Republic of Sudan
Ministry of high education and scientific research
Shendi University
College of Graduate Studies

Pulmonary Function Values in Association with Variable Tests for Healthy Adult Eritrean Population – Eritrea

A thesis Submitted in Fulfillment for the Requirements of the Ph.D. degree in medical sciences - Human Physiology

By

Barakat Mohazab Bakhit Ahmed

Supervisor

Prof. Omer Abdalaziz Musa

Co-Supervisor

Dr. Salwa Ahmed Alsayed Mohammed

2022

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيم

قال تعالى:

(وَفِي أَنفُسِكُمْ أَفَلا تُبْصِرُونَ)

صدق الله العظيم

(سورة الذاريات : الايه 21)

Declaration and statement

I, Barakat Mohazab Bakhit, undersigned, declare this thesis is an original our original work. I have followed all ethical and technical principles in the preparation, data collection, analysis, and compilation of this thesis. Any scholarly matter included in this thesis has been recognized through citation. I solemnly declare that this thesis has not been submitted to any other institutions anywhere for a word of any academic degree- diploma, or certification.

Signature………………

Date …………………...

DEDICATION

To all my family in particularly my mother, wife, and son

Acknowledgments

I want to thank Allah for everything and for making this effort possible.

Prof. Omer Abdalaziz Musa has provided me with the best direction and supervision, taught me that anything is possible with patience and perseverance, and sparked my interest in physiology. In addition, I would like to thank my co-supervisor, Dr. Salwa Ahmed Alsayed Mohammed, for her aid and encouragement.

I'm also thankful to my colleague, Dr. Ahmed Osman Noury Saleh, for being careful, patient, and working hard while gathering data. I want to thank the president of Eritrea's National Council for Higher Education for helping and making things easier.

This work was only completed with the help of the dean of Orotta College of Medicine and Health Sciences. This thankfulness is extended to everyone who participated and to my colleague, Kamal Mohamad Awad Osman, for his assistance.

Abstract

Background: The high prevalence of respiratory diseases in Eritrea is a primary concern in the country's public health sector, as is the global spread of covid-19 and World Health Organization guidelines for the importance of deriving lung function prediction equations and filling the gap in the availability of lung function data in East Africa.

Objectives: To establish the Eritreans' normal pulmonary test reference values by constructing new reference equations based on age and height for forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and peak expiratory flow rate (PEFR) in 18–60-year-old Eritreans of both sexes. In addition, to predict lung function using a flexible and effective statistical technique.

Research design and method: This was a cross-sectional community-based study carried out in all geographical areas of Eritrea in the year 2020. A questionnaire, and pulmonary function tests which done by a portable spirometer to measure FVC, FEV1, and PEFR, and anthropometric measurements were used to collect the data. Polynomial regression and generalized additive models for location, scale, and shape (LMS) were used to predict the FVC, FEV1, and PEFR using age, height, weight, and altitude as variables.

Results: In total, 566 (200 males, 366 females) healthy, non-smoking adult Eritreans aged (18 to 60) were recruited from three cities in Eritrea. Spirometry prediction equations were developed by correlating lung function with anthropometric measurements. Age negatively correlates with lung function, while height and weight have a positive correlation. The average FVC, FEV1, and PEFR in Asmara are higher than in other cities. Eritreans had higher values than Sudanese and lower than African Americans (GLI) compared to their prediction values.

Conclusion: The study establishes the FVC, FEV1, and PEFR reference values for pulmonary function in healthy, nonsmoking adult Eritrean. These values can be used as references in respiratory clinics throughout Eritrea. Predicted values from the Sudanese reference equation and the global lung initiative for African Americans show that they need to be better fitting for Eritreans.

الخلاصة

المقدمه : يعد الانتشار الواسع لأمراض الجهاز التنفسي في إريتريا مصدر قلق رئيسي في قطاع الصحة العامة بالبلاد ، كما هو الحال مع الانتشار العالمي لكورونا ، وكذلك إرشادات منظمة الصحة العالمية لأهمية استنتاج معادلات التنبؤ بوظائف الرئة وايضا لمعالجة الفجوة في توافر بيانات وظائف الرئة في شرق أفريقيا.
الأهداف: للتنبؤ بمعادلات وظائف الرئة باستخدام تقنية إحصائية مرنة وفعالة للإريتريين الذين تتراوح أعمارهم بين 18-60 عامًا. لتطوير معادلات تنبؤ السعة الحيوية القصوى ، وحجم الزفير الأقصى في ثانية واحدة ، ومعدل تدفق الزفير الأقصى للإريتريين.

الطريقه: هذه دراسة مقطعية مجتمعية أجريت في جميع المناطق الجغرافية لإريتريا في عام 2020 ، تم استخدام نماذج الانحدار متعدد الحدود وتعميم النماذج المضافة للموقع والمقياس والشكل للتنبؤ بالقدرة الحيوية القسصوي ، وحجم الزفير الأقصى في ثانية واحدة ، ومعدل تدفق الزفير الأقصى باستخدام العمر والطول والوزن والارتفاع كمتغيرات. تم استخدام استبيان واختبار وظائف الرئة وقياسات الطول والوزن لجمع البيانات.

النتائج: شملت الدراسه في المجموع 566 (200 ذكر ، 366 أنثى) من الإريتريين البالغين الأصحاء الغير مدخنين تتراوح أعمارهم بين 18 و 60 عامًا من ثلاث مدن في إريتريا. تم تطوير معادلات تنبؤ قياس التنفس من خلال ربط وظيفة الرئة بالقياسات البشرية. يرتبط العمر ارتباطًا سلبيًا بوظيفة الرئة ، بينما يرتبط الطول والوزن ارتباطًا إيجابيًا . متوسط السعة الحيوية القصوى و حجم الزفير الأقصى في ثانية واحدة ، ومعدل تدفق الزفير الأقصى في أسمرة أعلى منه في المدن الأخرى. كان للإريتريين قيم أعلى من السودانيين وأقل من واقل من الأمريكيين من أصل أفريقي مقارنة بقيم تنبؤاتهم.

الخلاصة: تحدد الدراسة القيم المرجعية للسعة الحيوية القصوى ، وحجم الزفير الأقصى في ثانية واحدة ، ومعدل تدفق الزفير الأقصى لوظيفة الرئة لدى البالغين الأصحاء وغير المدخنين. يمكن استخدام هذه القيم كمراجع في عيادات الجهاز التنفسي في جميع أنحاء إريتريا. تظهر القيم المتوقعة من المعادلة المرجعية للسودانيين والعالمية للأميركيين الأفارقة أنها لا تناسب الإريتريين.

Table of contents

1. Chapter 1 Introduction 1
1.1. Eritrea Profile 2
1.1.1. Population 2
1.1.2. Health Care Services 3
1.2. Spirometry 4
1.2.1. Spirometry Indices 4
1.2.2. Spirometry Reference Equations and Lower Limit of Normal (LLN) ... 5
1.3. Research Objectives 6
1.3.1. General objective 6
1.3.2. Specific objectives 6
Chapter 2 Literature Review 1
2. Literature Review 10
2.1. Spirometry's Historical Background 10
2.2. The influence of ethnicity on the prediction of spirometry reference equations 11
2.3. The impact of altitude on spirometry reference values 12
2.4. The impact of Exercise on spirometry reference values 13
2.5. Polynomial regression 13
2.6. Generalized Additive Models 16
2.7. African country: spirometric predictive equations in Africa 19
3. Chapter 3 Materials and Methods 25
3.1. Methods 25
3.1.1. Study Design 25
3.1.2. Study area/setting 25
3.1.3. Study Population 25

3.1.4. Inclusion criteria: 25
3.1.5. Exclusion criteria: 26
3.1.6. Sampling 26
3.1.7. Sample Size 26
3.1.8. Sample size criteria: 26
3.1.9. Sample size equation: 27
3.2. Materials 27
3.2.1. Questionnaire: 27
3.2.2. Consent form 28
3.2.3. Anthropometric Measurement 28
3.2.4. Blood Pressure (BP) Measurement 28
3.2.5. Pulmonary functions: 28
3.2.6. Recording of PFTs: 29
3.3. Variables: 29
3.3.1. Dependent variables 29
3.3.2. Independent Variables 29
3.3.3. Background variables 30
3.4. Statistical Analysis 30
3.4.1. Derivation of Reference equation: Polynomial regression method 30
3.4.2. Derivation of Reference equation: LMS method 31
3.4.3. Comparisons of Reference Equations 31
4. Chapter 4 Results 33
4.1. Lung function values in adult Eritreans by age group 35
4.1.1. Lung function values for adult Eritrean males 35
4.1.2. Lung function values for adult Eritrean females 37
4.2. Socio-demographic Characteristics 39
4.3. Anthropometric and Spirometric Characteristics 41

4.4. Distribution of Participants' height and weight by gender and age 43
4.5. Lung function values in Eritreans by gender 43
4.6. Comparing age and height across altitude for male 45
4.7. Comparing age and height across altitude for females 46
4.8. Correlation of the Lung Function parameters with Anthropometric and socio-demographic variables 47
4.8.1. Correlation with quantitative variables 47
4.8.2. Comparison of mean lung function parameters with qualitative variables 49
4.8.3. Bonferroni Post-hoc Pairwise comparisons 53
4.9. Correlation of the Lung Function Parameters 55
4.10. Development of new Reference Equation for Spirometry 57
4.10.1. Male new spirometric equations 59
4.10.2. Female new spirometric equations...60
4.11. Comparison of the predicted values by age 61
4.12. Predicted spirometer variables with varying age and height 63
4.12.1. Predicted spirometer parameters with varying age and height for males 63
4.12.2. Predicted spirometer parameters with varying age and height for females65
4.13. Reference Equation using LMS method 66
4.13.1. Reference equation of FVC for males and females 67
4.13.2. Model fit for FVC reference equations by LMS method 68
4.13.3. Reference equation of FEV1 for males and females 71
4.13.4. Model fit for FEV1 reference equations by LMS method....................72
4.13.5. Reference equation of PEFR for males and females 75

4.13.6. Model fit for PEFR reference equations by LMS method....................76
4.14. Predicted spirometer parameters with variable age and height for males using the LMS method..79
4.15. Predicted spirometer parameters with variable age and height for females using the LMS method..81
4.16. Difference between measured and predicted values 83
4.16.1. Difference between measured and predicted values among males 83
4.16.2. Difference between measured and predicted values among females ...85
4.16.3. Differences between Altitudes using LMS method 86
Chapter 5. Discussion, ...32
5. Discussion...74
5.1. Conclusion...79
5.2. Recommendation..80
References...81
Appendix 1...90
Appendix 2...92
Appendix 3...93
C-1: FVC age-specific spline values for males and females...............................93
C-2 FEV1 age-specific spline values for males and females 97
C-3 PEFR age-specific spline values for males and females 101
Appendix 4..106
Appendix 5..107
Appendix 6..108
Appendix 7..110

List of Tables

Table 4. 1 Lung function values in adult Eritrean males by age, height, weight, and altitude groups 35

Table 4. 2 Lung function values in adult Eritrean females by altitude, age, height, and weight groups 37

Table 4. 3 Socio-demographic characteristics of the study participants (n=566) ... 39

Table 4. 4 Anthropometric, health-related, and spirometric characteristics of the study participants 41

Table 4. 5 Distribution of participants' height and weight by age category 43

Table 4. 6 Comparing age and height across altitude for male 45

Table 4. 7 Comparing age and height across altitude for females 46

Table 4. 8 Correlation of males' lung function parameters with quantitative anthropometric and health variables 47

Table 4. 9 Correlation of females' lung function parameters with quantitative health-related, anthropometric measures 48

Table 4. 10 Comparison of mean lung function parameters of males across the categories of the qualitative variables 49

Table 4. 11 Comparison of mean lung function parameters of females across the categories of the qualitative variables 51

Table 4. 12 Further comparison of the mean lung function parameters across the categories of Altitude and Educational Level 53

Table 4. 13: Pearson's correlation matrix of the lung function parameters of male participants 55

Table 4. 14 Pearson's correlation matrix of the lung function parameters of female participants 56

Table 4. 15 The combination of independent variables comparing the adjusted R^2 and standard error of the estimate 57

Table 4. 16 Estimates during the development of new prediction equations for spirometry among males 59
Table 4. 17 Estimates during the development of new prediction equations for spirometry among females 60
Table 4. 18 Selected reference equations for FVC, FEV1, and PEFR 61
Table 4. 19 Predicted spirometer variables with varying age (year) and height (cm) for males 63
Table 4. 20: Predicted spirometer variables with varying age (year) and height (cm) for females 65
Table 4. 21 Comparison of the FVC models with L≠1 and L=1 for both males and females 67
Table 4. 22 LMS reference equations for FVC stratified by gender 68
Table 4. 23 Comparison of the FEV1 models with L≠1 and L=1 for both males and females 71
Table 4. 24 LMS reference equations for FEV1 stratified by gender 72
Table 4. 25 Comparison of the PEFR models with L≠1 and L=1 for both males and females 75
Table 4. 26 LMS reference equations for PEFR stratified by gender 76
Table 4. 27 Predicted spirometer parameters with variable age and height for males using the LMS method 79
Table 4. 28 Predicted spirometer parameters with variable age and height for females using the LMS method 81
Table 4. 29 Differences for FVC, FEV1, and PEFR between observed and predicted values according to different reference equations among males 83
Table 4. 30 Differences for FVC, FEV1, and PEFR between observed and predicted values according to different reference equations among females 85

Table 4. 31 The significance of the inclusion of additional variable altitude in the derived equations using LMS for the spirometric parameters.................................86

List of Figures

Figure 4.1 Flow chart showing eligibility of study participants..............................33
Figure 4.2: Histogram of the spirometric parameters stratified by age and Kolmogorov-Smirnov measures..34
Figure (4.3) Error plots comparing the spirometric parameters by gender. 44
Figure (4.4) Scatter plot showing the predicted values of the current equation and that of Sudan by age...62
Figure 4.5 FVC model diagnosis for males using residual plot (left) and worm plot (right)...69
Figure (4.6) FVC model diagnosis for females using residual plot (left) and worm plot (right) 70
Figure (4.7) FEV1 model diagnosis for males using residual plot (left) and worm plot (right) 73
Figure 4.8 FEV1 model diagnosis for females using residual plot (left) and worm plot (right) 74
Figure (4.9) PEFR model diagnosis for males using residual plot (left) and worm plot (right) 77
Figure (4.10) PEFR model diagnosis for females using residual plot (left) and worm plot (right)...78

List of Abbreviations

A	Age (years)
AIC	Akaike information criterion
ATS	American thoracic society
BMI	Body mass index
COPD	Chronic obstructive pulmonary disease
Cm	Centimeter
DBP	Diastolic blood pressure
ERS	European respiratory society
ECSC	European Coal and Steel Community
EXP	Function in Excel meaning e raised to the power of the number
FVC	Forced vital capacity
FEV1	Forced expiratory volume in the first second
FEF25–75%	Forced expiratory flow at 25–75 percent of FVC
GAM	Generalized additive models
GAMLSS	Generalized Additive Models for Location, Scale and Shape
GLI	Global lungs initiative
H	Height (cm)
HIV	Human immunodeficiency virus
L	lameda (index of skewness) – shape

LMS	Lambda-mu-sigma method
LLN	Lower limit of normal
LN	Natural logarithm of the number
M	Meter
M	Mean
M	mu (predicted value) – location
MMEF	Maximal mid-expiratory flow
M-S	M-spline
NSO	National statistics office
PEF	Peak expiratory flow
PEFR	Peak expiratory flow rate
PFTs	Pulmonary function tests
S	sigma (coffecint of variation) – scale
SBC	Schwartz's bayesian criterion
SBP	Systolic blood pressure
S-S	S-spline.
SD	Standard deviations
Sq	Square kilometer
SPSS	Statistical package for the social sciences
VC	Vital capacity

VCT	Voluntary counseling and testing

Chapter 1

Introduction

1. Introduction

Respiratory disorders have been one of the most severe diseases affecting humans since antiquity, but their significance has lately risen due to the discovery of COVI D19. Spirometry is an essential diagnostic tool for general practice.

It should play a vital role in diagnosing and managing chronic respiratory diseases, such as diagnosing or ruling out chronic obstructive pulmonary disease (COPD), confirming asthma, determining the efficiency of asthma treatment, and correctly staging COPD patients (Derom et al., 2008).

Furthermore, this research is critical. Because it is the first study of its kind for East Africa and the first study of its kind for Eritrea. According to a study conducted in Sudan, most African countries have spirometry reference values significantly different from those of the world average. Aside from ethnicity, height, gender, and weight, altitude was one of the variables we used in our research (Bashir and Musa, 2012). For this reason, we divide the country into three different regions.

Spirometry is a physiological test that determines how much air a person can take in and expel while exerting maximum effort (Graham et al., 2019).

The primary signal in spirometry is either volume or flow as a function of time (Graham et al., 2019). The forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) are the parameters measured by the spirometer(Graham et al., 2019).

It can also assess the vital capacity (VC) and (FEV1/FVC) ratio. Peak expiratory flow rate (PEFR), and inspiratory vital capacity (IVC) at (25%, 50%, and 75%) of FVC, respectively. Inspiratory vital capacity (IVC) (Moore, 2012).

1.1. Eritrea Profile

Eritrea is in the Horn of Africa, between (12 00'N) and (36 026'21″E). It covers (122,000) sq. km ((NHERI), 2021). The Red Sea runs (1,212) kilometers east of the country, from Ras Kasar to Dar Elwa. Eritrea shares borders with Djibouti, Ethiopia, and Sudan. Eritrea is divided into (6) zobas (regions):Anseba, Debub, Debubawi Keih Bahri, Gash-Barka, Maekel, and Semenawi Keih Bahri ((NHERI), 2021). Eritrea is a land of contrasts, rising from sea level to (3,000) meters. The Western Lowlands, Central and Northern Highlands, and Eastern Lowlands are the major physiographic zones (also referred to as the Coastal Plains)((NHERI), 2021). Temperatures in the Highlands range from (16°-18°C) to (18° - 28°C) in the Lowlands and more than (30°C) in the Coastal Plains. The Western Lowlands and Coastal Plains are hot and dry, while the Highlands are relatively cool. Several major cities in Eritrea, including Asmara, are in the Central Highlands((NHERI), 2021). The Coastal Plains are Eritrea's (2) major port towns, Massawa and Assab((NHERI), 2021). The Central Highlands are the most densely populated, while the lowlands are the least populated. Rainfall in Eritrea ranges from less than (200 mm) to over (1,000 mm) per year. Eritrea has (2) main rainfall periods; one covers both the Western Lowlands and the Highlands from June to September. The second covers the Eastern Lowlands from October to March ((NHERI), 2021).

1.1.1. Population

Eritrea has never conducted a population census; however, according to the Ministry of Local Government's population count and NSO estimates, Eritrea's total resident population in 2010 was around (3.2) million ((NHERI), 2021). The population is primarily rural, with approximately (65%) of the people residing in the countryside. The urban population is rapidly growing, partly because of returning refugees from bordering and other countries; this is partly because of high rural-urban migration((NHERI), 2021). Eritrea's population is not evenly distributed, and the highlands are home to (50-60%) of the population ((NHERI), 2021). The age distribution is typical of a high fertility regime, with a large proportion of the people in the younger age groups than in the older age groups ((NHERI), 2021). Eritrea is a multi-ethnic society with (9) ethnic groups speaking (9) different languages and (2) major religions, Christianity and Islam ((NHERI), 2021).

1.1.2. Health Care Services

Eritrea currently has (343) health facilities, including (60) clinics (VCT, clinics in various industries, dental clinics, etc.), (190) health stations, (54) health centers, (14) community hospitals, (16) hospitals, and eight maternal and child health care centers ((NHERI), 2021).

In 2018, the most common diseases were, acute upper respiratory infections, pneumonia, acute pharyngitis/tonsillitis, gastroenteritis/duodenitis, urinary system diseases, diarrhea without dehydration, disorders of skin/subcutaneous tissues, dental caries, conjunctivitis, and another conjunctival disease ((NHERI), 2021).

According to the ranking, the most common diseases treated at inpatients in 2018 were: severe pneumonia, pneumonia, diarrhea with severe dehydration, urinary system diseases, soft tissue injury, no nerve/blood vessel involvement, skin/subcutaneous tissue infections, asthma, fracture of limb bones, cataracts, and other lens disorders diabetes mellitus, as well as gastroenteritis/duodenitis ((NHERI), 2021).

There is currently an epidemiological shift in Eritrea, as it is in many developing countries. because life expectancy has increased, the move from communicable to non-communicable diseases is primarily due to demographic and nutritional changes (from 48 years at birth in 1990 to over 67 in 2013). in 2018, Eritrea's maternal mortality ratio was reduced to (486/100,000) or as per the health facility based maternal mortality ratio(126/100,000), neonatal mortality rate to (18.2/1,000), under (5) mortality rate to (45.9/1,000), HIV prevalence to (0.65%), HIV incidence to (0.11%)((NHERI), 2021).

1.2. Spirometry

1.2.1. Spirometry Indices

The most measured indices in spirometry include FVC, FEV1, (FEV1/FVC), and (PEF). Obstruction and restriction of lung function are measured with the help of these parameters (Moore, 2012, Pellegrino et al., 2005, Anand et al., 2003, Wanger et al., 2005). Lung volume decreases due to restrictive disorders (or restrictions)(Moore, 2012, Hallett et al., 2018, Naji et al., 2006, Irvin, 1998). The following is a brief description of FVC, FEV1, FEV1/FVC, and PEF: FVC, which is the volume delivered during a forceful and complete expiration beginning with full inspiration (Graham et al., 2019, Lassenius et al., 2020, Chhabra, 1998). FVC is measured in liters. A reduced FVC means restrictive disorders(Vaz Fragoso et al., 2010, Hnizdo et al., 2006, Hansen et al., 2007).

Forced Expiratory Volume (FEV1). FEV1 measures how much air can be exhaled during a forced expiration at the first second(Moore, 2012). This index is also measured in liters. A reduced value of FEV1 means an obstructive disease (Moore, 2012, Hansen et al., 2007).

The ratio of (FEV1/FVC), is also known as the Tiffeneau Pinelli index (Yao et al., 2013). This ratio is used in the diagnosis of restrictive and obstructive lung diseases. It represents the proportion (or %) of a person's VC that can be expired in the first second of exhalation; in healthy people, this should be above the lower limit of normal(LLN) 70 % (Swanney et al., 2008).

Peak expiratory flow (PEF) is the maximal flow exhaled when blowing out at a steady rate (Moore, 2012).

1.2.2. Spirometry Reference Equations and Lower Limit of Normal (LLN)

Individuals' ethnic and demographic characteristics create spirometry reference equations, such as age, height, weight, and sex. (Quanjer et al., 2012a). These equations are developed through statistical techniques such as regression models. Only healthy nonsmokers were used as references in the development of these equations. This standard, healthy state is then compared to the lung function values of an individual's test results to evaluate their lung function. As a result, developing appropriate reference values is critical for interpreting PFT and assessing lung function and respiratory diseases.

When interpreting LFT results, the first thing to see is within the expected normal range. A (5 %) error rate (i.e., $P < 0.05$ for the probability of a chance association and ± (2) standard deviations (SD) to encompass (95 %) of a value customarily distributed in the population) has been widely accepted in both research and clinical medicine. The lower limit of normal (LLN) is defined as the 5^{th} percentile of a healthy population in PFT. After similar statements appeared in the early 1980s, this was confirmed in the 2005 document by the American Thoracic Society and the European Respiratory Society (ATS/ERS)(Culver, 2012).

1.3. Research Objectives

1.3.1. General objective

- To establish the normal pulmonary function test reference values for all Eritreans.

1.3.2. Specific objectives

- To establish new reference equations according to age and height for (18-60) years old Eritreans of both sexes for FVC, FEV1, and PEFR.
- To predict lung function using a flexible and effective statistical technique for Eritreans ages between (18 and 60) years old.

The study's objectives result in two main research questions, which are as follows:

1. What are the available spirometry reference equations for lung function variables in adult Eritreans between (18 and 60) years from various ethnic groups?

(a) How are the FVC, FEV1, FEV1/FVC ratios, and PEFR modeled statistically?

(b) What adult ages are considered for predicting lung function?

(c) Which variables are used as predictors for lung function indices modeling?

(d) What statistical procedure or technique was conducted to analyze the LLN?

(e) What is the optimal technique for predicting lung function variables?

2. What would be the reference equations for predicting lung function indices (FVC, FEV1, FEV1/FVC) for adult Eritreans aged between (18 and 60) years old?

(a) What data and variables will be analyzed in this study?

(b) Which approach/model is being evaluated for lung function indices modeling?

(c) How can the best models be selected?

(d) What is the lower limit of normal (LLN) for each spirometry indices?

This study's initial objective accomplished a detailed literature review on spirometry reference equations and LLN. Using the information gleaned from the literature review, we used the best technique for modeling spirometry prediction equations and LLN in Eritreans aged (18–60).

Chapter 2

Literature Review

2. Literature Review

A vital goal of this study is to develop a statistical method for calculating lung function prediction equations and LLN in Eritreans ages (18 to 60). This chapter addresses the spirometry theory and research questions for the first objective. Additionally, this will help identify any theoretical gaps in spirometry, both scientifically and statistically. Researchers focus on how different ethnic groups differ in their lung function, while statisticians concentrate on the best method for predicting lung function values for each group. In general, it is recommended to create separate equations for male and female lungs because of the wide range of differences in lung function between genders.

2.1. Spirometry's Historical Background

To determine if observed spirometry readings are normal or pathological, one must first define "normal" and "healthy. Hutchinson, who published the first work in lung function tests, measured the VC of (2000) healthy men in the mid-1840s and determined that the VC grew with height (H) and declined with age (A)(Hutchinson, 1846). This was the starting point for today's spirometry reference values. The 1920s saw the next significant advancement in pulmonary function testing. Physicians such as Georges Dreyer pioneered spirometry in pre-operative thoracic surgery assessments, occupational lung disease evaluation, and the respiratory problems encountered by pilots during first world war I and documented the growing recognition of asthma and emphysema complications(Spriggs, 1978).

Two French physicians, Robert Tiffeneau and André Pinelli made a significant advance in 1947 when they proposed the forced expiratory volume in one second (FEV1) which is defined as the largest forced volume that can be expired in one second following a maximum inspiration (Tiffeneau and Pinelli, 1947). The

Tiffeneau–Pinelli index, which represents the ratio of FEV1 to VC and is today known as the FEV1/ (FVC) ratio, was also introduced(Kouri et al., 2021).

Spirometry reference equations' scientific context is briefly explained by different scientists (Kory et al., 1961, Cole, 1975, Wang et al., 1993, Miller et al., 2005, Milanzi et al., 2019). Recently, there has been a lot of work done in spirometry and will go over some of it in this study.

2.2. The influence of ethnicity on the prediction of spirometry reference equations

Lung function can be measured in various ways based on a person's ethnicity, sex, age, and Height (Graham et al., 2019, Kory et al., 1961), and (Quanjer et al., 2012c, Stocks et al., 2014, Sachs et al., 2009, Quanjer et al., 2011). There is a growing recognition that ethnicity might be an independent predictor of spirometry values, which are sex-specific and influenced by age and height(Quanjer et al., 1993, Hankinson et al., 1999, Quanjer et al., 2012b). It is impossible to create a spirometry reference equation for all ethnicities because of the wide range of social, environmental, and economic variables and the predictors describing lung function values (Trabelsi et al., 2008, Mannino et al., 2003, Lujan and DiCarlo, 2018, Elmaleh-Sachs et al., 2021). Numerous studies show that pulmonary function in Caucasians, South and Northeast Asians and African Americans differs proportionally from other races. (Quanjer et al., 2012c), (Yan et al., 2018). Another study discovered that height and age were the most important explanatory variables in spirometry reference equations when the fit of 13 prediction equations was tested against the Global Lungs Initiative (GLI) dataset using spirometry data from (55,136) healthy Caucasians (54 % female)(Quanjer et al., 2012c).

2.3. The impact of altitude on spirometry reference values

There have also been numerous studies that have used altitude as a variable in developing spirometry prediction equations. The study conducted by R. Aristizabal-Duque et al. illustrates this type of study. Their goal was to find the most appropriate spirometry equations for children and adolescents in Bogota, Colombia. A total of (326) spirometry tests (149 boys and 177 girls) have been used to create the reference data set. They recommended using Knudson and GLI-2012 spirometry reference equations for most spirometry parameters when evaluating the respiratory function of children in Bogota, Colombia, at an altitude of (2640) meters (Aristizabal-Duque et al., 2019).

The researchers in Argentina compared the spirometry values of residents of the Andean Plateau's high elevations to those predicted for the lowland population. They recruited (172) healthy men and (235) healthy women between the ages of (20 and 70) to obtain spirometry prediction equations from a healthy subgroup of persons living at (3440 m) above sea level in Argentina. Spirometry was performed following ATS/ERS guidelines. Their findings indicated that supra-normal values were discovered for this population when compared to the values predicted by lowland Caucasian equations (López Jové et al., 2018).

In another research, the Himalayan Sherpas, an isolated racial group living at an altitude of (3840 M), had their spirometry values compared to those expected for the European Coal and Steel Community (ECSC). The study included (146) healthy adult Sherpas (64 males and 82 females) and 103 teenagers (10–18 years, 37 females and 66 males). To determine anticipated values for each adult individual, the EC and S reference equations were employed, as well as distinct Caucasian values for youngsters and new predictive equations for the Sherpa’s population. Boys, adult males, and female Sherpas all have much more FVC than predicted. The FVC is less different in Sherpa girls. They concluded that Sherpas have significantly higher spirometry values than Caucasians (Havryk et al., 2002).

2.4. The impact of Exercise on spirometry reference values

Exercise and physical activity can also affect spirometry readings (Mälkiä and Impivaara, 1998, Doherty and Dimitriou, 1997, Cheng et al., 2003, Watsford et al., 2005). For the Mälkiä and Impivaara study, a representative sample of Finns aged (30) and up (n=8000) was selected from the population record. Asthma was determined based on chronic disease self-reports. In asthmatic men, the results revealed evident and substantial relationships between spirometry values and intensities of physical activity at work and during leisure time(Mälkiä and Impivaara, 1998). In a study of (61) healthy, non-smoking females (age 66 ± 4.4 years), women who met physical activity requirements for health had significantly higher values for FVC and FEV1 (Nawrocka and Mynarski, 2017). A cross-sectional study of (161) adult male police officers aged (20–50) was conducted in Khartoum (June–December 2012).

Police officers were trained by extensive exercise their Lung function values were higher than the normal values, exercise, and height correlated positively, while age correlated negatively (Bakhit et al., 2020).

Another study showed that the factors like sports conditioning could alter VC, maximal breathing capacity, and diffusing capacity. Athletics has a higher mean vital capacity, breathing capacity, and airflow than the rest of the subjects(George et al., 2014).

2.5. Polynomial regression

In simple terms, regression means "finding the best fit line or the regression equation that’s use to make predictions."(Pant, 2019). Polynomial regression is a type of regression that's use because there is a non-linear relationship between the dependent and independent variables (Pant, 2019, Brown, 2001, Adesanya et al., 2018). FEV1, for example, often has a curved relationship with age: before puberty, it usually rises, then it rises quickly during puberty, and then it falls back down again afterward. In this case, the polynomial regression model is more appropriate for modeling the curved relationship between a lung function measure and a predictor (such as age) (Pant, 2019).

Polynomial regression is a subset of multiple linear regression. This model has been used in several studies to predict spirometry equations.

According to a study conducted in Iran, spirometry measures were taken on (4,341) healthy nonsmokers in Isfahan, Iran, following ATS criteria and a rigorous quality control program. Multiple regression approaches were utilized to develop prediction equations for spirometry variables using measured data from (3,213) people; the remaining (1,128) respondents acted as a control group to assess the resultant equations' validity. In addition, predicted values for the United States have been compared to values derived from recently published equations. For the majority of spirometry parameters, the derived prediction equations performed well. Adult Persians have limitedly lower forced vital capacities than USA Whites, while children's values are comparable to USA Whites (Golshan et al., 2003). A study also reported spirometry reference values for 5 Latin American cities. 906 (17%) people aged (40 to 90) years old have been chosen from (5315) people who had spirometry in the platino study in Caracas, Mexico City, Santiago, Sao Paulo, and Montevideo to provide reference values. Multiple regression models were built using the spirometry parameters FEV1/FEV6, FEV1/FVC,

and forced mid-expiratory flow rate. The model included height, sex, and age. Their result is that the subjects' average values were like those of the 3rd National Health and Nutrition Examination Survey's white North American and Mexican American populations, but 20% higher than those of the same survey's black people (Perez-Padilla et al., 2006).

In 2002-05, 2250 Sudanese aged 7-86 years were studied cross-sectionally. Lung function and anthropometric measurements were correlated. Arab Sudanese had significantly higher FVC, FEV1, and PEFR than African Sudanese. In adults, lung function is significantly associated with height and negatively with age. Sudanese lung function varies by gender and ethnicity and the values were less than reference Caucasian values by (15%) (Bashir and Musa, 2012).

Research was conducted in Kigali and Huye, both in Southern Rwanda. The variables studied were FEV1, FVC, and PEF. Age, height, weight, and BMI were used as independent variables in multiple regression to predict equations for both sexes. (740) were used healthy nonsmokers (394 females and 346 males) to provide predicted equations for normal lung functions. There were minor differences in FEV1 and FVC between Africans, African Americans, Chinese, and Indians. FEV1 and FVC were (9–12%) and (16–18%) lower in men than Caucasians and white Americans, respectively (Musafiri et al., 2013).

A study done in Malagasy has predicted the spirometry equations for a healthy adult Malagasy population and compared Malagasy population measurements with the ECSC, NHANES III, and GLI 2012 prediction equations. Age and height were used as independent variables in a linear model for everyone. FEV1, FVC, and FEV1/FVC were best predicted by NHANES III black male and female equations and GLI 2012 black male and southeast Asian female equations in this model. ECSC-predicted FEV1, FVC, and FEV1/FVC did not match Malagasy measurements (Ratomaharo et al., 2015a).

Another study in Korea for Healthy children aged 4 to 17 was recruited from three regions in Korea, totaling 5590 (2607 males and 2983 females). Using age, height, and weight as variables, simple and multiple regression analyses were used to predict FVC, FEV1, FEV1, MMEF, and PEFR (Kim et al., 2020).

2.6. Generalized Additive Models

The generalized additive models (GAM) are a type of regression model that can characterize curved relationships more flexibly (Hastie and Tibshirani, 1986, Austin, 2007). Generalized additive models for location, scale, and shape embedded in GAMLSS were used to develop prediction models. The FEV1, FVC, (FEV1/FVC), and PEFR spirometric parameters can be classified according to their mean (location, M, or mu), coefficient of variation (scale, S, or sigma), and skewness coefficient (shape, L, or lambda). The acronym LMS summarizes these characteristics (Rigby and Stasinopoulos, 2005). The LMS procedure (lambda, mu, sigma) allows for continuous population analyses, avoiding gaps in the transition from childhood to adulthood. It also allows for more precise calculations of average values, dispersion, and 5^{th} percentiles commonly thought to be the lower limit of normality (Martinez-Briseno et al., 2021).

Numerous studies employed the LMS method to develop prediction equations for spirometric reference values.

Philip H. Quanjer et al. researched to derive continuous prediction equations and their lower normal limits for spirometric indices applicable globally. The European Respiratory Society's Global Lung Function Initiative received over (160,000) data points from (72) centers in (33) countries. After removing data that could not be used (mostly missing ethnic groups and some outliers), (97,759) records of healthy nonsmokers (55.3% females) aged 3–95 years remained. The LMS method, which allows simultaneous modeling of the mean (mu), coefficient of variation (sigma), and skewness (lambda) of a distribution family, was used to collect lung function data, and derive prediction equations (Quanjer et al., 2012c).

Another German study developed new reference values and a lower limit of normal (LLN) for children in small-range age and height categories in Germany, were conducted field tests on (4 to 18) year-old children in three German communities. The (1943) children were in good health and had visually proper lung function that met international quality standards. They used the LMS regression model, which Stanojevic and Quanjer introduced in this context. According to the old equation, the measured lung function and the predicted values differed significantly. The child's lung function was affected by their height and their age in a non-linear manner. Age had no significant impact on the variation coefficient (Huls et al., 2014).

A Japanese researcher updated the spirometric reference values for Japanese individuals, including VC, and compares them to earlier Japanese reference values. Spirometric data were gathered from (12) sites across Japan from (20341) healthy nonsmokers aged (17–95) years (67% females), and reference equations were constructed using the LMS approach. This approach calculates LMS as a function of gender, age, and height. In addition, the age-specific lower limits of normal (LLN) were calculated. Spirometric reference values and age dependent LLN were computed for Japanese adults aged (17 to 95). The new reference values for FEV1 in males are lower, while those for VC and FVC in middle-aged and elderly males and FEV1, VC, and FVC in females are higher. For females, the LLN of the FEV1 / FVC is higher than the previous values. In the elderly, the FVC is significantly smaller than the VC (Kubota et al., 2014).

A study carried out in China, determined the best approach for developing all-age spirometric reference values by comparing 3 regression approaches multiple linear regression, quantile regression, and GAMLSS. In the Second National Lung Function Survey (male, n=3279), spirometric data from healthy, never-smoking Chinese children and adults aged 5 to 80 years were analyzed. They discovered that GAMLSS appears to be more appropriate for modeling spirometric reference values than multiple linear regression and quantile regression, making it a powerful approach for constructing such references (Jiang et al., 2016).

A study was conducted in Korea using the LMS approach to update the prediction equations for spirometry and their lower limits of normal (LLN) and compare the results to the values of prior spirometric reference equations. Spirometric data on (10,249) healthy nonsmokers were collected in the 4th and 5th Korean National Health and Nutrition Examination Surveys (8,776 females). The new projected values for spirometry and associated LLN obtained using the LMS method were shown to depict transitions more correctly in pulmonary function in young people than previous prediction equations derived using conventional regression analysis. There were many differences between the revised reference values and the GLI reference values from 2012 (Jo et al., 2018).

In a population-based cohort study, spirometric data were collected from (757) healthy Taiwanese children aged (5 to 18) years to establish reference equations for spirometry in healthy Taiwanese children and assess the applicability of the GLI 2012 equations to Taiwanese children. Linear regression and the GAMLSS were utilized to generate prediction equations. It demonstrates that the GLI-2012 reference equations do not match spirometric data in a modern Taiwanese child population. It gives up-to-date spirometry reference values in healthy Taiwanese children aged 5 to 18 years using GAMLSS modeling. (Chang et al., 2019).

Another study was carried out in Mexico City to compare the results obtained using the LMS method to those obtained using standard multiple linear regression and international GLI equations. Several studies were used to compile data from (9835) healthy residents of Mexico City's metropolitan area, ages ranging from (8 to 80) years. The LMS residuals had a median closer to zero and a smaller dispersion than the linear models, but these differences. While statistically significant, they were minor and of questionable practical significance. The population's average spirometric values for a given height were higher than those predicted by the GLI study. Continuous reference equations calculated using the LMS technique for the Mexican population had a slightly better fit than linear regression models (Martinez-Briseno et al., 2021).

Spirometric data from healthy Cameroonians aged (4–89) years old were utilized to develop reference equations using LMS. (625) children and adolescents (290 boys and 335 females) and (1152) adults participated in the study (552 males and 600 females). When the GLI standards were applied to African Americans, the overall values for FEV1, and FVC were higher than in this study FVC. Compared to the GLI equations, these values were similar in children and adolescents but much higher in adults. The FEV1/FVC ratio was equal in adult men but lower in adult females when the Nigerian norm was employed (88 % vs 85 %, difference = + 3.5 %) (Pefura-Yone et al., 2021).

2.7. African country: spirometric predictive equations in Africa

In Africa, more than (30) spirometric studies were conducted. Some created spirometric prediction equations, while others validated global prediction equations in various African regions.

It was in 2012 that Bashir and Musa first came up with the Sudanese prediction formula. In all, (2,250) people aged (7 to 86) years were included in their study. It is possible to use the values of FVC, FEV1, FEV1/FVC percentage, and PEFR established in this study in Sudan's respiratory clinics(Bashir and Musa, 2012). Bakhit and Musa also developed athlete prediction equations for Sudanese(Bakhit et al., 2020).

Researchers in Ethiopia measured the FVC and the FEV1, (FEV1/FVC)$FEF_{200-1200}$ (forced expiratory flow), and PEFR. These indices were correlated with anthropometric variables using multiple linear regression analysis. FVC, FEV1, and PEFR show significant correlations with age and height in both sexes, according to the findings. Percentage of body fat, weight, and fat-free mass (FFM) all have significant regression coefficients with these indices in men, but only PEFR has a significant regression coefficient in women. Ethiopians have lower FVC and FEVI levels than Caucasians but higher than other Africans, Chinese, and Indians. For future use in obtaining reference values for lung function indices in similar subjects, prediction equations are provided (Mengesha and Mekonnen, 1985).

The purpose of a study conducted in Djibouti was to establish the spirometric values in Djiboutian children and compare them to those reported in African studies and the 2012 GLI. Spirometric data from healthy Djiboutian children were collected, and reference equations were developed using the generalized additive models, which include modeling skewness (k, L), mean (l, M), and coefficient of variation (r, S) based on gender, age, and height. Additionally, they calculated the age-dependent lower limits of normal. Spirometric values were lower in Djiboutian children than in other black children. However, these differences were small and clinically insignificant, representing differences of approximately (3%) of the predicted.

However, significant differences in their equations were observed compared to those for Tunisian children(Idleh Abar et al., 2018).

The current study sought to establish normal lung function in Rwandans. The research was conducted in Kigali and Huye, both in southern Rwanda. The variables studied were FEV1, FVC, and PEF. Age, height, weight, and BMI were used as independent variables in multiple regression to predict equations for both sexes. (740) healthy, nonsmoking (394 females and 346 males) predicted normal lung function equations. There were minor differences in FEV1 and FVC between Africans, African Americans (less than 5%), Chinese, and Indians. When compared to selected Caucasians and white Americans studies, their FEV1 and FVC were (9–12%) and (16–18%) lower in men, and (12–23%) and (17–28%) lower in women (Musafiri et al., 2013).

Another study sought to establish reference values among Kinshasa's healthy adults. They conducted a cross-sectional study with (7443) participants (3208 women, 43 percent). Anthropometric data is being used to correlate FEV1, FVC, and PEF. 5 age groups have been formed, and comparisons have been made based on sex, age, BMI, and participation in sports. Differences in FEV1 (3.00 vs 2.21 L), FVC (3.19 vs 2.38), and PEF (6.8 vs 5.70 L/s) between sexes are evident, as are differences in the outer age categories. FEV1 ranged between (2.33) and (4.54 L vs 1.93-3.3 L) in the age group (20-29) years and (1,76-3,39 L vs 1,60 vs 2,53 L) in the age group (60-70) years; FVC ranged between (2,44-4,89 L vs 1,96-3,56 L) and (1,79-3,78 L vs 1,66-2,74 L) ; PEF ranged between (4,34-12.2 L/s vs 3,62-8.58) Gender, age, anthropometric data, and participation in sports all show significant differences (Kamanga et al., 2019).

A systematic review of studies involving healthy children and adults in Africa that reported spirometry results the Z-scores for forced expiratory volume in one second (zFEV1), forced vital capacity (zFVC), and zFEV1/FVC were calculated using data from these studies and compared to GLI reference equations. (9) studies were reviewed, encompassing (4750) North, South, East, West, and Central Africa (52% female). Individuals from North Africa and Sub-Saharan Africa demonstrated significant differences. Southern African zFEV1 (0.12 6 0.98), zFVC (0.15 6 0.98) and zFEV1/FVC (0.05 6 0.89), Central African zFEV1 (0.16 6 0.79), zFVC (0.09 6 0.83) and zFEV1/FVC (0.17 6 0.71) cohorts, and East African zFEV1 (0.10 6 0.88), zFVC (0.16 6 0.85) and zFEV1/F All reference equations had a poor fit for the West African. The North African group best fits the GLI Caucasian zFEV1 (0.12 / 1.37), zFVC (0.26 / 1.36) and zFEV1/FVC (0.25 / 1.11) data. All populations had stable zFEV1/FVC ratios. They conclude that GLI 2012 reference values can be used in North African and Sub-Saharan African populations after ethnic correction factors are applied (Masekela et al., 2019).

An Alighieri researcher conducted the study. A cross-sectional study of (300) healthy non-smoking adults (50 % men, age range: 18–85 years) recruited from the Algiers region general population was conducted to determine how well the GLI-2012 norms fit contemporary adult Algerian spirometric data. All participants were subjected to a clinical examination as well as plethysmography measurements. Z-scores were calculated for some spirometric data [FEV1, FVC, FEV1/FVC, and forced expiratory flow at (25–75%) of FVC (FEF25–75 percent)]. The GLI-2012 norms would be considered reflective of the contemporary Algerian spirometer if the average Z-score deviated by " 0.5" from the overall mean. The mean SDS for age, height, weight, FVC, FEV1, FEV1/FVC, and FEF25-75 percent of the participants were (48 ±17) years, (1.65±0.10 m), (73±14 kg), (4.04±1.04 L), (3.18±0.82 L), (0.79±0.05), and (4.09±1.09 L/s), respectively. Almost a quarter of those who took part were obese. The total sample means SDs Z-scores for FVC were (0.22±0.87), (0.04±0.88) for FEV1, (-0.34±0.67) for (FEV1/FVC), and (0.93±0.79) for $FEF_{25-75\ \%}$. Only the means SDs of the FEF25-75 percent Z-scores for men and women exceeded the "±0.5" threshold, (1.13±0.77) and (0.73± 0.76), respectively. The findings of this study supported the use of the GLI-2012 norms to interpret FEV1, FVC, and (FEV1/ FVC) but not FEF25-75 percent (Ketfi et al., 2018).

In Maputo, Mozambique, a cross-sectional study was carried out. Participants who met the inclusion criteria were subjected to a brief interview, anthropometric measurements, and LFT. Various modeling approaches were used to generate new Mozambican prediction equations and compare them to

GLI and South African equations. Participants' pulmonary function performance was measured against various reference standards. (212) men and women were recruited, with (155) usable spirometry results obtained. The participants' mean age was (35.20) years (SD 10.99), and females made up (93 of 155) (59.35 t). The Mozambican equations predicted lower values for FVC, FEV1, and the (FEV1/FVC) ratio than the South African and GLI equations. Their study provides the first data on pulmonary function in healthy Mozambican adults and compares it to the GLI and South African spirometry reference values (Ivanova et al., 2020).

So far, no research has been conducted on spirometry reference equations for adult Eritreans. As discussed in Chapter 1, the prevalence of respiratory diseases is high among the Eritrean population, so reference equations for other ethnic groups may not be applicable.

Chapter 3

Materials and Methods

3. Materials and Methods

3.1. Methods

3.1.1. Study Design

This was a cross-sectional community-based study. A quantitative approach was used to create these spirometric reference values.

3.1.2. Study area/setting

This research has been carried out in all geographical areas of Eritrea in the year 2020. The study area (Eritrea) is classified into three groups based on their altitude as follows:

- The first group includes cities that are higher than (2000) meters above sea level (Asmara and Adi keyh).
- The second group includes cities that have a maximum elevation of close to (1500) meters (Keren, Mendefera, and Barentu).
- The third group includes sea-level cities (Massawa, Assab, and Akordat).

One city from each group was selected randomly. Then three neighborhoods from each town were randomly selected, and samples were collected from inside the houses in these neighborhoods. All data were collected during the period from eight in the morning until noon (convenience sampling),during the 1st quarter of 2021. Asmara from the first group, Karen from the second group, and Massawa from the third group were chosen for this study.

3.1.3. Study Population

The Eritrean general population of male and females aged (18 to 60) years old is the target population for this study based on the inclusion and exclusion criteria listed below:

3.1.4. Inclusion criteria:

1. Healthy Eritrean subjects of both sexes
2. Age group (18 -60) years.
3. Residence for at least (1) year in the specific city to be considered

3.1.5. Exclusion criteria:

1. Any chronic disease (rheumatoid arthritis, hypertension cardiopulmonary diseases. …etc.)
2. People who were smokers or smoking currently
3. Pregnant ladies
4. Lactation
5. Subject not consenting

3.1.6. Sampling

two-stage stratified random sampling was used. The population was divided into three strata (Asmara, Keren, and Massawa), and then each stratum was subdivided into two strata (Males and Females).

3.1.7. Sample Size

Males and females of Eritrean descent, ages (18 to 60), comprised (566) of the total population of Eritreans surveyed. The population has been divided into four age groups: (18–30) years old, (30-39) years old, (40–49) years old, and (50) years old and over.

3.1.8. Sample size criteria:

- Eritrea's Population Size
- The level of precision is (5%) sampling error.
- The level of confidence: In a normal distribution, approximately (95%) of the sample values are within two standard deviations of the true population value (confidence interval CI level of (95%), meaning (95 out of 100) samples have the true population value within the range of precision specified earlier).
- The degree of variability in the data measured: usually (0.05%)

3.1.9. Sample size equation:

The sample size was calculated using the one-sample numerical dependent variable formula as: $= \frac{Z^2 S^2}{d^2}$,

where z = standard normal score of 1.96, d = margin of error of 0.05, and S = standard deviation of 0.49 The standard deviation was taken from the maximum of the standard deviations of FVC, FEV1, PEFR, and (FEV1/FVC) to give the maximum sample size needed to estimate the 4 spirometric parameters. Accordingly, the initial sample size was found to be (370). Since the population was not small, the population correction factor was not taken into consideration. Hence, adjustment to the multistage sampling technique was done by multiplying the initial sample size by (1.5) to account for the variation introduced. After adjusting for the design effect, the sample size was modified to (554) normal individuals to get more participants. Finally, adjustment for the potential non-response of the individuals selected in the sample was made assuming that(5%). Hence, the final sample size for this study was (583) normal individuals.

3.2. Materials

The following tools were used to conduct this study:

1. Portable spirometer model 6800 (Vitalograph Ltd- Ireland)
2. Digital Weight scale (Etekcity – China)
3. Height scale
4. Disposable mouthpieces (Vitalograph Ltd- Ireland)
5. Mercury Sphygmomanometers
6. Stethoscopes (Littman- Germany)

3.2.1. Questionnaire:

An interview or structured questionnaire was completed by all volunteers containing the following information: (Appendix 1)

1- Personal information (name optional , age, sex, height, weight)
2- Demographic information (residences, tribe, religion, level of education, marital status, occupation)
3- Past medical history

3.2.2. Consent form

After a thorough explanation of the project, all participants signed a written consent form. (Appendix 2)

3.2.3. Anthropometric Measurement

Bodyweight and height were measured using standard scales, with participants in lightweight clothing without shoes and standing in an upright position. BMI was calculated as weight (kg)/height (m)2.

3.2.4. Blood Pressure (BP) Measurement

The subjects' blood pressure was measured by trained personnel using a mercury sphygmomanometer and stethoscope. Participants were instructed to sit in a chair for (5) minutes. The upper arm has been used to obtain the measurements. Different cuff sizes were used for different body types, and the cuffs were placed on the left arm at the level of the heart. The subjects' systolic and diastolic blood pressures were measured (3) times, with at least a (5) minute interval between each measurement. It was decided to use the average of the (3) readings for the analysis

3.2.5. Pulmonary functions:

The pulmonary function tests were performed with a portable spirometer. The device measures spirometric values (FVC, FEV1, FEV1/FVC, and PEFR). A detailed history has been documented following each subject's briefed written consent to rule out the exclusion criteria. The pulmonary function tests were repeated (3) times for each subject, with the best results chosen for analysis. Each subject must relax for a minimum of (2-5) minutes before performing the next PFT procedure. The parameters listed below have been recorded:

1. FVC: The maximum volume of air expired after a maximum inspiration.
2. FEV1: Forced expiratory volume in the first second) the fraction of vital capacity expired during the first second of forced expiration.
3. PEFR: Peak expiratory flow rate.
4. FEV1/FVC ratio.

3.2.6. Recording of PFTs:

The subject was sitting upright in an armed chair with their feet flat on the floor and legs uncrossed. The relaxed subject gripped the sterile mouthpiece as demonstrated to him/her before the recording. When the subject was confident and comfortable with the procedure, he was requested that tidal (normal) breaths be taken first, followed by a deep breath maximum in while still using the mouthpiece, followed by another quick, full expiration. The mouthpiece was then removed, and the FVC, FEV1 and PEFR values were printed for analysis.

3.3. Variables:

3.3.1. Dependent variables

1. FVC, in litter
2. FEV1, in litter
3. FEV1/FVC calculated in percentage terms.
4. PEFR Peak expiratory flow in liters per minute (L/min)

3.3.2. Independent Variables

1. The age in years
2. The weight in kilogram
3. The height in centimeters

4. Gender
5. Altitude

3.3.3. Background variables

1. Educational Level
2. Marital Status
3. Ethnicity
4. Occupation

3.4. Statistical Analysis

Statistical analysis was done using SPSS (Version- 26, IBM Corp.) and R (Version 4.0.1; www.r-project.org). The spirometric variables, for both males and females, were normally distributed as per Komogrov-Smirnov Test (the histograms are shown in Figure 3.1). The Kolmogorov–Smirnov test is used to determine the degree to which a particular set of data fits a theoretical distribution, making it a one-sample test (Berger and Zhou, 2014). Hence, descriptive analysis of FVC, FEV1, PEFR, and (FEV1/FVC) was made using mean ± SD for the categories of age, height, weight, and town/altitude. Correlation coefficients were computed to find out the relationship between the spirometric variables and quantitative anthropometric and health-related characteristics. Moreover, independent samples T-test and ANOVA were also used to find out the difference in spirometric parameters across the categories of height, weight, age, and town. In such types of studies, residuals are considered as outliers if the standardized residuals are less than (-5 or greater than 5). Tables and graphs were used to present the findings. Two-sided P-values of (0.05) were considered statistically significant for all tests.

3.4.1. Derivation of Reference equation: Polynomial regression method

Spirometric equations were derived using polynomial regression methods. The prediction equations were derived for males and females separately. Using the paired t-test and relative mean differences, predicted values from the equations were compared to values from neighboring countries and the global one.

3.4.2. Derivation of Reference equation: LMS method

For statistical modeling, the Generalized Additive Models for Location (Mu, M), Scale (Sigma, S) and Shape (Lambda, L), GAMLSS package (version 5.1-6) in R software was used. The mean (mu), coefficient of variance (sigma), and skewness (lambda) was modeled with Box-Cox–Cole–Green (BCCG) distribution. However, the B-splines were added as functions of age to make age-specific adjustments. Parsimonious models were selected eventually according to Schwarz Bayesian Criterion. The goodness of fit for predictive models was examined through consulting normal Q–Q plots, worm plots, and centile plots. The final predictive equations were built in the following forms:

$$\text{Log (mu)} = \beta_0 + \beta_1 \times \log(\text{age}) + \beta_2 \times \log(\text{height}) + \text{age-spline for mu (M\_spline)}$$

$$\text{Log (sigma)} = \beta_0 + \beta_1 \times \log(\text{age}) + \text{age-spline for sigma (S\_spline)}$$

$$\text{Log (Lambda)} = \beta_0 + \beta_1 \times \log(\text{age}) + \text{age-spline for lambda (L\_spline)}$$

where age (year) and height (cm) were used as independent predictive variables.

3.4.3. Comparisons of Reference Equations

To explore the difference in the performance of the previously fitted models (in Sudan), comparisons were performed. Paired t-tests were used to examine the differences in spirometry variables (FVC, FEV1, PEFR, and FEV1/FVC) between Sudanese predicted values and the predicted values, as well as between Eritrean predicted values and global spirometric values.

Chapter 4

Results

4. Results

The total number of participants in the study area who gave consent were (726) subjects. Out of these total participants, (99) had invalid spirometric data. However, (61) subjects were excluded from the study (Cardiopulmonary problems (n=45) and smoking (n=16)). Finally, 566 subjects were eligible for the study with (200) meters and the remaining (366) females.

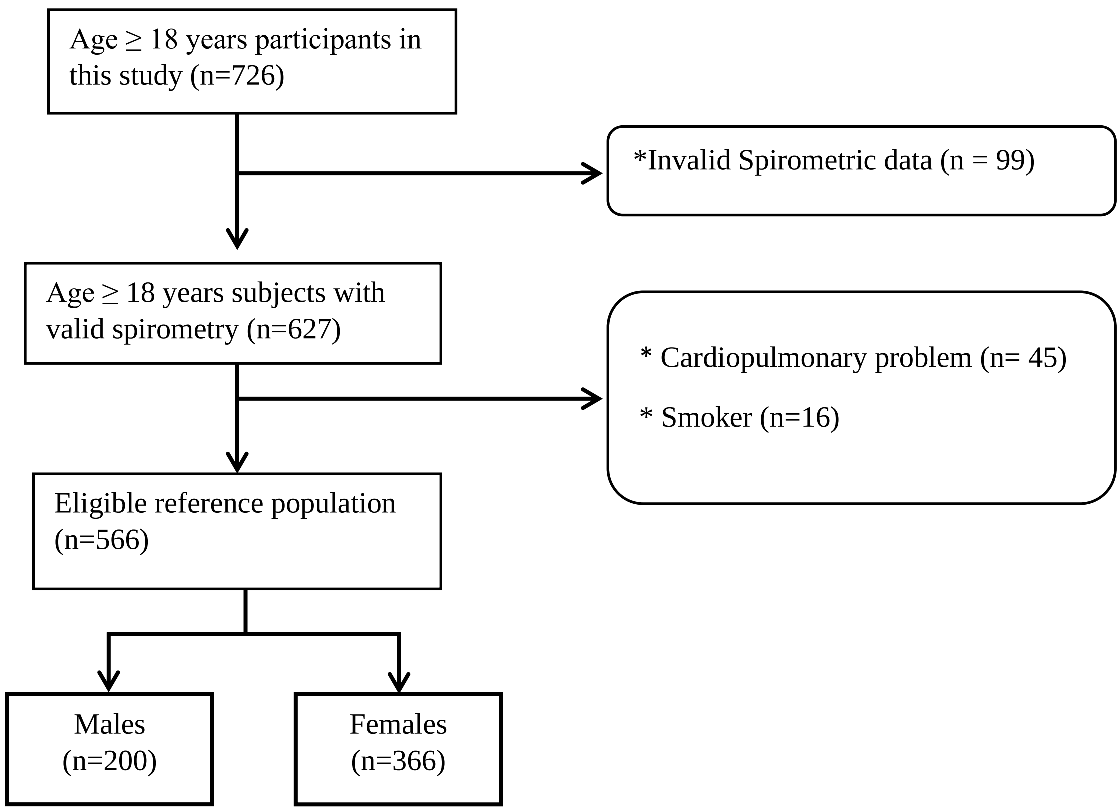
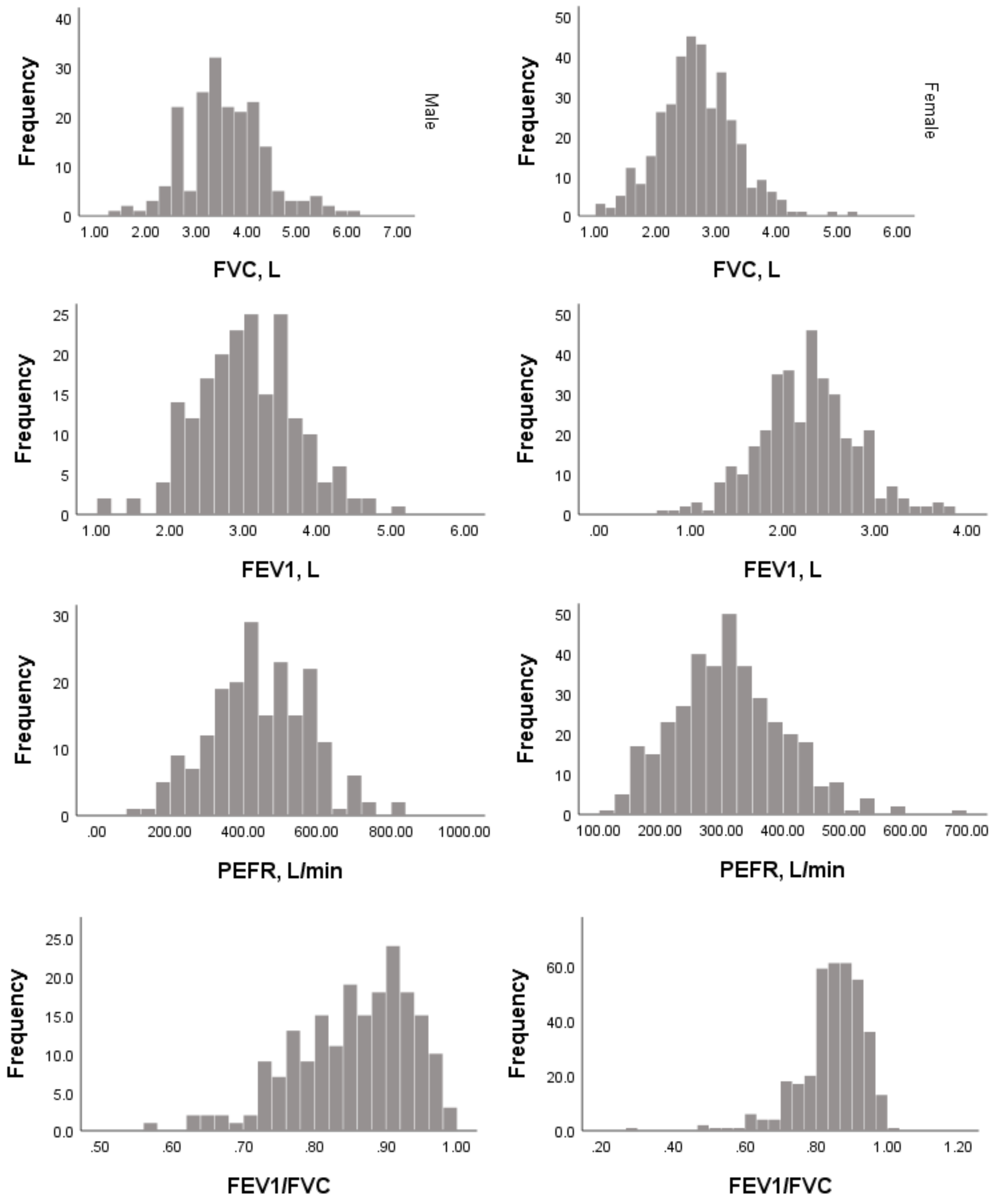


Figure 4.1 Flow chart showing eligibility of study participants

Two tests (skewness and Kolmogorov Smirnov) were performed to determine the normality of our data. The test result was significant if the p-value was less than or equal to 0.05.



FVC: Forced Vital Capacity, Kolmogorov-Smirnov's p-value for both males and females =0.200; FEV1: Forced Expiratory Volume in 1 second, Kolmogorov-Smirnov's p-value for both males and females =0.200*; PEFR: peak expiratory flow rate, Kolmogorov-Smirnov's p-value for males=0.200* and for females=0.092*; FEV1/FVC, Kolmogorov-Smirnov's p-value for males=0.005 (Skewness=-0.702, slightly negatively skewed) and females =0.000 (Skewness=-1.566, moderately positively skewed).*

Figure 4.2: Histogram of the spirometric parameters stratified by age and Kolmogorov-Smirnov measures

4.1. Lung function values in adult Eritreans by age group

4.1.1. Lung function values for adult Eritrean males

Table (4. 1) Lung function values in adult Eritrean males by age, height, weight, and altitude groups

Variables	Number	FVC, L M ± SD	FEV1, L M ± SD	PEFR, L/min M ± SD	FEV1/FVC M ± SD
Age, years					
18 to 30	56	3.78 ± 0.75	3.28 ± 0.66	448.68 ± 141.91	87 % ± 0.09
31 to 40	42	3.82 ± 0.60	3.25 ± 0.58	483.31 ±133.49	85 % ± 0.09
41 to 50	49	3.39 ± 0.88	2.88 ± 0.70	414.98 ± 126.36	85 % ± 0.08
51 to 60	53	3.29 ± 0.83	2.76 ± 0.65	433.55 ± 136.44	85 % ± 0.09
Height, cm					
141.00 - 150.00	2	3.39 ± 0	2.69 ± 0	501.50 ± 126.57	79 % ± 0
151.00 - 160.00	23	2.98 ± 0.55	2.57 ± 0.46	371.13 ± 116.69	86 % ± 0.09
161.00 - 170.00	94	3.41 ± 0.78	2.95 ± 0.68	453.89 ± 140.83	87 % ± 0.08
171.00 - 180.00	73	3.91 ± 0.76	3.28 ± 0.67	452.59 ± 132.26	84 % ± 0.08
181.00 - 190.00	6	3.94 ± 0.72	3.22 ± 0.50	415.17 ± 125.96	82 % ± 0.07
191.00 - 200.00	1	4.46 ± 0	3.78 ± 0	628 ± 0	85 %
Weight, kg					
30.01 - 40.00	3	2.86 ± 0.43	2.42 ± 0.61	255.00 ± 89.27	84% ± 0.10
40.01 - 50.00	24	3.15 ± 0.69	2.81 ± 0.67	399.08 ± 115.91	88% ± 0.09
50.01 - 60.00	62	3.51 ± 0.82	2.98 ± 0.67	447.11 ± 143.25	86 %± 0.09
60.01 - 70.00	53	3.66 ± 0.78	3.12 ± 0.69	451.81 ± 133.21	85 % ± 0.09
70.01 - 80.00	31	3.79 ± 0.82	3.20 ± 0.69	468.90 ± 132.99	85 % ± 0.07
80.01 - 90.00	21	3.67 ± 0.80	3.06 ± 0.67	459.05 ± 143.12	83 % ± 0.06
90.01 - 100.00	5	3.84 ± 0.95	3.28 ± 0.76	435.40 ± 119.76	86 % ± 0.02
Town/Altitude					
Asmara	131	3.68 ± 0.74	3.10 ± 0.66	450.35 ± 140.24	84 % ± 0.08
Keren	25	3.23 ± 0.88	2.85 ± 0.85	421.20 ± 123.06	88 % ± 0.10
Massawa	44	3.40 ± 0.91	2.96 ± 0.67	436.61 ± 131.61	88 % ± 0.08

M: Mean, SD: Standard deviation, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

The lung function values for the males by age, height, weight and altitude groups were summarized in Table (4.1) A decline in mean FVC (L) and FEV1 (L) was observed with an increase in the level of age group. However, a rise in the mean level of PEFR (L/min) was found in the (31- 40) age group, followed by a decline in the following age groups. The (FEV1/FVC) ratio was observed to decline from the (18 – 30) (M ± SD, 0.87 ± 0.09) to (31 – 40) (M ± SD 0.85 ± 0.09) age group but was constant thereafter.

There was a constant increase in the mean level of FVC (L) and FEV1 (L) with an increase in categories of height (cm). However, such trends were not clear for the PEFR (L/min) and (FEV1/FVC), among the male participants. On the other hand, there was a clear inclining trend of the FVC (L), FEV1 (L), and PEFR (L/min) across the increase in weight, with a slightly different pattern in that of (FEV1/FVC). FVC (L) was highest in Asmara (M ± SD, 3.68 ± 0.74) as compared to Massawa (M ± SD, 3.40 ± 0.91) and Keren (M ± SD, 3.23 ± 0.88). the elevation of the Asmara is about (2325) meters above the sea level, Keren sits at an elevation (1445) meters and Massawa at the sea level. A similar higher measurement in FEV1 and PEFR was observed in Asmara, followed by that of Massawa and Keren. However, a relatively low measurement of (FEV1/FVC) was observed in Asmara as compared to those of Keren and Massawa.

4.1.2. Lung function values for adult Eritrean females

Table (4. 2) Lung function values in adult Eritrean females by altitude, age, height, and weight groups

Variables	Number	FVC M ± SD	FEV1 M ± SD	PEFR M ± SD	FEV1/FVC M ± SD
Age, years					
18 to 30	88	2.93 ± 0.53	2.52 ± 0.45	320.35 ± 89.91	87 % ± 0.09
31 to 40	86	2.80 ± 0.60	2.37 ± 0.51	328.36 ± 78.47	85 % ± 0.10
41 to 50	107	2.62 ± 0.65	2.18 ± 0.51	303.36 ± 92.75	85 % ± 0.08
51 to 60	84	2.40 ± 0.58	1.97 ± 0.47	296.43 ± 94.08	83 % ± 0.09
Height, cm					
131.00 - 140.00	4	2.16 ± 0.76	1.73 ± 0.34	269.75 ± 75.92	84 % ± 0.13
141.00 - 150.00	50	2.32 ± 0.58	1.97 ± 0.48	280.00 ± 75.16	86 % ± 0.09
151.00 - 160.00	189	2.62 ± 0.57	2.21 ± 0.52	314.08 ± 90.71	85 % ± 0.10
161.00 - 170.00	117	2.96 ± 0.61	2.47 ± 0.48	323.41 ± 93.22	84 % ± 0.08
171.00 - 180.00	5	2.72 ± 0.20	2.34 ± 0.23	309.20 ± 67.19	86 % ± 0.04
181.00 - 190.00	1	4.11 ± 0	-	379.00 ± 0	-
Weight, kg					
30.01 - 40.00	16	2.72 ± 0.56	2.20 ± 0.42	284.69 ± 76.17	82 % ± 0.14
40.01 - 50.00	87	2.53 ± 0.57	2.14 ± 0.56	298.85 ± 88.99	85 % ± 0.11
50.01 - 60.00	117	2.81 ± 0.66	2.37 ± 0.53	318.92 ± 85.38	85 % ± 0.08
60.01 - 70.00	89	2.61 ± 0.60	2.19 ± 0.49	306.61 ± 93.44	84 % ± 0.08
70.01 - 80.00	45	2.89 ± 0.58	2.44 ± 0.44	346.60 ± 93.42	86 % ± 0.08
80.01 - 90.00	11	2.31 ± 0.74	1.98 ± 0.64	284.18 ± 90.25	86 % ± 0.07
90.01 - 100.00	1	2.66 ± 0	2.32 ± 0	329.00 ± 0	87 % ± 0
Town/Altitude					
Asmara	247	2.78 ± 0.66	2.31 ± 0.55	317.66 ± 96.63	84 % ± 0.08
Keren	80	2.48 ± 0.48	2.17 ± 0.46	303.92 ± 76.99	88 % ± 0.10
Massawa	39	2.47 ± 0.49	2.10 ± 0.46	292.74 ± 63.09	86 % ± 0.12

M: Mean, SD: Standard deviation, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

The lung function values for the females by age, height, weight, and altitude groups were summarized in Table (4.2). Among female participants, there was a constant decline in the mean level of the FVC (L), FEV1 (L), and PEFR (L/min), and FEV1/FVC with an increase in the age group level. On the contrary, a rise in FVC (L), FEV1 (L), and PEFR (L/min) were observed with an increase in height. A rise in PEFR (L/min), and (FEV1/FVC) was also observed with increased weight, but no such trend was observed in FVC (L) and FEV1 (L).

FVC (L) was highest in Asmara (M ± SD, 2.78 ± 0.66) followed by that of Keren (M ± SD, 2.48 ± 0.48) and Massawa (M ± SD, 2.47 ± 0.49) among females. A similar higher measurement in FEV1 and PEFR was observed in Asmara followed by that of Keren and Massawa. However, relatively low measurement in (FEV1/FVC) was observed in Asmara, as compared to those of Keren and Massawa.

4.2. Socio-demographic Characteristics

Table (4. 3)Socio-demographic characteristics of the study participants (n=566)

Variable	Male n(%)	Female n(%)	Total n(%)
Town			
Asmara	131 (65.5)	247 (67.5)	378 (66.8)
Keren	25 (12.5)	80 (21.9)	105 (18.6)
Massawa	44 (22.0)	39 (10.7)	83 (14.7)
Educational Level			
Illiterate	20 (10.0)	81 (22.1)	101 (17.8)
Primary School	20 (10.0)	61 (16.7)	81 (14.3)
Junior	54 (27.0)	105 (28.7)	159 (28.1)
Secondary	82 (41.0)	95 (26.0)	177 (31.3)
College	24 (12.0)	24 (6.6)	48 (8.5)
Marital Status			
Married	143 (71.5)	255 (69.7)	398 (70.3)
Living together	0 (0)	3 (0.8)	3 (0.5)
Widowed	1 (0.5)	16 (4.4)	17 (3.0)
Divorced	0 (0)	20 (5.50	20 (3.5)
Separated	1 (0.5)	5 91.4)	6 (1.1)
Single/Never married	55 (27.5)	67 (18.3)	122 (21.6)
Ethnicity			
Afar	4 (2.0)	9 (2.5)	13 (2.3)
Blen	18 (9.0)	32 (8.7)	50 (8.8)
Hedareb	0 (0)	0 (0)	0 (0)
Kunama	1 (0.5)	0 (0)	1 (0.2)
Nara	1 (0.5)	0 (0)	1 (0.2)
Rashaida	0 (0)	0 (0)	0 (0)
Saho	5 (2.5)	10 (2.7)	15 (2.7)
Tigre	42 (21.0)	35 (9.6)	77 (13.6)
Tigrigna	129 (64.5)	280 (76.5)	409 (72.3)
Occupation			
Government Employee	10 (50.0)	119 (32.5)	219 (38.7)
Merchant	28 (14.0)	14 (3.8)	42 (7.4)
Farmer	7 (3.5)	1 (0.3)	8 (1.4)
Retired	2 (1.0)	1 (0.3)	3 (0.5)
Homemaker	2 (1.0)	161 (44.0)	163 (28.8)
Others	61 (30.5)	70 (19.1)	131 (23.1)

The socio-demographic characteristics of the sample population are shown in Table (4.3) Out of the total (566) study participants, two-thirds (66.8%) of them were residents of Asmara, and the remaining one-third belonged to Keren (18.6%) and Massawa (14.7%). The majority (31.3%) were in secondary school, followed by junior (28.10%), illiterate (17.8%), primary school (14.3%), and college (8.5%). Almost seventy percent (70.3%) were married and 21.6% were single. The predominant ethnic group was Tigrigna (72.3%) followed by Tigre (13.6%). With regards to education, the participants were mostly government employed (38.7%), housewives (28.8%), and others (23.1%).

4.3. Anthropometric and Spirometric Characteristics

Table (4. 4) Anthropometric, health-related, and spirometric characteristics of the study participants

Variables	Male (n=200)		Female (n=366)		Overall (n=566)	
	M ± SD	Min., Max.	M ± SD	Min., Max.	M ± SD	Min., Max.
Age	40.32 ± 12.66	18, 60	41.25 ± 11.81	18, 60	40.92 ± 12.12	18.00, 60.00
Height, cm	168.53 ± 7.55	144, 199	157.54 ± 6.55	136, 185	161.41 ± 8.68	136.00, 199.00
Weight, kg	63.77 ± 12.69	40, 100	58.45 ± 12.36	32.40, 151.00	60.32 ± 12.72	32.40, 151.00
BMI, kg/m^2	22.50 ± 4.07	14.87, 37.95	23.59 ± 4.86	14.10, 58.25	23.21 ± 4.63	14.10, 58.25
SBP	120.90 ± 16.19	90, 180	118.35 ± 17.96	84.00, 210.00	119.24 ± 17.38	84.00, 210.00
DBP	78.88 ± 9.39	60, 118	75.92 ± 10.73	58.00, 140.00	76.96 ± 10.37	58.00, 140.00
Sleeping, hours	7.63 ± 0.80	5, 10	7.74 ± 1.06	4.00, 12.00	7.70 ± 0.98	4.00, 12.00
FVC, L	3.57 ± 0.81	1.42, 6.03	2.68 ± 0.62	1.09, 5.18	2.99 ± 0.81	1.09, 6.03
FEV1, L	3.04 ± 0.69	1.03, 5.02	2.26 ± 0.53	0.70, 3.76	2.53 ± 0.69	0.70, 5.02
PEFR, L/min	443.68 ± 136.68	111, 831	312.03 ± 89.84	103.00, 678.00	358.63 ± 125.36	103.00, 831.00
FEV1/FVC	0.86 ± 0.08	0.57, 1.00	0.85 ± 0.09	0.30, 1.00	0.85 ± 0.09	0.30, 1.00

M: Mean, SD: Standard deviation, BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate, Min., Minimum, Max., Maximum

The anthropometric and spirometric characteristics of the sample population are presented in Table (4.4). This study included (566) non-smokers (200 males and 366 females). The age range and mean ± SD age of male subjects were (18–60) and (40.32 ± 12.66) years, respectively; and those of female subjects were (18–60) and (41.25 ± 11.81) years, respectively. The mean ± SD heights of male and female participants were (168.53 ± 7.55) cm and (161.14 ± 8.68) cm, respectively. The mean ± SD weights of male and female participants were (63.77 ± 12.69) kg and (58.54 ± 6.55) kg, respectively. The mean ± SD of body mass index (BMI) for males and females were (22.50 ± 4.07) kg/m^2 and (23.59 ± 4.86) kg/m^2, respectively.

The SBP and DBP range for males was (90 – 180) and (60 – 118) respectively; and those of female subjects were(84 – 210) and (58 – 140), respectively. The mean ± SD SBP and DBP of male subjects were (120.90 ± 16.19) and (78.88 ± 9.39) respectively; and those of female subjects were (118.35 ± 17.96) and (75.92 ±

10.73), respectively. The mean ± SD sleeping hours of male and female participants were (7.63 ± 0.80) hours and (7.74 ± 1.06) hours, respectively.

The mean ± SD values of FVC, FEV1, PEFR, and FEV1/FVC for males were (3.57 ± 0.81 L), (3.04 ± 0.69 L), (443.68 ± 136.68 L/min), and (0.86 ± 0.08), respectively. The mean ± SD values of FVC, FEV1, PEFR, and FEV1/FVC for females were (2.68 ± 0.62 L), (2.26 ± 0.53 L), (312.03 ± 89.84) L/min, and (0.85 ± 0.10), respectively.

4.4. Distribution of Participants' height and weight by gender and age

Table (4. 5) Distribution of participants' height and weight by age category

Age Category	Height, cm		Weight, kg	
	Male M±SD	**Female M±SD**	**Male M±SD**	**Female M±SD**
18 – 30	169.11±8.54	158.02±5.47	59.14±11.37	52.79±9.92
31 – 40	170.86±6.13	158.45±6.54	63.86±11.51	60.07±10.82
41 – 50	167.77±7.98	157.45±6.60	64.80±12.67	59.61±11.68
51 – 60	166.77±6.66	156.20±7.39	67.40±13.84	59.92±11.41
Total	168.53±7.55	157.54±6.55	63.77±12.69	58.42±12.37

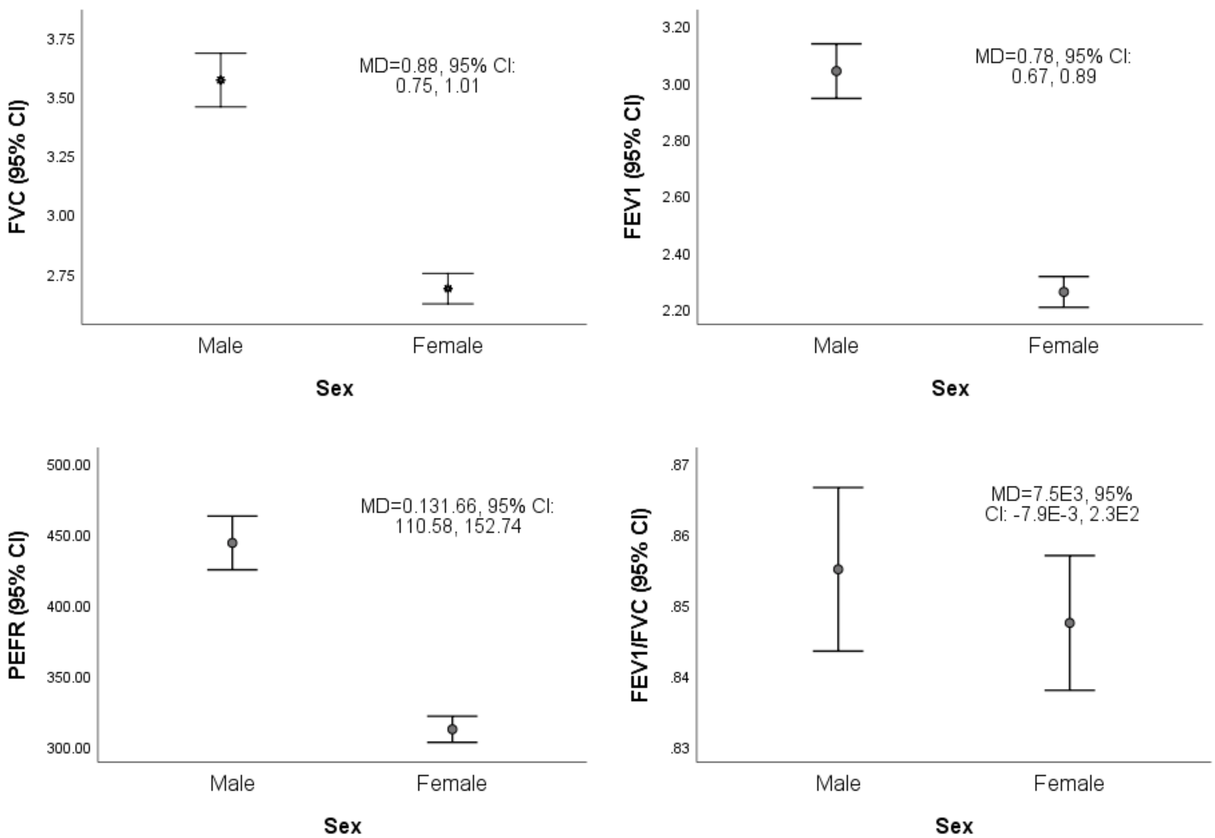
M: Mean, SD: Standard deviation

The distribution of participant's height and weight by gender and age group is presented in Table (4.5). Males in the age group (51 – 60) were found to have a relatively lower height (166.77±6.66 cm), while those in the age group (31 – 40) were higher (170.86±6.13 cm). A similar trend was observed among females regarding height. On the other hand, there was a constant increase in the mean weight of males starting from the age group of (18 – 30) (59.14±11.37 kg) to (51 – 60) (67.40±13.84 kg). However, an abrupt increase in mean weight was observed among females in the age group (31 – 40) (M ± SD, 60.07 ± 10.82) as compared to that of 18 – 30 (M ± SD, 52.79 ±9.92), and then was almost constant.

4.5. Lung function values in Eritreans by gender

To assess the difference in FVC, FEV1, PEFR, and FEV1/FVC between males and females, an independent sample T-test was used after confirmation of normality (Figure 4.2). The results of these findings will give a clue as to whether separate prediction equations are needed for each gender category. The result revealed that there was a significant difference ($P<0.001$) in FVC between males ($M=3.57$, $SD=0.81$) and females ($M=2.68$, $SS=0.62$). Similarly, a significant difference

(P<0.001) was observed in FEV1 between males (M=3.04, SD=0.69) and females (M=2.26, SD=0.53). PEFR among males (M=443.69, SD=136.09) was also significantly different (P<0.001) from PEFR of females (M=312.03, SD=89.84). However, in the (FEV1/FVC) ratio, there was no statistically significant difference (p=0.336) between males (M=0.56, SD=0.08) and females (M=0.85, SD=0.09).



MD: Mean Deviation, CI: Confidence Interval, σ_M^2: Variance of the males, σ_F^2: Variance of females; $\sigma_M^2 \neq \sigma_F^2$: Variance of males is significantly different from females, $\sigma_M^2 = \sigma_F^2$: Variance of males is not significantly different from females.

Figure 4.3) Error plots comparing the spirometric parameters by gender.

4.6. Comparing age and height across altitude for male

Table (4. 6) Comparing age and height across altitude for male

Males			
Altitude		Age	Height
	Number	M, SD	M, SD
Asmara	131	38.55 ±11.57	169.98 ± 7.58
Keren	25	47.28 ±11.96	167.00 ± 6.42
Massawa	44	41.64 ± 14.77	165.11 ± 6.91
Overall	200	40.32 ± 12.66	168.53 ± 7.55
p-value		0.005	<0.001

4.7. Comparing age and height across altitude for females

Table (4. 7) Comparing age and height across altitude for females

Females			
Altitude		**Age**	**Height**
	Number	**M, SD**	**M, SD**
Asmara	247	41.59 ±11.42	158.19 ± 6.32
Keren	80	42.61 ± 12.41	156.00 ± 7.03
Massawa	39	36.33 ± 12.10	156.62 ± 6.45
Overall	366	41.25 ± 11.81	157.54 ± 66.55
p-value		0.018	0.021

4.8. Correlation of the Lung Function parameters with Anthropometric and socio-demographic variables

4.8.1. Correlation with quantitative variables

Table (4. 8) Correlation of males' lung function parameters with quantitative anthropometric and health variables

Variable	FVC (L)		FEV1 (L)		PEFR (L/min)		FEV1/FVC	
	R	***p-value***	**R**	***p-value***	**R**	**p-value**	**R**	**p-value**
Age	-0.298	***<0.001***	-0.349	***<0.001***	-0.110	*0.122*	-0.097	*0.178*
Height, cm	0.429	***<0.001***	0.389	***<0.001***	0.156	***0.028***	-0.099	*0.170*
Weight, kg	0.216	***0.002***	0.170	***0.018***	0.124	*0.081*	-0.113	*0.114*
BMI, kg/m2	0.038	*0.600*	0.007	*0.926*	0.069	*0.331*	-0.074	*0.306*
SBP	0.012	*0.873*	-0.005	*0.946*	-0.025	*0.734*	-0.032	*0.676*
DBP	-0.058	*0.441*	-0.059	*0.433*	-0.072	*0.337*	-0.016	*0.836*
Sleeping hours	-0.126	*0.106*	-0.093	*0.234*	-0.022	*0.778*	0.079	*0.314*

r: Pearson's correlation coefficient; FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

After assessing the normality, bivariate correlation of the lung function parameters and quantitative anthropometric, as well as health-related variables, were computed as shown in Table (4.8). A significant decline in FVC (r=-0.298, p<0.001), and FEV1 (r=-0.349, P<0.001) was observed with an increase in age among male participants. However, a significant rise in FVC (r=0.429, P<0.001), FEV1 (r=0.389, P<0.001), and PEFR (r=0.156, P=0.028) was found with an increase in height. On the other hand, a significant rise in FVC (r=0.216, P=0.002) and FEV1 (r=0.17, P=0.018) was found with an increase in the weight of the male participants. Bivariate correlation results showed no significant association of the four lung function parameters with BMI, SBP, DBP, and sleeping hours.

Table (4. 9) Correlation of females' lung function parameters with quantitative health-related, anthropometric measures

Variable	FVC (L)		FEV1 (L)		PEFR (L/min)		FEV1/FVC	
	R	***p-value***	**R**	***p-value***	**R**	***p-value***	**R**	***p-value***
Age	-0.332	***<0.001***	-0.395	***<0.001***	-0.119	***0.023***	-0.122	***0.021***
Height, cm	0.357	***<0.001***	0.327	***<0.001***	0.155	***0.003***	-0.042	*0.427*
Weight, kg	0.063	*0.235*	0.078	*0.138*	0.11	***0.035***	0.046	*0.386*
BMI, kg/m2	-0.099	*0.060*	-0.062	*0.238*	0.044	*0.405*	0.073	*0.169*
SBP	-0.224	***<0.001***	-0.245	***<0.001***	-0.082	*0.132*	-0.041	*0.454*
DBP	-0.199	***<0.001***	-0.195	***<0.001***	-0.056	*0.312*	0.012	*0.832*
Sleeping hours	-0.075	*0.179*	-0.054	*0.334*	-0.017	*0.768*	0.044	*0.437*

r: Pearson's correlation coefficient; FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

Table (4.9) shows the bivariate correlation of female lung function parameters with quantitative anthropometric and health background characteristics. A significant negative relationship was observed between the four lung function parameters and age. A decline in FVC (r=-0.332, P<0.001), FEV1 (r=-0.390, P<0.001), PEFR (r=-0.119, P=0.023), and (FEV1/FVC) (r=-0.117, P=0.026) was observed with an increase in age. On the other hand, a rise in FVC (r=0.357, P<0.001), FEV1 (r=0.332, P<0.001) and PEFR (r=0.155, p=0.003) was observed with an increase in height. A positive significant relationship was also observed between PEFR (r=0.110, P=0.035) and weight. Moreover, a significant decrease in both FVC and FEV1 was observed with an increase in SBP and DBP.

4.8.2. Comparison of mean lung function parameters with qualitative variables

Table (4. 10) Comparison of mean lung function parameters of males across the categories of the qualitative variables

Variables	FVC (L)	FEV1 (L)	PEFR (L/min)	FEV1/FVC
	M ± SD	**M ± SD**	**M ± SD**	**M ± SD**
Altitude				
Asmara	3.68 ± 0.74	3.10 ± 0.66	450.35 ± 140.24	0.84 ± 0.08
Keren	3.23 ± 0.88	2.85 ± 0.85	421.20 ± 123.06	0.88 ± 0.10
Massawa	3.40 ± 0.91	2.96 ± 0.67	436.61 ± 131.61	0.88 ± 0.08
p-value	***0.015***	*0.203*	*0.575*	***0.009***
Educational Level				
Illiterate	3.37 ± 0.90	2.92 ± 0.67	452.15 ± 129.82	0.88 ± 0.10
Primary School	3.23 ± 0.72	2.66 ± 0.61	378.20 ± 116.39	0.83 ± 0.08
Junior	3.45 ± 0.83	2.94 ± 0.69	420.61 ± 133.19	0.86 ± 0.09
Secondary	3.66 ± 0.79	3.13 ± 0.67	451.28 ± 137.43	0.86 ± 0.07
College	3.91 ± 0.65	3.31 ± 0.66	517.17 ± 129.09	0.85 ± 0.08
p-value	***0.027***	***0.013***	***0.008***	*0.398*
Marital Status				
Ever married†	3.47 ± 0.81	2.95 ± 0.69	435.00 ± 133.43	0.85 ± 0.08
Never married	3.8 ± 0.74	3.27 ± 0.61	466.58 ± 141.58	0.87 ± 0.08
p-value	***0.011***	***0.002***	*0.143*	*0.210*
Ethnicity				
Tigrigna	3.61 ± 0.78	3.03 ± 0.68	443.15 ± 140.37	0.84 ± 0.08
Others*	3.48 ± 0.86	3.05 ± 0.69	444.66 ± 128.93	0.88 ± 0.08
p-value	*0.288*	*0.873*	*0.94*	***0.001***
Occupation				
Government employed	3.65 ± 0.74	3.04 ± 0.64	437.77 ± 133.00	0.84 ± 0.08
Non-government employed**	3.49 ± 0.86	3.03 ± 0.73	449.60 ± 139.54	0.87 ± 0.07
p-value	*0.159*	*0.905*	*0.54*	***0.002***

Ever married†: includes married, living together, divorced, separated, and widowed; Others: includes Tigre, Saho, Blen, Afar, Nara, and Kunama; Non-government employed**: includes merchant, farmer, retired, and housewife.*

Independent samples T-test and one-way ANOVA were used to make comparisons of the mean lung function parameters across the categories of the qualitative variables (Table 4.10). One-way ANOVA revealed a significant difference in at least one of the cities for FVC (P=0.015) and FEV1/FVC (P=0.009). Similarly, at least one of the categories of the educational level was found to have significantly different in FVC (P=0.027), FEV1 (P=0.01), and PEFR (P=0.008). Post hoc results are depicted (Table 4.10) Independent samples T-test also had significantly higher FVC (P=0.011) and FEV1 (P=0.002) as compared to the ever-married. On the other hand, other ethnic groups had significantly higher (FEV1/FVC) (P=0.001) as compared to the Tigrigna ethnic group. Similarly, participants employed in non-governmental jobs were found to have significantly higher (FEV1/FVC) (P=0.002) as compared to government-employed ones.

Table (4. 11) Comparison of mean lung function parameters of females across the categories of the qualitative variables

Variables		FVC (L)	FEV1 (L)	PEFR (L/min)	FEV1/FVC
		M ± SD	M ± SD	M ± SD	M ± SD
Altitude					
	Asmara	2.78 ± 0.66	2.31 ± 0.55	317.66 ± 96.63	0.87 ± 0.10
	Keren	2.48 ± 0.48	2.17 ± 0.46	303.92 ± 76.99	0.87 ± 0.14
	Massawa	2.47 ± 0.49	2.10 ± 0.46	292.74 ± 63.09	0.86 ± 0.12
	p-value	***<0.001***	***0.015***	*0.182*	***0.002***
Educational Level					
	Illiterate	2.39 ± 0.57	2.02 ± 0.46	301.99 ± 76.44	0.85 ± 0.09
	Primary School	2.52 ± 0.56	2.11 ± 0.45	289.92 ± 87.44	0.85 ± 0.10
	Junior	2.76 ± 0.57	2.34 ± 0.53	316.17 ± 95.96	0.85 ± 0.08
	Secondary	2.90 ± 0.66	2.42 ± 0.55	331.00 ± 90.61	0.84 ± 0.10
	College	2.94 ± 0.49	2.44 ± 0.44	308.71 ± 96.44	0.83 ± 0.09
	p-value	***<0.001***	***<0.001***	*0.055*	*0.917*
Marital Status					
	Ever married†	2.65 ± 0.63	2.23 ± 0.53	309.81 ± 90.50	0.85 ± 0.09
	Never married	2.82 ± 0.59	2.36 ± 0.47	321.91 ± 86.81	0.85 ± 0.09
	p-value	*0.051*	*0.062*	*0.320*	*0.871*
Ethnicity					
	Tigrigna	2.74 ± 0.64	2.28 ± 0.54	314.18 ± 92.37	0.84 ± 0.09
	Others*	2.48 ± 0.52	2.17 ± 0.46	304.95 ± 81.05	0.88 ± 0.09
	p-value	***0.001***	*0.089*	*0.408*	***<0.001***
Occupation					
	Government employed	2.63 ± 0.62	2.13 ± 0.49	285.62 ± 94.02	0.82 ± 0.10
	Non-government employed**	2.71 ± 0.63	2.31 ± 0.53	324.80 ± 85.03	0.86 ± 0.09
	p-value	*0.245*	***0.002***	***<0.001***	***<0.001***

Ever married†: includes married, living together, divorced, separated, and widowed; Others: includes Tigre, Saho, Blen, Afar, Nara, and Kunama; Non-government employed**: includes merchant, farmer, retired, and housewife.*

As per the one-way ANOVA results, the mean FVC (p<0.001), FEV1 (p=0.009), and (FEV1/FVC) (P=0.047) for females were significantly different in at least one of the three study sites possessing different altitudes. Similarly, at least one of the categories of the educational level was found to have significantly different FVC (P<0.001) and FEV1 (P<0.001).

Independent samples T-test also showed that Tigrigna ethnic group had significantly higher FVC ($P=0.001$) as compared to other ethnic groups. Contrarily, the other ethnic groups were found to have significantly higher (FEV1/FVC) ($P<0.001$) as compared to Tigrigna ethnic group. With regards to occupational categories, non-government employed were found to have significantly higher FEV1 $p=0.003$), PEFR ($P<0.001$), and (FEV1/FVC) ($p=0.003$).

4.8.3. Bonferroni Post-hoc Pairwise comparisons

Table (4. 12) Further comparison of the mean lung function parameters across the categories of Altitude and Educational Level

Variables		**Male**	**Female**
Altitude			
	FVC, L	A>K*$^{\zeta}$	A>K***, A>M**
	FEV1, L	-	A>K**
	PEFR, L/min	-	-
	FEV1/FVC	M>A*	-
Educational Level			
	FVC, L	-	J>I***, S>I***, S>P**, C>I**, C>P*
	FEV1, L	C>P*	J>I***, J>P*, S>I***, S>P**, C>I**
	PEFR, L/min	C>J*, C>P**	-
	FEV1/FVC	-	-

*A: Asmara; K: Keren; M: Massawa; I: Illiterate; P: Primary; J: Junior; S: Secondary; C: College;*p<0.05; **p<0.01; ***p<0.001; A>K$^{\zeta}$: Participants from Asmara had significantly greater FVC, L as compared to those of Keren.*

A more conservative measure of the pair-wise comparison test tool, the Bonferroni post-hoc test, was used to identify which pairs of categories were significantly different for the altitude and educational level for both males and females (Table 4.12) The result revealed that Asmara has significantly greater FVC (P=0.042) as compared to that of Keren among males. On the other hand, Massawa has significantly higher FEV1/FVC (P=0.021) as compared to Asmara. With regards to educational level, college-level male participants had significantly greater FEV1 (P=0.021) compared to those at the primary level. Similarly, male participants at the college level had significantly greater PEFR as compared to those at the junior (P=0.034) and primary (P=0.007) levels.

Among females, Asmara had significantly greater FVC as compared to both Keren (P<0.001) and Massawa (P=0.008). Moreover, Asmara had significantly higher FEV1 as compared to Keren (P=0.043). With regards to educational level, female participants at junior level had significantly greater FVC as compared to illiterate

(P<0.001), the secondary had higher compared to illiterate (P<0.001), and primary (P=0.001), the college had higher compared to illiterate (P=0.001) and primary (P=0.043). Moreover, FEV1 was significantly higher among junior as compared to illiterate (P<0.001), the secondary had higher compared to illiterate (P<0.001), and primary (P=0.007), and junior had higher compared to illiterate (P=0.006).

4.9. Correlation of the Lung Function Parameters

Table (4. 13) Pearson's correlation matrix of the lung function parameters of male participants

Parameters	FVC	FEV1	PEFR	FEV1/FVC
	r (p-value)	**r (p-value)**	**r (p-value)**	**r (p-value)**
FVC	1	-	-	-
FEV1	0.897 (<0.001)	1	-	-
PEFR	0.516 (<0.001)	0.670 (<0.001)	1	-
FEV1/FVC	-0.183 (0.010)	0.255 (<0.001)	0.370 (<0.001)	1

r: Pearson's correlation coefficient; FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

Bivariate correlation among the four lung function parameters for the male participants is shown in Table 4.13.

The result showed a significant increase in FVC with an increase in FEV1 (r=0.897, P<0.001) among male participants. Moreover, significant positive relationship was observed between PEFR and FVC (r=0.516, P<0.001), and FEV1 (r=0.670, P<0.001). However, the relationship between (FEV1/FVC) and FVC (r=-0.183, P=0.010) was negative, even though positive was observed with FEV1 (r=0.255, P<0.001), and PEFR (r=0.370, P<0.001).

Table (4. 14) Pearson's correlation matrix of the lung function parameters of female participants

Parameters	FVC	FEV1	PEFR	FEV1/FVC
	r (p-value)	**r (p-value)**	**r (p-value)**	**r (p-value)**
FVC	-			
FEV1	0.874 (<0.001)	-		
PEFR	0.471 (<0.001)	0.583 (<0.001)	-	
FEV1/FVC	-0.224 (<0.001)	0.266 (<0.001)	0.248 (<0.001)	-

r: Pearson's correlation coefficient; FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

Bivariate correlation among the four lung function parameters for the female participants is shown in Table 4.14. The result showed a significant increase in FVC with an increase in FEV1 (r=0.857, P<0.001) among female participants. Moreover, a significant positive relationship was observed between PEFR and FVC (r=0.471, P<0.001), and FEV1 (r=0.570, P<0.001). However, the relationship between FEV1/FVC and FVC (r=-0.200, P<0.001) was negative, even though a contrarily positive relationship was observed with FEV1 (r=0.323, P<0.001), and PEFR (r=0.224, P<0.001).

4.10. Development of new Reference Equation for Spirometry

Table (4. 15) The combination of independent variables comparing the adjusted R^2 and standard error of the estimate

Spirometry Parameter	Combination	Male		Female	
		Adjusted R^2	**SEE**	**Adjusted R^2**	**SEE**
FVC	A H	0.225	0.7118	0.213	0.555
	A^2, H	0.229	0.7096	0.214	0.5546
	A^2, H^2	0.229	0.7098	0.215	0.5543
	A, H^2	0.224	0.712	0.214	0.5548
	A^2, H^3	0.228	0.7103	***0.216***	***0.554***
	A^3, H^2	0.232	0.7086	0.214	0.5549
	A^3, H	***0.232***	***0.7085***	0.213	0.5552
	A, H^3	0.223	0.7125	0.215	0.5546
FEV1	A H	0.222	0.6049	0.233	0.472
	A^2, H	0.226	0.6032	***0.236***	***0.4711***
	A^2, H^2	0.225	0.6037	0.236	0.4711
	A, H^2	0.221	0.6054	0.233	0.472
	A^2, H^3	0.223	0.6044	0.235	0.4711
	A^3, H	***0.227***	***0.6028***	0.234	0.4717
	A^3, H^2	0.226	0.6033	0.234	0.4717
	A, H^3	0.227	0.6061	0.232	0.4721
PEFR	A H	***0.021***	***134.887***	***0.035***	***88.424***
	A^2, H	0.023	134.774	0.031	88.374
	A^2, H^2	0.022	134.816	0.03	88.380
	A, H^2	0.021	134.931	0.029	88.431
	A^2, H^3	0.022	134.862	0.03	88.388
	A^3, H	0.024	134.688	0.031	88.343
	A^3, H^2	0.024	134.73	0.031	88.348
	A, H^3	0.020	134.977	0.029	88.439
FEV1FVC	A H	0.014[£]	0.0816	0.009	0.0914
	A^2, H	0.012[£]	0.0817	***0.015***	***0.0913***
	A^2, H^2	0.013[£]	0.0816	0.015	0.0913
	A, H^2	0.014[£]	0.0816	0.012	0.0914
	A^2, H^3	0.013[£]	0.0816	0.02	0.0913
	A^3, H	0.011[£]	0.0817	0.017	0.0912
	A^3, H^2	0.011[£]	0.0817	0.017	0.0912
	A, H^3	0.015[£]	0.0816	0.012	0.0914

[£]*All the multiple regressions were not significant predictors of FEV1/FVC among males and hence, the constant mean was used as a predictor. A: Age, H: Height.*

The combination of the independent variables that might predict the spirometry parameters was optimally selected based on the adjusted R^2 and SEE as shown in Table 4.15. Weight and BMI were individually significantly correlated with some of the spirometry parameters; however, they were excluded for two reasons. Firstly, they showed multicollinearity with height and secondly, they did not increase explanatory power significantly when added to the polynomial regression equations.

Hence, based on the statistical significance, clinical significance, and guidelines approved by the European Respiratory Society and the American Thoracic Society, in this study age and height selected as potential determinants for equations of FVC, FEV1, PEFR, and (FEV1/FVC). The combinations of age and height resulting in higher adjusted R2, and lower SEE are summarized in Table (4.15).

4.10.1. Male new spirometric equations

Table (4. 16) Estimates during the development of new prediction equations for spirometry among males

Variable		Estimate	SE	t-value	p-value	R (Adj. R^2)
FVC						
	Intercept	-3.109	1.209	-2.57	0.011	0.490 (0.232)
	Age^3	-3.08E-6	8.18E-7	-3.77	<0.001	
	Height	0.041	0.007			
FEV1						
	Intercept	-1.770	1.029	-1.72	0.087	0.485 (0.227)
	Age^3	-3.19E-6	6.96E-7	-4.60	<0.001	
	Height	0.030	0.006	5.00	<0.001	
PEFR						
	Intercept	52.096	225.233	0.23	0.817	0.177 (0.021)
	Age	-0.905	0.768	-1.18	0.240	
	Height	2.542	1.290	1.97	0.050	
FEV1/FVC						
	Intercept	-	-	-	-	-
	Age	-	-	-	-	
	Height	-	-	-	-	

FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate; SE: Standard Error, R: Coefficient of correlation, Adj.R2: Adjusted Coefficient of determination; [§]FEV1/FVC cannot be predicted the independent variables.

For FVC and FEV1, the combination of age cubed, and height had the highest explanatory power with no multicollinearity among the independent variables incorporated in the polynomial regression. For PEFR, the combination of age and height had the highest adjusted R2. Table 4.16 shows the final selected spirometric reference equations derived from the reference population of this study for males.

4.10.2. Female new spirometric equations

Table (4. 17) Estimates during the development of new prediction equations for spirometry among females

Variable		Estimate	SE	t-value	p-value	R (Adj. R^2)
FVC						
	Intercept	1.427	0.248	5.76	<0.001	0.469 (0.216)
	Age^2	-2.01E-04	3.10E-05	-6.53	<0.001	
	$Height^3$	4.15E-07	5.95E-08	6.96	<0.001	
FEV1						
	Intercept	-1.015	0.608	-1.67	0.096	0.492 (0.238)
	Age^2	2.05E-04	2.60E-05	-7.97	<0.001	
	Height	0.023	0.004	6.08	<0.001	
PEFR						
	Intercept	33.694	114.982	0.29	0.77	0.187 (0.029)
	Age	-0.797	0.394	-2.02	0.044	
	Height	1.973	0.712	2.77	0.006	

FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate; SE: Standard Error, R: Coefficient of correlation, Adj.R2: Adjusted Coefficient of determination

Among females, polynomial regression models had shown higher explanatory power and high goodness of fit relative to linear regression for FVC and FEV1. For FVC among females, age squared, and height cubed was the combination with the highest adjusted R^2. The polynomial regression model for FEV1 was age squared and height. However, multiple linear regression resulted in better-adjusted R^2, for PEFR.

Table (4. 18) Selected reference equations for FVC, FEV1, and PEFR

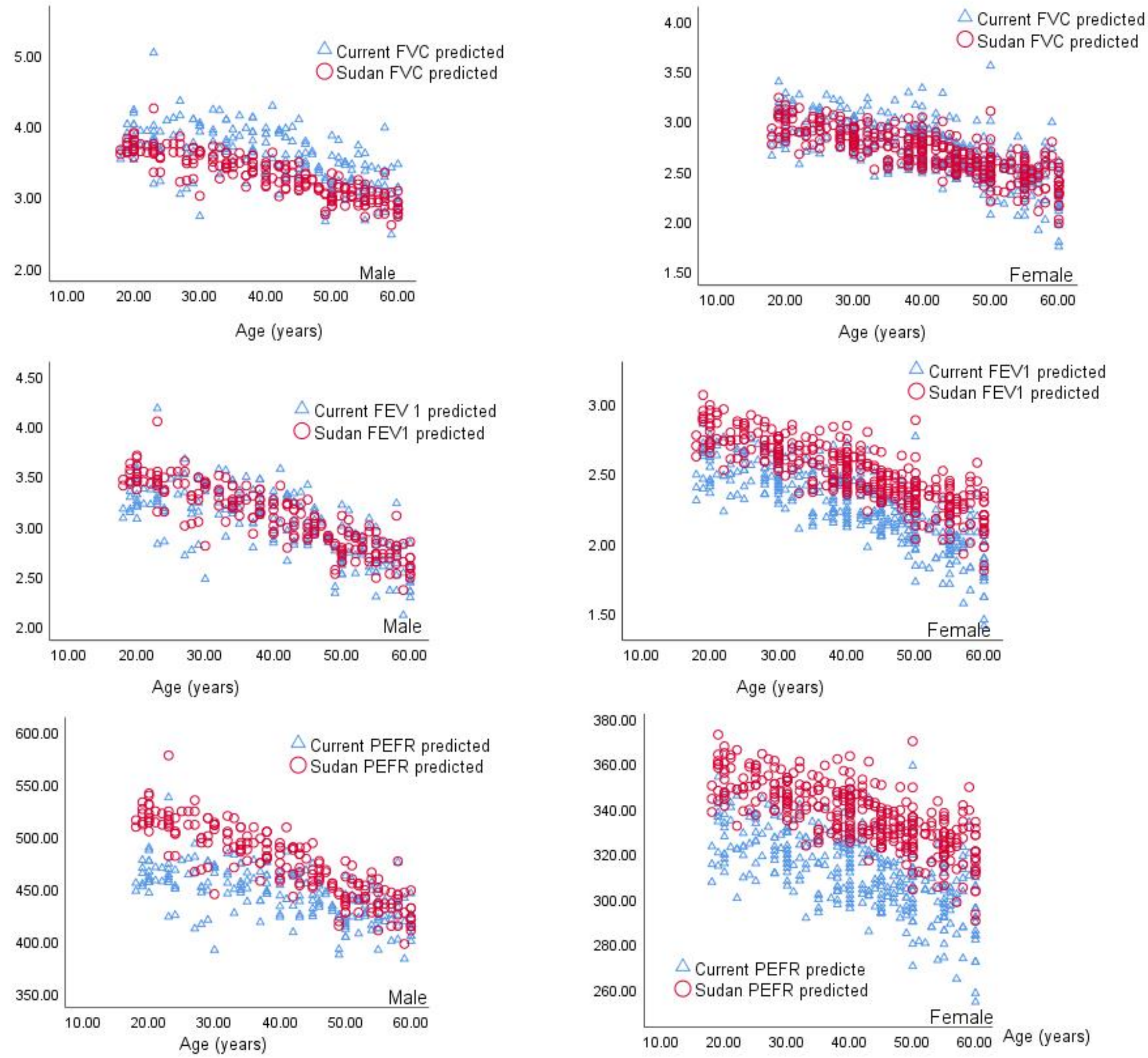
Present	**Sex**	**Indices**	**Reference Equations**
	Male	FVC	$-3.109 - 0.00000308*Age^3 + 0.041 * Height$
		FEV1	$-1.770 - 3.19E\text{-}6*Age^3 + 0.030 * Height$
		PEFR	$52.096 – 0.905 * Age + 2.542 * Height$
	Female		
		FVC	$1.427 – 0.000201*Age^2 + 4.145E\text{-}7 * Height^3$
		FEV1	$-1.178 – 0.000205 * Age^2 + 0.024 * Height$
		PEFR	33.6 94 – 0.797 * Age + 1.973 * Height

FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

Hence, the new prediction equations are written in Table (4.18).

4.11. Comparison of the predicted values by age

The predictions made using the currently estimated equation and that of Sudan were compared based on age (Figure 4.4). The scatter plot showed that the predictions made by the regression equation derived from Sudan were lower than those of the current study in both males and females. On the other hand, the predictions for FEV1 made by the equation derived from the Sudan population were higher as compared to those of the current prediction equation among females. Similar higher predictions were observed using the equations from the Sudan population as compared to that of the Eritrean population from PEFR among both males and females.



FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate.

Figure (4.4) Scatter plot showing the predicted values of the current equation and that of Sudan by age.

4.12. Predicted spirometer variables with varying age and height

4.12.1. Predicted spirometer parameters with varying age and height for males

Table (4. 19) Predicted spirometer variables with varying age (year) and height (cm) for males

Age	Height	150	155	160	165	170	175	180	185	190
20	FVC	3.02	3.22	3.43	3.63	3.84	4.04	4.25	4.45	4.66
20	FEV1	2.70	2.85	3.00	3.15	3.30	3.45	3.60	3.75	3.90
20	PEFR	415	428	441	453	466	479	492	504	517
25	FVC	2.99	3.20	3.40	3.61	3.81	4.02	4.22	4.43	4.63
25	FEV1	2.68	2.83	2.98	3.13	3.28	3.43	3.58	3.73	3.88
25	PEFR	411	423	436	449	462	474	487	500	512
30	FVC	2.96	3.16	3.37	3.57	3.78	3.98	4.19	4.39	4.60
30	FEV1	2.64	2.79	2.94	3.09	3.24	3.39	3.54	3.69	3.84
30	PEFR	406	419	432	444	457	470	483	495	508
35	FVC	2.91	3.11	3.32	3.52	3.73	3.93	4.14	4.34	4.55
35	FEV1	2.59	2.74	2.89	3.04	3.19	3.34	3.49	3.64	3.79
35	PEFR	402	414	427	440	453	465	478	491	503
40	FVC	2.84	3.05	3.25	3.46	3.66	3.87	4.07	4.28	4.48
40	FEV1	2.53	2.68	2.83	2.98	3.13	3.28	3.43	3.58	3.73
40	PEFR	397	410	423	435	448	461	473	486	499
45	FVC	2.76	2.97	3.17	3.38	3.58	3.79	3.99	4.20	4.40
45	FEV1	2.44	2.59	2.74	2.89	3.04	3.19	3.34	3.49	3.64
45	PEFR	393	405	418	431	444	456	469	482	494
50	FVC	2.66	2.86	3.07	3.27	3.48	3.68	3.89	4.09	4.30
50	FEV1	2.33	2.48	2.63	2.78	2.93	3.08	3.23	3.38	3.53
50	PEFR	388	401	414	426	439	452	464	477	490
55	FVC	2.53	2.73	2.94	3.14	3.35	3.55	3.76	3.96	4.17
55	FEV1	2.20	2.35	2.50	2.65	2.80	2.95	3.10	3.25	3.40
55	PEFR	384	396	409	422	434	447	460	473	485
60	FVC	2.38	2.58	2.79	2.99	3.20	3.40	3.61	3.81	4.02
60	FEV1	2.04	2.19	2.34	2.49	2.64	2.79	2.94	3.09	3.24
60	PEFR	379	392	405	417	430	443	455	468	481

FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

For fixed height, the predicted FVC was found to decrease with increasing age (Table 4.19). Furthermore, the FVC was found to increase with increasing height for a given age. Predicted FEV1 and PEFR showed a similar pattern.

4.12.2. Predicted spirometer parameters with varying age and height for females

Table (4. 20): Predicted spirometer variables with varying age (year) and height (cm) for females

Age	**Height**	**150**	**155**	**160**	**165**	**170**	**175**	**180**	**185**	**190**
20	**FVC**	2.75	2.89	3.04	3.21	3.38	3.57	3.76	3.97	4.19
20	**FEV1**	2.34	2.46	2.58	2.70	2.82	2.94	3.06	3.18	3.30
20	**PEFR**	314	324	333	343	353	363	373	383	393
25	**FVC**	2.70	2.84	3.00	3.16	3.34	3.52	3.72	3.93	4.14
25	**FEV1**	2.29	2.41	2.53	2.65	2.77	2.89	3.01	3.13	3.25
25	**PEFR**	310	320	329	339	349	359	369	379	389
30	**FVC**	2.65	2.79	2.94	3.11	3.28	3.47	3.66	3.87	4.09
30	**FEV1**	2.24	2.36	2.48	2.60	2.72	2.84	2.96	3.08	3.20
30	**PEFR**	306	316	325	335	345	355	365	375	385
35	**FVC**	2.58	2.72	2.88	3.04	3.22	3.40	3.60	3.81	4.02
35	**FEV1**	2.17	2.29	2.41	2.53	2.65	2.77	2.89	3.01	3.13
35	**PEFR**	302	312	321	331	341	351	361	371	381
40	**FVC**	2.50	2.65	2.80	2.97	3.14	3.33	3.52	3.73	3.95
40	**FEV1**	2.09	2.21	2.33	2.45	2.57	2.69	2.81	2.93	3.05
40	**PEFR**	298	308	317	327	337	347	357	367	377
45	**FVC**	2.42	2.56	2.72	2.88	3.06	3.24	3.44	3.64	3.86
45	**FEV1**	2.01	2.13	2.25	2.37	2.49	2.61	2.73	2.85	2.97
45	**PEFR**	294	304	314	323	333	343	353	363	373
50	**FVC**	2.32	2.47	2.62	2.79	2.96	3.15	3.34	3.55	3.77
50	**FEV1**	1.91	2.03	2.15	2.27	2.39	2.51	2.63	2.75	2.87
50	**PEFR**	290	300	310	319	329	339	349	359	369
55	**FVC**	2.22	2.36	2.52	2.68	2.86	3.04	3.24	3.44	3.66
55	**FEV1**	1.80	1.92	2.04	2.16	2.28	2.40	2.52	2.64	2.76
55	**PEFR**	286	296	306	315	325	335	345	355	365
60	**FVC**	2.10	2.25	2.40	2.57	2.74	2.92	3.12	3.33	3.55
60	**FEV1**	1.68	1.80	1.92	2.04	2.16	2.28	2.40	2.52	2.64
60	**PEFR**	282	292	302	311	321	331	341	351	361

FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

The predicted FVC was observed to decline with an increase in age for fixed height (Table 4.20). Moreover, with an increase in height for a fixed age, the FVC was observed to rise. A similar pattern was observed for predicted FEV1, PEFR, and (FEV1/FVC) among females.

4.13. Reference Equation using LMS method

The LMS method allows for modeling of the expected mean (mu, M), coefficient of variation (sigma, S), and skewness (lambda, L). A continuous smooth fit over the entire age range is achievable by spline functions, which allow the dependent variables to vary smoothly but non-linearly as a function of the independent variables.

Generally, the reference equations are expressed in terms of age and height as in the following form:

$M=e\{a + b*\ln(\text{height}) + c*\ln(\text{Age}) + \text{M-Spline}\}$, where M is the reference mean value; a, b, and c are coefficients, and M-spline is the age-specific contribution of the spline function.

The Lower Limit Normal (LLN) is also calculated from L, M, and S as follows:

$$LLN = e\{\ln(M) + \ln(1-1.645*L*S)/L)\}$$

4.13.1. Reference equation of FVC for males and females

Table (4. 21) Comparison of the FVC models with L≠1 and L=1 for both males and females

Parameters	Male		Female	
	Model 1 (L≠1)	**Model 2 (L=1)**	Model 1 (L≠1)	**Model 2 (L=1)**
Degrees of Freedom	7.9403	**5.7718**	9.1322	**7.2218**
AIC	428.7225	**427.9545**	598.2156	**601.1555**
SBC	454.5921	**446.8457**	633.7041	**629.2201**

AIC: Akaike information criterion, SBC: Schwartz's Bayesian Criterion, L: Lambda

The spirometric equations were also obtained by LMS models using the biologically plausible and conventionally accepted explanatory variables of age and height. As a result, the equations for the M, S, and L values of each spirometric index were estimated. In the LMS method, any age transformation should be applied in the same manner to M, S, and L. If the distribution is normal, as is in this data (except for PEFR among females and FEV1/FVC among both males and females), then L=1. Two optimum equations were compared based on the skewness of the FVC; the first was a transformation applied to M, S, and L, while the second was with no transformation for L, which is equivalent to normal distribution. The Schwartz's Bayesian Criterion (SBC) for the age transformed M, S, and L was greater than SBC for the normal distribution in which L=1; hence the later model was chosen. A comparison of the summary of the degrees of freedom, AIC, and SBC is given in Table (4.21).

Table (4. 22) LMS reference equations for FVC stratified by gender

Parameter	Sex	Indices	Reference Equations
FVC	Male	M	exp {-8.1197 + 1.9226*ln (H) – 0.1319*ln (A) + m-s}
		S	exp {-2.7885 + 0.3223*ln (A) + s-s}
		L	1
	Female		
		M	exp {-6.2682 + 1.5954*ln (H) – 0.2238*ln (A) + m-s}
		S	exp {-3.0967 + 0.4118*ln (A) + s-s}
		L	1

Age-specific spline values for males and females are indicated in appendix 2. LMS=Lambda, mu, sigma, FEV1=Forced Expiratory Volume in 1 second, M=mu as predicted value, S=Sigma as the coefficient of variation, L=lambda as an index of skewness, H=Standing height (cm), A=Age (years), m-s=M-spline, s-s=S-spline.

The simplest and most parsimonious model (L=1) required 2 degrees of freedom less than the model with L≠1 and produced slightly lower SBC for both males and females. The reference equation of FVC for males and females is given in Table (4.22).

4.13.2. Model fit for FVC reference equations by LMS method

After fitting a model, an assessment of the validity of the derived reference equations was performed. The standardized quantile residuals were evenly distributed over the predicted values. The plot also revealed that there was no age dependency in the variability. The frequency density plot was compatible with normal distribution, which confirmed in the Q-Q plot with very slight positive skewness. Furthermore, the worm plots are nearly horizontal, depicting the normality of the residuals. With regards to the standardized residuals score, a very satisfactory fit was concluded because the z-scores were between (-2.851 to 4.047) for males, and (-2.521 to 3.665) for females, (compared to the standard that required being between -5 and 5). The plots for males are shown in Figures (4.5) and (4.6).

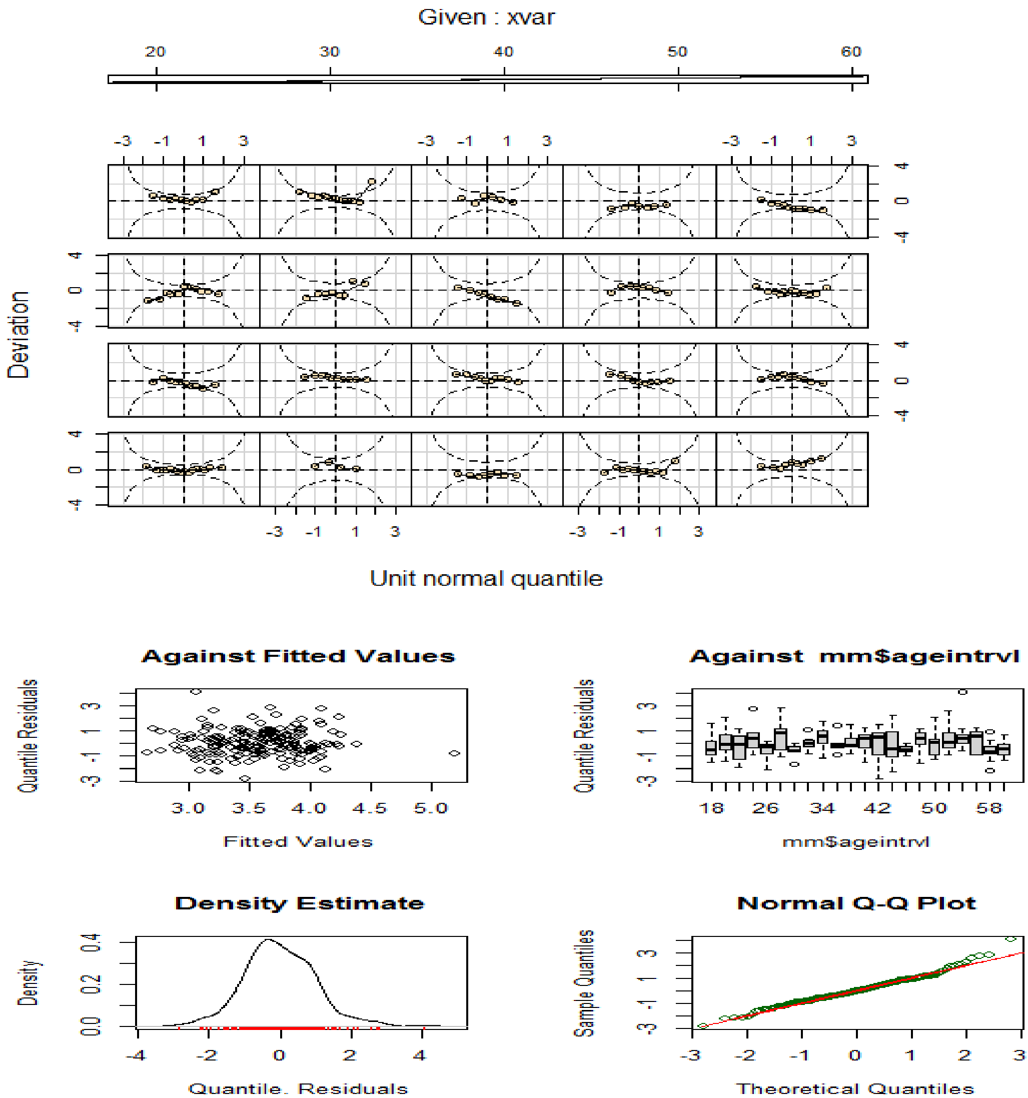
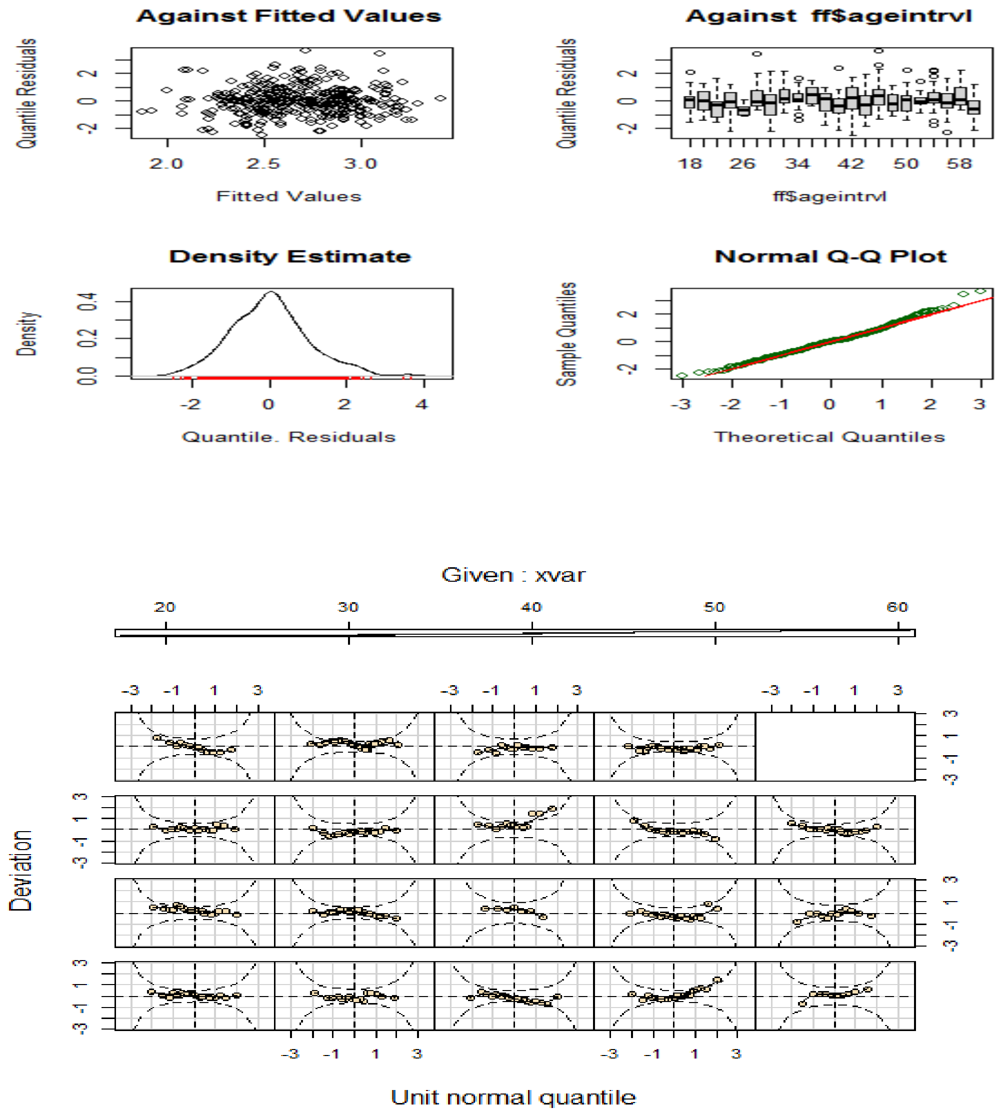


Figure 4.5 FVC model diagnosis for males using residual plot (left) and worm plot (right)



<u>Figure 4.6) FVC model diagnosis for females using residual plot (left) and worm plot (right)</u>

4.13.3. Reference equation of FEV1 for males and females

Table (4. 23) Comparison of the FEV1 models with L≠1 and L=1 for both males and females

Parameters	**Male**		**Female**	
	Model 1 (L≠1)	**Model 2 (L=1)**	Model 1 (L≠1)	**Model 2 (L=1)**
Degrees of Freedom	8.049197	**6.081836**	8.73609	**7.17754**
AIC	367.0829	**363.408**	474.2131	**470.4022**
SBC	393.4279	**383.3138**	508.1624	**498.2948**

AIC: Akaike information criterion, SBC: Schwartz's Bayesian Criterion, L: Lambda

The simplest and most parsimonious model (L=1) required (2) degrees of freedom less for males and (1) degree of freedom less for females than the model with L≠1 and produced slightly lower SBC for both males and females.

Table (4. 24) LMS reference equations for FEV1 stratified by gender

Parameter	Sex	Indices	Reference Equations
FEV1	Male	M	exp {-7.1560 + 1.7364*ln (H) – 0.1767*ln (A) + m-s}
		S	exp {-2.317 + 0.1908*ln (A) + s-s}
		L	1
	Female		
		M	exp {-6.2047 + 1.5749*ln (H) – 0.2597*ln (A) + m-s}
		S	exp {-2.6104 + 0.2768*ln (A) + s-s}
		L	1

Age-specific spline values for males and females are indicated in appendix 2. LMS=Lambda, mu, sigma, FEV1=Forced Expiratory Volume in 1 second, M=mu as predicted value, S=Sigma as the coefficient of variation, L=lambda as an index of skewness, H=Standing height (cm), A=Age (years), m-s=M-spline, s-s=S-spline.

4.13.4. Model fit for FEV1 reference equations by LMS method

The standardized quantile residuals were evenly distributed over the predicted values. The plot also revealed that there was no age dependency in the variability. The frequency density plot was compatible with normal distribution, which is confirmed in the Q-Q plot. Generally, the distribution appears to be good because the z-scores were between -3.065 and 3.079, which is a very satisfactory fit to the distribution (compared to the standard that required being between -5 and 5). The plots for males are shown in Figure 4.7 and Figure 4.8.

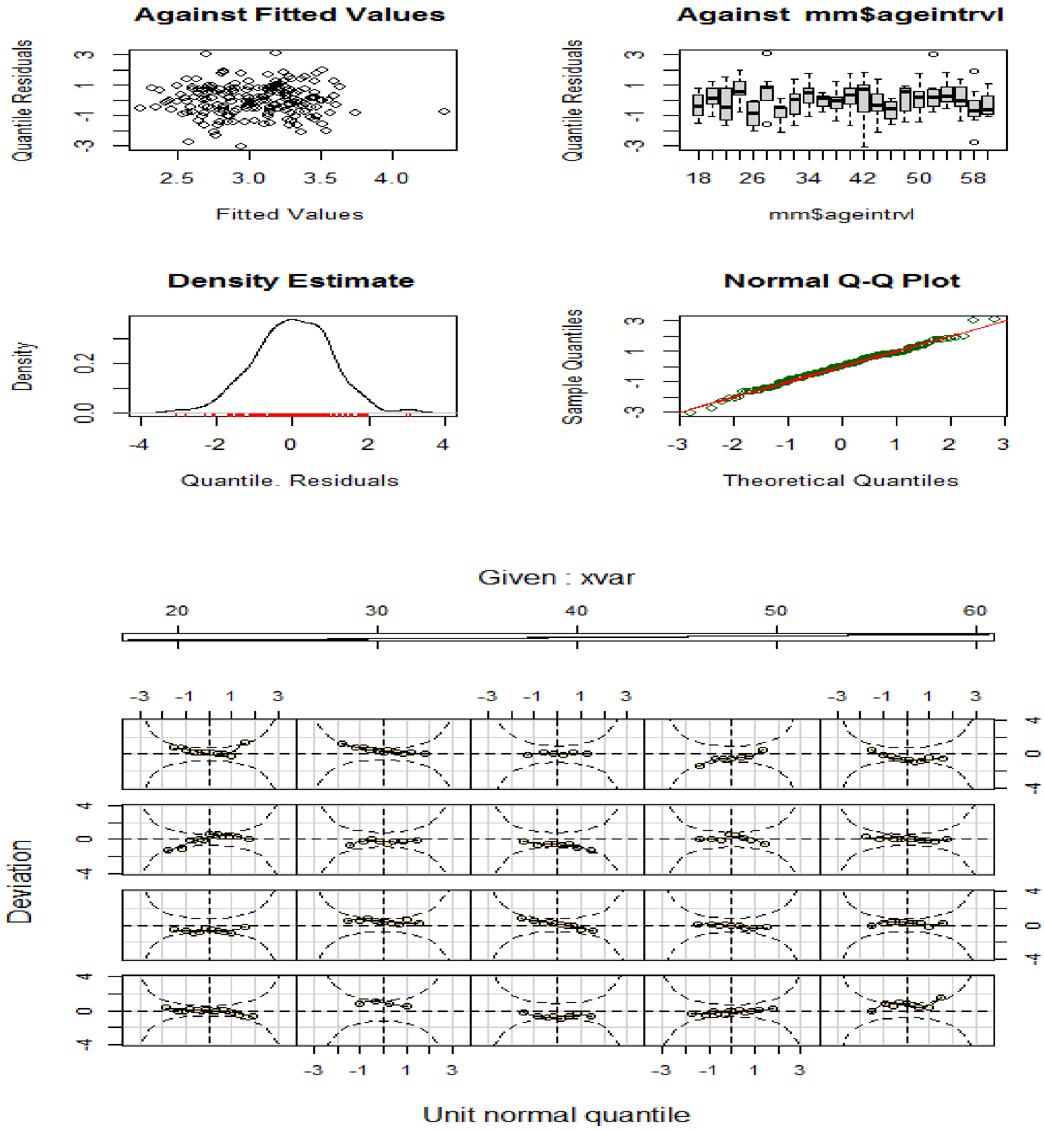


Figure 4.7) FEV1 model diagnosis for males using residual plot (left) and worm plot (right)

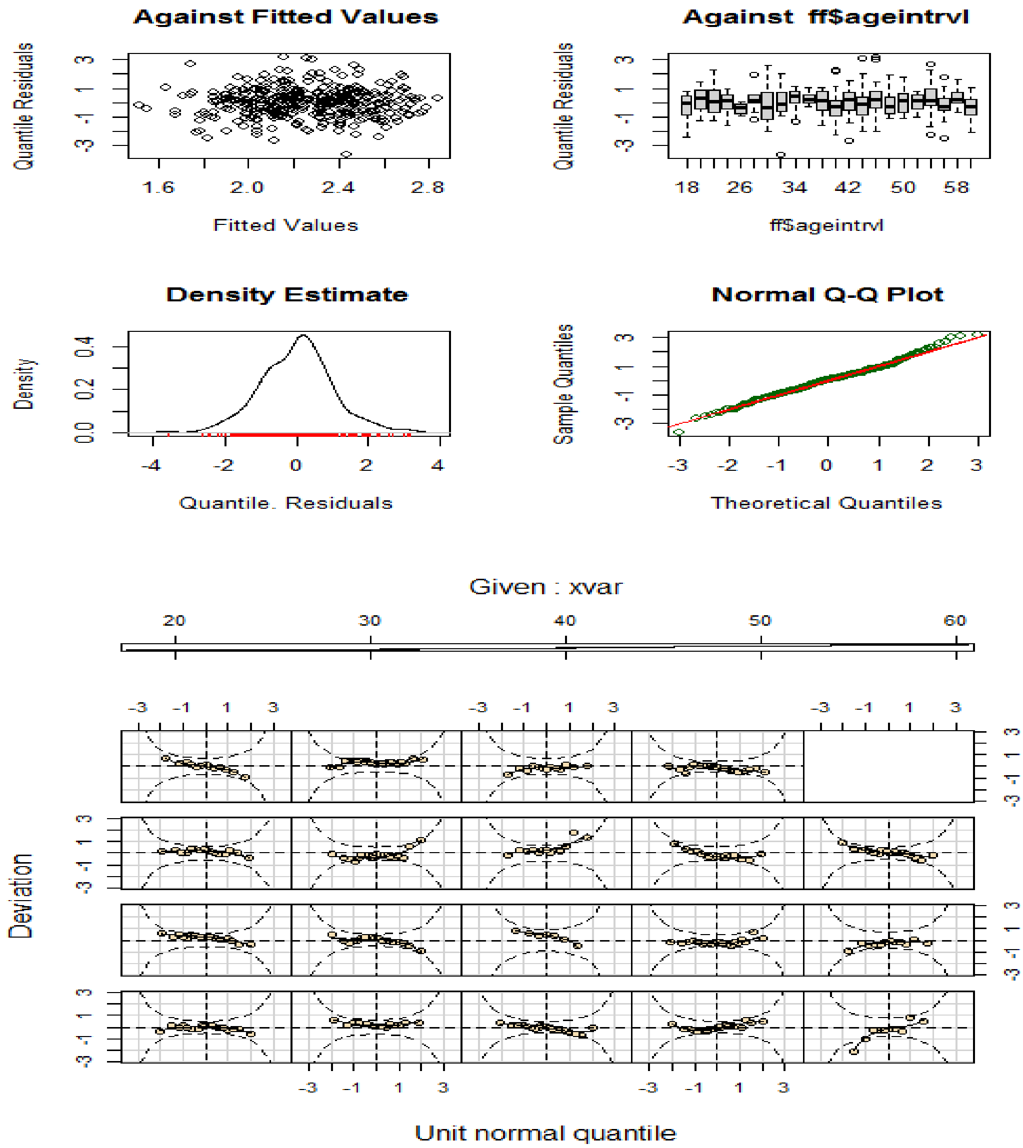


Figure 4.8 FEV1 model diagnosis for females using residual plot (left) and worm plot (right)

4.13.5. Reference equation of PEFR for males and females

Table (4. 25) Comparison of the PEFR models with L≠1 and L=1 for both males and females

Parameters	**Male**		**Female**	
	Model 1 (L≠1)	**Model 2 (L=1)**	**Model 1 (L≠1)**	Model 2 (L=1)
Degrees of Freedom	7.0049	**5.0052**	**7.0092**	5.0142
AIC	2477.695	**2474.419**	**4247.268**	4256.883
SBC	2500.622	**2490.802**	**4274.354**	4276.354

AIC: Akaike information criterion, SBC: Schwartz's Bayesian Criterion, L: Lambda

The reference equations of PEFR for both males and females were obtained similarly to those of FVC and FEV1. For males, no transformation for L was the optimum reference equation, while for females the transformation applied equation was chosen due to less Schwartz's Bayesian Criterion (Table 4.25).

Among males, the SBC was smaller in Model 2 as compared to Model 1. Besides, the degree of freedom for Model 2 was smaller in Model 2. Among females, the SBC was smaller in model 1 and hence chosen as an equation for the prediction of the reference, though the degrees of freedom are bigger

Table (4. 26) LMS reference equations for PEFR stratified by gender

Parameter	Sex	Indices	Reference Equations
FEV1	Male	M	exp {0.3264 + 1.15823*ln (H) – 0.0470*ln (A) + m-s}
		S	exp {-1.5118 + 0.0884*ln (A) + s-s}
		L	1
	Female		
		M	exp {0.8284 + 1.0347*ln (H) – 0.0943*ln (A) + m-s}
		S	exp {-1.8175 + 0.1559*ln (A) + s-s}
		L	exp {0.9998 – 0.1479*ln (A) + l-s}

Age-specific spline values for males and females are indicated in appendix 2. LMS=Lambda, mu, sigma, FEV1=Forced Expiratory Volume in 1 second, M=mu as predicted value, S=Sigma as the coefficient of variation, L=lambda as an index of skewness, H=Standing height (cm), A=Age (years), m-s=M-spline, s-s=S-spline; l-s= L-Spline.

4.13.6. Model fit for PEFR reference equations by LMS method

Model fit of the PEFR reference equations was assessed using the standardized quantile residual, variability, density plot, and Q-Q plots. Besides, the centile plot of the model constructed along with the observed values is displayed showing a very satisfactory fit to the distribution. The z-scores for the residuals ranged from (-2.3553 to 2.9326) for males, and (-2.3887 to 3.1815) for females. The plots for males and females are shown in Figure (4.9) and Figure (4.10).

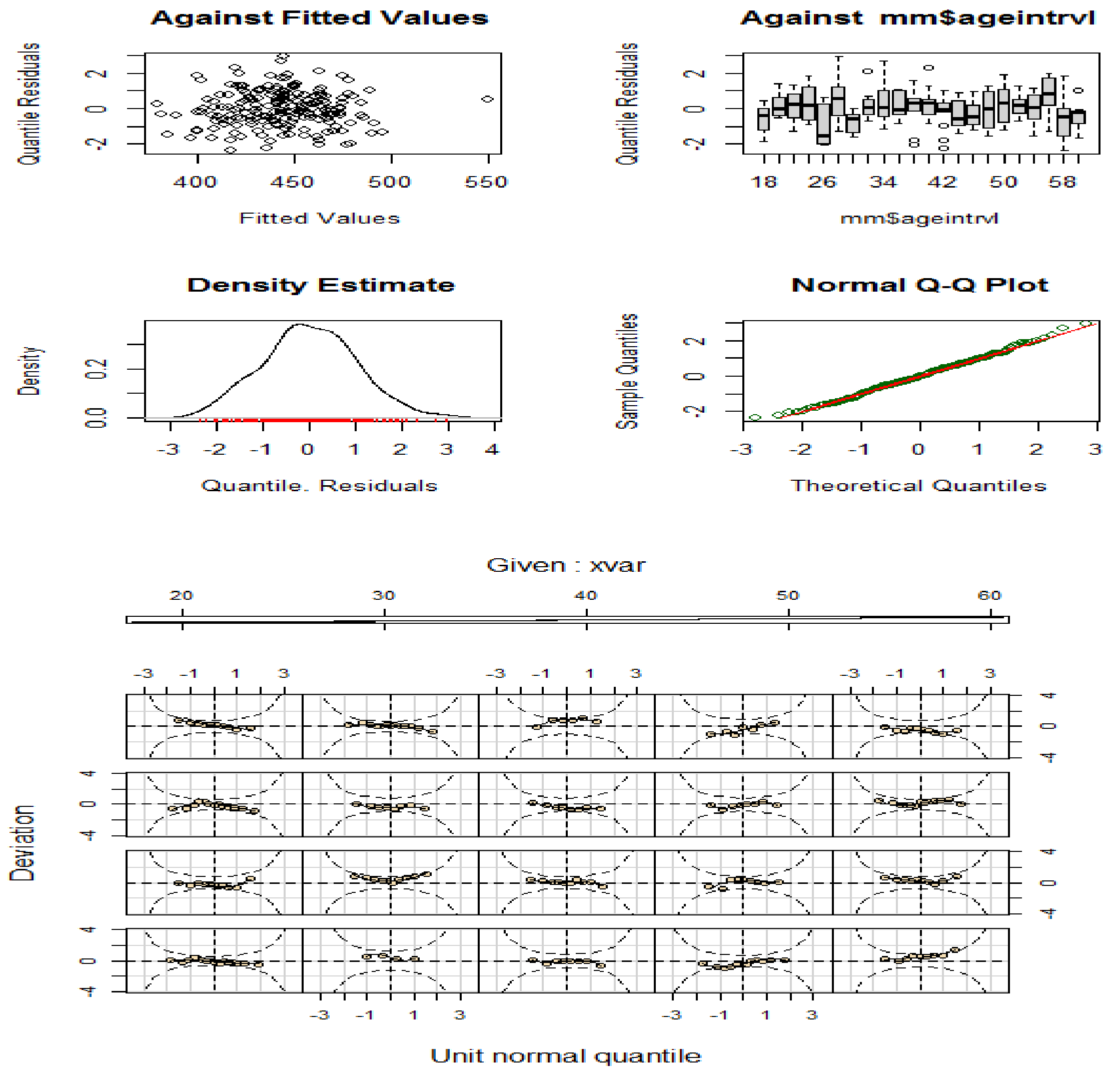


Figure (4.9) PEFR model diagnosis for males using residual plot (left) and worm plot (right)

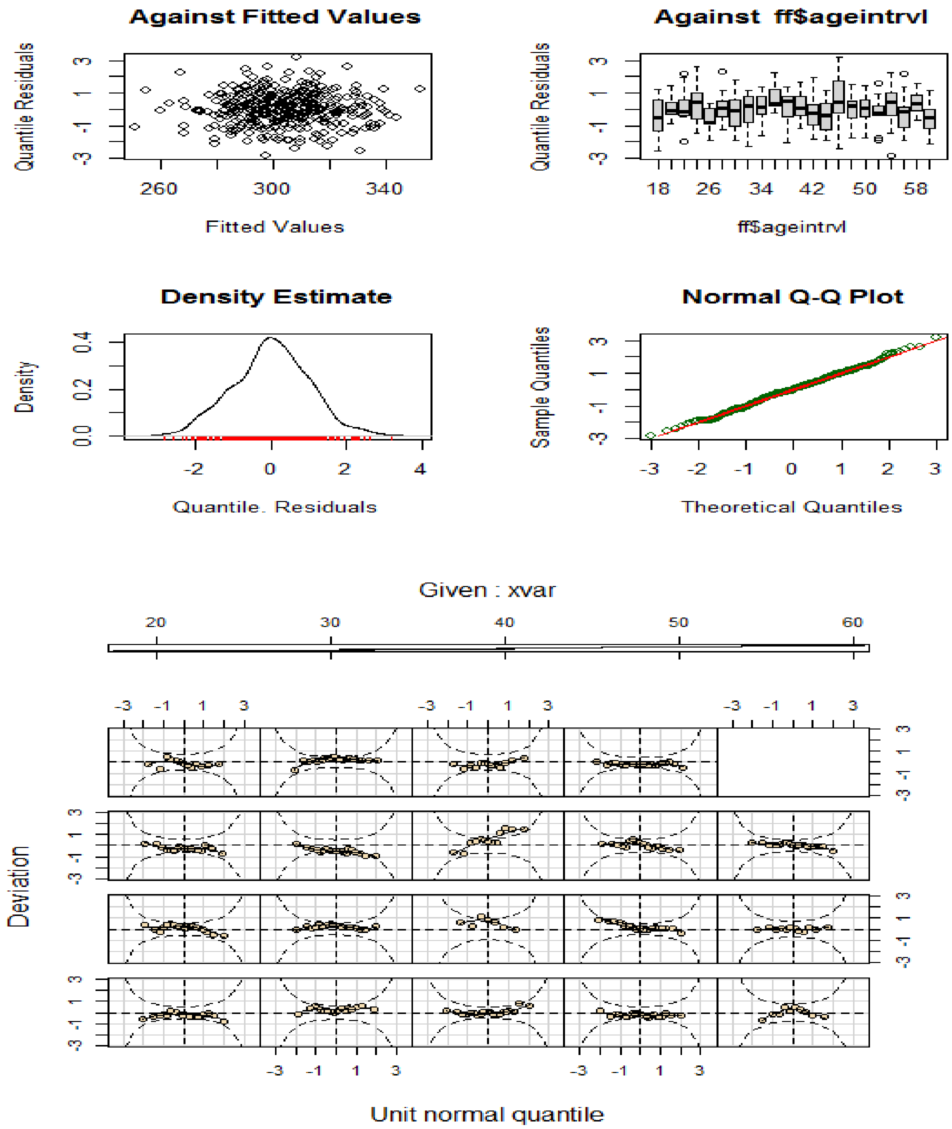


Figure 4.10) PEFR model diagnosis for females using residual plot (left) and worm plot (right)

4.14. Predicted spirometer parameters with variable age and height for males using the LMS method

Table (4. 27) Predicted spirometer parameters with variable age and height for males using the LMS method

Age	Height	150	155	160	165	170	175	180	185	190
20	**FVC**	3.00	3.19	3.39	3.60	3.81	4.03	4.25	4.48	4.72
20	**FEV1**	2.67	2.83	2.99	3.15	3.32	3.49	3.67	3.85	4.03
20	**PEFR**	399	414	430	446	461	477	493	509	525
25	**FVC**	2.98	3.18	3.38	3.58	3.79	4.01	4.23	4.46	4.70
25	**FEV1**	2.67	2.82	2.98	3.15	3.32	3.49	3.66	3.84	4.02
25	**PEFR**	395	410	426	441	456	472	488	503	519
30	**FVC**	2.95	3.14	3.34	3.54	3.75	3.96	4.18	4.41	4.64
30	**FEV1**	2.63	2.78	2.94	3.10	3.27	3.44	3.61	3.78	3.96
30	**PEFR**	392	407	422	437	453	468	484	499	515
35	**FVC**	2.89	3.08	3.28	3.48	3.68	3.89	4.11	4.33	4.56
35	**FEV1**	2.57	2.72	2.87	3.03	3.19	3.35	3.52	3.69	3.87
35	**PEFR**	389	404	419	434	449	465	480	496	511
40	**FVC**	2.83	3.02	3.21	3.40	3.60	3.81	4.02	4.24	4.46
40	**FEV1**	2.49	2.64	2.79	2.94	3.10	3.26	3.42	3.59	3.76
40	**PEFR**	386	401	416	431	447	462	477	492	508
45	**FVC**	2.77	2.95	3.13	3.32	3.52	3.72	3.93	4.14	4.36
45	**FEV1**	2.41	2.55	2.70	2.85	3.00	3.15	3.31	3.47	3.64
45	**PEFR**	384	399	414	429	444	459	474	490	505
50	**FVC**	2.71	2.88	3.06	3.25	3.44	3.64	3.84	4.05	4.26
50	**FEV1**	2.34	2.48	2.62	2.76	2.91	3.06	3.21	3.37	3.53
50	**PEFR**	382	397	412	427	442	457	472	487	503
55	**FVC**	2.65	2.82	3.00	3.18	3.37	3.56	3.76	3.96	4.17
55	**FEV1**	2.27	2.40	2.54	2.68	2.82	2.97	3.11	3.27	3.42
55	**PEFR**	380	395	410	425	440	455	470	485	500
60	**FVC**	2.59	2.76	2.93	3.11	3.29	3.48	3.68	3.88	4.08
60	**FEV1**	2.20	2.33	2.46	2.60	2.74	2.88	3.02	3.17	3.32
60	**PEFR**	379	394	408	423	438	453	468	483	498

FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

The predicted FVC for a fixed height was found to decline with age (Table 4.27). Furthermore, for a given age, the FVC was observed to rise with increasing height. The predicted FEV1 and PEFR both followed the same pattern.

4.15. Predicted spirometer parameters with variable age and height for females using the LMS method

Table (4. 28) Predicted spirometer parameters with variable age and height for females using the LMS method

Age	Height	150	155	160	165	170	175	180	185	190
20	**FVC**	2.78	2.93	3.08	3.24	3.39	3.55	3.72	3.88	4.05
20	**FEV1**	2.34	2.46	2.59	2.72	2.85	2.98	3.11	3.25	3.39
20	**PEFR**	308	319	329	340	351	361	372	383	393
25	**FVC**	2.71	2.86	3.01	3.16	3.31	3.47	3.63	3.79	3.96
25	**FEV1**	2.32	2.44	2.56	2.69	2.82	2.95	3.09	3.22	3.36
25	**PEFR**	302	312	322	333	343	354	364	375	385
30	**FVC**	2.65	2.80	2.94	3.09	3.24	3.39	3.55	3.71	3.87
30	**FEV1**	2.30	2.42	2.54	2.67	2.80	2.93	3.06	3.19	3.33
30	**PEFR**	297	307	317	327	338	348	358	368	379
35	**FVC**	2.58	2.72	2.86	3.01	3.15	3.30	3.45	3.61	3.76
35	**FEV1**	2.22	2.34	2.46	2.58	2.71	2.83	2.96	3.09	3.23
35	**PEFR**	292	302	312	323	333	343	353	363	373
40	**FVC**	2.50	2.63	2.77	2.91	3.05	3.20	3.34	3.49	3.64
40	**FEV1**	2.13	2.25	2.36	2.48	2.60	2.72	2.84	2.97	3.10
40	**PEFR**	289	299	309	319	329	339	349	359	369
45	**FVC**	2.42	2.55	2.68	2.81	2.95	3.09	3.23	3.38	3.53
45	**FEV1**	2.04	2.15	2.26	2.38	2.49	2.61	2.72	2.84	2.97
45	**PEFR**	285	295	305	315	325	335	345	355	365
50	**FVC**	2.34	2.46	2.59	2.72	2.85	2.99	3.13	3.27	3.41
50	**FEV1**	1.95	2.06	2.16	2.27	2.38	2.49	2.60	2.72	2.83
50	**PEFR**	283	292	302	312	322	331	341	351	361
55	**FVC**	2.26	2.38	2.51	2.63	2.76	2.89	3.02	3.16	3.30
55	**FEV1**	1.86	1.96	2.06	2.16	2.27	2.37	2.48	2.59	2.70
55	**PEFR**	280	290	299	309	319	328	338	348	358
60	**FVC**	2.19	2.31	2.43	2.55	2.68	2.80	2.93	3.06	3.20
60	**FEV1**	1.77	1.87	1.96	2.06	2.16	2.26	2.36	2.47	2.57
60	**PEFR**	278	287	297	307	316	326	335	345	355

FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate

The predicted FVC for a fixed height was found to decline with age (Table 4.28). Furthermore, for a given age, the FVC was observed to rise with increasing height. The predicted FEV1 and PEFR both followed the same pattern.

4.16. Difference between measured and predicted values

4.16.1. Difference between measured and predicted values among males

<u>Table (4. 29) Differences for FVC, FEV1, and PEFR between observed and predicted values according to different reference equations among males</u>

		Prediction equation (Reference Number)	Mean ± SD	p-value[§]	Relative difference (%)
FVC, L	Measured		3.57 ± 0.81		
	Predicted	Present study	3.55 ± 0.40	1.000	-0.56
		LMS method	3.54 ± 0.40	0.622	-0.70
		Sudan [Bashir & Musa]	3.15 ± 0.31	<0.001	-11.76
		GLI	3.88 ± 0.50	<0.001	8.69
FEV1, L	Measured		3.04 ± 0.69		
	Predicted	Present study	3.03 ± 0.69	1.000	-0.33
		LMS method	3.01 ± 0.37	0.500	-0.97
		Sudan[Bashir & Musa]	2.93 ± 0.32	0.166	-3.62
		GLI	3.19 ± 0.44	<0.001	5.59
PEFR, L	Measured		443.68 ± 136.09		
	Predicted	Present study	444.04 ± 24.08	1.000	0.08
		LMS method	442.95 ± 27.20	0.896	-0.29
		Sudan[Bashir & Musa]	458.25 ± 33.99	0.002	3.28
		GLI	-	-	-
FEV1FVC	Measured		0.86 ± 0.08		
	Predicted	Present study	0.86 ± 0.00	0.999	0.00
		LMS method	0.86 ± 0.01	0.199	0.88
		Sudan[Bashir & Musa]	-	-	-
		GLI	0.82 ± 0.03	<0.001	-3.57

A paired T-test was used to compare the predicted values of the present study (both polynomial regression and the LMS method), a study from Sudan, and the global lung initiative (GLI) with the measured values. Relative difference (%) is computed by (mean of predicted values – mean of measured values)/mean of measured values; '-' No such measurement was found in the studies, and hence comparisons were not possible.

In this research, the means of the observed values of FVC, FEV1, and PEFR were not statistically significantly different from the means of the predicted values for the reference population among males (Table 4.29). However, the anticipated values from the reference equation produced in Sudan (Bashir & Musa) and the global lung initiative for African Americans (GLI) were considerably different from the observed values, suggesting a lack of fit of the reference equations save for the FEV1 (L) in Sudan. For example, the predicted FVC was (11.76%) higher than predicted in Sudan and (8.69%) lower than predicted for African Americans globally. To make comparisons between the reference equations derived from this study and others, the relative difference was computed. The result showed that the relative differences (%) were much lower for all the parameters, showing the higher goodness of fit of the constructed polynomial regression models.

4.16.2. Difference between measured and predicted values among females

Table (4. 30) Differences for FVC, FEV1, and PEFR between observed and predicted values according to different reference equations among females

Parameter		Prediction equation (Reference Number)	Mean ± SD	p-value§	Relative difference (%)
FVC, L	Measured		2.68 ± 0.62		
	Predicted	Present study	2.69 ± 0.29	1.000	0.37
		LMS method	2.69 ± 0.29	0.751	0.35
		Sudan	2.75 ± 0.26§	<0.001	2.61
		GLI	2.87 ± 0.32	<0.001	6.88
FEV1, L	Measured		2.26 ± 0.53		
	Predicted	Present study	2.26 ± 0.26	1.000	0.00
		LMS method	2.27 ± 0.28	0.532	0.67
		Sudan	2.57 ± 0.26§	<0.001	13.72
		GLI	2.39 ± 0.31	<0.001	5.59
PEFR, L	Measured		312.03 ± 89.84		
	Predicted	Present study	311.67 ± 16.73	1.000	-0.12
		LMS method	304.61 ± 18.47	0.176	-2.05
		Sudan	342.63 ± 16.92§	<0.001	9.81
		GLI	-	-	-
FEV1/FVC	Measured		0.85 ± 0.09		
	Predicted	Present study	0.85 ± 0.01	1.000	0.00
		LMS method	0.86 ± 0.01	0.003	1.68
		Sudan	-	-	-
		GLI	0.83 ± 0.03	0.007	-1.58

§Paired T-test was used to compare the predicted values of the present study (both polynomial regression and LMS method) and another study from Sudan with the measured values. Relative difference (%) is computed by (mean of predicted values – mean of measured values)/mean of measured values; '-'No such measurement was found in the studies and hence comparisons were not possible.

As in males (Table 4.29), the observed values of FVC, FEV1, and PEFR were not statistically substantially different from expected values in females. All the anticipated values from the reference equation produced in Sudan (Bashir & Musa) and the global lung initiative for African Americans were considerably different from the measured values, indicating a lack of fit of the reference equations. The relative differences (%) were also much smaller for all parameters, which means that the polynomial regression models that were made had a better fit.

4.16.3. Differences between Altitudes using LMS method

Table (4. 31) The significance of the inclusion of additional variable altitude in the derived equations using LMS for the spirometric parameters

	Parameter	Spline	Coefficients				
Males			**β_0**	**β_1**	**β_2**	**A Vs K**	**A Vs M**
	FVC	M	-7.7566ζ	1.4848ζ	-0.1201§	-0.0870	-0.0184
		S	-2.7433ζ	-	0.2964	0.1410	0.1146
	FEV1	M	-7.1456ζ	1.7399ζ	-0.1855ζ	-0.0211	0.0230
		S	-2.2530ζ	-	0.1747	0.2287	-0.1987
	PEFR	M	0.3538	1.1513*	-0.0433	-0.0358	-0.0047
		S	-1.5628§		0.1093	-0.0912	-0.0078
	FEV1/FVC	M	1.0127§	-0.2041ζ	-0.0362	0.0592	0.0488
		S	-3.2755*	-	0.2847	-0.1723	-0.4820
		L	16.1730	-	-2.409	-	-
Females							
	FVC	M	-5.0863ζ	1.3745ζ	-0.2321ζ	**-0.0910ζ**	**-0.1525ζ**
		S	-3.3370ζ	-	0.4800ζ	**-0.1908***	-0.0833
	FEV1	M	-5.6156ζ	1.4726ζ	-0.2734ζ	-0.0358	**-0.1310§**
		S	-2.9614ζ	-	0.3737*	-0.1314	0.0253
	PEFR	M	0.9979	0.9973§	-0.0849	-0.0196	-0.0722
		S	-1.7396ζ	-	0.11514	**-0.2129***	**-0.3494***
		L	1.2518	-	-0.2064	**-**	**-**

*β0: Intercept; β1: Coefficient of ln (H); β2: Coefficient of ln (A); A Vs K: Asmara Versus Keren; A Vs M: Asmara Versus Massawa; ζ: significance is less than 0.001; §: less than 0.01; *: less than 0.05; FVC: Forced Vital Capacity; FEV1=Forced Expiratory Volume in 1 second, PEFR: peak expiratory flow rate; M=mu as predicted value, S=Sigma as a coefficient of variation*

The derived reference equations were collated from the dataset that consist of (3) different towns located at (3) different altitudes of the country. One possible concern is that there are systematic differences between cities located at (3) different altitudes. The potential difference between the (3) cities was assessed using LMS by incorporating the center at which the data was collected as an independent variable.

Analysis for the differences in altitude was performed for FVC, FEV1, PEFR, and (FEV1/FVC) stratified by gender. The summary of the effect of inclusion of the additional independent variable for the spirometric parameters using LMS is shown in Table (4.31).

The results portray that the inclusion of the variable altitude is not necessary for the prediction of the spirometric parameters for females. However, all spirometric parameters were found to have been significantly affected by the variable ‘altitude’.

The coefficients, A Vs K and A Vs M respectively, compare Keren with Asmara and Massawa with Asmara (Asmara acting as the baseline). Since FVC is natural log-transformed data, the coefficients are multiplied by (100) and treated as percentage differences. The coefficients shown for the comparison of FVC in Keren and Massawa with Asmara are not significant among males. However, for females, Keren has (9.1%) lower FVC as compared to Asmara and Massawa (15.2%)lower as compared to that of Asmara, as per the results about M (mu spline). According to the results from S (sigma spline), Keren has a (19.08%) larger variability as compared to Asmara. Comparison of the Schwartz’s Bayesian Criterion (SBC) of the model for FVC with and without the inclusion of altitude showed improvement (SBC without A=629.2201 to SBC with A = 615.8718) at the expense of 2 degrees of freedom (df without A =7.2217 to df with A = 9.9252).

Similar interpretations follow for the other significant parameters.

Chapter 5

Discussion,
Conclusion
&
Recommendations

5. Discussion

The high prevalence of respiratory diseases in Eritrea is a primary source of concern in the country's public health sector ((NHERI), 2021), as is the global spread of covid-19, as well as World Health Organization guidelines for the importance of deriving lung function prediction equations and addressing the gap in the availability of lung function data in East Africa. This study was done to help filling these gaps. The two objectives of this study were:

- To predict lung function values using a flexible and effective statistical technique for Eritreans ages between (18 and 60) years old.
- To develop FVC, FEV1, FEV1/FVC, and PEFR prediction equations for Eritreans.

Spirometry prediction equations for children and adults of different ethnicities have been extensively studied. For the development of the lung function prediction equations, statistical techniques focused on linear regression, ranging from the most basic form of simple linear regression to a more flexible way of generalized additive models for location, scale, and shape (GAMLSS).

According to the researchers, choosing the best prediction equations for lung function indices is very important because it has a considerable impact on the clinical interpretation of the data (Rosenfeld et al., 2001, Subbarao et al., 2004, Mehrparvar et al., 2012).

Most studies addressed participants to be adults between (18 and 60), with some studies going beyond (60) years (Paul et al., 2018, Ratomaharo et al., 2015b, Ketfi et al., 2018, Quanjer et al., 2012a, Kainu et al., 2016, Pereira et al., 2007, Quanjer et al., 2013, Jo et al., 2018, Kamanga et al., 2019, Wang et al., 2020).

The adult Eritrean's spirometry reference equations were the subject of this study. Models should be adaptable enough to generalize to multiple contexts, such as creating equations for all age groups and across different ethnic backgrounds.

Following an exhaustive literature review, the flexible and efficient statistical procedures was selected. The lung function prediction equations were derived using these chosen methods. Polynomial regression and a generalized additive model are on the table as research options for this project.

Polynomial regression is the sort of regression we apply when the dependent and independent variables have a non-linear relationship. Many studies employ polynomial regression to generate spirometric prediction equations(Eom and Kim, 2013, Kim et al., 2020, Ratomaharo et al., 2015a, Musafiri et al., 2013, Bashir and Musa, 2012, Golshan et al., 2003, Perez-Padilla et al., 2006, Lee et al., 2008). The adult Eritrean FVC, FEV1, and PEFR prediction equations were driven by this model for males and females. Males and females were found to have significantly different lung functions (FVC, FEV1, and PEFR) and anthropometric measurements. Thus, unique fitting equations for each sex are appropriate.

Based on age, the predictions generated using the polynomial regression equation and those made in Sudan were compared. In both males and females, the regression equation generated from Sudan produced lower values than those found in the current study in Eritrea. In females, the FEV1 predictions made using the equation generated from the Sudan population were higher than those made by the present prediction equation. As for the PEFR, both Eritrean males and females have higher values. This is possibly because most of the data came from the highland cities (Asmara and Karen). As people who live in the highlands have a higher lung parameter when compared to people who live in the lowlands (White et al., 1994, López Jové et al., 2018, Havryk et al., 2002, Aristizabal-Duque et al., 2020, Aristizabal-Duque et al., 2019). This may be an adaptation to chronic hypoxia and high levels of habitual exercise.

The generalized additive model was the second method we employed in this study to build the adult Eritrean spirometry prediction equations. This approach was chosen since Quanjer et al. employed it in earlier generations of GLI equations and because most recent research has recommended it over the earlier method. (Quanjer et al., 2012c, Ratomaharo et al., 2015b, Jo et al., 2018, Pefura-Yone et al., 2021, Huls et al., 2014, Chang et al., 2019, Zhang et al., 2017, Martinez-Briseno et al., 2021, Aristizabal-Duque et al., 2020, Agarwal et al., 2019, Havryk et al., 2002, Jiang et al., 2016). The one that is the most appropriate prediction equation for adults Eritrean were used.

A significant difference was observed between males' and females' pulmonary functions (FVC, FEV1, and PEFR). As a result, it is customary to use separate equations for each gender.

Both models have shown efficacy in producing spirometry values for adult Eritreans because there is no statistically significant difference between them, and the values were collected. Comparing the values produced by the two methods within them, the polynomial regression equation is better for Eritrean adults than the generalized additive model because it is the least different from the data we collected. It would be the opposite of what we discovered in the literature review, but we accept this finding because the difference is not substantial or impactful. All the research that compared them did not use the same data.(Martinez-Briseno et al., 2021, Kubota et al., 2014).

The average lung function parameters of males and females were compared across altitudes. The average FVC, FEV1, and PEFR in Asmara are higher than in other cities. Which aligns with what in the previous studies (Ulrich et al., 2020, López Jové et al., 2018, Aristizabal-Duque et al., 2020). It's also a good fit with hypoxia adaptation in the highlands and exercises habituation. On the other hand, comparing the lung parameters FVC, FEV1, and PEFR comparison in Keren and Massawa females found that this parameter is higher in Keren females than in Massawa females. This outcome is in line with the findings of the literature review. When were looked at the males, the Massawa males had higher spirometry parameters than the Keren males, contradicting the literature analysis. This is probably related to ethnic differences, and it’s also possible because the Keren males in this study were older on average than the Massawa males, and spirometry values decreased with age. (Aristizabal-Duque et al., 2020, Aristizabal-Duque et al., 2019, Ulrich et al., 2020).

Most previous research bases its prediction equations on age and height as the two most closely independent variables to vital capacity (Morris et al., 1971, Silva, 2007). There was a significant inverse relationship between age and ventilatory function in adult Eritrean males. A decline in FVC (r=-0.298, p0.001) and FEV1 (r=-0.349, p0.001) were observed with increasing age. The 4 lung function indices and age were shown to have a strong negative relationship with females. With increasing age, there was a decrease in FVC (r = -0.332, p0.001), FEV1 (r = -0.390, p0.001), PEFR (r = -0.119, p=0.023), and FEV1/FVC (r = -0.117, p=0.026). which is consistent with the findings in the literature (Janssens, 2005). Respiratory function declines with age, primarily due to structural changes in the respiratory system caused by aging, including the chest wall, respiratory muscle, and respiratory airway (Janssens, 2005).

Height, on the other hand, was associated with increases in FVC, FEV1, and PEFR in both males and females. With an increase in the Height of the male participants, there was a significant increase in FVC and FEV1. In addition, PEFR and Height were also found to have a positive, significant relationship in females, which is similar to the previous studies (Bashir and Musa, 2012, Bakhit et al., 2020, Mengesha and Mekonnen, 1985).

Researchers found that among males in this study, the means of measured FVC, FEV1, and PEFR were statistically indistinguishable from the average of the projected values. A significant discrepancy existed between the predicted values of the reference equation derived in Sudan (Bashir and Musa) and the global lung initiative for African Americans (GLI), suggesting that the reference equations do not accurately represent actual lung function adults Eritrean. For example, the predicted FVC was 11.76% higher than anticipated in Sudan and (8.69%) lower than predicted for African Americans globally (Bashir and Musa, 2012, Quanjer et al., 2012c).

In adult Eritrean females, the observed FVC, FEV1, and PEFR values were not statistically significantly different from predicted values. Predicted values from the Sudanese reference equation (Bashir & Musa) and the global lung initiative for African Americans differed substantially from the observed values, showing that the Sudanese and GLI reference equations were not well-fitting for Eritrean (Quanjer et al., 2012c, Bashir and Musa, 2012).

This study is the first of its kind in Eritrea and has the potential to close the enormous reference equation gaps that exist in the North African population. Recent models (e.g., GAMLSS and polynomial regression models) which were used worldwide to model lung function values were employed in this study to derive reference models for lung function indices. Doctors can use the projected results to evaluate adult Eritreans' lung function, the severity of respiratory diseases, and responsiveness to treatment. To guarantee that the equations in this study were functional, all lung function evaluations were reviewed by a respirologist to ensure that they were acceptable for use in developing prediction equations.

The limitation of this study is that the spirometry reference values should be utilized exclusively for the age group examined (18 to 60 years). Extrapolations below or above the minimum and maximum values are not justified. The data collected from the Keren and Massawa are small, and the spirometer used is an olde

5.1. Conclusion

- This study establishes the FVC, FEV1, FEV1/FVC%, and PEFR reference values **for pulmonary function in a healthy, nonsmoking adult Eritrean.** These values can be used as references in respiratory clinics throughout Eritrea.
- According to this study, the lung function measures FVC, FEV1, and (FEV1/FVC) ratio decreased with increasing age in Eritrean males and females. Females' PEFR lowers as their age.
- Height rise was associated with increases in FVC, FEV1, and PEFR in both males and females in this study.
- An increase in the weight of male participants resulted in a significant rise in FVC and FEV1. Furthermore, PEFR and weight had a positive, significant relationship in females.
- The average FVC, FEV1, and PEFR in Asmara are higher than in other cities.
- Predicted values from the Sudanese reference equation and the global lung initiative for African Americans differed substantially from the observed values in this study, showing that the Sudanese and GLI reference equations

were not well-fitting for Eritreans and normal values for each African country should be establish by using polynomial regression.

- The regression equation from Sudan predicted lower values than those in Eritrean males and females, except for FEV1 in females.
- According to this study, the GLI reference values were higher than those in Eritrean males and females.
- Current research showed that Eritreans have different pulmonary function values than people from other races and ethnicities, which shows how important it is to have different reference equations for different races and ethnicities.

5.2. Recommendation

- We recommend using these FVC, FEV1, and PEFR values for healthy Eritrean adults in all respiratory clinics as reference values.
- More research is needed to validate these values in all cites and all ages.
- Because this study excluded people under the age of (18) and those over the age of (60), it would be valuable to conduct a similar study on a national scale.
- More research is recommended to link the spirometry reference values and respiratory disorders.
- An African project for stablishing normal reference values is recommended.

References

National Higher Education- Eritrea (NHERI), N. H. E. A. R. I. 2021. ERITREAN PROFILE. Eritrea OROTTA COLLEGE OF MEDICINE AND HEALTH SCIENCES.

ADESANYA, K. K., TAIWO, A. I., ADEDODUN, A. F. & OLATAYO, T. O. 2018. Modeling continuous non-linear data with lagged fractional polynomial regression. *Asian Journal of Applied Sciences,* 6.

AGARWAL, D., PARKER, R., ROY, S., PINNOCK, H., GHORPADE, D., SALVI, S., KHATAVKAR, P. & JUVEKAR, S. 2019. Development of spirometry predictive values for Western Indian population (NIHR-RESPIRE Study). Eur Respiratory Soc.

ANAND, D., STEVENSON, C., WEST, C. & PHAROAH, P. 2003. Lung function and respiratory health in adolescents of very low birth weight. *Archives of disease in childhood,* 88**,** 135-138.

ARISTIZABAL-DUQUE, R., CASTIBLANCO, E., RODRIGUEZ, I., SOSSA-BRICEÑO, M. P. & RODRIGUEZ-MARTINEZ, C. E. 2019. Reference values for spirometric parameters in healthy children living in a Colombian city located at 2640 m altitude. *Pediatr Pulmonol,* 54**,** 886-893.

ARISTIZABAL-DUQUE, R., SOSSA-BRICEÑO, M. P. & RODRIGUEZ-MARTINEZ, C. E. 2020. Development of spirometric reference equations for children living at high altitude. *Clin Respir J,* 14**,** 1011-1017.

AUSTIN, P. C. 2007. A comparison of regression trees, logistic regression, generalized additive models, and multivariate adaptive regression splines for predicting AMI mortality. *Statistics in medicine,* 26**,** 2937-2957.

BAKHIT, B. M., MUSA, O. A. E. & MALLA, M. H. 2020. Normal Sudanese Spirometeric Values for Police Men. Research Square.

BASHIR, A. A. & MUSA, O. A. 2012. Reference spirometric values in a Sudanese cohort. *East Mediterr Health J,* 18**,** 151-8.

BERGER, V. W. & ZHOU, Y. 2014. Kolmogorov–smirnov test: Overview. *Wiley statsref: Statistics reference online*.

BROWN, A. M. 2001. A step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet. *Computer methods and programs in biomedicine*, 65**,** 191-200.

CHANG, S.-M., TSAI, H.-J., TZENG, J.-Y., YEH, K.-W., CHEN, L.-C., LAI, S.-H., LIAO, S.-L., HUA, M.-C., TSAI, M.-H. & HUANG, J.-L. 2019. Reference equations for spirometry in healthy Asian children aged 5 to 18 years in Taiwan. *World Allergy Organization Journal,* 12**,** 100074.

CHENG, Y., MACERA, C., ADDY, C., SY, F., WIELAND, D. & BLAIR, S. N. 2003. Effects of physical activity on exercise tests and respiratory function. *British journal of sports medicine,* 37**,** 521-528.

CHHABRA, S. 1998. Forced vital capacity, slow vital capacity, or inspiratory vital capacity: which is the best measure of vital capacity? *Journal of Asthma,* 35**,** 361-365.

COLE, T. 1975. Linear and proportional regression models in the prediction of ventilatory function. *Journal of the Royal Statistical Society: Series A (General),* 138**,** 297-325.

CULVER, B. H. 2012. How should the lower limit of the normal range be defined? *Respir Care,* 57**,** 136-45; discussion 143-5.

DEROM, E., VAN WEEL, C., LIISTRO, G., BUFFELS, J., SCHERMER, T., LAMMERS, E., WOUTERS, E. & DECRAMER, M. 2008. Primary care spirometry. *European Respiratory Journal,* 31**,** 197-203.

DOHERTY, M. & DIMITRIOU, L. 1997. Comparison of lung volume in Greek swimmers, land based athletes, and sedentary controls using allometric scaling. *British journal of sports medicine,* 31**,** 337-341.

ELMALEH-SACHS, A., BALTE, P., OELSNER, E. C., ALLEN, N. B., BAUGH, A. D., BERTONI, A. G., HANKINSON, J. L., PANKOW, J., POST, W. S. & SCHWARTZ, J. E. 2021. Race/Ethnicity, Spirometry Reference Equations and Prediction of Incident Clinical Events: The Multi-Ethnic Study of Atherosclerosis (MESA) Lung Study. *American journal of respiratory and critical care medicine*.

EOM, S. Y. & KIM, H. 2013. Reference values for the pulmonary function of Korean adults using the data of Korea National Health and Nutrition Examination Survey IV (2007-2009). *J Korean Med Sci,* 28**,** 424-30.

GEORGE, J. M., SEN, K. & RAVEENDRAN, C. 2014. Evaluation of the effect of exercise on pulmonary function in young healthy adults. *International Journal of Biomedical and Advance Research,* 5**,** 308-312.

GOLSHAN, M., NEMATBAKHSH, M., AMRA, B. & CRAPO, R. O. 2003. Spirometric reference values in a large Middle Eastern population. *Eur Respir J,* 22**,** 529-34.

GRAHAM, B. L., STEENBRUGGEN, I., MILLER, M. R., BARJAKTAREVIC, I. Z., COOPER, B. G., HALL, G. L., HALLSTRAND, T. S., KAMINSKY, D. A., MCCARTHY, K., MCCORMACK, M. C., OROPEZ, C. E., ROSENFELD, M., STANOJEVIC, S., SWANNEY, M. P. & THOMPSON, B. R. 2019. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med,* 200**,** e70-e88.

HALLETT, S., TORO, F. & ASHURST, J. V. 2018. Physiology, tidal volume.

HANKINSON, J. L., ODENCRANTZ, J. R. & FEDAN, K. B. 1999. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med,* 159**,** 179-87.

HANSEN, J. E., SUN, X.-G. & WASSERMAN, K. 2007. Spirometric criteria for airway obstruction: Use percentage of FEV1/FVC ratio below the fifth percentile, not< 70%. *Chest,* 131**,** 349-355.

HASTIE, T. & TIBSHIRANI, R. 1986. Generalized Additive Models. *Statistical Science,* 1**,** 297-310.

HAVRYK, A. P., GILBERT, M. & BURGESS, K. R. 2002. Spirometry values in Himalayan high altitude residents (Sherpas). *Respir Physiol Neurobiol,* 132**,** 223-32.

HNIZDO, E., GLINDMEYER, H. W., PETSONK, E. L., ENRIGHT, P. & BUIST, A. S. 2006. Case definitions for chronic obstructive pulmonary disease. *COPD: Journal of Chronic Obstructive Pulmonary Disease,* 3**,** 95-100.

HULS, A., KRAMER, U., GAPPA, M., MULLER-BRANDES, C., SEITNER-SORGE, G., VON BERG, A., SCHUSTER, A., BECKMANN, C., ILLI, S., WISBAUER, M. & BERDEL, D. 2014. [New spirometric reference values for children and adolescents in Germany considering height and non-linear age effects: the LUNOKID-Study]. *Pneumologie,* 68**,** 393.

HUTCHINSON, J. 1846. On the capacity of the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer. *Medico-chirurgical transactions,* 29**,** 137.

IDLEH ABAR, A., AOUICHAOUI, C., CHERIF, J., TRABELSI, Y. & TABKA, Z. 2018. Prediction equations for spirometry in healthy children from Djibouti. *The International Journal of Tuberculosis and Lung Disease,* 22**,** 1233-1238.

IRVIN, C. G. Lung volumes. Seminars in respiratory and critical care medicine, 1998. Copyright© 1998 by Thieme Medical Publishers, Inc., 325-334.

IVANOVA, O., KHOSA, C., BAKULI, A., BHATT, N., MASSANGO, I., JANI, I., SAATHOFF, E., HOELSCHER, M. & RACHOW, A. 2020. Lung Function Testing and Prediction Equations in Adult Population from Maputo, Mozambique. *International journal of environmental research and public health,* 17**,** 4535.

JANSSENS, J.-P. 2005. Aging of the respiratory system: impact on pulmonary function tests and adaptation to exertion. *Clinics in chest medicine,* 26**,** 469-484.

JIANG, M., GAO, Y., XIE, Y., ZHENG, J. & CHEN, W. 2016. Comparison of regression models for developing spirometric reference values. Eur Respiratory Soc.

JO, B. S., MYONG, J. P., RHEE, C. K., YOON, H. K., KOO, J. W. & KIM, H. R. 2018. Reference Values for Spirometry Derived Using Lambda, Mu, Sigma (LMS) Method in Korean Adults: in Comparison with Previous References. *J Korean Med Sci,* 33**,** e16.

KAINU, A., LINDQVIST, A. & SOVIJÄRVI, A. R. 2016. Prevalence of abnormal findings when adopting new national and international Global Lung Function Initiative reference values for spirometry in the Finnish general population. *Eur Clin Respir J,* 3**,** 30658.

KAMANGA, B. M., KAYEMBE, J. M. N., NKIAMA, C. E., KAYEMBE, P. K., KIKONTWE, L. K. & LENGA NKOY, M. J. 2019. [Spirometric reference values in the Bantu population aged 20-70 years in Kinshasa]. *Pan Afr Med J,* 33**,** 295.

KETFI, A., GHARNAOUT, M., BOUGRIDA, M. & BEN SAAD, H. 2018. The multi-ethnic global lung initiative 2012 (GLI-2012) norms reflect contemporary adult's Algerian spirometry. *PLoS One,* 13**,** e0203023.

KIM, D., KIM, J. H. & LIM, D. 2020. Normal Predicted Reference Values for Spirometry in Korean Children and Adolescents. *Children,* 7**,** 105.

KORY, R. C., CALLAHAN, R., BOREN, H. G. & SYNER, J. C. 1961. The Veterans Administration-Army cooperative study of pulmonary function. I. Clinical spirometry in normal men. *Am J Med,* 30**,** 243-58.

KOURI, A., DANDURAND, R. J., USMANI, O. S. & CHOW, C.-W. 2021. Exploring the 175-year history of spirometry and the vital lessons it can teach us today. *European Respiratory Review,* 30.

KUBOTA, M., KOBAYASHI, H., QUANJER, P. H., OMORI, H., TATSUMI, K. & KANAZAWA, M. 2014. Reference values for spirometry, including vital capacity, in Japanese adults calculated with the LMS method and compared with previous values. *Respir Investig,* 52**,** 242-50.

LASSENIUS, M. I., TOPPILA, I., PÖNTYNEN, N., KASSLIN, L., KAUNISTO, J., KILPELÄINEN, M. & LAITINEN, T. 2020. Forced Vital Capacity (FVC) decline, mortality and healthcare resource utilization in idiopathic pulmonary fibrosis. *European Clinical Respiratory Journal,* 7**,** 1702618.

LEE, C. H., LEE, J. Y., JANG, E. J., PARK, K. Y. & HAN, H. W. 2008. New predictive equations for spirometric reference values and comparison with Morris equation in a Korean population. *Respirology,* 13**,** 365-71.

LÓPEZ JOVÉ, O. R., ARCE, S. C., CHÁVEZ, R. W., ALANIZ, A., LANCELLOTTI, D., CHIAPELLA, M. N., JULIÁN, A., DE VITO, E. L. & SALA, H. L. 2018. Spirometry reference values for an andean high-altitude population. *Respir Physiol Neurobiol,* 247**,** 133-139.

LUJAN, H. L. & DICARLO, S. E. 2018. Science reflects history as society influences science: brief history of "race,""race correction," and the spirometer. *Advances in physiology education*.

MÄLKIÄ, E. & IMPIVAARA, O. 1998. Intensity of physical activity and respiratory function in subjects with and without bronchial asthma. *Scandinavian journal of medicine & science in sports,* 8**,** 27-32.

MANNINO, D., BUIST, A., PETTY, T., ENRIGHT, P. & REDD, S. 2003. Lung function and mortality in the United States: data from the First National Health and Nutrition Examination Survey follow up study. *Thorax,* 58**,** 388-393.

MARTINEZ-BRISENO, D., GOCHICOA-RANGEL, L., TORRE-BOUSCOULET, L., CID-JUAREZ, S., FERNANDEZ-PLATA, R., MARTINEZ-VALDEAVELLANO, L., CHAPELA-LARA, S., DEL RIO-HIDALGO, R. & PEREZ-PADILLA, R. 2021. Comparing Spirometric Reference Values From Childhood to Old Age Estimated by LMS and Linear Regression Models. *Arch Bronconeumol (Engl Ed),* 57**,** 172-178.

MASEKELA, R., HALL, G. L., STANOJEVIC, S., SARTORIUS, B., MACGINTY, R., SAAD, H. B., TRABELSI, Y., MESSAN, F., ARIGLIANI, M., KETFI, A. & GRAY, D. 2019. An urgent need for African spirometry reference equations: the Paediatric and Adult African Spirometry study. *Int J Tuberc Lung Dis,* 23**,** 952-958.

MEHRPARVAR, A. H., RAHIMIAN, M., MIRMOHAMMADI, S. J., GHEIDI, A., MOSTAGHACI, M. & LOTFI, M. H. 2012. Comparison of FEV3, FEV6, FEV1/FEV3 and FEV1/FEV6 with usual spirometric indices. *Respirology,* 17**,** 541-546.

MENGESHA, Y. A. & MEKONNEN, Y. 1985. Spirometric lung function tests in normal non-smoking Ethiopian men and women. *Thorax,* 40**,** 465-468.

MILANZI, E. B., KOPPELMAN, G. H., OLDENWENING, M., AUGUSTIJN, S., AALDERS-DE RUIJTER, B., FARENHORST, M., VONK, J. M., TEWIS, M., BRUNEKREEF, B. & GEHRING, U. 2019. Considerations in the use of different spirometers in epidemiological studies. *Environmental Health,* 18**,** 1-8.

MILLER, M. R., CRAPO, R., HANKINSON, J., BRUSASCO, V., BURGOS, F., CASABURI, R., COATES, A., ENRIGHT, P., VAN DER GRINTEN, C. M. & GUSTAFSSON, P. 2005. General considerations for lung function testing. *European Respiratory Journal,* 26**,** 153-161.

MOORE, V. C. 2012. Spirometry: step by step. *Breathe,* 8**,** 232-240.

MORRIS, J. F., KOSKI, A. & JOHNSON, L. C. 1971. Spirometric standards for healthy nonsmoking adults. *American Review of Respiratory Disease,* 103**,** 57-67.

MUSAFIRI, S., VAN MEERBEECK, J. P., MUSANGO, L., DEROM, E., BRUSSELLE, G., JOOS, G. & RUTAYISIRE, C. 2013. Spirometric reference values for an East-African population. *Respiration,* 85**,** 297-304.

NAJI, N. A., CONNOR, M. C., DONNELLY, S. C. & MCDONNELL, T. J. 2006. Effectiveness of pulmonary rehabilitation in restrictive lung disease. *Journal of Cardiopulmonary Rehabilitation and Prevention,* 26**,** 237-243.

NAWROCKA, A. & MYNARSKI, W. 2017. Objective assessment of adherence to global recommendations on physical activity for health in relation to spirometric values in nonsmoker women aged 60–75 years. *Journal of aging and physical activity,* 25**,** 123-127.

PANT, A. 2019. *Introduction to Linear Regression and Polynomial Regression* [Online]. Available: https://towardsdatascience.com/introduction-to-linear-regression-and-polynomial-regression-f8adc96f31cb [Accessed].

PAUL, D. W., LAGAT, D. K., MACINTYRE, N., EGGER, J. R., MURDOCH, D. M., QUE, L. G. & KUSSIN, P. S. 2018. Validation of spirometry prediction equations in western Kenya. *Int J Tuberc Lung Dis,* 22**,** 112-118.

PEFURA-YONE, E. W., BALKISSOU, A. D., POKA-MAYAP, V., DJENABOU, A., MASSONGO, M., OFIMBOUDEM, N. A., MAYOH-NGUEMFO, C. F., TSALA, A. G., HADJARA, H. & AMOUGOU, F. 2021. Spirometric reference equations for Cameroonians aged 4 to 89 years derived using lambda, mu, sigma (LMS) method. *BMC Pulm Med,* 21**,** 344.

PELLEGRINO, R., VIEGI, G., BRUSASCO, V., CRAPO, R., BURGOS, F., CASABURI, R., COATES, A., VAN DER GRINTEN, C., GUSTAFSSON, P. & HANKINSON, J. 2005. Interpretative strategies for lung function tests. *European respiratory journal,* 26**,** 948-968.

PEREIRA, C. A., SATO, T. & RODRIGUES, S. C. 2007. New reference values for forced spirometry in white adults in Brazil. *J Bras Pneumol,* 33**,** 397-406.

PEREZ-PADILLA, R., VALDIVIA, G., MUINO, A., LOPEZ, M. V., MARQUEZ, M. N., MONTES DE OCA, M., TALAMO, C., LISBOA, C., PERTUZE, J., JR, B. J. & AM, B. M. 2006. [Spirometric reference values in 5 large Latin American cities for subjects aged 40 years or over]. *Arch Bronconeumol,* 42**,** 317-25.

QUANJER, P., STOCKS, J., COLE, T., HALL, G. & STANOJEVIC, S. 2011. Influence of secular trends and sample size on reference equations for lung function tests. *European Respiratory Journal,* 37**,** 658-664.

QUANJER, P. H., BRAZZALE, D. J., BOROS, P. W. & PRETTO, J. J. 2013. Implications of adopting the Global Lungs Initiative 2012 all-age reference equations for spirometry. *Eur Respir J,* 42**,** 1046-54.

QUANJER, P. H., HALL, G. L., STANOJEVIC, S., COLE, T. J. & STOCKS, J. 2012a. Age- and height-based prediction bias in spirometry reference equations. *Eur Respir J,* 40**,** 190-7.

QUANJER, P. H., STANOJEVIC, S., COLE, T. J., BAUR, X., HALL, G. L., CULVER, B. H., ENRIGHT, P. L., HANKINSON, J. L., IP, M. S. & ZHENG, J. 2012b. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. Eur Respiratory Soc.

QUANJER, P. H., STANOJEVIC, S., COLE, T. J., BAUR, X., HALL, G. L., CULVER, B. H., ENRIGHT, P. L., HANKINSON, J. L., IP, M. S., ZHENG, J. & STOCKS, J. 2012c. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J,* 40**,** 1324-43.

QUANJER, P. H., TAMMELING, G., COTES, J., PEDERSEN, O., PESLIN, R. & YERNAULT, J. 1993. Lung volumes and forced ventilatory flows. *European Respiratory Journal,* 6**,** 5-40.

RATOMAHARO, J., LINARES PERDOMO, O., COLLINGRIDGE, D. S., ANDRIAMIHAJA, R., HEGEWALD, M., JENSEN, R. L., HANKINSON, J. & MORRIS, A. H. 2015a. Spirometric reference values for Malagasy adults aged 18-73 years. *Eur Respir J,* 45**,** 1046-54.

RATOMAHARO, J., PERDOMO, O. L., COLLINGRIDGE, D. S., ANDRIAMIHAJA, R., HEGEWALD, M., JENSEN, R. L., HANKINSON, J. & MORRIS, A. H. 2015b. Spirometric reference values for Malagasy adults aged 18–73 years. *European Respiratory Journal,* 45**,** 1046-1054.

RIGBY, R. A. & STASINOPOULOS, D. M. 2005. Generalized additive models for location, scale and shape. *Journal of the Royal Statistical Society: Series C (Applied Statistics),* 54**,** 507-554.

ROSENFELD, M., PEPE, M. S., LONGTON, G., EMERSON, J., FITZSIMMONS, S. & MORGAN, W. 2001. Effect of choice of reference equation on analysis of pulmonary function in cystic fibrosis patients. *Pediatric pulmonology,* 31**,** 227-237.

SACHS, M. C., ENRIGHT, P. L., STUKOVSKY, K. D. H., JIANG, R. & BARR, R. G. 2009. Performance of maximum inspiratory pressure tests and maximum inspiratory pressure reference equations for 4 race/ethnic groups. *Respiratory care,* 54**,** 1321-1328.

SILVA, L. C. 2007. Spirometry: what's normal? *J Bras Pneumol,* 33**,** xxi-xxii.

SPRIGGS, E. A. 1978. The history of spirometry. *Br J Dis Chest,* 72**,** 165-80.

STOCKS, J., SONNAPPA, S. & LUM, S. 2014. Lung function testing in children: importance of race and ethnic-specific reference equations. Taylor & Francis.

SUBBARAO, P., LEBECQUE, P., COREY, M. & COATES, A. L. 2004. Comparison of spirometric reference values. *Pediatr Pulmonol,* 37**,** 515-22.

SWANNEY, M. P., RUPPEL, G., ENRIGHT, P. L., PEDERSEN, O. F., CRAPO, R. O., MILLER, M. R., JENSEN, R. L., FALASCHETTI, E., SCHOUTEN, J. P., HANKINSON, J. L., STOCKS, J. & QUANJER, P. H. 2008. Using the lower limit of normal for the FEV1/FVC ratio reduces the misclassification of airway obstruction. *Thorax,* 63**,** 1046-51.

TIFFENEAU, R. & PINELLI 1947. [Circulating air and captive air in the exploration of the pulmonary ventilator function]. *Paris Med,* 37**,** 624-8.

TRABELSI, Y., PARIES, J., HARRABI, I., ZBIDI, A., TABKA, Z., RICHALET, J. & BUVRY, A. 2008. Factors affecting the development of lung function in Tunisian children. *American Journal of Human Biology: The Official Journal of the Human Biology Association,* 20**,** 716-725.

ULRICH, S., FURIAN, M., ESTEBESOVA, B., TOKTOGULOVA, N., BEISHEKEEVA, G., ULRICH, S., BURNEY, P. G., SOORONBAEV, T. M. & BLOCH, K. E. 2020. Spirometry in Central Asian Lowlanders and Highlanders, a Population Based Study. *Frontiers in medicine,* 6**,** 308.

VAZ FRAGOSO, C. A., CONCATO, J., MCAVAY, G., VAN NESS, P. H., ROCHESTER, C. L., YAGGI, H. K. & GILL, T. M. 2010. The ratio of FEV1 to FVC as a basis for establishing chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine,* 181**,** 446-451.

WANG, W. T., KO, H. K., LIN, C. C., SHU, J. H., HSU, H. C., LIANG, Y., HSU, P. F., LIN, C. C., WANG, Y. J., DIN, Y. Z., LIOU, T. L., WANG, Y. W., HUANG, S. S., LU, T. M., LEU, H. B., CHAN, W. L. & LIN, S. J. 2020. Spirometric reference values in heathy Chinese adults in Taiwan: The secular changes and comparison with other Asian populations. *J Formos Med Assoc,* 119**,** 290-299.

WANG, X., DOCKERY, D. W., WYPIJ, D., GOLD, D. R., SPEIZER, F. E., WARE, J. H. & FERRIS JR, B. G. 1993. Pulmonary function growth velocity in children 6 to 18 years of age. *American Journal of Respiratory and Critical Care Medicine,* 148**,** 1502-1508.

WANGER, J., CLAUSEN, J., COATES, A., PEDERSEN, O., BRUSASCO, V., BURGOS, F., CASABURI, R., CRAPO, R., ENRIGHT, P. & VAN DER GRINTEN, C. 2005. Standardisation of the measurement of lung volumes. *European respiratory journal,* 26**,** 511-522.

WATSFORD, M. L., MURPHY, A. J., PINE, M. J. & COUTTS, A. J. 2005. The effect of habitual exercise on respiratory-muscle function in older adults. *Journal of aging and physical activity,* 13**,** 34-44.

WHITE, N. W., HANLEY, J. H., LALLOO, U. G. & BECKLAKE, M. R. 1994. Review and analysis of variation between spirometric values reported in 29 studies of healthy African adults. *Am J Respir Crit Care Med,* 150**,** 348-55.

YAN, R., TSE, L. A., LIU, Z., BO, J., CHAN, E. Y., WANG, Y., YIN, L. & LI, W. 2018. Ethnic differences in spirometry measurements in China: Results from a large community-based epidemiological study. *Respirology,* 23**,** 704-713.

YAO, G. Y., MA, Y. L., ZHANG, M. Q. & GAO, Z. C. 2013. Macrolide therapy decreases chronic obstructive pulmonary disease exacerbation: a meta-analysis. *Respiration,* 86**,** 254-60.

ZHANG, J., HU, X. & SHAN, G. 2017. Spirometry reference values for population aged 7–80 years in China. *Respirology,* 22**,** 1630-1636.

Appendix 1

Questionnaire on the Study of Pulmonary Function Values in Association with Variable Tests for Healthy Adult Eritrean Populations Normal Spirometry Values

Questionnaire ID:________________

A. Demographic Data

Code	Questions	Response	Skip
A1	Respondent No.		
A2	Address	A1.1 Zoba ____________________ A1.2.Subzoba _______________	
A3	Sex	1. Male 2. Female	
A4	Age	**In complete years: _____________**	
A5	Height	**In centimeters: _______________**	
A6	Weight	**In kilograms: ______________**	
A7	Blood pressure	**In mmHg: ______/______**	
A7	What is your religion?	**1.** Orthodox **2.** Catholic **3.** Protestant **4.** Muslim **5.** Traditional believer **99.** Other ,Specify__________	
A8	To which ethnic group do you belong?	**1.** Afar **2.** Bilen **3.** Hedareb **4.** Kunama **5.** Nara **6.** Rashaida **7.** Saho **8.** Tigre **9.** Tigrigna 96.Others,Specify_____________	

A9	What is the highest level of school you attended?	1. Illiterate 2. Primary school 3. Junior 4. Secondary **5.** College	
A10	What is your Marital Status?	**1.** Married **2.** Living together **3.** Widowed **4.** Divorced **5.** Separated **6.** Single/Never Married	
A11	What is your Occupation?	**1.** Gov't employee **2.** .Merchant **3.** Farmer **4.** Retired **5.** Housewife **99.** Others specify	
A12	History of Chronic respiratory Diseases	**1.** Yes **2.** No	If No Skip to B1
A13	Type of Diseases	1. Pulmonary TB 2. Chronic Bronchitis 3. Asthma 4. Others Specify____________	
A14	Duration of Disease	1. <5 2. 5-9 3. 10-14 4. 15-19 5. >20	

B. Ventilatory Lung Function Tests

Code	Questions	Values	
B1	FVC		
B2	FEV1		
B3	PEFR		
B4	FEV1/FVC		

Appendix 2

CONSENT

I have listened to and understood the provided information and have had the opportunity to ask questions about the research title Pulmonary Function Values in Association with Variable Tests for Healthy Adult Eritrean Population – Eritrea. I know that my participation is voluntary and that I can withdraw at any time without giving a reason or cost. I voluntarily agree to take part in this study.

Participant's signature _______________________________ Date ___________

Investigator's signature ______________________________ Date ___________

Appendix 3

Age-specific B splines of FVC, FEV1, PEFR, and FEV1/FVC for males and females

C-1: FVC age-specific spline values for males and females

		Male			**Female**		
S.No.	**Age**	**Mspline**	**Sspline**	**Lspline**	**Mspline**	**Sspline**	**Lspline**
1	18	-0.0349	2.79E-05	0	-0.0445	-0.2003	0
2	18.25	-0.0331	2.68E-05	0	-0.043	-0.1918	0
3	18.5	-0.0314	2.57E-05	0	-0.0416	-0.1834	0
4	18.75	-0.0296	2.46E-05	0	-0.0402	-0.1751	0
5	19	-0.0279	2.36E-05	0	-0.0387	-0.1669	0
6	19.25	-0.0262	2.26E-05	0	-0.0373	-0.1587	0
7	19.5	-0.0246	2.16E-05	0	-0.0359	-0.1507	0
8	19.75	-0.0229	2.06E-05	0	-0.0345	-0.1427	0
9	20	-0.0214	1.96E-05	0	-0.0332	-0.1349	0
10	20.25	-0.0198	1.87E-05	0	-0.0318	-0.1271	0
11	20.5	-0.0183	1.77E-05	0	-0.0304	-0.1194	0
12	20.75	-0.0168	1.68E-05	0	-0.0291	-0.1118	0
13	21	-0.0153	1.59E-05	0	-0.0277	-0.1043	0
14	21.25	-0.0139	1.50E-05	0	-0.0264	-0.0969	0
15	21.5	-0.0125	1.41E-05	0	-0.025	-0.0896	0
16	21.75	-0.0112	1.33E-05	0	-0.0237	-0.0824	0
17	22	-0.0099	1.24E-05	0	-0.0223	-0.0753	0
18	22.25	-0.0086	1.16E-05	0	-0.021	-0.0684	0
19	22.5	-0.0073	1.07E-05	0	-0.0197	-0.0616	0
20	22.75	-0.0061	9.87E-06	0	-0.0184	-0.055	0
21	23	-0.0049	9.03E-06	0	-0.0171	-0.0485	0
22	23.25	-0.0037	8.20E-06	0	-0.0158	-0.0421	0
23	23.5	-0.0026	7.37E-06	0	-0.0145	-0.0359	0
24	23.75	-0.0015	6.53E-06	0	-0.0133	-0.0299	0
25	24	-0.0004	5.70E-06	0	-0.012	-0.024	0
26	24.25	0.00063	4.87E-06	0	-0.0108	-0.0183	0
27	24.5	0.00164	4.03E-06	0	-0.0096	-0.0127	0
28	24.75	0.00261	3.19E-06	0	-0.0084	-0.0073	0
29	25	0.00355	2.35E-06	0	-0.0072	-0.0021	0
30	25.25	0.00446	1.51E-06	0	-0.006	0.00297	0
31	25.5	0.00533	6.66E-07	0	-0.0049	0.00787	0

32	25.75	0.00617	-1.81E-07	0	-0.0037	0.01261	0
33	26	0.00698	-1.03E-06	0	-0.0026	0.01718	0
34	26.25	0.00776	-1.88E-06	0	-0.0015	0.02159	0
35	26.5	0.0085	-2.73E-06	0	-0.0004	0.02583	0
36	26.75	0.00921	-3.57E-06	0	0.0006	0.0299	0
37	27	0.00988	-4.42E-06	0	0.00162	0.03381	0
38	27.25	0.01053	-5.26E-06	0	0.00262	0.03754	0
39	27.5	0.01114	-6.09E-06	0	0.00359	0.04111	0
40	27.75	0.01172	-6.91E-06	0	0.00454	0.04451	0
41	28	0.01227	-7.73E-06	0	0.00545	0.04773	0
42	28.25	0.01279	-8.54E-06	0	0.00634	0.05078	0
43	28.5	0.01329	-9.33E-06	0	0.00719	0.05366	0
44	28.75	0.01375	-1.01E-05	0	0.00802	0.05636	0
45	29	0.01418	-1.09E-05	0	0.00881	0.05889	0
46	29.25	0.01459	-1.16E-05	0	0.00956	0.06123	0
47	29.5	0.01497	-1.23E-05	0	0.01029	0.06341	0
48	29.75	0.01533	-1.30E-05	0	0.01098	0.06541	0
49	30	0.01566	-1.37E-05	0	0.01164	0.06725	0
50	30.25	0.01597	-1.44E-05	0	0.01226	0.06893	0
51	30.5	0.01626	-1.50E-05	0	0.01286	0.07045	0
52	30.75	0.01652	-1.56E-05	0	0.01342	0.07183	0
53	31	0.01676	-1.62E-05	0	0.01395	0.07305	0
54	31.25	0.01698	-1.67E-05	0	0.01444	0.07414	0
55	31.5	0.01718	-1.72E-05	0	0.01491	0.07508	0
56	31.75	0.01736	-1.77E-05	0	0.01534	0.07591	0
57	32	0.01751	-1.81E-05	0	0.01574	0.07661	0
58	32.25	0.01765	-1.85E-05	0	0.01611	0.07719	0
59	32.5	0.01777	-1.89E-05	0	0.01645	0.07767	0
60	32.75	0.01787	-1.93E-05	0	0.01676	0.07805	0
61	33	0.01795	-1.96E-05	0	0.01704	0.07833	0
62	33.25	0.01801	-1.98E-05	0	0.0173	0.07852	0
63	33.5	0.01805	-2.01E-05	0	0.01752	0.07863	0
64	33.75	0.01807	-2.03E-05	0	0.01772	0.07866	0
65	34	0.01808	-2.04E-05	0	0.01789	0.07861	0
66	34.25	0.01807	-2.06E-05	0	0.01803	0.07849	0
67	34.5	0.01804	-2.07E-05	0	0.01815	0.0783	0
68	34.75	0.01799	-2.08E-05	0	0.01825	0.07805	0
69	35	0.01793	-2.08E-05	0	0.01832	0.07774	0
70	35.25	0.01785	-2.08E-05	0	0.01836	0.07737	0
71	35.5	0.01776	-2.08E-05	0	0.01839	0.07695	0

72	35.75	0.01765	-2.07E-05	0	0.01839	0.07647	0
73	36	0.01752	-2.06E-05	0	0.01837	0.07595	0
74	36.25	0.01739	-2.05E-05	0	0.01834	0.07538	0
75	36.5	0.01723	-2.04E-05	0	0.01828	0.07476	0
76	36.75	0.01707	-2.02E-05	0	0.01821	0.0741	0
77	37	0.01689	-2.00E-05	0	0.01812	0.07339	0
78	37.25	0.0167	-1.98E-05	0	0.01801	0.07265	0
79	37.5	0.0165	-1.95E-05	0	0.01789	0.07186	0
80	37.75	0.01628	-1.93E-05	0	0.01775	0.07104	0
81	38	0.01605	-1.90E-05	0	0.0176	0.07017	0
82	38.25	0.01582	-1.87E-05	0	0.01743	0.06927	0
83	38.5	0.01557	-1.83E-05	0	0.01725	0.06832	0
84	38.75	0.01531	-1.80E-05	0	0.01706	0.06734	0
85	39	0.01504	-1.76E-05	0	0.01686	0.06632	0
86	39.25	0.01476	-1.72E-05	0	0.01664	0.06526	0
87	39.5	0.01448	-1.68E-05	0	0.01641	0.06415	0
88	39.75	0.01418	-1.64E-05	0	0.01617	0.06301	0
89	40	0.01388	-1.60E-05	0	0.01593	0.06183	0
90	40.25	0.01356	-1.55E-05	0	0.01567	0.06061	0
91	40.5	0.01324	-1.51E-05	0	0.0154	0.05935	0
92	40.75	0.01291	-1.46E-05	0	0.01512	0.05805	0
93	41	0.01257	-1.42E-05	0	0.01483	0.0567	0
94	41.25	0.01223	-1.37E-05	0	0.01453	0.05532	0
95	41.5	0.01188	-1.32E-05	0	0.01422	0.05389	0
96	41.75	0.01152	-1.27E-05	0	0.0139	0.05242	0
97	42	0.01116	-1.22E-05	0	0.01357	0.05092	0
98	42.25	0.01079	-1.17E-05	0	0.01323	0.04936	0
99	42.5	0.01041	-1.12E-05	0	0.01288	0.04777	0
100	42.75	0.01003	-1.07E-05	0	0.01253	0.04614	0
101	43	0.00964	-1.02E-05	0	0.01216	0.04446	0
102	43.25	0.00925	-9.74E-06	0	0.01179	0.04275	0
103	43.5	0.00885	-9.24E-06	0	0.0114	0.04099	0
104	43.75	0.00845	-8.74E-06	0	0.01101	0.0392	0
105	44	0.00804	-8.24E-06	0	0.01061	0.03736	0
106	44.25	0.00763	-7.74E-06	0	0.0102	0.03549	0
107	44.5	0.00722	-7.25E-06	0	0.00978	0.03358	0
108	44.75	0.00681	-6.75E-06	0	0.00935	0.03164	0
109	45	0.00639	-6.26E-06	0	0.00891	0.02966	0
110	45.25	0.00597	-5.77E-06	0	0.00847	0.02764	0
111	45.5	0.00554	-5.28E-06	0	0.00801	0.0256	0

112	45.75	0.00512	-4.79E-06	0	0.00755	0.02352	0
113	46	0.00469	-4.30E-06	0	0.00708	0.02142	0
114	46.25	0.00425	-3.81E-06	0	0.00661	0.01929	0
115	46.5	0.00382	-3.33E-06	0	0.00613	0.01714	0
116	46.75	0.00339	-2.84E-06	0	0.00564	0.01497	0
117	47	0.00295	-2.36E-06	0	0.00514	0.01278	0
118	47.25	0.00251	-1.88E-06	0	0.00464	0.01058	0
119	47.5	0.00207	-1.40E-06	0	0.00413	0.00836	0
120	47.75	0.00162	-9.14E-07	0	0.00361	0.00613	0
121	48	0.00118	-4.33E-07	0	0.00309	0.00388	0
122	48.25	0.00073	4.82E-08	0	0.00256	0.00163	0
123	48.5	0.00028	5.28E-07	0	0.00203	-0.0006	0
124	48.75	-0.0002	1.01E-06	0	0.00149	-0.0029	0
125	49	-0.0006	1.49E-06	0	0.00095	-0.0052	0
126	49.25	-0.0011	1.96E-06	0	0.0004	-0.0074	0
127	49.5	-0.0015	2.44E-06	0	-0.0001	-0.0097	0
128	49.75	-0.002	2.91E-06	0	-0.0007	-0.0119	0
129	50	-0.0025	3.39E-06	0	-0.0013	-0.0142	0
130	50.25	-0.0029	3.86E-06	0	-0.0018	-0.0164	0
131	50.5	-0.0034	4.33E-06	0	-0.0024	-0.0187	0
132	50.75	-0.0039	4.80E-06	0	-0.003	-0.0209	0
133	51	-0.0043	5.26E-06	0	-0.0035	-0.0231	0
134	51.25	-0.0048	5.73E-06	0	-0.0041	-0.0253	0
135	51.5	-0.0053	6.19E-06	0	-0.0047	-0.0275	0
136	51.75	-0.0058	6.65E-06	0	-0.0053	-0.0297	0
137	52	-0.0062	7.11E-06	0	-0.0058	-0.0319	0
138	52.25	-0.0067	7.57E-06	0	-0.0064	-0.034	0
139	52.5	-0.0072	8.02E-06	0	-0.007	-0.0362	0
140	52.75	-0.0077	8.47E-06	0	-0.0076	-0.0383	0
141	53	-0.0082	8.92E-06	0	-0.0082	-0.0404	0
142	53.25	-0.0087	9.36E-06	0	-0.0088	-0.0425	0
143	53.5	-0.0092	9.80E-06	0	-0.0094	-0.0446	0
144	53.75	-0.0096	1.02E-05	0	-0.01	-0.0467	0
145	54	-0.0101	1.07E-05	0	-0.0106	-0.0488	0
146	54.25	-0.0106	1.11E-05	0	-0.0112	-0.0508	0
147	54.5	-0.0111	1.15E-05	0	-0.0118	-0.0529	0
148	54.75	-0.0116	1.20E-05	0	-0.0124	-0.0549	0
149	55	-0.0121	1.24E-05	0	-0.013	-0.0569	0
150	55.25	-0.0126	1.28E-05	0	-0.0136	-0.0589	0
151	55.5	-0.0131	1.32E-05	0	-0.0142	-0.0609	0

152	55.75	-0.0136	1.36E-05	0	-0.0148	-0.0629	0
153	56	-0.0141	1.40E-05	0	-0.0154	-0.0648	0
154	56.25	-0.0147	1.44E-05	0	-0.016	-0.0668	0
155	56.5	-0.0152	1.49E-05	0	-0.0166	-0.0687	0
156	56.75	-0.0157	1.53E-05	0	-0.0172	-0.0706	0
157	57	-0.0162	1.57E-05	0	-0.0178	-0.0725	0
158	57.25	-0.0167	1.60E-05	0	-0.0184	-0.0744	0
159	57.5	-0.0172	1.64E-05	0	-0.019	-0.0763	0
160	57.75	-0.0177	1.68E-05	0	-0.0196	-0.0782	0
161	58	-0.0182	1.72E-05	0	-0.0202	-0.0801	0
162	58.25	-0.0187	1.76E-05	0	-0.0208	-0.082	0
163	58.5	-0.0192	1.80E-05	0	-0.0214	-0.0838	0
164	58.75	-0.0197	1.84E-05	0	-0.022	-0.0857	0
165	59	-0.0202	1.87E-05	0	-0.0226	-0.0875	0
166	59.25	-0.0207	1.91E-05	0	-0.0232	-0.0893	0
167	59.5	-0.0212	1.95E-05	0	-0.0238	-0.0912	0
168	59.75	-0.0217	1.99E-05	0	-0.0244	-0.093	0
169	60	-0.0222	2.02E-05	0	-0.025	-0.0948	0

C-2 FEV1 age-specific spline values for males and females

		Male			Female		
S. No.	**Age**	**Mspline**	**Sspline**	**Lspline**	**Mspline**	**Sspline**	**Lspline**
1	18	-0.05393	-5.76E-05	0	-0.09329	-0.06687	0
2	18.25	-0.05103	-5.46E-05	0	-0.08891	-0.06412	0
3	18.5	-0.04817	-5.16E-05	0	-0.08459	-0.06141	0
4	18.75	-0.04537	-4.86E-05	0	-0.08034	-0.05873	0
5	19	-0.04261	-4.56E-05	0	-0.07615	-0.05609	0
6	19.25	-0.03991	-4.27E-05	0	-0.07204	-0.05349	0
7	19.5	-0.03726	-3.99E-05	0	-0.068	-0.05092	0
8	19.75	-0.03468	-3.70E-05	0	-0.06404	-0.04839	0
9	20	-0.03215	-3.42E-05	0	-0.06017	-0.04589	0
10	20.25	-0.02968	-3.15E-05	0	-0.05639	-0.04343	0
11	20.5	-0.02728	-2.88E-05	0	-0.0527	-0.041	0
12	20.75	-0.02494	-2.61E-05	0	-0.0491	-0.03861	0
13	21	-0.02267	-2.34E-05	0	-0.0456	-0.03626	0
14	21.25	-0.02046	-2.09E-05	0	-0.04219	-0.03394	0
15	21.5	-0.01831	-1.83E-05	0	-0.03888	-0.03165	0
16	21.75	-0.01623	-1.59E-05	0	-0.03567	-0.02941	0
17	22	-0.0142	-1.34E-05	0	-0.03255	-0.0272	0

18	22.25	-0.01224	-1.11E-05	0	-0.02953	-0.02503	0	
19	22.5	-0.01033	-8.78E-06	0	-0.02661	-0.0229	0	
20	22.75	-0.00848	-6.56E-06	0	-0.02378	-0.02081	0	
21	23	-0.00669	-4.40E-06	0	-0.02104	-0.01876	0	
22	23.25	-0.00496	-2.31E-06	0	-0.0184	-0.01676	0	
23	23.5	-0.00327	-2.86E-07	0	-0.01584	-0.0148	0	
24	23.75	-0.00165	1.66E-06	0	-0.01337	-0.01287	0	
25	24	-7.49E-05	3.54E-06	0	-0.01098	-0.01099	0	
26	24.25	0.001442	5.34E-06	0	-0.00867	-0.00916	0	
27	24.5	0.002906	7.06E-06	0	-0.00643	-0.00736	0	
28	24.75	0.004316	8.71E-06	0	-0.00427	-0.00561	0	
29	25	0.005674	1.03E-05	0	-0.00217	-0.00389	0	
30	25.25	0.006981	1.18E-05	0	-0.00014	-0.00222	0	
31	25.5	0.008237	1.32E-05	0	0.001821	-0.00059	0	
32	25.75	0.009442	1.45E-05	0	0.003722	0.000997	0	
33	26	0.010599	1.58E-05	0	0.005563	0.002543	0	
34	26.25	0.011707	1.70E-05	0	0.007344	0.004047	0	
35	26.5	0.012767	1.81E-05	0	0.009066	0.005508	0	
36	26.75	0.013779	1.91E-05	0	0.010729	0.006926	0	
37	27	0.014744	2.01E-05	0	0.012333	0.008301	0	
38	27.25	0.015663	2.10E-05	0	0.013877	0.009631	0	
39	27.5	0.016536	2.18E-05	0	0.015363	0.010917	0	
40	27.75	0.017364	2.26E-05	0	0.01679	0.012158	0	
41	28	0.018147	2.32E-05	0	0.018158	0.013352	0	
42	28.25	0.018888	2.39E-05	0	0.019467	0.014499	0	
43	28.5	0.019587	2.44E-05	0	0.020719	0.015599	0	
44	28.75	0.020244	2.49E-05	0	0.021911	0.016651	0	
45	29	0.02086	2.53E-05	0	0.023046	0.017654	0	
46	29.25	0.021437	2.57E-05	0	0.024122	0.018607	0	
47	29.5	0.021976	2.60E-05	0	0.025141	0.019511	0	
48	29.75	0.022478	2.63E-05	0	0.026103	0.020365	0	
49	30	0.022946	2.65E-05	0	0.027011	0.021169	0	
50	30.25	0.02338	2.67E-05	0	0.027864	0.021923	0	
51	30.5	0.02378	2.68E-05	0	0.028665	0.022628	0	
52	30.75	0.024149	2.69E-05	0	0.029412	0.023283	0	
53	31	0.024486	2.70E-05	0	0.030109	0.023889	0	
54	31.25	0.024793	2.70E-05	0	0.030755	0.024445	0	
55	31.5	0.025069	2.70E-05	0	0.031352	0.024955	0	
56	31.75	0.025316	2.69E-05	0	0.031899	0.025418	0	
57	32	0.025534	2.68E-05	0	0.032398	0.025837	0	
58	32.25	0.025724	2.67E-05	0	0.032849	0.026213	0	
59	32.5	0.025885	2.66E-05	0	0.033253	0.026547	0	
60	32.75	0.026019	2.64E-05	0	0.033611	0.026842	0	

61	33	0.026124	2.62E-05	0	0.033923	0.027097	0
62	33.25	0.026202	2.60E-05	0	0.03419	0.027314	0
63	33.5	0.026253	2.58E-05	0	0.034414	0.027496	0
64	33.75	0.026277	2.56E-05	0	0.034594	0.027643	0
65	34	0.026275	2.53E-05	0	0.034734	0.027758	0
66	34.25	0.026248	2.50E-05	0	0.034833	0.027842	0
67	34.5	0.026196	2.48E-05	0	0.034894	0.027896	0
68	34.75	0.026119	2.45E-05	0	0.034916	0.027923	0
69	35	0.026018	2.42E-05	0	0.034901	0.027923	0
70	35.25	0.025894	2.39E-05	0	0.034851	0.027897	0
71	35.5	0.025747	2.35E-05	0	0.034766	0.027847	0
72	35.75	0.025579	2.32E-05	0	0.034649	0.027774	0
73	36	0.025389	2.29E-05	0	0.034501	0.027677	0
74	36.25	0.02518	2.25E-05	0	0.034324	0.027559	0
75	36.5	0.024951	2.22E-05	0	0.03412	0.027418	0
76	36.75	0.024704	2.18E-05	0	0.03389	0.027258	0
77	37	0.024439	2.15E-05	0	0.033635	0.027077	0
78	37.25	0.024157	2.11E-05	0	0.033358	0.026877	0
79	37.5	0.023859	2.08E-05	0	0.033058	0.026658	0
80	37.75	0.023545	2.04E-05	0	0.032737	0.02642	0
81	38	0.023214	2.00E-05	0	0.032397	0.026164	0
82	38.25	0.022869	1.96E-05	0	0.032037	0.025889	0
83	38.5	0.022509	1.92E-05	0	0.031659	0.025596	0
84	38.75	0.022134	1.89E-05	0	0.031264	0.025285	0
85	39	0.021745	1.85E-05	0	0.030853	0.024956	0
86	39.25	0.021343	1.81E-05	0	0.030427	0.024609	0
87	39.5	0.020927	1.77E-05	0	0.029985	0.024245	0
88	39.75	0.020498	1.72E-05	0	0.029529	0.023863	0
89	40	0.020056	1.68E-05	0	0.029059	0.023464	0
90	40.25	0.019602	1.64E-05	0	0.028574	0.023047	0
91	40.5	0.019136	1.60E-05	0	0.028076	0.022614	0
92	40.75	0.018659	1.55E-05	0	0.027563	0.022163	0
93	41	0.018171	1.51E-05	0	0.027035	0.021696	0
94	41.25	0.017671	1.46E-05	0	0.026493	0.021212	0
95	41.5	0.017162	1.42E-05	0	0.025935	0.020711	0
96	41.75	0.016643	1.37E-05	0	0.025364	0.020193	0
97	42	0.016114	1.32E-05	0	0.024777	0.019659	0
98	42.25	0.015575	1.27E-05	0	0.024175	0.019108	0
99	42.5	0.015028	1.22E-05	0	0.023558	0.018542	0
100	42.75	0.014473	1.17E-05	0	0.022925	0.017959	0
101	43	0.013911	1.12E-05	0	0.022277	0.017362	0
102	43.25	0.013342	1.07E-05	0	0.021613	0.016748	0
103	43.5	0.012767	1.01E-05	0	0.020932	0.01612	0

104	43.75	0.012186	9.59E-06	0	0.020235	0.015477	0
105	44	0.011601	9.04E-06	0	0.01952	0.01482	0
106	44.25	0.011011	8.49E-06	0	0.018789	0.014148	0
107	44.5	0.010417	7.93E-06	0	0.01804	0.013462	0
108	44.75	0.00982	7.37E-06	0	0.017274	0.012763	0
109	45	0.00922	6.80E-06	0	0.016491	0.012051	0
110	45.25	0.008617	6.23E-06	0	0.015691	0.011326	0
111	45.5	0.008011	5.65E-06	0	0.014873	0.010589	0
112	45.75	0.007403	5.06E-06	0	0.014038	0.00984	0
113	46	0.006792	4.48E-06	0	0.013187	0.009081	0
114	46.25	0.006179	3.89E-06	0	0.01232	0.008313	0
115	46.5	0.005564	3.30E-06	0	0.011437	0.007535	0
116	46.75	0.004946	2.71E-06	0	0.010539	0.006748	0
117	47	0.004326	2.12E-06	0	0.009624	0.005953	0
118	47.25	0.003705	1.53E-06	0	0.008695	0.005151	0
119	47.5	0.003082	9.33E-07	0	0.00775	0.004342	0
120	47.75	0.002456	3.40E-07	0	0.006791	0.003527	0
121	48	0.001829	-2.52E-07	0	0.005817	0.002706	0
122	48.25	0.001199	-8.43E-07	0	0.00483	0.001879	0
123	48.5	0.000567	-1.43E-06	0	0.003829	0.001049	0
124	48.75	-6.81E-05	-2.02E-06	0	0.002814	0.000215	0
125	49	-0.00071	-2.61E-06	0	0.001788	-0.00062	0
126	49.25	-0.00135	-3.19E-06	0	0.000748	-0.00146	0
127	49.5	-0.00199	-3.78E-06	0	-0.0003	-0.00231	0
128	49.75	-0.00264	-4.36E-06	0	-0.00137	-0.00315	0
129	50	-0.00329	-4.94E-06	0	-0.00244	-0.00399	0
130	50.25	-0.00395	-5.51E-06	0	-0.00352	-0.00484	0
131	50.5	-0.00461	-6.09E-06	0	-0.00462	-0.00568	0
132	50.75	-0.00527	-6.66E-06	0	-0.00572	-0.00653	0
133	51	-0.00594	-7.23E-06	0	-0.00684	-0.00737	0
134	51.25	-0.00661	-7.79E-06	0	-0.00797	-0.00822	0
135	51.5	-0.00729	-8.35E-06	0	-0.0091	-0.00906	0
136	51.75	-0.00797	-8.91E-06	0	-0.01025	-0.0099	0
137	52	-0.00865	-9.47E-06	0	-0.0114	-0.01074	0
138	52.25	-0.00934	-1.00E-05	0	-0.01256	-0.01158	0
139	52.5	-0.01003	-1.06E-05	0	-0.01373	-0.01241	0
140	52.75	-0.01073	-1.11E-05	0	-0.01492	-0.01325	0
141	53	-0.01142	-1.17E-05	0	-0.01611	-0.01408	0
142	53.25	-0.01213	-1.22E-05	0	-0.0173	-0.01492	0
143	53.5	-0.01283	-1.27E-05	0	-0.01851	-0.01574	0
144	53.75	-0.01354	-1.33E-05	0	-0.01973	-0.01657	0
145	54	-0.01426	-1.38E-05	0	-0.02095	-0.0174	0
146	54.25	-0.01497	-1.43E-05	0	-0.02218	-0.01822	0

147	54.5	-0.01569	-1.49E-05	0	-0.02342	-0.01904	0
148	54.75	-0.01642	-1.54E-05	0	-0.02466	-0.01986	0
149	55	-0.01714	-1.59E-05	0	-0.02591	-0.02068	0
150	55.25	-0.01787	-1.64E-05	0	-0.02717	-0.02149	0
151	55.5	-0.01859	-1.69E-05	0	-0.02843	-0.02231	0
152	55.75	-0.01932	-1.74E-05	0	-0.02969	-0.02311	0
153	56	-0.02005	-1.79E-05	0	-0.03096	-0.02392	0
154	56.25	-0.02078	-1.85E-05	0	-0.03223	-0.02473	0
155	56.5	-0.02151	-1.90E-05	0	-0.03351	-0.02553	0
156	56.75	-0.02224	-1.95E-05	0	-0.03479	-0.02633	0
157	57	-0.02297	-2.00E-05	0	-0.03607	-0.02712	0
158	57.25	-0.0237	-2.05E-05	0	-0.03735	-0.02792	0
159	57.5	-0.02443	-2.09E-05	0	-0.03863	-0.02871	0
160	57.75	-0.02516	-2.14E-05	0	-0.03991	-0.0295	0
161	58	-0.02589	-2.19E-05	0	-0.0412	-0.03028	0
162	58.25	-0.02662	-2.24E-05	0	-0.04248	-0.03106	0
163	58.5	-0.02734	-2.29E-05	0	-0.04376	-0.03184	0
164	58.75	-0.02807	-2.34E-05	0	-0.04504	-0.03262	0
165	59	-0.02879	-2.39E-05	0	-0.04632	-0.0334	0
166	59.25	-0.02951	-2.43E-05	0	-0.0476	-0.03417	0
167	59.5	-0.03023	-2.48E-05	0	-0.04888	-0.03494	0
168	59.75	-0.03095	-2.53E-05	0	-0.05015	-0.0357	0
169	60	-0.03166	-2.58E-05	0	-0.05142	-0.03646	0

C-3 PEFR age-specific spline values for males and females

		Male			**Female**		
S.No.	**Age**	**Mspline**	**Sspline**	**Lspline**	**Mspline**	**Sspline**	**Lspline**
1	18	-0.00028	-5.79E-05	0	-0.00038	0.000107	1.939075
2	18.25	-0.00027	-5.48E-05	0	-0.00037	0.000103	1.934884
3	18.5	-0.00026	-5.18E-05	0	-0.00035	9.91E-05	1.930751
4	18.75	-0.00025	-4.89E-05	0	-0.00034	9.51E-05	1.926673
5	19	-0.00023	-4.60E-05	0	-0.00033	9.11E-05	1.922648
6	19.25	-0.00022	-4.31E-05	0	-0.00031	8.71E-05	1.918677
7	19.5	-0.00021	-4.02E-05	0	-0.0003	8.32E-05	1.914757
8	19.75	-0.0002	-3.73E-05	0	-0.00029	7.93E-05	1.910886
9	20	-0.00019	-3.45E-05	0	-0.00027	7.55E-05	1.907065
10	20.25	-0.00017	-3.17E-05	0	-0.00026	7.18E-05	1.90329
11	20.5	-0.00016	-2.89E-05	0	-0.00025	6.82E-05	1.899563
12	20.75	-0.00015	-2.62E-05	0	-0.00024	6.45E-05	1.89588
13	21	-0.00014	-2.35E-05	0	-0.00022	6.10E-05	1.892241
14	21.25	-0.00013	-2.08E-05	0	-0.00021	5.75E-05	1.888646
15	21.5	-0.00012	-1.82E-05	0	-0.0002	5.41E-05	1.885092

16	21.75	-0.00011	-1.56E-05	0	-0.00019	5.07E-05	1.88158
17	22	-0.0001	-1.31E-05	0	-0.00018	4.74E-05	1.878108
18	22.25	-9.11E-05	-1.06E-05	0	-0.00017	4.41E-05	1.874675
19	22.5	-8.18E-05	-8.18E-06	0	-0.00016	4.08E-05	1.87128
20	22.75	-7.28E-05	-5.80E-06	0	-0.00015	3.76E-05	1.867923
21	23	-6.40E-05	-3.48E-06	0	-0.00014	3.45E-05	1.864602
22	23.25	-5.54E-05	-1.22E-06	0	-0.00013	3.14E-05	1.861318
23	23.5	-4.70E-05	9.77E-07	0	-0.00012	2.84E-05	1.858068
24	23.75	-3.89E-05	3.10E-06	0	-0.00011	2.53E-05	1.854853
25	24	-3.11E-05	5.16E-06	0	-0.0001	2.24E-05	1.851672
26	24.25	-2.34E-05	7.14E-06	0	-9.15E-05	1.95E-05	1.848524
27	24.5	-1.60E-05	9.05E-06	0	-8.30E-05	1.66E-05	1.845408
28	24.75	-8.87E-06	1.09E-05	0	-7.48E-05	1.37E-05	1.842323
29	25	-1.94E-06	1.26E-05	0	-6.68E-05	1.10E-05	1.83927
30	25.25	4.75E-06	1.43E-05	0	-5.91E-05	8.23E-06	1.836247
31	25.5	1.12E-05	1.59E-05	0	-5.15E-05	5.54E-06	1.833253
32	25.75	1.74E-05	1.74E-05	0	-4.42E-05	2.91E-06	1.830289
33	26	2.34E-05	1.88E-05	0	-3.70E-05	3.23E-07	1.827354
34	26.25	2.92E-05	2.01E-05	0	-3.01E-05	-2.21E-06	1.824446
35	26.5	3.48E-05	2.13E-05	0	-2.34E-05	-4.68E-06	1.821566
36	26.75	4.01E-05	2.25E-05	0	-1.68E-05	-7.10E-06	1.818714
37	27	4.52E-05	2.35E-05	0	-1.04E-05	-9.45E-06	1.815887
38	27.25	5.01E-05	2.45E-05	0	-4.25E-06	-1.17E-05	1.813087
39	27.5	5.48E-05	2.54E-05	0	1.76E-06	-1.40E-05	1.810313
40	27.75	5.92E-05	2.62E-05	0	7.59E-06	-1.61E-05	1.807563
41	28	6.35E-05	2.69E-05	0	1.33E-05	-1.83E-05	1.804838
42	28.25	6.75E-05	2.76E-05	0	1.88E-05	-2.03E-05	1.802138
43	28.5	7.13E-05	2.82E-05	0	2.41E-05	-2.23E-05	1.799461
44	28.75	7.50E-05	2.87E-05	0	2.93E-05	-2.42E-05	1.796807
45	29	7.84E-05	2.91E-05	0	3.43E-05	-2.60E-05	1.794177
46	29.25	8.17E-05	2.94E-05	0	3.92E-05	-2.78E-05	1.791569
47	29.5	8.48E-05	2.97E-05	0	4.39E-05	-2.95E-05	1.788983
48	29.75	8.76E-05	3.00E-05	0	4.84E-05	-3.11E-05	1.786419
49	30	9.03E-05	3.02E-05	0	5.29E-05	-3.26E-05	1.783877
50	30.25	9.29E-05	3.03E-05	0	5.71E-05	-3.41E-05	1.781356
51	30.5	9.52E-05	3.03E-05	0	6.13E-05	-3.56E-05	1.778855
52	30.75	9.74E-05	3.04E-05	0	6.52E-05	-3.69E-05	1.776375
53	31	9.94E-05	3.03E-05	0	6.91E-05	-3.82E-05	1.773915
54	31.25	0.000101	3.03E-05	0	7.28E-05	-3.94E-05	1.771475
55	31.5	0.000103	3.01E-05	0	7.63E-05	-4.06E-05	1.769054
56	31.75	0.000105	3.00E-05	0	7.97E-05	-4.16E-05	1.766652
57	32	0.000106	2.98E-05	0	8.30E-05	-4.27E-05	1.764269
58	32.25	0.000107	2.95E-05	0	8.61E-05	-4.36E-05	1.761905

59	32.5	0.000108	2.93E-05	0	8.91E-05	-4.45E-05	1.759559
60	32.75	0.000109	2.90E-05	0	9.20E-05	-4.53E-05	1.757231
61	33	0.00011	2.86E-05	0	9.47E-05	-4.60E-05	1.75492
62	33.25	0.000111	2.83E-05	0	9.72E-05	-4.67E-05	1.752627
63	33.5	0.000111	2.79E-05	0	9.96E-05	-4.73E-05	1.750351
64	33.75	0.000112	2.74E-05	0	0.000102	-4.79E-05	1.748092
65	34	0.000112	2.70E-05	0	0.000104	-4.84E-05	1.74585
66	34.25	0.000112	2.65E-05	0	0.000106	-4.88E-05	1.743625
67	34.5	0.000112	2.60E-05	0	0.000108	-4.92E-05	1.741415
68	34.75	0.000112	2.55E-05	0	0.00011	-4.95E-05	1.739221
69	35	0.000111	2.50E-05	0	0.000111	-4.97E-05	1.737043
70	35.25	0.000111	2.45E-05	0	0.000113	-4.99E-05	1.734881
71	35.5	0.000111	2.39E-05	0	0.000114	-5.00E-05	1.732734
72	35.75	0.00011	2.33E-05	0	0.000115	-5.00E-05	1.730602
73	36	0.000109	2.27E-05	0	0.000116	-5.00E-05	1.728485
74	36.25	0.000109	2.21E-05	0	0.000117	-5.00E-05	1.726382
75	36.5	0.000108	2.15E-05	0	0.000118	-4.99E-05	1.724294
76	36.75	0.000107	2.09E-05	0	0.000118	-4.97E-05	1.72222
77	37	0.000106	2.03E-05	0	0.000119	-4.95E-05	1.72016
78	37.25	0.000105	1.97E-05	0	0.000119	-4.92E-05	1.718114
79	37.5	0.000103	1.90E-05	0	0.00012	-4.89E-05	1.716082
80	37.75	0.000102	1.84E-05	0	0.00012	-4.85E-05	1.714063
81	38	0.000101	1.77E-05	0	0.00012	-4.80E-05	1.712058
82	38.25	9.92E-05	1.71E-05	0	0.00012	-4.76E-05	1.710066
83	38.5	9.77E-05	1.64E-05	0	0.00012	-4.70E-05	1.708086
84	38.75	9.61E-05	1.58E-05	0	0.000119	-4.64E-05	1.70612
85	39	9.45E-05	1.51E-05	0	0.000119	-4.58E-05	1.704166
86	39.25	9.28E-05	1.45E-05	0	0.000119	-4.51E-05	1.702225
87	39.5	9.10E-05	1.38E-05	0	0.000118	-4.44E-05	1.700296
88	39.75	8.93E-05	1.32E-05	0	0.000117	-4.36E-05	1.698379
89	40	8.74E-05	1.25E-05	0	0.000117	-4.28E-05	1.696474
90	40.25	8.55E-05	1.19E-05	0	0.000116	-4.20E-05	1.694581
91	40.5	8.36E-05	1.13E-05	0	0.000115	-4.11E-05	1.6927
92	40.75	8.16E-05	1.06E-05	0	0.000114	-4.02E-05	1.69083
93	41	7.96E-05	9.99E-06	0	0.000113	-3.92E-05	1.688972
94	41.25	7.76E-05	9.37E-06	0	0.000112	-3.82E-05	1.687125
95	41.5	7.55E-05	8.75E-06	0	0.000111	-3.72E-05	1.685289
96	41.75	7.34E-05	8.13E-06	0	0.000109	-3.61E-05	1.683465
97	42	7.13E-05	7.53E-06	0	0.000108	-3.50E-05	1.681651
98	42.25	6.92E-05	6.93E-06	0	0.000107	-3.39E-05	1.679848
99	42.5	6.70E-05	6.34E-06	0	0.000105	-3.27E-05	1.678055
100	42.75	6.49E-05	5.75E-06	0	0.000104	-3.16E-05	1.676273
101	43	6.27E-05	5.18E-06	0	0.000102	-3.04E-05	1.674502

102	43.25	6.05E-05	4.61E-06	0	0.000101	-2.91E-05	1.672741
103	43.5	5.83E-05	4.05E-06	0	9.90E-05	-2.79E-05	1.670989
104	43.75	5.61E-05	3.50E-06	0	9.73E-05	-2.66E-05	1.669248
105	44	5.38E-05	2.96E-06	0	9.56E-05	-2.54E-05	1.667517
106	44.25	5.16E-05	2.42E-06	0	9.39E-05	-2.41E-05	1.665796
107	44.5	4.94E-05	1.89E-06	0	9.21E-05	-2.28E-05	1.664084
108	44.75	4.71E-05	1.38E-06	0	9.03E-05	-2.15E-05	1.662382
109	45	4.49E-05	8.68E-07	0	8.84E-05	-2.01E-05	1.66069
110	45.25	4.26E-05	3.69E-07	0	8.65E-05	-1.88E-05	1.659006
111	45.5	4.04E-05	-1.22E-07	0	8.46E-05	-1.74E-05	1.657332
112	45.75	3.81E-05	-6.04E-07	0	8.26E-05	-1.61E-05	1.655668
113	46	3.59E-05	-1.08E-06	0	8.06E-05	-1.47E-05	1.654012
114	46.25	3.36E-05	-1.54E-06	0	7.86E-05	-1.33E-05	1.652365
115	46.5	3.14E-05	-2.00E-06	0	7.66E-05	-1.19E-05	1.650727
116	46.75	2.91E-05	-2.45E-06	0	7.45E-05	-1.05E-05	1.649098
117	47	2.69E-05	-2.89E-06	0	7.23E-05	-9.16E-06	1.647478
118	47.25	2.46E-05	-3.32E-06	0	7.02E-05	-7.76E-06	1.645866
119	47.5	2.23E-05	-3.74E-06	0	6.80E-05	-6.36E-06	1.644263
120	47.75	2.01E-05	-4.16E-06	0	6.58E-05	-4.96E-06	1.642668
121	48	1.78E-05	-4.56E-06	0	6.35E-05	-3.56E-06	1.641082
122	48.25	1.56E-05	-4.96E-06	0	6.12E-05	-2.15E-06	1.639503
123	48.5	1.33E-05	-5.35E-06	0	5.89E-05	-7.48E-07	1.637933
124	48.75	1.11E-05	-5.74E-06	0	5.66E-05	6.60E-07	1.636371
125	49	8.80E-06	-6.11E-06	0	5.42E-05	2.07E-06	1.634817
126	49.25	6.53E-06	-6.48E-06	0	5.18E-05	3.48E-06	1.633271
127	49.5	4.27E-06	-6.84E-06	0	4.94E-05	4.89E-06	1.631733
128	49.75	2.00E-06	-7.20E-06	0	4.69E-05	6.30E-06	1.630202
129	50	-2.72E-07	-7.55E-06	0	4.45E-05	7.71E-06	1.628679
130	50.25	-2.55E-06	-7.89E-06	0	4.20E-05	9.13E-06	1.627164
131	50.5	-4.82E-06	-8.22E-06	0	3.94E-05	1.05E-05	1.625656
132	50.75	-7.10E-06	-8.55E-06	0	3.69E-05	1.20E-05	1.624156
133	51	-9.39E-06	-8.87E-06	0	3.43E-05	1.34E-05	1.622663
134	51.25	-1.17E-05	-9.19E-06	0	3.18E-05	1.48E-05	1.621177
135	51.5	-1.40E-05	-9.50E-06	0	2.92E-05	1.62E-05	1.619699
136	51.75	-1.63E-05	-9.80E-06	0	2.65E-05	1.76E-05	1.618227
137	52	-1.85E-05	-1.01E-05	0	2.39E-05	1.90E-05	1.616763
138	52.25	-2.08E-05	-1.04E-05	0	2.13E-05	2.04E-05	1.615306
139	52.5	-2.31E-05	-1.07E-05	0	1.86E-05	2.18E-05	1.613856
140	52.75	-2.54E-05	-1.10E-05	0	1.59E-05	2.32E-05	1.612413
141	53	-2.77E-05	-1.12E-05	0	1.32E-05	2.46E-05	1.610976
142	53.25	-3.01E-05	-1.15E-05	0	1.05E-05	2.60E-05	1.609546
143	53.5	-3.24E-05	-1.18E-05	0	7.75E-06	2.74E-05	1.608123
144	53.75	-3.47E-05	-1.20E-05	0	5.01E-06	2.88E-05	1.606707

145	54	-3.70E-05	-1.23E-05	0	2.25E-06	3.02E-05	1.605297
146	54.25	-3.93E-05	-1.25E-05	0	-5.13E-07	3.15E-05	1.603894
147	54.5	-4.16E-05	-1.28E-05	0	-3.29E-06	3.29E-05	1.602497
148	54.75	-4.39E-05	-1.30E-05	0	-6.07E-06	3.43E-05	1.601106
149	55	-4.62E-05	-1.33E-05	0	-8.87E-06	3.56E-05	1.599722
150	55.25	-4.85E-05	-1.35E-05	0	-1.17E-05	3.70E-05	1.598344
151	55.5	-5.08E-05	-1.37E-05	0	-1.45E-05	3.83E-05	1.596973
152	55.75	-5.31E-05	-1.39E-05	0	-1.73E-05	3.97E-05	1.595607
153	56	-5.54E-05	-1.42E-05	0	-2.01E-05	4.10E-05	1.594248
154	56.25	-5.77E-05	-1.44E-05	0	-2.29E-05	4.24E-05	1.592895
155	56.5	-6.00E-05	-1.46E-05	0	-2.57E-05	4.37E-05	1.591547
156	56.75	-6.23E-05	-1.48E-05	0	-2.86E-05	4.50E-05	1.590206
157	57	-6.46E-05	-1.50E-05	0	-3.14E-05	4.64E-05	1.58887
158	57.25	-6.68E-05	-1.53E-05	0	-3.42E-05	4.77E-05	1.587541
159	57.5	-6.91E-05	-1.55E-05	0	-3.70E-05	4.90E-05	1.586217
160	57.75	-7.14E-05	-1.57E-05	0	-3.98E-05	5.03E-05	1.584899
161	58	-7.37E-05	-1.59E-05	0	-4.26E-05	5.16E-05	1.583587
162	58.25	-7.59E-05	-1.61E-05	0	-4.55E-05	5.29E-05	1.58228
163	58.5	-7.82E-05	-1.63E-05	0	-4.83E-05	5.42E-05	1.580979
164	58.75	-8.04E-05	-1.65E-05	0	-5.11E-05	5.55E-05	1.579683
165	59	-8.27E-05	-1.67E-05	0	-5.39E-05	5.67E-05	1.578393
166	59.25	-8.49E-05	-1.69E-05	0	-5.67E-05	5.80E-05	1.577108
167	59.5	-8.71E-05	-1.71E-05	0	-5.94E-05	5.93E-05	1.575829
168	59.75	-8.94E-05	-1.73E-05	0	-6.22E-05	6.06E-05	1.574555
169	60	-9.16E-05	-1.75E-05	0	-6.50E-05	6.18E-05	1.573287

Appendix 4

Eritrea map

Appendix 5
Ethical Clearance

Health Research Proposal Review and Ethical Clearance Result

Name of researchers: Barakatb Mohazab Bakhit Ahmed B.Sc.: Basic Medical Sciences Omdurman Islamic University; MSc in Human Physiology National Ribat University

Address: OSMD

Title of Research: Normal Spirometric reference values for Eritrean Population

Sponsor: OSMD Department of Physiology

Letter of Reference: 15/08/2017

The Health Research Proposal Review and Ethical Committees have reviewed your paper for its research relevance and ethical soundness and come up with the following conclusion. Based on their deliberations

1. The research proposal is accepted ✓
2. The research proposal is not accepted ____

Signed and approved on date 25/08/2017

1. Dr. Berhane Debru
2. Mr Salih Gemam
3. Mr. Mehari Woldu

Appendix 6
Photo during Data collection

When I showed one participant how to perform the pulmonary function test in Keren

.


In Keren, the volunteer conducted the pulmonary function test.

This image was captured in Asmara during a data collection mission.

This image was captured on the beach in Massawa, next to the Eritrean flag.

Appendix 7

R-code used for deriving the reference equation (Sample - FVC of males)

```
ALL<-read.csv("data3.csv", header = TRUE)

head(ALL)

cutbin <- function(x, k) {

        # x = variable, k = bin width

        # mid-bin labels are multiples of k

        k2 <- k / 2

        b <- seq(floor(min((x - k2) / k)) * k, ceiling(max((x - k2) / k)) * k, k)

        cut(x, breaks=b + k2, labels=b[-1])

 }

ALL$ageintrvl <- cutbin(ALL$age, 2)

ALL$heightintrvl <- cutbin(ALL$height, 5)

mm <- ALL[ALL$sex == 1, ]

length(mm$sex)

ff <- ALL[ALL$sex == 2, ]

windows(6,5)

plot(mm$age, mm$fvc)

plot(mm$ageintrvl, mm$fvc)

plot(mm$ageintrvl, mm$fvc, xlab="Age interval (yr)", ylab="FVC (L)", main="Males", las = 1, cex.main=1.1,
cex.lab=1.3, cex.axis = 1.1)

summary(mm$heightintrvl)

plot(mm$heightintrvl, mm$fvc, xlab="Age interval (yr)", ylab="FVC (L)", main="Males", las = 1, lwd=1,
cex.main=1.1, cex.lab=1.3, cex.axis = 1.1)

plot(mm$ageintrvl, xlab="Age interval (yr)", ylab="Number of subjects", main="Males", cex.lab=1.1,
las=1)

mm.fvc <- gamlss(fvc ~ log(height) + pb(log(age)), sigma.fo =~ pb(log(age)), nu.fo =~log(age), family =
BCCG(mu.link = "log"), data=na.omit(mm))

summary(mm.fvc)
```

```
mm.fvc2 <- gamlss(fvc ~ log(height) + pb(log(age)), sigma.fo =~ pb(log(age)), nu.fix=T, nu.start=1, family = BCCG(mu.link = "log"), data=na.omit(mm))

summary(mm.fvc2)

GAIC(mm.fvc, mm.fvc2, k=log(length(mm$fvc)))

op <- par(mfrow = c(2, 2), mar = par("mar") + c(0, 1, 0, 0), bg = "white")

plot(mm.fvc2, xvar=mm$ageintrvl, par=op)

length(mm.fvc2)

length(mm$ageintrvl)

range(resid(mm.fvc2))

windows(6,5)

mm$fvchtk <- mm$fvc / (mm$height / 100) ^ mm.fvc$mu.coeff[2]

mm.corr1 <- gamlss(fvchtk ~ pb(log(age)), sigma.fo =~ pb(log(age)), nu.fo =~log(age), family = BCCG(mu.link = "log"), data=mm)

mm.corr2 <- gamlss(fvchtk ~ pb(log(age)), sigma.fo =~ pb(log(age)), nu.fix=TRUE, nu.start=1, family = BCCG(mu.link = "log"), data=mm)

centiles.com(mm.corr1,mm.corr2,xvar=mm$age)

centiles(mm.corr2, xvar=mm$age, cent=c(0.4, 2, 5, 50, 95, 98, 99.6), col.centiles=c("green","blue","red","black","red","blue","green"), legend=FALSE, ylab="FVC (L)", xlab="(Age (yr)", main="", points=TRUE, pch=20, col="gray", plot=TRUE, lwd=2, las=1)

wp(mm.fvc2, xvar=mm$age, n.inter=20)

term.plot(mm.fvc2, what="mu", se=TRUE, partial=FALSE, col.term="black", col.se="black", las=1)

mm$predictfvc <- exp(predict(mm.fvc2))

mm$zfvc <- resid(mm.fvc2)

mm.height <- gamlss(height ~ pb(log(age)), sigma.fo =~pb(log(age)), family = NO, data=mm)

summary (mm.height)

windows (6, 5)

p <- data.frame(age=18:60)

p$height <- predict(mm.height, newdata=p, data=mm)

plot(p$age, p$height, las=1, xlab="Age (yr)", ylab="Predicted height (cm)", type="l", lwd=3)

p <- data.frame(age=seq(18,60,0.25))
```

```
p$height <- 1

M <- predict(mm.fvc2, newdata=p, data=mm)

S <- predict(mm.fvc2, what="sigma", newdata=p, data=mm)

p$Mspline <- M - (mm.fvc2$mu.coeff[1] + mm.fvc2$mu.coeff[3] * log(p$age))

p$Sspline <- S - (mm.fvc2$sigma.coeff[1] + mm.fvc2$sigma.coeff[2] * log(p$age))

p$Lspline <- 0

p$height <- NULL

write.csv(p, file="LookupTablefvcmale.csv")

windows(6,5)

plot(p$age, p$Mspline, xlab="Age (yr)", ylab="FVC Mspline", type="l", lwd=3, las=1)

abline(h=0,lty=3)

windows(6,5)

plot(p$age, p$Sspline, xlab="Age (yr)", ylab="Sspline", type="l", lwd=3, las=1)

abline(h=0,lty=3)

mm.bcpe <- gamlss(fvc ~ log(height) + pb(log(age)), sigma.fo =~ pb(log(age)), nu.fo =~log(age), tau.fo=~log(age),family = BCPE(mu.link = "log"), data=ff)

summary (mm.bcpe)

mm$centre <- factor(mm$centre)

mm.centre <- gamlss(fvc ~ log(height) + pb(log(age)) + centre, sigma.fo =~ pb(log(age)) + centre, nu.fix=TRUE, nu.start=1, family = BCCG(mu.link = "log"), data=mm)

summary (mm.centre)

GAIC(mm.fvc2, mm.centre, k=log(length(mm$fvc)))
```