http:// ijp.mums.ac.ir
Original Article (Pages: 10335-10341)

Investigating the Effect of TNF α (-863) and TNF α (-308) genes Polymorphism on the Progression of Disease in Patients with Cystic Fibrosis

Maryam Hassanzad[1], *Golnaz Hajiesmail[2], Poopak Farnia[3,4], Jalaledin Ghanavi[3], Ghamartaj Khanbabayi[2], Mohammad Feshangchi-Bonab[2], Ehsan Razeghi[5], Ali Akbar Velayati[3]

[1]Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran. [2]Department of Pediatrics, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Mycobacteriology Research Centre (MRC), National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran. [4]Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [5]Student Research Committee, School of Medicine, Shahid Beheshti University of Medical Siences, Tehran, Iran.

Abstract

Background: Recent studies have shown that the course of cystic fibrosis in patients with this disease differs despite the same mutation in CFTR gene. We aimed to investigate the role of polymorphism in TNF α (-308) and TNF α (-863), and its effect on the phenotype of the patients with cystic fibrosis and progression of disease.

Materials and Methods: In this case-control study, 50 children with cystic fibrosis and 50 healthy children were examined for TNF-α-308 GA and TNF α - 863CA polymorphism. Four ml of citrated blood was taken from the patients in order to perform the DNA purifying and PCR-RFLP. With custom designed primers, PCR was done. Then with restriction enzymes PCR-RFLP was performed on the product of previous PCR. Changes were analyzed taking the following into consideration: diagnosis age, starting point of the pulmonary disease. Hb O2 saturation level, FEV1, and FVC. Also, for each of them, a Schwachman index basis score was calculated and results are mentioned.

Results: Patient's average age was 21±5.1 years old (ranged 5-26 years), and 48.9% (n=24) of patients were females. The average age at diagnosis was also 39.78 ±13.51 months. Patients with genotype TNF-α-308GA were older in diagnostic time compared to TNF-α-308GG genotype. However, for other variables, such as O_2 sat, FEV1, FVC no difference was observed. Patients Heterozygote genotypes for "C" allele (CA) of TNF-863 have better Schwachman score than CC genotype.

Conclusion

The results of this research emphasize the importance of genetic factors affecting inflammatory processes. Identifying these variables is helpful in treating patients with cystic fibrosis disease.

Key Words: Cystic fibrosis, FEV1, TNF-α promoter polymorphism.

*Please cite this article as: Hassanzad M, Hajiesmail G, Farnia P, Ghanavi J, Khanbabayi Gh, Feshangchi-Bonab M, et al. Investigating the Effect of TNF α (-863) and TNF α (-308) genes Polymorphism on the Progression of Disease in Patients with Cystic Fibrosis. Int J Pediatr 2019; 7(11): 10335-341. DOI: **10.22038/ijp.2019.41707.3513**

Corresponding Author:

Golnaz Hajiesmail (M.D), Department of Pediatrics, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: hajiesmail.golnaz@gmail.com

Received date: Mar.29, 2019; Accepted date: Sept. 22, 2019

1- INTRODUCTION

Cystic Fibrosis (CF) is the most common life-limiting autosomal recessive disease in the white race, it is usually caused by mutation in gene kb230 on chromosome 7 that codes 1480 amino acids of chlorine channel in epithelial cells, the so called CFTR (Cystic Fibrosis Trans membrane conducting Regulator). In ~~the~~ most cases, phenylalanine elimination on 508 happens (1-2-3). This mutation causes impaired chloride ion and dysfunction of the mucocilliary system. It leads to inflammation of the airways, infections, and damage to the lungs and other organs. As a matter of fact, the main cause of mortality in CF patients is lung-related dysfunctions such as bronchiectasis, airway obstructions and progressive lung involvement (4-5).

It is important to note that the course of cystic fibrosis is different in patients with the same mutations in the CFTR gene, and the possibility of interfering with changes in the environmental factors and other genes involved in host immunity is suggested. Most influencing factors are: TNF-a, TNF-b, MHC, nitric oxide syntheses and mannose binding lectin that have a role in immunity. Among these factors, TNF-a has a noticeable role (6, 7).

TNF alpha protein acts as an immune response regulator and plays a role in biological processes such as cell proliferation and differentiation, apoptosis, lipid metabolism, and blood coagulation. The TNF-a protein is synthesized as an inflammatory cytokine by macrophage which is the main mediator in response to acute inflammation. It also has a main role to stimulate neutrophils and monocytes to the site of infection and activate these cells to remove microorganisms and also stimulate endothelial cells of the vessels to express adhesion molecules to attract white blood cells at the infection site (8). As seen in other studies, the change in the expression of this gene affects the progression of the disease in patients with cystic fibrosis. For example, the region -308 is at the beginning of the expression of this gene and has a crucial role in the secretion of TNFα. There are two alleles in this area: TNF alpha 308 "A" and TNF alpha 308 "G". In a normal homozygous population, the "G" allele forms the dominant genotype. The heterozygosis of "GA" in the region of -308 is seen in few individuals, but in terms of performance, it has a significant effect on the function of this gene, so that the "A" allele expresses more of this gene (9, 10). Recent studies have shown that pulmonary function is significantly lower in patients with cystic fibrosis with -308A polymorphism than in other patients. In addition, in other proven studies, polymorphism in other regions of the TNF-α gene was associated with the severity of pulmonary involvement in patients (6, 8). In patients with a higher level of cytokines and secretion of TNF-α, despite the lower incidence of infectious agents, however, long-term lung destruction and eventually prognosis have been worse.

The aim of this study was to determine the effect of TNF promoter region polymorphism and its expression and its effect on the clinical signs and symptoms of patients with cystic fibrosis with the same mutation in the CFTR gene (homozygote for ΔF508), so that if these changes are observed, it is possible to find newer therapies to improve the quality of life in patients. In order to do that, we studied the polymorphism in -308 and -863 regions of the TNF-a gene, in patients diagnosed with CF, and its relationship with clinical prognosis in Masih Daneshvari hospital of Tehran, Iran, from October 2015 to 2018.

2- MATERIALS AND METHODS

2-1. Subjects

In this study, 50 children with cystic fibrosis (that are positive due to clinical

symptoms, sweat's chlorine test and CFTR gene mutation; all CF patients included in this study were homozygous for ΔF508 mutation), and 50 healthy children were examined. This study was conducted in the infant section of Masih Daneshvari hospital, October 2015-2018. 41 CF patients and 47 participants of the control group remained in the study. Patients were evaluated for FEV1, FVC, O2 saturation level, age at diagnosis time, starting point of the pulmonary disease and Schwachman index during admission and nursing visits.

2-2. Genotyping and DNA extraction

Four ml of citrated blood was taken from the patients and placed in tubes that contained an EDTA anticoagulant in order to perform the DNA purifying and PCR-RFLP. With custom designed primers, PCR was done. Then with restriction enzymes, PCR-RFLP was performed on the product of previous PCR.

DNA samples were genotyped for a promoter polymorphism in the -863C/A and -308A/G region position of the TNF-α gene and polymerase chain reaction (PCR-RFLP). For the amplification of polymorphism at the -863C/A, antisense'5′- GGC TCT GAG GAA TGG GTT AC-3′ and 5′-CTA CAT GGC CCT GTC TTC GTT ACG-3′ were used and for -308A/G region 5′-AGG CAA TAG GTT TTG AGG GCC AT-3′ and 5′-TCC TCC CTG CTC CGA TTC CG-3′ were used. PCR conditions were as follows:

- -863: denaturation at 4 min 94 ℃, 30 cycles at 94 ˚C for 40 s, 57 ˚C for 40 s, and 72 ˚C for 55 s, followed by one cycle of final extension at 72 ˚C for 6 min.
- -308: denaturation at 94 ˚C for 4 min, 30 cycles at 94 ˚C for 60 s, 56 ˚C for 60 s, and 72 ˚C for 60 s, followed by one cycle of final extension at 72 ˚C for 10 min.

2-3. Statistical analysis

Changes were analyzed with consideration for dependents like diagnosis age, the starting point of the pulmonary disease. Haemoglobin–oxygen saturation levels, FEV1, and FVC. Also, for each of them, a Schwachman index basis score was calculated and results are mentioned. The instrument used for data analysis was GraphPad Prism v8.1. For quantity variants, Chi^2 test, and for qualitative variants, Fisher's exact test was done.

2-4. Ethics

The study was approved by the Institute's Ethics Committee with the reference number of IR.SBMU.NRITLD.REC.1395.246.

3- RESULTS

In this study, we observed 50 patients diagnosed with Cystic Fibrosis and 50 members of control group (41 patients and 47 members of control group remained until the end of the study), that were hospitalized in Masih Daneshvari hospital (Tehran, Iran), between October 2015 to 2018. The average age of patients at diagnosis point was 39.78± 13.51 months. The results of patients' demographic factors are presented in **Table.1**.

3-1. Genotype and allele frequencies of TNFα (-308, -863) gene polymorphisms

First of all, it is worth mentioning that in order to remove the effect of CFTR genotype on phenotype of CF patients, all CF patients included in this research were homozygous for ΔF508 mutation. Genotype and allele frequencies in CF patients and healthy controls are delineated in **Tables 2, 3**. The genotypes are observed in each of the loci as well as the frequency of each in the patient population and control group. In examining the age of diagnosis in patient samples, some specimens were excluded due to lack of information. According to the **Table.4** which presents the relationship between different genotypes and age of diagnosis in

each of the genotypes, genotype GA in locus -308 in comparison to genotype GG in the same locus in patient samples shows an increase in age of diagnosis ($p<0.01$).

Table-1: Demographic data, actual FVC, FEV1 and O2 saturation in different groups of CF patients.

Participant Groups	Age (at diagnose), onth	FVC %	FEV1 %	O2 Sat %
TNF α-308 Allele "GA"	11.7 (2.4)	33.22 (4.74)	32.33 (4.95)	86.9 (3.86)
TNF α-308 Allele "GG"	4.69 (1.11)	45.48 (3.78)	44.69 (4.03)	89.52 (1.62)
TNF α-863 Allele "CA"	1.83 (1.09)	30	19	93.5 (2.5)
TNF α-863 Allele "CC"	6.93 (1.2)	43.03 (3.27)	42.67 (3.45)	88.34 (1.71)

TNF-α: Tumor necrosis factor alpha; FVC: Forced vital capacity; FEV1: Forced expiratory value in one score; O2 Sat: Oxygen saturation.

Table-2: Allele frequency in each polymorphism with separation in the patients and control group (n=97).

		All subjects		Case		Control	
SNP	Allele	Count	Proportion	Count	Proportion	Count	Proportion
-308	G	177	0.91	87	0.87	90	0.96
	A	17	0.09	13	0.13	4	0.04
-863	C	169	0.87	93	0.93	76	0.81
	A	25	0.13	7	0.07	18	0.19

SNP: Single nucleotide polymorphism.

Table-3: Genotype frequency in each of the studied polymorphisms with separation in the patients and control group (n=97).

		All subjects		Case group		Control group	
SNP	Genotype	Count	Proportion	Count	Proportion	Count	Proportion
-308	A/A	1	0.01	1	0.02	0	0
	G/A	15	0.15	11	0.22	4	0.09
	G/G	81	0.84	38	0.76	43	0.91
-863	A/A	4	0.04	1	0.02	3	0.06
	C/A	17	0.18	5	0.1	12	0.26
	C/C	76	0.78	44	0.88	32	0.68

SNP: Single nucleotide polymorphism.

Table-4: Age of diagnosis of disease in different genotypes in studied polymorphisms (n=41).

SNP	Genotype	Number	Response mean (SE)	Difference (95% CI)	P-value
-308	G/G	31	4.69 (1.11)	0.00	0.0055
	G/A	10	11.7 (2.4)	7.01 (2.34 - 11.68)	
-863	C/C	37	6.93 (1.2)	0.00	0.36
	C/A	3	1.83 (1.09)	-5.10 (-13.50 - 3.31)	
	A/A	1	0.6 (0)	-6.33 (-20.52 - 7.87)	

SNP: Single nucleotide polymorphism; CI: Confidence interval; SE: standard error.

Table.4 shows the comparison of Schechman score.This indicator is designed to evaluate patients with cystic fibrosis. Patients are classified according to criteria including: general activity, physical examination, nutrition, lung imaging to severe, moderate, good, and excellent groups. We observed no meaningful difference between Schwachman scores in different genotype in locus-308. But a higher score was recorded for heterozygote genotypes in comparison to homozygote ones in the locus -863 ($p<0.05$). The result of Schwachman scores is shown in the **Table.5**. We observed no meaningful connection between Hb O2 saturation level and different genotypes. We did not see a meaningful difference in FVC and FEV1 in different genotypes in different loci.

Table-5: The comparison between different Schwachman scores in different genotypes (n=41).

Model	Genotype	Number	Response mean (SE)	Difference (95% CI)	P-value
-308	G/G	31	40.81 (2.49)	0.00	0.08
	G/A	10	32.5 (2.5)	-8.31 (-17.38 - 0.77)	
-863	C/C	37	37.16 (1.48)	0.00	0.01
	C/A	3	60 (20.21)	22.84 (8.84 - 36.83)	
	A/A	1	35 (0)	-2.16 (-25.79 - 21.47)	

CI: Confidence interval; SE: standard error.

4- DISCUSSION

Our goal in this study was to investigate the effect of TNF-a gene polymorphism in regions -308 and -863 in patients with cystic fibrosis and to examine the course of the disease due to different expression of this gene. The results of this study indicated that patients with an A allele in the 863 region had better prognosis than others. A lot of scientific studies have looked at the relationship between the CFTR genotype and the phenotype of cystic fibrosis, and have pointed to the complexity of this relationship. Recent studies have been conducted to find out the role of other genes affecting immune response and their role in the severity of the disease has become more prominent. The TNF-a gene and its receptors have an important role in inflammatory processes, cell deviation, and differentiation and apoptosis in order to stop bacterial growth in macrophages. Several researches showed a connection between this gene's polymorphism and potential to be affected by diseases and severity of diseases (i.e. Cystic Fibrosis, Rheumatoid Arthritis, Crohn's disease, pulmonary tuberculosis, and osteoporosis). In people who had the most expression of the TNF gene, the severity of the disease was worse (11-12). In a study on TNF-α gene polymorphism in different regions, no effect on region-238 polymorphism was observed, but other areas studied in this study revealed the association between the 857 region polymorphism and the symptoms of the patients (13). In another study on -308 regions, it was shown lung function in patients with "A" allele was significantly lower than those with "G" allele (14). In studies of other diseases TNFa308A allele increases the susceptibility to asthma in Asian-born children, on the other hand, SO_2 gas inhalation has been shown to further decrease the lung function in individuals with this genotype (15-16). In our study, we investigated polymorphisms in 2 regions of TNF-a gene's promoter area (-308, -863) in patients with Cystic Fibrosis and healthy controls from the population. The aim of this study was to investigate the association between this polymorphism and the severity of

pulmonary symptoms, the age at which signs and symptoms were diagnosed, oxygen saturation, pulmonary function and, finally, examination of the Schwachman index in the subjects. This study was performed on 50 patients with cystic fibrosis. The results showed us that: the analysis of frequency of different alleles of polymorphism of -308 and -863 in the extracted DNA of 41 patients and 47 members of the control group showed no meaningful difference between different genotypes in patient samples nor normal ones. We observed no meaningful connection between age of diagnosis in patient samples and different genotype of loci -863. Although it seems that heterozygote genotype (GA) in locus -308 has a meaningful effect on increasing the age of diagnosis ($p<0.01$).

In the study, more patients with 308A suffered from asthma (17); but we found no meaningful relationship between different genotypes in different loci that we studied and FEV1, FVC, Hb O2 saturation level in patient samples. In another study on -863 gene polymorphism, individuals with AA genotype expressed less TNF gene expression and in studies done on other diseases, less gene expression has been associated with fewer autoimmune and inflammatory diseases. For example, the prevalence of Alzheimer's and glaucoma in patients with "A" allele has been lower in subjects with "C" alleles (18-19). Similar results were found in patients with JIA and patients with genotype -863 C/C showed a higher risk of juvenile idiopathic arthritis (JIA) (20).

5- CONCLUSION

In our study of Schwachman score in patient samples, no meaningful connection was observed in different genotypes of locus -308 but heterozygote genotype in locus -863 (CA) was accompanied with a meaningful increase in Schwachman score in patients with Cystic Fibrosis. As noted above, allele"A" in the 863 region is associated with a lower expression of TNFα gene and a lower inflammatory process in these patients has led to a better clinical course. These regions can be an important factor in determination of the phenotype of patients diagnosed with Cystic Fibrosis, which implies that the inflammation process has a further effect on the destruction of lung tissues. So we can get a better prognosis in the patients by controlling this inflammation process. Of course, to verify these results in the gene expression, further studies are needed to be done in greater populations. In conclusion, determination of TNF-a genotype in patients diagnosed with CF can be used to predict the prognosis and to select the most suitable treatment.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENT

The authors would like to thank National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences for supporting this project. We would like to appreciate all the parents who helped us.

8- REFERENCES

1. Ratjen F, Döring G. Cystic fibrosis. Lancet. 2003; 361(9358):681-9.

2. Geographical distribution of cystic fibrosis; the past 70 years of data analyzis. Mirtajani SB, Farnia P, Hassanzad M, Ghanavi J, Parissa Farnia1, Velayati AA. 2017; 1(2): 105-12.

3. Hassanzad, M., Ansari, M., Valinejadi, A., Sayedi, S., Velayati, A. Cystic fibrosis Patients Evaluation in the Last Decades in Iran. International Journal of Pediatrics, 2019; 7(9): 9701-9710.

4. Hassanzad M, Orooji A, Valinejadi A, Velayati A. A fuzzy rule-based expert system

for diagnosing cystic fibrosis. Electronic physician. 2017; 9(12):5974.

5. Hassanzad M, Farnia P, Samadani AA, Sayedi SJ, Velayati AA. Bone Mineral Density and Cystic Fibrosis: A Review Int J Pediatr 2019; 7(7): 9701-10.

6. Yarden J, Radojkovic D, De Boeck K, Macek M Jr, Zemkova D, Vavrova V, et al. Association of tumor necrosis factor alpha variants with the CF pulmonary phenotype. Thorax 2005; 60:320–25.

7. Laki J, Laki I, Németh K, Ujhelyi R, Bede O, Endreffy E, et al. The 8.1 ancestral MHC haplotype is associated with delayed onset of colonization in cystic fibrosis. Int Immunol. 2006; 18(11):1585-90.

8. Boyle MP, Cheng S, Steiner LL, McDougal K, Fallin MD, Merlo C, et al. Variants in mannose-binding lectin and tumour necrosis factor alpha affect survival in cystic fibrosis. J Med Genet 2007; 44(3):209-14.

9. Sanchez-Dominguez CN, Reyes-Lopez MA, Bustamante A, Cerda-Flores RM, Villalobos-Torres MdC, Gallardo-Blanco HL, et al. The Tumor Necrosis Factor α (-308 A/G) Polymorphism Is Associated with Cystic Fibrosis in Mexican Patients. PLoS ONE 2014; 9(3): e90945.

10. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor α promoter on transcriptional activation. Proceedings of the National Academy of Sciences 1997; 94(7):3195-9.

11. Elborn JS, Cordon SM, Western PJ, Macdonald IA, Shale DJ. Tumour necrosis factor-alpha, resting energy expenditure and cachexia in cystic fibrosis. Clin Sci (Lond) 1993; 85 (5):563-8.

12. Pierik M, Vermeire S, Steen K.V, Joossens S, Claessens G, Vlietinck R, Rutgeerts P. Tumor necrosis factor-alpha receptor 1 and 2 polymorphisms in inflammatorybowel disease and their association with response to infliximab. Aliment.Pharmacol. Ther 2004; 20, 303–10.

13. Hassanzad M, Farnia P, Ghanavi J, Parvini F, Saif S, Velayati AA. TNFα− 857 C/T and TNFR2+ 587 T/G polymorphisms are associated with cystic fibrosis in Iranian patients. Eur J Med Genet. 2019; 62(11):103584.

14. Hull J, Thomson AH. Contribution of genetic factors other than CFTR to disease severity in cystic fibrosis. Thorax 1998; 53 (12):1018-21.

15. Kang SW, Kim SK, Han YR, Hong D, Chon J, Chung JH, et al. Promoter Polymorphism (-308G/A) of Tumor Necrosis Factor-Alpha (TNF-α) Gene and Asthma Risk: An Updated Meta-Analysis.Genet Test Mol Biomarkers. 2019; 23(6):363-72.

16. Makamure MT, Reddy P, Chuturgoon A, Naidoo RN, Mentz G, Batterman S, et al. Tumour necrosis factor α polymorphism (TNF-308α G/A) in association with asthma related phenotypes and air pollutants among children in KwaZulu-Natal. Asian Pac J Allergy Immunol. 2016; 34(3):217-22.

17. Zhang Y, Zhang J, Tian C, Xiao Y, He C, Li X, Bogati A, Huang J, Fan H. The− 308 G/A polymorphism in TNF-α gene is associated with asthma risk: an update by meta-analysis. Journal of clinical immunology 2011; 1; 31(2):174-85.

18. Ramos EM, Lin MT, Larson EB, Maezawa I, Tseng LH, Edwards KL, et al. Tumor necrosis factor α and interleukin 10 promoter region polymorphisms and risk of late-onset Alzheimer disease. Archives of neurology 2006; 1; 63(8):1165-69.

19. Wang CY, Shen YC, Wei LC, Lin KH, Feng SC, Yang YY, Chiu CH, Tsai HY. Polymorphism in the TNF-α (− 863) locus associated with reduced risk of primary open angle glaucoma. Molecular vision. 2012; 18:779.

20. Batar B, Özman S, Barut K, Kasapçopur Ö, Güven M TNF-alpha 863C > A promoter and TNFRII 196T > G exonic variationsmay be risk factors for juvenile idiopathic arthritis Turk J Med Sci. 2017;47(6): 1819-25.