Original Research Article

Correlation between upper airway and lower airway function in current smokers, never smokers and former smokers.

Abstract

Introduction: Smoking is the major risk factor for the development of chronic lung disease and airway malignancy. The development of biomarkers for disease onset and early progression is hindered by the accessibility of the primary tissue in the lungs, so there is a need to evaluate alternative sites for surrogate biomarkers. The harmful effects seen in the lower and distal airways are also mirrored in the nasal epithelium as one airway and one disease.

Objective: To study the correlation between nasal mucosal cytology, mucociliary function, nasal airflow and lung function among the current smokers, never smokers, and former smokers.

Methods: Cross sectional, observational study from a tertiary care hospital.105 subjects were randomly distributed on the basis of smoking pattern into 3 groups, never smoker, current smoker and former smoker. Nasal mucosal cytology and function were assessed by saccharin transit time test (STT), peak nasal inspiratory flow(PNIF) and nasal ciliated cells & goblet cell ratio. The lower airway was assessed by spirometey.

Results: The increase in saccharin transit time is statistically significant ($p < .001$) in current smokers and former smokers compared to never smokers. The lower mean goblet cell count of the former smoker group was statistically significant when compared to the never smoker and current smoker groups, (p.023) while the change in ciliated cell/ goblet cell ratio remained statistically insignificant. The decrease in FEV1/FVC is statistically significant (p 0.036) in former smokers compared to both never smokers and current smokers.

Conclusion: Nasal mucociliary function is reduced in smokers and this reduction is permanent as cessation of smoking does not improve the mucociliary function.

Lay Summary: The study is focused to find out whether harmful effects seen in the lower and distal airways are also mirrored in the nasal epithelium as one airway and one disease in current smokers, never smokers and former smokers and thus to find out an early predictor of chronic lung disease so that intervention might be initiated. To counsel and help the smokers taking part in the study to quit smoking. It was a Cross sectional, observational study from a tertiary care hospital.105 subjects were randomly distributed on the basis of smoking pattern into 3 groups, never smoker, current smoker and former smoker. Nasal mucosal cytology and function were assessed by saccharin transit time test (STT), peak nasal inspiratory flow

(PNIF) and nasal ciliated cells & goblet cell ratio (CC/GC). Lower airway was assessed by spirometry.The increase in saccharin transit time is statistically significant (p <.001) in current smoker and former smoker compared to never smoker which implied that nasal mucociliary function is reduced in smokers. The lower mean goblet cell count of the former smoker group was statistically significant when compared to the never smoker and current smoker groups. (p.023) while the changes in ciliated cell and goblet cell ratio remained statistically insignificant. The decrease in FEV1/FVC is statistically significant (p 0.036) in former smokers compared to both never smokers and current smoker swhich lead us to the conclusion that this reduction is permanent and cessation of smoking does not improve the mucociliary function.

Key words: Saccharin transit time, Peak nasal inspiratory flow, Nasal cytology, Ciliated cell:goblet cell ratio, Pulmonary function test, Spirometry, Current smokers, Never smokers, Former smokers.

Introduction

Smoking is a major risk factor for the development of chronic lung diseases worldwide leading to significant morbidity and mortality. Cigarette smoke contains a number of toxicologically significant chemicals including polycyclic aromatic hydrocarbons (Benzopyrene), tobacco-specific nitrosamines, aldehydes, carbon monoxide, hydrogen cyanide, nitrogen oxides, benzene, toluene, phenols, aromatic amines and harmala alkaloids.A person's increased risk of disease is directly proportional to the length of time that a person continues to smoke as well as the amount smoked[1]. Pulmonary Function Tests (PFT) and various diagnostic techniques including the assessment of gas transfer and High-Resolution Computed Tomography (HRCT) assess the lung function and structural damage to it[2,3].

Although valuable, these methods do not allow for the identification of subjects at risk of developing lower airway disease early or those who have subclinical disease, when intervention might still be effective. The development of biomarkers for disease onset and early progression is hindered by the accessibility of the primary tissue in the lungs, so there is a need to evaluate alternative sites for surrogate biomarkers. The appreciation of the nasal epithelium as a surrogate for the lower airways has grown in recent years[4]. Not only is it the passage through which airborne toxicants travel to the lower airways, but it also mimics the bronchus with respect to cellular composition i.e., pseudostratified columnar ciliated

epithelium. The harmful effects seen in the lower and distal airways are also likely to be mirrored in the nasal epithelium as one airway and one disease[5,6].

Aims :

1. To compare the nasal mucosal cytology, nasal muco-ciliary function, nasal airflow and lung function among the current smokers (CS), never-smokers (NS) and former smokers (FS).
2. To determine the correlation between upper airway and lower airway functions among current smokers (CS), never-smokers (NS) and former smokers (FS).
3. To determine whether upper airway function and cytology can be a predictor for lower airway function among smokers.

Materials And Methods

Ours was across sectional observational study among patients attending the Otorhinolarygology and Pulmonology outpatient clinics at a tertiary referral hospital in West Bengal, India, over a period of 16 months. A total of 105 individuals were examined and data collected from them for the study. All the participants were above 19 years of age and free from any apparent nasal and paranasal sinus disease. The study subjects were broadly classified into three groups i.e. never smokers(NS), former smokers (FS) and current smokers (CS). The three groups were defined based on the following criteria:

1. Never-Smoker (NS): Defined as a subject who has never smoked or who has smoked less than 100 cigarettes in his life time.
2. Current Smoker (CS): Defined as a subject who had smoked at least 100 cigarettes in his life time and who currently smoked at least one cigarette per day.
3. Former Smoker (FS) : Defined as a subject who smoked at least 100 cigarettes in his life time but who had quit smoking at the time of interview[7].

The exclusion criteria included the presence of nasal or paranasal sinus disease/trauma/surgery, subjects on medication for nasal or paranasal sinus disease and subjects having a high SNOT20 score (≥ 10). The total sample size was 105. The calculation was based on consecutive sampling technique. The description of the procedure is shown in figure 1.

After getting a signed informed consent following a detailed explanation of the procedure, a detailed history and the patient's clinical examination findings were recorded. The peak nasal inspiratory flow (PNIF) of the selected patients was measured with an In-Check inspiratory flow meter manufactured by Clement-Clark (UK). Each subject received detailed instructions and a total of five measurements were taken under supervision of one of the investigators. The highest of the five recorded measurements (PNIF MAX) was recorded for the study[8]. The pulmonary function of the subjects was assessed and recorded by spirometry performed according to the guidelines of the American Thoracic Society using a portable spirometer (RMS, Helios 702)[9].

The nasal muco-ciliary clearance function was measured using the saccharin test described by Andersen. Two saccharin tablets (each 1mm in size) were gently placed on the floor of the nose about 1cm behind the anterior edge of inferior turbinate with a pair of forceps. Participants were then instructed to remain seated and to swallow every 30 seconds. They were instructed to breathe normally and not to cough, sniff or blow their nose. The time from saccharin placement until the participant reports the sensation of sweetness is recorded with stopwatch. The test is supposed to be terminated if nothing had been tasted up to 40 minutes[10].

The above were followed by a nasal cytological examination. The samples this cytological examination were however collected before the Saccharin transit time test. First each subject was asked to blow his/her nose to get rid of any excess secretions. Then under direct vision the medial surface of the inferior turbinate was rubbed with a sterile cotton swab soaked in saline. The swabs were then smeared on the middle third of a glass slide, air dried and stored for cytological examination. These slides were later stained by May Grunwald Giemsa stain, washed in tap water, air-dried and mounted in a synthetic resin with cover glass. The slides were then examined under a light microscope with a 3100 objective lens in oil immersion. At least 50 microscopic field were examined, ciliated cells (CC) and goblet cells (GC) were counted in these fields and the average calculated. The CC: GC ratio was calculated and recorded[11].

The participants were then given a Sino nasal outcome test (SNOT 20) questionnaire for the assessment and recording of nasal symptom severity. The collected data was recorded and analysed using SPSS Version 22 software.

The study was aimed to find out whether harmful effects seen in the lower and distal airways are also mirrored in the nasal epithelium as one airway and one diseasein Current Smokers,

Never Smokers and Former Smokers and thus to find out an early predictor of Chronic lung disease so that intervention might be still possible by counselling the smokers to quit smoking.

Results:

The age of our subjects ranged from 20 to 52 years, 21 to 60 years and 23 to 62 years among never smokers (NS) group, current smokers (CS) and former smokers (FS) respectively.(Table 1). The mean age of the NS group, CS group and FS groups were 30.82(± 9.01) years ,31.69(± 10.69) years and 40.34(± 13.22)years respectively.(Table 2) .Mean age of NS and CS groups were in the same range while FS group had a slightly higher mean. However, the mean age in 3 groups are within comparable limits.

The mean PNIF value of NS group in our study was 101.2 L/min (± 4.07).In CS, it was higher at 106.05 L/min (± 4.11) but lower in FS group where it was 94.17 L/min (± 2.91) (Table 3). However, these differences were, not statistically significant. (p 0.083) indicating no significant nasal obstructive features in any of the groups.

Forced Expiratory Volume at 1st second/ Forced Vital Capacity (FEV1/FVC) findings comparing the NS,CS and FS groups are 83.50% (± 0.51), 83.40%(± 0.54) and 81.62% (± 0.64) (Table 4) .There is statistically significant decrease in FEV1/FVC values of FS group compared to NS and CS group(p .036).

In our study, there wasn't any statistically significant difference in FEV1/FVC between NS and CS groups. However, the decrease in FEV1/FVC is statistically significant (p.036) in FS group compared to both NS and CS groups. This could can that most of the FS group quit smoking after developing associated symptoms or complications. Adding to this observation, there is statistically significant reduction in goblet cell count in FS group compared to NS and CS groups which indicated that there is a change at cellular level in former smokers who quit after reaching a certain point.

The NS group had a mean STT of 921.48(± 46.96) seconds. The STT in CS and FS groups were comparatively elevated at 1338.11(± 67.82) seconds and 1322.50 (± 60.56) seconds respectively. This increase is statistically significant ($p < .001$) (Table 3). There isn't any statistically significant difference in STT between CS and FS groups. This indicates a strong

correlation between smoking and elevated STT. Thus, there is statistically significant decrease in mucociliary function in CS/FS group compared to NS group manifested by an increased STT.

The mean ciliated cells/goblet cells (CC:GC) ratio in the NS group was 1.26 (± 0.17), while CS group had a mean ratio of 1.20 (± 0.19) and FS group had a mean ratio of 1.54(± 0.17). These differences were however, not statistically significant. (p.327)

The mean goblet cell (GC) count of NS group was 16.43 (± 1.42)/HPF (High power field) while in the CS group the mean GC count was 18.11(± 2.29) /HPF. Interestingly, in FS group the mean GC count was lowest at 12.14 (± 1.11). This lower mean GC count of the FS group was statistically significant when compared to the NS and CS groups. (p.023) (Table 7) i.e. on an average the FS group had fewer GC per unit area compared to NS and CS groups. However, CC/GC ratio showed no statistically significant variations between the 3 groups (p 0.327)(Table 6).

In our study, the Mean ± Standard Error of all parameters in rural and urban population among NS group, CS group and FS group are not statistically significant. This indicates 3 groups are having no cellular level and structural changes in response to urban pollution and thus ruling it out as a confounding factor.

DISCUSSION

The nose plays a crucial role in warming, humidifying and filtering air before it enters the lower airways[12]. Impairment in nasal function can therefore impact the lower airways[12].The upper and lower airways not only interact via their anatomical connection and common mucosal lining but there may also be neural reflexes and systemic mechanisms[13].

Simple peak flow instruments such as the Wright, mini-Wright, and Youlten flow meters are often used to measure peak nasal inspiratory flow (PNIF) with the use of a face mask[14]. PNIF is reported to be the best validated technique for evaluation of nasal airflow[12].Normal PNIF values of healthy individuals ranges from 130L/min to 140L/min[15] . Kjaergaard *et. al.* [16] observed that smokers exhibit lower minimal nasal cross-sectional areas and nasal cavity volumes, achieve lower PNIF-values and have a less compliant nasal mucosa compared to non smokers. Another study by Gomes *et al.* concluded that PNIF is affected by lower

airway function and has been reported to positively correlate with peak expiratory flow (PEF) in healthy children and adults[17]. In our study, there are no statistically significant variations of PNIF values between the 3 groups (p 0.083) indicating no significant nasal obstructive features in any of the groups which contradicts the two former studies. (Table 3). It was also noted that there was no statistically significant difference in PNIF values between rural and urban population among the 3 groups indicating no role for urban pollution and cigarette smoking on PNIF values.

Pulmonary function testing is an important diagnostic tool for assessing lower airway status particularly with regard to diseases such as COPD, asthma, and interstitial lung disease[18].A study done by *Nawafleh HA et. al.*found that mean pulmonary function were found to be lower in smokers than the non-smokers, there were significant differences between mean spirometric values of smoking and non-smoking individuals in the age groups of 20-30 years and 30-39 and 40-49 years[19]. A comparative study of pulmonary function done by Kumar *et. al.* between rural smokers and rural non-smokers showed significant decreased value (p value < 0.05) in smokers of rural population[20].*Yunus Çolak et. al.*concluded that a combination of baseline lung function and smoking exposure yielded a higher predictive capability for subsequent clinical COPD development than lung function and smoking exposure separately[21].In an US based study, smokers had accelerated lung function decline compared with never-smokers and the accelerated decline in lung function persisted for decades after smoking cessation[22].

In our study the mean FEV1/FVC values were in the normal range for NS, CS and FS groups.There was no significant difference in FEV1/FVC findings among NS and CS groups. However, the decrease in FEV1/FVC is statistically significant (p value =0.036) in FS which tallies with the US based study. We do not have any conclusive evidence to explain this. But we can presume that it can be due to the quitting of smoking among FS group after developing significant associated symptoms or complications. Especially since the bulk of the subjects in our study consisted of patients attending the pulmonology out patient department. It was also noted that, there is a statistically significant reduction in goblet cell count in FS compared to NS and CS which indicates that there was a significantly higher cellular damage in this group of former smokers. However, the average CS seems to have better CC/GC population compared to an average FS. In FS, cell counts reduce due to structural damage after certain point when the patient is compelled or is more motivated to stop smoking. There is further scope of study in this regard. There was no statistical

significance on comparison of FEV1/FVC values between rural and urban population among the 3 groups.

The STT test was first described in 1974 by Anderson *et. al.*[23]. It is a method for scientific research widely used to assess nasal mucociliary clearance as it is reproducible, simple and non-invasive, besides being low cost[24-28]. Studies done by Paglicua *et. al.*[29] and Xavier *et. al.*[30] observed a positive correlation between STT and cigarette smoking i.e., nasal mucociliary transport time is significantly higher in smokers than non smokers. Meanwhile, Nicole *et. al.*[31] found a faster STT in young healthy smokers compared to healthy non-smokers. They speculate that young subjects who are early or light smokers may have a protective increase in ciliary beat frequency and transport in response to cigarette smoking. Juliana T *et. al.*[32] studied nasal mucociliary clearance in subjects with COPD . They observed that after cessation of smoking, even in people with full blown COPD, there is an improvement in mucociliary clearance within 1 year of smoking cessation.

Our study showed STT of NS group close to the accepted upper limit of normal value of 10.99 min[33] compared to the CS and FS groups who had raised STT(Table 5). Our observations suggests a statistically significant decrease in mucociliary function and an increase in STT among current smokers and former smokers compared to never smokers due to the impaired mucociliary function following exposure to tobacco smoke($p < .001$). It was also noted that there is no improvement in STT with cessation of smoking as the values of former smokers was similar to that of current smokers. So we conclude, that the mucociliary function impairment is of a more permanent nature with no reversal to normal levels after cessation of smoking (in the FS group).Comparison of STT values between rural and urban population among the 3 groups was statistically insignificant. This probably indicates that urban pollution (smoke) doesn't have a significant impact on nasal mucociliary function unlike tobacco smoke.

Nasal cytology, being cheap, non-invasive and repeatable, can easily be considered as part of rhino-allergologic diagnostics[34]. Pagliuca *et. al.* found the ratios between Ciliated Cells(CCs) and Goblet Cells(GCs) to be 0.745 in smokers, 0.825 in ex-smokers, and 0.83 in non-smokers. Thus, reduction of number of CCs compared to GCs[29]. Another study[35] showed variable degrees of regeneration of the ciliated cells and decreased vascular congestion post cessation of smoking. Numerous goblet cells and sero-mucinous acini were seen. It concluded that quitting smoking may help smokers to overcome their recalcitrant disease.

But, we didn't observe any significant variations in the CC:GC ratio between the 3 groups (p value = 0.327).(Table 6) .Interestingly there is a statistically significant reduction in Goblet cell count in FS compared to NS and CS.(Table 6). We would have expected an increase in CC/GC ratio in FS following the reduction in goblet cells. However CC/GC ratio is not significantly reduced which leads us to conclude that there was corresponding reduction in ciliated cells too among FS group.

There was no statistically significant difference on comparison of CC/GC ratio values between rural and urban populations among the 3 groups which signifies no cellular level change in response to urban pollution. Although, as nasal biopsy is a better method for assessing ciliated and goblet cells, in our study we used nasal scrapings which is taken from the mid portion of inferior turbinate for nasal cytology as it is still quite an effective method and gave a good representative sample for cytology and also in view of it's non-invasive nature[34].

CONCLUSION:

1. Nasal mucociliary function is reduced in smokers.

2. This reduction is permanent and cessation of smoking does not improve the mucociliary function.

3.Nasal mucosal cytology can be used as a reliable surrogate marker for assessment of lower airway function

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LIST OF ABBREVIATIONS

CC - Ciliated Cells

COPD – Chronic Obstructive Pulmonary Disease

CS – Current smokers

FEV1 –Forced Expiratory Volume at 1st second

FS – Former smokers

FVC- Forced Vital Capacity

GC – Goblet Cells

NS – Never smokers

PFT – Pulmonary Function Test

PNIF- Peak Nasal Inflow meter

Spo2- Oxygen Saturation(%)

STT – Saccharin Transit Time Test

Figures 1

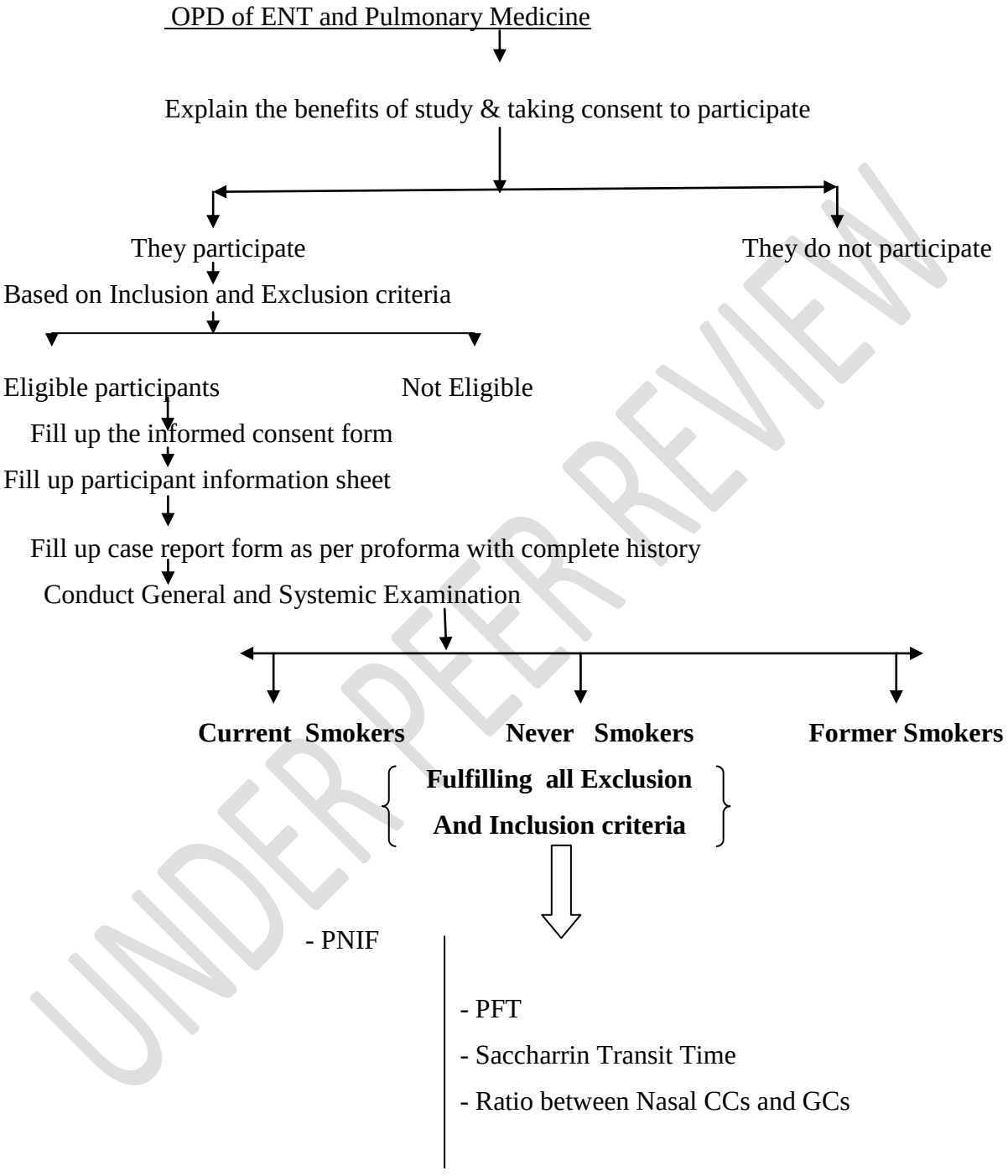

Figure. 1 Consecutive sampling technique

LIST OF TABLES

Age	Never Smoker	Current Smoker	Former Smoker
Maximum Age	52	60	62
Minimum Age	20	21	23

Table 1 : Maximum and minimum age in different groups

Never Smoker	Current Smoker	Former Smoker
30.82 ± 9.01	31.69 ± 10.69	40.34 ± 13.22

Table 2: Mean age of different groups

Never Smoker	Current Smoker	Former Smoker	P-value
101.2±4.07	106.05±4.11	94.17±2.91	.083

Table 3 : Comparison of PNIF in (Mean± Standard Error) between three groups

Group / Parameter	Never Smoker	Current Smoker	Former Smoker	P-value
FEV1	2.71±.09	2.72±.07	2.70±.08	.988
FVC	3.23±.10	3.25±.08	3.29±.08	.854
FEV1:FVC(%)	83.50±.51	83.40±.54	81.62±.64	.036*

Table 4: Comparison of FEV1, FVC and FEV1:FVC (Mean± Standard Error) between three groups

Never Smoker	Current Smoker	Former Smoker	P-value
921.48 ±46..96[b]	1338.11± 67.82[a]	1322.50 ±60.56[a]	<.001**

Table 5 :Comparison of STT in seconds (Mean± Standard Error) between three groups

Different superscripts (a,b,c) differ significantly according to Tukey's HSD test

Group / Parameter	Never Smoker	Current Smoker	Former Smoker	P-value
CC	15.94±1.48	17.08±2.14	15.25±1.36	.703
GC	16.43[ab] ±1.42	18.11[a]±2.29	12.14[b] ±1.11	.023*
CC:GC	1.26±.17	1.20±.19	1.54±.17	.327

Table 6: Comparison of CC:GC (Mean± Standard Error) between three groups