ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 28(1): 139-145, 2023
DOI: 10.5505/ejm.2023.03264

The Association Between Platelet-Derived Growth Factor Receptor-β Polymorphisms and Idiopathic Pulmonary Fibrosis

Zehra Kaya[1*], Seren Duran[1], Hulya Gunbatar[2], Elif Sena Sahin[1], Burak Mugdat Karan[1]

[1]Van Yuzuncu Yil University, Faculty of Medicine, Department of Medical Biology, Van Turkey
[2]Van Yuzuncu Yil University, Faculty of Medicine, Department of Chest Diseases, Van, Turkey

ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease with a dismal prognosis. Platelet-derived growth factor (PDGF) receptor-β (PDGFR-β) are a receptor tyrosine kinase that PDGFs activate. It has been reported that inhibiting PDGFR-β in IPF patients can slow and improve disease progression. However, the effects of PDGFR-β on IPF remain unknown, and no studies on PDGFR-β polymorphisms for IPF have been conducted.

The purpose of this study is to look into the relationship between PDGFR-β gene polymorphisms (rs246395, rs2302273, rs3828610, rs138008832) and IPF disease.

The study included eleven patients with IPF and twelve healthy controls. DNA was isolated from blood samples taken from all participants, and genotyping was performed using a StepOne plus real time PCR device.

There was no statistically significant difference between the variables (age, gender, smoking, alcohol, and gastroesophageal reflux (GER). There was no statistically significant difference between the patient and control groups in the allele and genotype frequencies of these polymorphisms. Furthermore, no statistically significant difference was found between patients' smoking, forced vital capacity (FVC) (normal, low), and GER data, as well as PDGFR-β variants (rs246395, rs2302273, rs3828610 and rs138008832). In order to determine the relationship between PDGFR-β gene polymorphisms and the risk of IPF, larger studies with more participants are required.

Keywords: Idiopathic pulmonary fibrosis, PDGF, PDGFR-β, polymorphism

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive interstitial lung disease with a poor prognosis and a prevalence of 2 to 29 per 100,000 people worldwide (1). IPF, which develops as a result of abnormal wound healing after alveolar injury, causes an excess of extracellular matrix (ECM) components, primarily collagen, as well as scarring and fibrosis of lung tissue (2). Lung fibrosis results in impaired pulmonary function, decreased gas exchange, and progressive respiratory failure (3). IPF, which is more commonly seen in men than in women, is typically diagnosed in adults over the age of 60 and has a 5-year survival rate of 20% (4, 5).

The cause of IPF is unknown, but environmental factors (smoking, radiation, chemical exposure such as asbestos, silica, some chemotherapy drugs, microorganisms, viral infections), metabolic changes (apoptosis, autophagy, aging, oxidative stress), and some genetic factors are known to play a role in the disease's development by causing damage to the alveolar epithelium (6, 7). Epithelial damage is the first step in the disordered healing of the alveolar epithelium and the excessive stimulation of fibroblasts leads to scarring. Many chemokines, cytokines, and growth factors are involved in these metabolic processes (7, 8).

While one of the growth factors, platelet-derived growth factor (PDGF), is expressed in fibroblasts and macrophages, it plays a paracrine role in wound healing, angiogenesis, and cell cycle events, but an autocrine role in tumor cells (9, 10). In response to tissue injury, PDGFs, the most potent proliferative stimulator identified for fibroblasts, are activated to promote scar formation and closure (9, 11). The PDGF family functions by interacting with two receptor tyrosine kinases, PDGFR-α and PDGFR-β. PDGFR-α is primarily expressed in mesenchymal cells, whereas PDGFR-β is primarily expressed in vascular smooth muscle

***Corresponding Author:** Zehra Kaya, Van Yuzuncu Yil University, School of Medicine, Department of Medical Biology, Van, Turkey
E-Mail: zkaya@yyu.edu.tr, Tel: +90 (432) 225 17 01/25167
Zehra Kaya and Seren Duran contributed equally to this work
ORCID ID: Zehra Kaya: 0000-0001-6222-7882, **Seren Duran:** 0000-0001-6063-4628, **Hulya Gunbatar:** 0000-0002-3504-8915, **Elif Sena Sahin:** 0000-0001-6645-2630, Burak **Mugdat Karan:** 0000-0002-9362-9267
Received: 24.11.2022, **Accepted:** 12.12.2022

cells (VSMC) (10). Because of its activation in mesenchymal cells in the tumor microenvironment, PDGFR-β is used as a stromal biomarker in various types of cancer (12). So far, research into the relationship between PDGFRs and IPF has mostly focused on gene expression. Inomata et al. showed that PDGF expression was higher in the epithelial cells and alveolar macrophages of IPF patients' lungs than in healthy lung cells (9). Also, it has been reported that inhibiting PDGFR- β in IPF patients can slow and improve the disease's progression (13). Although these studies, no research on polymorphisms in the PDGFR-β gene in IPF disease was found. Single nucleotide polymorphisms (SNPs) in the PDGFR-β gene have been linked to a variety of diseases. rs246395 (T>C, B19) is a polymorphism found in the 19th exon region of the PDGFR-β gene on chromosome 5. It has been associated with increased gene expression and a lower 5-year survival rate in colorectal cancer (14). rs2302273 (G>A) is a polymorphism found in the 5'UTR region of the PDGFR-β gene on chromosome 5 and it has been reported that it causes a poor prognosis in renal cell carcinoma (15). rs3828610 (-202A/C), a polymorphism found in the promoter region of the PDGFR-β gene, has been studied in moyamoya disease in China, but it was found to have no effect (16). Another polymorphism found in the PDGFR-β gene is rs138008832 (c.2083 C>T, pR695C), which is located in the tyrosine kinase domain in the 15th exon of the gene and causes an amino acid change from arginine to cysteine (p.Arg695Cys). It has been reported that the pR695C polymorphism causes partial loss of auto phosphorylation in idiopathic basal ganglia calcification (IBGC) (17).

The purpose of this study was to look into the relationship between PDGFR-β gene polymorphisms (rs246395, rs2302273, rs3828610, rs138008832), which have been linked to various diseases, and IPF disease. A person's risk of developing IPF is likely to be increased by genetic variations. The detection of variations is thought to aid in the determination of individual diagnoses and treatment approaches.

Material and Methods

Study Group: In Turkey, the incidence of IPF has been reported to be 5/100,000 people (18). The effective sample size we will include in our study is for the incidence of IPF, which has a reported incidence of 5/100000; it was found that the total (patient-control) number of subjects should be at least 22 when calculated with a minimum of 80% power and dual hypothesis, within the 95% confidence interval and 5% confidence limits (19, 20). The study included 11 (7 men, 4 women) patients and 12 (8 men, 4 women) healthy (control) volunteers who were diagnosed with IPF at Van Yuzuncu Yil University Dursun Odabas Education and Research Hospital Chest Diseases Department between 2020 and 2021. The diagnosis of idiopathic pulmonary fibrosis was made based on the official ATS/ERS/JRS/ALAT clinical practice guidelines' idiopathic pulmonary fibrosis diagnostic criteria (21). Demographic information such as age, gender, smoking, family history, finger stick and occupational exposure, and gastroesophageal reflux (GER) status were recorded in the patient and control groups. Because studies have identified GER disease (GERD) as one of the potential external factors that predispose to IPF (22), it has been included among the factors investigated. The study included patients aged 18 or older who were diagnosed with IPF based on clinical and imaging findings and were in the stable period. Patients who were in the attack period but were not diagnosed with IPF based on clinical and imaging findings were not included in the study. The research conforms to the provisions of the Declaration of Helsinki (as revised in Brazil 2013). All participants gave informed consent for the research. The study was approved by Van Yuzuncu Yil Universtiy's non-interventional clinical research ethic committee (2020/02-05 21/02/2020).

Blood Samples, DNA Extraction, and Genotyping: During routine examinations of the patients and control groups, peripheral venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. Genomic DNA isolation was performed using the PureLinkTM Genomic DNA Mini Kit (Invitrogen, Catalog No: K182002). DNA purity and quantity were measured after isolation using the NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples with a measurement ratio of DNA A260/A280 ≅ 1.8 were considered pure. DNA samples were stored at + 4 °C until used for genotyping.

TaqMan probes (Applied Biosystems, Waltham, MA, USA) were chosen for each SNP (rs2302273, rs246395, rs3828610, rs138008832), and genotyping was done using StepOne plus real time PCR system (Applied Biosystems), with the assay IDs shown in Table 1. The genotyping analysis was carried out using the reaction mix created

Table 1. PDGFR-β Polymorphisms Probe Sequences and Polymorphic VIC/FAM Alleles

rs number	Probe sequences [VIC/FAM]	Assay ID
246395	AGATGCTCTCCGGAGCCATCCACTT[C/T]AAAGGCAAAAA AGGTCTGTAGGGAGG	C___7507227_10
2302273	CAGAGGGGCCGCCCTGGGTCTGGCT[A/G]TCTGCGTTGG GCAGGGCGAGCACAG	C___2599411_1_
3828610	CAAGTTTCTTGTTTTTCTTCTTTTC[A/C]CTCTGCTTACTCC CTCCCATCGCCC	C___2599412_10
138008832	TGCTGCAGGAAGGTGTGTTTGTTGC[A/G]GTGCAGGTAG TCCACCAGGTCTCCG	C_170971889_10

with the assay mix and genotyping kit [ABT 2X SNP Genotyping Probe Master Mix (with UDG), Catalog No: Q10-02-05]. qPCR was performed with a total volume of 10 μL for each sample using 2,55 μL of dH2O, 5 μL of master mix kit (ABT Genotyping Probe Master Mix 2X), 0,2 μl Rox dye (50X), 0.25 μL of genotyping assay kit (TaqMan, Applied Biosystems, Waltham, MA, ABD) and 2 μL DNA. QPCR conditions included a 2 min pre-PCR (UDG treatment) step at 50 °C, followed by a 10 min initial denaturation at 95 °C. The conditions were then set to 95 °C for 15 sec (denaturation), 60 °C for 1 min, and 50 °C for 30 sec for 40 cycles. Allele discrimination was accomplished using two probes labeled with the TaqMan FAM and VIC dyes.

Statistical Analysis: Graphad-prism 8 was used to conduct all the statistical analysis. To calculate sample size, a numerical results analysis on bilateral confidence intervals for single ratio was performed. The relationships between demographic information from the patient and control groups and PDGFR-β polymorphisms were examined using Chi-square and Fisher's exact tests. The relationship between SNPs and PDGFR- β was determined using the odds ratio (OR) and 95% confidence interval (95% CI). Statistical significance level was considered as 5% for all statistical computations.

Results

Clinical Samples and Characterization: Table 2 shows a comparison of demographic data (age, gender, smoking, alcohol) and GER between the patient and control groups. No statistically significant difference was found in any of the variables (Table 2). Only IPF patients provided information on factors such as clubbing, pharmacological treatment, and occupation (Table 2).

Distribution of Allele and Genotype Frequencies: The allele and genotype frequencies for the PDGFR-β gene polymorphisms (rs246395, rs3828610, rs2302273 and rs13800883) are listed in Table 3. Mutant allele frequencies in the control group were 29, 37.5, and 21 % for the rs246395, rs3828610 and rs2302273 SNPs, respectively. This distribution in the patients was 18, 64 and 23 %, respectively. There was no statistically significant difference in the allelic frequencies of these polymorphisms between the patient and control groups. The A allele frequency was found to be 100% in both the patient and control groups for the rs138008832 polymorphism. In the study group, there was no G allele change for this SNP.

The genotype frequency distributions for the variants were in the range of Hardy-Weinberg equilibrium (HWE) (rs246395 T/C p = 0.197; rs3828610 A/C p= 0.553; and rs2302273 G/A p=0.458). Mutant genotype frequencies in the control group were 17, 0 and 0 % for the rs246395, rs3828610 and rs2302273 SNPs, respectively. This distribution in the patients was 9, 36 and 9 %, respectively. Despite the fact that the mutant genotype of the rs3828610 and rs2302273 polymorphisms was never found in the control group, there was no significant difference between the patient and control groups. The T-carriers (TT+TC) of the rs246395 were found in 91% of the patients and in 83% of the controls, with no statistically significant difference. The p-value between rs3828610 A-carriers (AA+AC) and non-A-carriers (CC) in patients and controls was <0.05, however the comparison was not statistically significant because the 95% confidence interval included the 1 value. The A-carriers (GA+AA) of the rs2302273 were found in 36% of the patients and in 42% of the controls, with no statistically significant difference. Other genotype combinations were also compared, but no statistically significant difference was found (data not shown).

Table 2. Demographic and Clinical Data In The Patient and Control Groups

Characteristics		IPF n (%)	Controls n (%)	P / OR; 95%CI
Sample size		11	12	
Gender	Female	4 (36.4)	4 (33.3)	
	Male	7 (63.6)	8 (66.7)	1/ 1.14; 0.20-6.37
Age	<70	5 (45.5)	5 (41.7)	
	≥70	6 (54.5)	7 (58.3)	1/ 1.16; 0.22-6.08
	Positive	2 (18.2)	2 (16.7)	
Smoking	Negative	9 (81.8)	10 (83.3)	1/ 1.11; 0.13-9.6
Alcohol	Positive	0	0	
	Negative	11(100)	12 (100)	-
	Positive	5 (45.5)	4 (33.3)	
GER	Negative	5 (45.5)	8 (66.7)	
	Unknown	1	-	0.66/2; 0.36-11.23
Clubbing	Positive	6 (54.5)	-	
	Negative	5 (45.5)	-	-
Occupation	Farmer	5 (45.4)	-	
	Housewife	4 (36.4)	-	-
	Retired	2 (18.2)	-	
Other diseases	Positive	7 (63.6)	-	
	Negative	4 (36.4)	-	-
Pharmacological Treatment	Nintedanib	4 (36.4)	-	
	Pirfenidone	7 (63.6)	-	-

GER gastroesophageal reflux, Significant level = $p < 0.05$ by fisher exact test.

Discussion

It is believed that a number of risk factors play a role in the development of the disease because the etiology of IPF is not entirely understood. Changes in the pathogenesis of IPF are caused by risk factors such as GER, genetic factors, epigenetic factors, smoking, aging, occupational exposures, and various comorbidities (23-26). Previous research has reported that smoking, age, and gender are associated with IPF (7, 27, 28). Smoking, age, and gender, on the other hand, were not found to be significantly associated with IPF in our study. IPF is more common in men worldwide, and in our study, the incidence in men was higher than in women.

It has been reported that PDGF isoforms are overexpressed in epithelial and macrophage cells in the lungs of IPF patients (9). However, the underlying causes of increased PDGF expression and activity in lung fibrosis remain unknown, and research is ongoing. PDGFR-specific tyrosine kinase inhibitors have been shown in animal models and cell culture to reduce pulmonary fibrosis. It was also stated that, while PDGFR inhibition reduces pulmonary fibrosis, it is insufficient to treat IPF on its own (9, 29). RTKs that target PDGFR-β inhibit and block signal transduction pathways involved in cell proliferation (30). PDGFR-β extracellular domain has been shown to reduce bleomycin (BLM)-induced pulmonary fibrosis in gene-transferred cells in vivo (9). In a more recent study, it was shown that inhibiting PDGFR-β suppressed apoptosis and proliferation of epithelial cells and fibroblasts in mouse models of BLM-induced pulmonary fibrosis, and it was reported that inhibiting PDGFR-β may be beneficial for the treatment of pulmonary fibrosis (13). As a result, the PDGF/PDGFR signaling pathways are considered therapeutic targets in pulmonary fibrosis. (9). PDGFR gene expression has previously been studied extensively in human, animal, and cell culture studies. However, no studies have been conducted to investigate the link between PDGFR- gene polymorphism/mutation and IPF. For the first time, we investigated at the PDGFR- gene SNPs (rs246395, rs2302273, rs3828610, and

Table 3. Genotype and Allele Frequencies for PDGFR-β SNPs in Patients and Controls.

SNPs	Genotype	Allele	Patients n (%)	Controls n (%)	OR (95% CI)	P
rs246395	TT		8 (73)	7 (58)	-	0.76
	TC		2 (18)	3 (25)		
	CC		1 (9)	2 (17)		
	T-carriers (TT+TC)		10 (91)	10 (83)	2 (0.15-25.77)[a]	1[a]
		T	18 (82)	17 (71)		
		C	4 (18)	7 (29)	1.85 (0.46-7.49)	0.49
rs3828610	AA		1 (9)	3 (25)	-	0.06
	AC		6 (55)	9 (75)		
	CC		4 (36)	0 (0)		
	A-carriers (AA+AC)		7 (64)	12 (100)	0.06 (0.003-1.42)[b]	0.03[b]
		A	8 (36)	15 (62.5)		
		C	14 (64)	9 (37.5)	0.34 (0.10-1.13)	0.14
rs2302273	GG		7 (64)	7 (58)		0.48
	GA		3 (27)	5 (42)		
	AA		1 (9)	0 (0)	-	
	A-carriers (GA+AA)		4 (36)	5 (42)	0.8 (0.14-4.29)[c]	1[c]
		G	17 (77)	19 (79)		
		A	5 (23)	5 (21)	0.89 (0.22-3.63)	1
rs138008832	AA		11 (100)	12 (100)	-	-
	AG		0 (0)	0 (0)		
	GG		0 (0)	0 (0)		
		A	22 (100)	24 (100)		
		G	0	0	-	-

[a]rs246395 T-carriers (TT+TC) vs non-T carriers (CC), [b]rs3828610 A-carriers (AA+AC) vs. non-A carriers (CC), [c]rs2302273 A-carriers (AA+GA) vs. non-A carriers (GG), SNP single nucleotide polymorphism, Significant level = $p < 0.05$ by fisher exact test (column value <5), Chi square test (column value >5), OR Odds ratio, CI confidence interval.

rs138008832), which have been linked to a variety of diseases, in IPF patients.

IPF patients are not affected at the same rate by the aforementioned risk factors and comorbidities. This could be due to genetic differences between people. In our study, no significant relationship was found between patients and controls for allele/genotype frequencies and clinical/demographic characteristics after analyzing PDGFR-β in 11 IPF patients and 12 healthy individuals without a history of the disease. The small number of people in our study's sample group limits our study to some extent, but our sample size is adequate based on the disease's incidence rate. We believe that PDGFR-β polymorphism studies will be critical in elucidating the etiology of IPF, which is one of the rare diseases, and in identifying new therapeutic targets in diagnosis and treatment. As a result, larger-scale studies with a larger number of participants are required to determine the relationship between PDGFR-β gene polymorphisms and IPF risk.

Funding This work was supported by grants from the Research Foundation of Van Yuzuncu Yil University (BAP) (TYL-2020-9122).

Declarations

Ethics approval and consent to participate The study was approved by Van Yuzuncu Yil Universtiy's non-interventional clinical research ethics committee (Approval number: 2020/02-05, Approval date: Feb 21, 2020) in accordance with the 1964 Helsinki declaration and its subsequent amendments or comparable ethical standards. All study participants provided informed consent.

Acknowledgements We would like to thank Prof.Dr. Sıddık Keskin (Ph.D.), a faculty member at Van Yuzuncu Yil Universtiy's Faculty of Medicine, Department of Biostatistics, for his assistance in statistical analysis.

Conflict of Interest: The authors declare no competing interests.

Table 4. The Association Between Demographic Data and PDGFR-β Variants in Patients.

SNPs	Characteristics n=11 (%)								
	Smoking			Normal FVC	Low FVC		GER (n=10)		
	Positive	Negative	P/OR (95%CI)	(%≥70)	(%<70)	P/OR (95%CI)	Positive	Negative	p/OR (95%CI)
rs246395									
T-carriers (TT+TC)	2 (%18)	8 (%72)		3 (%27)	7 (%63)	0.36/0.15 (0.049-4.87)	5 (%50)	4 (%40)	1/ 3.66 (0.11-113.8)
Non-T carriers (CC)	0	1 (%10)	1/0.88 (0.26-29.17)	1 (%10)	0		0	1 (%10)	
rs3828610									
A-carriers (AA+AC)	2 (%18)	5 (%45)	0.49/4.1 (0.15-109)	2 (%18)	5 (%45)	0.57/ 0.4 (0.031-5.15)	3 (%30)	3 (%30)	1 / 1 (0.08-12.57)
Non-A carriers (CC)	0	4 (%37)		2 (%18)	2 (%18)		2 (%20)	2 (%20)	
rs2302273									
A-carriers (AA+AG)	0	4 (%37)	0,49/0.24 (0.09-6.51)	2 (%18)	2 (%18)	0.57/ 2.5 (0.19-32.2)	1 (%10)	3 (%30)	0.52/ 0.16 (0.01-2.82)
Non-A carriers (GG)	2 (%18)	5 (%45)		2 (%18)	5 (%45)		4 (%40)	2 (%20)	

FVC forced vital capacity, GER gastroesophageal reflux, SNP single nucleotide polymorphism, OR Odds ratio, CI confidence interval, Significant level = $p < 0.05$ by fisher exact test.

References

1. Behr J. The diagnosis and treatment of idiopathic pulmonary fibrosis. Dtsch Arztebl Int. 2013 Dec 23; 110: 875-881.
2. Waters DW, Blokland KEC, Pathinayake PS, et al. Fibroblast senescence in the pathology of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2018; 315: L162-L172.
3. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M. et al. Idiopathic pulmonary fibrosis. Nat Rev Dis Primers 2017; 20:3: 17074.
4. Selman M, Pardo A. The leading role of epithelial cells in the pathogenesis of idiopathic pulmonary fibrosis. Cell Signal. 2020; 66: 109482
5. Spagnolo P, Lee JS, Sverzellati N, Rossi G, Cottin V. The Lung in Rheumatoid Arthritis: Focus on Interstitial Lung Disease. Arthritis Rheumatol 2018; 70: 1544-1554.
6. Shioya M, Otsuka M, Yamada G, Umeda Y, Ikeda K, Nishikiori H, Kuronuma K, Chiba H, Takahashi H. Poorer Prognosis of Idiopathic Pleuroparenchymal Fibroelastosis Compared with Idiopathic Pulmonary Fibrosis in Advanced Stage. Can Respir J 2018; 2018: 6043053.
7. Phan THG, Paliogiannis P, Nasrallah GK, et al. Emerging cellular and molecular determinants of idiopathic pulmonary fibrosis. Cell Mol Life Sci 2021; 78: 2031-2057.
8. King TE Jr., Pardo A, Selman M. Idiopathic pulmonary fibrosis. *Lancet* 2011; 378: 1949-1961.
9. Inomata M, Nishioka Y, Azuma A. Nintedanib: evidence for its therapeutic potential in idiopathic pulmonary fibrosis. Core Evid 2015; 10: 89-98.
10. Ouyang L, Zhang K, Chen J, Wang J, Huang H. Roles of platelet-derived growth factor in vascular calcification. J Cell Physiol 2018; 233: 2804-2814.
11. Grimminger F, Günther A, Vancheri C. The role of tyrosine kinases in the pathogenesis of idiopathic pulmonary fibrosis. *Eur Respir J*. 2015; 45: 1426-1433.
12. Kanzaki R,Ose N, Kawamura T, Funaki S, Shintani Y, Minami M, et al. Stromal PDGFR-β expression is associated with postoperative survival of non-small cell lung cancer patients receiving preoperative chemo-or

chemoradiotherapy followed by surgery. World Journal of Surgery. 2018; volume 42, Issue 9, pp-2879-86
13. Kishi M, Aono Y, Sato S, et al. Blockade of platelet-derived growth factor receptor-β, not receptor-α ameliorates bleomycin-induced pulmonary fibrosis in mice. *PLoS One*. 2018;13(12):e0209786.
14. Estevez-Garcia P, Castaño A, Martin AC, Lopez-Rios F, Iglesias J, Muñoz-Galván S. et al. PDGFRα/β and VEGFR2 polymorphisms in colorectal cancer: incidence and implications in clinical outcome. BMC Cancer 2012; 12; 12: 514.
15. Garrigós C, Espinosa M, Salinas A, et al. Single nucleotide polymorphisms as prognostic and predictive biomarkers in renal cell carcinoma. *Oncotarget* 2017; 8: 106551-106564.
16. Wang X, Zhang Z, Liu W, Xiong Y, Sun W, Huang X, et al. Impacts and interactions of PDGFR-β, MMP-3, TIMP-2 and RNF213 polymorphims on the risk of moyamoya disease in han Chinese human subjects. Gene. 2013;1 0[526(2]:437-42
17. Sanchez-Contreras M, Baker MC, Finch NA, et al. Genetic screening and functional characterization of PDGFRB mutations associated with basal ganglia calcification of unknown etiology. *Hum Mutat*. 2014;35(8):964-971.
18. Okumuş G. Türk Toraks Derneği İdiyopatik Pulmoner Fibrozis (İpf) Tanı Ve Tedavi Uzlaşı Raporu. Okumuş G, Bingöl Z, editörler. İdiyopatik Pulmoner Fibrozis (İpf) Tanı Ve Tedavi Uzlaşı Raporu. Ankara; Bilimsel Tıp Yayınevi; 2018.
19. Hintze, J. (2011). PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com.
20. Newcombe, R. G. 1998. 'Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods.' Statistics in Medicine, 17, pp. 857-872.
21. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, Behr J, Cottin V, Danoff SK, Morell F et al., American Thoracic Society ERSJRS, Latin American Thoracic S. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. American journal of respiratory and critical care medicine 2018; 198: e44-e68.
22. Lee JS, Ryu JH, Elicker BM, et al. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011; 184: 1390-1394.
23. Barratt SL, Creamer A, Hayton C, Chaudhuri N. Idiopathic Pulmonary Fibrosis (IPF): An Overview. J Clin Med 2018; 7: 201.
24. Zaman T, Lee JS. Risk factors for the development of idiopathic pulmonary fibrosis: A review. Curr Pulmonol Rep 2018; 7: 118-125.
25. Sack C, Raghu G. Idiopathic pulmonary fibrosis: unmasking cryptogenic environmental factors. Eur Respir J 2019; 53: 1801699
26. Krishna R, Chapman K, Ullah S. Idiopathic Pulmonary Fibrosis. In: StatPearls. Treasure Island (FL) StatPearls Publishing; 2022 Jan-. Bookshelf ID: NBK448162PMID: 28846333
27. Ley B, Collard HR, King TE Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011; 183: 431-440.
28. Zhou LL, Wang M, Liu F, et al. Cigarette smoking aggravates bleomycin-induced experimental pulmonary fibrosis. Toxicol Lett 2019; 303: 1-8.
29. Wollin L, Wex E, Pautsch A, Schnapp G, Hostettler KE, Stowasser S, Kolb M. Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis.Eur Respir J 2015; 45: 1434-45.
30. Qian Y, Yu L, Zhang XH, Yuan ZQ, Zhao P, Sun LN et al. Genetic Polymorphism on the Pharmacokinetics and Pharmacodynamics of Platelet-derived Growth Factor Receptor (PDGFR) Kinase Inhibitors.Curr Drug Metab. 2018; 19: 1168-1181.