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The role of IL10 as predictor in Tuberculosis patients with Aspergillosis

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Abstract

Background: In present study found that IL-10 play an important role in diagnosis of Aspergilosis in the patient with pulmonary tuberculosis

Coinfection between aspergillus fungi and tuberculosis disease is one of the most prevelant disease in the world and one of the most high risk of mortality and significant morbidity

Objective: To evlauate the role of IL-10 in pullmonary aspergillosis fungi coinfection with tuberculosis Patients by measuring the concentrations of IL -10.

Patients and Methods: The presented case-controlled study registered the examination finding of blood serum specimens and sputum specimens belonging 90 person, 45 patients (28males, 17 females) diagnosed with tuberculosis disease and 45 healthy controls (28 males, 17 females). The serum concentrations of interlukin 10 markers were analyzed using enzyme linked immunosorbent assay (ELISA).

Results: In respect to the immunological parameters among study's groups, it has been found that the mean level IL-10 were significantly lower among cases group as compared to controls, suggesting that these markers may be useful to diagnosis pulmonary aspergilosis with the tuberculosis patient .

Conclusion: In conclusion Tuberculosis is a prevalent health issue in numerous countries, including Iraq, with varying severity and spread across different nations, T.B patient is more evident among males pulmonary aspergillosis recorded high frequency among T.B patient (patient with second line drug or new T.B patient) with majority of them were of *A. fumigatus*, followed by *A. niger*, *A. flavus*, and *A. terreus* respectivelyin immunlogy the present study shows there was significant the decreased levels of IL-10 in the blood serum of TB patients compared to healthy controls, suggesting potential biomarkers for the diagnosis of TB.

Key words:tuberculosis, pulmonary aspergillosis , interlukin10

Introduction:

The main bacterial pathogen responsible for deaths worldwide is Mycobacterium tuberculosis (Mtb), which causes tuberculosis (TB) in humans. When droplets containing bacteria are inhaled, the lungs become infected with Mtb, an intracellular pathogen[1]. Over 90% of people with Mtb infection have latent TB, a protracted and asymptomatic stage of the illness. Less than 10% of infected people have active TB, highlighting the significance of the interaction between bacterial pathogenesis and host defense in the avoidance of active illness [2]. Lung fungal infections are rather common in persons with impaired immune systems (immunocompromised patients) [45].This infection frequently manifests clinically as a fungal ball known as a mycetoma[41].Aspergillosis and tuberculosis frequently co-occur in immunocompromised people; in some studies, this co-occurrence has been as high as 80%.[3].Aspergillus species are opportunistic pathogens and saprophytes that are found throughout the world .Aspergillus fumigatus has been labeled as an 'accidental' pathogen primarilydue to its independence of a host for survival, and its pathogenic potential may have evolved to facilitate survival in the environment [4]. Co-infection is defined as the simultaneous presence of two or more infections, which may worsen the severity and duration of one or both, our goal was to assess the prevalence of Invasive Aspergillosis in suspected patient T.B. [5]. Aspergillus species and Mycobacterium tuberculosis can co-infect a host's lungs, leading to additional difficulties in pulmonary aspergillosis co-infection[6].The interplay between *Aspergillus* spp. and various components of the host immune system influences disease progression. Agent factors such as conidia size, temperature tolerance, hydrophobin /melanin expression etc.

In immunocompetent hosts, the key defense against Aspergillus spp. The recruitment and activation of cellular immunity, notably neutrophils and monocytes capable of engulfing and eliminating the conidia, is a sign of infection [7]. It has been demonstrated that cytokine production is essential for fungus clearance[8].NK cells release IL-10 during systemic infection[9]. When it comes to the immune system, IL-10 is a two-edged sword. While it has been shown to be a powerful anti-inflammatory and immunosuppressive cytokine, and have immunostimulatory effects [10],[11].

IL-10 and tuberculosis:

CD4+, Th2, Th1 and Th17 cells, and, DCs, Treg cells monocytes and macrophages are main producers of IL-10 [12], In CD4+ Th cells, IL-10 production occurs downstream of T cell receptor (TCR) activation and the subsequent activation of Ras, ERK1/2 and transcription factor AP1 [12][10] In order to drive Th0 to Th1 cell polarization, IL-10 inhibits the death of macrophages, the uptake, processing, and presentation of dendritic cells, as well as the migration of DCs from the infection site to the lymph nodes. Moreover, it contributes to the inhibition of chemokines that facilitate Th1 migration from lymph nodes to the lungs. It has been observed that IL-10 affects both innate and adaptive immune responses, which may foster microbial persistence, intracellular infections, and chronic infection. Since IL-10 has been found to lower immunity, it has been shown in vivo that its production can reactivate chronic pulmonary tuberculosis. [13].The antimicrobial response of activated macrophages, which produces low levels of IL-10, helps to restrict MTB development and prevent lung injury. Therefore, it is believed that adjusting IL-10 levels during TB treatment will reduce treatment time and hasten the elimination of bacteria. In the early stages of infection, the immune system responds better and eliminates

MTB when IL-10 is absent. [14]. Agenetic predisposition to tuberculosis susceptibility is indicated by the prevalence of tuberculosis in specific racial/ethnic groups and families. This relates to the intricate way that MTB interacts with host genetic and environmental factors, explaining why certain individuals are more or less prone to contracting tuberculosis. [15].

IL-10 and aspergillusis

Strong immunosuppressive effects of IL-10 are seen in monocytes, dendritic cells, and macrophages—the cells that express IL10R at higher levels [19] It suppresses these cells' differentiation, maturation, and migration to lymphoid organs as well as their capacity to produce pro-inflammatory cytokines (IL-1a and b, IL-6, IL-12, IL-18, and TNF-a) and chemokines (CCL2, CCL12, CCL5, IL-8, CXCL10, and CXCL2) [12]. co-stimulatory molecules CD80, CD56, and CD54 (intercellular adhesion molecule-1, or ICAM-1) [42]. Additionally, it can influence CD4+ T cells by preventing their lymph node-based antigen-specific activation and proliferation, as well as decreasing the amount of cytokines they can secrete, including IL-2, IFN-g, IL-4, IL-5, and TNF-a, as well as their ability to cause cytotoxicity [16]. The role of IL-10 during infection appears to vary for different microorganisms, a largely detrimental the role has been attributed this cytokine during fungal disease[17]. Given the variable risk of infection and its outcome among patients with comparable predisposing factors, susceptibility to invasive aspergillosis (IA) is thought to rely largely on genetic predisposition. [18].

Subjects and Method patient Study design

This case-controlled study included (100)suspected patient were collected in this study and the positive patient 45 were taken from patient with pulmonary tuberculosis The samples were taken during the period from April to July 2023 and are collected from the National Institute of Tuberculosis in Baghdad.Fuorty five (45%) sputum sample and forty five(45%) serum sample from Iraqi patients (28 males, 17females) diagnosed with pulmonary tuberculosis Disease with ages (12-70) information (obtained during the collection of sputum from lung and blood samples), the questionnaire data sheet that included: patient name, sample number, sex, age, jop,sample type,type of fungi ,number of bacilli, symptoms accommodation area ,if countryside or city, Smoking,if the patient on treatment 0r not, and disease that patient suffer from, x ray. so,The patients' ages ranged from (12_70) years and male/female ratios were collected through a questionnaire data sheet from both patients and controls

Healthy control Group: (45)serum sample from healthy individuals were collected (29males, 16females).with ages (12-70) from people who had negative history or clinical evidence for TB

Collecting of Blood samples

90 of 1:1 of collected 45% cases and 45% controls samples Cytokine measurement, Five millilitres of venous blood were drawn from both T.B Patients and control group member using a five-milliliter disposable syringe.The blood sample was immediately transformed into jell plain tube and left to clot for 15 minutes in room temperature (20-25) °C. Then, it was centrifuge from 3000 rpm for 10 min period to isolate serum , then serum was put into foure Eppendorf tubes & kept in the deep freezer (-80C˚)

for later examination. TNF- and IL-10 levels in the blood are measured using a sandwich enzyme-linked immunosorbent assay .

Sputum collection

Each patient had a loud cough in the morning, and specimens were taken in sterile, clean, dry containers. The specimens were processed right away for direct microscopy and culture.

Laboratory Diagnosis of Fungi:

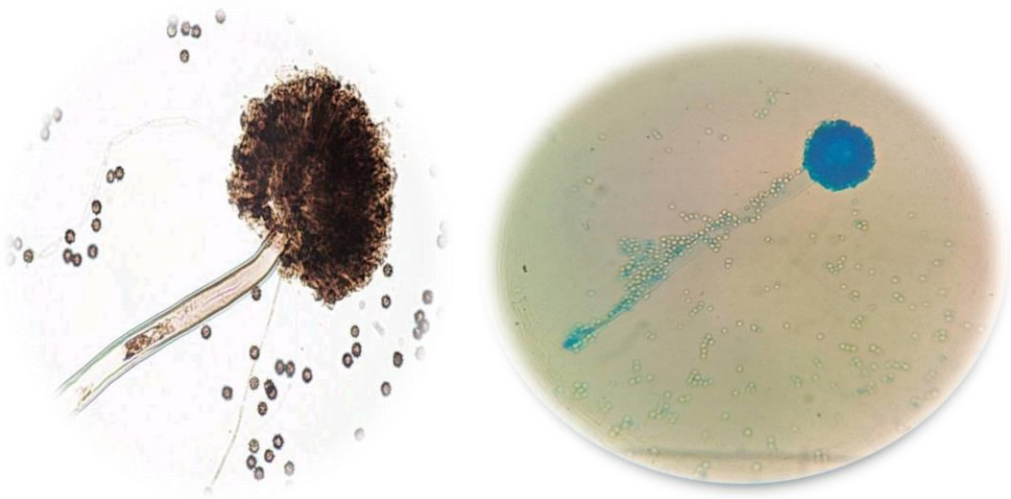
Culture media preparation:

Sabouraud Dextrose agar, To ensure sterility, the medium was either autoclaved at 121 C for 15 minutes at 15 pounds per inch2 or incubated at 37 C for 24 hours The media was then spread into sterile Petri plates [20].

Culture and Isolation: Two sets of Sabouraud's Dextrose Agar slope media with chloramphenicol were used to cultivate all of the sputum samples. as previously stated by the authors, incubated at 37 C°, aerobically for up to 14-21 days, with daily inspection. [21].

Direct Microscopy: A big drop of KOH was put in the center of a spotless glass slide using Pasteur's pipette. Justatiny percentageofthe Using a sterile wire loop, the sputum was transferred and thoroughly mixed within the KOH drop. After being flattened down and covered with a cover slip, the preparation was maintained at room temperature for half an hour. The slide was then inspected for the presence of fungal components using low power (10x and 40x objective)

Microscopic Examination with Lacto phenol Cotton Blue: The fungal parts are stained with lactophenol aniline blue stain, which produces a blue tint that makes it easier to see different fungal structures such hyphae, conidia, and spores. In clinical laboratories, this staining method is frequently used to identify fungus infections. Healthcare practitioners can identify and evaluate the distinctive characteristics of the fungi by looking at the stained samples under a microscope, which helps with the precise diagnosis and categorization of fungal diseases [22].

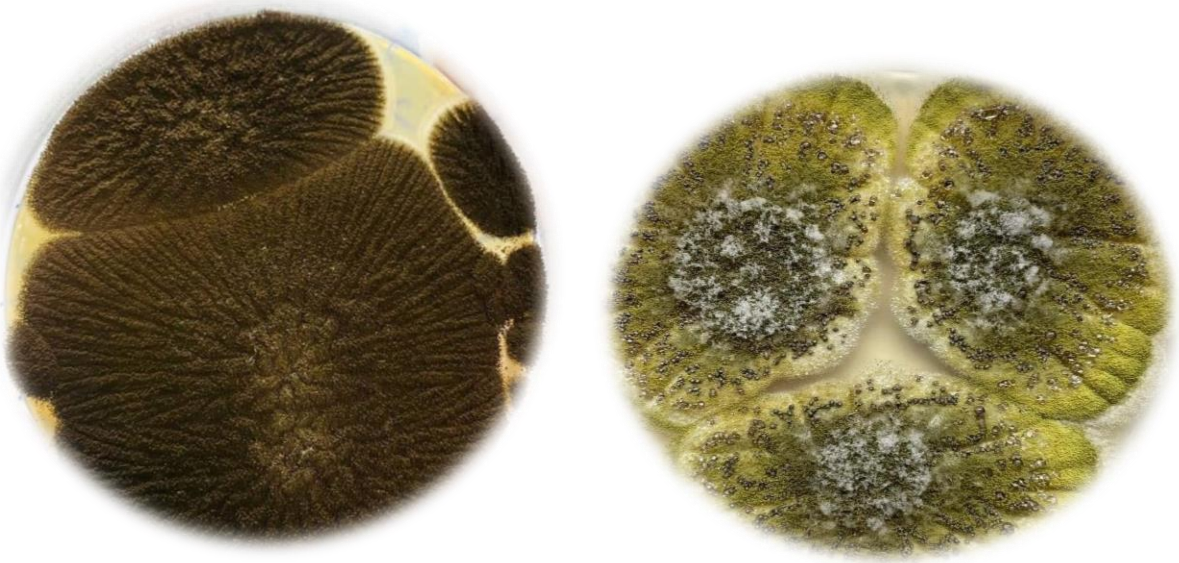


(1) **(2)**

Figer(1)*Aspergillus niger*, Figer(2)*Aspergillus flvus* under Microscopic

Macroscopic Analysis of Culture: Surface topography, texture, and pigment were used to identify and isolates. The colony's diameter, The characteristics of the colony include the color of the conidia, the mycelia, the texture and shape of the reverse colony, and more[21] , A positive result on both microscopy and culture serves as laboratory evidence of broncho pulmonary aspergillosis[23].

Microscopic Analysis of the Culture: The cultures were identified using a preparation of lactophenol cotton blue. Microscopic characteristics such as conidial heads, color, size, and length, vesicles shape, serration, and roughness were utilized to identify them [21].



(3) **(4)**

Figer(3)*Aspergillus niger,* Figer(4)*Aspergillus flvus* Macroscopic examination on Sabouraud Dextrose Agar (SDA).

Cytokine measurement : At the time of patient enrolment, blood samples for cytokine analysis were taken T.B patients' serum samples were isolated right away, divided into aliquots, and stored in a -80°C freezer for later examination. TNF- levels in the blood are measured using a sandwich enzyme-linked immunosorbent assay.

Result

The age of study samples was ranged from 12 to 70 years with a mean of 35.49 ± 16.434,

In respect to the sex of the study sample, the entire study's sample were male dominant with male to female ratio of 1.5:1 (60%: 40%), as well as cases and control groups were also male dominant sex with male to female ratio of 1.36:1 (57.8%; 42.2%) and 1.64:1 (62.2%; 37.8%) respectively without significant differences ($P >$ 0.05)

Comaprsion of immunological parameters among study's groups

Regarding the immunological parameters among study's groups, it has been found that the mean level of interlukin-10 (IL-10) was significantly lower among cases group (TB) than that of the controls (97.47984 ± 14.033652 vs. 288.28304 ± 105.388622) with significant difference of 190.803200 (t= 12.039, df:88, P= 0.000) respectively (Table1) (Figure1).

Table (1)Mean comparison of immunological parameter of interlukin-10 among study's groups (n=90)

Immunological Parameters (Mean ± SD)	Study groups (TB) (n=90)		Mean differences	Significance [a]
	Cases (Yes, n=45)	Control (No, n=45)		
Interlukin-10	97.47984 ± 14.033652	288.28304 ± 105.388622	190.803200	t= 12.039, df:88, P= 0.000

a: Unpaired T-Test

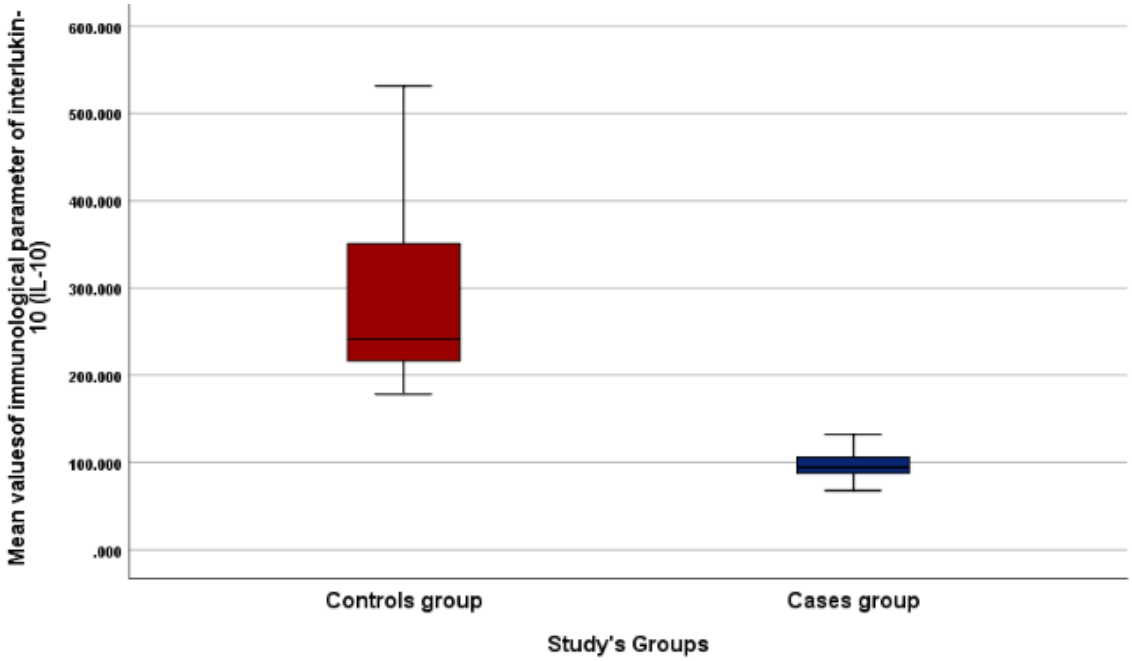


Figure (1) Mean comparison of immunological parameter of interlukin-10 among study's groups (n=90)

Comaprsion of immunological parameters among cases group of study's samples

When comparing immunological parameter of interlukin-10 (IL-10) among patients of cases groups regarding their positive test of fungal infection with *Aspergillus,* significant differences were also identified as the mean value of interlukin-10 (IL-10) was significantly lower among patient with positive *Aspergillus* test than those with negative one (91.49953 ± 7.296140 vs. 100.57504 ± 15.752657) respectively with significant difference of 9.075506 (*t*= 2.227, df:43, *P*= 0.031) (Table2) (Figure2).

Table (2) Mean comparison of immunological parameter of interlukin-10 among cases group of study sample (n=45)

Immunological Parameters (Mean ± SD)	Cases group (n=45) *Aspergillus* test		Mean differences	Significance [a]
	Yes (n=17)	No (n=28)		
Interlukin-10	91.49953 ± 7.296140	100.57504 ± 15.752657	9.075506	*t*= 2.227, df:43, *P*= 0.031

a: Unpaired T-Test

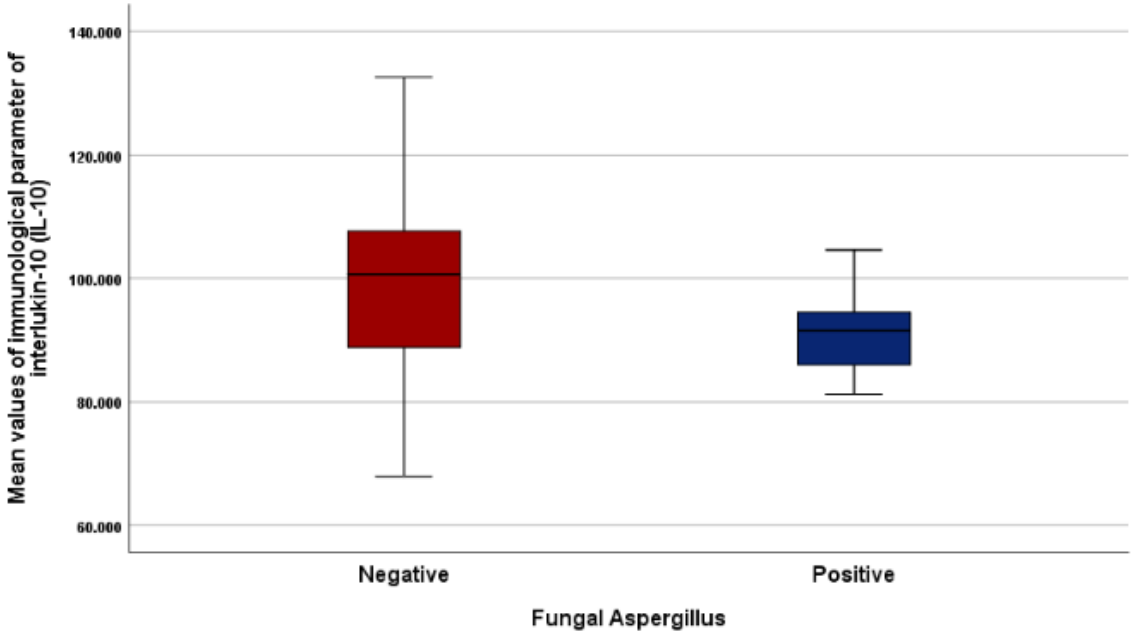


Figure (2) Mean comparison of immunological parameter of interlukin-10 among cases group of study samples (n=45)

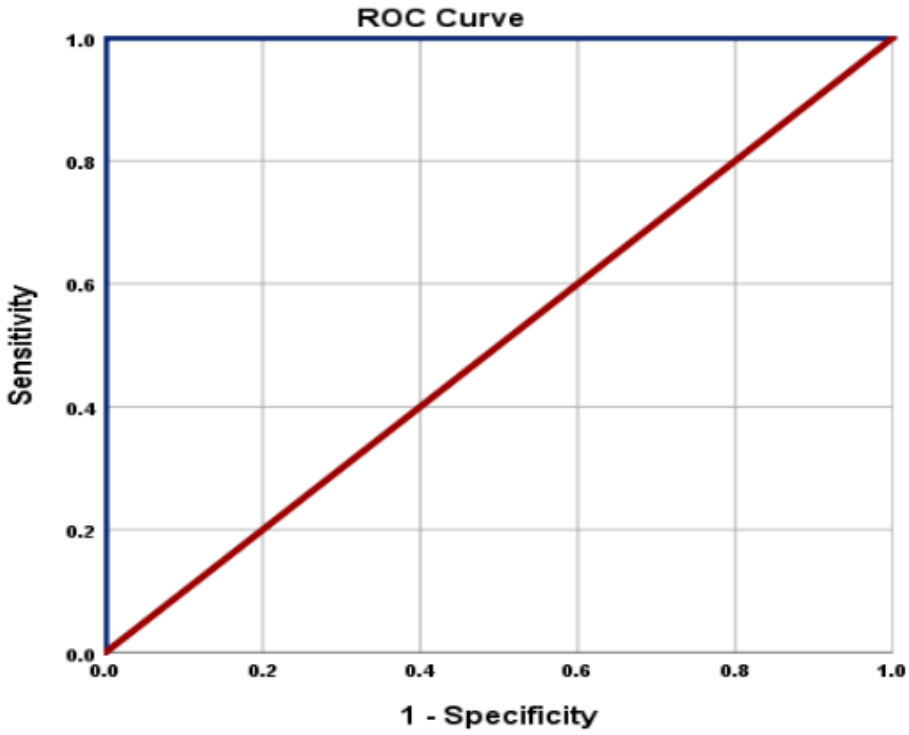
Immunological parameters as predictive diagnostic test

Interlukine-10 (IL-10) as predictive diagnostic test for TB diseases among

Among a 90-study sample, the optimal cutoff value of interlukine-10 (IL-10) for detect patients with reducing risk (low risk) of developing tuberculosis diseases was 106.56250 with sensitivity of 100%, specificity of 97.8%, and excellent area under the ROC curve (AUC) of 1.00 ± 0.000 (*P*= 0.000) (Table3) (Figure3).

Table (3) Predective value of interlukine-10 (IL-10) as protective from developing tuberclosis diseases among study's sample (n=90)

Paramter	Validity of model				
	Sensitvity (Sn)	Specificity (Sp)	Accuracy	Area Under the curve (AUC)	Significancy (*P*-value)
Interlukine-10 (IL-10)	100	97.8	100	1.00	0.000



Figure(3) ROC Curve of protective development of tubercluosis diseases predicted by immunological parameter of interlukine-10 (IL-10) among study's sample (n=90)

Disscusion:

Pulmonary Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*, which frequently invades the lungs Aspergillus-related lung fungal infection was found to be highly prevalent. The current study to evaluated the serum cytokine profile of pulmonary aspergillosis In patients with tuberculosis patients and controls were age and sex matched. These parameters were controlled to reduce their impact of them on serum cytokine levels ,In the current study showed that the serum level of IL-10 among patients had significant relation with the tuberculosis disease with aspergillosis .

Data on baseline IL-10 concentrations were available for 90 pateints it has been found that the mean level of interlukin-10 (IL-10) was significantly lower among cases group (T.B) than that of the controls it has been found that the mean level of interlukin-10 (IL-10) was significantly lower among cases group (TB) than that of the controls (97.47984 ± 14.033652 vs. 288.28304 ± 105.388622) with significant difference of 190.803200 (t= 12.039, df:88, P= 0.000) respectively

A recent study agreement has demonstrated a consistent reduction in IL-10 levels in patients with active tuberculosis at all periods during therapy, indicating that patients who maintain elevated levels of IL-10 after the conclusion of treatment may be at risk for tuberculosis recurrence[40] . Reduced IL-10 production during TB therapy was also noted by Sahiratmadja et al.[24]. indicating that this cytokine may be a helpful biomarker signature to gauge the course of the disease. Neutralization of endogenous IL-10 has been shown in studies using peripheral blood mononuclear cells from tuberculosis patients to boost T-cell proliferation and interferon production,All of these

investigations came to the same conclusion: IL-10 may play a role in the pathophysiology of tuberculosis and was limiting the immune response to Mtb.
IL-10 was limiting the immune response to Mtb and might be involved in the pathophysiology of tuberculosis [25]

And this Study agreement by ,Lago and his colleagues illustrated that after six months TB treatment. The finding showed that IL10 level decreased during treatment. In agreement with the present study, [40]

additionally noted decreased IL-10 production following TB treatment, indicating that this cytokine could be a helpful biomarker signature to gauge the course of the illness. All of these investigations came to the conclusion that IL-10 may play a role in the pathophysiology of tuberculosis and was limiting the immune response to Mtb.

Other study disagreement are consistent with earlier research that found higher levels of IL-10 in the group with active tuberculosis than in the control group, and that also found an association between IL-10 and tuberculosis susceptibility [41] Increased production of IL-10 may have a key function in stimulating tuberculosis reactivation during the chronic/latent stage of pulmonary tuberculosis. [26] The body's defense against M. tuberculosis includes this immunological reaction, but the bacterium has ways to avoid the harmful immune response. M. tuberculosis produces interleukin 10 (IL-10), which is one method it suppresses immunological responses. Anti-inflammatory cytokine IL-10 is secreted by immune cells of many kinds, such as B cells, T cells, and macrophages. Inhibiting excessive inflammation and preserving immunological response balance are the primary roles of IL-10. M. tuberculosis has the ability to induce macrophages to produce more IL-10 when these cells become infected. [27] ,Inhibiting the synthesis of pro-inflammatory cytokines like interleukin 1 (IL-1), interleukin 6 (IL-6), and nuclear factor kappa B (NF-κB) is the main role of IL-10 in the control of immunological responses. This lessens inflammation that could harm lung tissue, but it also makes it more difficult for the body to get rid of M. tuberculosis fast. [9]. Another study conducted by Jamil and his colleagues illustrated that IL-10 has been associated with increased susceptibility to *M. tuberculosis* infection in humans [28] Moreover, our findings suggest that by harling and his colleagues illustrated that concurrent analysis of many cytokines may better capture differences between research groups. We demonstrated that while TB-patients had lower amounts of IL-6 in their plasma, TB-Pb patients had higher levels of IL-10. It has been observed by us and others that patients with tuberculosis i increased expression of IL-10 [29]. IL-10 is a crucial cytokine for immune regulation with anti-inflammatory qualities [30].Although the exact role of IL-10 (and IL-10 family cytokines) is not clear yet [31]. ecpecially in tuberculosis patient with aspergillosis at the same time

Although beneficial in some bacterial infections, exogenous IL-10 has been shown deleterious in models of fungal infection. Our data indicate IL-10 is deleterious during systemic aspergillosis infection, increasing the host susceptibility to lethal infection.

interlukin-10 (IL-10) among patients of cases groups regarding their positive test of fungal infection with *Aspergillus,* significant differences were also identified as the mean value of interlukin-10 (IL-10) was significantly lower among patient with positive *Aspergillus* test than those with negative one (91.49953 ± 7.296140 vs.

100.57504 ± 15.752657) respectively with significant difference of 9.075506 (t= 2.227, df:43, P= 0.031)

The phenomenon of co-infection with *Mtb* and *A. fumigatus* is clinically common and difficult to diagnose, due to shared pulmonary symptoms associated with both types of infection. Consequently, misdiagnoses of co-infected patients can occur that can prevent these patients from receiving early and accurate treatments [44]

Agreement study Suggest s to analyze cytokine changes during the development of cronic pulmonary aspergillosis CPA from Allergic bronchopulmonary aspergillosis (ABPA). We showed that IL-10, levels decreased with VRCZ treatment in this patient. ABPA is a disease caused by an excessive immune response to Aspergillus spp. [32],[33]

Disagrement study by ,Robinet and his colleagues illustrated that Th2 response is the main immune reaction, and the cytokines IL-10 were increased in peripheral blood[34] . Pathogens linked to both tuberculosis and cronic pulmonary aspergillosis cause a Th1 response, whereas the cronic pulmonary aspergillosis pathogen also causes a Th2 response, indicating that the immune responses of cronic pulmonary aspergillosis and tuberculosis patients differ, according to other research [35]. The number of Th1 and Th2 cells and the balance of Th1/Th2 play major roles in maintaining the body's normal immune function and have an important impact on the onset and prognosis of tuberculosis [43]. *A. fumigatus* infection can lead to elevated patient blood leukocyte and neutrophil counts, while also inducing pulmonary epithelial cells to release inflammatory cytokines that, in turn, promote lymphocyte recruitment and trigger other inflammatory responses. [36],[37].

In contrast, high IL-10 levels and IL-10/ IL-5 ratio have been reported in patients with croronic pulmonary aspergillosis [38]. Severe allergic inflammation is known to induce exhausted ILC2s with a high expression of IL-10 [39].In this instance, antifungals decreased antigen levels, which would have alleviated exaggerated inflammation and lowered IL-10 levels. Following the antifungal medication, the patient's clinical course improved and cytokine levels decreased. Right now

Conclusion: In this study, cytokines interlukine -10 were identified that may serve as potential biomarkers for use in detecting Tubeculosis patients with Pulmonary Aspergillosis., our results should enhance understanding of how immune system dysfunctions influence susceptibility to *Mtb* and/or *A. fumigatus* infections.

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