Journal for Research in Applied Sciences and Biotechnology

www.jrasb.com

ISSN: 2583-4053

Volume-3 Issue-1 || February 2024 || PP. 187-192

https://doi.org/10.55544/jrasb.3.1.30

Expression of IL32 and IL33 Genes in Tuberculosis Patients

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www.jrasb.com || Vol. 3 No. 1 (2024): February Issue

Received: 23-01-2024	**Revised:** 01-02-2024	**Accepted:** 13-02-2024

Abstract

In the present case-control study, blood specimens were obtained from (60) patients infected with TB from TB center in Baghdad city, and from (60) healthy persons as a control group during the period from January 2023 to December 2023. Results of demographic picture showed that the distribution of infections in males was 31(51.7%) compared to the control group 3(51.7%) and in females was 29 (48.3%) compared to the control group 29 (48.3%). The infection distribution according to age revealed that the highest infection rate was shown to be within the age group (<20 -29), followed by (30-39) then (40-50) years, which matched with the control group (<20 -29). According to residency, there were no significant differences between rural and urban residents. Mean ±Std anti TB IgM antibodies was (2.40±1.44) in comparison with controls (0.08±0.17), with highly significant difference (P<0.01). Also, Mean ±Std anti TB IgG antibodies was (1.42±0.59) in comparison to controls (0.11±0.21), with highly a significant differences P< 0.01. Mean±Std IL-33 was (20.38±6.53) in comparison to the controls (2.28±2.48), with highly significant differences P<0.01. Also, mean ±Std IL-32 was (10.61±2.24) in comparison to the control group (1.89±2.043) with highly significant differences P< 0.01. Expression of IL32 in TB patients showed positive reaction and it was highly affected in patients with TB compared to the control group, while expression of IL33 in TB patients showed positive reaction and was highly affected in patients with TB compared to the control group.

***Keywords*-** Tuberculosis, IL32, and IL33, gene expression.

I. INTRODUCTION

Tuberculosis was the major infectious agent that was killing humans. Tuberculosis can be treated when diagnosed early, screened for drug resistances and completely treated with suitable short course regimen [1]. In 2019 , and prior to COVID-19 pandemic spread, it has been estimated that 10 million individuals were infected with tuberculosis [2]. Out of the 10.6 million patients who were infected with tuberculosis, only 6.4 million were diagnosed and observed in 2021 to the national tuberculosis programs in the world. Moreover, initially, in more than 10 years, there was an increase in both the mortality and the estimated incidence of tuberculosis [3]. A decrease in detection of tuberculosis cases, increased transmissions and worsening of poverty is a possible explanation. Despite short drug regimens are available at present for treatment of all tuberculosis forms, none of them may realize their possible public health effect until the improvement of its diagnosis. In simple words, if tuberculosis cannot be found, it cannot be treated [4]. If TB cannot be treated, it cannot be ended. To accept that, time is appropriate to act on the chances created by COVID-19 pandemic, and to call on the global tuberculosis community for making such transitions with urgencies [5]. In in vitro IL-32 transgenic mice, it was suggested that IL-32 can develop protective impacts against *Mycobacterium tuberculosis*, however, few studies on humans are available. In the current study, the roles of IL-32 and its splice variant in TB in vitro and in vivo were detected [6]. In comparison with the latent tuberculosis patients and healthy control group, blood transcriptional investigations revealed low serum levels of IL-32 mRNA in pulmonary disease patients [7]. The reduction in IL-32γ is reflected by an elevation of other splice variants, such as IL-32β. Moreover, the high IL-32γ/IL-32β ratio is related to the production of IFN-γ. These information indicate that IL-32 participates in

Journal for Research in Applied Sciences and Biotechnology
www.jrasb.com
ISSN: 2583-4053
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protection against tuberculosis, and that such impact can rely on the relative abundances of various IL-32 isoforms [8]. Interleukin-33 is a major cytokine that is involved in type-2 immunity and allergic diseases. IL-33 has essential roles in adaptive and innate immune response in mucosal organs at the pulmonary epithelial cell levels, where it is evidently expressed [9]. It was clearly shown that in cases of respiratory viral infection, there will be an increase in IL-33 release, with resulting pro-inflammatory impacts and deterioration of clinical features in chronic respiratory disorders like TB [10]. Our study aimed to determine IL-32, IL-33 gene expression.

II. MATERIAL AND METHODS

In this case-control study, blood specimens were obtained from (60) patients infected with TB from the TB center in Baghdad city and same number were collected from healthy individuals as a control group during the period from January 2023 to December 2023. The measurement of IgM and IgG antibodies were done according to the qualitative enzyme immunoassay technique, while the IL-32 and IL-33 were measured according to quantitative sandwich enzyme immunoassay technique. For gene detection and expression, the following primers were used: IL33_exp-F GCCTGTCAACAGCAGTCTACTG
IL33_exp-R TGTGCTTAGAGAAGCAAGATACTC
IL32_exp-F CAAAGAGGGCTACCTGGAGAC
IL32_exp-R TCTGTTGCCTCGGCACCGTAAT
Analysis of gene expression was done using Livak method

Relative quantifications

Foldings =2-ΔΔCT
ΔCT =CT gene - CT House Keeping Genes
ΔΔCT =ΔCT Treated or Control - ΔCT Control

Statistical analysis: For data analysis**,** SPSS-20 program was used, i.e. T-test and (Mean±S.D). The $p<0.05$ value is regarded significant.

III. RESULT

The demographic data showed that the infection distribution in males was 31(51.7%) compared to the control group 3(51.7%), and in females was 29 (48.3%) compared to the control group 29 (48.3%). The highest infection rate was shown to be within the age group (<20 -29), followed by (30-39) then (40-50) years, which matched with the control group (<20 -29) as observed in in the table (1).

Table (1): Distributions of the study group in accordance with demographic characteristics

Demographic characteristics		Study groups	
Gander		Control(n=60)	Patients(n=60)
Male	No.	31	31
	%	51.7%	51.7%
Female	No.	29	29
	%	48.3%	48.3%
Total	No.	60	60
	%	100%	100%
Residency		Controls	Patients
Urban	No.	29	29
	%	48.3%	48.3%
Rural	No.	31	31
	%	51.7%	51.7%
Total	No.	60	60
	%	100%	100%
Age group (years)		Controls	Patients
(<20 -29)	No.	37	37
	%	61.7%	61.7%
(30-39)	No.	16	16
	%	26.7%	26.7%
(40-50)	No.	7	7
	%	11.7%	11.7%
Total	No.	60	60
	%	100%	100%

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Mean ±Std anti TB IgM antibodies was (2.40±1.44) in comparison with controls (0.08±0.17), with highly significant difference (P<0.01). Also, Mean ±Std anti TB IgG antibodies was (1.42±0.59) in comparison to controls (0.11±0.21), with highly a significant differences P< 0.01 as illustrated in the table (2).

Table (2): Comparison between Studied groups according to TB IgM &TB IgG

	Study group	(Mean ±Std)	Levine Test (F)	P Values
TB IgM	Controls	(0.08±0.17)	53.013	P=0.000 P< 0.01(HS)
	Patients	(2.40±1.44)		
TB IgG	Controls	(0.11±0.21)	24.857	P=0.000 P< 0.01(HS)
	Patients	(1.42±0.59)		

Mean±Std IL-33 was (20.38±6.53) in comparison to the controls (2.28±2.48), with highly significant differences P<0.01. Also, mean ±Std IL-32 was (10.61±2.24) in comparison to the control group (1.89±2.043) with highly significant differences P< 0.01 as seen in the table (3).

Table (3): Comparison between the studied groups according to IL-33& IL-32

	Study group	(Mean ±Std)	Levine Test (F)	P Values
IL-33	Controls	(2.28±2.48)	37.34	P=0.000 P< 0.01(HS)
	Patients	(20.38±6.53)		
IL-32	Controls	(1.89±2.043)	0. 145	P=.705 P> .05(NS)
	Patients	(10.61±2.24)		

Table (4) and figure (1) showed the ROC curve between anti- IgM antibodies with sensitivity 100% and Specificity 0.00 %, while anti- IgG anti bodies showed 93.3% Sensitivity and 6.7% specificity.

Table (4) : Analysis of receiver operating characteristic curve (ROC) of TB IgG & IgM

Variables	Areas	P Values	Cut offs	Sensitivity	Specificity
TB IgM	1.000	0.000	> 0.92	100%	0.000 %
TB IgG	.989	0.000	> 0.44	93.3%	6.7%

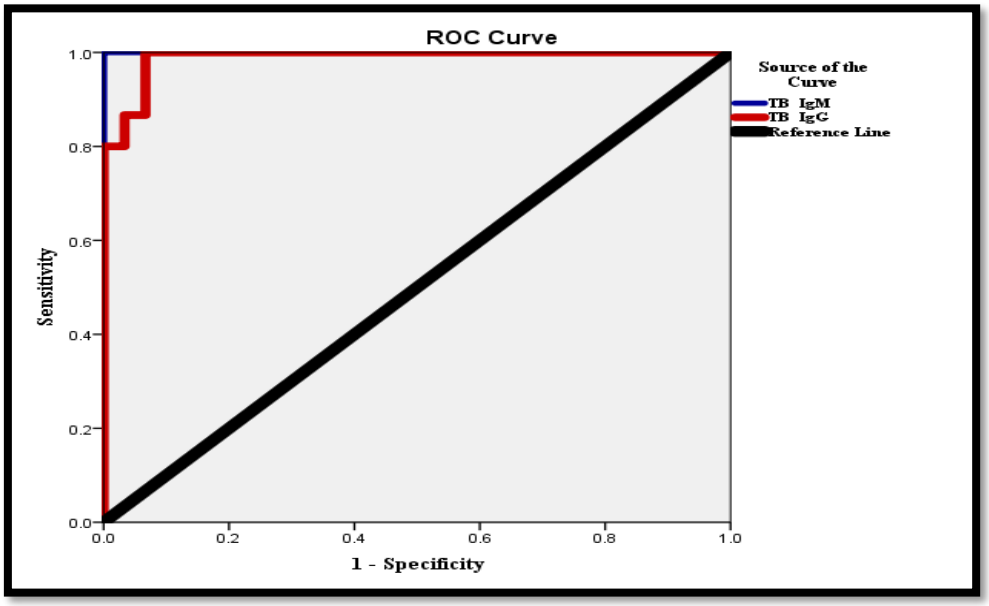

Figure. (1): Analysis of receiver operating characteristic curve (ROC) of TB IgG & IgM

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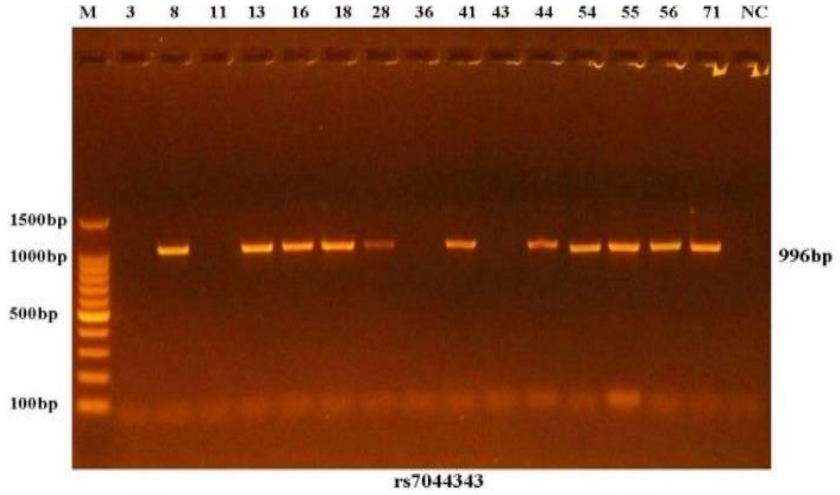

Figure (2): Results of amplification of rs7044343 specific IL-32regions. Species of human specimens were fractionated in 1.5% agarose gel electrophoresis and stained with ethedium brom. M: 100bp ladder marker. Lane 3-71 resembling 996bp PCR product.

The expression of IL32 in TB patients: There was a positive reaction and it was highly affected by TB diseases compared to the control group, as shown in figure (3).

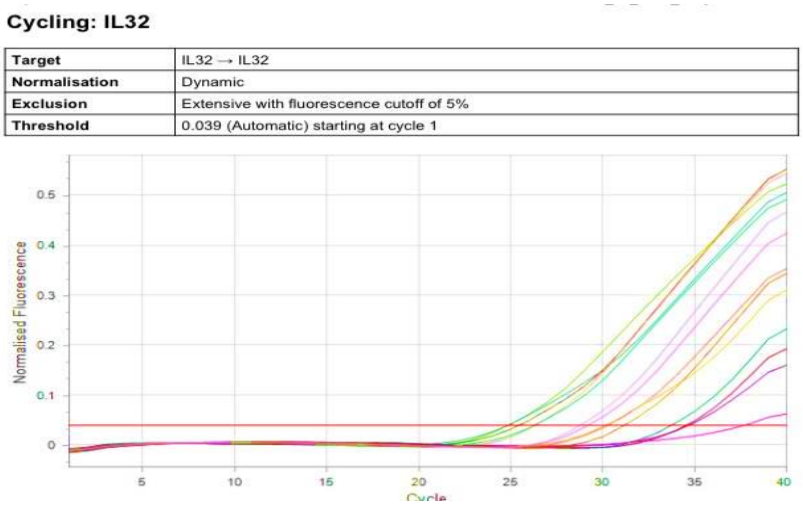

Figure 3: Cycling of IL-32 gene expression

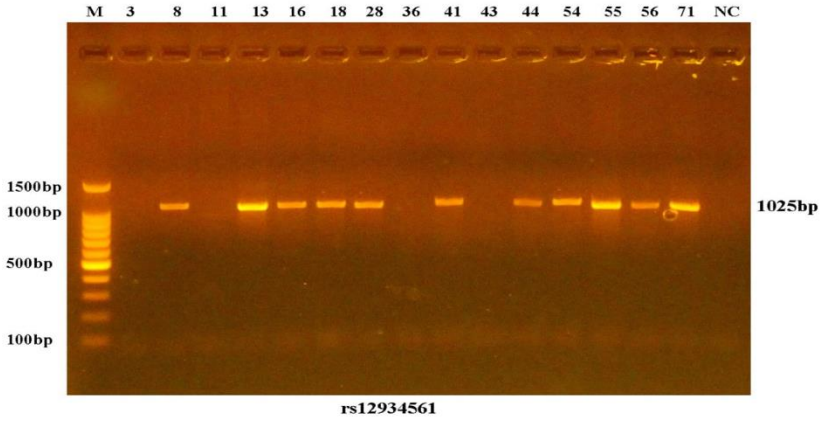

Figure (4): Results of amplification of rs12934561 specific region of IL-33. Species of human samples were fractionated in 1.5% agarose gel electrophoresis stained with ethedium brom. M: 100bp ladder marker. Lane 3-71 resembling 1025bp PCR product.

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The expression of IL33 in TB patients: There was a positive reaction and it was highly affected by TB diseases compared to the control group as shown in figure (5).

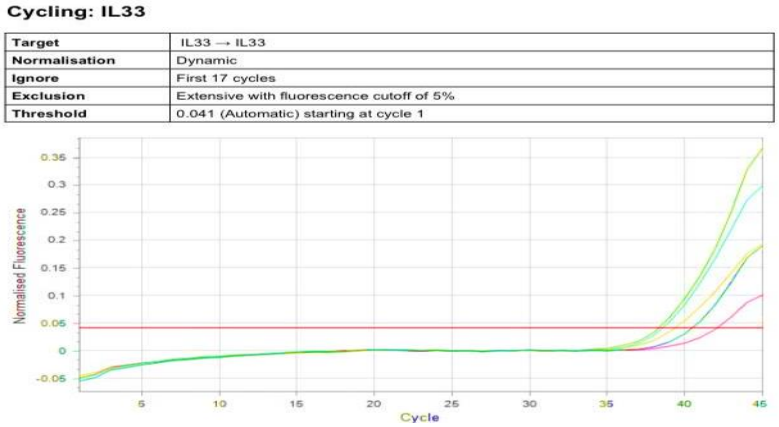

Figure (5): Cycling of IL-33 gene expression

IV. DISCUSSION

Tuberculosis is a very serious disease that is transmitted from sick to healthy people by means, including breathing, and its spread is epidemic. According to the results, a non-significant difference was found in infection distribution between patients and controls in terms of age, gender, and geographical distribution. The lack of differences may be due to the equality of both groups. The Mean ±Std of ant IgM antibodies TB infections was (2.40±1.44) when compared with the controls (0.08±0.17) with highly significant differences. Tran, et al, (2023) reported that there is a very noticeable increased levels of IgM antibodies in the sera of individuals with TB disease in comparison with the control groups before they were given the vaccine against this disease [11]. Mean ±Std anti TB IgG antibodies was (1.42±0.59) in comparison to controls (0.11±0.21), with highly significant differences. Zhao, et al, (2017) proved the increase in IgG titer with the chronic period in cases of TB disease in the sera of infected patients and that there is a response to antibodies compared to healthy people who are considered as controls [12]. The Mean±Std of IL-33 was (20.38±6.53) in comparison to the control group (2.28±2.48) with a highly significant difference. Sá, et al, (2022) stated that IL-33 has essential roles in adaptive and innate immune response in mucosal organ. It was clearly shown that in cases of respiratory viral infection, there will be an increased IL-33 secretion, with resulting pro-inflammatory impacts and deterioration in clinical features in chronic respiratory disorders. In this study, we investigated prognostic with pathogenic IL-33 contribution in time of the major respiratory viral infection [13]. Also, the Mean ±Std of IL-32 was (10.61±2.24) in comparison to the control group (1.89±2.043) with a highly significant difference. These findings agreed with (Li, 2018) who concluded that IL-32 was highly observed as an essential host molecule against TB. In the current study, it was focused on the pro-inflammatory characteristics of IL-32 with its action mode in mycobacterial infection for inspiring new immunity development countermeasure and host-directed treatments for TB. The pleiotropic cytokine, IL-32 is able to induce pro-inflammatory cytokines like TNFα & IL1β through activations of NF-κB with p-38 MAPK signaling. IL32 is principally present just in human primate, and such gene is present on chromosome 16p-13.3 and composed of 8 exons. The existence of IL32 m-RNA in immune and non-immune cells & tissues, such as T-cells, NK cells, dendritic cell, endothelial cell and epithelial cell indicates that this gene has many functions like inflammatory responses [15]. These results agreed with (Netea, 2016) who reported that other studies provided many essential insights into the IL-32 biology. The results confirmed that IL-32 is a key element in the body's defense against TB. These data help to understand the disease manner to spread and those are most vulnerable to it. Finally, it may help in searching for novel methods to treat and prevent the disease [16]. Also, there was positive IL-33 expression with TB diseases. The expressions of IL-33 elevated in pleural spaces of patients with tuberculosis pleural effusion that are induced by TNF and IFN-γ. The development of tumor led to downregulation of IL33 in epithelial cell, but caused upregulation of IL33 in serum and tumor stromas. Expression of IL33 in a tumor cell increased immunogenicity and promoted type 1 anti-tumor immune response.

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ISSN: 2583-4053
Volume-3 Issue-1 || February 2024 || PP. 187-192
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