Evaluation of mTOR Activity in COPD Patients with Emphysema

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ABSTRACT

Objective: To evaluate the function of the mammalian target of the rapamycin (mTOR) pathway in chronic obstructive pulmonary disease (COPD) patients with emphysema.

Study Design: Observational study.

Place and Duration of Study: Department of Pulmonology, Mugla Training and Research Hospital, Turkey, from January to March 2022.

Methodology: Thirty COPD patients and thirty healthy volunteers were included. Demographic data, pack-year of cigarette, spirometric values, and emphysema percentage (calculated with CT scan) were recorded. mTOR, raptor, and deptor were measured with ELISA method. Statistical significance was accepted as $p<0.05$.

Results: The mean value of mTOR in the COPD group was 3.48±2.01 ng/ml and it was significantly higher than the control (1.51±0.44 ng/ml). The mTOR was positively correlated with MMRC, annual exacerbation rate, emphysema percentage, and pack/year of cigarette and negatively correlated with SpO_2 and FEV1. The significant relationship was found with only emphysema (B=0.067, SE=0.020, 95% CI=0.027-0.107, p=0.002). The cut-off value of mTOR for COPD was found as 1.815 ng/ml (sensivity=77%).

Conclusion: Overexpression of mTOR and its signalling proteins have a significant role in emphysema development. Reduction of mTOR expression/activity might be helpful to control dyspnea severity, number of exacerbations, loss of FEV1, and progression of emphysema.

Key Words: *COPD, Emphysema, mTOR.*

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common lung disease with persistent respiratory symptoms and airflow limitation. The major aetiological factors are exposure to noxious particles or gases, genetics, airway hyper-responsiveness and poor lung growth. The incidence of COPD is increasing due to the increase in cigarette smoking and environmental pollution. Inflammation responsible for exacerbations in COPD is characterised by cellular activation, production of cytokines, cellular relationships, and tissue destruction. The inflammation has local and systemic effects. Chronic inflammation is the cause of structural changes, narrowing of the small airways and destruction of the lung parenchyma with loss of elasticity. Emphysema is a pathological term that is used for the destruction of the gas-exchanging surfaces of the alveoli. Chronic inflammation is also responsible for the development of comorbidities in patients with COPD.[1] Understanding the mechanisms leading to airway and/or alveolar abnormalities and inflammation in COPD may help to prevent and treat the disease.

The mammalian target of the rapamycin (mTOR) signalling pathway integrates intracellular and extracellular stimuli. MTOR is considered as a central mediator of cell metabolism. Inflammation, autophagy, apoptosis, protein synthesis, and cytoskeleton organisation are the most important metabolic processes that have been regulated by the mTOR.[2] It is formed by mTORC1 and mTORC2 which are two distinct protein kinase complexes. These two complexes may act either synergistically, independently or antagonistically, according to the prevalence of the various stimuli.[3] MTORC1 consists of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal

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with SEC13 protein 8 (mLST8)/G-protein β-subunit-like protein (GβL), PRAS40, deptor, and scaffold protein TTI1/TEL2 complex.[4] MTORC1 is sensitive to rapamycin, it regulates protein synthesis through the activation of ribosomal protein S6 (RPS6) kinase and inhibition of the eukaryotic transcriptional initiation factor 4E binding protein 1. MTORC2 regulates the actin cytoskeleton of cell growth. The mTOR pathway is shown in Figure 1. The response of mTORC2 to rapamycin is not clear and may vary in different cell types.[5] Both mTORC1 and mTORC2 are negatively influenced by deptor, and both complexes negatively regulate deptor expression for their activity modulation.

Since the mTOR regulates physiological cell processes such as cell autophagy, this could be relevant for both the development and therapy of COPD. It is known that specifically, mTORC1 inhibits cell autophagy in the presence of nutrients.[6] The mTOR activation, inhibition and function of rapamycin as an mTOR inhibitor have been evaluated in lung diseases mostly in *in-vitro* pre-clinical studies. In a study on transgenic mice inflammation, lung cell senescence, emphysema and pulmonary hypertension in COPD have been reported as related to the increase of mTOR activity.[7] The researchers in a different study reported that rapamycin had restored corticosteroid sensitivity on isolated mononuclear cells from COPD patients.[8]

The data about mTOR activity in COPD is controversial and it has to be confirmed with clinical studies. The aim of this study was to evaluate the mTOR activity of patients with COPD by using the major subunits (mTOR, raptor, deptor) of the mTORC1 complex *via* enzyme-linked immunosorbent assay (ELISA).

METHODOLOGY

After obtaining the study approval for this observational study from the Clinical Research Ethics Committee of Mugla Sitki Kocman University, Faculty of Medicine (protocol number: 05/01/2022-1/VI), COPD patients and healthy volunteers were enrolled for the study between January and March 2022. The sample size was calculated using G-Power 3.1.9.4. Considering possible losses (10%), it was planned to reach approximately sixty people (thirty COPD patients and thirty healthy volunteers). COPD patients of the pulmonology outpatient clinic at Mugla Training and Research Hospital, who were accepted to participate, were included to the study. All COPD patients were in a stable phase, were free from acute exacerbations and/or they did not receive anti-inflammatory drugs or systemic corticosteroids in the last 4 weeks. The case group were with emphysema phenotype confirmed by thorax computerised tomography. The participants who have any chronic pulmonary disease other than COPD were excluded. The control group was consisted of volunteers who admitted to the smoking cessation outpatient clinic. It is well known that cigarette smoke exposure plays an important role in the development of emphysema. The authors chose them because they were mostly healthy individuals although they had a smoking history. Controls did not have any diagnosed (clinical and radiological) chronic pulmonary disease. Presence of emphysema is the primary outcome of this study.

Demographic data, smoking history, oxygen saturation (SpO_2) values and blood samples were collected from all partcipants. Modified Medical Research Council (mMRC) dyspnea score, spirometric measurements and thorax tomography findings of the COPD patients were evaluated. After taking the blood samples to the yellow-top jel test tubes, they were centrifuged at 2000-3000 RPM for 20 minutes. The supernatant was collected without sediment and stored at -80°C. ELISA test kits for the accurate quantitative detection of mTOR, raptor and deptor in serum were performed due to the instructions of the manufacturer. BT Lab brand, Human Mammalian Target of Rapamycin ELISA Kit with product code E3693HU (96 tests), Human Regulatory-associated Protein of mTOR ELISA Kit with product code E6623HU (96 tests), Human DEP Domain-containing mTOR-interacting Protein ELİSA with product code E6973HU kit (96 tests) ELISA kits were used.

The thoracic computerised tomography (CT) scans of the COPD patients were analysed in the radiology software system analysed. Radiological images were obtained with 256-slice Toshiba-TCT-60 AX and 4-slice Siemens Somatom device. CT images were evaluated on high-resolution medical screen. Right lung 3 lobes left lung 2 lobes were examined separately. Each lobe was accepted as 20% and lobe volume was measured first. Then, the emphysema areas were calculated by calculating the volumetric voxel on the computer, and they were calculated over the total volume. The total emphysema percentage was found by summing the percentage values of all lobes.[9]

Statistical analysis was calculated by using SPSS 22.0. The descriptive variables were presented as frequency and percentages. The normality test was performed and it was observed that some variables were normally distributed, and some were not normally distributed. The Shapiro-Wilk test was used in those with $n < 50$, while the Kolmogorov-Smirnov test was used in those with $n > 50$. The association between the categorical variables and the outcome was tested using a chi-square test. Independent samples t-test was used for normally distributed continuous variables and Mann-Whitney U test was used for not-normally distributed continuous variables. The cut-off value was determined by examining the area under the receiver operating characteristic (ROC) curve for the mTOR value, and this value was obtained as 1.815 ng/ml. Since mTOR, raptor and deptor values, emphysema percentage, mMRC score, annual exacerbation rate and SpO_2 did not show normal distribution, the Spearman correlation analysis was used to evaluate the correlation between these parameters. Rho $\geq$0.20 was accepted as a small effect, rho $\geq$0.40 was accepted as a medium effect, rho $\geq$0.60 was accepted as a large effect and $p<0.05$ value was accepted as significant for statistical analysis. A multivariate logistic regression model was fit with the variables (independent variable was mTOR value, dependent variables were emphysema percentage, COPD exacerbation rate, dyspnea severity, pack/year of cigarette, FEV1 and SpO_2) which are associated with the outcome.

Table I: Descriptive statistics of demographic data and levels of mTOR, raptor and deptor.

	COPD patients n=30	Control n=30	p-value
Age, mean±SD	72.06±6.89	69.30±8.11	0.160[a]
Gender			
Male, n (%)	29 (97.7%)	28 (93.3%)	0.554[c]
Female, n (%)	1 (3.3%)	2 (6.7%)	
Pack year of cigarette, mean±SD	44.10±14.81	29.63±8.20	<0.001[a]
SpO_2, % (interquartile range)	90.00 (78-97)	96.00 (92-99)	<0.001[b]
mTOR, ng/ml (interquartile range)	3.31 (1.24-10.49)	1.58 (0.15-2.16)	<0.001[b]
Raptor, ng/ml (interquartile range)	392.20 (296.48-1323.54)	347.48 (313.89-436.61)	0.020[b]
Deptor, ng/ml (interquartile range)	3.40 (2.35-13.70)	2.83 (1.68-16.88)	0.010[b]

[a] T test; [b] Mann–Whitney U test; [c] Chi-square; SpO_2: Oxygen saturation; mTOR: Mammalian target of rapamycin.

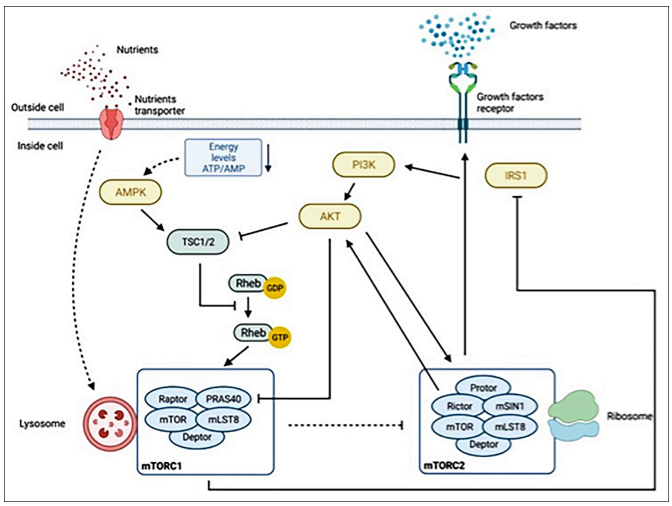
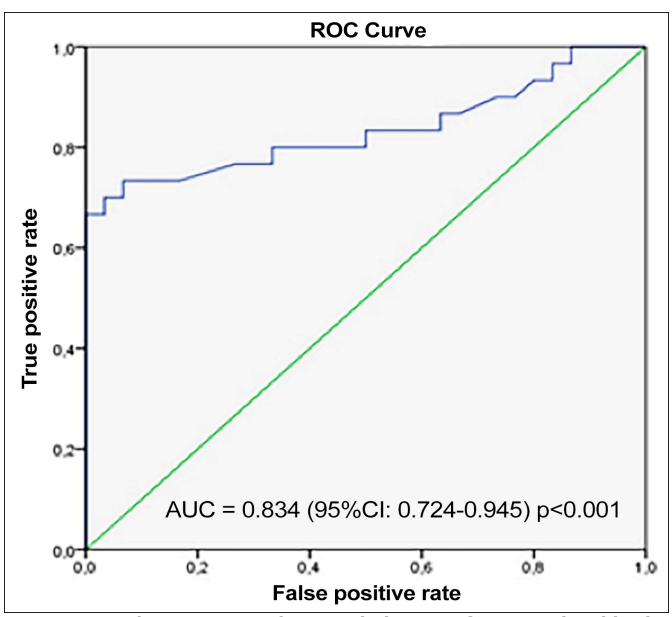


Figure 1. The mTOR pathway with all involved complex. The figure has been modified from that shown in Ref 6.
AMPK: AMP-activated protein kinase: AKT (PKB): Protein kinase B; GDP: guanosine dipbosphate; GTP: guanosine triphosphate; IRS1: insulin receptor substrate 1; mTORC1/2: mammalian target of rapamycin complex 1/2; PI3K: phosphoinositide 3-kinase; PRAS40: proline-rich Ak-t substrate of 40 kDa; TSC1/2: Tuberous sclerosis proteins 1 and 2.



Fgure 2: Receiver operator characteristic curve for mTOR level in the prediction of COPD patients with emphysema.

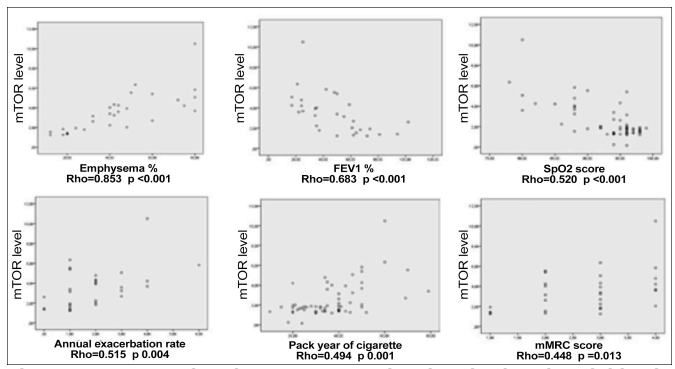


Figure 3: Scatter plots between mTOR level and related variables in patients with COPD.

RESULTS

Thirty patients with COPD who matched the inclusion criteria of the study and 30 healthy controls who accepted to attend the study were enrolled. The mean age and gender of the COPD patients and healthy controls were similar (Table I). The mean pack-year of cigarette smoking of the COPD patients was significantly higher, whereas, the mean oxygen saturation in the room air was significantly lower (Table I). It was found that; mTOR, raptor, and deptor levels were significantly increased in the peripheral blood samples of patients with COPD compared with those in normal subjects (Table I).

The cut-off value of mTOR was calculated as 1.815 ng/ml (sensivity:77%) (AUC=0.834 (95% CI:0.724-0.945) p<0.001, Figure 2).

The mean forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) values of patients with COPD were 59.76±20.13% and 48.10±22.86%, respectively. The median mMRC score was 2.70±0.98, the median annual exacerbation rate was 1.90±1.37 and the median emphysema percentage in the thoracic CT scan was 43.76±21.7 of the patients with COPD. The mTOR value of the COPD patients had significant correlations with mMRC, number of annual exacerbations, emphysema percentage positively; SPO_2 in the room air and FEV1 negatively (Figure 3). As a result of multivariable regression analysis model with mTOR value, emphysema percentage, FEV1 %, SPO_2 %, annual exacerbation rate, pack year of cigarette, and mMRC score; mTOR value had a significant association only with emphysema percentage (B=0.067, SE=0.020, 95% CI 0.027-0.107, p=0.002).

Raptor value of the patients with COPD had significant correlations with emphysema percentage (rho=0.599, p<0.001), pack year of cigarette (rho=0.283, p=0.028), FEV1% (rho=-0.568, p=0.001) and oxygen saturation (rho=-0.511, p<0.001) in the room air. However, the authors could not find any significant correlation between deptor and the listed variables above.

DISCUSSION

According to this study results, mTOR activity in COPD patients with emphysema was significantly higher than the healthy controls at the same age. The results support that the overexpression of mTOR and some of its signalling proteins are associated with emphysema. Increased mTOR activity in COPD patients with emphysema was found to be related with FEV1, mMRC score, annual exacerbation rate, oxygen saturation and pack-year of cigarette. As a result of this study, the authors think that mTOR may be used as a predictor of emphysema in blood tests and it can be used for monitoring the disease progress.

The role of mTOR signalling pathway has been established in several age-related diseases.[10] Lung cancer, pulmonary hypertension and COPD are some of the diseases that have been investigated in experimental studies.[11,12] To the authors knowledge, this study is the first clinical study for the evaluation of mTOR activity in peripheral blood samples by using the ELISA method.

Several features of COPD have different prognoses and/or responses to treatment.[13] Evaluation of these features may help to guide clinical management. In some studies, the correlation between cell senescence criteria and emphysema score has been shown.[7,14] In a study by Houssaini *et al.*, they showed the development of lung emphysema in mice with the mTOR pathway activation and they declared that emphysema developed without telomere shortening.[7] This result suggests that various stresses may affect the mTOR pathway for cell senescence in COPD. Oxidative stress occurring as a result of smoking exposure may be one of the inducers.[15] Haruna *et al.* reported that emphysematous changes assessed by radiological diagnosis predicted respiratory mortality in various stages of COPD.[16] Moreover, mTOR activation may be related to mortality. Further studies are needed to evaluate the role of mTOR on mortality in COPD.

In several studies, it had been reported that autophagy was necessary for apoptosis, necroptosis and mucus hyperproduction due to cigarette smoke exposure in the airway epithelium.[17,18] One of the functions of mTOR is to suppress the autophagy that performs vital functions in cellular homeostasis.[19] Wang *et al.* had shown that cigarette smoke decreased the mTOR expression and activity in the lung by using cultured bronchial cell lines and lung specimens from patients with COPD.[20] The results of the study showed a positive correlation between pack year of cigarette and mTOR activity. This is supporting data for the hypothesis that the eventual functions of mTOR signalling in COPD pathogenesis may be cell-specific. Based on this theory, mTOR activity in chronic bronchitis may differ from emphysema.

The annual loss of FEV1 is higher in patients with severe emphysema and adversely affects the prognosis.[21] The mean FEV1 percentage of our patients showed a negative correlation with mTOR activity. This result showed us that mTOR activity was more active in patients with lower FEV1. In a study about COPD-associated lung cancer, the interaction between cancer and FEV1 had been explained with two opposing translational programs.[22] The program was related to mTOR activity that was more active in stromal lung tissue adjacent to lung cancer in normal to moderate COPD. In lung cancer patients with more severe COPD (FEV1pp <60) this program was likely to be switched off.

COPD exacerbation is an acute clinical feature of COPD that is seen by a change in the patient's initial dyspnea, cough, or sputum resulting in additional therapy.[1] Exacerbations are the results of various factors and they are associated with acutely worsening of existing airway inflammation. T cell-mediated immunity has been demonstrated in sputum samples of patients with severe exacerbations that require hospitalisation.[23] Lymphocyte cellular immunity is regulated by the mTOR. The mTOR stimulates cytokine release from inflammatory cells. The differentiation and activation of CD4+ T cell subsets are regulated by the mTOR pathway, and rapamycin treatment led to T cell anergy.[24] Zhang *et al.* claimed that treatment with mTOR inhibitors did not show statistically significant differences from treatment with budesonide in patients with asthma.[25] One of the results of this study, mTOR activity showed a positive correlation with the annual COPD exacerbation rate. Since inhaled steroids are commonly used in COPD exacerbations, mTOR inhibitors may be considered as potential agents for the treatment.

Raptor and deptor are the other subunits of mTORC1 complex. Their direct effects on COPD is not known, they were evaluated in this study to determine whether the mTOR pathway worked in patients with COPD. It seemed that the pathway worked in the study population but the results of deptor were not clear.

The limitations of this study were the small sample size, experimential technique and the lack of COPD patients with chronic bronchitis. Maybe further studies with large sample size will be planned for comparing the mTOR activity between patients with emphysema and chronic bronchitis. In the future, mTOR may be determined as a biomarker for monitoring patients with COPD. Clinical studies that will evaluate the effect of mTOR inhibitors will contribute to the development of alternative treatment methods for COPD.

CONCLUSION

Increased mTOR activity in COPD patients with emphysema negatively correlated with FEV1 and oxygen saturation, and positively correlated with MMRC score and annual exacerbations suggests that the mTOR pathway is effective in the clinical course of the disease. The present data suggest that reducing mTOR expression/activity in emphysema-type COPD patients may be beneficial to control dyspnea severity, number of exacerbations, loss of FEV1, and progression of emphysema.

ETHICAL APPROVAL:
Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Mugla Sitki Kocman University (Date: January 5, 2022; Issue: 1/VI).

PATIENTS' CONSENT:
All of the participants signed the informed consent.

COMPETING INTEREST:
The authors declared no competing interest.

AUTHORS' CONTRIBUTION:
OOT: Concept, design, definition and intellectual content, literature search, data collection, data analysis, manuscript preparation and editing.
UT: Literature search, data collection, data analysis.
TE: Design, definition and intellectual content, literature search.
ED: Data collection, intellectual content.
All the authors have approved the final version of the manuscript to be published.

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