How to Cite:
Raju, D. S. S. K., Munta, A. K., & Lalitha, D. L. (2021). A study on the serum arginase and nitrate levels in association with Fev1/Fvc ratio in chronic obstructive pulmonary disease. *International Journal of Health Sciences*, *6*(S9), 4803–4811. https://doi.org/10.53730/ijhs.v6nS9.14067

A study on the serum arginase and nitrate levels in association with Fev1/Fvc ratio in chronic obstructive pulmonary disease

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***Abstract*---**COPD is generally associated with chronic inflammation in peripheral airway and lung parenchyma which finally leads to obstruction of the airways. The diagnostic criteria for COPD is FEV1/FVC ratio which is less than 0.7. Arginase being the key enzyme of urea cycle, is also expressed in extra hepatic tissue including lung in the bronchial epithelial cells and endothelial cells. The main function of arginase here, is to regulate the formation of nitric oxide, indirectly competing for the common substrate L-arginine. The NO forms peroxynitrite on reaction with reactive oxygen species due to oxidative stress. Peoxynitrite is unstable molecule and breaks down to nitrite and nitrate. The present research work was approved by Institutional Ethical committee. Informed consent was obtained from the patients and controls prior to the study. It is a case control study and it included 45 COPD patients between the age group of 40-60 years who attended the outpatient department of pulmonary medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram. These are labeled cases. 45 normal healthy age and sex matched individuals with normal FEV1/FVC ratio are considered as controls. we observed that FEV1/FVC ratio decreased in COPD patients, where as, serum Arginase and Serum Nitrate levels showed an increase. Serum arginase and nitrate are negatively correlated with FEV1/FVC. COPD is due to profound inflammatory changes in respiratory airways with higher neutrophil count and NLR in the blood.

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2021.
Manuscript submitted: 18 May 2021, Manuscript revised: 08 June 2021, Accepted for publication: 12 August 2021

Keywords---Chronic Obstructive Pulmonary Disease, FEV1/FVC ratio, Arginase, nitrate and Neutrophil Lymphocyte Ratio.

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is characterized by irreversible air flow limitation and deterioration of pulmonary function [1]. Prevalence of COPD is increasing globally and it is the fourth leading cause of death. It is predicted to be the third leading cause of the death by 2020 [2]. The main cause of COPD is cigarette smoking followed by other risk factors like indoor and outdoor air pollution, dust and chemicals.

COPD is generally associated with chronic inflammation in peripheral airway and lung parenchyma which finally leads to obstruction of the airways. It causes four anatomical lesions such as chronic bronchitis, pulmonary hypertension, emphysema and small airway remodeling resulting in Chronic cough, production of sputum, chest tightness, dyspnea and wheezing [3].

COPD remains undiagnosed during early stages due to its slow onset and usually manifest after 40 years. COPD can be assessed by measuring forced expiratory volume in one second (FEV 1) and forced vital Capacity (FVC) by using spirometer. The diagnostic criteria for COPD is FEV1/FVC ratio which is less than 0.7. Based on Global Initiate for Chronic Obstructive Lung Disease (GOLD) criteria, the COPD patients are categorized into 4 stages based on severity [1].

Arginase being the key enzyme of urea cycle, is also expressed in extra hepatic tissue including lung in the bronchial epithelial cells and endothelial cells. The main function of arginase here, is to regulate the formation of nitric oxide, indirectly competing for the common substrate L- arginine [4]. Serum arginase levels increase in COPD due to neutrophilia and smoking (Nicotine induces arginase). Hence, Arginase acts on arginine to produce ornithine which is a precursor for synthesis of polyamines and proline that are involved in cell proliferation and collagen synthesis.This causes pulmonary remodeling [5].

Inducible nitric oxide synthase (iNOS) contains oxygenase and reductase moieties. Reduced availability of arginine to iNOS will produce nitric oxide (NO) and superoxide by oxygenase andreductase moieties of enzyme [6]. The NO forms peroxynitrite on reaction with reactive oxygen species due to oxidative stress. Peoxynitrite is unstable molecule and breaks down to nitrite and nitrate [7]. The final stable end product of NO metabolism is nitrate which will be estimated along with arginase in the present study.

Material and Methods

Study design

The present research work was approved by Institutional Ethical committee. Informed consent was obtained from the patients and controls prior to the study.

It is a case control study and it included 45 COPD patients between the age group of 40-60 years who attended the outpatient department of pulmonary medicine, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram. These are labeled cases. 45 normal healthy age and sex matched individuals with normal FEV1/FVC ratio are considered as controls. Demographic data of all the participants was collected followed by history regarding current health status, history of medications, alcoholism, history of active smoking (regular cigarette smoking more than 6 months), exposure to atmospheric dust and at work place and history of COPD by personal interview.

Selection Criteria

Inclusion Criteria
The patients diagnosed with COPD and FEV1/FVC ratio less than 0.7 attended pulmonary medicine department was included in the study.

Exclusion Criteria
Known Subjects with obesity, alcoholics, hypertension, diabetes mellitus, liver diseases, cardiac diseases, renal diseases, stroke, bleeding disorders, clotting disorders, thyroid disorders, HIV, cancers and autoimmune diseases, history of repeated blood transfusion.

Pulmonary function test
In all the subjects forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) was measured by using spirometer (Spirowin). It was conducted after giving instructions to the participants and the subjects were in sitting position and highest value FEV1 and FVC were obtained. Two reproducible spirometric data was obtained from three acceptable curves [8].

Sample collection
From each subject 5 ml of venous blood was drawn by venipuncture and blood was distributed into sodium citrate vacutainer tube for cell count and vacutainer plain tubes for biochemical analysis.

Sample Analysis
Blood samples was analyzed by using Nihon Kohden (Cell tac ES - 5 part) for cell count. Serum Arginase was estimated by ELISA method and expressed in ng/ml. Serum nitrate was estimated by colorimetric Griess assay method and expressed in µmol/L [9].

Statistical analysis
Data was expressed in Mean and Standard deviation (mean ±SD). Z test was performed using SPSS software version 22.0. The statistical significance was determined at 5% ($p < 0.05$) level. Spearmen correlation conducted between FEV1/FVC with Serum Arginase and Serum Nitrate

Observation and Results

Table 1: Demographic data between Control and Cases

Parameter	Control (n=45)	Cases (n=45)
Age (years) Mean ± SD	44.10±4.32	44.07±4.26
Sex: Male/female	37/8	39/6
Smoking Status: Smokers/Non smokers	0/45	44/1

The above table shows age and sex matched individuals were considered for the study. Amongthe cases all are smokers except one individual

Table 2: Comparison of FEV1/FVC ratio in Control and Cases

Parameter	Control (n=45)	Cases (n=45)	p value
	Mean ± SD	Mean ± SD	
FEV1/FVC ratio	80.81 ± 6.55	58.58 ± 7.79	<0.001

The above table shows FEV1/FVC ratio was decreased in Cases when compared with Control.This decrease is statistically significant (p value <0.001).

Table 3: Comparison of Complete Blood Count in Control and Cases

Parameter	Control (n=45)	Cases (n=45)	p value
	Mean ± SD	Mean ± SD	
Hemoglobin (g/dL)	13.40 ± 1.63	12.99 ± 1.87	N.S
RBC (10^6/µL)	4.65 ± 0.39	4.53 ± 0.44	N.S
WBC (10^3/µL)	7.41 ± 1.51	9.60 ± 1.11	<0.001
Neutrophils count (10^3/µL)	4.9 ± 1.05	6.86 ± 0.92	<0.001
Eosinophils cont (10^3/µL)	0.32 ± 0.11	0.35 ± 0.11	N.S
Lymphocytes(10^3/µL)	2.19 ± 0.69	2.38 ± 0.53	N.S
Platelets (10^3/µL)	262.24 ± 60.15	271.28 ± 74.65	N.S
Neutrophil Lymphocyte Ratio (NLR)	2.41± 0.82	3.02 ± 0.78	<0.001

The above table shows WBC count, Neutrophil count, NLR were increased in Cases whencompared to controls. This increase is statistically significant (p value <0.001).

Table 4: Comparison of Serum Arginase and Serum Nitrate in Control and Cases

Parameter	Control (n=45)	Cases (n=45)	p value
	Mean ± SD	Mean ± SD	
Serum Arginase (ng/mL)	18.06 ± 2.69	27.64 ± 4.96	<0.001
Serum Nitrate (µmol/L)	16.80 ± 3.28	24.96 ± 2.11	<0.001

The above table shows Serum Arginase and Serum Nitrate were increased in Cases whencompared to controls. This increase is statistically significant (p value <0.001).

Table 5: Correlation of FEV1/FVC ratio with Serum Arginase and Serum Nitrate

Parameter	Serum Arginase	Serum Nitrate
FEV1/FVC ratio	r= -0.8145(p<0.001)	r= -0.6707(p<0.001)

The above table shows FEV1/FVC ratio was negatively correlated with Serum Arginase andSerum Nitrate. It is statistically significant (p<0.001).

Discussion

In the present study Age and sex matched individuals are taken as controls and cases. Among the cases all are smokers except one individual. The mean FEV1/FVC ratio is decreased in cases (58.58 ± 7.79) when compared to controls (80.81 ± 6.55) (Table-2) and it is found to be statistically significant. The diagnosis of COPD was done using Global Initiative for Chronic Obstructive Lung Disease guidelines basing on which, if forced expiratory volume in 1 s to forced vital capacity (FEV1/FVC) was <70 % it is considered as COPD [1, 10].

In the present study, neutrophil count and Neutrophil Lymphocyte Ratio (NLR) were significantly higher in cases (6.86 ± 0.92, 3.02 ± 0.78 respectively) when compared to controls (4.9 ± 1.05, 2.41± 0.82 respectively) (Table-3). Leukocytes and its subtypes are markers of inflammation. [11, 12]. Besides other inflammatory markers like ESR and CRP, NLR ratio is a significant marker derived from Complete blood count. It is a rapid and easy method and many studies reported that increased NLR is associated with inflammatory conditions [13, 15]. Our study coincides with the above statement that NLR increased in COPD cases. This could be due to an overwhelming neutrophil response alone rather than an insignificant change in the lymphocyte count. In a study by Cockayne et al., (2012) the increased neutophil count was associated with the GOLD stages of COPD [15].

In COPD, there is secretion of chemoattractants from the epithelial cells and production of macrophages in the alveoli. The chemoattractants promote the recruitment of neutrophils [16]. Increased neutrophils are also responsible for the release of proteolytic enzymes like matrix metalloproteinases, neutrophil elastase and free radicals, which will cause lung tissue destruction [17]. In addition to increased neutrophil influx there is also impairment in the clearance of neutrophils which contributes to the inflammatory phase of the disease [18]. Smoking impairs degradation of apoptotic neutrophils and TNF-α impairs the uptake of apoptotic cells by macrophages which leads to secondary necrosis in apoptotic cells releasing proinflammatory substances [19, 20]. These circulating neutrophils cause neutrophil-mediated tissue injury [21, 22].

In the present study arginase and nitrate were significantly higher in cases (27.64 ± 4.96, 24.96 ±

2.11 respectively) when compared to control (18.06 ± 2.69, 16.80 ± 3.28 respectively) (Table-4). In our study there was a negative correlation between FEV1/FVC to arginase (r= -0.8145 and p<0.001) and FEV1/ FVC to nitrate (r= -0.6707 and p<0.001) (Table-5).

Arginase level in airway smooth muscles is usually below the detection limit. Recent studies have shown that nicotine present in tobacco smoke may increase arginase1 in airway smooth muscles. As demonstrated in recent studies, there is an increase in the neutrophil count [23]. Human neutrophils liberate azurophilic granules in patients with COPD which contain high levels of arginase1 [24]. Expression of arginase 1 is associated with peroxynitrate production and airway hyperresponsiveness [25]. Constitutive nitric oxide synthase which includes endothelial and neuronal nitric oxide synthases present in the airway cells produce low amount of nitric oxide [26]. But inducible NOS present in macrophages and epithelial cells is activated during inflammation in response to the gene expression induced by proinflammatory cytokines produced in COPD [27].

Smoking decreases NO synthesis by three mechanisms. These are i) Tobacco induced toxins downregulating nitric oxide synthase (NOS). [28] ii) NOS activity decreased because of high concentration of NO in cigarette smoke [29] iii) Oxidants like superoxides in cigarette smoke inactivate NO by combining with it forming peroxynitrite and damaging NO producing epithelial cells [30]. Reduction in NO may also occur due to hypoxia or mucus hypersecretion which prevent stimulation of airway walls to produce NO. As a result of the decrease in NO formation there is reduction in mucociliary clearance and decrease in bactericidal action of phagocytes [31,32].

This decrease in NO triggers activation of iNOS which produces NO in excess .It is found in epithelial cells, neutrophils and macrophages [33, 34]. Neutrophils and macrophages synthesize a cytokine IL-8 which is an activator of neutrophils. This cytokine induces release of superoxide ion from neutrophils which leads to the formation of peroxynitrite. This in turn activates neutrophil derived matrix metallo proteniases which degrade the components of extracellular matrix in the lung [35].

The peroxynitrite formed from NO and superoxide produces nitrotyrosine by adding nitro group to tyrosine. NO also forms nitrite directly by reacting with oxygen [36, 37]. This nitrite is oxidized by myeloperoxidase producing nitryl chloride and nitrogen dioxide [38]. These reactive nitrogen species damage the lung tissue. Peroxynitrite further forms stable nitrate and nitrite and other nitrate derivatives.

In summary, we observed that FEV1/FVC ratio decreased in COPD patients, whereas, serum Arginase and Serum Nitrate levels showed an increase. Serum arginase and nitrate are negatively correlated with FEV1/FVC. COPD is due to profound inflammatory changes in respiratory airways with higher neutrophil count and NLR in the blood.

Conclusion

This study can support the utilization of arginase and nitrates as early alarming markers during the initial stages of COPD. Their estimation can provide an insight into the prevention of the rapid progession of COPD. An arginase

antagonist may be developed in future as a new therapeutic option to target the deleterious effects of COPD.

Conflict of interest: No conflict of interest

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