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Relationship between chronic obstructive pulmonary disease and telomerase, agmatine, deubiquitinase and sirtuin in the pathway of aging

COPD and aging

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Abstract
Aim: Chronic Obstructive Pulmonary Disease (COPD) is a progressive disease that has both chronic bronchitis-related changes in airways and differences of emphysema in the lung parenchyma. Aging is a complex and irreversible process involving cells, tissues, organs and systems resulting from numerous endogenous and exogenous factors. Agmatine, telomerase, deubiquitinase and sirtuin molecules appear during aging process. A better understanding of the role of these molecules in aging process may lead to advancing therapies for COPD and comorbidities.
In the present study, it was aimed to investigate whether there was a relationship between COPD, a chronic progressive disease, and agmatine, telomerase, sirtuin and deubiquitinase, which are molecules involved in the aging pathway.
Material and Methods: Thirty-five patients with COPD and 35 healthy persons without chronic diseases participated in this study. Serum agmatine levels were measured by a fluorescence detector using a high-performance liquid chromatography method (HPLC). Serum Sirtuin, deuibiquitinase and telomerase levels were performed using the ELISA method.
Results: A result of the statical analysis in the terms of the deubiquitinase showed no differences between patients and control groups (p>0.05). In the patient group, sirtuin values were lower than in controls (P<005). Telomerase and agmatine values were higher (P<0.05).
Discussion: As a result, the increase in telomerase activity may be to prolong the telomere shortening in COPD patients. The increase in agmatine may also be caused by depression and hypoxia in COPD. Decreased sirtuin in COPD can be explained by the increase in aging and inflammation.

Keywords
COPD, Sirtuin, Telomerase, Deubiquitnase, Agmatine

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Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive disease that includes changes in the airways and emphysema in the lung parenchyma. COPD is an increasing cause of morbidity and mortality worldwide. Among the risk factors for COPD are exposure to environmental differences and genetic predisposition. Active smoking is the main risk factor for COPD. In addition, air pollution, infections, bronchial hyperactivity, and social and economic factors also play a significant role in the development of COPD [1]. In those with COPD, as the age increases, not only epigenetic changes but also increased lipid peroxidation, decreased immunity, decreased protein synthesis and DNA damage are observed [2].

Telomeres are heterochromatic regions consisting of specialized DNA repeat sequences found at the chromosome ends of eukaryotic organisms [3]. Telomeres stabilize chromosomes and protect them against abnormal conditions. The length of the telomere shortens with each cell division [4]. Telomerase enzyme prevents losses in some cells, and prevents aging [5]. Telomere length was measured in some patients with lung diseases and it was observed that they were shortened compared to controls [6].

Sirtuins (SIRT's), which were initially discovered in yeast are a family of proteins containing protein deacetylase and ADP-ribosyl transferase activity [7]. There are seven variants of the SIRT family. They provide regulation of gene expression [8]. SIRT1 plays an important role in gene expression by affecting transcription factors [9]. SIRT1 and SIRT7 prevent the formation of cancer by activating the p53 gene [10]. The SIRT family is also effective in programmed cell death [11]. SIRT1 regulates apoptosis by deacetylating DNA repair factor Ku 70 [12]. The SIRT1 gene prolongs life by stimulating the insulin-like growth factor (IGF) [11]. SIRT1 regulates glycolysis and suppresses gluconeogenesis and affects oxidative phosphorylation [13].

Ubiquitin is a low molecular weight heat stable protein found in abundance in the cell. Ubiquitin-labeled proteins are rapidly degraded by proteases [14]. Proteins with coding errors are degraded in proteasomes by the effect of ubiquitin. The major role of deubiquitinating enzymes (DUBs) is the removal of ubiquitin from the substrate. Thus, proteins are reactivated. DUBs, which are related to many pathological conditions, are one of the candidates used for drug development [15].

Agmatine is a polyamine formed from the decarboxylation of arginine [16]. Nitric oxide forms peroxynitrite, which is a powerful oxidant. NOS activity increases with aging. Agmatine is a strong NOS inhibitor. Therefore, agmatine has an antioxidant effect. It also has a protective effect against endothelial dysfunction [17].

In the present study, it was aimed to investigate whether there was a relationship between COPD, a chronic progressive disease, and agmatine, telomerase, sirtuin and deubiquitinase, which are molecules involved in the aging pathway.

Material and Methods

In the present study, the patient group consisted of 35 individuals diagnosed with COPD in the Chest Diseases Clinic of Cumhuriyet University Faculty of Medicine Research and Training Hospital. The patients were randomly selected without taking into account such factors as age and sex. Disease grading was performed according to the GOLD 2017 report. Patients were classified as group A (low risk, few symptoms), group B (low risk, many symptoms), group C (high risk, few symptoms) and group D (high risk, many symptoms) according to the number of symptoms and exacerbations [18]. Thirty-five people who presented to Cumhuriyet University Medical Faculty Research and Training Hospital and did not have any systemic disease comprised the control group.

Collection of blood samples

From each of the individuals included in the patient and control groups, 10 ml of blood samples were drawn into sterile biochemistry tubes. These blood samples were centrifuged at 4000 rpm for 15 minutes. The serums obtained were portioned for the determination of agmatine, telomerase, SIRT 1 and deubiquitinase, and stored at -40°C until the analysis was performed. In our study, agmatine levels were determined by high-performance liquid chromatography (HPLC) [18]. Sirtuin, deubiquitinase and telomerase levels were measured through the ELISA method.

Statistical Method

The data obtained from our study were analyzed using the SPSS (Statistical Package for the Social Sciences) (version 22.0) program. When the parametric test assumptions were fulfilled (Kolmogorov-Smirnov) in the analysis of the data, the test for the significance of the difference between the pairs in independent groups was used, and when the parametric test assumptions were not fulfilled, the Mann-Whitney U test used. To determine the relationship between the variables, correlation analysis and logistic regression analysis were used. The chi-square test was used in the analysis of the quantitative data. The margin of error was 0.05.

Ethical issues

Every stage of the study was carried out in accordance with ethical principles. Ethical approval from the ethics committee (dated July 11, 2017, numbered 2007-07/01) (Annex 1) and permission from the institution where the study was to be conducted was obtained prior to the study. An informed consent form was obtained from the study participants.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

In our study, the difference between the participants in the patient and control groups was not significant in terms of age, sex and body mass index ($p>0.05$). Of the participants in the patient group, 28.6% were graded as Gold A, 22.9% as Gold B, 8.6% as Gold C, and 40% as Gold D.

The difference between biomarker levels in the participants in the patient group in terms of the degree of COPD was not significant ($p>0.05$).

The comparison of the sirtuin, telomerase and agmatine measurements of the participants in both groups demonstrated that the difference between the groups was significant ($p<0.05$). While sirtuin values were lower in the patient group than were those in the control group, telomerase and agmatine values were higher ($p<0.05$). The difference between the groups in terms of deubiquitinase levels was not significant. According to the inter-group logistic regression analysis, agmatine was determined as a risk factor. According to the analysis, the risk of having a high agmatine value was 1.33 times higher in the participants in the patient group. The values were as follows: ODDS=1.33, 95% CI 1.03-1.60. The ODDS value was significant ($p<0.05$)

Discussion

In the present study, we investigated aging-related parameters in various metabolic pathways in patients with COPD. COPD is one of the major causes of morbidity, mortality and incapacity to work worldwide. Therefore, the molecular and cellular properties of COPD have been questioned for years. In patients with COPD, chronic inflammation, narrowing of the airways and alveolar vessel destruction are observed [19].

Aging is the result of damage to cells and tissues over time. This damage is a process that starts early in life and continues throughout life. Most eukaryotic cells undergo a limited number of cell divisions. This division is limited by the replicative and cellular aging process [20]. Aging can develop many pathological mechanisms in many chronic diseases, including COPD. In our study, in the participants in the patient group, agmatine and telomerase levels increased, sirtuin levels decreased, and deubiquitinase levels did not change compared to controls.

The process of lung aging occurs in all its tissue sections in response to environmental stress. Aging is a risk factor for various lung diseases such as COPD. Signs of cellular senescence intersect with the pathology of age-related lung diseases. Cellular senescence and signs of aging produce chronic inflammatory and pulmonary distress in patients with COPD [21]. The biomarkers we study are in the aging pathway. Decrease or increase in biomarkers may cause lung aging in patients with COPD.

Table 1. COPD grading of the participants in the patient group

COPD grading	S	%
Gold A	10	28,6
Gold B	8	22,9
Gold C	3	8,6
Gold D	14	40

Table 2. Serum sirtuin, telomerase, deubiquitinase and agmatine levels of the participants in the patient and control groups

Biomarker	Control Group (N=35)	Patient Group (N=35)	p
Sirtuin (pg/ml)	2,54 ± 1,03	1,49 ± 1,17	<0,005
Telomerase (ng/ml)	1,36 ± 0,34	1,65 ± 0,045	<0,005
Deubiquitinase (ng/ml)	45,00 ± 21,35	43,78 ± 28,99	0,462
Agmatine (ng/ml)	17,87 ± 5,84	41,04 ± 18,54	<0,005

Table 3. Inter-group logistic regression analysis

Variables	B	S.E.	Wald	df	Sig.	Exp (B)	95% C.I.for EXP(B)	
							Lower	Upper
Agmatine	0,28	0,09	8,79	1	0,003	1,33	1,1	1,6
Constant	-19,33	7,93	5,93	1	0,015	0		

It was observed that telomeres were significantly shortened in patients with COPD compared to the participants in the control group. However, smoking had no effect on their shortening. In those with COPD, this marker may be an indicator of biological aging. As aging increases, telomeres shorten. There is an association between short leukocyte telomere length and impaired lung function [22]. In our study, telomerase levels increased significantly in the participants in the patient group compared to controls. The increase was probably due to shortened telomeres. To lengthen shortened telomeres, metabolism can increase the synthesis of telomerase. Our study is the first study conducted to investigate telomerase levels in the serum of patients with COPD.

Sirtuin1 is a biomarker related to longevity. Expression of SIRT 1 appears to be decreased in the lungs of people with COPD. SIRT1 is a biomarker related to longevity. Expression of SIRT1 appears to be decreased in the lungs of people with COPD. SIRT1 expression was found to be low in the lymphocytes of patients with COPD. SIRT1 may be an important therapeutic target in patients with COPD because it affects the inhibition of cellular senescence, inflammation, autophagy and matrix metalloproteinase. In the aforementioned studies, it was stated that the expression of SIRT1 decreased [22]. In other words, we found that the serum SIRT1 levels were significantly lower in the participants in the patient group than in the controls, because the production of SIRT1 was reduced.

In our study, agmatine levels were significantly higher in the participants in the patient group than in the controls. Our search for studies in which agmatine levels were investigated in patients with COPD revealed a gap in the literature. In some studies in the literature, agmatine levels in patients with several diseases have been investigated and it has been determined that agmatine levels vary from one disease to another.

This increase in patients with COPD may be due to some changes in the polyamine pathway. The level of polyamine in the cell occurs by the co-regulation of synthesis, degradation and use. COPD may have affected polyamine metabolism and thus increased agmatine levels. Polyamine metabolism in cancer cells was investigated. Polyamine synthesis in cells exposed to chronic hypoxia in the primary tumor may decrease. However, an increase is observed in the external intake. Especially, the increase in extracellular sperm causes a decrease in the expression of the hypoxia-dependent adhesion molecule CD44. This decrease increases the migration of tumor cells. An increase in polyamine synthesis begins in cells that participate in the circulation as a result of migration [23]. Patients with COPD also have varying degrees of hypoxia. In addition, epithelial cells and many cells are activated [24]. Therefore, the aforementioned mechanisms may have worked in the same way in COPD, and increased the agmatine level.

DUBs are essential for the regulation and balance of the ubiquitin system, which controls inflammatory processes. In studies, DUBs have been shown to have effects on some diseases related to inflammatory processes, and cancer development. In our study, no changes were determined in deubiquitinase levels in the participants with COPD in the patient group compared to controls.

According to the COPD grading, among the participants in the patient group, 28.6% were graded as Gold A, 22.9% as Gold B, 8.6% as Gold C, and 40% as Gold D. According to the COPD grading, the difference between the biomarker levels in the participants in the patient group was insignificant. In the participants in the patient group, agmatine and telomerase levels increased, SIRT1 level decreased, and DUBs did not change compared to controls. In our study, aging parameters, other than DUBs, changed in COPD. Shortening of telomere is a major indicator of aging.

Conclusion

In several studies, it has been demonstrated that a person with COPD is biologically older than a person of the same age without COPD [25]. The decrease in SIRT 1 levels may be related to inflammation and oxidation in old age. Agmatine is responsible for oxidative stress, telomerase cell cycle and senescence. Thus, changes in these biomarkers can affect COPD metabolism. In the literature, there are no studies similar to our study, which have been conducted on patients with COPD. The major limitation of our study is that the sample size was not large enough. We recommend that future studies be more comprehensive and include a higher number of patients in groups formed by COPD grading. Knowing the molecular pathways can help health professionals to find the right biomarkers for the pathogenesis of COPD and develop new pharmacological treatments. Thus, more detailed and long-term clinical studies should be conducted.

Scientific Responsibility Statement
The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Conflict of interest
The authors declare no conflict of interest.

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