipid profile, values for total cholesterol and LDL Cholesterol but triglycerides are significantly higher ($P<0.05$) in male subjects as compared to female subjects shown in table 3.19. In contrast HDL Cholesterol are significantly higher ($P<0.05$) in female subjects as compared to male subjects shown in table 3.17. Higher triglycerides in males than females are supported by the study YC LO, David A. Armb YC LO, David A. Armbruster *et al*.[63] where was shown upper limit of triglyceride are significantly higher in males than females. The estrogen produced by female has been found to raise HDL cholesterol, generally giving female higher HDL levels than male.

In hematology parameters, the significantly higher values of the reference ranges for RBC, hemoglobin, HCT,MCV, MCH and MCHC in males compared to females indicates sex differences as shown in table from 3.26 to 3.31 . In contrast platelets in hematology are the significantly higher values in females compared to males as shown in table 3.33.These findings were documented for

the RBC parameters (haemoglobin, haematocrit and RBC), and this is consistent with an already-established knowledge that males have higher values for these parameters than females [64,.65,67,68.69]. Females had a higher platelet count compared to men, comparable to a study which looked at ethnic and sex differences in WBC and platelet counts [84]. The reasons for these differences have been attributed to factors such as the influence of the androgen hormone on erythropoiesis and menstrual blood loss in females [61,69].

The demonstration of significantly higher platelet values in females than males supports findings from previous three studies (1)Kibaya RS, Bautista CT, Sawe FK, Shaffer DN, Sateren WB, et al. (2008)[61] , (2)Koram K, Addae M, Ocran J, Adu-Amankwah S, Rogers W, et al. (2007)[60] and (3)Wakeman L, Al-Ismail S, Benton A, Beddall A, Gibbs A, et al. (2007)[65]

Age based partitioning of reference range are not applicable for the following biochemistry and all hematology parameter, since reference values of these parameters did not vary between less than 40 years and more than 40 years old subjects with consider statistical significance with 90% confidence interval and Clinical consideration.

The biochemistry parameters are included Glucose, Sodium, Potassium, Chloride, Calcium, Magnesium, Inorganic phosphate, Total Protein, Albumin, Total Bilirubin, Alanine aminotransferase*(*ALT*)*,Aspartate aminotransferase (AST) and Alkaline phosphatase(ALP) and Uric acid .The observed significant increase of some biochemical analytes and decrease of others as age progressed is an indication that these analytes are age dependent. Analysis between the two age groups showed higher reference intervals creatinine, urea, total cholesterol, LDL cholesterol and triglycerides in the above 40 years age group compare to less than 40 years age group shown in table 3.7, table 3.8,table 3.16, table 3.18 and table 3.19.

The increase in serum reference range for creatinine and urea in males and females with progression of age could be due to the decrease of renal integrity with advancing age; similar results have been reported by Verma *et al*. (1992).[66] Lipid Profile included total cholesterol, LDL cholesterol and triglyceride showed higher reference range in the above 40 years age group compare to less than 40 years age group could be due to taking rich food and decreasing of physical movement or labor similar results have been reported by the AHERF Apollo Hospital.[68]

From above findings it could be recommend that, separate reference values for urea, creatinine, total cholesterol, LDL cholesterol and triglycerides for less than 40 years and more than 40 years subjects.

Compare reference values between Bangladeshi population obtained from the present study and non Bangladeshi population found in literature:

Table from 4.1 to 4.6 compares reference range values for Bangladeshi population and non Bangladeshi population. Comparisons were based on the lower and upper reference limits and interval values of each analyte.

Reference values of glucose for present studied Bangladeshi population was similar to North Indian study[70] and slightly higher than Standard Text Book[35] shown in table 6.1. The World Health Organization (WHO) has said that someone may have diabetes if they have a fasting blood glucose of 7 mmol/L or more where the present study obtained 4.0 - 6.1 mmol/L in Bangladeshi population.

Table-4.1 Compare the observed reference values of fasting glucose, creatinine, urea and uric acid for Bangladeshi population and other populations.

Analyte (Unit)	Sex	Present Study	North Indian study[70]	Hong Kong study[63]	Hinduja National Hospital[71]	Standard text Book of clinical chemistry
Fasting Glucose (mmol/L)	M&F	4.0 -6.1	4.0-6.27	NA	NA	4.1- 5.9 [35]
	M	4.01 -6.1	4.04-6.27	NA	4.44-6.44	NA
	F	3.99 – 6.1	4.0-6.24	NA	4.38-6.38	NA
Creatinine (mg/dL)	M&F	0.57– 1.35	0.4-1.2	0.64-1.187	NA	NA
	M	0.76 -1.37	0.49-1.2	0.78-1.24	0.7-1.3	0.8- 1.3 [72]
	F	0.52– 1.08	0.4-1.19	0.62-0.94	0.6-1.0	0.6-1.1 [72]
Urea (mg/dL)	M&F	11 - 37	20-43	16.18-43.16	NA	17 – 43[35]
	M	13 – 38.3	20-40.62	19.18-44.95	10.7-36.38	NA
	F	10.5 - 34	20-45	13.78-38.36	10.7-34.4	NA
Uric Acid (mg/dL)	M&F	2.85-7.22	2.50-7.70	2.88-8.81	NA	NA
	M	3.48-7.5	3.44-7.81	3.73-9.32	3.8-7.8	3.5- 7.2[72]
	F	2.5-6.41	2.40-6.84	2.71-6.27	2.6-6.3	2.3 -6.6[72]

In the study values of Creatinine separately male and female in agreement with Hinduja National Hospital study[71]and standard Text Book [72] and slightly variation were obtained with Hong Kong study [63] and North Indian study[70]. Other renal function tests Urea were similar to Hinduja National Hospital study[71] but were varied from standard Text Book [35] and with Hong Kong study [63] and North Indian study[70]. Also Uric acid are almost similar to standard text book [72] and Hinduja National Hospital study[71] but some variation were occurred with Hong Kong study [63] and North Indian study[70] shown in table 6.1.

The observed variation in reference range values creatinine, urea and uric acid developed in this study compared to reference range values from other locations such as north India and Hong Kong a suggest variations in analytical methods in addition to ethnic composition and ecological parameters as stated by Saathoff *et al*. (2008). The higher reference range value urea and uric acid, compared to those of other locations could be due to genetic factors, dietary and environmental factors. Since urea is influenced by factors such as a high protein diet, variables in protein synthesis. Increased serum uric acid may reflect the

higher prevalence of metabolic syndrome in the population. Diet rich in purines as observed in animal proteins increases serum uric acid.

Table-4.2 Comparethe observed reference values for lipid profiles for Bangladeshi population andotherpopulations.

Analyte (Unit)	Sex	Present Study	North Indian[70]	Hong Kong[63]	Hinduja National Hospital[71]	National Cholesterol Education Program [75]
Chol (mg/dL)	M&F	122-249	117.4-247.6	115.7-254.2	NA	<200 Desirable
	M	118-244	115.5-255.7	115.0-257.3	126-267	
	F	132-250	118.6-244.0	116.9-247.7	123-263	
HDL.C (mg/dL)	M&F	25-55.51	32.17-64.0	30.76-72.69	NA	<40
	M	24-52	33.25-67.25	30.0-72.3	35-55	
	F	27-59	32-64	33.07-72.69	45-65	
LDL.C (mg/dL)	M&F	69-167	47.22-170.0	NA	NA	100 -139 Near optimal
	M	62-168	50-170.55	NA	67-192	
	F	71.5-167	42.87-170.0	NA	65-185	
TG (mg/dL)	M&F	47-277	48-190	45.45-390	NA	<150
	M	62.5-297	50.60-190.67	47.27-450	41-256	
	F	45-237	45.82-177.0	43.6-285.4	35-218	

Reference range of lipid profile in the present study were markedly vary than reference range assigned by National cholesterol education program expert panel(NCEP)[75].

The currently used recommended values are adapted from experts panel(NCEP)[75] decision arrived at upon analysis of cardiovascular event associated prospective studies performed on the caucasians largely. Data of Bangladeshi population is absent or lacking. As the proportion of persons with above recommended values is high and prospective studies among Bangladeshi is currently lacking, one cannot make definitive guidelines or recommendations at this juncture. Large prospective cardiovascular event associated studies are urgently needed.

Although reference range of lipid profile obtained from the present study were almost similar to others study such as North Indian[70], Hinduja National Hospital study[71] and Hong Kong study[63] shown in table 4.2.

In the present study values of serum cholesterol separately male and female are generally supported by other studies such as YC LO, David A. Armbruster*et al*[63][115.0-257.3 mg/dl for males and 116.9-247.7 for females] and Hinduja National Hospital study[71][126-267 mg/dl for males and 123 -263 mg/dl for females]as well as North Indian study[70][115.47-255.75 mg/dl for males, 118.6-244.0 mmol/L for females] shown in table 4.2.

Values of serum HDL.Cholesterol in combined and separately male and female were obtained variation with others studies such as YC LO, David A. Armbruster *et al*[63][30.0-72.3 mg/dl for males , 33.07-72.69 for females and 30.76-72.69 for combined] and North Indian study[70][33.25-67.25 mg/dl for males, 32.0-64.0 mmol/L for females and 32.17-64.0 for combined]as well as Hinduja National Hospital study[71][35-55 mg/dl for males and 45 -65 mg/dl for females] as shown in table 4.2. In some case upper limit for females are similar to our study but variation occurred in males reference range and females lower limit due to possible cause using different methodology, different instrument and calibrator traceability

Serum LDL.Cholesterol in combined and separately male and female are generally supported by the study North Indian study[70][50-170.55 mg/dl for males, 42.87-170.0 mg/dl for females and 47.22-170.0] where upper limit were similar to our study and lower limit were lower than our study and another study Hinduja National Hospital study[71][67-192 mg/dl for males and 65-185 mg/dl for females]

where lower limit were similar to our study and upper limit were higher than our study in both males and females.

Observed triglyceride values separately male and female are generally supported by the study Hinduja National Hospital study[71][41-256 mg/dl for males and 35 - 218 mg/dl for female] and YC LO, David A. Armbruster *et al*[63][47.27- 450.0 mg/dl for males 43.0- 285.4 mg/dl for females and 45.45-390.0 mg/dl for

combined] where female reference range were similar to our study but male reference range of YC LO, David A.

Armbruster *et al*[63] were in higher than our study and male reference range of Hinduja National Hospital study[71]were in lower than our study shown in table 4.2.

Although variation were obtained with other study such as North Indian study[70][50.60 – 190.67 mg/dl for males, 45.82-177.0 mg/dl for females and 48.0-190.0 mg/dl for combined] and USA population[105](<159.0 mg/dl).

Different food habit and life style as well as using different method and instrument may possible cause for this variation.

Reference range of electrolyte such as sodium, potassium and chloride were obtained similar to standard text book[36]withUSA population[77][Sodium 136-145 mmol/L, potassium 3.5-5.0 mmol/L and chloride 98-106 mmol/L] although some variation were shown with other study such as Hong Kong study [63] and North Indian study[70] .Observed values of total calcium and magnesium were comparable with Standard text book[35]but slightly difference with others study although this difference is not clinically significant. Also Phosphate values were almost similar to North Indian study[70]but slightly variation were obtained from other study[63] and standard text book [72] shown in table 4.3.

Table-4.3 Compare the observed reference values for Na, K, Cl, Ca, Mg and $PO_4$for Bangladeshi population and other populations.

Analyte (Unit)	Sex	Present Study	North Indian study[70]	Hong Kong study[63]	Singapore study[79]	Standard text Book of clinical chemistry
Sodium (mmol/L)	M&F	143-144	130-148	137-143	135-145	136-145 [36]
	M	134.8-145	129.9-147.1	137-144	NA	NA
	F	134.8-142	130-148	137-143	NA	NA
Potassium (mmol/L)	M&F	3.4-5.0	3.20-5.38	3.6-5.2	3.3 -4.9	3.5-5.1[36]
	M	3.4-5.0	3.18-5.30	3.5 -5.2	NA	NA
	F	3.48-4.8	3.14-5.50	3.6-5.2	NA	NA
Chloride (mmol/L)	M&F	99-108	93-107	102-109	96 - 108	98 - 107 [36]
	M	98-108	93.7-107	102-109	NA	NA
	F	97-107	92.2-107.8	103-109	NA	NA
Total Calcium (mmol/L)	M&F	2.2-2.65	2.05-2.65	2.19-2.50	2.10- 2.60	2.20-2.65 [35]
	M	2.2-2.65	2.12-2.70	2.19-2.49	2.15-2.55	NA
	F	2.2-2.63	2.05-2.57	2.19-2.51	2.12-2.55	NA
Magnesium (mmol/L)	M&F	0.75-1.05	0.69-1.03	0.8-1.14	NA	NA
	M	0.75-1.06	0.67-1.024	0.82-1.15	NA	0.73-1.06 [35]
	F	0.75-1.03	0.66-1.057	0.77-1.13	NA	0.77-1.03 [35]
PO_4 (mmol/L)	M&F	0.85-1.59	0.97-1.61	0.76-1.40	0.8-1.40	0.81- 1.45 [72]
	M	0.81-1.58	0.969-1.64	0.75-1.36	NA	NA
	F	0.87-1.59	0.92-1.647	0.77-1.41	NA	NA

Observed differences in reference range values for electrolyte, Ca, Mg and phosphate developed in this study of Bangladeshi population compared to those of other adult population could be due to differences in either the lower reference limit or the upper reference limits or both [80]. Differences in the lower and upper reference limits could be due to differences in the geographical location, methods and equipments used, sample size, posture, race, regional differences in the dietary intakes of foods rich in these analytes, and genetics [81,82,83].

Table-4.4 Compare the observed reference values for TP, Alb, Bil-T ALT, AST and ALP for Bangladeshi population and other populations.

Analyte (Unit)	Sex	Present Study	North Indian[70]	Hong Kong[63]	Hinduja National Hospital[71]	Standard text Book of clinical chemistry
TP (g/L)	M&F	64-82	62.3-85.0	63.0-79.1	NA	64-83[35]
	M	64-82	66.0-85.2	62.9-79.3	64-80	NA
	F	64-82	60.5-84.4	63.5-79.0	63-80	NA
Alb(g/L)	M&F	33-50	32.3-48.0	37.9-48.9	NA	33-50 [72]
	M	33-50	33.8-48.0	38.1-49.4	35-49	NA
	F	33-49	31.0-48.4	37.4-48.6	34-48	NA
Bil-T (mg/dL)	M&F	0.34-1.21	0.7-1.40	0.23-1.22	NA	0.3-1.2 [72]
	M	0.35-1.35	0.7-1.40	0.26-1.33	0.2-1.5	NA
	F	0.33-0.97	0.6-1.40	0.216-0.95	0.1-1.1	NA
ALT (U/L)	M&F	10-55	NA	7-49	NA	NA
	M	10-57	NA	8-57	12-72	<50[74]
	F	9-48	NA	7-39	9-50	<35[74]
AST (U/L)	M&F	12-38	NA	11-33	NA	NA
	M	12-39	NA	12-47	NA	<50[75]
	F	12-34	NA	11-26	NA	<35[75]
ALP (U/L)	M&F	44-120	NA	39-142	NA	30-120[73]
	M	45.03-125	NA	47-168	38-123	NA
	F	41.47-118	NA	36-105	35-120	NA

Reference values for total Protein and Albumin were similar with the Hinduja National Hospital study[71] and Standard Text book[35,72] but slightly variation obtained from other study[63,70], this variation do not affect on clinical decision.

Liver function test in the study only the reference value of total Bilirubin and alkaline Phosphatase were comparable with Standard text [72,73] as well as ALT and AST values were higher than Standard text book[74]. Higher levels of the liver enzymes alanine transaminase and aspartate transaminase are commonly discovered in asymptomatic in subjects. Evidence to guide the diagnostic workup is limited. If the history and physical examination do not suggest a cause. The most common cause is nonalcoholic fatty liver disease, which can affect up to 30 percent of the population. ALP and Bilirubin total value also similar to Hinduja National Hospital study[70] and Hong Kong study[63]respectively.

Although obtained ALT value almost similar to Hong Kong study[63].(shown in table 3.4).The higher reference range value for TP, and ALP, compared to those of other locations could be due to genetic factors, dietary and environmental factors Manolio *et al*. The differences in the reference range value for AST compared to those determined from other literature sites could be explained by differences in genetic factors and muscular exertion; these results agree with those reported in studies in six Asian cities and Ghana (Ichihara *et al*., 2008; Koram *et al*., 2007).

In hematology the results obtained from the present study demonstrated that the red blood cell parameters (haemoglobin, haematocrit MCV and MCH) were lower than values set as standards text book[104] of the clinical hematology. Also our study obtained these value lower than USA population.[77]Although study obtained RBC values almost similar to standard text and USA population[77] shown in table 4.5 as well as Platelets count of our study are lower than Standard text book of hematology [76] but similar to USA population [77]. Such variations are expected for populations in different geographical/ecological locations. Primarily nutritional factors and socioeconomic condition being the possible causes of lower value of hemoglobin, HCT, MCV and MCH parameters. This needs to be further addressed especially as this population is more reflective of the middle and upper socio-economic class.

This difference could be due to environmental or genetic factors or a combination of both or to several other factors. Such differences indicate the need to develop reference values that are appropriate for the applicable population.

Table 4.5Compare the observed reference values for hematology parameters RBC, Hb, HCT, MCV, MCH, MCHC, RDW-CV, PLT for Bangladeshi population and other populations.

Analyte (Unit)	Sex	Present study	Standard text Book of Hematology[76]	Diagnostic Perspectives[78]	USA[77]
RBC (10^6/µl)	M	4.4 - 5.6	4.5 - 5.5	4.44 -5.63	4.5-5.9
	F	3.9 - 5.2	3.8 - 4.8	3.92- 5.08	4.0-5.2
Hb (gm/dl)	M	12.4 - 16.55	13 - 18	13.5-16.9	13.5-17.5
	F	10.3 - 13.6	11.5 - 16.5	11.9-14.6	12.0-16.0
HCT (%)	M	36.5 - 48.8	40- 54	40.0-49.4	41.0-53.0
	F	32.1 - 41.7	37-47	36.6-44.0	36.0-46.0
MCV (fl)	M&F	73.4 - 94.2	76-94	NA	80-100
	M	NA	NA	81.8-95.5	NA
	F	NA	NA	82.9-98.0	NA
MCH (pg)	M&F	23.4 - 32.2	27-32	27.0-32.3	26.0-34.0
	M	NA	NA	NA	NA
	F	NA	NA	NA	NA
MCHC (gm/dl)	M&F	31.2 - 35.6	31-35	NA	31.0-37.0
	M	NA	NA	20.1-21.7	NA
	F	NA	NA	19.7-21.5	NA
RDW-CV (%)	M&F	12.2 - 15.1	11-14	NA	NA
	M	NA	NA	12.0-13.6	NA
	F	NA	NA	12.1-14.3	NA
Platelets (10^3/µl)	M&F	155.4- 344	150 - 400	NA	150-350
	M	151 - 331	NA	NA	150-350
	F	175 - 420	NA	NA	150-350
MPV (fl)	M&F	9.2 - 12.7	5 -11	NA	NA
	M	NA	NA	9.3-12.1	NA
	F	NA	NA	9.1-11.9	NA

Table 4.6Compare the observed reference values for hematology parameters white blood cell and it's differential counts for Bangladeshi and other populations.

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Analyte (Unit)	Sex	Present study	Standard text Book of Hematology[76]	Diagnostic Perspect[78]	USA[77]
WBC (10^3/ µl)	M&F	4.5 - 11.0	4- 11	NA	4.5-11.0
	M	NA	NA	3.91-10.9	NA
	F	NA	NA	4.49-12.68	NA
Neutrophil (%)	M&F	45.6 - 72.1	40- 75	NA	NA
	M	NA	NA	41.0-70.7	NA
	F	NA	NA	42.9-74.3	NA
Lymphocyte (%)	M&F	21.6 - 47.7	20- 50	NA	NA
	M	NA	NA	19.1-47.9	NA
	F	NA	NA	18.3-45.7	NA
Monocyte (%)	M&F	2.2 - 6.7	2 -10	NA	NA
	M	NA	NA	5.2-15.2	NA
	F	NA	NA	18.3-45.7	NA
Eosinophil (%)	M&F	0.7 -6.9	1 -6	NA	NA
	M	NA	NA	0.6-7.6	NA
	F	NA	NA	0.2-5.3	NA
Basophil (%)	M&F	0.0 - 0.3	<1	NA	NA
	M	NA	NA	0.1-1.2	NA
	F	NA	NA	0.1-1.0	NA

Total white blood cell count and percentage count of neutrophil lymphocyte and eosinophil occurred from the present study were in agreement with Standard text book of hematology [76] but monocyte count are lower .Total white blood cell count developed by present study similar to USA population [77].

4.2 Conclusion

The reference values developed for the Bangladeshi population will be of immense benefit to most clinical trials requiring monitoring of hematological and biochemical parameters and patient care in general. The reference interval established here ,which is differed from the currently used (standard text book, guideline such as NECP and reagent literature) reference values.

The 2.5% lower reference limit was lower for hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin .The 97.5% upper reference interval was higher for fasting glucose, inorganic Phosphate total cholesterol, HDL. cholesterol ,LDL. cholesterol triglycerides, alanine aminotransferase(ALT) and aspartate aminotransferase (AST).

Gender based partitioning is required for Urea, Creatinine, Uric acid, Sodium, Triglyceride, HDL Cholesterol Total Bilirubin, Alanine aminotransferase (ALT),Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in biochemistry and Red blood cell (RBC) count, Hemoglobin, Hematocrite, MCV (Mean Corpuscular volume) Mean Corpuscular Hemoglobin(MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and Platelets count (PLT) in hematology. Age based reference intervals for renal function and lipid profile need to be considered.

Reference values established by this study could have top importance for quality of health care in the clinical management of patients. Further nationwide study where included large sample volume should be carried out to establish the biochemical and hematological reference values of the Bangladeshi population as a whole.

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Annex I

CONSENT FORM FOR RESEARCH WORK

I, the undersigned, Mr. / Mrs. ……..
have been given all information about the nature of the research being undertaken in this Laboratory.

I give my consent willingly to this Laboratory to perform all kinds of test on my blood sample

Donar's' Name/Legal Guardian: _____ **Witness Name: _____**

Signature: _____ **Signature: _____**

Date: _____ **Date: _____**

Consent form in Bangla

রিসার্চ কাজের জন্য সম্মতি পত্র

আমি নিম্ন স্বাক্ষরকারী জনাব/জনাবা ---------- এই ল্যাবরেটরীতে বাংলাতে রিসার্চ সম্পর্কে সবিশেষ অবহিত হয়েছি। আমি এই ল্যাবরেটরী কর্তৃপক্ষকে আমার রক্তের নমুনায় বিভিন্ন পরীক্ষার জন্য সম্মতি প্রদান করলাম।

রক্তদাতার নাম/ রক্তদাতার অভিভাবকের নাম : স্বাক্ষীর নাম: ----------

স্বাক্ষর: ---------- **স্বাক্ষর: ----------**

তারিখ: ---------- **তারিখ: ----------**

Annex II

QUESTIONNAIRE FORM

Name of investigator :

Name of Volunteer: Age: Gender: Male/Female
ID No-
BP: Height: Weight:
Contact No: District/Division:

SN	Questionnaire	Response	
1.	Citizen of Bangladesh	Yes	No
2.	Age range 18 -65 Years	Yes	No
3.	Obese	Yes	No
4.	Hypertensive	Yes	No
5.	Diabetic	Yes	No
6.	Pregnant(in case of female)	Yes	No
7.	Involved in excessive Exercise	Yes	No
8.	Under any medication	Yes	No
9.	Taken any oral contraceptive (for female)	Yes	No
10.	Smoker	Yes	No
11.	Any kind of tobacco user	Yes	No
12.	Alcohol user	Yes	No
13.	Fasting Condition	Yes	No
14.	Virus screening result, if done 1.HbsAg Positive Negative 2.HCV Positive Negative 3.HIV Positive Negative		Not done
15.	Syphilis VDRL/RPR Positive Negative		Not done

Acceptance: YES/ NO

Signature:

Date: