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RESEARCH ARTICLE

Smoking-induced oxidant/antioxidant imbalance in chronic obstructive pulmonary disease: Assessment of auditory evoked potential: A comparative study of smokers and non-smokers with or without chronic obstructive pulmonary disease

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ABSTRACT

Background: Oxidative stress, inflammation, and hypoxia, which are associated with chronic obstructive pulmonary disease (COPD) may contribute to auditory impairment. **Aims and Objectives:** The present study was undertaken to assess the brainstem auditory evoked potential (BAEP) of smokers and non-smokers with or without COPD. **Materials and Methods:** The study comprised smokers with ($n = 25$) or without COPD ($n = 15$) and also healthy non-smokers ($n = 30$). Oxidative stress was assessed by malondialdehyde (MDA) and ferric reducing antioxidant power (FRAP). Pulmonary functions were determined. Data were analyzed using ANOVA and Pearson's correlation tests. $P < 0.05$ was considered statistically significant. **Results:** Spirometric values were significantly lower in COPD group in comparison with other two groups. Oxidant/antioxidant imbalance was more in smokers with COPD. BAEP result (both right and left ear) showed no significant difference between non-smokers and smokers without COPD in Wave I ($P = 0.885$ and 0.085, respectively) and in wave II ($P = 0.554$ and 0.396, respectively). Significant difference (both ears) was found between non-smokers and smokers with and without COPD in waves III, IV, V, and IPL I-V. MDA showed significant negative and positive correlations with FRAP, wave latencies, and IPL. Significant negative correlation was found between pack years and FRAP. However, non-significant positive correlation was found with MDA. Significant positive correlation was found between pack years and wave latencies III, IV, and V and interpeak latency I-V in both ears. **Conclusion:** Smoking-induced oxidative stress as well as increased susceptibility to COPD, which in turn led to hypoxia and effects the auditory mechanisms and these are responsible for BAEP abnormalities.

KEY WORDS: Smokers; Non-smokers; Chronic Obstructive Pulmonary Disease; Pulmonary Functions; Brainstem Auditory Evoked Potential; Oxidant/Antioxidant

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a disease state, characterized by progressive airflow limitation, which is not fully reversible. The disease burden is rising and the WHO estimates that it is to be the third leading cause of mortality by the year 2030.[1] It is a multietiological disease. An imbalance between oxidant/antioxidant capacities is reported to play an important role in the pathophysiology of COPD.[2,3] COPD is reported to have systemic effects. Almost one-third of patients with COPD have clinical evidence of peripheral neuropathy, and two-thirds have electrophysiological abnormalities. Brainstem hypoxia, associated with COPD contributes to the brainstem auditory evoked potential (BAEP) abnormalities. The systemic effects of COPD contribute to BAEP changes also.[4-6] Cigarette

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smoking is a major risk factor in the development of COPD and COPD-related peripheral neuropathy.[7] Various studies have shown differences between hearing status of COPD patients and the required controls. However, the existing paucity of data[8] renders it difficult, and therefore challenging. Moreover, very few studies have been done to assess the oxidant antioxidant imbalance and also the comparison of the BAEP, findings between non-smokers and smokers with or without COPD. Even though smoking is a major risk factor in COPD, yet not all smokers develop COPD. The crucial factor seems to be the amount smoked and also the extent of inhalation.[9] Although the data are insufficient, as found hypoxia, hypercapnia, and respiratory acidosis due to smoking and respiratory insufficiency affect the brainstem.[10] Association of smoking with hearing loss may be due to its effect on the vascular supply of auditory apparatus and also due to oxidative stress.[11] Thus, the present study was undertaken to compare oxidant antioxidant imbalance and BAEP changes between smokers (with or without COPD) and non-smokers. Some studies have shown that hearing loss increases with increase in pack years while other studies have found that the so-called "dose effect" changes with age.[12,13] Further, some studies have found no relation of hearing loss with smoking.[14] Thus, in the present study, correlation of pack years with malondialdehyde (MDA), ferric reducing antioxidant power (FRAP), and BAEP waves was also analyzed in smokers with COPD.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Physiology, Jawaharlal Nehru Medical College, AMU, Aligarh, Uttar Pradesh. The study period was from January to June 2013. Smokers were selected from TB and Chest Diseases OPD. On the basis of pulmonary function tests, they were then divided into two groups: Smokers with COPD ($n = 25$) and smokers without COPD ($n = 15$). Healthy non-smokers ($n = 30$) were selected from in and around JNMC. Only those who willingly participated in the study were selected.

Inclusion and Exclusion Criteria

All the participants included were more than 18 years of age. Smoker group comprised those with a history of (cigarette, bidi) smoking. Smokers with a history of chronic cough or sputum production on most days for at least 3 months of the two consecutive years were included. FEV1 % predicted <70% and FEV1/FVC % <70% on the basis of pulmonary function test (the results of MIR Spirolab in TB and chest diseases department JNMC) were included in smoker group with COPD while the remaining were labeled as smokers without COPD. Healthy participants with normal PFT and without any history of smoking were also included in this study. Participants taking antioxidants or having any diseases, namely, asthma, hypertension, carcinoma, diabetes, cardiovascular diseases, or renal disease in which oxidative stress is implicated in pathophysiology were excluded from the study. Those having medical, surgical trauma, history of altered nerve conduction, and BAEP were also excluded.

Under aseptic conditions and prior consent of participants, 5 ml blood was drawn from the peripheral vein. It was centrifuged at 3000 rpm for 15 min. The serum was subjected to the estimation of MDA and FRAP assay. MDA was estimated by the method of Philpot[15] in which one molecule of MDA reacts with two molecules of thiobarbituric acid at pH 3.5, yielding pink color chromagen, measured spectophotometrically at 532 nm. Total antioxidant activity was measured by FRAP assay of Benzie and Strain,[16] in which at low pH, reduction of ferric tripyridyl triazine (Fe III TPZ) complex to ferrous form is measured by the change in absorption at 593 nm.

BAEP was done in the Neurophysiology Laboratory of the Department of Physiology, JNMC, using Neuroperfect Software (Medicaid), which has default settings. BAEP was recorded from ipsilateral ear, referred as vertex, using surface electrodes. Ipsilateral (Ai) and contralateral ear (Ac) channels were used. The consistent waveforms, produced by relaxed or sleeping participants, were marked as I, II, III, IV, and V and evaluated for wave latencies and interpeak latency (I-V). The importance of BAEP lies in the fact that hearing thresholds can be detected accurately.[17]

Statistical Analysis

The data were analyzed, using SPSS 19.0 for Microsoft Windows. MDA, FRAP, and BAEP waves were compared, using ANOVA with appropriate *post hoc* Test. In smokers with COPD correlation of pack years with MDA, FRAP, and BAEP waves (latencies and interpeak latency) was done, using Pearson's correlation. $P < 0.05$ was considered statistically significant.

RESULTS

Participants having a history of smoking were selected and divided into COPD and non-COPD groups. The mean age of the former group was 53.01 ± 10.56 years while that of the latter group was 49.56 ± 9.45 years (Table 1). Thirty healthy non-smokers were having mean age of 41.67 ± 8.64 years. The spirometric values, namely, FVC, FEV1, FEV1/FVC %, and FEV1 % predicted were significantly lower in COPD group in comparison with other two groups. The estimated values of MDA were significantly higher in smokers with COPD, but the levels of FRAP were lower in the same group in comparison to non-smokers and smokers without COPD. In comparison with non-smokers the levels of FRAP were lower in smokers group (with or without COPD). However, there was no significant difference between smokers with

Table 1: Age and pack years of study participants

Parameters	Non-smokers (*n*=30)	Smokers without COPD (*n*=15)	Smokers with COPD (*n*=25)	*P* value
Age (years)	41.67±8.64	49.56±9.45	53.01±10.56	<0.05
Pack years	-	19.68±3.24	27.69±6.60	<0.05

COPD: Chronic obstructive pulmonary disease

Table 2: PFT, MDA, and FRAP

Parameter	Non smokers	Smokers without COPD	Smokers with COPD
FEV1	3.61±0.28*	3.25±0.41*	2.84±0.32*
FVC	4.52±0.63*	3.87±0.45*	2.88±0.40*
FEV1/FVC	85.40±4.92*+	80.60±4.10*+	66.37±4.37*
FEV1% predicted	80.70±3.74*	79.09±4.16*	63.82±2.70*
MDA	1.03±0.15*	3.10±0.45*	4.59±0.41*
FRAP	2.58±0.50*	1.95±0.48*+	1.55±0.30*+

**P* value is significant in all groups, +*P* value is not statistically significant (FEV1/FVC % not significant between non-smokers and smokers without COPD). FRAP is not significant between smokers without COPD and smokers with COPD, COPD: Chronic obstructive pulmonary disease, MDA: Malondialdehyde, FRAP: Ferric reducing antioxidant power

COPD and smokers without COPD (Table 2). BAEP result analysis showed that in both the right and left ears, there was no significant difference between non-smokers and smokers without COPD in Wave I (*P* = 0.885 and 0.085, respectively) and II (*P* = 0.554 and 0.396, respectively). There was significant difference between non-smokers and smokers with or without COPD in both the right and left ears in waves II, III, IV, V, and IPLI-V (Table 3). Correlation of MDA with BAEP waves and FRAP showed significant negative correlation of MDA with FRAP and significant positive correlation of MDA with wave latencies and IPL (Table 4). The data in Table 5 show non-significant positive correlation between pack years and MDA. Significant negative correlation was found between pack years and FRAP. Significant positive correlation was found between pack years and wave latencies III, IV, V and interpeak latency I-V in both the right and left ears.

DISCUSSION

Lungs by virtue of large surface area, high blood supply, and high exposure to environmental oxidants are susceptible to oxidative stress.[18] COPD is a multietiological disease, but smoking appears to be a major risk factor. The National Health and Nutrition Examination Survey found that COPD was five times more in smokers.[19] In cigarette smoke oxidants and free radicals are present in large quantities, which in turn induce inflammation and alter repair mechanisms.[18] Several Studies have reported oxidant antioxidant imbalance in COPD: Reportedly 90% of patients with COPD are smokers, but all smokers do not develop COPD. This may be attributed to various reasons, namely, genetics, extent, and duration of smoking.[20] In the present study, pack years in smokers with COPD were found to be more than in those without COPD. Similarly, oxidant and antioxidant imbalance were implicated by elevated levels of MDA and lower levels of FRAP, and the lung functions deterioration was found to be more in smokers with COPD. Pack years correlated positively with MDA and negatively with FRAP, indicating that smoking-induced oxidant and antioxidant imbalance alters lung function. This increases susceptibility to COPD.

In this study, oxidative stress was more in smokers with or without COPD, and latencies of BAEP waves and interpeak latency I-V are increased, as compared to non-smokers. Various Studies have shown that mean interpeak latency of wave I-V is prolonged in males and in comparison to non-smokers, the mean latency of wave V is more in smokers.[21] The present study is also in contrast to the study done by Martins et al., who found no statistically significant difference in interlatencies I-V in smokers and non-smokers.[22]

In the present study, correlation of pack years with BAEP waves and interpeak latency was found to be significant. Correlation between BAEP waves and smoking has been found in a Study done by Shalabi et al.[23] The study done by Shabina et al. found prolonged latencies of BAEP waves between smokers and non-smokers.[24] Kumar and Tandon reported prolonged latencies of Waves I and III in tobacco smokers, as compared to non-smokers.[25] Noxious stimuli lead to excess production of free radicals in the cochlea, which, in turn, affects hearing.[26] In fact, cochlea is highly sensitive to nicotine, which disrupts auditory function pathway by interfering with cholinergic transmission and processing of auditory impulses.[24] In this present study, positive correlation of MDA with BAEP wave latencies and negative correlation of MDA with FRAP were found, indicating that oxidant antioxidant imbalance affects the wave latencies and alter the neural processing of auditory information. The latencies were increased in smokers group, as compared to non-smokers. However, there was no significant difference between latencies of Waves I and II between non-smokers and smokers without COPD. The results can be attributed to the extent and duration of smoking along with other environmental exposure to smoke such as biomass fuel or passive smoking. Further, in the present study, it was found that smokers, who had COPD showed significant difference in BAEP waves compared to non-smokers and smokers without COPD. Hafez et al. have reported prolonged latencies in both the right and left ears of COPD patients.[27] BAEP abnormalities in COPD patients

Table 3: BERA waves latencies and interpeak latency in study participants

Parameters	Non-smokers (*n*=30)		Smokers without COPD (*n*=15)		Smokers with COPD (*n*=25)	
	Right ear	Left ear	Right ear	Left ear	Right ear	Left ear
Latencies (msec)						
I	1.54±0.054	1.54±0.07	1.57±0.06	1.55±0.09	1.71±0.09	1.72±0.09
II	2.68±0.09	2.66±0.10	2.74±0.08	2.75±0.09	2.86±0.08	2.89±0.10
III	3.59±0.07	3.59±0.13	3.77±0.09	3.77±0.12	3.86±0.06	3.88±0.06
IV	4.67±0.41	4.68±0.08	4.82±0.06	4.81±0.07	4.99±0.06	4.99±0.09
V	5.62±0.07	5.63±0.08	5.76±0.06	5.77±0.07	5.91±0.08	5.95±0.07
Interpeak latencies (msec)						
I-V	3.06±0.17	3.03±0.05	3.21±0.18	3.22±0.17	3.87±0.26	3.90±0.24

Right ear: No significant difference between non-smokers and smokers without COPD in Wave I and Wave II (*P*=0.885 and 0.554)). Left ear: No significant difference between non-smokers and smokers without COPD in Wave I and Wave II (*P*=0.085 and 0.396). Significant difference between Waves III, IV, and V and IPL I-V in both the right and left ears, COPD: Chronic obstructive pulmonary disease

Table 4: Correlation of MDA with BAEP waves and FRAP

Values	Wave I#	Wave II#	Wave III#	Wave IV#	Wave V#	IPL I-V#	FRAP*
r	0.399	0.315	0.396	0.553	0.517	0.339	−0.682
p	0.001	0.008	<0.001	<0.001	<0.001	0.004	<0.001

*Significant negative correlation of MDA with FRAP, #significant positive correlation of MDA with Wave latencies and IPL, MDA: Malondialdehyde, FRAP: Ferric reducing antioxidant power

Table 5: Correlation of pack years with MDA, FRAP, and BAEP waves in right and left ears

Parameter	Right ear		Left ear	
	r	*P*	*r*	*P*
Wave III*	0.512	0.01	0.298	0.04
Wave IV*	0.398	0.001	0.485	0.001
Wave V*	0.398	0.01	397	0.01
IPL I-V*	0.398	0.01	373	0.01
FRAP##	−0.563	<0.001	−0.211	<0.001
MDA#	0.211	0.19	0.563	0.19

#Non-significant positive correlation, ##significant negative correlation, *significant positive correlations, MDA: Malondialdehyde, FRAP: Ferric reducing antioxidant power

could be attributed to hypoxia. Although the extent of hypoxemia is not known in the present study, careful analysis of pulmonary functions shows that smokers had moderate COPD as per the GOLD criterion.[18] Thus, combination of hypoxia due to smoking and hypoxia expected to be associated with COPD could possibly explain the BAEP abnormalities. El-Kady et al. showed significant changes in audiological parameters between controls and presumptive hypoxic COPD subgroups.[28] Apart from hypoxia, systemic effects of COPD could also possibly lead to neuropathy.[29] The BAEP changes may also be attributed to drugs, used in the treatment of COPD.[30] Interestingly, smoking and inflammation both are implicated in the pathophysiology of COPD. Levels of TNF-α are associated with both smoking and hypoxia.[31,32] Systemic inflammation results in hearing defects in COPD.[22]

The present study found that the age group smokers with or without COPD was higher than non-smokers. Oxidant/antioxidant imbalance and BAEP abnormalities were more in smokers with COPD. Latencies of BAEP waves tend to increase in older adults.[33] Reportedly, hearing loss increases with age[34] and smoking influences age-related hearing loss.[11] The role of oxidative stress, ageing, hypoxia, associated with COPD and effects of oxidative stress on auditory functions are a matter of further research and debate.

In the present study, only male participants were included. Data from previous works indicate that changes in BAEP waves are attributed to differences in brain size of male and female both.[35,36] Thus, a comparative study of males and females will further improve the results and would yield more evidence.

Limitations and Improvements

Assessment of hypoxia and subdivision of COPD patients on the basis of smoking severity and hypoxemia will yield better results. Measurement of inflammatory cytokines in COPD patients with BAEP abnormalities will also yield better results.

CONCLUSION

It is concluded that smoking-induced oxidative stress as well as increased susceptibility to COPD, in turn lead to hypoxia effects on the auditory mechanisms and are responsible for BAEP abnormalities.

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