Study of compounds in exhaled breath for detection of obstructive lung disease

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A thesis submitted in partial fulfillment of the requirements for the degree of

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Thesis Supervisor

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I am thankful to my Creator Allah Subhana-Watala to have guided me throughout this work at every step and for every new thought which Your setup in my mind to improve it. Indeed, I could have done nothing without Your priceless help and guidance. Whosoever helped me throughout the course of my thesis, whether my parents or any other individual was Your will, so indeed none be worthy of praise but You.

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Dedicated to

Holy and Last Prophet MUHAMMAD (Peace and Blessings of ALLAH be upon him and his family) who is, ever a torch of guidance and knowledge for humanity.

Abstract

Background and Objective:

Breath sensors technology can be used as a tool for medical diagnostics. Compounds of exhaled breath are important markers of lung health. Exhaled nitric oxide is a FDA approved marker for monitoring of obstructive lung disease. Studies have shown a correlation of breath carbon monoxide, hydrogen per oxide and hydrogen sulfide for detection of obstructive lung disease. Besides these compounds, ammonia, acetone and alcohol are possible marker of obstructive lung disease. This study is aimed to find the level of hydrogen sulfide, ammonia, acetone and alcohol in exhaled breath of patients and healthy to find the efficacy of compounds of exhaled breath for detection of obstructive lung disease.

Methods:

This clinical study is conducted on 105 subjects, here 60 subjects are patients of obstructive lung disease and 45 subjects are healthy individuals. PFT is measured for all the participants. The obstructive lung patients are screened on the basis of PFT results by a healthcare professional. Subjects are divided into two groups i.e. patients and healthy. Patients are further ranked on the scale of mild, moderate, moderate severe, severe and very severe obstruction on the basis of PFT results. Finally, compounds of exhaled breath are recorded for all the subjects one by one.

Results:

It was found that level of hydrogen sulfide, ammonia and acetone in exhaled breath were significantly different ($p<0.05$) between healthy subjects (63.98±21.63 ppb), (103.41±33.43 ppb), (0.65±0.27 ppm) and obstructive lung patients (34.12±20.17 ppb), (70.37±34.71 ppb), (0.495±0.24 ppm) respectively. While no significant difference was found for level of breath alcohol ($p>0.05$). Positive correlation was found between breath ammonia with respect to FEV1 ($r=0.74$), FVC ($r=0.61$) and FEF ($r=0.63$). Moreover, positive correlation was found between H_2S with respect to FEV1 ($r=0.54$), FVC ($r=0.41$) and FEF ($r=0.37$). Whereas, weak correlation was found for acetone and alcohol with respect to FVC, FEV1 and FEF.

Conclusion:

Level of ammonia and hydrogen sulfide are promising exhaled breath markers of obstructive lung disease. Further studies are required to compare the performance of breath ammonia and hydrogen sulfide with respect to breath nitric oxide for monitoring of obstructive lung disease.

List of Abbreviations:

VOC, Volatile organic compounds
EBC, Exhaled Breath Condensates
PFT, pulmonary function test
H_2S, hydrogen sulfide
NH_3, ammonia
FEV1, forced expiratory volume in 1s
FVC, forced vital capacity
PEF, peak expiratory flow
COPD, chronic obstructive pulmonary disease
GERD, gastro esophageal reflux disease
CO_2, carbon dioxide
C_2H_5OH, alcohol
C_2H_6CO, Acetone
FDA, food and drug regulatory authority
DHT22, digital temperature and humidity sensor
SnO_2, tin Oxide
FeNO, fractional exhaled nitric oxide
QCM, Quartz Crystal Microbalance
SAW, Surface Acoustic Wave

Keywords: *Obstructive Lung Disease, Biomarkers, Pulmonary Function Test, Ammonia, Hydrogen sulfide, Acetone and Alcohol in exhaled breath*

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Chapter 1: Introduction

Technology plays an active role in medical applications, while making use of information for making important decisions [1]. Breath sensors technology can be used as a tool for medical diagnostics [2]. Exhaled breath markers are various compounds found in the exhaled breath of a person, while breath analysis is a way of detecting various lung, metabolic and digestive system diseases by examining the level of various compounds found in human breath. The major compounds are oxygen, CO_2, water vapor, and nitrogen, with 500 different kinds of components. The exhaled breath analysis can see the diseases of the respiratory system with metabolic system and digestive system that significantly impact the exhaled breath markers with dysfunction [1]. Breath compounds are produced, as a result of various metabolic processes that are released into blood. Once the blood reaches the lungs, they are passed on to the airways, for exchange of gases through mouth [3].
Obstructive lung diseases are several diseases, where lungs are obstructed due to asthma, Chronic Obstructive Pulmonary Disease (COPD), infection, allergen, irritants, air pollution, tobacco smoke, exercise, cold air, Gastro Esophageal Reflux Disease (GERD), strong emotions, medications and preservatives [4-5]. The lung obstruction disease is more escalating among the residents of Islamabad due to the presence of moderate amounts of mulberry tree pollen in the air [6]. Some risk factors for lung obstructive disease are things in the environment around us, with asthma and COPD in family, genes, races, sex, job, lung infection, allergies or obesity. The lung diagnosis tests are conventionally invasive, time consuming, hazardous to health due to exposure to radiation. At present, lung disease is diagnosed through Spirometry, X-Ray, CT scan, Methacholine and blood or skin allergy tests [5], [7]. According to the US center for disease control and prevention (CDC), people having moderate to severe obstructive lung disease are at higher risk to develop complications from Covid-19 infection [8].

Exhaled breath analysis is a novel, easy, instant and noninvasive method to diagnose lung obstruction [1]. Some possible exhaled breath markers for detection of obstructive lung diseases are nitric oxide (NO), hydrogen per oxide (H_2O_2), carbon monoxide (CO), hydrogen sulfide (H_2S), alcohol (C_2H_5OH), acetone(C_2H_6CO) and ammonia (NH_3) [9-14], [15]. Previous studies have revealed that breath concentration of nitric oxide, hydrogen peroxide, carbon monoxide and hydrogen sulfide are important for diagnosis of lung obstruction. Moreover, the nitric oxide has been approved by Food and Drug Administration (FDA) for clinical monitoring of asthma [9-10], [11], [16]. Fast raise of exhaled breath temperature is reported for obstructive lung patients by Paredi et al in 2002 [17]. Breath level of ammonia, acetone and alcohol can be important for diagnosis of lung obstruction [13], [14], [15]. However, limited studies are conducted for determining the efficacy of ammonia, alcohol and acetone for detection of obstructive lung disease.

In this thesis, we have fabricated a device to measure level of various compounds of exhaled breath in an individual. This clinical study is aimed to find the level of exhaled breath hydrogen sulfide, ammonia, acetone and alcohol in obstructive lung patients and healthy individuals. It also finds correlation between exhaled breath compounds and pulmonary function test variables.

Furthermore, this clinical study helps to find efficacy of studied breath compounds for detection of obstructive lung disease and sets a reference value for breath acetone, alcohol and ammonia for obstructive lung patients.

1.1 Problem Statement

The obstructive lung disease detection tests are conventionally Invasive, time consuming and hazardous to health due to exposure of radiation.

1.2 Objectives

1. To build a device to measure the level of ammonia, hydrogen sulfide, acetone and alcohol in the exhaled breath
2. To find the efficacy of above breath compounds for detection of obstructive lung disease
3. To correlate level of exhaled breath compounds with pulmonary function test

1.3 Areas of Application

1. Hospitals
2. Clinics
3. Laboratories
4. personal use at homes

1.4 Thesis Overview

Chapter 1 is introduction which defines the problem and its solution with procedures and recent work on it. It also contains objective of our work and where it can be implemented. Chapter 2 contains literature review which describes some other common technologies to detect exhaled breath and quantities of exhaled gases in disease and healthy state. Chapter 3 is about methodology we adopt to successfully build that system. It defines MQ series gas sensors and how it works. Complete design of our system is also discussed in this chapter. Chapter 4 is about statistical results we achieved from this system and it matches with work of other authors as described in introduction. Chapter 4 includes statistical and graphical analysis of discussion on it. Chapter 5 is about conclusion of the whole work. At the end references are given from where we gather data

Chapter 2: Literature Review

Major compounds and its concentrations for healthy persons.

Exhaled Breath	Concentration
Nitrogen	78.04%
Oxygen	16%
Carbon dioxide	4%–5%
Hydrogen	5%
Carbon monoxide	0–6 ppm
Ammonia	0.5 ppm–2 ppm
Inert gases and VOCs: Acetone, Isoprene and Ethanol	0.9% <1 ppm
Hydrogen sulphide	0–1.3 ppm
Nitric oxide	10 ppb–50 ppb
Nitrous oxide	1 ppb–20 ppb
Carbonyl Sulphide	0–10 ppb
Ethane	0–10 ppb
Pentane	0–10 ppb
Methane	2 ppm–10 ppm

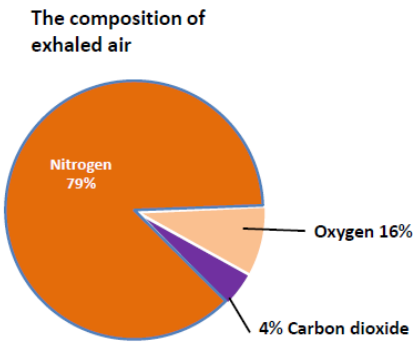

Figure 1. Major Compounds concentration and its composition for healthy individuals.

Figure 1 displays the major compound in exhaled breath with composition of exhaled breath in the pie chart [18]. Most of the human exhaled breath is composed of inorganic compounds like nitrogen, oxygen, carbon dioxide, water, and inert gases like argon. The major volatile organic compounds in exhaled breath are isoprene, acetone, ethanol, methanol and other type of alcohols. Exhaled breath condensate (EBC) is the liquid form of the exhaled air, which is sampled by condensation, and reflects the composition of fluid in airway lining [19].

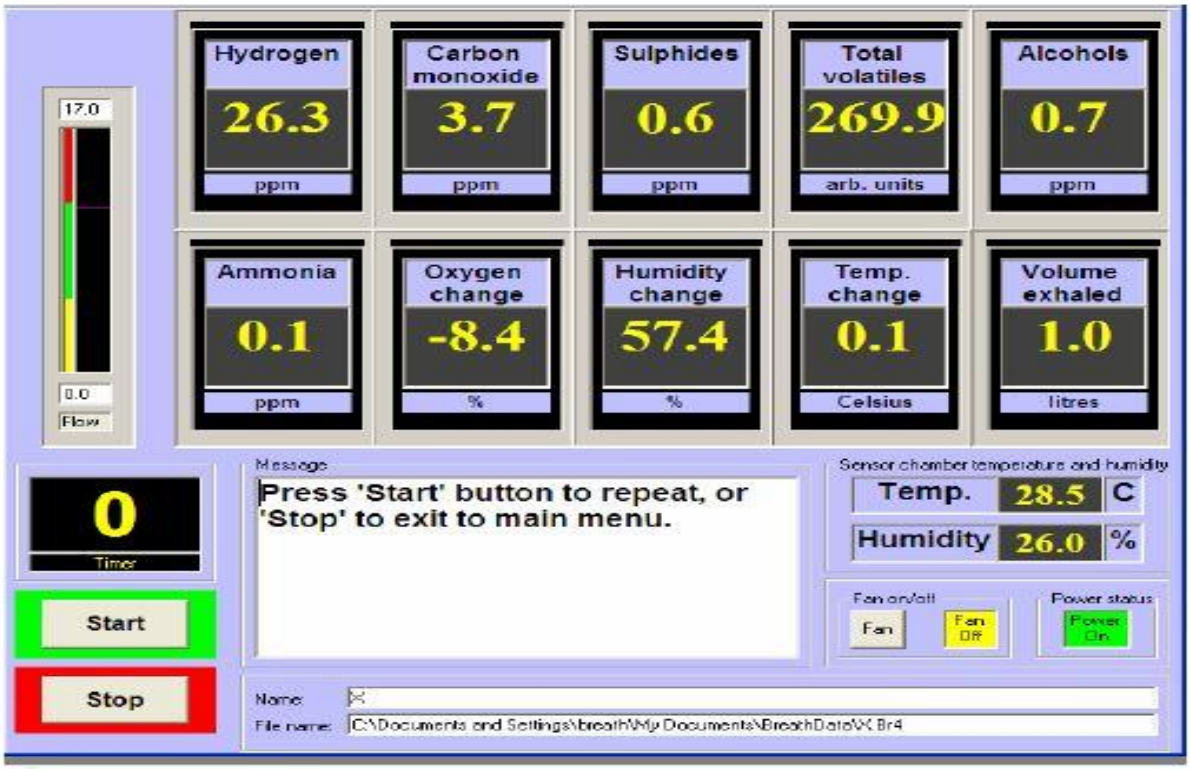

Figure 2. GUI of system for measuring hydrogen, carbon monoxide, alcohol and ammonia in exhaled breath

Figure 2 display the graphical user interface of a sensor system for measuring hydrogen, carbon monoxide, hydrogen sulfide, alcohol and ammonia in exhaled breath.

De Lacy Costello el al constructed a sensor array containing electrochemical sensors of hydrogen, hydrogen sulfide, carbon monoxide and ethanol. Ceramic based sensor was used for volatiles, while optical sensor was used for ammonia. Large variation was shown by breath hydrogen level from day to day. Fasting range of level was (0.6-34.1ppm with average 9.1ppm). The level of carbon monoxide for non-smokers was (0.6-4.9ppm with average 2.1ppm), while level for smoker (8.3-18.7ppm with average 12.8ppm). The level measured for hydrogen sulfide (0-1.3ppm with average 0.33ppm). Furthermore, level for ethanol (0-3.9ppm, average 0.62ppm) and ammonia (0-1.3ppm, average 0.42ppm) [20].

Electronic devices for the detection and management of diseases are becoming more popular and receiving more attention because these devices provide rapid and early detection of diseases without pain and non-invasive procedures that allow earlier treatments prescribed by doctors' or healthcare professionals. Traditional methods are painful, invasive, time-consuming, expensive, damaging to body tissues and organs, discouraging patients from testing for disease. These electronic devices are easy to use, give many results in a short time, are portable, less expensive and have a low operating cost. Many diseases can be detected by detecting the amount of metabolites produced by body organs or tissues and released by exhaled human breath. Biomarkers of diseases in exhaled breath are called volatile organic compounds (VOCs) that are produced within the body due to the metabolic process of different organs or tissues and are present in blood,

body fluids, and breath. The normal or abnormal amount of VOC in exhaled air predicts the health status of humans and the effects of treatments or medications on a given disease [21].

Some methods used to measure exhaled breath compounds are described below:

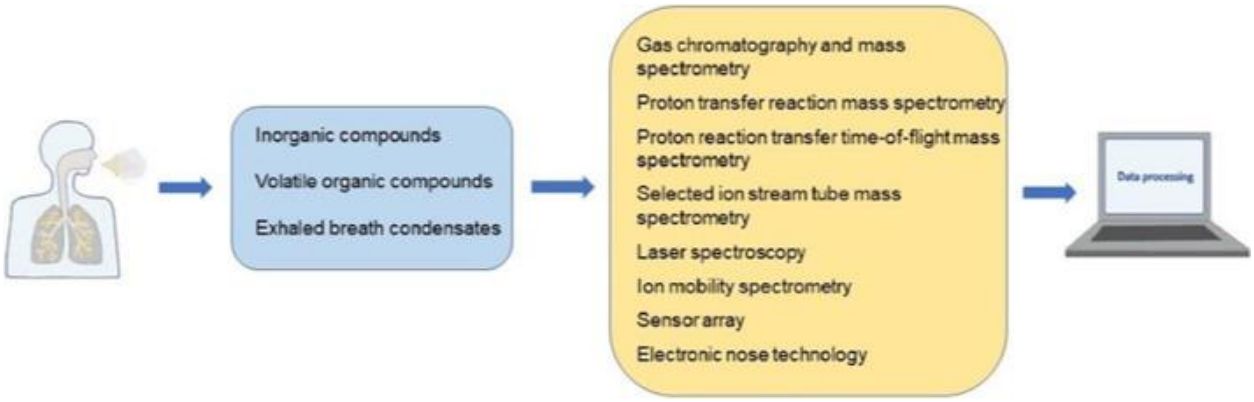

Figure 3. Method used for detection of different types of compounds in exhaled breath

Figure 3 displays the method used for detection of various compounds in exhaled breath [22].

2.1 QCM:

In this technique quartz crystal surface is exposed to gaseous mixture and due to absorption of gas molecules in crystal surface, its mass and resonant frequency changes. It is very sensitive to changes in mass to area ratio and it can measure up to Nano grams [23].

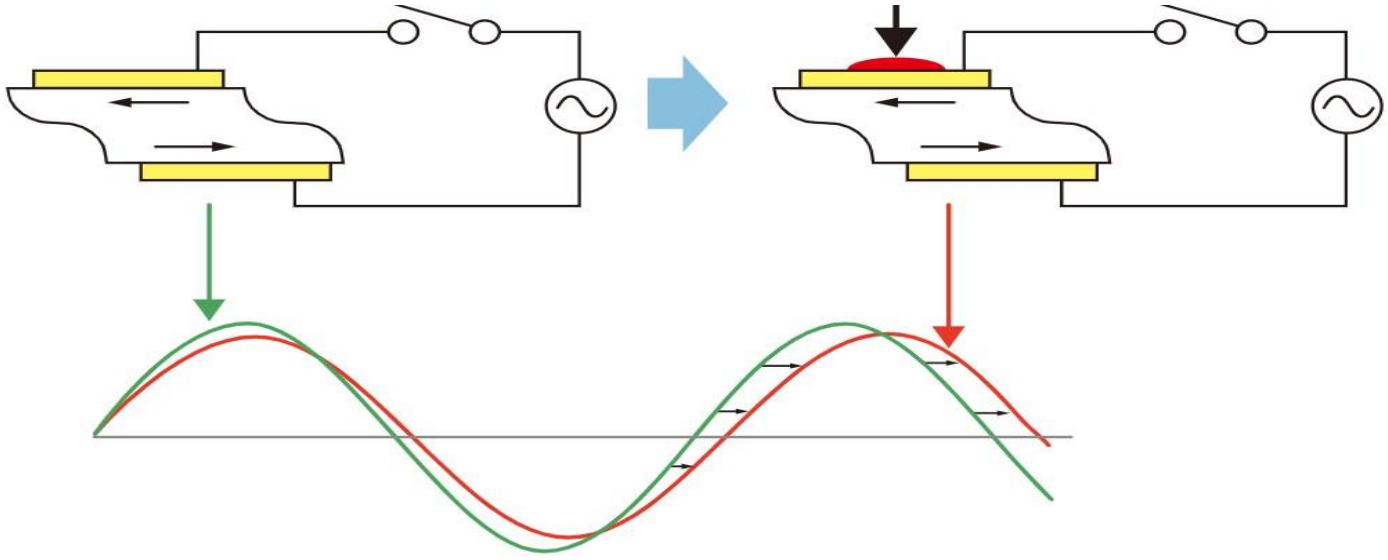

Figure 4. Schematic diagram of QCM setup.

2.2 SAW

In this technique firstly an electrical signal is converted into a mechanical wave and then passes parallel to solid surface. When gaseous sample get absorb on solid surface then frequency of mechanical wave altered which is received at receiver end. At receiver end this mechanical wave

convert back to electrical signal and through this signal we came to know about composition of gaseous sample [23].

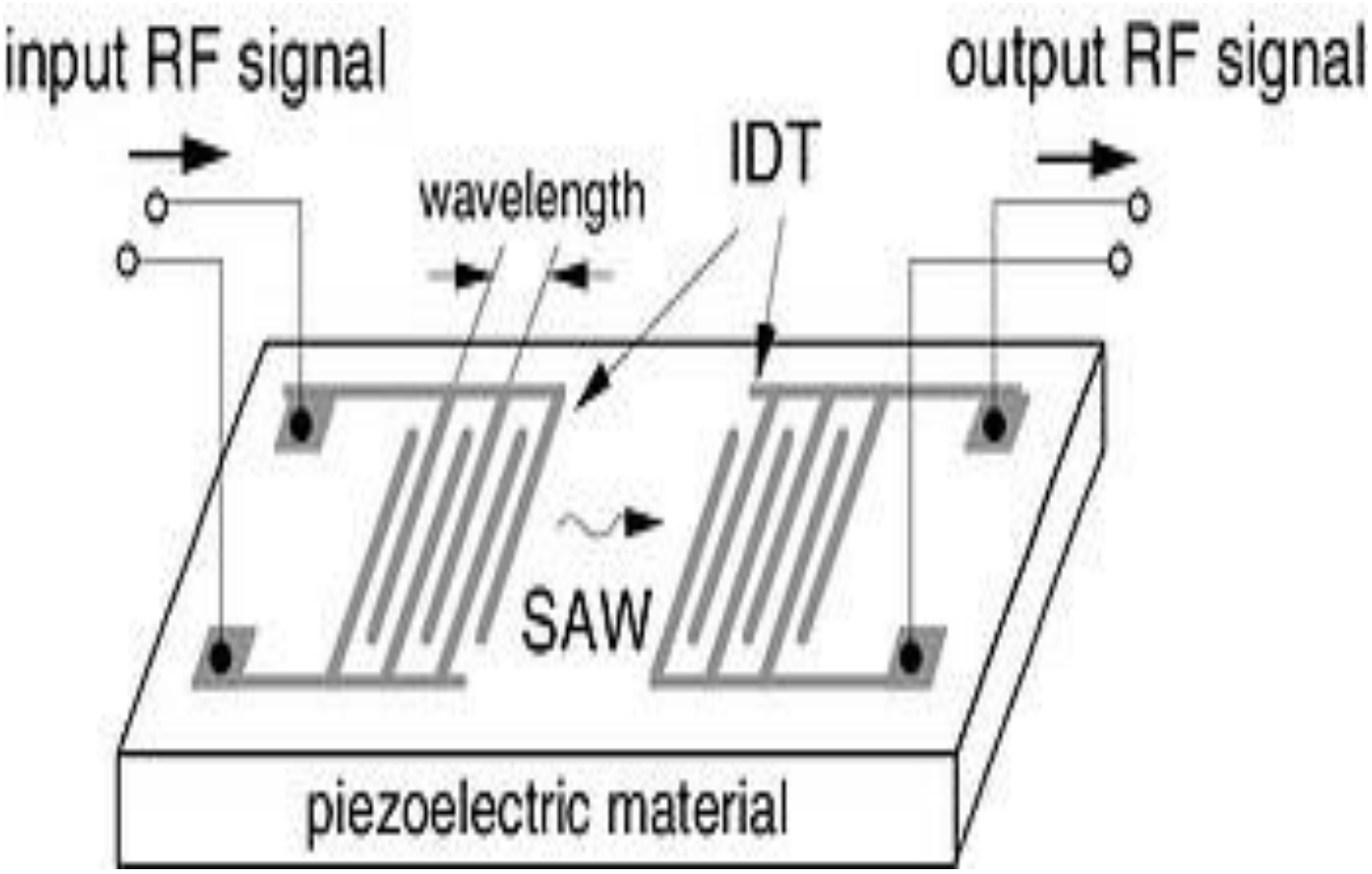

Figure 5. Schematic diagram of SAW setup.

2.3 Colorimetric Gas Detection Tubes

In this technique a chemical reagent (enclose in a glass tube) is used. This tube comes with both ends closed from manufacturer. At time of gas analysis on end is broken and specific volume of gaseous sample in induce in it with the help of hand pump. Reaction between reagent in tube and gaseous sample takes place and color of reagent changes. The length of the color change in accordance with markings on tube tells about the concentration of target gas. it has many advantages like low cost, simplicity, fast response, no need of electronic instruments, no need of calibration [24].

Figure 6. Colorimetric Gas Detection Tubes.

Like nitric oxide and carbon monoxide, hydrogen sulfide is called third gasotransmitter. Hydrogen sulfide is produced as a result of relaxing action of vascular smooth muscles.
Small amount of hydrogen sulphide is produced by the cells in the mammalian body. Where it has many signalling functions. It relaxes smooth muscles as vasodilators and also active in brain for memory formation [26]. Zhang et al established in 2014 that exhaled hydrogen sulfide level is positively correlated with percentage of predicted forced expiratory volume (FEV) and asthma control test (ACT) score [27].

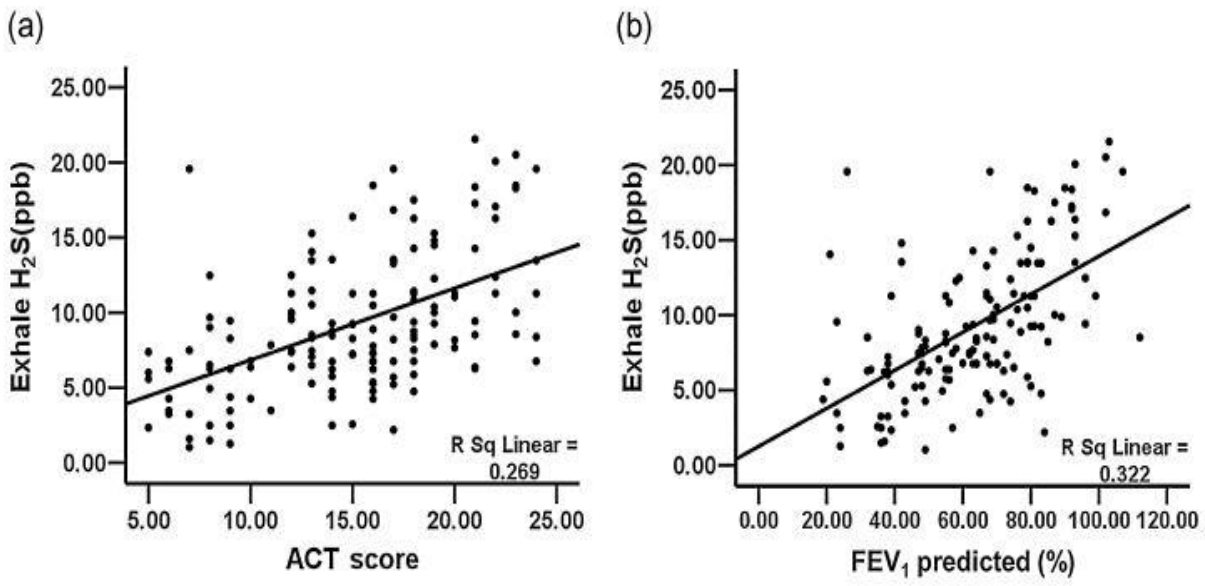

Figure 7. . Relationship between exhaled hydrogen sulfide (H2S) and variables of chronic persistent asthma correlation analysis was performed using the Spearman test. (a) Asthma Control Test (ACT) score; (b) forced expiratory volume in 1 s (FEV1) (% of predicted)

Similarly, our study demonstrates that the level of exhaled hydrogen sulfide in healthy individuals (63.98±21.63 ppb) is significantly higher than the level of exhaled hydrogen sulfide in obstructive lung patients (34.12±20.17 ppb). Moreover, a positive correlation is observed for ammonia and hydrogen sulfide with FEV1, FVC and PEF. Acetone is by product of fat metabolism process. It may be used for detection of ketosis. Ketosis is raised in level of ketone bodies in the human tissues. Which can be either increased due to diabetes or diet that is very low in carbohydrates.

Acetone is by product of fat metabolism process. It may be used for detection of ketosis. Ketosis is raised in level of ketone bodies in the human tissues. Which can be either increased due to diabetes or diet that is very low in carbohydrates. Kinoyama et al established the average value of acetone in healthy individuals 0.53±0.45 ppm in 2008 [28]. Our study highlights that exhaled level of acetone in healthy individuals (0.65±0.27ppm) is significantly more than level of exhaled acetone in patients with obstructive lung disease (0.495±0.25ppm); $p<0.05$.

Ammonia is produced as a by-product after digestion of protein. In normal functioning of liver, it is excreted automatically. Production of ammonia takes place in small intestine. It is moved to liver by a vein. Where ammonia is transformed into urea by the urea cycle enzymes, that is excreted by the kidney. Carraro et al in 2005 measured condensed ammonia from saliva of asthmatic children. Exhaled ammonia condensate for asthmatic children (Treated: 476.17 uM, Non-treated: 253.24 uM) are lower as compared to healthy children (Healthy: 788.3 uM) [13]. Similarly, our study identifies a significant decrease in exhaled breath ammonia for obstructive lung patients (70.37±34.71 ppb) than healthy individuals (103.41±33.43 ppb) ($p<0.05$). Most research conducted on breath ammonia is related to detection of kidney disease and it is an accepted breath marker for monitoring of kidney disease [29].

Fermentation of carbohydrates by yeast and bacteria in small intestine produces alcohol. An average alcohol produced by digestive system in human is 3g a day. Yates et al in 1996 measured

the effect of alcohol ingestion on level of exhaled nitric oxide. They found a significant decrement in exhaled NO level occurred in asthmatic patients (204±58 ppb) without ingestion of alcohol (from 204±58 ppb to 158±59 ppb). While no significant decrement occurred in healthy individuals (from 122±14 ppb to 114±15 ppb) [14]. Our study demonstrates a significant difference in the level of exhaled breath alcohol for obstructive lung patients (0.945±0.25) and healthy people (1.21±0.42) ($p<0.05$).

Chapter 3: Methodology

3.1 Study Subjects:

This clinical study is conducted on a sample size of 109 individuals, with 62 subjects i.e. patients of obstructive lung disease and 47 subjects i.e. healthy individuals. Adults are recruited among patients attending the Pulmonology Outpatient's Department (OPD) of Military Hospital (MH), Rawalpindi, Pakistan. This experimental protocol is approved by ethical committee of National University of Science and Technology (NUST) Islamabad, Pakistan (NUST/SMME-BME/REC/000512/32612020). Consents of all the participants is taken prior to execution of the experiment.

3.2 Protocol:

Pulmonary function test (PFT) is measured for all the individuals by large MR spirometer (Spriolab, Italy). Measured parameters include: forced vital capacity (FVC), forced expiratory volume in 1 s "FEV1" and "FVC/FEV1" along with peak expiratory flow "PEF" and forced expiratory flow "FEF2575". A standard protocol is followed to conduct the pulmonary function test. Based on pulmonary function test results, obstructive lung patients are screened by healthcare professional. Individuals are divided into two groups of patients and healthy individuals. Patients are further ranked on the scale of mild, moderate, moderate severe, severe and very severe obstruction by healthcare professional. None of the patient is treated with glucocorticoids. Level of exhaled breath markers are recorded for patients and healthy individuals one by one. All individuals are asked to take a deep inhale breath and strongly blow to the sensors, placed in front of them for at least 5 s (in one breathing cycle). Individuals, who could not blow on the sensors by protocol, were asked to blow on the sensors again, to make the breath recording process uniform.

3.3 Health Status:

Clinical data is collected for all the individuals comprising of enrollment: gender, age, weight, smoking history and medical history. Furthermore, spirometry test is conducted to confirm the health status of the patients.

3.4 Experimental Setup:

3.4.1 Device Components:

A device is fabricated to measure level of exhaled breath concentrations of various compounds (ammonia, hydrogen sulfide, acetone, alcohol). This device consists of a circuit of four Mingan Qi lai (MQ) sensors, Arduino UNO, load resistor and Digital temperature humidity sensor (DHT22). The MQ sensors are used to find concentration level of four different gases. A digital temperature humidity sensor is connected to feed digital signal of humidity and temperature to Arduino UNO. Arduino UNO is used to get analog and digital values from sensors, compute corresponding results by performing mathematical calculations and to display these results on laptop through serial monitor. Load resistor is connected to adjust sensor sensitivity and accuracy.

3.4.2 Working of MQ Sensor:

MQ sensor series contains tin oxide (SnO_2) based electrochemical gas sensors. It computes concentration of the specific gas using voltage divider network present in the sensor. It also contains a small heater fabricated along with sensor to help in measuring different gases. Electrical resistance of tin oxide changes according to concentration of measured gas. As these sensors work on 5V DC voltage, therefore, corresponding analog value of the sensor is resulted from voltage divider circuit [25].

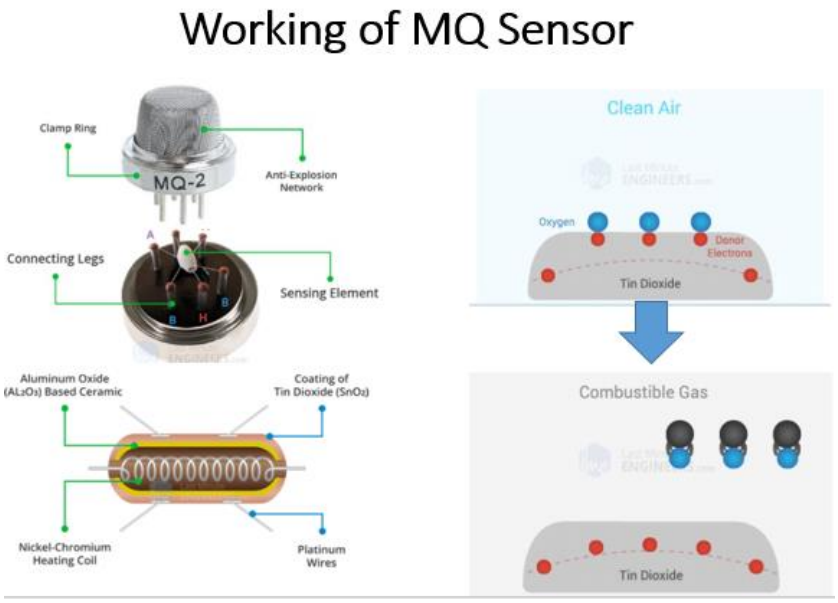

Figure 8.Working of the MQ sensor

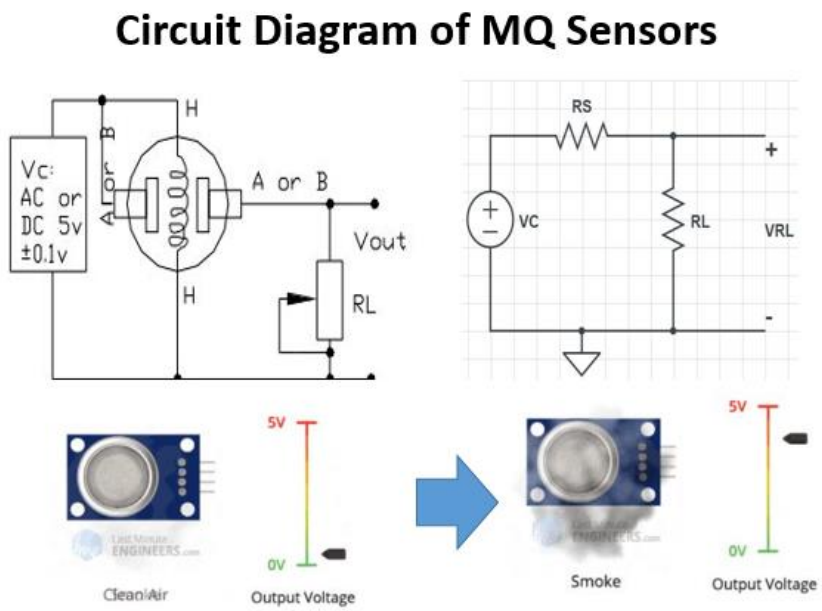

Figure 9. Circuit diagram of MQ sensor

3.4.3 Components Interfacing:

Gas sensors are placed on the Vero board & Breadboard. All connections are made with the Arduino UNO. Power and ground pins of gas sensors are attached to +5V and ground terminals of the Arduino respectively. Arduino UNO is directly connected with the laptop, through which, we can control and access data.

Figure 10 displays the device design of the system.

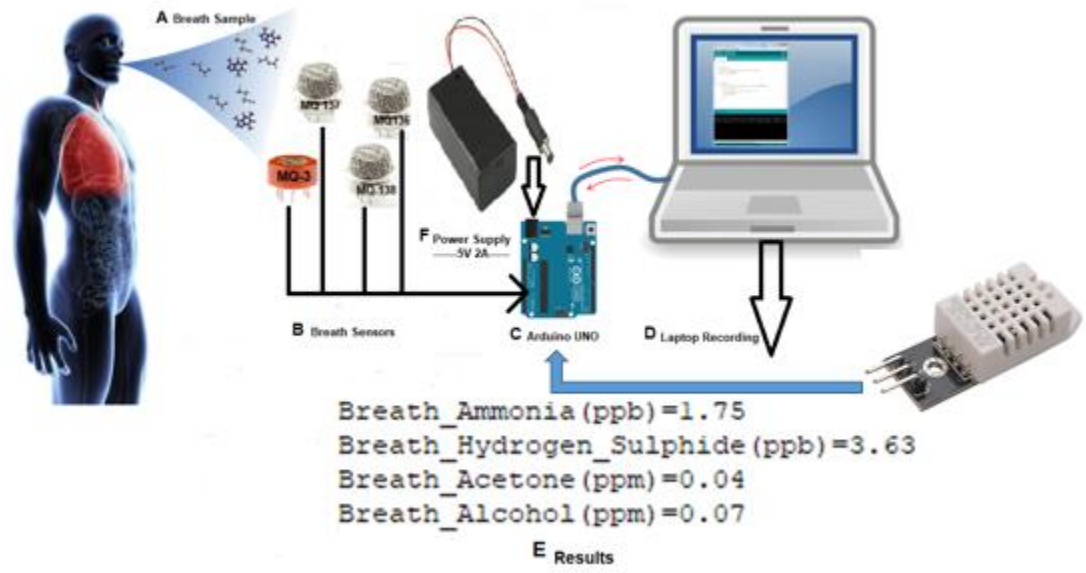

Figure 10. Diagram of Device Design

Figure 11 shows the image of the device.

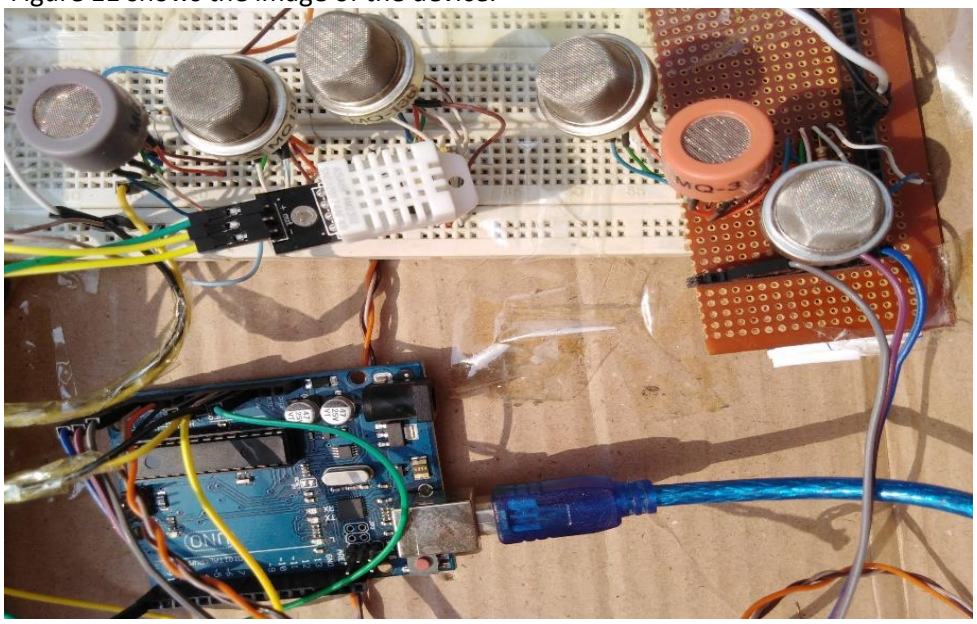

Figure 11. Image of the Device

Figure 12 shows the recording of the pulmonary function test.

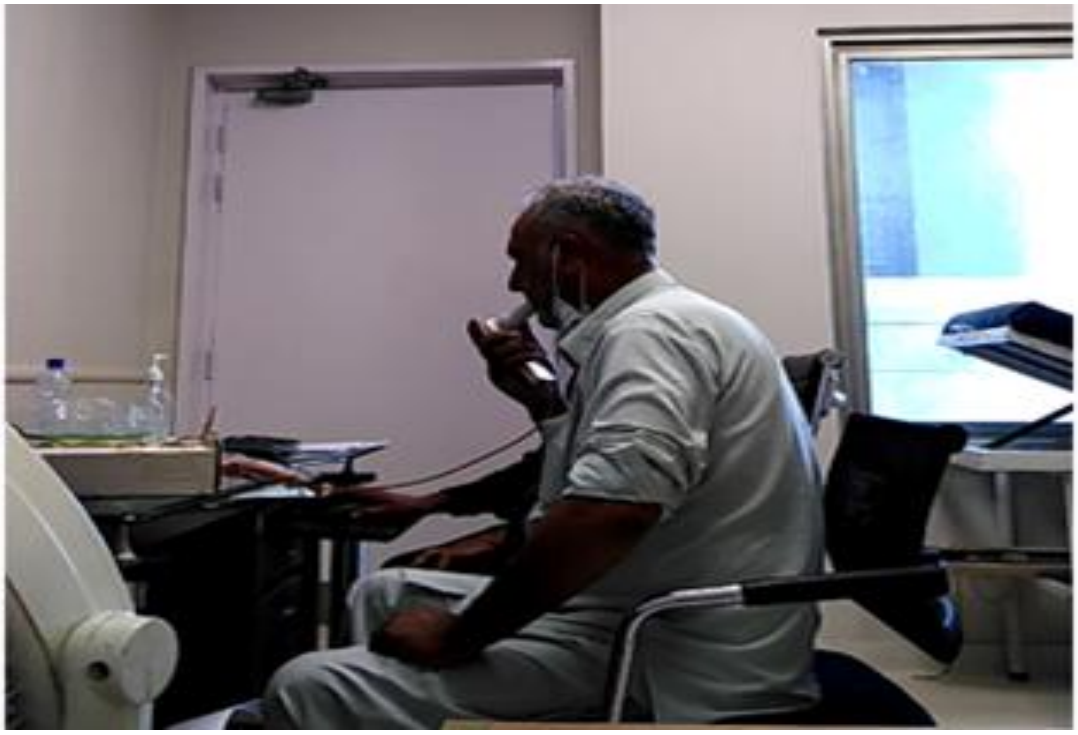

Figure 12. Recording of Pulmonary Function Test

Figure 13 shows the recording of compounds in exhaled breath.

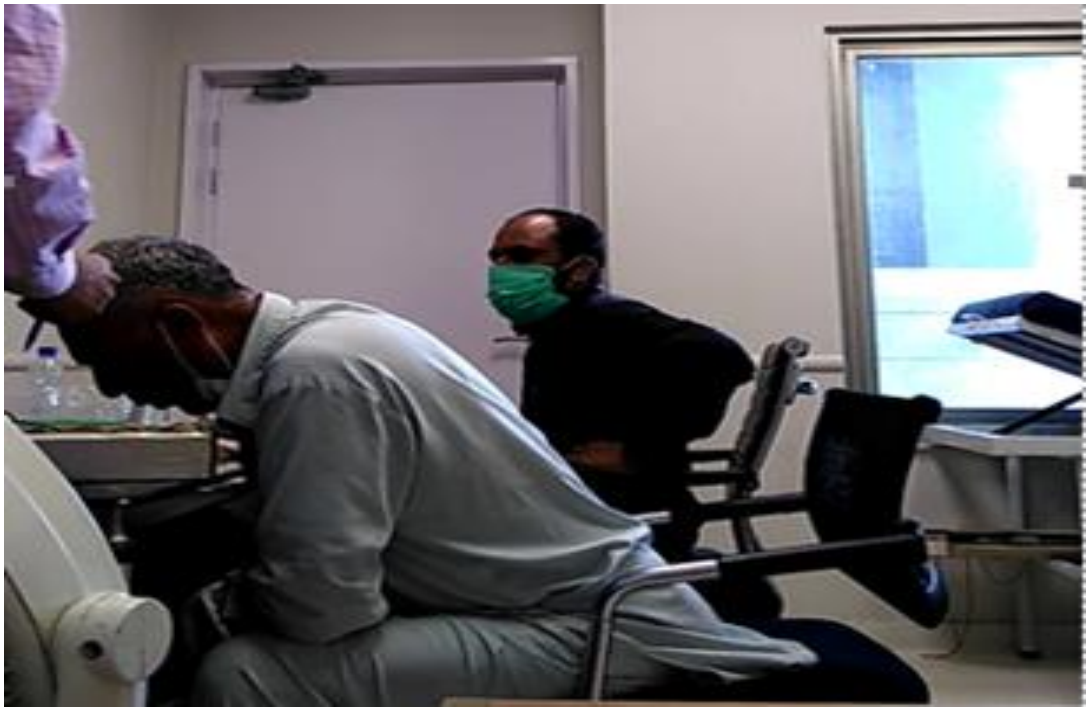

Figure 13. Recording of Exhaled Breath Compounds

Figure 14 below shows the calculation for measuring gas concertation.

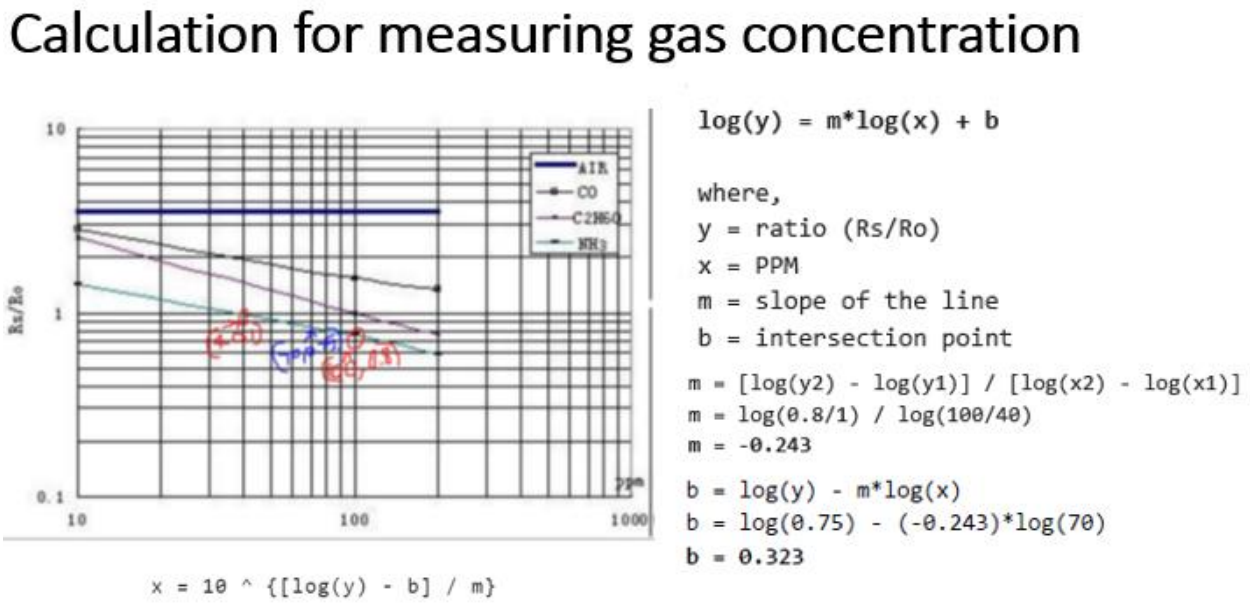

Figure 14. Calculation for measuring gas concentration

Figure 15 shows the calculation for measuring Rs and Ro

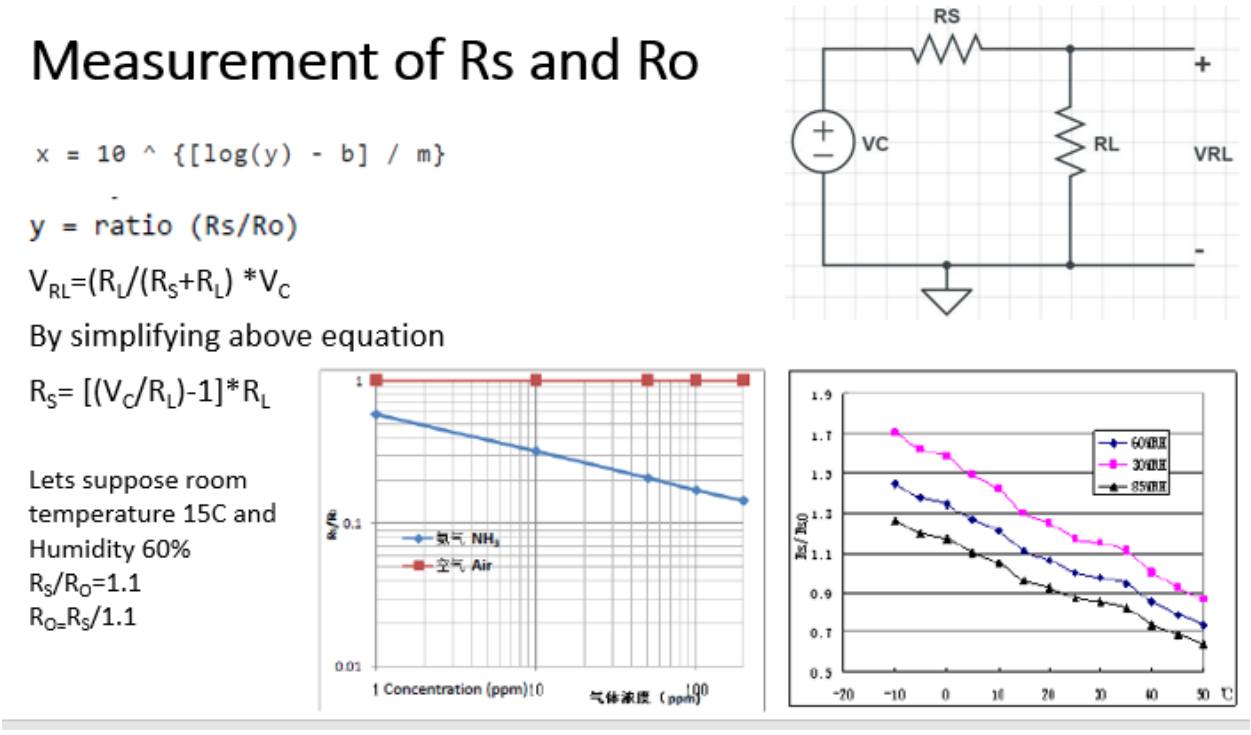

Figure 15. Calculations for measuring Rs and Ro

3.5 Data Analysis Statistics:

All datasets are stored in the spread sheet. Normality of all datasets is tested by Kolmogorov-Smirnov (S-R) test. Normally distributed data is represented by mean and standard deviation while non-normally distributed data is represented by median and standard deviation. For correlation analysis, datasets of both groups are compared using analysis of variance (ANOVA-I), tables and bar plots. Scatter plots are drawn to show relationship between level of exhaled breath compounds and level of pulmonary function test variables. Finally, conclusion is drawn from the results of the statistical analysis.

3.5 Program for measuring gas concentration:

```
#define RL_ammonia 4.7

#define RL_acetone 4.7

#define RL_hydrogen_sulphide 4.7

#define RL_alcohol 4.7

#define m_ammonia -0.264

#define b_ammonia -0.236

#define m_acetone -0.36

#define b_acetone 0.114

#define m_hydrogen_sulphide -0.264

#define b_hydrogen_sulphide -0.236

#define m_alcohol -1

#define b_alcohol 0.93


#include <Adafruit_Sensor.h>

#include <DHT.h>

// Set DHT pin:

#define DHTPIN 3

// Set DHT type, uncomment whatever type you're using!

#define DHTTYPE DHT11   // DHT 11

//#define DHTTYPE DHT22   // DHT 22  (AM2302)
```

```
//#define DHTTYPE DHT21   // DHT 21 (AM2301)
// Initialize DHT sensor for normal 16mhz Arduino:
#define DHTTYPE DHT11   // DHT 11
#define DHTTYPE DHT22   // DHT 22  (AM2302), AM2321
#define DHTTYPE DHT21   // DHT 21 (AM2301)
// Connect pin 1 (on the left) of the sensor to +5V
// NOTE: If using a board with 3.3V logic like an Arduino Due connect pin 1
// to 3.3V instead of 5V!
// Connect pin 2 of the sensor to whatever your DHTPIN is
// Connect pin 4 (on the right) of the sensor to GROUND
// Connect a 10K resistor from pin 2 (data) to pin 1 (power) of the sensor
// Initialize DHT sensor.
// Note that older versions of this library took an optional third parameter to
// tweak the timings for faster processors.  This parameter is no longer needed
// as the current DHT reading algorithm adjusts itself to work on faster procs.
DHT dht(DHTPIN, DHTTYPE);
void setup()
{
  Serial.begin(9600);
    dht.begin();
}

void loop() {    //1.5 mins loop

 float thc=0.92;

 float sensormax_temp=0.0;
 float sensormin_temp=100.0;
 float difference_temp=0.0;
```

```
float difference_ammonia=0.0;
 float sensorMax_ammonia = 0.0;
 float sensorMin_ammonia = 1023.0;

 float difference_acetone=0.0;
 float sensorMax_acetone= 0.0;
 float sensorMin_acetone= 1023.0;

 float difference_hydrogen_sulphide=0.0;
 float sensorMax_hydrogen_sulphide=0.0;
 float sensorMin_hydrogen_sulphide=1023.0;

  float analogvalue_ammonia=0;
  float analogvalue_acetone=0;
  float analogvalue_hydrogen_sulphide=0;

  float VRL_ammoniaRO=0;
  float VRL_acetoneRO=0;
  float VRL_hydrogen_sulphideRO=0;

  float Rs_ammoniaRO=0;
  float Rs_acetoneRO=0;
  float Rs_hydrogen_sulphideRO=0;

  float Ro_ammonia=0;
  float Ro_acetone=0;
  float Ro_hydrogen_sulphide=0;
```

```
 float difference_alcohol=0.0;
float sensorMax_alcohol=0.0;
float sensorMin_alcohol=1023.0;
float analogvalue_alcohol=0;
float Rs_alcoholRO=0;
float Ro_alcohol=0;
float VRL_alcoholRO=0;

 for(int test_cycle = 1 ; test_cycle <= 500 ; test_cycle++)
 { analogvalue_ammonia = analogvalue_ammonia + analogRead(A1);
   analogvalue_hydrogen_sulphide = analogvalue_hydrogen_sulphide + analogRead(A2);
   analogvalue_acetone = analogvalue_acetone + analogRead(A3);
   analogvalue_alcohol = analogvalue_alcohol + analogRead(A4);  }
 analogvalue_ammonia =  analogvalue_ammonia/500.0;
analogvalue_acetone =  analogvalue_acetone/500.0;
analogvalue_hydrogen_sulphide =  analogvalue_hydrogen_sulphide/500.0;
VRL_ammoniaRO = analogvalue_ammonia*(5.0/1023.0);
VRL_acetoneRO = analogvalue_acetone*(5.0/1023.0);
VRL_hydrogen_sulphideRO = analogvalue_hydrogen_sulphide*(5.0/1023.0);
Rs_ammoniaRO = ((5.0/ VRL_ammoniaRO)-1) * RL_ammonia;
Rs_acetoneRO = ((5.0/ VRL_acetoneRO)-1) * RL_acetone;
Rs_hydrogen_sulphideRO = ((5.0/VRL_hydrogen_sulphideRO)-1) *  RL_hydrogen_sulphide;
Ro_ammonia = Rs_ammoniaRO/thc;
Ro_acetone = Rs_acetoneRO/thc;
Ro_hydrogen_sulphide = Rs_hydrogen_sulphideRO/thc ;
analogvalue_alcohol =  analogvalue_alcohol/500.0;
 VRL_alcoholRO = analogvalue_alcohol*(5.0/1023.0);
 Rs_alcoholRO = ((5.0/ VRL_alcoholRO)-1) * RL_alcohol;
  Ro_alcohol = Rs_alcoholRO/thc;
```

```
 for(int a=0;a<15;a++){   //take 20 seconds to read from sensors
  float VRL_alcohol=0;
  float Rs_alcohol=0;
  float ratio_alcohol=0;
  float ppm_alcohol=0;
  float VRL_ammonia=0;
  float VRL_acetone=0;
  float VRL_hydrogen_sulphide=0;
  float Rs_ammonia=0;
  float Rs_acetone=0;
  float Rs_hydrogen_sulphide=0;
  float ratio_ammonia=0;
  float ratio_acetone=0;
  float ratio_hydrogen_sulphide=0;

  float ppm_ammonia=0;
  float ppm_acetone=0;
  float ppm_hydrogen_sulphide=0;

 VRL_ammonia = analogRead(A1)*(5.0/1023.0);
 Rs_ammonia = ((5.0*RL_ammonia)/VRL_ammonia)-RL_ammonia;
 ratio_ammonia = Rs_ammonia/Ro_ammonia;
 ppm_ammonia= pow(10, ((log10(ratio_ammonia)-b_ammonia)/m_ammonia));
ppm_ammonia= ppm_ammonia*1000;

 VRL_hydrogen_sulphide = analogRead(A2)*(5.0/1023.0);
 Rs_hydrogen_sulphide = ((5.0*RL_hydrogen_sulphide)/VRL_hydrogen_sulphide)-
RL_hydrogen_sulphide;
 ratio_hydrogen_sulphide = Rs_hydrogen_sulphide/Ro_hydrogen_sulphide;
```

```
 ppm_hydrogen_sulphide= pow(10, ((log10(ratio_hydrogen_sulphide)-
b_hydrogen_sulphide)/m_hydrogen_sulphide));
 ppm_hydrogen_sulphide=ppm_hydrogen_sulphide*1000;

  VRL_acetone = analogRead(A3)*(5.0/1023.0);
 Rs_acetone = ((5.0*RL_acetone)/VRL_acetone)-RL_acetone;
 ratio_acetone = Rs_acetone/Ro_acetone;
 ppm_acetone= pow(10, ((log10(ratio_acetone)-b_acetone)/m_acetone));

 VRL_alcohol = analogRead(A4)*(5.0/1023.0);
 Rs_alcohol = ((5.0*RL_alcohol)/VRL_alcohol)-RL_alcohol;
 ratio_alcohol = Rs_alcohol/Ro_alcohol;
 ppm_alcohol= pow(10, ((log10(ratio_alcohol)-b_alcohol)/m_alcohol));

Serial.print("Ammonia air Concentration(ppb)=");
Serial.println( ppm_ammonia);

Serial.print("Hydrogen_sulphide air Concentration(ppb) =");
Serial.println( ppm_hydrogen_sulphide);

Serial.print("Acetone air Concentration(ppm) =");
Serial.println( ppm_acetone);

Serial.print("Alcohol air Concentration(ppm) =");
Serial.println( ppm_alcohol);

  float h = dht.readHumidity();
  // Read temperature as Celsius (the default)
  float t = dht.readTemperature();
```

```
// Read temperature as Fahrenheit (isFahrenheit = true)
float f = dht.readTemperature(true);
// Check if any reads failed and exit early (to try again).
if (isnan(h) || isnan(t) || isnan(f)) {
  Serial.println(F("Failed to read from DHT sensor!"));
  return;
}
// Compute heat index in Fahrenheit (the default)
float hif = dht.computeHeatIndex(f, h);
// Compute heat index in Celsius (isFahreheit = false)
float hic = dht.computeHeatIndex(t, h, false);

Serial.print(F("Humidity: "));
Serial.print(h);
Serial.print(F("%  Temperature: "));
Serial.print(t);
Serial.print(F("°C "));
Serial.print(f);
Serial.print(F("°F  Heat index: "));
Serial.print(hic);
Serial.print(F("°C "));
Serial.print(hif);
Serial.println(F("°F"))
if (ppm_ammonia > sensorMax_ammonia) sensorMax_ammonia = ppm_ammonia;
if (ppm_ammonia < sensorMin_ammonia) sensorMin_ammonia = ppm_ammonia;

if (ppm_acetone > sensorMax_acetone) sensorMax_acetone = ppm_acetone;
if (ppm_acetone < sensorMin_acetone) sensorMin_acetone = ppm_acetone;
```

```
 if (ppm_alcohol > sensorMax_alcohol) sensorMax_alcohol = ppm_alcohol;
 if (ppm_alcohol < sensorMin_alcohol) sensorMin_alcohol = ppm_alcohol;

 if (ppm_hydrogen_sulphide > sensorMax_hydrogen_sulphide) sensorMax_hydrogen_sulphide =
ppm_hydrogen_sulphide;
 if (ppm_hydrogen_sulphide < sensorMin_hydrogen_sulphide) sensorMin_hydrogen_sulphide =
ppm_hydrogen_sulphide;
delay (1500); }       //20 second loop
difference_ammonia= sensorMax_ammonia-sensorMin_ammonia;
difference_acetone= sensorMax_acetone-sensorMin_acetone;
difference_hydrogen_sulphide= sensorMax_hydrogen_sulphide-sensorMin_hydrogen_sulphide;
difference_alcohol= sensorMax_alcohol-sensorMin_alcohol;
difference_hydrogen= sensorMax_hydrogen-sensorMin_hydrogen;
difference_temp= sensormax_temp-sensormin_temp;

 Serial.print("sensorMax_ammonia=");
 Serial.println(sensorMax_ammonia);
 Serial.print("sensorMin_ammonia=");
 Serial.println(sensorMin_ammonia);
 Serial.print("Patient_Breath_Ammonia(ppb)=");
 Serial.println(difference_ammonia);

  Serial.print("sensorMax_hydrogen_sulphide=");
 Serial.println(sensorMax_hydrogen_sulphide);
 Serial.print("sensorMin_hydrogen_sulphide=");
 Serial.println(sensorMin_hydrogen_sulphide);
 Serial.print("Patient_Breath_Hydrogen_Sulphide(ppb)=");
 Serial.println(difference_hydrogen_sulphide);
```

```
 Serial.print("sensorMax_acetone=");
 Serial.println(sensorMax_acetone);
 Serial.print("sensorMin_acetone=");
 Serial.println(sensorMin_acetone);
 Serial.print("Patient_Breath_Acetone(ppm)=");
 Serial.println(difference_acetone);

 Serial.print("sensorMax_alcohol=");
 Serial.println(sensorMax_alcohol);
 Serial.print("sensorMin_alcohol=");
 Serial.println(sensorMin_alcohol);
 Serial.print("Patient_Breath_Alcohol(ppm)=");
 Serial.println(difference_alcohol);
  delay(1000000);
}
```

Chapter 4: Results and Discussion

A total of 109 individual (89 males and 19 females) are enrolled. All the hypotheses are tested by ANOVA-I analysis for 62 patients and 47 healthy individuals, that used exhaled breath hydrogen sulfide, ammonia, acetone and alcohol as dependent variable and obstructive lung disease as independent variable. ANOVA-I analysis is done by finding the significance of variance between dependent and independent variables. It is used to determine the correlation between obstructive lung disease and tested exhaled breath markers.

4.1 Exhaled Hydrogen Sulfide: ANOVA-I analysis of exhaled breath hydrogen sulfide for obstructive lung patients (34.12±20.17 ppb) and healthy people (63.98±21.36 ppb) shows strong correlation between exhaled level of hydrogen sulfide and obstructive lung disease ($p<0.001$).

4.2 Exhaled Alcohol: ANOVA-I analysis of exhaled breath alcohol for obstructive lung patients (0.945±0.42 ppm) and healthy people (1.21±0.42 ppm) shows correlation between exhaled level of alcohol and obstructive lung disease ($p<0.01$).

4.3 Exhaled Ammonia: ANOVA-I analysis of exhaled breath ammonia for obstructive lung patients (70.37±34.71 ppb) and healthy people (103.41±33.43 ppb) results strong correlation between exhaled level of ammonia and obstructive lung disease ($p<0.001$).

4.4 Exhaled Acetone: ANOVA-I analysis of exhaled breath acetone for obstructive lung patients (0.495±0.24 ppm) and healthy people (0.65±0.27 ppm) shows strong correlation between exhaled level of acetone and obstructive lung disease ($p<0.05$).

4.5 Statistical Comparison Analysis:

S-R normality test of all datasets show that they are normally distributed.

Table 1 displays analysis of variance of independent variables for obstructive lung patients and healthy individuals. A significant difference of level is observed for all the exhaled breath compounds for patients and healthy individuals

Table 1. ANOVA single factor analysis of exhaled breath compounds for obstructive lung patients and healthy individuals

S.NO	Dependent Variable	Independent Variables (Exhaled Breath Compounds)	P-value	Conclusion
1	**Obstructive Lung Disease**	**Alcohol (C_2H_5OH)**	**P < 0.001**	**Significant Difference**
2		**Acetone (CH_3COCH_3)**	**P < 0.001**	**Significant Difference**
4		**Ammonia (NH_3)**	**P < 0.001**	**Significant Difference**
5		**Hydrogen Sulfide (H_2S)**	**P < 0.001**	**Significant Difference**

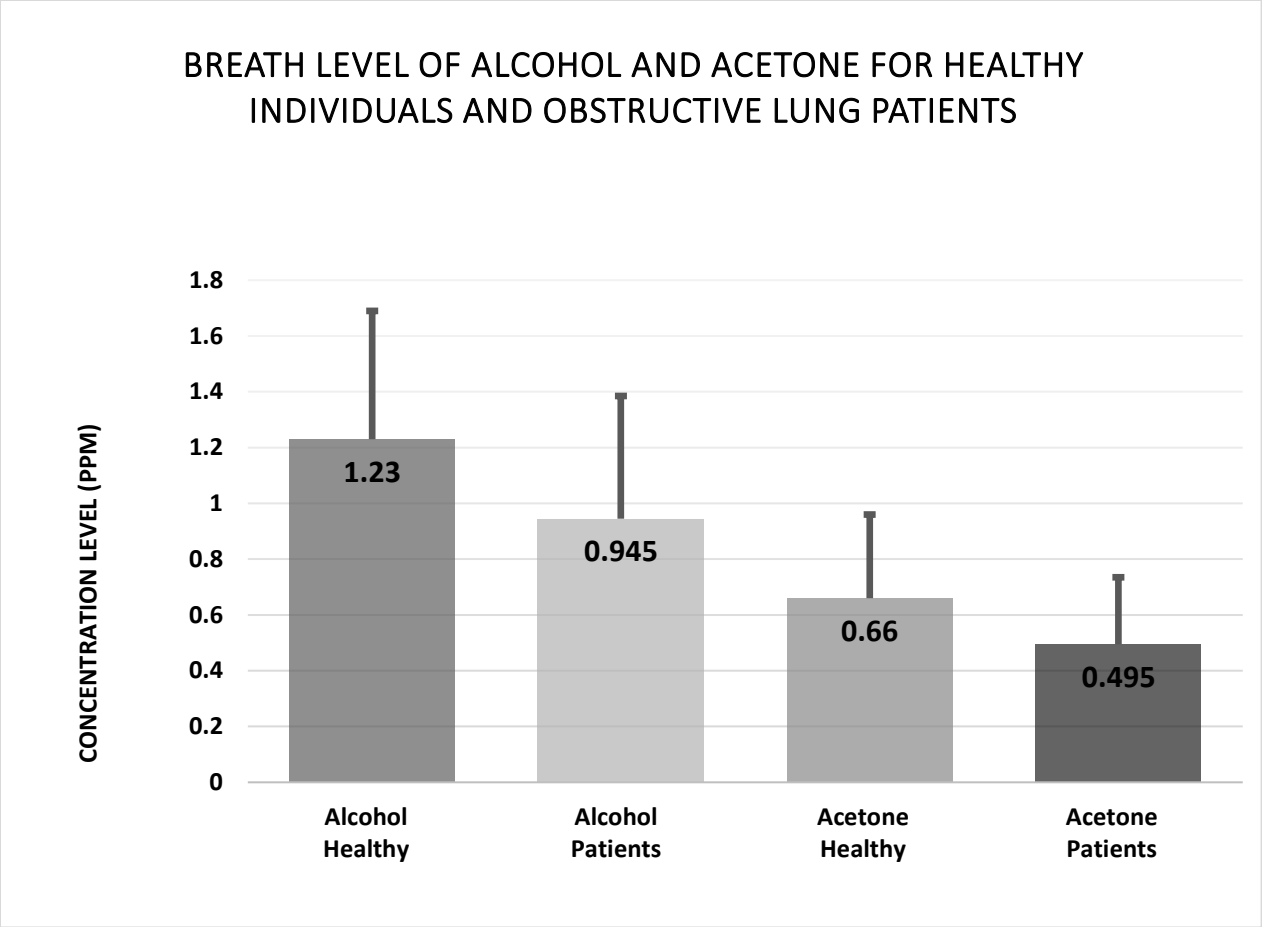

Figure 16. Displays the mean and standard deviation level of exhaled breath alcohol and acetone between patients and Healthy

In figure 16. The bar plots display the mean and standard deviation level of alcohol and acetone for obstructive lung patients and healthy people.

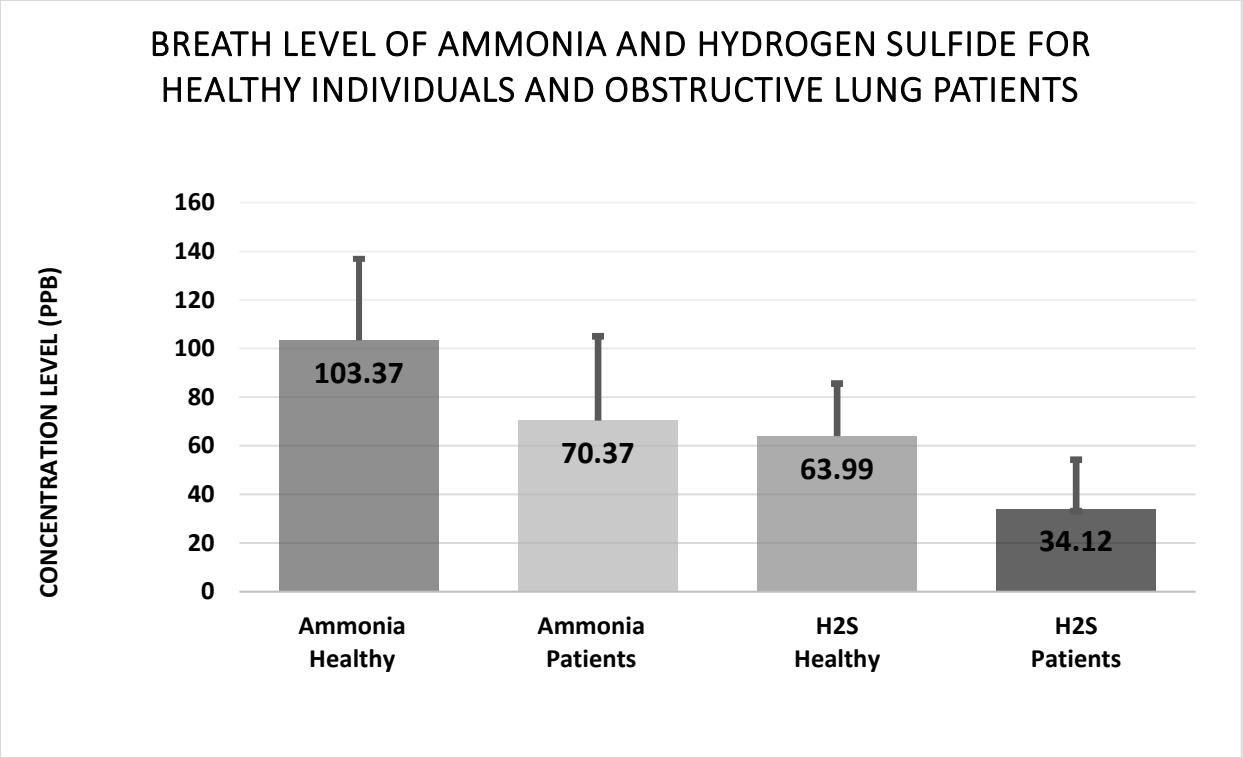

Figure 17. Displays the mean and standard deviation level of exhaled breath ammonia and hydrogen sulfide between patients and Healthy persons

In figure 17. The bar plots display the mean and standard deviation level of ammonia and hydrogen sulfide on the right, for obstructive lung patients and healthy people.

Table 2. Comparison of mean, standard deviation and range of exhaled breath compounds

S.NO	Independent Variable	Dependent Variables (Exhale Breath compounds)	Patients Average ± STD (Range)	Healthy Average ± STD (Range)
1	Obstructive Lung Disease	Ammonia (NH_3)	70.37±34.71 ppb (9.97-136.6 ppb)	103.41±33.43 ppb (10.32-171 ppb)
2		Hydrogen Sulfide (H_2S)	34.12±20.17 ppb (3.52-80.68 ppb)	63.98±21.63 ppb (10.31-113.63 ppb)
3		Acetone (CH_3COCH_3)	0.495±0.24 ppm (0.15-1.2 ppm)	0.65±0.27 ppm (0.11-1.51 ppm)
4		Alcohol (C_2H_5OH)	0.945±0.25 ppm (0.25-1.99 ppm)	1.21±0.42 ppm (0.29-2.13 ppm)

Table 2 displays mean, standard deviation and range level of exhaled breath ammonia, hydrogen, acetone and alcohol for patients and healthy individuals.

In table 3. The general characteristics of subjects: number, gender, age, weight, smoking status and clinical characteristics: severity of disease, exhaled breath level of ammonia, hydrogen sulfide, acetone and alcohol; forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), FVC/FEV1 and peak expiratory flow (PEF) are highlighted for all subjects.

Table 3. Comparison of General and Clinical Characteristics

Information Group	Patients	Healthy
Number	62	47
Gender (M/F)	46/16	43/3
Average Age (Years)	46.12±15.14	41.8±14.78
Average Weight (Kg)	67.80±11.32	70.04±14.78
Smoking Status (Y/N)	12/50	11/36
Severity of obstructive lung Disease and exhaled breath compound values		
Mild Obstruction (number, breath compound values)	23, NH_3(88.11±7.12 ppb), H_2S(37±4.32 ppb), C_2H_6CO(0.50±0.05 ppm), C_2H_5OH(0.91±0.11 ppm)	47 NH_3(103.41±33.43 ppb) H_2S(63.98±21.63ppb) C_2H_6CO(0.65±0.27) C_2H_5OH(1.21±0.42)
Moderate Obstruction (number, breath compound values)	10, NH_3(62.87±31.60 ppb), H_2S(38.1±19.20 ppb), C_2H_6CO(0.51±0.20 ppm), C_2H_5OH(1.02±0.57 ppm),	same as above
Moderate Severe Obstruction (number, breath compound values)	10, NH_3(55.18±22.8 ppb), H_2S(23.23±15.72 ppb), C_2H_6CO(0.46±0.28 ppm), C_2H_5OH(1±0.63 ppm)	same as above
Severe Obstruction (number, breath compound values)	14, NH_3(52.25±33.72 ppb) H_2S(22.43±16.12 ppb) C_2H_6CO(0.45±0.27 ppm) C_2H_5OH(0.92±0.42 ppm)	same as above

Very Severe Obstruction (number, breath compound values)	**5, NH_3(43.32±34.31 ppb) H_2S(21.36±19.52 ppb) C_2H_6CO(0.50±0.34 ppm) C_2H_5OH(1.41±0.51 ppm)**	**same as above**
% of Predicted FVC	**77.74±22.55**	**101±10.50**
% of Predicted FEV1	**61.27±18.59**	**99.78±12.80**
% of Predicted FVC/FEV1	**82.71±17**	**101.56±8.77**
% of Predicted PEF	**52.19±24.42**	**87.54±19.85**

In table 3 the comparison analysis shows that for all the independent variables a significant difference of concentration level is found between patients and healthy individuals. Moreover, breath level of ammonia and hydrogen sulfide decreases with severity of obstructive lung disease. Lower level of breath ammonia, hydrogen sulfide, acetone and alcohol is observed for patients of severe and very severe obstruction.

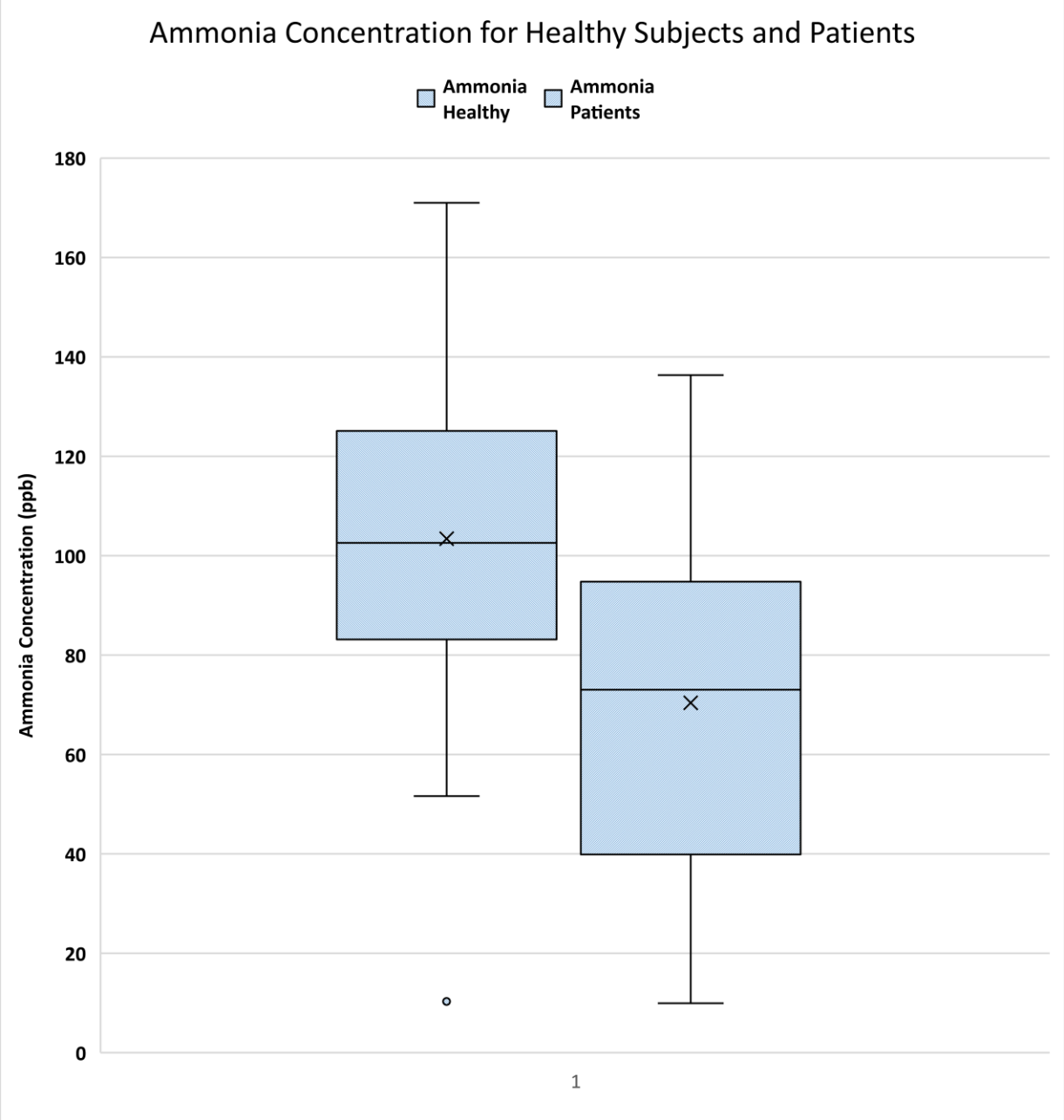

Figure 18. Box plot of concentration of ammonia for healthy persons and obstructive lung patients

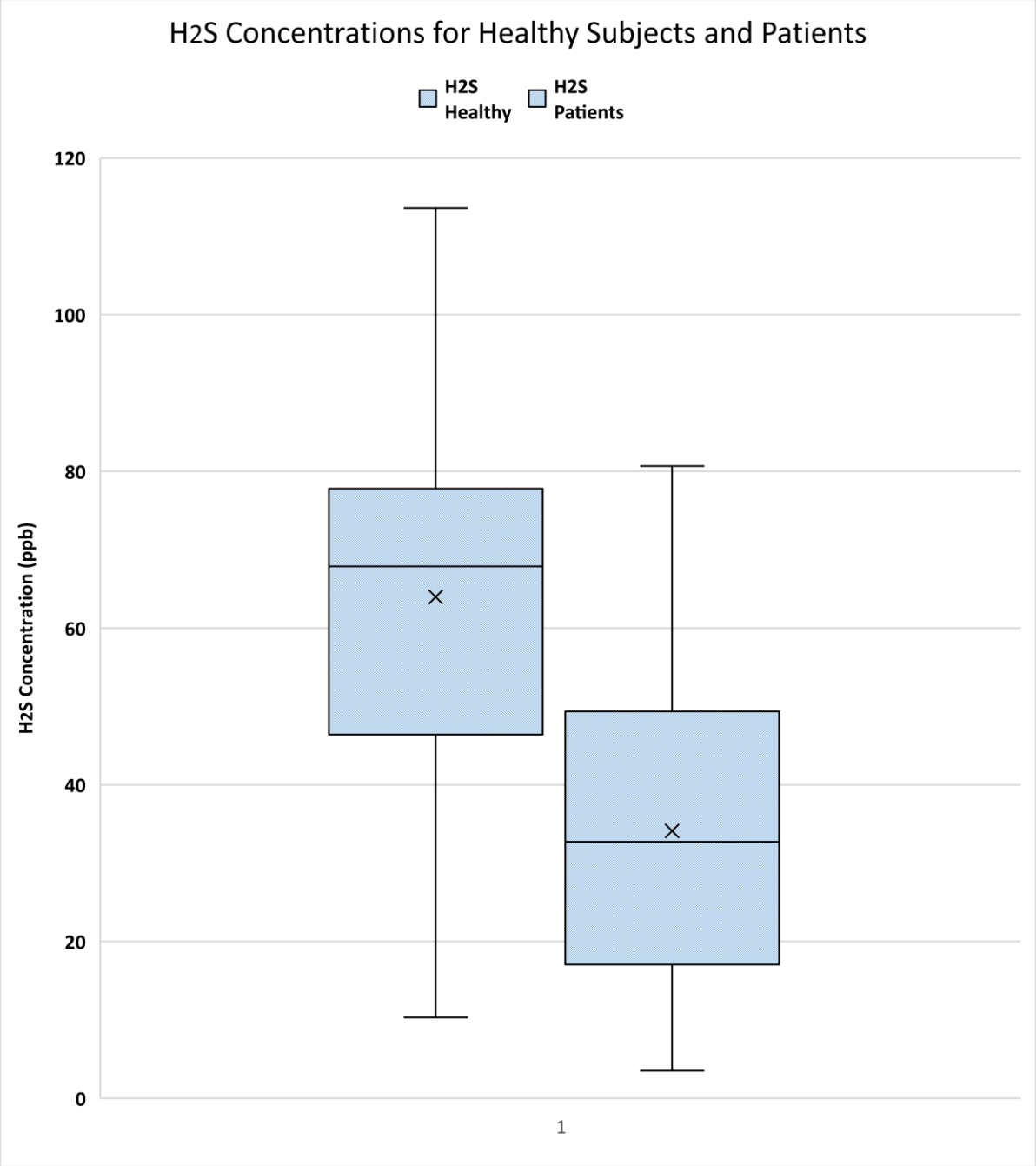

Figure 19. Box plot of concentration of hydrogen sulfide for healthy persons and obstructive lung patients

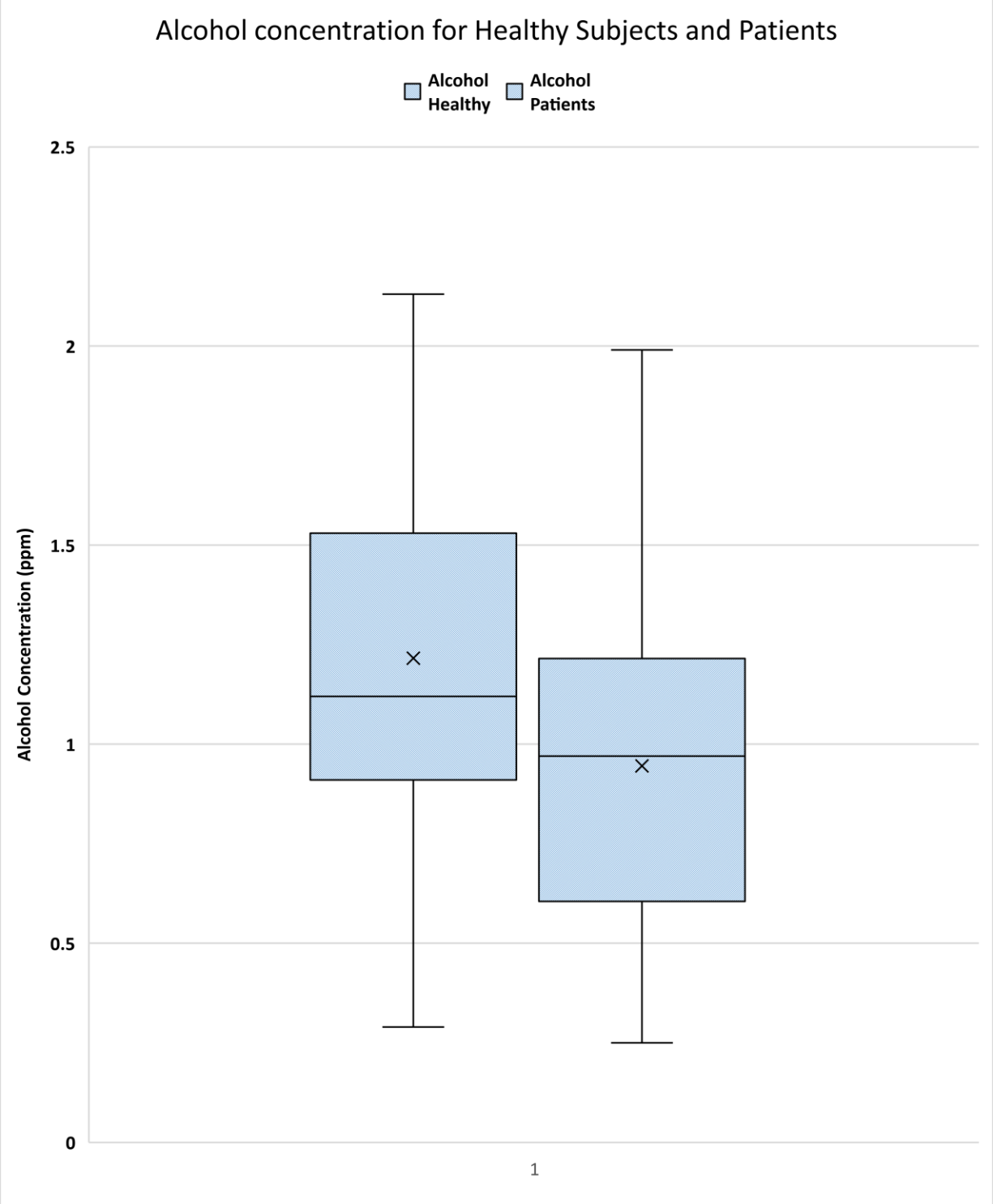

Figure 20. Box plot of concentration of alcohol for healthy persons and obstructive lung patients

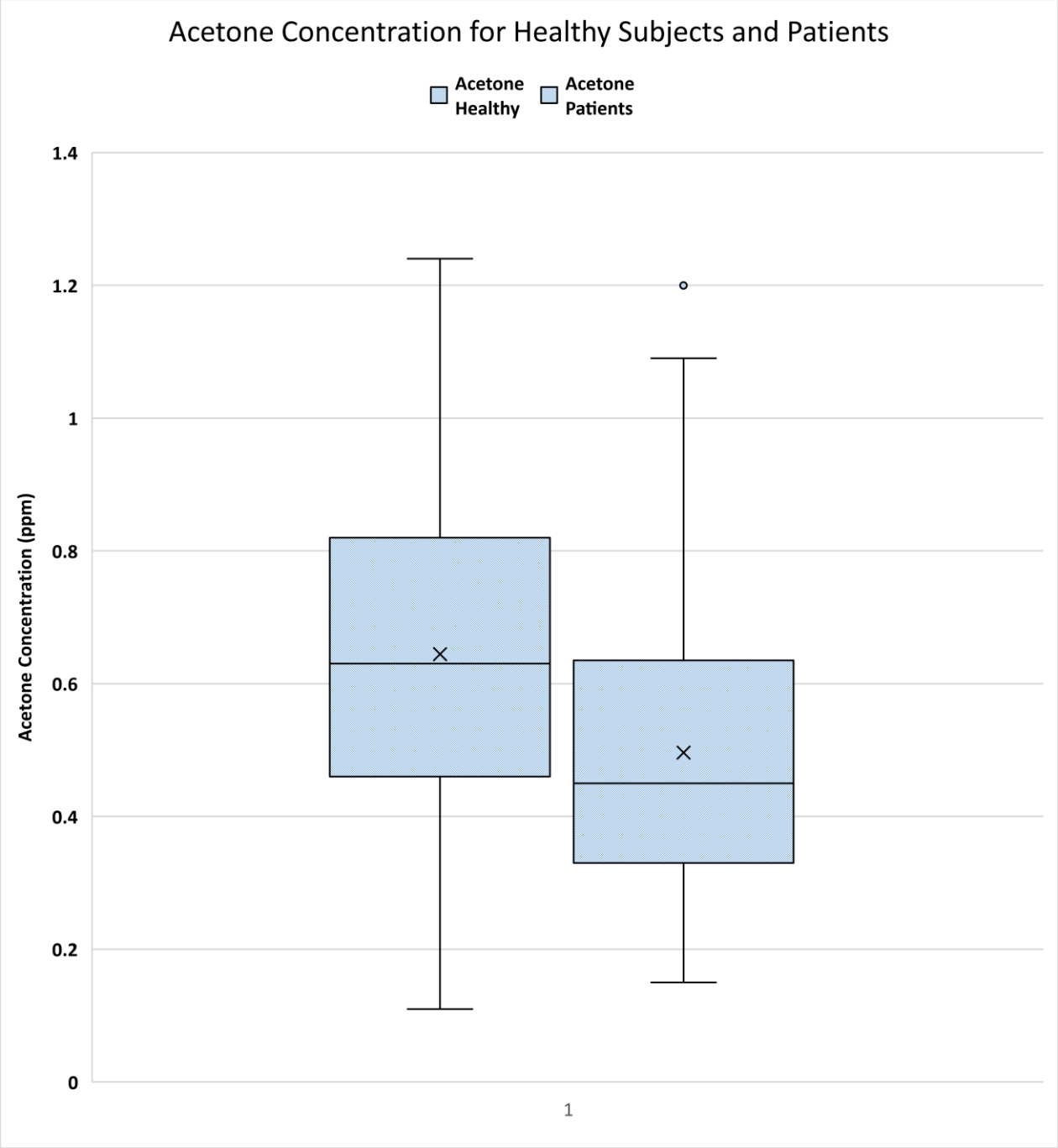

Figure 21 Box plot for concentration of acetone for healthy persons and obstructive lung patients

Box plot analysis in figure 18-21 shows the level of acetone, alcohol, ammonia and hydrogen sulfide in exhaled breath of healthy persons and patients of obstructive lung. A significant difference of level is found between two groups for most of the compounds.

In this correlational study, analysis of various exhaled breath compounds for obstructive lung disease is performed. Our study demonstrates that exhaled breath concentration of hydrogen sulfide, alcohol, acetone and ammonia found in a healthy individual is different than in patients of

obstructive lung disease. Similarly, lung function test parameters (FVC, FEV1, PEF) found in healthy individuals are different than in patients of obstructive lung disease.

4.6 Statistical Correlation Analysis:

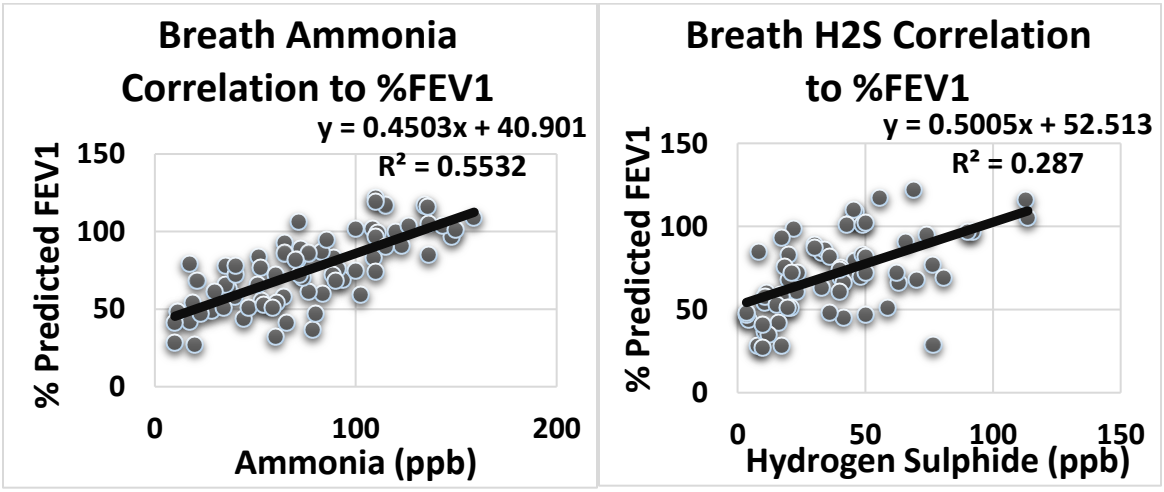

Figure 22. Correlation between exhaled breath ammonia and hydrogen sulfide to forced expiratory volume in 1 s (FEV1) (% of predicted).

In figure 22. Medium positive correlation is detected between exhaled breath level of ammonia and FEV1 (R^2=0.553) while medium correlation is observed between exhaled breath level of hydrogen sulfide and FEV1 (R^2=0.287).

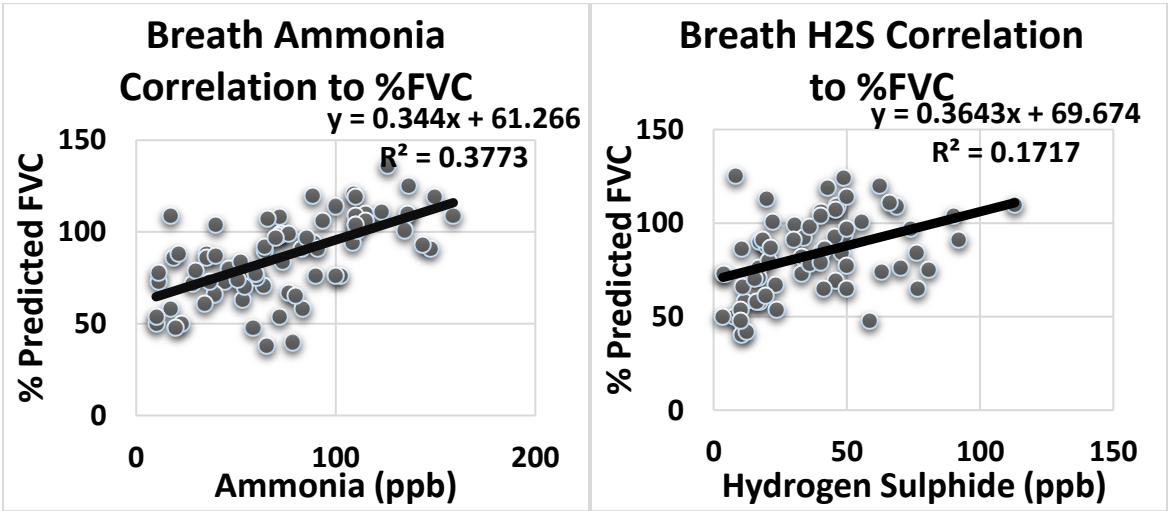

Figure 23. Correlation between exhaled breath ammonia and hydrogen sulfide to forced vital capacity (FVC) (% of predicted).

In figure 23. Medium positive correlation is detected between exhaled breath level of ammonia and FVC (R^2=0.55) and medium positive correlation is observed between exhaled breath level of hydrogen sulfide and FVC (R^2=0.287).

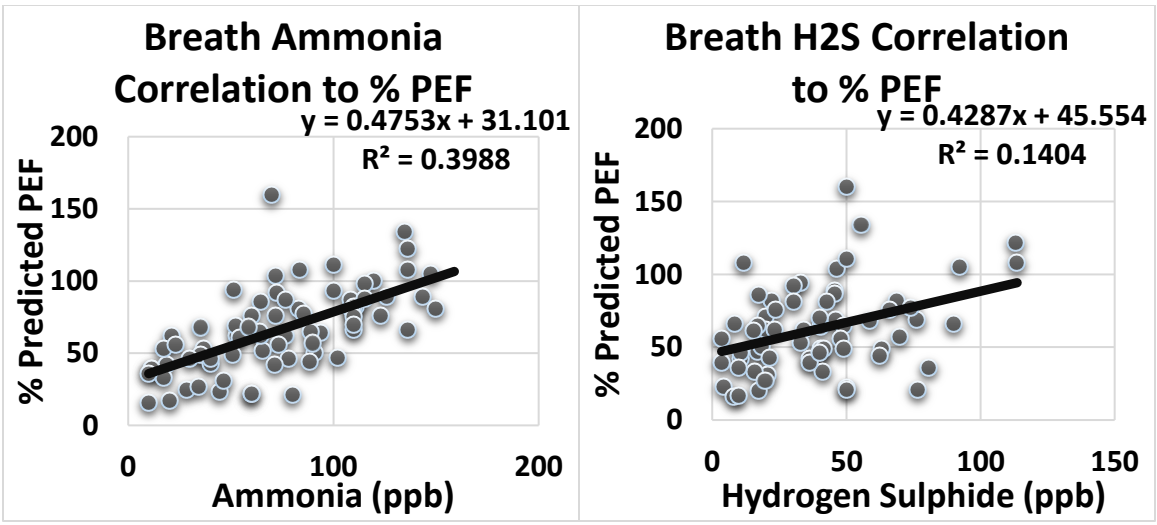

Figure 24. Correlation between exhaled breath ammonia and hydrogen sulfide to peak expiratory flow (PEF) (% of predicted)

In figure 24. Medium positive correlation is detected between exhaled breath level of ammonia and PEF (R^2=0.398) while small positive correlation is observed between exhaled breath level of hydrogen sulfide and PEF (R^2=0.140).

Table 4. Pearson Linear Regression Analysis of Exhaled Breath variables and Lung Function Test Variables

S.N	Variables	Equation	r (Correlation Coefficient)	R^2 (Determination Coefficient)	Summary
1	NH_3- FEV1	y=0.45x+40.90	0.74	0.553	Large Positive Correlation
2	H_2S-FEV1	y=0.50x+52.51	0.54	0.287	Medium Positive Correlation
3	NH_3- FVC	y=0.34x+61.27	0.61	0.377	Medium Positive Correlation
4	H_2S-FVC	y=0.36x+69.67	0.41	0.172	Small Positive Correlation
5	NH_3- PEF	y=0.47x+31.10	0.63	0.398	Medium Positive Correlation
6	H_2S-PEF	y=0.429x+45.55	0.37	0.14	Small Positive Correlation

Table 4 displays the results of Pearson linear regression analysis. Which is performed between exhaled breath variables and pulmonary function test variables. Medium correlation is observed between exhaled breath ammonia and FEV1, FVC and PEF. Small correlation is observed between hydrogen sulfide and FEV1, FVC and PEF. No significant correlation is observed for acetone and alcohol with FEV1, FVC and PEF.

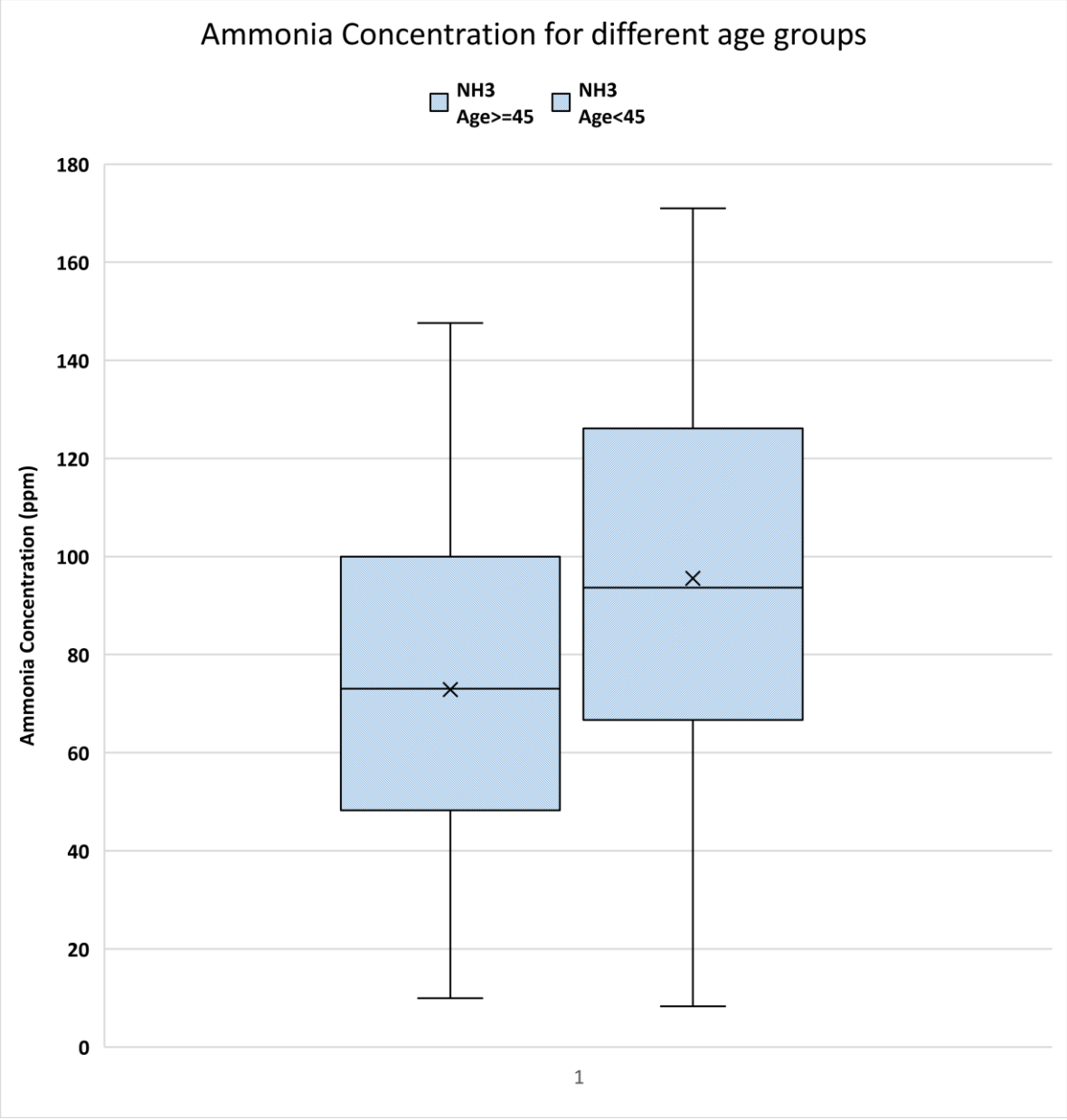

Figure 25. Box plot of Breath ammonia concentration for different age groups

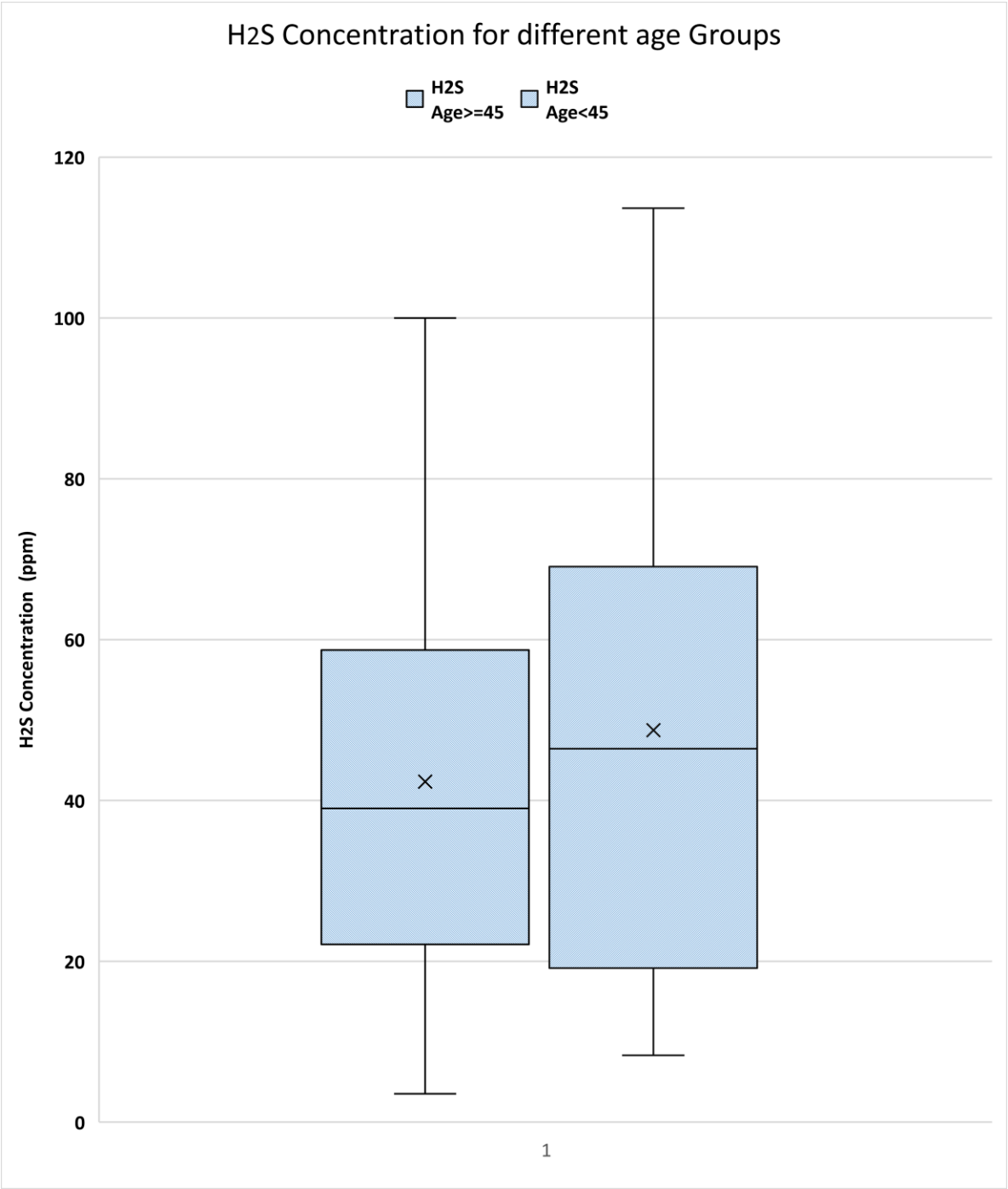

Figure 26. Box plot of breath hydrogen sulfide concentration for different age groups

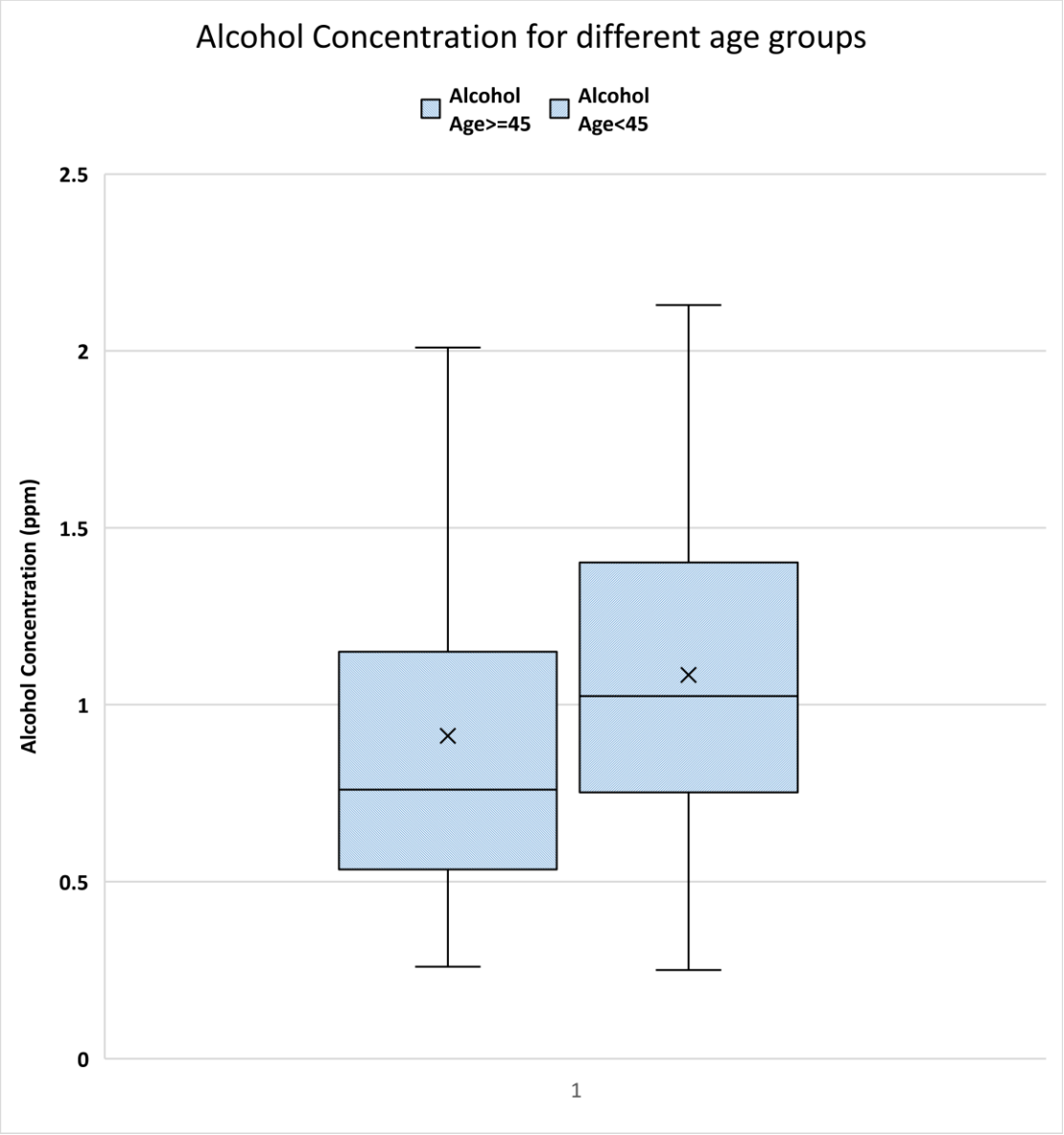

Figure 27. Box plot of the level of breath alcohol for different age groups.

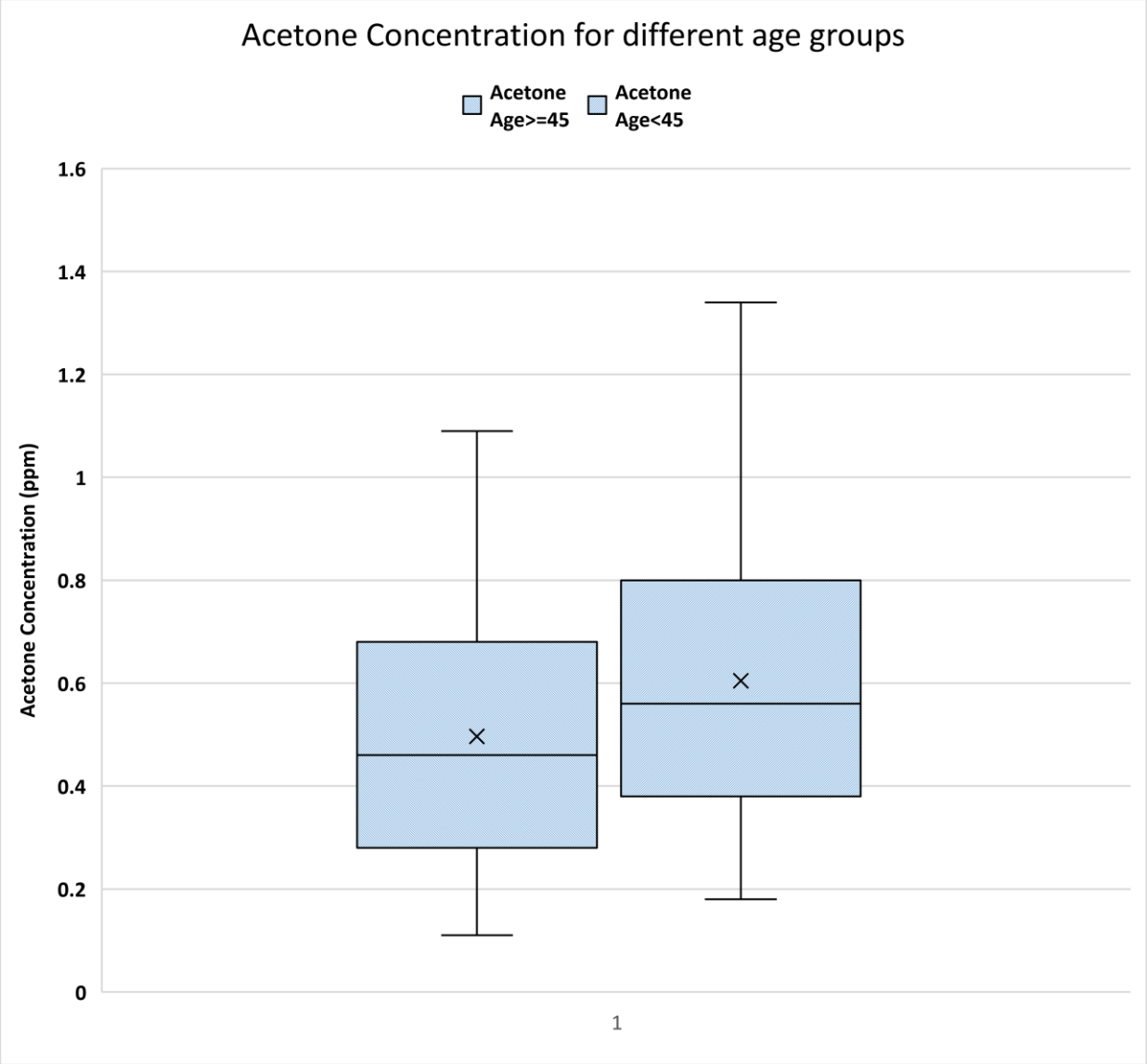

Figure 28. Box plot of breath acetone concentration for different age groups.

Box plot analysis in figure 25-28 shows the level of acetone, alcohol, ammonia and hydrogen sulfide in exhaled breath of subjects with age groups. Level of exhaled breath compounds in subjects of age less than 45 is more than the level of compounds for subjects of age greater than or equal to 45.

This clinical study finds correlation between studied exhaled breath compounds and obstructive lung disease. It also finds reference values for those exhaled breath markers that are rarely studied for lung obstruction. These findings are important in the use of other exhaled breath compounds other than nitric oxide for the monitoring of obstructive lung disease. As lung obstructive disease causes narrowing of airways, therefore exhaled breath level of exhaled breath compounds are lower for obstructive lung patients.

Chapter 5: Conclusion

5.1 Conclusion:

1. Exhaled ammonia and hydrogen sulfide are potential markers for detection of obstructive lung disease
2. This study provides reference values of exhaled breath ammonia, hydrogen sulfide, acetone and alcohol for severity of obstructive lung disease
3. Our device can screen patients with obstructive lung disease

5.2 Future Perspective:

Further studies are required to compare the performance of breath ammonia and hydrogen sulfide to breath nitric oxide for monitoring of obstructive lung disease.

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