"ASSESSMENT OF BREATH CARBON MONOXIDE AND SALIVARY COTININE LEVELS AMONG SMOKERS AND NON-SMOKERS"

By

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Under the Guidance of

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"Showing gratitude is one of the simplest yet most powerful things humans can do for each other". ***-Randy Rausch***

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Dr. ARUN PANWAR.

LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	EXPANSION
1.	CO	Carbon monoxide
2.	CL	Cotinine level
3.	COHb	Carboxy Hemoglobin
4.	ETS	Environmental Tobacco Smoke
5.	BCO	Breath Carbon Monoxide
6.	WHO	World Health Organization
7.	GC	Gas Chromtography
8.	GATS	Global Adult Tobacco Survey
9.	GCF	Gingival Crevicular Fluid
10.	HCN	Hydrogen Cyanide
11.	IARC	International Agency for Research on Cancer
12.	LSD	Lysergic Acid Diethylamide
13.	OR	Odds Ratio
14.	PAH	Poly Aromatic Hydrcarbon
15.	PCP	Phencyclidine
16.	PDA	Photo Diode Array

ABSTRACT

Study Title: Assessment of breath carbon monoxide and salivary cotinine levels among smokers and non-smokers.

Background and Objectives: Tobacco kills up to half of its users which estimate to more than 8 million people each year. Cotinine, the major metabolite of nicotine is generally regarded as the best biomarker for monitoring tobacco exposure in both actively and passively exposed individuals but it is time consuming, expensive and time consuming. Exhaled CO is a useful and validated marker for identifying smokers because high levels of Carbon Monoxide (CO) are produced during tobacco combustion. As a result, the study's goal was to see how smoking intensity affected breath carbon monoxide (BCO) levels, salivary cotinine levels and determine reliable CO cut-off levels for smokers and non-smokers.

Materials and methods: Breath analysis was done using an automated Smokerlyser to estimate breath CO levels and to quantify the level of smoking exposure. The smoking group was compared to age and anthropometric parameters matched non-smokers.

Saliva samples were collected, and Cotinine was determined by comparing HPLC peak heights to those of a genuine standard. An L-7100 pump, an L-7400 UV detector, an L-7200 auto sampler, an L-7500 integrator, and an 865-CO column oven were used for chromatography.

Results: Smokers had significantly higher values of breath carbon monoxide and salivary cotinine levels than non smokers. Age of the subject, number of cigarettes smoked per day, and the duration of smoking habit all had a positive correlation with breath CO and salivary cotinine levels. Mean breath carbon monoxide levels obtained for smoker and non smokers were 3.40 ± 1.80 ppm and 15.46 ± 3.63 ppm, respectively. Mean salivary cotinine levels obtained for

smoker and non smokers were 1.19 ± 1.10 ng/dl and 28.73 ± 19.04 ng/dl, respectively.

Conclusion: The severity of smoking can be quantified by breath carbon monoxide and salivary cotinine levels as these parameters have a correlation with the number of cigarettes smoked per day and the duration of smoking habit. Our study shows the advantages of using CO breath analyzers for distinguishing smokers from non smokers, as they are relatively cost effective, simple, portable, noninvasive, and provide quick and immediate real time results.

Keywords: Cigarette smoke, cotinine, High profile liquid chromatography, carbon monoxide, breath analyzer

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INTRODUCTION

Smoking tobacco, is major cause of mortality and it is estimated that by 2030, it will lead to 10 million deaths.[1] Tobacco contains 4000 carcinogens like tobacco-specific N-nitrosamines (TSNAs) leading to an increased risk of cancers of oral cavity, pharynx and esophagus.[2]

Tobacco use is currently the primary reason of disability and death among the world's population. Every day, one out of every ten adults dies from smoking-related diseases around the world, and the WHO predicts that if current trends continue, smoking will kill one out of every six people by the next decade.[3]

According to GATS 2007, India's current tobacco smokers account for 14.9 percent of the population, while cigarette smokers account for 5.7 percent. The overall male prevalence is 24.3 percent, with 10.3 percent of current cigarette smokers.[1]

Nicotine is the substance that causes cigarette addiction. Cotinine is a metabolite of Nicotine and therefore always used as a 'gold standard' tobacco exposure biomarker.[4]

Most of the nicotine which enters the body (> 80%) is metabolized to cotinine.[5] It is the most valuable biochemical marker for distinguishing smokers from non smokers, estimating tobacco intake and non tobacco user's second-hand smoke exposure.[6]

The biological half-life of cotinine is 15 - 40 hours. Its concentration in the body is relative to the nicotine ingested in the previous few days.[7]

Rejection and underreporting the amount of tobacco use is very common practices among smokers. Therefore, biochemical validation is very important in such situations but estimating cotinine levels necessitates time-consuming, expensive, and technique-sensitive investigations (such as HPLC, GS/MS), which may not be feasible during camps and community service programmes. Finding a reliable, simple, and quick method of assessing a person's smoking status in a hospital setting, as well as during camps, is critical.[8]

HPLC is a chromatography based technique used for analytical estimation of cotinine in saliva. HPLC has been considered as a complex, technique sensitive, expensive and time consuming biochemical method.[8]

High levels of CO gases are produced during tobacco usage, hence it makes exhaled Carbon monoxide a useful and reliable marker for identifying individuals who use smoking tobacco. Carbon monoxide (CO) a colorless gas which has no odor but highly poisonous, is produced when carbon is burnt incompletely.[7]

CO after entering the body, binds with Hb in RBC's to form carboxyhemoglobin (COHb), which is then transported to the blood. CO binds immediately with RBC's but very slowly exits the body. Increased levels CO in the blood starve the body of oxygen and affect all the body system.[9]

CO breath analyser is a very handy and non-invasive monitor to determine blood CO level. It has been used as a tool to assess tobacco usage in hospital settings and response to smoking cessation therapy.[7]

CO breath analyzer is extremely useful in a smoking cessation clinic setup – from a single breath; the results are right away displayed in parts per million (PPM)

and % carboxyhaemoglobin (%COHb). Depending on conc. of exhaled carbon monoxide colored indicators such as red, yellow and green, provide clear visual guidance, indicating whether the person is a non-smoker, light smoker or heavy smoker.[9]

Knowing the burden of oral malignant conditions due to the use of smoked tobacco in North Karnataka and ease of assessing CO levels by breath analyzer and comparing it with the levels of cotinine by HPLC method was planned to undertake in this study. This study would help to establish a reliable, simple, and quick method of assessing a person's smoking status by estimating BCO and determining cut-off levels to distinguish tobacco users from non users using a CO breath analyzer.

AIM AND OBJECTIVES

AIM OF THE STUDY:

Assessment of breath carbon monoxide (BCO) and salivary cotinine levels among smokers and non-smokers.

OBJECTIVE OF THE STUDY:

- To assess carbon monoxide (CO) levels in breath among smokers and non-smokers.
- To assess cotinine levels in saliva among smokers and non-smokers.
- To determine mean cut – off levels for breath carbon monoxide (CO) to distinguish smokers and non- smokers.
- To determine mean cut – off levels for salivary cotinine to distinguish smokers and non-smokers.

REVIEW OF LITERATURE

SMOKING

HISTORY

Smoking's history dates back to 5000 BC, when Native Americans used it for ceremonial purposes. The first cigarette machine was built in 1870, after which cigarette smoking became very popular. This was also where all tobacco companies got their start.[11]

Smoke released from cigarettes is a complex chemical mixture containing nitrogen oxides, certain TSNA and PAHs which can contribute to multiple negative health effects.

EPIDEMIOLOGY[13]

Despite the fact that smoking is becoming less popular in the Western world, it still has an image of toughness and sophistication, and around 10,000 new young smokers are recruited every day. In total, about one third of the adult population smokes, and the WHO estimates that 1000 cigarettes are produced per person per year, including women. In men, there is an early two-fold difference in rate of tobacco smoked across WHO regions, with the lowest rate (34.2 %) in the Eastern World and the highest in the Western Pacific Region (62.3 %). According to these weighted prevalence estimates, there are over 1.2 billion smokers across the six WHO regions, with women accounting for a small percentage of those in developing countries.

Tobacco use can be reduced by employing a number of tobacco prevention and control strategies. When it was discovered that passive smoking was also a health hazard in the 1980s, the anti-smoking debate was accelerated. The WHO and the European Union have devised rules and recommendations for combating the "smoking pandemic." Tobacco control is a low-cost solution. Many countries agreed to have laws on smoke free areas, rules for cigarette commerce and public health interventions to control tobacco use.[14]

In Finland, for example, the Tobacco Control Act was enacted in 1976. It outlawed smoking in most public places, limited tobacco advertising, and set a tobacco purchase age limit of 16 years old. The Act was amended again in 1995, when the age limit for purchasing tobacco was raised to 18 years, and again in 2000, when ETS was added to the national list of carcinogenic substances. Smoking prevalence among Finnish adult males is now one of the lowest in Europe. In general, smoking trends indicate that tobacco policy is reducing youth smoking initiation; for example, the legislation appears to have reduced minors' purchases from commercial sources.[15]

FORMS OF TOBACCO

There is an extensive variety of smoking tobacco products in the world market.

1. **Cigarette** is a type of tobacco wrapped in paper along with filter-tipped approx. 8 millimeters in diameter, 70–120 millimeters in length.
2. **Cigar** is a tobacco roll wrapped in leaf tobacco or any other tobacco-containing substance. Cigars are divided into four categories:

a. **Small cigars** or cigarillos are small, narrow cigars that do not contain cigarette paper or an acetate filter.

b. **Regular cigars** have a diameter of up to 17 mm and a length of 110-150 mm.[16]

3. **Bidis** are the most popular tobacco smoking method. Teenagers in the United States are also becoming more interested in them. Some people believe that this type of smoking is a better tasting, less expensive, safer, or more natural alternative to traditional cigarettes.[17]

4. **Cheroot** is a tobacco leaf-based roll. Cheroots were widely smoked in South India by both men and women.

5. **Dhumti** unlike bidis and chuttas, are not sold by vendors and are made by the smokers for their own selves.[19]

6. **Kreteks** are native to Indonesia, but they can also be found in the United States.

7. **Pipe** smoking is being since old times. The different kinds of pipes used for smoking range from the small – stemmed types made up of wood to long ones made from metal.

8. **Hookah** is white Indian pipe in which tobacco smoke is passed through water before being inhaled. It used to be more common among women, the reason being that it was inconvenient for men to carry a hookah, whereas women remain at home for most of the time.

9. **Hooklis** are clay pipes that are popular in Western India. The pipe is smoked intermittently once it is lit. On a daily basis, 15 g of tobacco is smoked.[20]

10. **Chillum** is a vertically held straight conical clay pipe that is 10–14 centimeters long. It is confined to the northern states of India, primarily rural areas, and is exclusive and common among men.[21]

BIOCHEMICAL METHODS

The interaction of a biological system with any agent, whether physical, chemical, or biological, is known as a 'biomarker.' As a result, assessment of any of the aforementioned components can be used to quantify tobacco use in smoke form, such as cigarettes, bidi, and so on. The smoke produced by the use of these products contains particles that can be in the form of gas or particles. Carbon monoxide, room air, nicotine, and other hydrocarbons are gas constituents, whereas tar is the primary particle constituent. Carbon monoxide, nicotine, cotinine, thiocyanate, and other alkaloid-containing substances can be used as biomarkers to determine whether a person is an active smoker or has been exposed to secondhand smoke.[22]

NICOTINE

Nicotine, which is abundant as an active component of tobacco, can be used to determine a person's smoking habit's duration or extent due to its pharmacological conversion into cotinine before excretion. Nicotine estimation can thus be performed using body samples such as saliva, blood, urine, toe nails, and hair. However, nicotine testing cannot be used as a reliable biomarker due to individual differences in metabolism, as well as its short half life of about 2 hours.[23]

A REVIEW OF SALIVA AS DIAGNOSTIC FLUID[27]

Saliva, the most readily available and non-invasive bio-fluid in the human body, "bathes" the oral cavity on a continuous basis. Because of its twofold function, the oral cavity is exposed to the external environment, where complex interactions between various surfaces such as host soft and hard tissues, food, air, and microorganisms occur.

Saliva is produced by a variety of salivary glands. Saliva is secreted by each type of salivary gland and has a distinct composition and properties. The activity of these three pairs of glands accounts for roughly 90% of total salivary volume, with the rest coming from minor salivary glands located at various oral mucosal sites.

Tobacco use or exposure (via "passive" or "second-hand" smoke) is now routinely measured by quantifying salivary nicotine levels with clearance and half-life values comparable to plasma. A sufficient intake of anti - oxidants may aid smokers in avoiding oxidative damage caused by cigarette smoke and preventing degenerative disease.

Saliva can detect nicotine levels, opioids, barbiturates, diazepams, amphetamines, cannabinoids, cocaine, phencyclidine, , and ethanol.[25]

COTININE

Comparative data on the relative sensitivities of cotinine measurements in serum, salivary, and urinary are required for the development of epidemiologic studies, but literature lacks such data.[28]

Cotinine is an extremely useful and most popular biomarker of tobacco use. The presence of cotinine can be an indication of exposure to nicotine, either from direct consumption or environmental tobacco exposure.

Cotinine has the added benefit of being a biomarker with a high sensitivity rate, allowing secondary exposure to cigarette smoking to be distinguished from regular smokers with very low levels in the former. Also, vegetables (potatoes, eggplants, cauliflower, and tomatoes) and other food items contain nicotine in trace amounts. Nicotine can be quantified using immunoassay or chromatography (gas or high performance liquid). Woodward et al. (1991) used cotinine estimation to distinguish between smokers and nonsmokers. Markers (thiocyanate, cotinine levels, exhaled CO, and self-reported tobacco exposure) were found to be higher in the smoking group when compared to nonsmokers, who had no significant association with markers. They claimed that cotinine is the best factor for distinguishing between smokers and nonsmokers. This was consistent with surveys that questioned the accuracy of cotinine in detecting cigarette smoking. Carbon monoxide gas, in addition to cotinine, can be analyzed due to its specificity and sensitivity.[6]

"ESTIMATATION OF COTININE LEVELS"

"High-performance liquid chromatography" (HPLC)

A HPLC separation column is filled with solid particles (e.g. silica, polymers, or sorbents) and the sample mixture is separated into compounds as it interacts with the column particles. HPLC separation is influenced by the liquid solvent's condition (e.g. pressure, temperature), chemical interactions between the sample mixture. Due to very high pressure separation conditions of HPLC, its columns have relatively

small internal diameter (e.g. 4.6 mm), are short (e.g. 25 mm) and packed more densely which helps to achieve better separations of a mixture compared to ordinary liquid chromatography.

HPLC instrument usually includes a sampler by which sample mixture is injected into the HPLC, one or more mechanical pumps for pushing liquid through a tubing system, a separation column, a digital analyte detector (e.g. a UV/Vis, or a photodiode array (PDA) for qualitative or quantitative analysis the separation, and a digital microprocessor for controlling the HPLC components. Some mechanical pumps in a HPLC instrument also mix multiple liquids together, and the recipe or gradient of those liquids can modify the chemical interactions that occur in HPLC's column, and thereby modify the chemical separation of the mixture.[10]

Haley et al (1983)[35] conducted a study to determine plasma and salivary cotinine and thiocyanate were used to differentiate tobacco users from non users and to follow their daily smoking habit.

Michael A wall et al (1988)[37] studied 98 subjects in age range of 24-66 years by gas liquid chromatography. The study concluded that active smokers of < 10 cigarettes per day had lower mean cotinine levels in both serum and saliva when compared to subjects who smoked >10 cigarettes.

CARBON MONOXIDE

Cigarette smoke contains a high concentration of carbon monoxide. When compared to nicotine, CO levels have a half life of 4-5 hours, which aids in identifying the same in blood or exhaled air. CO is converted into carboxyhemoglobin (COHb), the concentration of which varies from 5% to 9%

depending on the number of cigarettes smoked. When heavy smoking is done with the aid of a cigar, these levels can rise to 20%. As a result, COHb detection can be accomplished using an oximeter and a blood sample for analysis. Although it has advantages, this procedure is not used routinely because it requires specimen (saliva, blood, or urine) to be preserved after collection, is invasive, and requires expertise. So, using a hand-held breath analyzer, a simple yet efficient method of CO analysis can be performed. It provides instant CO level measurement, which can be used to educate the patient about the deliberate effects of smoking as part of an awareness programme.[25] Secker-Walker et al found a link between CO, urinary cotinine, and self-reported smoking, with CO being 100 percent concordant with self-reporting. However, CO levels cannot be distinguished between cigarette smoking and environmental exposure. This results in false identifications ranging from 2% to 16% of the time. Differences between nonsmokers and those who smoke infrequently or irregularly cannot be reliably established.[25]

"ESTIMATION OF BREATH CARBON MONOXIDE"

Wage *et al.* (1981)[38] compared CO analyzer and other biochemical tests. The conclusion was that the smoking indicator of choice is cotinine due to its excellent precision, but CO measurement has sufficient accuracy to be used in most clinical applications. In addition, it is available at a much lower cost, provides immediate real-time results, and is noninvasive, simple and easy to use, therefore should be considered.

Jarvis *et al.* (1987)[26] concluded that cotinine provides the best discrimination and must be the marker of choice for situations where accuracy is paramount. But high costs and the elaborate laboratory facilities necessary argue against cotinine for routine use. Carbon monoxide levels provide a more sensitive indication of smoking and are cheaper and easier to apply.

MATERIALS AND METHODS

TOPIC OF STUDY: Assessment of breath carbon monoxide and salivary cotinine levels among smokers and non-smokers.

Study design: Analytical case control study

Study duration: This study was carried out in the Dept. of Oral Medicine and Radiology, KLE V.K.I.D.S, Belagavi, between December 2019 - October 2021, with laboratory support from KLE Dr. Prabhakar Kore's Basic Science Research Centre (BSRC).

Study population: A total number of 104 patients (52 smokers and 52 non-smokers) were involved in the study.

Obtaining approval from the authorities: Permission from the Institutional Review Board of KLE V.K Institute of Dental Sciences, Belagavi was obtained before starting the study.

Consent letter from the participants of the study was obtained in patient's vernacular language.

MATERIALS ARMAMENTARIUM USED

Examination of the patient

1. Dental chair with halogen lamp
2. Plain mouth mirror
3. Dental probe
4. Mouth mask
5. Disposable latex gloves

Salivary sample collection

1. Disposable mouth mask
2. A pair of sterile gloves
3. Sterile plastic containers for collection of saliva
4. Refrigerator

CO breath sample collection

a. CO breath analyzer
b. Mouth piece
c. Sterile Cotton
d. 70% alcohol as surface disinfectant

Cotinine estimation

1. Cotinine analytical standard
2. Acetanilide
3. Sodium hydroxide

4. Dichloromethane
5. Conc. Hydrochloric acid
6. Vaccum evaporator
7. High performance liquid chromatography
    a. L – 7100 pumo
    b. L – 7400 u – v detector
    c. L – 7500 autosampler
    d. L – 7500 integrator
    e. 865 co- colum oven
    f. Phosphoric acid

METHODOLOGY

SAMPLE SIZE ESTIMATION

Sample size for the study was calculated as

$$N = \frac{2\,(S)^2\,(Z\ 1\text{-}\alpha/2 + Z\ 1\text{-}\beta)^2}{d^2}$$

Where standard deviation,

S1 in the 1st group= 17.4

S2 in the second group= 1.80

d is the detectable mean difference= 10.3

$Z_{1-\alpha}$ =1.96 at 5% α error

$Z_{1-\beta}$ = 1.682 at 5% β error

Power = 95%

So, the estimated sample size is 52 per group, which makes the sample size 104 in total. The study comprised of a total number of 104 patients. Out of the104 patients, 52 were non smokers and the other 52 were smokers.

STUDY GROUP (SMOKER'S GROUP)

The study group comprised 52 patients in the age group of 18 -50 yrs with cigarette smoking habit.

INCLUSION CRITERIA

- Patient consenting to be a part of the study with a habit of cigarette smoking only
- Both males and females subjects
- Subjects aged between 18-50 years

EXCLUSION CRITERIA

- Subjects using smokeless tobacco or in any other forms except smoking
- Subjects with history of undergoing nicotine replacement therapy in the past 3 months
- Other types of tobacco smokers
- Patients with Diabetes , hypertension and any known systemic diseases
- Patients who are currently under medication for habit cessation such as NRT

CONTROL GROUP (NON – SMOKER'S GROUP)

The control group comprised of 52 patients in the age group of18 -50 yrs with no smoking habit.

INCLUSION CRITERIA

- Male and female subjects aged between 18 - 50 yrs
- Subjects with no history of tobacco habit

EXCLUSION CRITERIA

- Patients with Diabetes, hypertension and any other known systemic diseases.
- Patients who were under any medication for habit cessation such as NRT.

INFORMED CONSENT

Permission from Institutional Review Board was obtained before startingthe study. Informed consent was obtained from all the subjects before including them in the study in their vernacular language.

EXAMINATION OF THE SUBJECTS

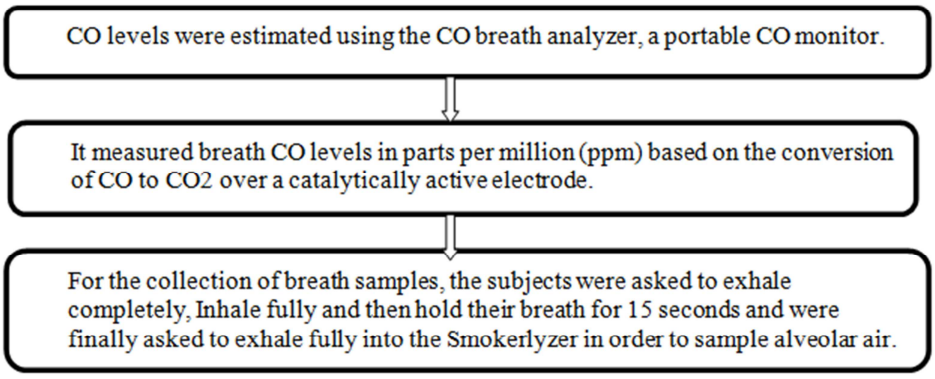
The patients included in the study were asked for demographic details and habits. An intraoral examination was carried under halogen light. The findings were recorded in the habit performa.

SALIVA SAMPLE COLLECTION

The subjects were instructed to abstain from drinking, smoking or using oral hygiene products for at least 1 hour before saliva collection. The patients were instructed to rinse their mouth with water and sit on a dental chair. The patients were instructed to pool the saliva in the mouth till the fullness was felt and then asked to spit in the given sterile plastic container with5 ml reading. This was repeatedly done for 2 times to collect 2 ml of saliva. The sample was freezed to - 20°c for the procedure to be

carried out. All samples were centrifuged at 3000 rpm for 10 min to remove particulate materials and the clean supernatant was processed immediately for estimation of cotinine which was then subjected to HPLC.

Breath Carbon Monoxide Estimation:



Subjects were asked to provide two breath samples into the Smokerlyzer.

The exhaled breath CO levels were recorded from the Smokerlyzer screen.

All the smokers were given tobacco cessation counseling and assistance by a tobacco cessation expert irrespective of their smoking status.

High – performance liquid chromatography[44]

1. Chromatography was performed using an L-7100 pump (Hitachi, Tokyo, Japan), an L-7400 UV detector (Hitachi), an L-7200 auto sampler (Hitachi), an L-7500integrator (Hitachi), and an 865-CO column oven (Jasco, Tokyo, Japan)

2. For thedetermination of the cotinine con-centration, the analytical column was a capecell Pak C UG 120 (2503.6 mm, 4mm) column (Shiseido, 18 Tokyo, Japan) and the mobile phase was 2% CH OH, 2 mm Nah PO, 0.1% phosphoric acid, 324 and 1 mm heptane sulfonate sodium.

3. Cotinine was quantified by comparing the HPLC peak heights to those of authentic standard.

The values were entered in the case sheet proforma and subjected to statistical analysis

STATISTICAL ANALYSIS

All the data were entered in Microsoft excel sheets. Statistical analysis was done using SPSS software version 21.

$$\text{Mean } (X) = \frac{\sum X}{N}$$

Chi Square Test

$$X^2 = \frac{\text{sum of (observed frequency – expected frequency)}^2}{\text{Expected frequency}} = \frac{\sum (O-E)^2}{E}$$

Mean: defined as sum of values (X) divided by the number of values (N) and denoted by.

P > 0.05 = Difference is not significantP ≤ 0.05 = Difference is significant (S)

P ≤ 0.01 = Difference is highly significant (S)

P ≤ 0.001 = Difference is very highly significant (HS)

Fig 1: Ependorff Tubes for saliva collection

Fig 2: Sample stored at – 20°C

Fig 3: Centrifuge Tubes

Fig 4: Centrifuge Machine

Fig 5: High Performance Liquid Chromatography (HPLC)

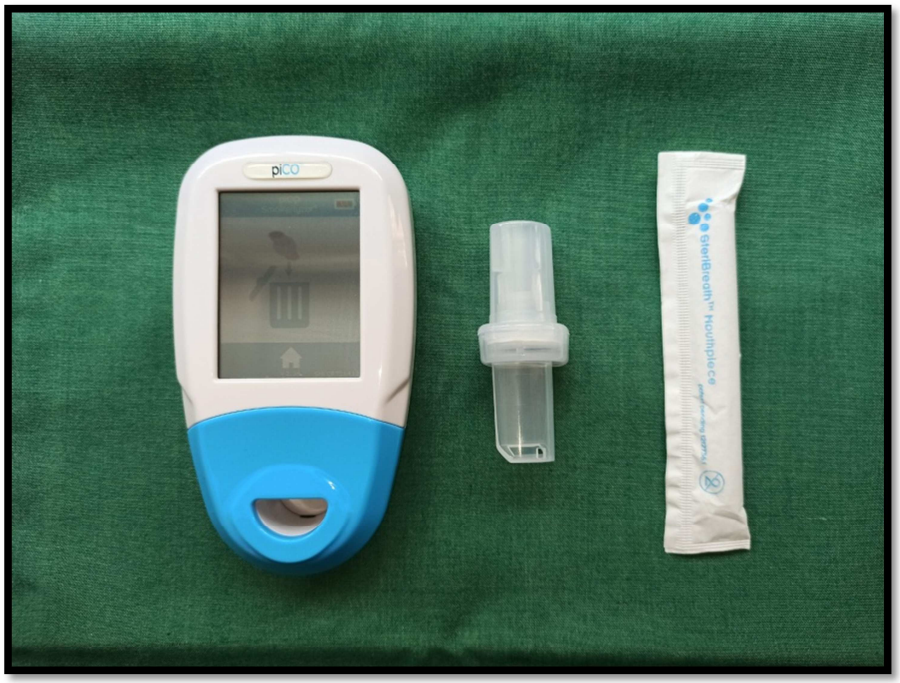


Fig 6: Breath Carbon Monoxide Analyzer

Fig 7: Breath assessment using CO breath analyser

RESULTS

This case control study was conducted in Dept. of Oral Medicine and Radiology, KAHER's KLE V.K.I.D.S, Belagavi. The purpose of the study was to assess the levels of carbon monoxide in breath and cotinine in saliva among smokers and non-smokers with HPLC and breath smokelyzer respectively. The study was conducted between December 2019 and October 2021 with 52 Smokers and 52 non Smokers. The data obtained from the study was statistically analyzed. The results extracted were compared with various variables included in the study and are presented here.

Table1: Mean age of subjects in study group and control group

The mean age of the subject among the study group was 31.73 ± 9.55 years and among the control group was 31.33 ± 8.93 years.

Table2: Age wise distribution of subjects in study group and control group

Subjects in the study group and control group were categorized based on their age into two groups of 21 to 35 years and 36 to 50 years.

Among the 52 subjects in the study group, 36 (69.2 %) subjects were between the age of 21 to 35 years and 16 (30.7 %) subjects were between the age of 36 to 50 years.

Among the 52 subjects in the control group, 35 (67.3 %) subjects were between the age of 21 to 35 years and 17 (32.7 %) subjects were between the age of 36 to 50 years.

Table3: Gender wise distribution of subjects in study and control group

Out of the 52 subjects in the study group, 44 (84.6 %) subjects males and 8 (15.4 %) subjects were females.

Out of the 52 subjects in the control group, 40 (76.9 %) subjects were males and 12 (23.1 %) subjects were females.

Table 4: Distribution of study group subjects according to duration of smoking habit

The subjects in the study group were divided into three groups depending on the duration of their smoking habit. The groups were 1 to 10 years of smoking habit, 11 to 20 years of smoking habit and 21 to 30 years of smoking habit.

Among the 52 subjects, 34 (65.4 %) subjects were smoking for 1 to 10 years, 10 (19.2 %) subjects were smoking for 11 to 20 years and 8 (15.4 %) subjects were smoking for 21 to 30 years.

Table 5: Distribution of study group subjects based on number of cigarettes smoked per day

The subjects in the study group were categorized into three groups based on the number of cigarettes they smoked per day. The groups were 1 to 5 cigarettes per day, 6 to 10 cigarettes per day and 11 to 15 cigarettes per day.

Among the 52 subjects, 39 (75.0 %) of them consumed 1 to 5 cigarettes, 10 (19.2 %) consumed 6 to 10 cigarettes and 3 (5.8 %) consumed 11 to 15 per day.

Table 6: Breath Carbon Monoxide (CO) levels in study group and control group.

The minimum level of CO obtained in the study group was 8 ppm and maximum was 28 ppm. The mean of CO levels was 15.46 ± 3.63 ppm.

The minimum level of CO obtained in the control group was 0 ppm and maximum was 7ppm. The mean of CO levels was 3.40 ± 1.80 ppm.

Table 7: Salivary cotinine levels in study group and control group.

The minimum leves of salivary cotinine obtained in the study group was 5.70 ng/dl and maximum was 84.70 ng/dl. The mean of salivary cotinine levels was 28.73 ± 19.04 ng/dl.

The minimum level of salivary cotinine obtained in the control group was 0 ng/dl and maximum was 3.83 ng/dl. The mean of salivary cotinine levels was 1.19 ± 1.10 ng/dl.

Table 8: Distribution of subjects in the study group depending on the levels of breath carbon monoxide.

The subjects in the study group were divided into four groups based on the breath CO levels which included 7 – 9 ppm (borderline smoker), 10 – 15 ppm (moderately addicted smoker), 16 – 35 ppm (heavily addicted smoker) and > 36ppm (very heavily addicted smoker) ppm.

Among the 52 subjects in the study group, 2 (3.8 %) subjects had breath CO levels between 7 - 10 ppm, 26 (50 %) subjects had breath CO levels between 11 -15 ppm and 24 (46.2 %) subjects had breath CO levels between 16 – 35 ppm.

Table9: Distribution of the study group subjects based on salivary cotinine levels.

The subjects in the study group were divided into 5 groups depending on their salivary cotinine levels which included 1 - 20 ng/dl of salivary cotinine levels, 21 - 40 ng/dl of salivary cotinine levels, 41 - 60 nd/dl of salivary cotinine levels, 61 - 80 nd/dl of salivary cotinine levels and 81 - 100 ng/dl of salivary cotinine levels.

Out of all the subjects in the study group, 23 (44.2 %) subjects had salivary cotinine levels in the range of 1 – 20 ng/dl, 15 (28.8 %) subjects has salivary cotinine levels in the range of 21 - 40 ng/dl, 9 (17.3 %) subjects had in the range of 41 – 60 ng/dl, only 1 (1.9 %) subject had salivary cotinine level between 61 - 80 ng/dl and 4 (7.7 %) subjects had salivary cotinine levels between 81 – 100 ng/dl.

Table 10: Distribution of the subjects in the study group based on the number of cigarettes smoked per day and Carbon Monoxide (CO) levels

Among the 52 subjects in the study group, 39 (75.0%) subjects smoked 1-5 cigarettes per day, out of which 2 subjects had breath CO levels of 7 – 9 ppm, 26 (50 %) subjects had breath CO levels of 10 - 15 ppm and 11 (21.2%) subjects had levels of 16 – 35ppm. All the subjects who smoked subjects smoked 6-10 cigarettes per day had breath CO levels between 16 – 35 ppm. 3 (5.8 %) subjects who smoked 11 – 15 cigarettes per day had breath CO levels between 16 – 35 ppm.

Table 11: Distribution of the study subjects based on number of cigarettes smoked per day and salivary cotinine levels

Among the 52 subjects in the study group, 39 (75.0%) subjects smoked 1-5 cigarettes per day, out of which 23 subjects had salivary cotinine levels between 1 – 20 ng/dl, 11(21.2 %) subjects had salivary cotinine levels of 21 - 40 ng/dl, 1 (1.9%) had levels

between 41 – 60 ng/dl and 4 (7.7 %) subjects had levels in the range of 80 – 100 ng/dl.

10 (19.2 %) subjects smoked around 6-10 cigarettes per day, out of which 4 (7.7%) subjects had salivary cotinine levels between 21 – 40 ng/dl and 6 (11.5 %) subjects had salivary cotinine levels between 41 – 60 nd/dl.

3 (5.8 %) subjects smoked around 11 – 15 cigarettes per day, out of which 2 (3.8 %) subjects had salivary cotinine levels between 41 – 60 ng/dl and only 1 (1.9 %) subject had salivary cotinine level between 61 – 80 nd/dl.

Table 12: Distribution of the subjects in the study group depending on duration of smoking habit and salivary cotinine levels

Among the 52 subjects in the study group, 34 (65.4 %) subjects who were smoking for 1 – 10 years, 18 subjects had salivary cotinine levels in the range of 1 –20 ng/dl, 10 (19.2) subjects had salivary cotinine levels between 21 – 40 ng/l, 3 (5.8%) subjects had levels between 41 – 60 ng/dl and 3 (5.8 %) subjects had salivary cotinine levels between 81 – 100 ng/dl. Out of 10 (19.2 %) subjects who were smoking for 11 - 20 years, 3 (5.8 %) had salivary cotinine levels between 1 – 20 ng/dl, 2 (3.8 %) subjects had levels between 21 – 40 ng/dl, 4 (7.7 %) subjects had salivary cotinine levels in the range of 41 – 60 ng/dl and only 1 (1.9 %) subject had salivary cotinine level between 81 – 100 ng/dl.

Out of 8 (15.4 %) subjects who were smoking for 21 - 30 years, 2 (3.8 %) subjects had salivary cotinine levels between 1 – 20 ng/dl, 3 (5.8 %) subjects had levels between 21 – 40 ng/dl, 2 (3.8 %) subjects had salivary cotinine levels in the range of 41 – 60 ng/dl, and only 1 (1.9 %) subject had salivary cotinine level between of 61 – 80 ng/dl.

Table 13: Distribution of the study group subjects based on duration of smoking habit and breath carbon monoxide levels

Among the subjects in the study group, 34 (65.4 %) subjects who smoked for 1 – 10 years, 2 (3.8 %) subjects showed breath carbon monoxide levels in the range of 7 – 9 ppm, 19 (36.5 %) subjects showed levels between 10 – 15 ppm, 13 (25 %) subjects showed breath carbon monoxide levels between 16 – 35ppm.

Out of 10 (19.2 %) subjects who smoked for 11 - 20 years, 6 (11.5 %) subjects had breath carbon monoxide levels between 10 – 15 ppm and 4 (7.7 %) subjects had levels in the range of 15 – 35ppm.

Among 8 (15.4 %) subjects who smoked for 21 - 30 years, 1 (1.9 %) subject had breath carbon monoxide levels between 10 – 15 ppm and 7 (13.5 %) subjects showed breath carbon monoxide levels between 16 – 35 ppm.

Normality was checked using Shapiro wilk test and the data of salivary cotinine was found to have a skewed distribution whereas the data of Carbon Monoxide was found to have normal distribution. Hence, non parametric tests were applied for salivary cotinine data and parametric tests were applied for Carbon monoxide data.

Table 15: Inter Group Comparison of salivary cotinine levels among smokers and non smokers

The Inter group comparison of salivary cotinine levels among smokers and non Smokers revealed highly significant difference with a p value of ≤ 0.001 using mann whitney test.

Table 16: Inter group comparison of breath carbon monoxide levels in between smokers and non smokers

The Inter group comparison of breath carbon monoxide levels among smokers and non smokers revealed highly significant difference with a p value of ≤ 0.001 using unpaired t- test.

Chi square test

In the study group, there was a highly significant association seen between the number of cigarettes smoked per day and salivary cotinine levels (p value <0.001). Also, there was a strong association seen between the number of cigarettes smoked and breath carbon monoxide levels (p value <0.001).

In the study group, duration of smoking habit had no association with the salivary cotinine levels or breath carbon monoxide levels (p >0.05).

Table 17: Correlation of parameters of the study group subjects which include salivary cotinine levels, age of the subjects, number of cigarettes smoked day, duration of smoking habit & breath CO levels

Among the study group subjects, a positive correlation was found between the age of the subjects and salivary cotinine levels ($r = 0.38$, $p < 0.001$); salivary cotinine levels and number of cigarettes smoked per day ($r = 0.89$, $p< 0.001$); salivary cotinine levels and breath CO levels ($r = 0.84$, $p < 0.001$) on using Spearman's correlation coefficient.

Table 18: Correlation of parameters of the control group subjects which include salivary cotinine levels, age of the subjects and breath CO levels

For control group subjects, a positive correlation was found between salivary cotinine levels and breath CO levels ($r = 0.83$, $p < 0.001$). However, age of the subjects and salivary cotinine level were not significantly correlated ($r = 0.21$, $p < 0.05$) on using Spearman's correlation coefficient

Table 19: Correlation of different parameters of study group subjects like breath CO levels, age of the subjects, number of cigarettes smoked day & duration of smoking habit

Among the study group subjects, a significant positive correlation was seen between the age of the subjects and breath CO levels ($r = 0.44$, $p < 0.001$); CO levels and number of cigarettes smoked per day ($r = 0.90$, $p< 0.001$); CO level and duration of smoking habit ($r = 0.48$, $p <0.001$) on using Karl pearson's correlation coefficient.

Table 20: Correlation between age of the subjects and breath CO level in study group

Among the study group subjects, a significant positive correlation was seen between age of the subjects and breath CO levels ($r = 0.44$, $p < 0.001$) on using Karl pearson's correlation coefficient

Table 1: Mean age of Subjects in study group and control group

Group	N	Age (Years)			
		Minimum	**Maximum**	**Mean**	**Std. Deviation**
Study	52	22	49	31.73	9.551
Control	52	21	49	31.33	8.935

Table 2: Age wise distribution of Subjects in study group and control group

Group	Age group (years)	N	Percent
Study	21 to 35	38	73.1
	36 to 50	14	26.9
	Total	52	100.0
Control	21 to 35	35	67.3
	36 to 50	17	32.7
	Total	52	100.0

Table 3: Gender wise distribution of subjects in study and control groups

Group	Gender	N	Percent (%)
Study	Male	44	84.6
	Female	8	15.4
	Total	52	100.0
Control	Male	40	76.9
	Female	12	23.1
	Total	52	100.0

Table 4: Distribution of study group subjects according to number of years of smoking

Years of smoking (years)	N	Percent
1 to 10	34	65.4
11 to 20	10	19.2
21 to 30	8	15.4
Total	52	100.0

Table 5: Distribution of study group subjects according to number of cigarettes smoked per day

No. of cigarettes per day	N	Percent
1 to 5	39	75.0
6 to 10	10	19.2
11 to 15	3	5.8
Total	52	100.0

Table 6: Breath Carbon Monoxide (CO) level in study group and control group

Group	N	Carbon Monoxide (ppm)			
		Minimum	Maximum	Mean	Std. Deviation
Study	52	8	24	15.46	3.63
Control	52	0	7	3.4	1.8

Table 7: Salivary cotinine levels in study group and control group

Group	N	Saliva cotinine level (ng/dl)			
		Minimum	**Maximum**	**Mean**	**Std. Deviation**
Study	52	5.7	84.7	28.73	19.04
Control	52	0	3.83	1.19	1.1

Table 8: Distribution of the study group subjects as per the level of addiction based on breath Carbon Monoxide (BCO) levels.

CO (ppm)	**N**	**Percent %**
Borderline (7 – 9 ppm)	2	3.8
Low addicted (10 – 15 ppm)	26	50.0
Moderately addicted (16 – 35 ppm)	24	4;6.2
Total	52	100.0

Table 9: Distribution of the study group subjects based on salivary cotinine levels.

Saliva Cotinine level (ng/dl)	**N**	**Percent %**
1 to 20	23	44.2
21 to 40	15	28.8
41 to 60	9	17.3
61 to 80	1	1.9
81 to 100	4	7.7
Total	52	100.0

Table 10: Distribution of the study group subjects based on the number of cigarettes smoked per day and breath carbon monoxide (BCO) levels

		CO			Total
		Borderline (7 – 9)	Low addicted (10 – 15)	Moderately addicted (16 – 35)	
No. of cigs per day	1 to 5	2	26	11	39
		(3.8)	(50.0)	(21.2)	(75.0)
	6 to 10	0	0	10	10
		.0	.0	(19.2)	(19.2)
	11 to 15	0	0	3	3
		.0	.0	(5.8)	(5.8
Total		2	26	24	52
		(3.8)	(50.0)	(46.2)	(100.0)

Data is given in frequency and percentage (in parentheses)

Table 11: Distribution of the study group subjects based on number of cigarettes smoked per day and salivary cotinine levels

		CL					Total
		1 to 20	21 to 40	41 to 60	61 to 80	81 to 100	
No of cigs per day	1 to 5	23	11	1	0	4	39
		(44.2)	(21.2)	(1.9)	(.0)	(7.7)	(75.0)
	6 to 10	0	4	6	0	0	10
		(.0)	(7.7)	(11.5)	(.0)	(.0)	(19.2)
	11 to 15	0	0	2	1	0	3
		(.0)	(.0)	(3.8)	(1.9)	(.0)	(5.8)
Total		23	15	9	1	4	52
		(44.2)	(28.8)	(17.3)	(1.9)	(7.7)	(100.0)

Data is given in frequency and percentage (in parentheses)

Table 12: Distribution of the study group subjects based on duration of smoking habit and salivary cotinine levels

		CL					Total
		1 to 20	21 to 40	41 to 60	61 to 80	81 to 100	
Years of smoking	1 to 10	18	10	3	0	3	34
		(34.6)	(19.2)	(5.8)	.0	(5.8)	(65.4)
	11 to 20	3	2	4	0	1	10
		(5.8)	(3.8)	(7.7)	.0	(1.9)	(19.2)
	21 to 30	2	3	2	1	0	8
		(3.8)	(5.8)	(3.8)	(1.9)	.0	(15.4)
Total		23	15	9	1	4	52
		(44.2)	(28.8)	(17.3)	(1.9)	(97.7)	(100.0)

Data is given in frequency and percentage (in parentheses)

Table 13: Distribution of the subjects in the study group based on years of smoking and breath carbon monoxide (BCO) levels

		CO			Total
		Borderline	Low addicted	Moderately addicted	
Duration of smoking	1 to 10	2	19	13	34
		(3.8)	(36.5)	(25.0)	(65.4)
	11 to 20	0	6	4	10
		.0	(11.5)	(7.7)	(19.2)
	21 to 30	0	1	7	8
		.0	(1.9)	(13.5)	(15.4)
Total		2	26	24	52
		(3.8)	(50.0)	(46.2)	(100.0)

Data is given in frequency and percentage (in parentheses)

Table 14: Exposure to passive smoking among control group

Exposure to passive smoking	N	Percent
Yes	25	48.1
No	27	51.9
Total	52	100.0

Table 15: Inter group comparison of salivary cotinine levels in between smokers and non - smokers

	Groups	N	Mean	Std. Deviation	*Z*	*p*-value
CL	Study group	52	28.7365	19.04201	1.378	**<0.001****
	Control group	52	1.1927	1.10522		

*** p – value ≤ 0.001- highly statistically significant; Salivary cotinine values are expressed as Mean and SD (mg/dl) Test applied – Mann-Whitney U test; 95 % CI*

Table 16: Inter group comparison of breath carbon monoxide (BCO) levels in between smokers and non - smokers

	Groups	N	Mean	Std. Deviation	t	p-value
CO	Study group	52	15.46	3.638	21.406	**<0.001****
	Control group	52	3.40	1.807		

*** p – value ≤ 0.001- highly statistically significant; Breath Carbon Monoxide values are expressed as Mean and SD (ppm) Test applied – Unpaired t- test; 95 % CI*

Table 17: Correlation of parameters of the study group subjects which iclude salivary cotinine levels, age of the subjects, number of cigarettes smoked day, duration of smoking habit &breath CO levels

Sl. No.		Spearman's correlation coefficient	
		r	p value
1	Age Saliva cotinine level	0.38	**0.006***
2	Saliva Cotinine level No. of cigs/day	0.89	**< 0.001****
3	Saliva Cotinine level No. of Years of smoking	0.52	**< 0.001****
4	Saliva Cotinine level CO level	0.84	**< 0.001****

Test applied - Spearman's Correlation coefficient

*** p – value ≤ 0.001- highly statistically significant*

Table 18: **Correlation of parameters of the control group subjects which include salivary cotinine levels, age of the subjects & breath CO levels**

Sl. No.		Spearman's correlation coefficient	
		r	p value
1	Age saliva cotinine level	0.21	**0.13**
2	Saliva Cotinine level CO level	0.83	<0.001**

Test applied - Spearman's Correlation coefficient

*** p – value ≤ 0.001- highly statistically significant*

Table 19. Correlation of different parameters of the study group subjects like breath CO levels, age of the subjects, number of cigarettes smoked day & duration of smoking habit

Sl. No.		Pearson's correlation coefficient	
		R	p value
1	CO level Age	0.44	**0.001****
2	CO level No. of cigs/day	0.90	< **0.001**
3	CO level No. of Years of smoking	0.48	< **0.001**

Test applied – Karl parson's Correlation coefficient

*** p – value ≤ 0.001- highly statistically significant*

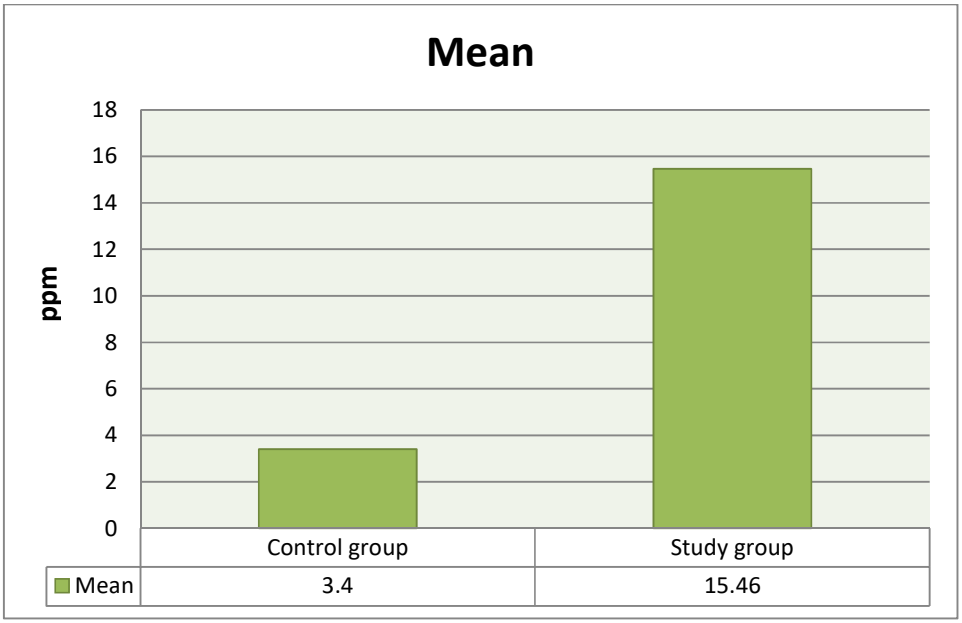
Table 20. Correlation between breath CO levels and age of the subject in control group

Sl. No.		Pearson's correlation coefficient	
		r	p value
1	CO level Age	0.44	**0.001****

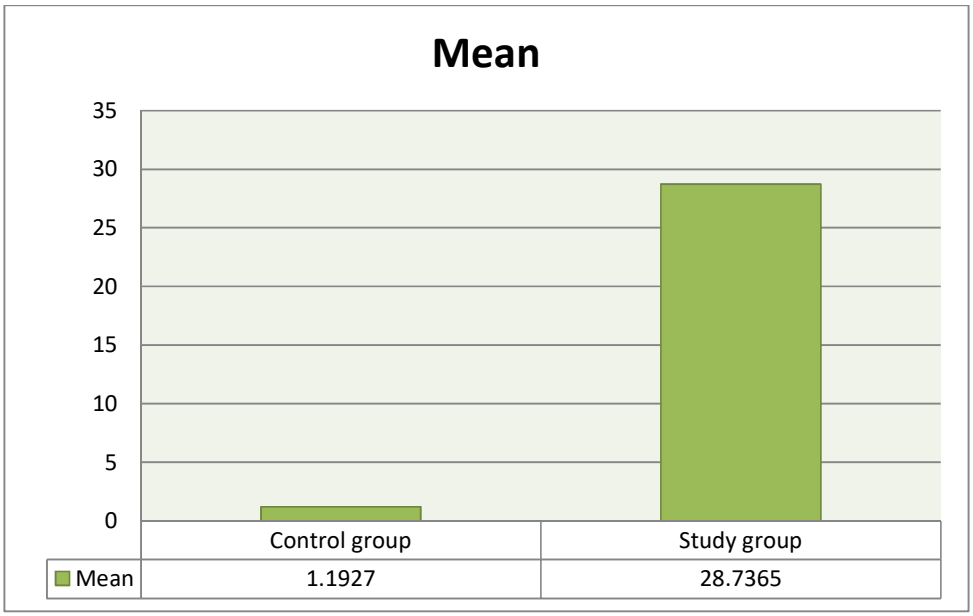
Test applied – Karl pearson's Correlation coefficient

*** p – value ≤ 0.001- highly statistically significant*

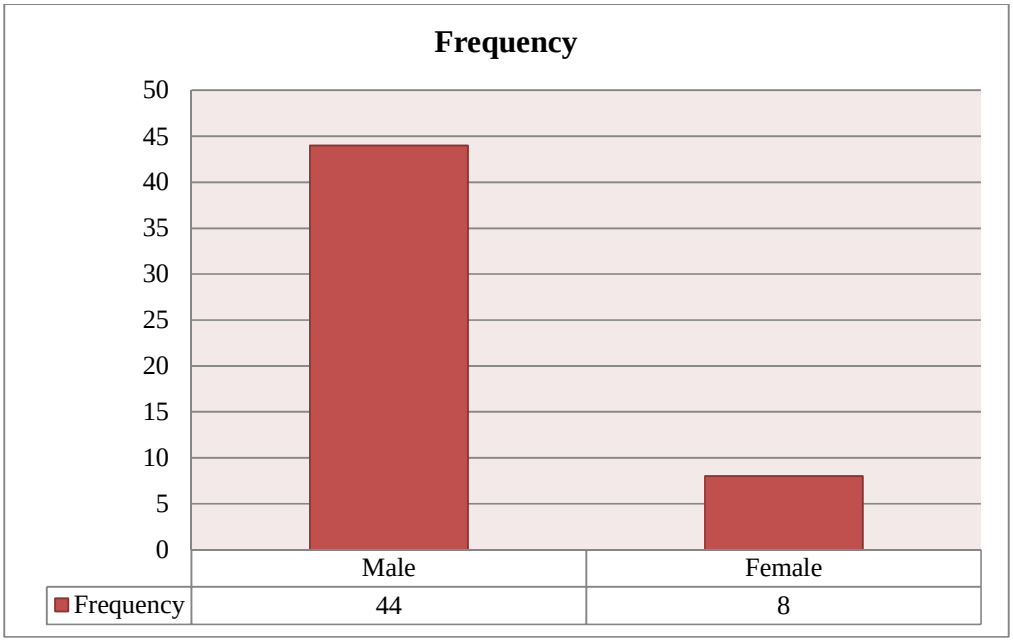
Graph 1: Mean CO level of study and control group



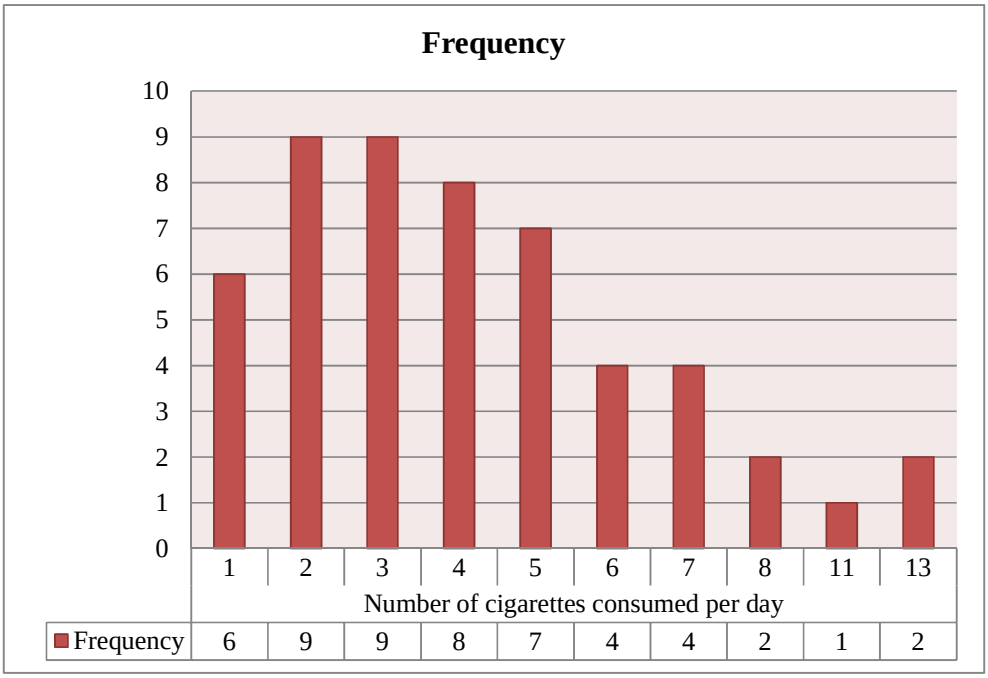
Graph 2: Mean cotinine level of study and control group



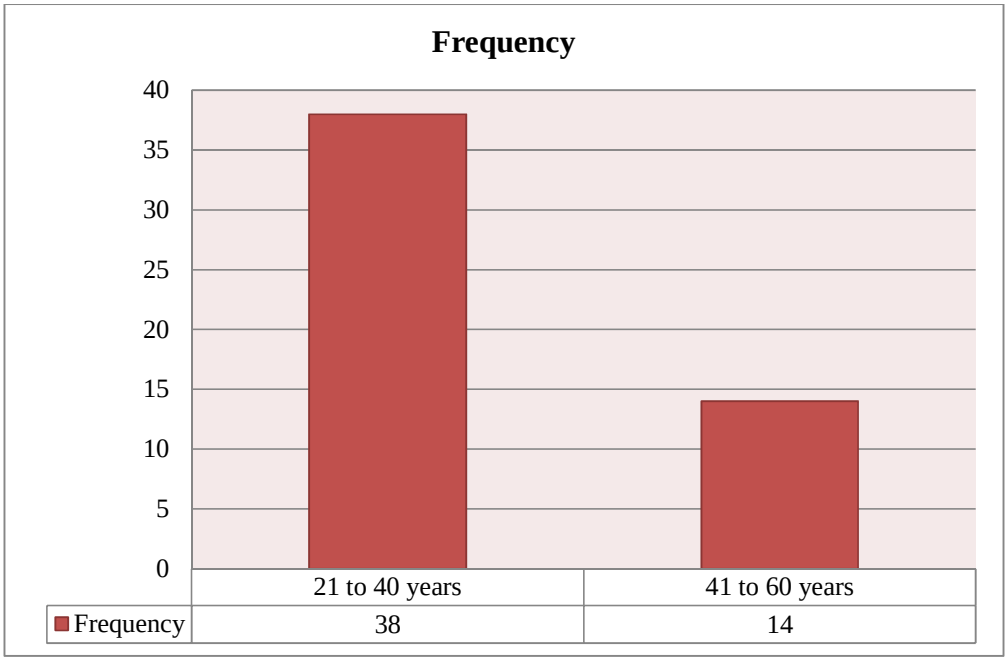
Graph 3: Frequency distribution of gender in study group



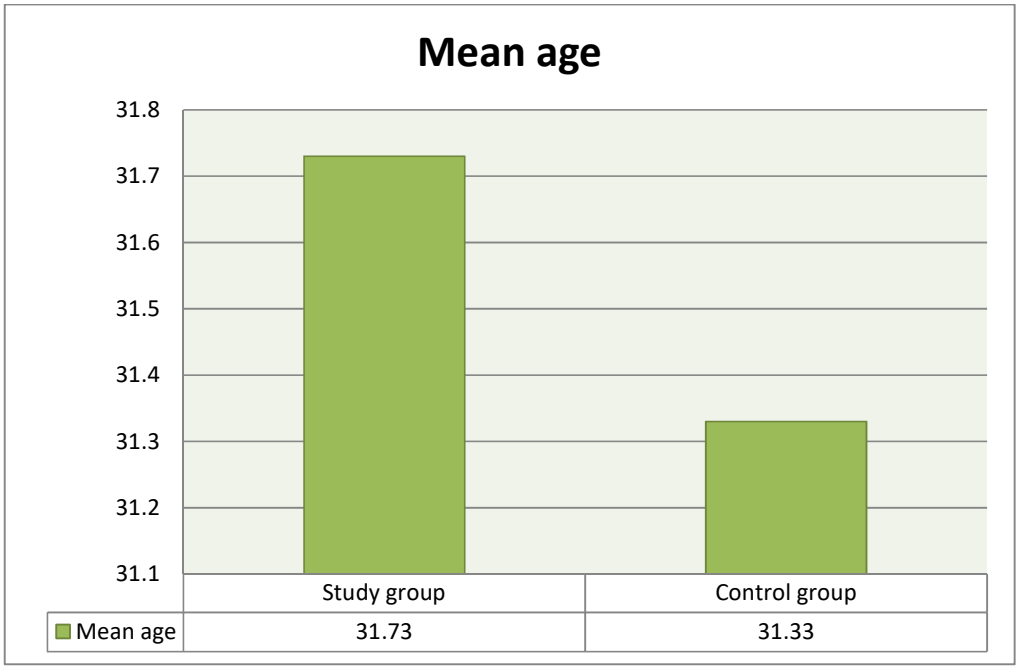
Graph 4: Frequency distribution of number of cigarettes consumed per day in study group



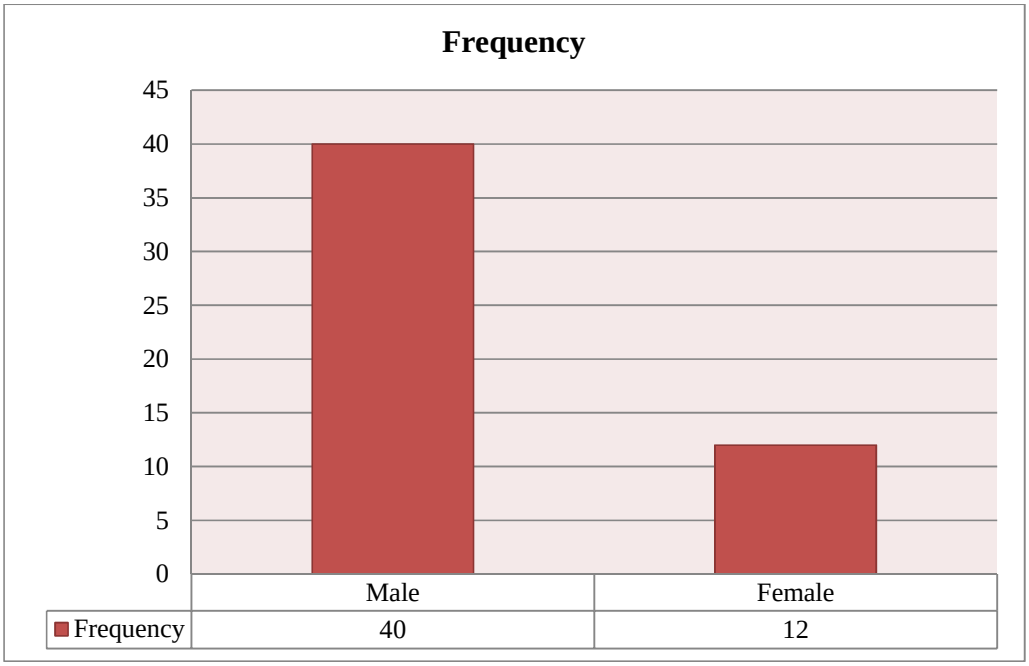
Graph 5: Frequency distribution of age groups in study group



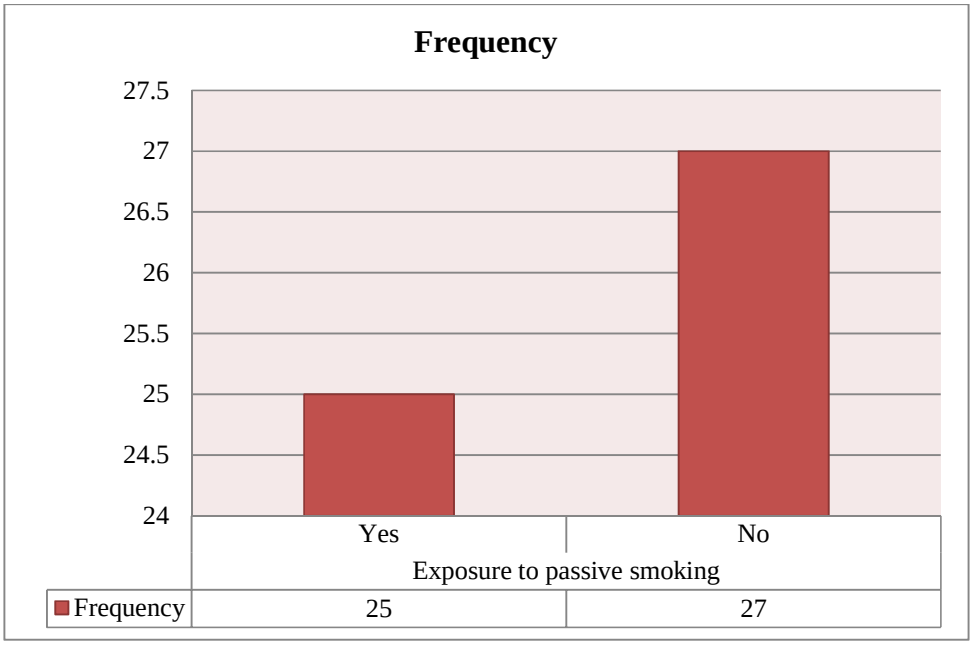
Graph 6: Mean age in study and control group



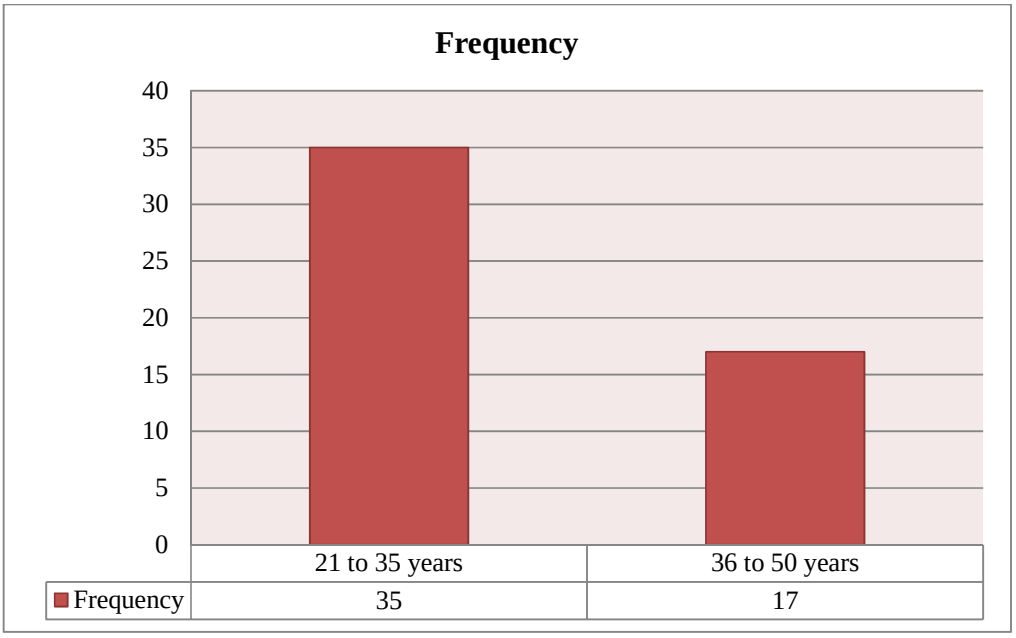
Graph 7: Frequency distribution of gender in control group



Graph 8: Frequency distribution of exposure to passive smoking in control group



Graph 9: Frequency distribution of age groups in control group



DISCUSSION

According to the WHO, the current tobacco consumption trends can lead to 8 million global tobacco-related deaths by 2030.[3]Tobacco consumption is a huge public health issue in India and controlling it should be a top priority. Tobacco smoking has both acute and chronic health consequences for smokers.[12]

Cigarette smoke contains a variety of chemicals that are known to be carcinogenic as a result of its complexity. Carbon monoxide, free radicals, HCN, nitrogen oxides and nicotine are believed to be the most important for the hazardous effects. [10] In past few years, concerns have also been raised regarding the health consequences faced by nonsmokers who are inadvertently exposed to tobacco smoke through passive smoking or environmental smoke.[44]

Given the high prevalence of oral premalignant and malignant conditions caused by the use of smoking tobacco, there is an urgent need to spread awareness about the consequences and control its usage.[1]

The most well known strategy for tobacco prevention and control are assessment of current smoking status and assisting tobacco users to quit. However, the various conventional modalities utilized for this purpose like cotinine estimation in saliva, serum, and urine are expensive and time consuming in spite of being reliable.[7]

Owing to the aforementioned challenges in assessing smoking status, there is a need for a non invasive, cheaper, and quick method for the same. Breath carbon monoxide is one such parameter which can be assessed to determine the severity of smoking and assist in the process of quitting.[9] Despite the development of various

breath carbon monoxide analyzers to assess breath carbon monoxide levels, there is a lack of sufficient evidence on the cut-off levels to distinguish smokers from non-smokers, owing to the environmental influences. Hence, the present study was conducted to assess the breath carbon monoxide levels among the smokers and non-smokers to determine the cut-off levels by correlating it to the highly reliable objective modality of salivary cotinine estimation.

The mean age of the subjects in smokers and non-smokers was 31.73 ± 9.55 years and 31.33± 8.93 years, respectively which was in accordance to **Marrone GF et al (2010)**[39] who reported the mean age of smokers to be 36.2 ± 8.5 years. The highest prevalence of smoking habit was noted in the age group of 21-35 years which was 73.1 %. This could be attributed to the higher indulgence of the younger age group in smoking because of the lifestyle changes and changing trends of stress.

In the current study, there was a male predominance in the smoking habit with 84.6 % males in the study group. This was in line with the studies conducted by **Deveci SE et al (2003)**[40] **and Herath P et al (2021)**[9] who reported a similar finding with 92.2 % and 100 % males, respectively. This is due to the fact that smoking tobacco by females is considered a cultural taboo in Indian subcontinent.

The majority (65.4 %) of the subject in the study group had a history of smoking for a duration of 1-10 years which is in line with the findings of **Marrone GF et al (2010).**[39] This could be due to the higher recruitment of younger population in the study and also, the fact that initiation of the habit occurs around 17-20 years of age. Additionally, most of the subjects (75 %) in study group smoked 1-5 cigarettes per day and only 5.8 % smoked 11-15 cigarettes per day. This is in accordance to the study conducted by **Herath P et al (2021)**[9] where the mean number of cigarettes per

day was reported to be 5.73 ± 4.88. The reason behind this finding is the busy and occupied working schedule of the younger population.

Smokers had statistically significant increased levels (15.46 ± 3.63 ppm) of breath carbon monoxide levels compared to the non-smokers who had 3.40 ± 1.80 ppm ($p < 0.001$). This was in concordance to the findings of **Deveci SE et al (2003)**[40] who reported the breath carbon monoxide levels of smokers to be 17.13 ± 8.5 ppm and of non-smokers to be 5.2 ± 3.38 ppm. The unusual levels of breath carbon monoxide among non-smokers can be due to the exposure to passive smoking or environmental air pollution.

The mean salivary cotinine level was found to be 28.73 ± 19.04 ng/ml among smokers and 1.19 ± 1.10 ng/ml among non smokers that was highly statistically significant. This is in accordance with **Polanska K et al (2016)**[41] who reported it to be 10 ng/ml and 1.5 ng/ml, respectively as well as with **Coutlas et al (1987)**[42] who reported it to be of 1.6 ± 2.8 ng/ml. The detection of considerable amounts of salivary cotinine among non-smokers can be attributed to the presence of cotinine in certain edible plants, particularly of *Solanaceae* family.

Both breath carbon monoxide levels and salivary cotinine levels had a highly significant association with the number of cigarettes smoked per day, whereas, it was not significant with the duration of smoking habit. These findings are line with that of **Herath P et al (2021)**[9]. This corresponds to the fact that the nicotine is excreted from the body after certain period of time. Hence, there is no influence of the duration of smoking habit on the breath carbon monoxide levels and salivary cotinine levels. Additionally, this can be attributed to limited sample size, variation in sampling time, assessment using single sample and variation in individual circadian

rhythm.

Similarly, there was a significant positive correlation found between breath carbon monoxide levels and salivary cotinine levels, which indicated that there was a parallel increase in both the parameters with increase in number of cigarettes smoked. To the best of our knowledge, this study is the first study conducted in Indian population to determine the severity of smoking by considering breath carbon monoxide levels as well as salivary cotinine levels among the smokers in comparison to non-smokers.

Breath carbon monoxide gave highly significant results and made assessment of smoking status more easier and more practical than compared to determining cotinine levels. Further, the results were immediately available to the investigator as well as to the patient. Therefore, a portable CO monitor is a highly reliable tool to assess smoking severity.

LIMITATIONS:

1. Breath CO measurement is only sensitive to recent smoking because CO has a short half-life (3 - 4 hours). Thus, breath CO readings following short and long term abstinence can be indistinguishable.
2. The specificity of CO as a biomarker of smoking is limited as it can be influenced by the environmental sources of CO from pollution, motor vehicle exhaust, and passive smoke exposure. Therefore, breath CO levels of nonsmokers exposed to environmental sources of CO (e.g. urban areas) can fall within the range of intermittent smokers.
3. This study involved a limited sample size of 104 participants.

FUTURE PROSPECTS OF THE STUDY:

1. The estimation of breath carbon monoxide and salivary cotinine levels could be done after successful tobacco cessation counseling programmes and NRT to assess the change in the levels post-cessation of the habit.
2. The study can be conducted involving a larger sample size.
3. Future studies should focus on refining the cutoff level for use in different clinical situations such as among pregnant women as a significant percentage of pregnant women are exposed to ETS.

SUMMARY AND CONCLUSION

Over 80% of the world's 1.3 billion tobacco smokers live in low- and middle-income nations, and tobacco use is a major public health concern in India, with over 20000 people dying each year which calls for an immediate attention towards assessing and helping smokers quit the habit.

Most of the available conventional biochemical methods to assess smoking habit in smokers are invasive, time consuming and expensive whereas assessing exhaled breath CO with a portable breath CO monitor is non invasive, quick and cheaper alternative.

Thus, in our study breath CO and salivary cotinine levels were assessed using breath CO analyzer and HPLC, respectively. A total of 104 subjects including 52 smokers and 52 non smokers were included in the study.

All the current smokers irrespective of being part of the study were given habit cessation counseling sessions. During the process, both the counselor and smoker had an outstanding quick assessment of smoking status and also success of quitting attempts.

Mean breath CO levels obtained among smokers and non smokers were 15.46 ± 3.63 ppm and 3.4 ± 1.8 ppm, respectively. Mean salivary cotinine levels obtained for smokers and non smokers were 28.73 ± 19.04 ng/dl and 1.119 ± 1.1 nd/dl, respectively.

The current study revealed significant correlation between breath CO and saliva cotinine levels among the smokers. Highly significant association was found

between number of cigarettes smoked per day and breath CO levels in the study group. Additionally, it was revealed that among the smokers, majority of the subjects (50%) were low addicted to the smoking habit.

To conclude, our study showed highly significant difference between breath CO and salivary cotinine levels between smokers and non smokers. Moreover, estimation of breath CO levels using a portable breath analyzer can provide a sensitive estimate of tobacco smoke exposure. Therefore, the measurement of smoking through the assessment of breath CO can be objectively used to assess the smoking status. In a primary care setup, it is very important to identify current smokers, in order to promote quitting and adherence to quitting amongst smokers. Measurement of breath CO using CO analyzer is more practical, suitable and easier to sustain in low-cost healthcare delivery settings such as India.

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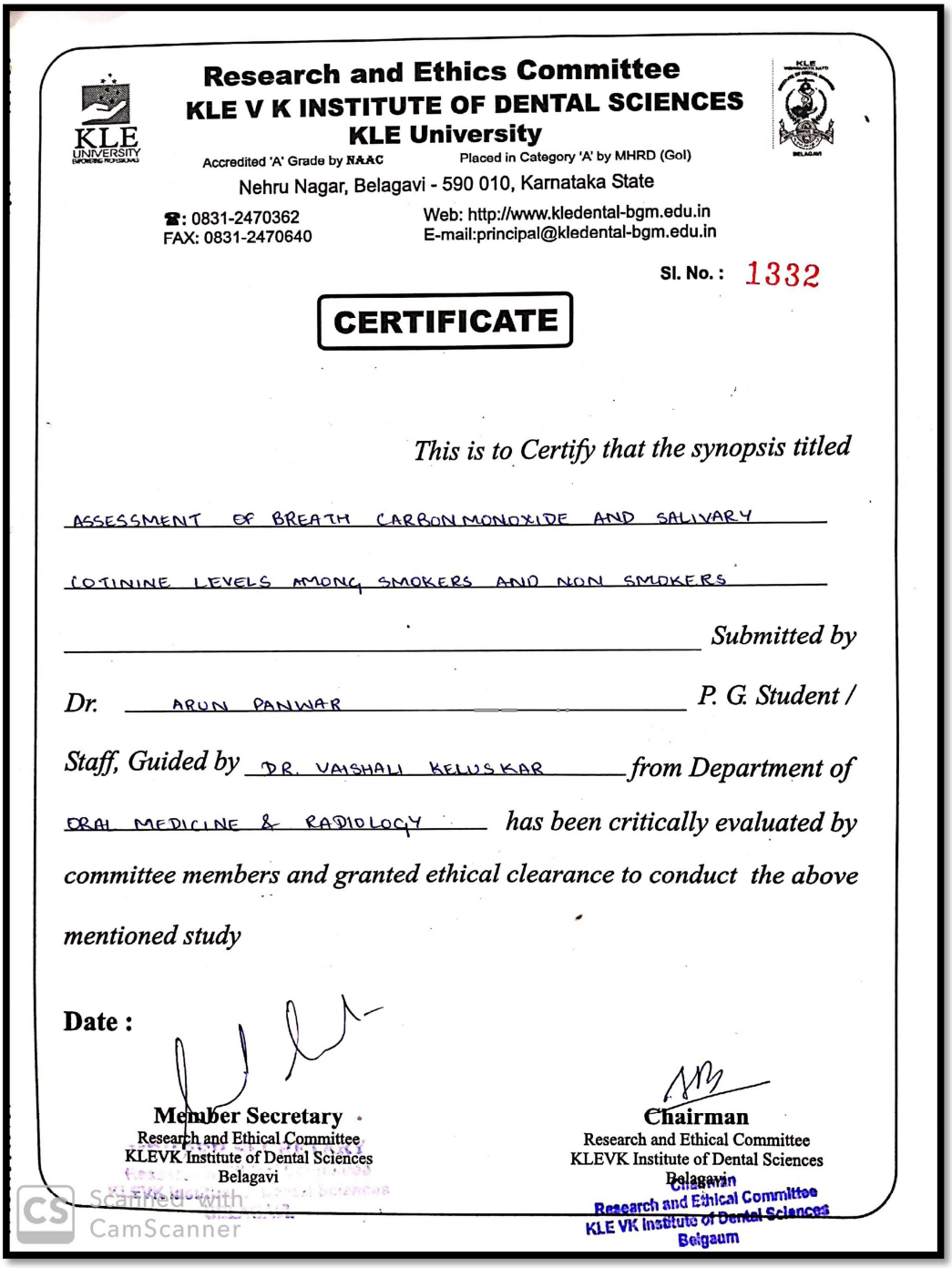
ANNEXURE I- ETHICAL CLEARANCE LETTER



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Sl. No. : 1332

CERTIFICATE

This is to Certify that the synopsis titled

ASSESSMENT OF BREATH CARBON MONOXIDE AND SALIVARY COTININE LEVELS AMONG SMOKERS AND NON SMOKERS

Submitted by

Dr. ARUN PANWAR *P. G. Student /*

Staff, Guided by DR. VAISHALI KELUSKAR *from Department of* ORAL MEDICINE & RADIOLOGY *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date :

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE II- CONSENT FORM

KLE VK Institute of Dental Sciences, Belagavi

Department Of Oral Medicine and Radiology

"Assessment of breath Carbon Monoxide and salivary cotinine levels among Smokers and Non-Smokers"

I, _____ aged___ _____years old have been informed about my involvement in the study.

1) I agree to give my details like name, age, sex and smoking history required for the study to the best of my knowledge.

2) I will willingly give breath test and my saliva sample to the dentist to assess breath carbon monoxide and salivary cotinine levels.

3) I permit the dentist to utilize the information given by me and results obtained from this study for presentation and publication purpose.

4) I will not claim any returns for my cooperation in the study, even if it is being sponsored by any agency. I am participating with my own will and wish.

In my full consciousness and presence of mind, after understanding all the procedure in my own language, I am willing and giving my consent to participate in this study.

Signature of the patient:……. **Signature of the dentist:……**

Date:…… **Name of the witness:……**

Place:…… **Signature of the witness:……**

ಕೆ ಲ್ ಇ ವಿಶ್ವನಾಥ್ ಕತ್ತಿ ದಂತ ಮಹಾವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ

ಮೌಖಿಕ ಔಷಧಿ ಮಾತು ವಿಕಾರಣಶಾಸ್ತ್ರ ಇಲಾಖೆ

ಒಪ್ಪಿಗೆ ಪತ್ರ

ಧೂಮಪಾನಿಗಳು ಮತ್ತು ಧೂಮಪಾನಿಗಳಲ್ಲದವರಲ್ಲಿ ಉಸಿರಾಟದಲ್ಲಿ ಕಾರ್ಬನ್ ಮಾನಾಕ್ಸೈಡ್ ಮತ್ತು ಲಾಲಾರಸದಲ್ಲಿ ಕೊಟಿನೈನ್ ಮಟ್ಟವನ್ನು ನಿರ್ಣಯಿಸುವುದು.

ನಾನು, _____ ವಯಸು _____ ವರುಷ ಅಧ್ಯಾನಧಾಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯ ಬಗ್ಗೆ ನನಗೆ ತಿಳಿಸಲಾಗಿದೆ.

೧) ನನ್ನ ಹೆಸರು, ವಯಸು, ಲಿಂಗ, ವಿಳಾಸ, ಹಲ್ಲಿನ ಇತಿಹಾಸ, ವೈದ್ಯಕೀಯ ಇತಿಹಾಸ ಮತ್ತು ಅಧ್ಯಯನಕ್ಕೆ ಅಗತ್ಯವಾಧ ವಿವರಗಳನ್ನು ನೀಡಲು ಒಪ್ಪುತ್ತೇನೆ.

೨) ಉಸಿರಾಟದ ಇಂಗಾಲದ ಮಾನಾಕ್ಸೈಡ್ ಮತ್ತು ಲಾಲಾರಸದ ಕೊಟಿನೈನ್ ಮಟ್ಟವನ್ನು ನಿರ್ಣಯಿಸಲು ನಾನು ಸ್ವಇಚ್ ingly ೆಯಿಂದ ಉಸಿರಾಟದ ಪರೀಕ್ಷೆ ಮತ್ತು ನನ್ನ ಲಾಲಾರಸದ ಮಾದರಿಯನ್ನು ದಂತವೈದ್ಯರಿಗೆ ನೀಡುತ್ತೇನೆ.

೩) ನಾನು ನೀಡಿದ ಮಾಹಿತಿ ಮತ್ತು ಫಲಿತಂಶಗಳನು ಪ್ರಸ್ತುತಿ ಮತ್ತು ಪ್ರಕಬನೆ ಉದ್ದೇಶಕ್ಕಾಗಿ ಬಳಸಿಕೊಳ್ಳಲು ದಂತ ವೈದ್ಯರಿಗೆ ನಾನು ಅನುಮತಿ ನೀಡುತ್ತೆನೆ .

೫) ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಸಹಕಾರಕ್ಕಾಗಿ ನಾನು ಯಾವುದೇ ತರಹದ ಏಜೆನ್ಸಿಯಿಂದ ಪ್ರಯೋಗಿನಾಗಲು ಆಧಾಯವನ್ನು ಪಡೆಯುವುದಿಲ್ಲ ಹಾಗು ನಾನು ಸ್ವಾಇಚೆಯಿಂದ ಅಧ್ಯಯನದಲ್ಲಿ ನಾನು ಭಾಗವಹಿಸುತಿದ್ದೇನೆ.

ನನ್ನ ಪೂರ್ಣಪ್ರಜ್ಞೆ ಮತ್ತು ಮನಸಿನ ಉಪಸ್ಥತಿಯಲ್ಲಿ ಎಲ್ಲಾ ಕಾರ್ಯವಿಧಾನಗೆಳನ್ನು ಸ್ಥಳೀಯ ಭಾಷೆಯಲ್ಲಿ ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ ಮತ್ತು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಂಪೂರ್ಣ ಒಪ್ಪಿಗೆಯನ್ನು ನೀಡುತ್ತಿದ್ದೇನೆ.

ರೋಗಿಯ ಸಹಿ:_____ ದಂತ ವೈದ್ಯರ ಸಹಿ:_____

ದಿನಾಂಕ:_____ ಸಾಕ್ಷಿದಾರರ ಹೆಸರು:_____

ಸ್ಥಳ:_____ ಸಾಕ್ಷಿದಾರರ ಸಹಿ:_____

के.एल.ईविश्वनाथकट्टीदंतविज्ञानसंस्था, बेलगावी

ओरलमेडिकलआणिरेडिओलॉजीविभाग

संमतीपत्र

"धूम्रपान करणाऱ्यांना आणि धूम्रपान न करणाऱ्यांमध्ये श्वासामध्ये कार्बन मोनोऑक्साईड आणि लाळ मध्ये कोटिनिनच्या पातळीचे मूल्यांकन करणे"

मी,_____वय _____वर्ष माझ्या सहभागाची माहिती मला देण्यात आली आहे।

१)मी माझे वैयक्तिकत पशील जसेकी नाव, वय, लिंग, पत्ता, मागील दंतइतिहास, वैद्यकीय इतिहास आणि अभ्यासासाठी आवश्यक असलेल्यात पशीलांना माझ्या सर्वोत्तम माहिती देण्यास सहमत आहे.

२) श्वासोच्छ्वास कार्बन मोनोऑक्साइड आणि लाळेच्या कोटिनाइन पातळीचे मूल्यांकन करण्यासाठी मी दंतवैद्याला स्वेच्छेने श्वासोच्छ्वास चाचणी व माझा लाळेचा नमुना देईन.

३)मी दंतचिकित्सकांकडून माझ्याद्वारे दिलेली माहिती आणि या अभ्यासामधूनप्राप्तझालेल्या सादरीकरणासाठी आणि प्रकाशनाच्या उद्देशाने वापरण्याची परवानगी देतो.

६)कोणत्याही अभ्यासानुसार प्रायोजित केलेगेले असले तरीही अभ्यासाच्या माझ्या सहकार्यासाठी मी परताव्याचादावा करणार नाही. मी माझ्यास्वत: च्याइच्छेने सहभाग घेतआहे.

माझ्यापूर्ण चेतने आणि मनाच्या उपस्थितीत, माझ्या स्थानिक भाषेतील सर्वप्रक्रिया समजल्यानंतर, मी या अभ्यासात भाग घेण्यास तयार आहेआणि मी संमतीदेतो.

रुग्णाची सही: .....दंतवैद्याची सही: .....

तारीख: .....साक्षीदाराचे नाव: .....

ठिकाण: .....साक्षीदाराची सही: .....

ANNEXURE III- PROFORMA

KLE V.K Institute of Dental Sciences

Department of Oral Medicine and Radiology

Smoking Habit Performa (WHO 1992)

Name:

Age:

Sex:

Occupation:

Address:

1) Do you smoke cigarettes?

a. Yes

b. No

2) On average, how many cigarettes do you smoke a day?

a. 1- 5

b. 5- 10

c. >10

3) On how many days a week do you smoke cigarettes?

a. On one day or less

b. On 2 to 4 days

c. Almost every day

4) How old were you when you began to smoke cigarettes regularly?

Age:

5) For how many years you have smoked cigarettes regularly?

a. Less than 5 years

b. 5- 10 years

c. More than 10 years

6) Have you ever used any other form of tobacco other than cigarettes/bidi ?

a. Yes

b. No

7) Were you exposed to passive smoke exposure?

a. Yes

b. No

8) For how many hours, on average each day, are you exposed to passive tobacco smoke?

Number: |__|__|__|

ANNEXURE IV- PATIENT INFORMATION SHEET

KLE V.K INSTITUTE OF DENTAL SCIENCES, BELAGAVI

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

"ASSESSMENT OF BREATH CARBON MONOXIDE AND SALIVARY COTININE LEVELS AMONG SMOKERS AND NON-SMOKERS"

Dear Patient,

You are invited to take part in a research study to assess your breath and collect your saliva for biochemical estimation. I would like to interview you to ask about your tobacco smoking habits. This research is a part of an MDS, main dissertation at KLE Academy of Higher Education and Research.

Please take time to read the information and discuss if you wish to. It is upto you to decide whether or not to take part. If you decide to take part you will be also asked to sign a consent form. You can change your mind at any time and withdraw from the study without giving any reason. The standard of care you receive will not change whether or not you decide to participate in this study.

The purpose of this research study is to assess breath carbon monoxide and salivary cotinine levels among smokers and non-smokers.

The information gained from this research will be used to publish in scientific platforms/ journals without revealing your identity to make recommendations for the best practice and the results.

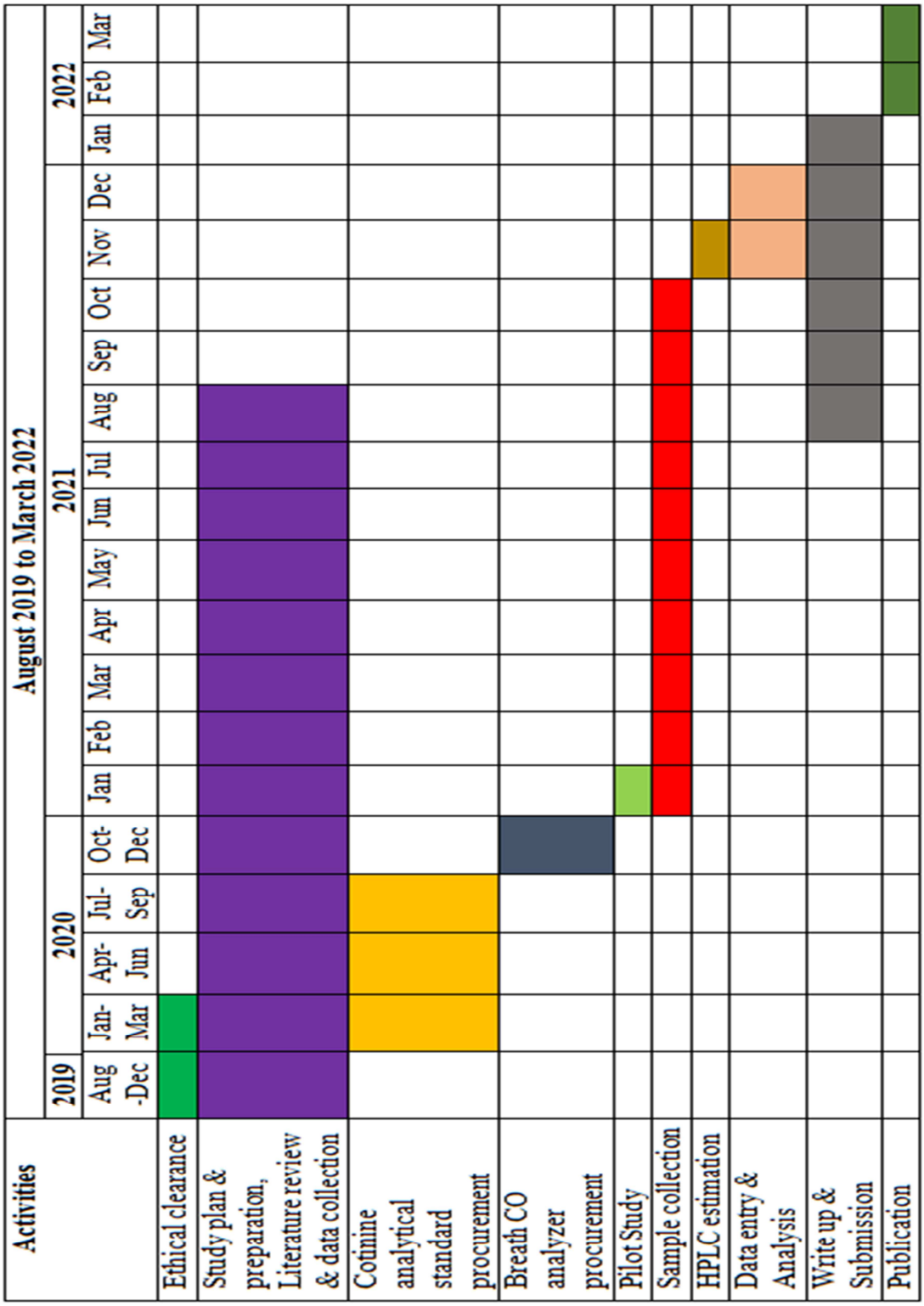
Dr. Arun Panwar

PG student

Dept.of Oral Medicine and Radiology

KLE VK I.D.S, Belagavi

ANNEXURE V- GANTT CHART



ANNEXURE VI- MASTER CHART SMOKERS – STUDY GROUP

S.No.	Name	Age	Sex	No. of cigarettes/ day	Years of smoking	Cotinine levels in saliva (ng/dl)	CO Levels in breath (ppm)
1	Gautam	24	M	4	5	25.7	16
2	Prasad	42	M	3	17	18.2	14
3	Siddesh J	23	M	6	4	38.2	18
4	Shekar	35	M	3	13	18.6	14
5	Saresh	23	M	8	4	48.7	20
6	Sarafaz	25	M	4	4	26.4	17
7	Charles	26	M	2	6	15.8	12
8	Abraham	41	M	5	24	33.5	19
9	Chetan	26	M	3	7	20.1	15
10	Ankita	25	F	2	3	17.9	11
11	Tanveer	48	M	11	21	57.8	22
12	Siddapa	54	M	5	25	36.5	17
13	Channapa	46	M	4	13	27.2	14
14	Abhishek	34	M	5	6	84.7	17
15	Gajendran	47	M	4	17	80.2	13
16	Shivam	36	M	7	12	43.1	19
17	Sanket	24	M	2	4	19.7	14
18	Pankaj	42	M	5	18	41.9	21
19	Kalra	23	M	6	4	51.2	17
20	Shrikant	47	M	13	23	77.3	23
21	Sushmita	27	F	1	5	12.6	10
22	Harsh	25	M	2	4	13.4	12
23	Swapnil	36	M	4	9	25.8	15
24	Tejan	27	M	5	5	33.1	17
25	Akin	26	M	3	4	20.4	16
26	Sonali	25	F	1	3	7.9	12
27	Moman	24	M	7	1	37.8	18
28	Sabeel	25	M	5	4	6.5	15
29	Akash	26	M	4	3	25.5	14
30	Paritosh	24	M	5	6	34.7	17
31	Abhilash	23	M	4	17	27.1	13
32	Chetan	26	M	2	3	13.8	12
33	Naveen	23	M	3	2	19.5	15
34	Siddhant	23	M	1	1	7.3	12
35	Prateek	32	M	6	8	39.9	19

36	Utkarsh	37	M	7	15	44.7	21
37	Ahaan	24	M	2	2	13.4	10
38	Sakshi	23	F	3	1	17.6	15
39	Divyal	29	F	6	3	33.8	18
40	Mihir	25	M	1	1	5.7	8
41	Daniya	43	F	2	16	14.1	13
42	Chandrashekar	45	M	13	24	58.2	24
43	Villas	46	M	7	19	45.2	20
44	Anand	49	M	3	26	19.8	16
45	Ashutosh	24	M	1	1	6.4	9
46	Manoj	47	M	3	29	17.2	14
47	Advait	22	M	2	1	9.9	12
48	Ritashna	31	F	3	5	16.9	15
49	Shubham	27	M	8	3	43.0	19
50	Fiaz	25	M	1	1	7.1	11
51	Simran	24	F	2	1	8.2	12
52	Tejraj	46	M	4	21	25.1	17

ANNEXURE VII- MASTER CHART NON SMOKERS – CONTROL GROUP

1	Mark	25	M			1.09	3
2	Parthiban	36	M			2.83	2
3	Savendra	25	M			0.09	1
4	Basvaraj	44	M			2	4
5	Vaibhav	25	M			3.83	6
6	Anurag	23	M			0.06	2
7	Saumya	25	F			0	1
8	Trinetra	27	F			3.5	5
9	Rijul	22	F			2.4	6
10	Mahantesh	48	M			0	1
11	Priyanca	25	F			0	02
12	Madhura	23	F			0.8	2
13	Hanosh	24	M			0.02	1
14	Joshua	28	M			2.4	6
15	Shaunak	24	M			0	0
16	Nagesh	38	M			1.9	4
17	Savita	42	F			2.1	6
18	Praveen	31	M			1.1	5
19	Neha	25	F			0.6	2
20	Asmi	24	F			1.2	4
21	Dikshant	27	M			0.8	5
22	Tejas	22	M			0	0
23	Vibhute	26	M			1.2	4
24	Roselle	24	F			3.6	7
25	Anubhav	27	M			0.2	1
26	Vikethono	21	F			0.1	1
27	Lokesh	26	M			0.2	3
28	Jayraj	27	M			1.6	4
29	Sridhar	25	M			0.3	3
30	Sulem	26	F			0	1
31	Anup	22	M			2.5	5
32	Himashu	28	M			1.2	4
33	Sankesh	46	M			2.9	6
34	Ayush	27	M			1.4	4
35	Mirajkar	49	M			0.2	3
36	Sajal	26	M			2.0	6
37	Guruprasad	29	M			3.2	8
38	Ramsurat	25	M			1.6	4

39	Mehul	32	M			1.0	3
40	Abhijeet	37	M			2.2	5
41	Namit	27	M			0	2
42	Mangesh	48	M			0.8	6
43	Basappa	44	M			1.2	4
44	Annaporna	22	F			0.5	3
45	Parashuram	47	M			3.3	7
46	Yellapa	41	M			2.1	4
47	Wasim	49	M			0.7	3
48	Anwar	40	M			0	2
49	Murlidhar	39	M			1.1	4
50	Krishnaprasad	41	M			0.1	3
51	Mahesh	31	M			2.3	5
52	Venkatesh	45	M			1.6	3