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# Evaluation of Interleukin 33 and ST2 Serum Concentration in Active Tuberculosis Patients

Masoud Youssefi (MD,PhD)[1,2], Gholsa Teimouri (MD)[1,2], Farnaz Zahedi Avval (MD,PhD)[3] , *Kiarash Ghazvini (MD,PhD)[1,2], Masoud Keikha (Ph.D)[1,2]

[1]*Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Science, Mashhad, Iran.*
[2]*Antimicrobial Resistance Research Center, Bu-Ali Research Institute, Mashhad University of Medical Science, Mashhad, Iran.*
[3]*Department of Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*

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**ABSTRACT**

The aim of this study was to determine the serum levels of ST2 protein and interleukin-33 in patients affected to tuberculosis and compare them with the control group. In the present study, at first 30 patients affected to TB were randomly selected and 52 healthy individuals, who were matched with respect to their age and gender, included in the study as the control group. After that, the serum levels of sST2 and IL33 were measured by ELISA sandwich method using commercial Quantikine Human ELISA kit (R&D Systems). The data were finally analyzed by SPSS software. IL-33 levels in the TB group were higher than the healthy controls and a statistically significant difference was observed in the IL-33 levels between the two groups (P = 0.021). Moreover, IL-33R (ST2) was slightly increased in the TB patients compared to the healthy controls, although statistical analysis showed no significant difference between the two groups (P = 0.083). Regarding the high normal variation of sST2 and the limitations of the present study, it is recommended that future studies of sST2 be performed in with higher number of TB patients.

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## Introduction

Despite passing over a century since Robert Koch's introduction of Mycobacterium tuberculosis, and since numerous therapeutic lines and global planning for the prevention and control of tuberculosis, tuberculosis is still remaining one of the top ten causes of mortality in the world (1).According to the World Health Organization (WHO), about 10 million new cases of TB and also 1.6 million deaths from the disease were reported in 2018.

More worrying issue is that a quarter of the world's population is infected with Mycobacterium tuberculosis, although these They have latent TB infection (LTBI) but about 5-10% of these people will develop the active disease (2-3).Numerous reactions have been mentioned in the progression from the latent-TB phase to the active form of the disease (active-TB), in which case the host immune system plays a crucial role, especially Cytokines, which affect numerous T cell lines, altering the cellular signaling network, and therefore plays a critical role in the pathogenesis of infection with Mycobacterium tuberculosis (4-5). In the meantime, the IL-33/ ST2 axis is considered a new approach in the

***Corresponding author:** Masoud Keikha,*
*Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*
***E-mail:** masoud.keykha90@gmail.com*
***Tel:** 09386836425*

pathogenesis and treatment of TB (tuberculosis), although it has received less attention by the researchers (6). Interleukin-33 (IL-33) is a member of the cytokine IL-1 family that is produced and secreted by a range of immune cells such as macrophages, dendritic cells as well as keratinocytes, fibroblast and epithelial cells of the respiratory tract, stomach, and salivary glands in response to the cellular damages and cell deaths; therefore, it is considered as a biomarker for the cellular pathogenesis (7-8).

Based on the available evidence, IL-33 plays an important role in inducing and enhancing the function of Th2/Th9 cells and T regulatory cells (Treg) (6). The specific receptor for IL-33 remained unknown until the early 2005; nevertheless, it was found after the in vitro and in sillico studies that it interacted with ST2 as a receptor (7,9).ST2 or IL-1 R4 is a member of the IL-1R family encoded by the IL1RL1 gene.

ST2 is divided into four isoforms, including 1) ST2L (cell surface receptor), 2) sST2 (secretory form), 3) ST2V (mostly secreted by gastrointestinal cells), and 4) ST2LV (derived from eye, heart, lung and liver cells is produced in the embryonic period). However, most of our information is limited to ST2L and sST2 (6,8).

ST2L is expressed in anchored form membrane on the surface of fibroblast cells, mast cells, eosinophils, dendritic cells, Th2, Th9, Treg and macrophages, whereas sST2 is produced by soluble mast cells and fibroblasts in response to TNF-α. (8,10). sST2 is differentiated from ST2L by an extracellular domain containing 5 amino acids (6,8). sST2 acts as a ST2L degradation factor and disrupts the IL-33/ST2L axis (10).

Based on the studies, lipopolysaccharide (LPS) as well as the inflammation and production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α lead to the increase in sST2 expression (11). extensive evidence has so far been observed of the dysregulation IL-33/ST2L axis, such as inflammatory bowel disease (IBD), diabetes type 2, small bowel transparent rejection, cardiac disease, graft-versus host disease (GVHD), asthma, dermatitis, rheumatoid arthritis, Alzheimer's, cancer and obesity and metabolic disorders (6,8,10).

Since ST2L is expressed on the surface of cells such as Th2, Th9 as well as T regulatory cells, the IL33/ST2L axis appears to be one of the most important risk factors for the development of latent-TB infection towards reactivation and the emergence of active-TB infection. The present study was the first document to determine the level of sST2 and IL33 in tuberculosis patients and the required comparisons with the healthy donors.

## Materials and Method

This study was performed on 30 patients affected to tuberculosis as the case group and 52 healthy individuals without tuberculosis as the control group, such that all the patients with TB showed positive results with regards to the sputum culture and pulmonary tuberculosis was confirmed by clinical, laboratory, and radiologic findings.

The control group was also screened and confirmed for the absence of infection and inflammation by CRP and ESR tests. Moreover, the healthy individuals were matched with the TB patients for age and gender. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences and received conscious consent from all the individuals in the case and control groups included in this study. Serum IL-33 and sST2 levels were measured in both groups of TB patients and healthy controls by the Quantikine Human ELISA kit (R&D Systems) based on the manufacturer's instructions. This assay is designed according to the quantitative sandwich enzyme immunoassay. All the samples were diluted 20 times and the optical density was measured and read at 450 nm.Statistical analysis was performed by SPSS software (Ver.18).

Normal distribution of data was first measured using the Kolmogorov-Smirnov test. Then, the descriptive statistics indices were calculated, which included the central and dispersion indices. The data were analyzed using chi-squared tests, t-test t-test or the nonparametric equivalents (ANOVA). The significance level of all statistical tests was considered to be less than 0.05 (12).

## Results

Out of 82 patients who entered into the study, 30 patients with tuberculosis were studied as case group and 52 healthy individuals as control group. The age range of these patients was between 22 and 88 years. The mean age of the case and control groups was 59.83 + 18.92 and 54.84 + 15.89, respectively, which was not statistically significant (Mann-Whitney test; P= 0.268). Frequency of male gender was 70% in the case group and 63.5% in the control group. The frequency distribution of males and females was similar in both groups and was not statistically significant (Chi-square test; P = 0.554). Also, the values of each of the variables are given in detail in Table 1.

**Table 1:** Descriptive demographic findings of the individual in the case and control groups

| Specification/Variable | Case Group (N=30) | Control Group (N=52) | P-Value | |
|---|---|---|---|---|
| Age (Year) | 59.83 + 18.92 | 54.84 + 15.89 | 0.268* | |
| Gender | Male | 21 (70%) | 33 (63.5%) | 0.548** |
| | Female | 9 (30%) | 19 (36.5%) | |

*: Mann-Whitney Test

The mean IL-33 levels of the serum in the case and control groups were 137.62 + 48.58 picogram/mL and 120.59 + 24.88 picogram/mL, respectively. Statistical analysis showed that the frequency of IL-33 was significantly different in the two groups (Mann-Whitney test; P = 0.021); i.e. the serum IL-33 level in the case group was significantly higher than in the control group. Also, the level of sST2 was higher in the case group (TB) than the control group (Figure 1).

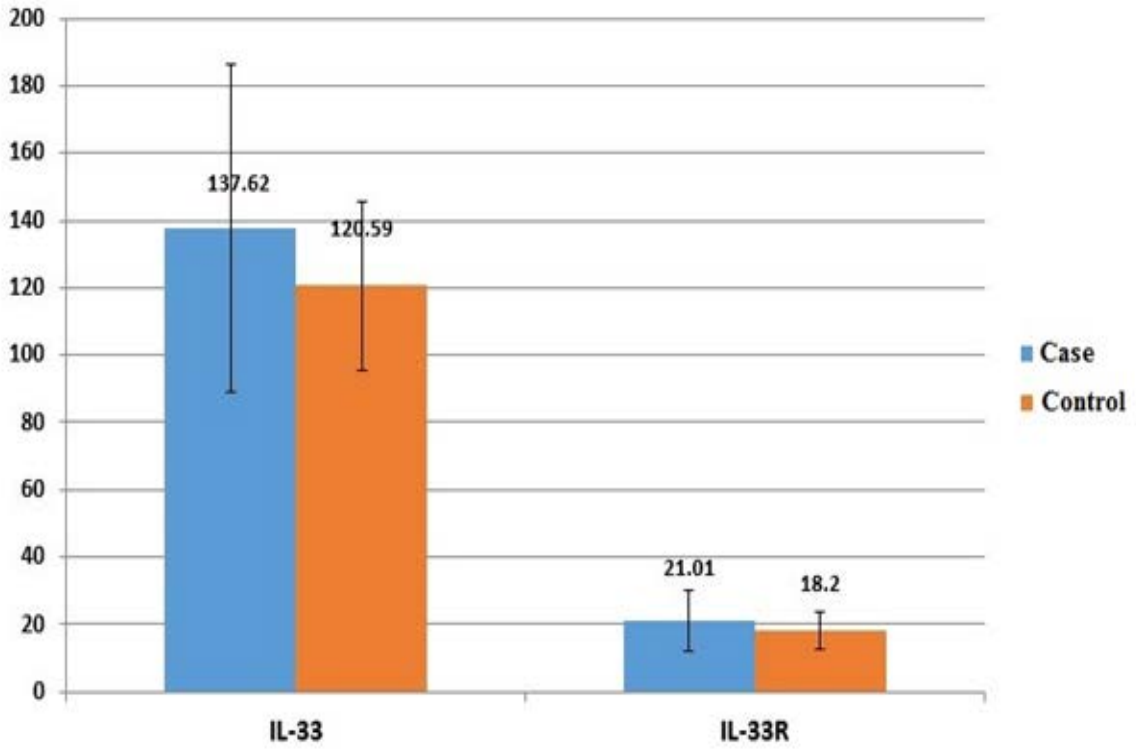


**Figure1.** shows the mean column diagram of IL-33 and IL-33R (ST2) in the case (blue) and control (orange) groups

The mean level of IL-33R (sST2) in the case group was 21.01 + 8.92 picogram/mL and that in the control group was 18.20 + 5.50 picogram/ mL. However, the statistical test showed no significant difference between the two groups (independent t-test; P = 0.03). The data values are given in Table 2.

| P-Value | Control Group (N=52) | Case Group (N=29) | Specification / Variable |
|---|---|---|---|
| IL-33 | 137.62 + 48.58 | 0.021* | 120.59 + 24.88 |
| IL-33R (sST2) | 0.083** | 18.20 + 5.50 | 21.01 + 8.92 |

*: Mann-Whitney Test

## Discussion

Tuberculosis is one of the most important infectious diseases, which annually kills millions of people all over the world (1-3). Although Th1 cells responses inhibit the development of infection, it should be noted that the host cells are also damaged during the Th1 cells activities, and Th1 alone is not

able to eradicate the infection. It is while the role of Th2 cells has not yet been elucidated. However, it seems that Th2 cells increase the activity of bacilli and promote infection by inhibiting the activities of Th1 cells (13). Based on the existing evidence, IL-33 is produced during the destruction and damage of host cells, which is able to react with ST2L expressed on the surface of Th2 cells, leading to development and shifting to greater Th2 production (13-14). Various reports have nowadays indicated the effect of IL-33 and ST2 on increasing the Th2 cell responses during different diseases (6-8).

In the present study, serum levels of IL-33 and sST2 were assayed between the two groups including the patients with TB and the healthy controls. Based on the results of this study, it was found that the serum level of IL-33 in TB patients had significant difference from healthy controls (Mann-Whitney test; P = 0.021). It was also found that the serum level of sST2 was slightly higher in the TB patients than the healthy controls, although no statistically significant difference was observed between the two groups. Perhaps this was due to normal variation above the level of sST2 and becomes significant with increasing the sample size. It should also be considered that the control group included in this study had no history of infection or inflammation, which in turn affects sST2 levels. Initial in vivo studies have shown that administration of the antibody against ST2 inhibits Th2 cell development and blocking the ST2 by IgG-ST2 recombinant protein reduces the airway allergic inflammation due to the over-activity of Th2 cells in the mice [13]. In another study, blocking the expressing T cells of ST2 potentiated the Th1 responses against Leishmania infection (15).

In another study by Blok et al., they found that sST2 in the TB patients was significantly higher than the healthy controls and that sST2 could be used as a biomarker to diagnose tuberculosis [16]. Moreover, based on the studies by Villarreal and Piñeros et al., it was shown that IL-33 can be used as an immunomodulator to treat tuberculosis (17-20). In contrast to the previous studies, Wieland et al. showed that in exposure to M. tuberculosis, the transgenic ST2 deficient mice experienced no significant changes in the produced levels of IFN-γ, bacterial loads and histopathological lesions in comparison with the controls, suggesting that the effect of Th2 activities on M. tuberculosis infection may be very limited (13).

## Conclusion

This study has provided new document about increasing of IL33/ST2 axis in active TB patients; However, to clarify the role of the IL33-ST2 axis in the pathogenesis of tuberculosis, more diverse studies on a larger demographic scale should be undertaken. In the present study, there were limitations such as few number of patients affected to TB and not determining the IL-33 / sST2 ratio. In order to complete the present study and determine the precise role of the IL-33 / ST2 axis, it is recommended that the future studies to be fulfilled with larger sample size for the changes in the IL-33/ST2 ratio in multiple patient groups, such as the ones with latent TB infection, previously treated TB patients, or new cases of them under treatment.

## Conflict of interest

The authors declare no conflicts of interest.

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