

**ORIGINAL ARTICLE**

Iran J Allergy Asthma Immunol  
April 2019; 18(2):218-224.

## **Is the Inflammasome Pathway Active in the Peripheral Blood of Sulfur Mustard-exposed Patients?**

Sholeh Etehad Asnaf<sup>1</sup>, Mitra Sabetghadam<sup>1</sup>, Habib Jaafarinejad<sup>2</sup>, Raheleh Halabian<sup>3</sup>, Shahram Parvin<sup>4</sup>,  
Ensieh Vahedi<sup>4</sup>, Nasrin pazoki<sup>1</sup>, and Jafar Salimian<sup>4</sup>

<sup>1</sup> *Department of Cell and Molecular Biology, Faculty of Biological Sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran*

<sup>2</sup> *Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran*

<sup>3</sup> *Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran*

<sup>4</sup> *Chemical Injuries Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran*

*Received: 4 November 2018; Received in revised form: 30 December 2018; Accepted: 6 January 2019*

### **ABSTRACT**

The mustard lung is a late consequence of exposure to sulfur mustard (SM) in veterans who had participated in the Iraq-Iran war. Three mechanisms are contributed in the pathogenesis of mustard lung including oxidative stress, protease-antiprotease imbalance, and dysregulated immune response. In the context of the immune response, the role of the inflammasome complex and their inflammatory cytokines are important. This study aims to investigate the inflammasome pathway and their inflammatory cytokine (i.e IL-1 and IL-18) in the peripheral blood of mustard lung patients as well as chronic obstructive pulmonary disease (COPD) patients.

This research was conducted as a cross-sectional analytical study on 15 SM patients and was compared with 15 COPD patients and 15 healthy controls. The real-time polymerase chain reaction was used to assess gene expression levels of inflammasome components (NLRP1, NLRP3, NLRC4, and ASC), inflammatory cytokines (IL-1 $\beta$ , IL-18, and IL-1 $\beta$ R), and IL-37 as an anti-inflammatory cytokine. Finally, the data were analyzed by SPSS version 21 software.

The gene expression level of molecules involved in inflammasome pathway showed a slight increase in the peripheral blood of SM and COPD patients compared to the control group. However, this difference was not statistically significant. Only IL-37 and NLRP1 had a significant increase in mustard lung and COPD patients; compared to healthy controls ( $p < 0.05$ ).

Due to the normal expression of genes involved in the inflammasome pathway, it can be stated that the inflammasome pathway is not active in the blood of mustard lung patients.

**Keywords:** Chronic obstructive pulmonary disease; Gene expression; Inflammasome; Mustard gas

---

**Corresponding Author:** Jafar Salimian, PhD;  
Chemical Injuries Research Center, Systems Biology and Poisonings  
Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.

---

Postal code: 14359-44711, Tel: (+98 21) 8248 2503, Fax: (+98 21) 8821 1524, E-mail: Jafar.salimian@gmail.com

\* Sholeh Etehad Asnaf and Mitra Sabetghadam contributed equally to this paper

### INTRODUCTION

Sulfur mustard (SM), the common name for bis (2-chloroethyl) Sulfide, is the most well-known chemical warfare agent that was used in the Iraq-Iran war. Near 100,000 Iranian victims were poisoned by SM and about 40,000 of them are now suffering from its late complications. The early sign and symptoms of SM appear in the eye, skin, and airways in the first 24 hours of exposure. However, in some patients the chronic pathologic effects of SM might initiate years after the first exposure and the patients may be referred with the diagnosis of SM lung.<sup>1,2</sup>

SM-exposed patients may show airway hyper-reactivity, chronic bronchitis, bronchiectasis, and lung subepithelial fibrosis as late complications of the chronic phase. Pathogenesis and clinical features of the mustard lung are similar to those of chronic obstructive pulmonary disease (COPD).<sup>3</sup>

While the pathophysiology of SM has not been completely characterized, DNA damage and epigenetic modifications, cell membrane damages, planned cell death, disruption of the balance between apoptosis and replenishment, oxidative stress, protease-antiprotease imbalance, and irregular immune system responses are well shown in these patients.<sup>4,7</sup> Although several mechanisms have been hypothesized for the pathogenesis of mustard lung and COPD, it seems that many of the local and systematic manifestations of the disease are associated with an increase in the level of inflammatory cells, cytokines, and proteins.<sup>8,9</sup>

In SM-lung patients, airway epithelial cell damage is accompanied by aberrant repair mechanisms. In addition, some studies indicated that dysbiosis in lung transient but not resident (TBNR) microbiome can cause a vicious cycle.<sup>10,11</sup> Sensing these damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) by inflammasome sensors leads to inflammasome complex activation and results in autoinflammatory cytokines release i.e. Interleukine-1 (IL-1 $\beta$ ) and IL-18. Therefore, Inflammasome complex, as the first line of the innate immunity, switches the innate immunity mechanisms on. This situation is called inflammasomeopathy or autoinflammation in chronic disease. Autoinflammation is a new term which describes the abnormal innate immunity in chronic diseases.<sup>12,13</sup> In innate immunity cells, inflammasome complex exist. This complex includes a sensor protein,

an adaptor protein, and procaspase-1. Sensor protein consists of a nucleotide-binding oligomerization domain (NOD) and a NODlike receptor (NLR). The adaptor protein is also known as an apoptosis-associated speck-like protein containing a C-terminal caspase recruitment domain (ASC). NALP1, NLRP3, and NLRC4 are large functional proteins of the NLR family. ProIL-1 $\beta$  (pro IL-1 $\beta$ , p35) changes into the mature active form (IL-1, p17) after enzymatic digestion and is released from the cell. Cleavage of pro-IL-1 $\beta$  is performed by caspase-1 and its activity depends on the activation of Pro-caspase-1. This enzyme is activated by C-terminal caspase recruitment domain (CARD) and ASC proteins.<sup>14,15</sup> On the other hand, IL-37 as an anti-inflammatory cytokine is able to downregulate inflammation and innate immunity. Previous reports revealed that IL-37 is a key cytokine in regulating inflammatory responses by binding to the  $\alpha$  chain of the IL-18 receptor and inhibiting IL-18 proinflammatory function.<sup>16,17</sup>

The aim of this study was to investigate the gene expression levels of inflammasome components, inflammatory cytokines (IL-1 $\beta$ , IL-18), and IL-37 as an anti-inflammatory cytokine in peripheral blood of COPD and mustard lung patients and compare the results with healthy subjects.

### MATERIALS AND METHODS

#### Ethical Considerations

This research was approved by the local ethical committee of Baqiyatallah Hospital (IR.BMSU.REC.1396.11). The subjects participated voluntarily in this study. Informed consent was completed by each case at the beginning of the project.

#### Study Type and Patients

The exclusion criteria of this cross-sectional-analytical study were acute bronchiolitis, pneumonia, history of pulmonary tuberculosis or resection of one or more lobes, history of smoking and participating in simultaneous clinical trials. All patients were in a moderate stable phase of the disease. According to medical documents and inclusion criteria, 3 groups of subjects were included in this study: Mustard lung group consisted of 15 patients who were exposed to SM in more than 30 years ago. Fifteen cases were included in the cigarette smoking COPD group and 15 healthy donors with normal lung function were chosen

as the control group. The peripheral blood was collected in EDTA-(purple top) tubes. Demographic data, type of illness, and spirometry indexes were obtained applying a confidential questionnaire. Patients with a history of exposure to SM during Iraq-Iran war and impaired spirometry indexes such as forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), and FEV1/ FVC ratio were included in this study.

### Quantitation of Genes mRNA

Patients were monitored by quantitative real-time polymerase chain reaction (qRT-PCR) for determining the transcription levels of genes in peripheral blood. Total RNA was extracted from whole blood by RNA extraction kit (Yekta Tajhiz Azma-Iran) according to the manufacturer's protocol. Then, complementary

DNA (cDNA) was synthesized from total RNA with the use of SuperScript III reverse transcriptase (Genall-Germany). qRT-PCR was carried out; using SYBR Green Master mix reagents (Genall, Germany) in Mini-8 Real-Time PCR system (Coyote Bioteck-Korea) with specific primers (Table 1).

A total reaction volume of 25  $\mu$ L was obtained by mixing 2  $\mu$ L of cDNA template corresponding to 50 ng of total RNA, 12.5  $\mu$ L of SYBR Green PCR Master Mix (1.5mmol/MgCl<sub>2</sub>), 1  $\mu$ L forward primer (10 pmol/l), 1  $\mu$ L reverse primer (10 pmol/l), and 8.5  $\mu$ L ddH<sub>2</sub>O. PCR condition was initially denaturated at 95° C for 5 minutes followed by 37 amplification cycles; consisting of denaturation at 94° C for 30 seconds, annealing at a suitable temperature for 30 seconds, and extension at 72° C for 30 seconds.

**Table 1. Sequences of PCR primers used for assaying gene expression level of desired cytokines and genes in a study on the inflammasome pathway active in the peripheral blood of sulfur mustard-exposed patients**

Designation		Sequence	Ann. Temp (° C)	Band length (bp)
IL-1B	Forward	5'- ACACATGGTATAGATGCAGC-3'	57	102
	Reverse	5'- TTCCAAGACCTCAGGCAAGA-3'		
IL-1B Receptor	Forward	5'- TCCGACCACCACTACAGCAA-3'	57	416
	Reverse	5'- ATCTTTCAACACGCAGGACA-3'		
IL-18	Forward	5'-GCTTCTTACTAAATTATCAGTC-3'	58	318
	Reverse	5'-GAAGAGGGTAATTGGATCTTAT-3'		
IL-37	Forward	5'- CTCCTGGGGTCTCTAAAG-3'	53.5	212
	Reverse	5'- TACAATTGCAGGAGGTGCAG-3'		
NLRP1	Forward	5'- CAGGCAGCACAGATCAACAT-3'	57.5	104
	Reverse	5'- GTGACCTTGAGGACGGAGAA-3'		
NLRC4	Forward	5'- TAGCCGAGCCCTTATTTCAA-3'	57	97
	Reverse	5'- ACCTTCTCGCAGCAAATGAT-3'		
NLRP3	Forward	5'- AAGGGCCATGGACTATTTCC-3'	57	101
	Reverse	5'- GACTCCACCCGATGACAGTT-3'		
ASC	Forward	5'- AACCCAAGCAAGATGCG-3'	60	82
	Reverse	5'- TTAGGGCCTGGAGGAGCAAG-3'		
Caspase-1	Forward	5'- GCTTTCTGCTCTTCCACACC-3'	61	160
	Reverse	5'- GATCTGGCTGCTCAAATGAA-3'		
GAPDH	Forward	5'- TCGACAGTCAGCCGCATCTTCTTT-3'	62	98
	Reverse	5'- ACCAAATCCGTTGACTCCGACCTT-3'		

## Inflammasome Pathway in Sulfur Mustard-exposed Patients

**Table 2. The demographic and clinical characteristics of mustard lung and COPD patients**

Variable	Healthy Control (n=15)	COPD (n=15)	Mustard lung (n=15)
Age (yrs.)	49.5 ± 3.12	51.03 ± 4.17	50.34 ± 6.83
FEV1 (%) Pred	89.95 ± 6.62	20.5 ± 8.12	41.30 ± 9.02
FVC (%) pred	85.89 ± 9.68	37.05 ± 4.13	52.65 ± 10.22
FEV1/FVC (%)	105.02 ± 6.30	55.09 ± 4.67*	65.71 ± 9.68*

All data are expressed as mean ± SD (range). COPD: the GOLD stage II COPD patients; Mustard lung: patients exposed to sulfur

The quantification of genes was normalized by Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) expression; using the 2- $\Delta\Delta$ Ct method.

### Statistical Analysis

Gene expression level was surveyed; using relative expression and calculated by  $\Delta\Delta$ CT formula and relative quantification (RQ) [RQ=2<sup>-( $\Delta\Delta$ CT)</sup>]. The data analyses were carried out with SPSS software for Windows (Version 21, SPSS Inc., Chicago, Illinois, USA). Data were analyzed; using analysis of variance (ANOVA). *p*-Value<0.05 was identified as statistical significance difference.

## RESULTS

### Clinical Findings

FEV1 (%), FVC (%), and FEV1/FVC (%) in the sulfur mustard-exposed patients were (41.30±9.02),

(52.65±10.22), and (65.71±9.68); respectively. The FEV1/FVC ratio was higher than the control group in both mustard lung and COPD groups (*p*<0.05) (Table2).

### Inflammasome and Cytokines mRNA Expression Level in mustard Lung, COPD, and Healthy Controls

In this study, the gene expression of cytokines related inflammation was investigated by qRT-PCR. Base on the results presented in Table 3, the expression level of all genes except IL-37, had a slight increase in the COPD group compared to the other two groups with no statistically significant relationship. IL-37 was highly expressed in mustard lung group compared to the COPD group and healthy controls. There was a significant relationship between the genes expression level of IL-37 and NLRP1 in mustard lung and COPD group compared to healthy controls (*p*<0.05).

**Table3. Comparison of the expression level of studied genes in a study on the inflammasome pathway active in the peripheral blood of sulfur mustard-exposed patients**

Gene names	Healthy Control (n=15)	COPD (n=15)	Mustard lung (n=15)	<i>p</i> value
IL-1B	0.01±0.12	0.05±0.02	0.1±0.22	NS*
IL-1BR	0.02±0.62	0.06±0.29	0.03±0.31	NS
IL-18	0.04±1.06	0.08±0.98	0.05±1.10	NS
IL-37	2.02±0.52	8.56±0.62 <sup>#</sup>	9.80±0.99 <sup>#</sup>	<i>p</i> value =0.03
NLRP1	0.07±0.64	14.33±0.68 <sup>##</sup>	7.36±0.42 <sup>##</sup>	<i>p</i> value =0.02
NLRC4	0.02±1.43	0.15±1.05	0.04±1.7	NS
NLRP3	0.04±0.78	0.17±0.89	0.05±0.73	NS
ASC	0.03±0.04	0.05±0.11	0.04±0.12	NS
Caspase	0.04±0.33	0.06±0.12	0.05±0.62	NS

All data are expressed as mean ± SD.

## DISCUSSION

Regarding the clinical interrelationship between pulmonary patients caused by SM and COPD phenotypes, it seems that the mechanisms of pathogenesis of these diseases are similar.<sup>2,18</sup> Molecular mechanisms in the pathophysiology of chemical injuries include an imbalance in the oxidative-antioxidant and protease-antiprotease system as well as dysregulated immune response which plays a major role in lung injury in this subgroup of patients.<sup>7,19</sup> The issue of inflammation and inherent immune responses that contribute to clinical complications of pulmonary patients needs to be studied more accurately. Inflammatory responses are involved in the pathogenesis of the lung which can lead to severe pulmonary dysfunction. So, one of the most important features that should be investigated among mustard lung patients is the immune response.<sup>10,20</sup>

In this study, inflammasomes were investigated in chemical warfare victims with pulmonary problems due to their role as sensors of innate immunity in the diagnosis of stress-induced and harmful messages, as well as their role in diagnosing the onset of inflammatory processes.

In the present study, the gene expression of inflammasome components (NLRP1, NLRP3, and NLRC4), certain inflammatory cytokines (IL-1 $\beta$ , IL-18, and IL-1 $\beta$ R), ASC, and IL-37 as an anti-inflammatory cytokine were studied in the peripheral blood of mustard lung and COPD groups and compared with healthy controls.<sup>21</sup>

The results of previous studies showed that inflammasome is active in COPD patients and causes a systematic inflammation.<sup>22</sup> Base on the present study, the genes involved in inflammatory pathway increase slightly in COPD group with no statistically significant difference. As a result, it seems that the inflammasome pathway is not active in the blood sample of mustard lung and COPD patients.

Eltom *et al.* showed the role of inflammasomes in airway inflammation and COPD by reporting an increase in IL-18 levels in mice exposed to the cigarette smoke. It was also mentioned that by inhibiting NLRP3, a decrease in the level of IL-18 has occurred.<sup>22</sup> Another study on the pathogenesis of inflammation in COPD patients revealed that the expression of NLRP3 gene in the lungs of COPD patients was significantly

increased compared to smokers and non-smokers. However, there was no significant difference between the two control groups. In addition, there was a significant correlation between the expression of NLRP3 and two evaluated cytokines (IL-1 $\beta$  and IL-18) in COPD patients.<sup>23</sup>

In a study by Levandowski *et al.* in 2013, NLRP1 was introduced as a positive regulator of inherent immunity in response to a variety of DAMPs and PAMPs.<sup>24</sup> Evaluating the role of inflammasome complex (NLRP3/ASC) in obstructive lung disease has shown that the up-regulation of NLRP3 is more effective in inflammatory conditions that can increase inflammatory cytokines. The results of a previous study revealed that the gene expression of IL-1 $\beta$  and IL-18 is unregulated in COPD and cigarette smoking patients.<sup>25</sup> These findings are in accordance with the findings of the current investigation.

In the present study, we observed no significant increase in the expression of proinflammatory cytokines (IL-1 $\beta$ , IL-18, and IL-1 $\beta$ R), ASC, and inflammasome components (NLRC4, NLRP3) in peripheral blood of mustard lung patients as compared to controls. However, there was a significant relationship between the gene expression level of IL-37 and NLRP1 in mustard lung and COPD patients compared with healthy controls. Various studies surveyed the role of inflammatory cytokines in bronchoalveolar lavage (BAL) samples of mustard lung patients. In one study the level of certain cytokines (IL-8, IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IL-12) in BAL samples were examined in chemical veterans and healthy donors. Their results showed that these cytokines have significantly increased in mustard lung patients as compared with healthy donors.<sup>26</sup>

Spirometry indexes have been studied in several studies both in mustard lung and COPD patients. According to Farahani *et al.* FEV1/FVC ration had a decrease in mustard-exposed and COPD patients in comparison with healthy donors. Their findings were in accordance with the spirometry results of this study; suggesting that there is an obstruction in lung airways both in mustard lung and COPD patients.<sup>27</sup> In the present study, we showed overexpression of IL-37 as an anti-inflammatory cytokine, in the blood sample of mustard lung patients. Overexpression of human IL-37 results in downregulation of inflammation. The increased expression of IL-37 seen in the bronchial

## Inflammasome Pathway in Sulfur Mustard-exposed Patients

submucosa, but not in BAL, of patients with stable COPD compared with control smokers suggests the counter-regulatory role of this molecule.<sup>25,28,29</sup>

In conclusion, regarding the lack of significant expression of genes involved in the inflammatory pathway, it can be stated that the inflammasome pathway is not active in the blood of mustard lung patients. It seems that upper gene expression level of IL-37 as an anti-inflammatory cytokine can reduce the expression level of other genes and inflammatory cytokines. However, it is suggested to evaluate the inflammasome gene expression level in a study of lung tissue, especially on lung epithelial cells. Furthermore, we recommend studying the inflammasome pathway in acute and chronic phases in the lung of SM-exposed animal models. Moreover, the *in vitro* effects of SM on the inflammasome complex in lung epithelial cell remains to be defined in ongoing studies in our laboratories.

### ACKNOWLEDGEMENTS

The authors sincerely appreciate of lung section of Baqiyatallah hospital for the kindly corporation.

### REFERENCES

1. Nourani MR, Mahmoodzadeh Hosseini H, Azimzadeh Jamalkandi S, Imani Fooladi AA. Cellular and molecular mechanisms of acute exposure to sulfur mustard: a systematic review. *Journal of Receptors and Signal Transduction* 2017;37(2):200-16.
2. Ghanei M, Mokhtari M, Mohammad MM, Aslani J. Bronchiolitis obliterans following exposure to sulfur mustard: chest high resolution computed tomography. *Eur J Radiol* 2004;52(2):164-9.
3. Sun M, Yang Y, Meng W, Xu Q, Lin F, Chen Y, et al. Advanced biotherapy for the treatment of sulfur mustard poisoning. *Chem Biol Interact* 2018; 111-118.
4. Imani S, Panahi Y, Salimian J, Fu J, Ghanei M. Epigenetic: A missing paradigm in cellular and molecular pathways of sulfur mustard lung: a prospective and comparative study. *Iran J Basic Med Sci* 2015;18(8):723.
5. Behboudi H, Nouredini SK, Ghazanfari T, Ardestani SK. DNA damage and telomere length shortening in the peripheral blood leukocytes of 20 years SM-exposed veterans. *Int Immunopharmacol* 2018;61:37-44.
6. Simons T, Steinritz D, Bölck B, Schmidt A, Popp T, Thiermann H, et al. Sulfur mustard-induced epigenetic modifications over time— a pilot study. *Toxicol Lett* 2018;293:45-50.
7. Shahriary A, Seyedzadeh MH, Ahmadi A, Salimian J. The footprint of TGF- $\beta$  in airway remodeling of the mustard lung. *Inhal Toxicol* 2015;27(14):745-53.
8. Imani S, Salimian J, Bozorgmehr M, Vahedi E, Ghazvini A, Ghanei M, et al. Assessment of Treg/Th17 axis role in immunopathogenesis of chronic injuries of mustard lung disease. *J Recept Signal Transduct Res* 2016;36(5):531-41.
9. Imani S, Salimian J, Fu J, Ghanei M, Panahi Y. Th17/Treg-related cytokine imbalance in sulfur mustard exposed and stable chronic obstructive pulmonary (COPD) patients: correlation with disease activity. *Immunopharmacol Immunotoxicol* 2016;38(4):270-80.
10. Iman M, Rezaei R, Azimzadeh Jamalkandi S, Shariati P, et al. Th17/Treg immunoregulation and implications in treatment of sulfur mustard gas-induced lung diseases. *Expert Rev Clin Immuno* 2017;13(12):1173-88.
11. Saedi P, Salimian J, Ahmadi A, Imani Fooladi AA. The transient but not resident (TBNR) microbiome: a Yin Yang model for lung immune system. *Inhal Toxicol* 2015;27(10):451-61.
12. Di Stefano A, Caramori G, Barczyk A, Vicari C, Brun P, Zanini A, et al. Innate immunity but not NLRP3 inflammasome activation correlates with severity of stable COPD. *Thorax* 2014; 69(6):519-24.
13. Scambler T, Holbrook J, Savic S, McDermott MF, Peckham D. Autoinflammatory disease in the lung. *Immunology* 2018.
14. Ather JL, Martin RA, Ckless K, Poynter ME. Inflammasome activity in non-microbial lung inflammation. *J Environ Immunol Toxicol* 2014;1(3):108.
15. Brusselle GG, Provoost S, Bracke KR, Kuchmiy A, Lamkanfi M. Inflammasomes in respiratory disease: from bench to bedside. *Chest* 2014;145(5):1121-33.
16. Boraschi D, Lucchesi D, Hainzl S, Leitner M, Maier E, Mangelberger D, et al. IL-37: a new anti-inflammatory cytokine of the IL-1 family. *Eur Cytokine Netw* 2011;22(3):127-47.
17. Tete S, Tripodi D, Rosati M, Conti F, Maccauro G, Saggini A, et al. IL-37 (IL-1F7) the newest anti-inflammatory cytokine which suppresses immune responses and inflammation. SAGE Publications Sage UK: London, England; 2012.
18. Ghanei M, Harandi AA. Long term consequences from exposure to sulfur mustard: a review. *Inhal Toxicol* 2007;19(5):451-6.
19. Ghanei M, Harandi AA. Molecular and cellular

- mechanism of lung injuries due to exposure to sulfur mustard: a review. *Inhal Toxicol* 2011;23(7):363-71.
20. Shohrati M, Ghanei M, Shamspour N, Jafari M. Activity and function in lung injuries due to sulphur mustard. *Biomarkers* 2008;13(7-8):728-33.
  21. Eghtedardoost M, Hassan ZM, Ghazanfari T, Sadeghipour A, Ghanei M. Evaluation of mRNA Expression Levels of TNF $\alpha$ , TNFR1 and IL1 $\beta$  in Lung Tissue 20 Years after Sulfur-mustard Exposure. *Iran J Allergy Asthma Immunol* 2018;17(4):379-87.
  22. Eltom S, Belvisi MG, Stevenson CS, Maher SA, Dubuis E, Fitzgerald KA, et al. Role of the inflammasome-caspase1/11-IL-1/18 axis in cigarette smoke driven airway inflammation: an insight into the pathogenesis of COPD. *PLoS one* 2014;9(11):e112829.
  23. Faner R, Sobradillo P, Noguera A, Gomez C, Cruz T, López-Giraldo A, et al. The inflammasome pathway in stable COPD and acute exacerbations. *ERJ Open Res* 2016;2(3):00002-2016.
  24. Levandowski CB, Mailloux CM, Ferrara TM, Gowan K, Ben S, Jin Y, et al. NLRP1 haplotypes associated with vitiligo and autoimmunity increase interleukin-1 $\beta$  processing via the NLRP1 inflammasome. *Proc Natl Acad Sci U S A* 2013;110(8):2952-6.
  25. Nold-Petry CA, Lo CY, Rudloff I, Elgass KD, Li S, Gantier MP, et al. IL-37 requires the receptors IL-18R $\alpha$  and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. *Nat Immunol* 2015;16(4):354-65.
  26. Emad A, Emad Y. Increased in CD8 T lymphocytes in the BAL fluid of patients with sulfur mustard gas-induced pulmonary fibrosis. *Respir Med* 2007;101(4):786-92.
  27. Farahani P, Halabian R, Vahedi E, Salimian J. Increased Genes Expression Levels of Cytokines Related to Th17/Treg Cells in Peripheral Blood Mononuclear Cell Correlate with Clinical Severity in COPD and Mustard Gas-exposed Patients. *Iran J Allergy Asthma Immunol* 2017;16(5):396-403.
  28. Dinarello CA, Nold-Petry C, Nold M, Fujita M, Li S, Kim S, et al. Suppression of innate inflammation and immunity by interleukin-37. *Eur J Immunol* 2016;46(5):1067-81.
  29. Bulau A-M, Nold MF, Li S, Nold-Petry CA, Fink M, Mansell A, et al. Role of caspase-1 in nuclear translocation of IL-37, release of the cytokine, and IL-37 inhibition of innate immune responses. *Proc Natl Acad Sci U S A* 2014;111(7):2650-5.