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Original Article

Vascular Responses to Post Occlusive Reactive Hyperemia and Systemic Inflammation in Overlap Syndrome of Chronic Obstructive Pulmonary Disease and Obstructive Sleep Apnea

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Abstract

Background : Post-occlusive reactive hyperemia (RH) is impaired in Chronic Obstructive Pulmonary Disease (COPD) and Obstructive Sleep Apnea (OSA). The aim of the present study was to examine systemic vascular response and endothelial function in patients of Overlap Syndrome (OS) of COPD and OSA and also to investigate whether OS has any additional effect on endothelial dysfunction when compared to dysfunction caused by COPD alone.

Methods : 31 COPD patients and 13 healthy controls participated in the study. Overnight Polysomnography was done to classify the patients into COPD only group (Apnea-Hypopnea Index <5) (n=15) and OS group (AHI >5) (n=16). Peripheral pulse waveform changes during reactive hyperemia were assessed using digital Photoplethysmography (PPG) technique in which pulse wave amplitude (PWA), Maximum slope of upstroke and Pulse Transit Time (PTT) were measured. C - reactive protein was assessed as marker of inflammation by ELISA.

Results : Maximum percentage changes in PWA during RH were significantly lower in the both COPD group [20.34(12.02-34.07)] ($p<0.001$) and Overlap Syndrome group [10.96(6.21-21.49)] ($p<0.0001$) as compared to Controls [49.79(46.03-65.32)], whereas amplitude responses were not significantly different in the COPD and OS group ($p>0.05$). Maximum percentage change in slope of upstroke showed similar responses in the three groups. CRP levels (mg/l) were raised in COPD [11.60(1.75-15.00] ($p<0.001$) and OS group [12.52(5.28-15.70))]($p<0.0001$) as compared to controls [0.59(0.58-0.91)]. Maximum percentage change in amplitude negatively correlated with serum CRP levels in COPD group ($r=-0.557$, $p=0.03$) and in OS group ($r=-0.552$, $p= 0.02$). FEV1% predicted positively correlated with maximum percentage change in amplitude in OS group

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(r=0.579, p=0.018). No correlation of AHI was found with any of the vascular function parameter in Overlap group.

Conclusion : The patients with Overlap Syndrome have systemic inflammation and impaired reactive hyperaemia response. However, no additive effect of OSA was observed on impaired RH in patients with co-existing COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) and Obstructive sleep apnoea (OSA) represent two of the most prevalent chronic respiratory disorders in clinical practice. Flenley coined the term “overlap syndrome” (OS) to describe, when both these disorders coexist in a patient (1).

It has been believed that, the presence of COPD could predispose to the development of OSA, since the two conditions share same etiologic factors, such as tobacco smoking. The prevalence of OSA associated with COPD has been found to vary between 14- 29% in COPD patients in various studies (2, 3).

COPD and OSA are independent risk factors for cardiovascular events and their co-existence in Overlap Syndrome probably increases this risk. Assessment of endothelial function has been used to determine risk for cardiovascular disease in its pre-atherogenic state (4). A number of recent studies have focused on using systemic vascular function indices as surrogate markers to measure increased cardiovascular risk in patients with stable COPD, using measurements of endothelial function and or arterial wall stiffness (5, 6). A number of studies also suggest that impaired endothelial function accompanies OSA (7, 8).

Patients with OS have a more prominent sleep-related oxygen desaturation than COPD patients with the same degree of bronchial obstruction and show an increased risk of developing hypercapnic respiratory failure and pulmonary hypertension when compared with patients affected by only one of the two diseases (9, 10).

The aim of the present study was to examine systemic vascular response and endothelial function in patients of Overlap syndrome. The other aim of the study was to investigate whether patients with OS have greater endothelial dysfunction when compared to dysfunction caused by COPD alone.

Another reason for evaluating COPD patients with OSA is that systemic inflammation develops in each disorder which may play an important role in underlying pathogenesis of cardiovascular diseases, like hypertension and atherosclerosis (11–13).

While systemic inflammation & endothelial dysfunction develop both in COPD & OSA (11), no study has earlier investigated the endothelial dysfunction in OS. Thus, noninvasive assessment of endothelial function in patients of overlap syndrome and its relation with inflammatory marker hs-CRP (high sensitivity C Reactive Protein) was investigated in the present study. Also, it was aimed to observe if there is any association between systemic inflammation and endothelial function in patients of OS.

Materials and Method

Selection of participants

Thirty-one diagnosed ex-smoker male stable patients of COPD were recruited in the study with mean smoking history of 16±6.67 pack years. These patients were classified into COPD (n=15) and Overlap syndrome (COPD and OSA, n=16). 13 age and gender matched non smoking healthy controls were also recruited. Patients diagnosed to have COPD, as defined by the GOLD guidelines were recruited from Medicine OPD after an informed written consent (14).

Stable COPD patients of 40-65 years, smoking history of at least 10 pack years or more, evidence of airflow limitation on spirometry ($FEV_1/FVC < 0.7$) were included. Patients receiving long-term oxygen therapy or oral corticosteroids, hypertension, heart disease, diabetes mellitus, chronic renal diseases, collagen vascular diseases, pulmonary hypertension, history of COPD exacerbation in last four weeks were excluded. The study protocol was approved by institutional ethics committee.

Study Protocol:

Spirometry was performed using a dry rolling spirometer (SPIROAIR, MEDISOFT, PK Morgan, Kent, UK) according to European Respiratory Society guidelines (15). The spirometer was calibrated before use to ensure that it records accurate values. The temperature and barometric pressure were entered into the spirometer, as variation in these measures does affect the final results. The patient were asked to sit straight, with head erect, nose clip in place, and holding the mouthpiece tightly between the lips. Initially, he was asked to breathe in and out normally to record the tidal flow–volume loop. He was then instructed to inhale maximally to TLC, and exhale as fast, as hard as and as completely as possible for six seconds. FEV_1 was calculated as the volume of air in litres that can be forcefully and maximally exhaled in the 1st second after a maximal inspiration. The test was repeated thrice and the best of the three results was reported. FEV_1 and FVC were reported as percentage predicted values.

Polysomnographic recording was carried out on all the participants. All the signals were acquired and amplified using computer based digital data acquisition system ALICE LE™ (Philips Respironics, Amsterdam, Netherlands).

An obstructive apnea was defined as complete cessation of respiratory airflow for at least 10 seconds accompanied by paradoxical respiratory thoracic and abdominal movements. A hypopnea was defined as decrease in amplitude of breathing for at least 10 seconds by 50% of the amplitude of preceding breaths or the drop in oxygen saturation by 3 percentage points. Differentiation between NREM and REM stages of sleep and analysis of breathing disorders during these stages was undertaken by pulmonary physician. Frequencies of apnea and hypopneas were calculated during sleep and expressed as Apnea Hypopnea Index (AHI). Fifteen COPD patients had AHI less than 5 and were included in COPD only group. Sixteen patients had AHI more than 5 and were included in Overlap Syndrome group. Control subjects had AHI less than 5 (16). Neck Circumference was measured in the midway of the neck, between mid-cervical spine and mid anterior neck, to within 1 mm, using non-stretchable plastic tape with the subjects standing upright. In men with a laryngeal prominence (Adam's apple), it was measured just below the prominence. While taking this reading, the subject was asked to look straight ahead, with shoulders down, but not hunched (17).

Systemic vascular response was assessed in patients as well as controls by recording pulse wave form and pulse transit time responses during reactive hyperemia (RH) using simultaneously acquired lead II ECG and finger Photoplethysmography (PPG) signals (18).

The PPG probe (comprised of an infrared reflection type photo electric sensor MLT1020PPG Plethysmograph, AD Instruments, Australia) records beat to beat changes in blood volume in the finger microvasculature. Both ECG and PPG signals were acquired using digital data acquisition system Powerlab 8/30, (AD Instruments, Australia).

Patients and controls were asked to fast overnight and report in the vascular function laboratory on the morning of the recording. Blood samples were collected for measuring CRP values after overnight fasting. After 15 minutes of supine rest, baseline blood pressure was recorded. Sphygmomanometer cuff was kept fastened to the right arm for producing arterial occlusion after baseline recording. Disposable Ag-AgCl electrodes were applied for recording standard bipolar limb lead II ECG. PPG probe was fixed to the middle finger of right hand using the Velcro strap without creating undue pressure. After appropriate placement of electrodes & PPG probe, signal acquisition was done using data acquisition

and analysis software Lab Chart7 (AD instruments, Australia). The entire recording period included 5 minutes of baseline recording, 5 minutes of arterial occlusion and 5 minutes of post occlusive reactive hyperemia. After acquiring baseline ECG and PPG signals, arterial occlusion was produced by raising the cuff pressure 50 mmHg above the baseline systolic blood pressure (18, 19).

The maintenance of arterial occlusion was verified real time by the absence of PPG signal from the monitor. Arterial occlusion was released after 5 minutes by deflating cuff pressure completely and recording was continued during the period of reactive hyperemia. All signals recorded were analyzed offline for the extraction and calculation of the following parameters with appropriate peak detection algorithms for ECG and PPG signals: 1) Amplitude of PPG pulse waveform: The magnitude of difference between maximum signal voltage and baseline signal voltage for each PPG pulse waveform; 2) Maximum slope of upstroke: Maximum positive value of the first derivative of PPG signal for each PPG pulse waveform; 3) Pulse Transit Time (PTT): Time interval between each R wave peak and foot of the corresponding PPG pulse waveform (Fig. 1).

Identification of R wave peaks of ECG and peaks and feet of PPG pulse waveform signal was done using the peak detection module of Lab chart software. Beat to beat values were extracted for the baseline and the reactive hyperemia data for all the above mentioned parameters. Mean values of each parameter were computed for the entire baseline recording and for every one minute period of RH (19).

The time course of responses during RH within each group in comparison to their respective baseline means was analyzed. To compare the responses between groups, normalization with respect to respective baseline means was done for the RH data of each subject. As PWA, slope and PTT values at baseline vary between individual, and the data after occlusion at different time points is relative to the baseline value before occlusion. Thus, in this study for inter-group comparison of the data at different time point, post occlusion data in an individual is considered in comparison to baseline value for that individual which is considered as 1 (One). To assess the magnitude of peak response, maximum percentage change in amplitude and slope response during RH as compared to baseline value was calculated and compared between groups. Venous blood sample (5 ml) was obtained from the subjects under aseptic precautions on the day of vascular function assessment. Serum was separated and stored at –80° centigrade. The hs-CRP was estimated

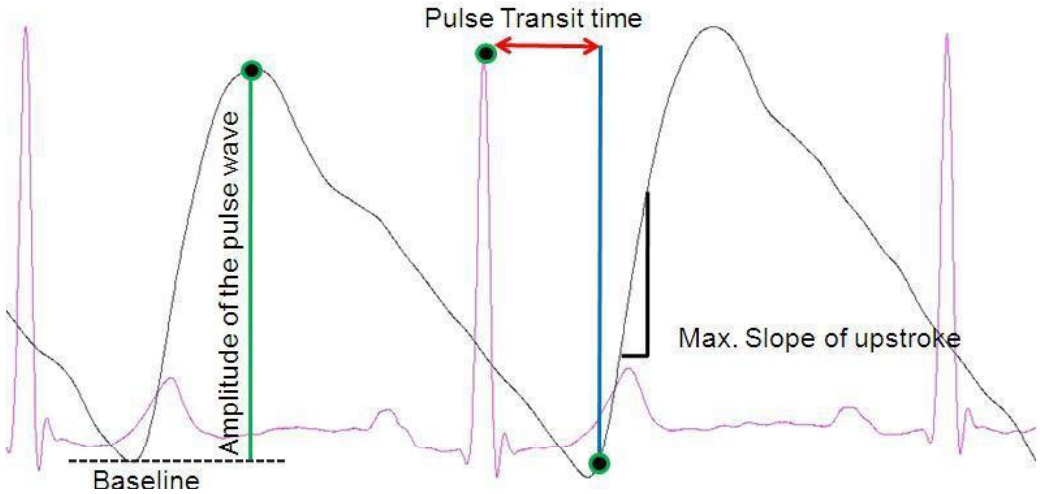

Fig. 1 : Figure shows overlapped and magnified image of simultaneously acquired lead II ECG and PPG signals labeled with various function parameters.

by ELISA using 96 well ELISA kit by Biocheck, inc. The Benchmark Plus™ Bio-Rad microplate reader was used for the measurement of absorbance of samples at 450 nm.

Statistical Analyses

Each parameter was tested for normality in the distribution of data using standard normality tests (D' Agostino-Pearson omnibus normality test and Shapiro-Wilk test), and subsequently appropriate parametric or non-parametric tests were applied. The absolute values of amplitude, slope and PTT of Control, COPD and Overlap patients has been documented as mean±SD. The CRP, normalized values of amplitude, slope and PTT, maximum percentage change in Pulse wave amplitude and slope in Control, COPD and Overlap patients has been documented as median with inter-quartile range.

To assess the significant trends in Pulse wave Amplitude and Pulse wave Slope during Reactive Hyperemia, repeated measures ANOVA was used whereas for Pulse Transit time, Friedman test with appropriate post hoc tests were applied within each group. Intergroup comparisons of all the parameters were performed using Kruskal Wallis test. The relationship between two parameters was evaluated using Spearman's rank correlation coefficient. The level of statistical significance was set at $p<0.05$. All statistical analyses were done using Graph Pad prism version 5.00 for Windows (Graph Pad Software, Inc., USA).

Results

Table I shows the demographic and clinical characteristics of the patients and controls.

Photoplethysmographic pulse wave amplitude and slope responses during reactive hyperemia were studied to assess the vascular reactivity and endothelial function in the three groups. For intragroup comparison, in the control group, it was observed that, averaged absolute pulse wave amplitudes during the 1st and 2nd minute post release of occlusion [(0.09±0.03) volts and (0.09±0.03) volts respectively] were significantly higher than the baseline values [(0.07±0.03) volts; $p<0.0001$]. In the COPD as well as Overlap Syndrome groups, there was no significant change in the averaged pulse wave amplitude values during any of the observed time periods following release of occlusion, in comparison to the baseline values (Table II).

For intergroup comparison, averaged pulse wave amplitudes at specific time periods during reactive hyperemia were first normalized to their respective baseline values for each participant in all the groups. Comparing between groups it was observed that pulse wave amplitude response was significantly lower in COPD group as compared to controls during the 1st [1.03(0.96-1.11)] vs [1.34(1.14-1.47)] ($p<0.01$) and 2nd [1.07(1.00-1.30)] vs [1.42(1.14-1.61)] ($p<0.01$) minutes of reactive hyperemia respectively (Fig. 2). It was also seen that amplitude response was significantly lower in Overlap group as compared to

TABLE I: Demographic and clinical characteristics of Controls, COPD and Overlap syndrome patients.

Parameters	*Controls (n=13)*	*COPD (n=15)*	*Overlap (n=16)*	*p value*
Age	55.85±2.19	56.86±4.99	57.75±2.67	N.S.
BMI	23.22±2.10	22.95±4.90	22.99±4.05	N.S.
FEV_1%	84.46±2.63 (FEV1/FVC>0.7)	41.33±15.39 (FEV1/FVC<0.7)	57.06±14.15 (FEV1/FVC<0.7)	$p<0.0001^{a,b}$ $p<0.01^{c}$
AHI*	2.4(2-4.5)	2.7(1.9-4)	16(12-26.5)	<0.001
Neck Circumference	14.61±0.76	14.47±2.47	15.06±1.76	N.S.

*Abbreviations: AHI-Apnea- Hypopnea Index. Values expressed as median with inter-quartile range
p^a-p value for comparison between controls and COPD
p^b-p value for comparison between controls and Overlap syndrome
p^c-p value for comparison between COPD and Overlap syndrome

TABLE II : Absolute values of Pulse wave amplitude(PWA) (volts), Pulse wave slope (PWS) (volts/sec) and Pulse transit time (PTT) (ms) in Control (n=13), COPD (n=15) and Overlap (n=16) patients.

	Groups	*Baseline*	*1st Minute*	*2nd Minute*	*3rd Minute*	*4th Minute*	*5th Minute*
PWA	Controls	0.07±0.03	0.09±0.03***	0.09±0.03***	0.08±0.03	0.07±0.03	0.06±0.02
	COPD	0.06±0.02	0.06±0.02	0.07±0.03	0.06±0.03	0.06±0.03	0.05±0.03
	Overlap	0.07±0.03	0.07±0.03	0.07±0.03	0.07±0.03	0.06±0.03	0.06±0.03
PWS	Controls	1.07±0.40	1.12±0.29	1.22±0.34*	1.07±0.37	1.01±0.31	0.93±0.30*
	COPD	0.91±0.31	0.88±0.30	0.97±0.36	0.89±0.33	0.88±0.34	0.85±0.35
	Overlap	0.98±0.35	0.92±0.33	1.00±0.35	1.00±0.37	0.96±0.37	0.95±0.39
PTT	Controls	200.39±14.61	206.96±14.89***	204.26±14.78***	202.50±14.51	201.50±13.68	201.13±14.26
	COPD	184.35±19.83	194.61± 22.57***	190.10±21.66	187.93±20.54	188.68±18.13	187.99±19.38
	Overlap	184.78±20.99	192.24±21.10***	186.83±19.75	185.22±19.75	185.43±20.91	183.65±20.24

Values are expressed as Mean±SD. * represents p value <0.01. *** represents p value <0.0001.

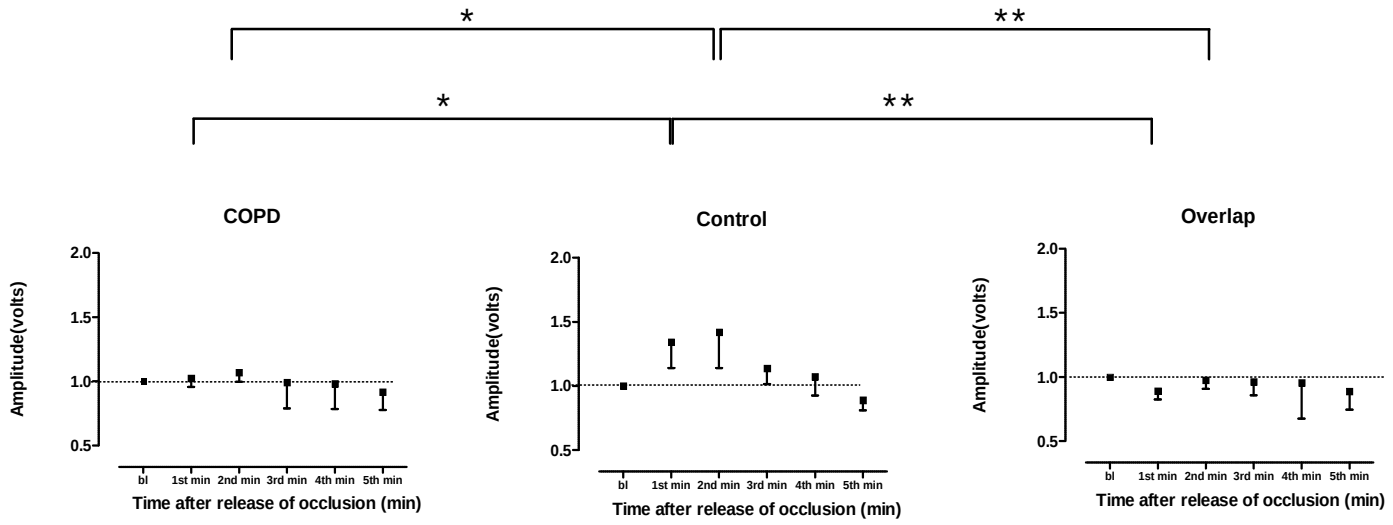

Fig. 2 : Graphical representation of normalized pulse wave amplitudes (y-axis) during baseline and different time periods during reactive hyperemia (x-axis) in controls, COPD and Overlap patients. Abbreviations: bl-baseline. Values are plotted as median with inter-quartile range. **represents p<0.001 and *represents p<0.01 for intergroup comparison. Dotted line represents baseline value taken as 1.0.

controls during the 1st [0.89(0.82-1.06)] vs [1.34(1.14-1.47)] (p <0.001) and 2nd minutes [0.98(0.91-1.12)] vs [1.42(1.14-1.61)] (p<0.0001) of reactive hyperemia, whereas there was no significant difference in amplitude responses during any time period of reactive hyperemia between COPD and Overlap group (p>0.05).

Similar findings were also observed in the pulse wave slope during RH. Following the release of arterial occlusion in the control group, averaged pulse wave slope during the 2nd minute [1.22±0.34 volts/s] was significantly higher than the baseline value [1.07±0.40 volts/s] (p<0.01) whereas averaged pulse wave slope during the 5th minute [0.93±0.30 volts/s] was significantly lower than the baseline value [1.07±0.40 volts/s] (p<0.01). Whereas, no significant change in the averaged pulse wave slope during any of the observed time periods following release of occlusion, in comparison to the baseline values were seen in COPD as well as Overlap Syndrome group (Table II). On intergroup comparison after normalization, (Fig. 3) it was observed that pulse wave slope during the 2nd minute [0.98(0.92-1.07) vs 1.16(1.06-1.28), p<0.01] of reactive hyperemia was significantly lower in overlap group as compared to controls, whereas there

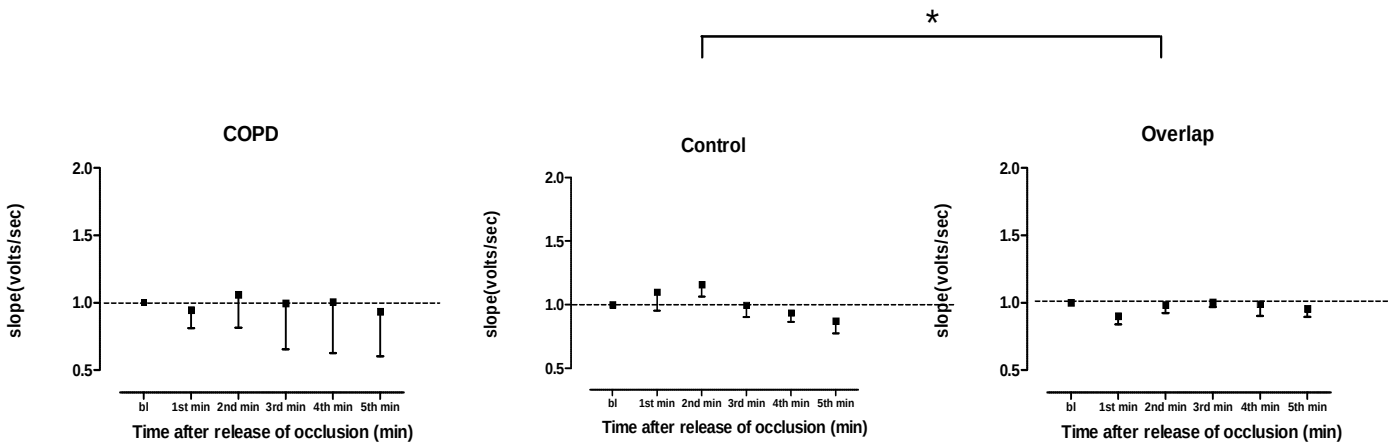

Fig. 3 : Graphical representation of normalized pulse wave slopes (y-axis) during baseline and different time periods during reactive hyperemia (x-axis) in controls, COPD and Overlap patients. Abbreviations: bl-baseline. Values are plotted as median with inter-quartile range. *represents p<0.01 for intergroup comparison. Dotted line represents baseline value taken as 1.0.

was no significant difference in slope during any time period of reactive hyperemia between COPD and Overlap group.

Maximal percentage changes in both pulse wave amplitude and slope were significantly lower in COPD as well as Overlap Syndrome as compared to Controls, whereas no significant difference was seen in the amplitude responses between the COPD and OS group (Table III).

It was observed that averaged pulse transit time was significantly higher than the baseline values during the first and second minutes of reactive hyperemia in controls and during the first minute in COPD and Overlap group. The pulse transit time returned to baseline at the end of fifth minutes in all the three groups (Table II). Fig. 4 shows the Normalized data of pulse transit time in Control, COPD and Overlap patients.

C Reactive Protein levels were measured in COPD group, Overlap group as well as in healthy subjects for comparison. C Reactive Protein levels (mg/L) were significantly increased in COPD [11.60(1.75-15.00] (p<0.001) and OS group [12.52(5.28-15.70))] (p<0.0001) as compared to controls [0.59(0.58-0.91)] (p<0.001), whereas there was no difference in CRP levels between the COPD and Overlap group.

The research team looked into univariate correlation of all the vascular function parameters with inflammatory markers, which is documented in Table IV.

We attempted Multivariate co-relation of all vascular function parameters with inflammatory markers though sample size is small. The co-relation with CRP was found to be significant with Max % change in Amplitude (r=-1.187, p=0.010) and Max % change in slope in COPD group, whereas it is significant

TABLE III : Maximum percentage change in Pulse wave amplitude (PWA) (volts) and Pulse wave slope (PWS) during reactive hyperemia in controls and COPD and Overlap patients

Parameter	*Controls (n=13)*	*COPD (n=15)*	*Overlap (n=16)*
Maximum %age change in PWA	49.79(46.03-65.31)	20.34(11.78-23.95)***[a]	10.96(6.21-21.50)***[b]
Maximum %age change in PWS	40.09(28.69-48.68)	26.69(11.48-35.38)***[a]	9.66(5.04-16.93)***[b]

Values are expressed as median with inter-quartile range (1st quartile- 3rd quartile). ***[a] represents p value <0.001 between Controls and COPD. ***[b] represents p value <0.0001 between Controls and Overlap patients.

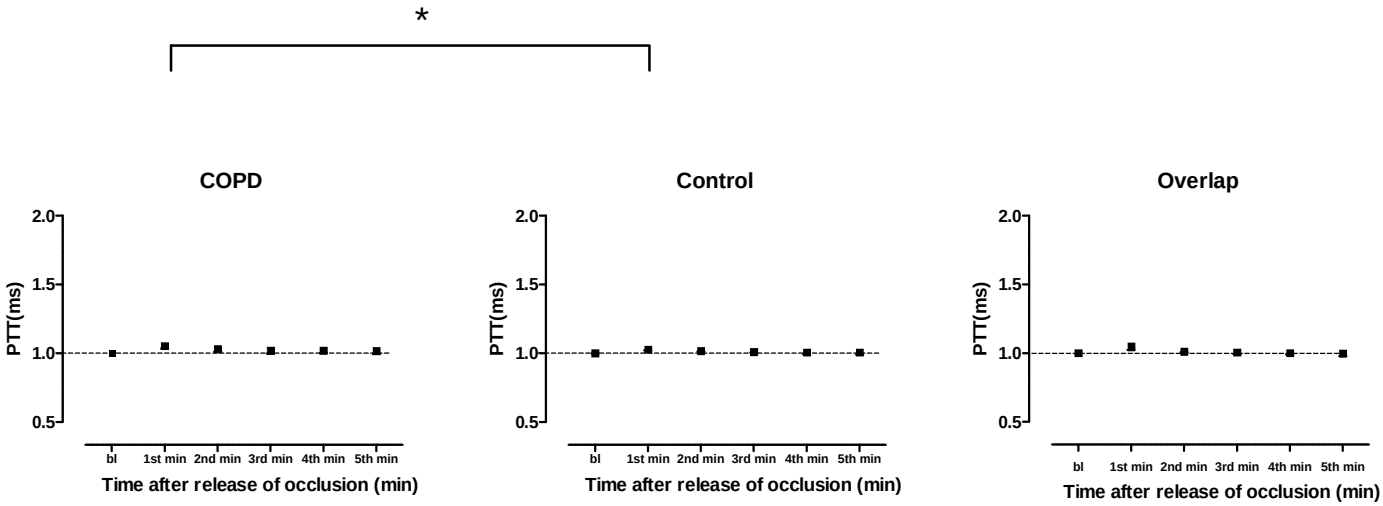

Fig. 4 : Graphical representation of normalized pulse transit time (y-axis) during baseline and different time periods during reactive hyperemia (x-axis) in controls, COPD and Overlap patients. Abbreviation: bl-baseline. Values are plotted as median with inter-quartile range. *represents p<0.01 for intergroup comparison. Dotted line represents baseline value taken as 1.0.

TABLE IV : Correlation of various parameters with maximum percentage change in amplitude and slope in COPD and Overlap syndrome patients.

Parameters	*COPD*		*Overlap*	
	Max. % change in Amplitude	*Max. % change in Slope*	*Max. % change in Amplitude*	*Max. % change in Slope*
Age (years)	r = 0.338 p = 0.216	r = 0.326 p = 0.235	r = 0.125 p = 0.642	r = -0.127 p = 0.638
BMI (kg/m^2)	r = -0.432 p = 0.107	r = -0.321 p = 0.243	r = -0.186 p = 0.488	r = 0.117 p = 0.664
FEV_1 (% predicted)	r = 0.198 p = 0.479	r = 0.264 p = 0.341	r = 0.579 p = 0.018	r = 0.272 p = 0.306
AHI	r = -0.091 p = 0.745	r = -0.080 p = 0.774	r = -0.220 p = 0.411	r = -0.128 p = 0.636
Neck Circumference (inches)	r = -0.407 p = 0.131	r = -0.310 p = 0.260	r = -0.168 p = 0.533	r = 0.194 p = 0.471
hs CRP (mg/l)	r = -0.557 p = 0.030	r = -0.600 p = 0.017	r = -0.552 p = 0.026	r = -0.520 p = 0.038

with only Max % change in Amplitude (r=-1.703, p=0.013) in Overlap group. It was not found significant with Max % change in Slope (r=-2.055, p=0.020) in this group.

Discussion

The present study was conducted in patients of COPD, patients of Overlap Syndrome and healthy controls to noninvasively assess the vascular response and endothelial function by digital Photoplethysmography technique. The present study demonstrates that the post occlusive reactive hyperemia is blunted in COPD as well as Overlap Syndrome patients.

We have used reactive hyperemia as a physiological model to assess the reactivity of blood vessels to the temporary arrest of circulation. The vascular tone responses during reactive hyperemia are better appreciated in terms of changes taking place in the proximal conduit vessels and distal resistance vessels supplying the tissue (20, 21).

The transient arterial occlusion leads to vasodilatation of resistance vessels and decrease in tone of both resistance and conduit due to myogenic and metabolic mechanisms (18). Restoration of perfusion pressure at the release of arterial occlusion leads to transient augmentation of blood flow to the ischaemic tissues and a flow mediated vasodilatory responses in the proximal conduit vessels (19).

The maximum percentage change in pulse wave amplitude and slope during reactive hyperemia have been shown to coincide with peak responses of flow mediated dilatation of brachial artery done by B mode ultrasound technique (19, 22). Also, the rise in peripheral digital pulse waveform amplitude during reactive hyperemia has been shown to be dependent on endothelial nitric oxide synthesis and thus endothelial function (23).

In the present study, on analyzing the time course of pulse wave amplitude and slope in healthy subjects, it was observed that both these parameters remained significantly higher than the baseline during the first and second minutes of reactive hyperemia indicating the normal flow mediated dilatation of proximal conduit vessels. These findings were in close agreement with the results obtained by Selvaraj et al in healthy subjects (18). It was also observed that there was no significant change in pulse wave amplitude and slope during first and second minutes of reactive hyperemia from the baseline mean values in COPD only group and Overlap Syndrome group. Using the same technique, Chandran et al also reported no significant change in amplitude and slope during reactive hyperemia from the baseline values in patients of Endogenous Hypercortisolism (19). On intergroup comparison of normalized data, it was observed that both the COPD and Overlap patients, had a significantly lower pulse wave amplitude and slope during the first and second minutes of reactive hyperemia. Also, the peak responses in pulse wave amplitude and slope measured as maximum percentage change from the baseline value during reactive hyperemia were lower in both the patient groups as compared to controls. Whereas, there was no significant difference in the pulse wave amplitude, slope during reactive hyperemia and maximum percentage change in these parameters between COPD and Overlap Syndrome patients. These findings clearly indicated that there is a significant impairment of flow mediated dilatation of conduit arteries in COPD patients as well as Overlap Syndrome patients.

Various studies have found similar results in impairment in flow mediated dilatation of brachial artery in COPD patients assessed by ultrasound technique (5, 6, 24). Vascular tone responses during reactive hyperemia were assessed in both COPD patients as well as Overlap Syndrome patients, by the time course of change of pulse transit time. Vascular tone changes in conduit and resistance vessels during reactive hyperemia are mainly due to myogenic vaso relaxation taking place during the period of arterial occlusion which gets reflected as a significant prolongation in the pulse transit time at the time of release of vessel occlusion (18).

On analyzing the time course of change in pulse transit time, it was observed that in control group, there was a significant rise in the pulse transit time values above the baseline values during the first and second minutes of reactive hyperemia which recovered to baseline values by the fifth minute. Similarly in patients group, it was observed that the pulse transit time values showed a significant rise above the baseline values during the first minute of reactive hyperemia and recovered to baseline values by the fifth minute. In patients of Overlap Syndrome mean arterial saturation was lower and time spent in desaturation was longer as compared to patient with OSA only (25, 26). Continuous Positive airway pressure (CPAP) therapy is treatment of choice of OSA in patients of OS. It not only decreases hospitalizations, but also improves the circulating levels of nitric oxide, which has vasodilator action and is found to be decreased in untreated patients of OS (27, 28).

In the present study though the systemic vascular response to reactive hyperemia was blunted in both COPD and OS groups, the presence of OSA does not result in greater impairment of vascular response in OS group.

The role of systemic inflammation has been well

established in the pathogenesis of endothelial dysfunction and atherosclerosis. There is growing epidemiologic evidence linking systemic inflammation to atherosclerosis, ischemic heart disease, stroke, and coronary death (29, 30).

In this study, the CRP levels are increased in COPD as well as Overlap Syndrome patients and is similar and comparable to other studies which have shown increased CRP levels in COPD patients (31, 32). In the present study, no significant difference was observed in CRP levels between COPD patients and Overlap Syndrome patients.

Systemic inflammation may have a bearing on systemic vascular function in COPD and Overlap Syndrome. A persistent low grade inflammatory response is present in COPD and this may explain the poor endothelial function in these patients (33, 34). This could be due to increased levels of pro-inflammatory cytokines, hypoxia and oxidative stress in COPD. Increased levels of CRP have been reported to down-regulate endothelial nitric oxide synthase in endothelial cells that had been incubated with C-reactive protein, resulting in a significant reduction in basal and stimulated nitric oxide release (35). This reduction, in turn, is associated with impaired endothelium-dependent vasodilation, one of the earliest detectable vascular changes before atherosclerotic plaque development (36).

Thus, we have also looked for the correlation between pulse waveform parameters and CRP levels in both the patient groups. On univariate correlation analysis, maximum percentage change in amplitude and slope during reactive hyperemia had a significant correlation with CRP levels in COPD patients as well as Overlap Syndrome patients.

Shiina et al in their study reported that arterial stiffness was related to exaggeration of nocturnal hypoxia and inflammation (CRP levels) in OSA patients as well as in Overlap syndrome patients. However, CRP levels between the two groups were comparable (34).

Many studies in the past have reported a link between FEV_1 and cardiovascular mortality in COPD patients (12, 37). In the present study, there was significant positive correlation between the maximum percentage change in amplitude during reactive hyperemia and FEV_1% predicted in overlap group. Few studies have found the significant correlation of FEV_1% predicted and maximum percentage change in flow mediated dilatation of brachial artery in COPD patients (5, 6). It is a well known fact that Obstructive Sleep Apnea leads to endothelial dysfunction, atherosclerosis and subsequently to cardiovascular diseases like coronary artery disease and heart failure (38, 39). Apnea Hypopnea Index is used not only to define OSA syndrome, but also serves to stratify disease severity (16). Many recent studies have proved helpful in OSA severity stratication by AHI as a marker for cardiovascular morbidity and mortality (28, 40, 41). In the present study, AHI did not correlate significantly with any of the vascular function parameters.

Neck Circumference measurement is another parameter used to assess severity of OSA (42, 43).

In the present study, we did not find any significant difference in neck circumference between COPD and Overlap Syndrome patients. Also, no significant correlation was observed between neck circumference and any of the vascular function parameter in either of the patient group.

Limitations of the study

One major limitation is the sample size. A larger sample size with an additional group with OSA alone would have been more meaningful. Also, the photoplethysmography as a technique to assess post occlusive reactive hyperemia is not the gold standard though it has been validated to assess it. Thus, assessment of vascular responses by flow mediated dilatation, a gold standard to assess reactive hyperemia could have been done.

Conclusion

In the present study, there was impairment of post-occlusive reactive hyperemia and endothelial function in both the COPD as well as Overlap Syndrome patients. But, no significant difference was observed

in any of the vascular function parameters between the two groups. The reason may be due to the significant difference in FEV_1% predicted between the two groups. The COPD patients had lower FEV_1% predicted as compared to Overlap syndrome patients. Even after FEV_1% matching between the two patient groups and re-analysis, there was no significant difference in vascular function parameters between the COPD as well as Overlap patients. It could also be due to the fact that in present study, the Overlap Syndrome patients had mainly mild and moderate OSA. Only three patients had severe OSA in Overlap Syndrome group. Therefore In the present study, the additive effect of OSA on endothelial function assessment was not observed as compared to dysfunction caused by COPD alone. Thus, future studies should aim at including patients with more severe OSA and a higher sample size.

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