Sindh Univ. Res. Jour. (Sci. Ser.) Vol.50 (002) 259-264 (2018)

http://doi.org/10.26692/sujo/2018.06.0045

SINDH UNIVERSITY RESEARCH JOURNAL (SCIENCE SERIES)

Plasma levels of anti-inflammatory cytokine Interleukin-10 predict tuberculosis risk in type 2 Diabetes Mellitus

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Received 11th June 2017 and Revised 13th January 2018

Abstract: Diabetes mellitus is a highly prevalent disease leading to weakening of immune system and leading to other health problems. In the present study it was aimed to evaluate the prediction of increased risk of getting tuberculosis in diabetic patients based on plasma levels of cytokine IL-10.For this purpose three groups of subjects: 100 healthy controls, 100 patients with diabetes only, and 100 patients with tuberculosis and diabetes were examined. The demographic characteristics such as age, BMI and hematological parameters i.e., total white blood cell count, lymphocytes, neutrophils and platelets were measured. By using ELIZA in-vivo production of interleukin 10 (IL-10) was measured. One way ANOVA was applied to the data with LSD as post hoc test for the comparison between various groups. Patients with type 2 diabetes showed increased IL-10production and patients with both active tuberculosis and diabetes were found to have lower IL-10 levels 7.96 ± 0.31. The mean difference found was significant at p value of 0.05.

Keywords: Type 2 Diabetesmellitus, tuberculoses, IL-10.

1. INTRODUCTION

Tuberculosis is considered the most important cause of death in developing countries and the prevalence of diabetes is also on rise (Stevenson *et al*., 2007). It is supposed that the influence of diabetes on tuberculosis could be equal to HIV in countries with high prevalence of diabetes (Alavi and Khoshkhoy, 2012). The risk of tuberculosis relapse and mortality is also increased if diabetes is developed in the patient (Bailey and Grant, 2011). A finding from recent report in Mexico concluded that 25% of pulmonary tuberculosis was attributable to diabetes (Alavi and Khoshkhoy, 2012).Some other observations suggest that diabetes has influence on radiological, clinical, and treatment failure of hospitalized tuberculosis patients (Sosman and Steidl, 1927). During diabetes immune system gets impaired and the main dysfunctions of the immune system are: uncharacteristic functions of polymorphonuclear cells, impaired phagocytosis, deprived lymphocytes transformation and stopping in complement opsonic function. In unison, tuberculosis triggers a state of latent diabetes by increasing blood glucose level. Stress hormones also raise the blood sugar level and these are stimulated by fever, malaise, and malnutrition. The plasma level of Interleukin (IL)-1 and tumor necrosis factor alpha (TNF-α) rise in tuberculosis, which have anti-insulin effect (Carreira *et al*., 2012).

In research and medical practice the use of biomarkers has become very important. Similarly, in tuberculosis identification biomarkers play a critical role in three areas. They can be used to predict non-relapsing treatment success in patients having active tuberculosis, to point out risk of reactivation and eradication of latent infection in patients with silent *M.tb* infection (LTBI) and to predict vaccine efficacy in people other than those with active disease (Wallis *et al*., 2010).

Cytokines regulate immune responses which play a key role in the pathogenesis of the disease such as: IL-10 down regulates immune responses and block phagosome maturation in macrophages consequently help in persistence of *M. tuberculosis* in humans (Liang *et al*., 2014). IL-10 increased expression is associated with stable disease. Studies have revealed that progression of tuberculosis is associated with IL-10 (Thye *et al.*, 2010).

In the present study we have demonstrated that if type 2 diabetic patients show increased chances of developing tuberculosis on the basis of plasma levels of anti-inflammatory cytokine IL-10**.**

2. MATERIAL AND METHODS

2.1. Subjects:

The study participants were divided into three groups, Group Iconsisted of healthy controls *n=100*. The group II with type 2 diabetes (without TB) *n=100*was recruited from Shalamar Hospital. T2DM was confirmed by Glycated hemoglobin (HbA1c), or fasting plasma glucose (FPG) test. The group III *n=100*

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consisted of newly diagnosed pulmonary tuberculosis and diabetic patients, who were contacted from Ghulab Devi Hospital, Lahore and Infectious disease Hospital, Lahore, Pakistan. Pulmonary TB confirmation was done by using sputum culture testing and tuberculin skin test in each patient on arrival by hospital staff.

HIV and HCV infected patients were excluded from all groups. The patients who were receiving other immunosuppressive therapies were also excluded.All of the patients were interviewed for their age, BMI, height, Disease History before the collection of blood samples.Informed consent was signed from all subjects. The ethical approval for this study was provided by Board of studies, Department of Zoology, GC University, Lahore, Pakistan.

2.2. Blood Sampling:

The blood sampling was done by venous puncture in EDTA tubes with the 5cc disposable syringe. Each sample (4 ml) was divided into two portions; one was utilized for studying hematological parameters and from the second portion of the blood plasma was isolated.

2.3. Assessment of Hematological Parameters:

Variousblood parameters were monitored like white blood cells, neutrophils, lymphocytes, platelets.

2.4. IL-10 Measurements:

Anti inflammatory cytokine IL-10 was measured using an ELISA kit (R & D system DY217B-05) according to the manufacturer's protocol.

2.5. Statistical Analysis:

The statistical analysis of data was done by using Statistical Package for the Social Sciences software (SPSS). One way ANOVA was applied to the data with LSD as post hoc test for the comparison between various groups. p value was taken significant at $p \leq 0.05$.

3. RESULTS

3.1. Demographic Characteristics:

It was observed in our study the individuals of group I with mean age 34.3 years ± 1.17 were younger than group II with mean age 47.9 years ± 1.26 and group III individuals with mean age 52.2 years ± 1.25as shown in **(Fig. 1a).** The mean value of BMI in group III was significantly lower when compared to the group II (30.11± 0.80) and I (23.52± 0.38) as shown in **(Fig. 1b).**

3.2. Hematological Parameters:

The number of WBCs observed in group I were $7.49\times10^3/\mu L \pm 0.20$, group II were $8.12\times10^3/\mu L \pm 0.24$ and in group III the number was $9.20\times10^3/\mu L \pm 0.36$. Significant increase in WBC count was observed group III, when compared with groupI **(Fig. 2a).**

Lymphocyte percentage observed in group I (control) was 32.14% ± 0.84 followed by group II with mean value 30.78% ± 0.97 and group III with mean value 23.54% ± 1.50. When compared with control subjects, significant decrease in lymphocyte percentage was observed in group III **(Fig. 2b).** The neutrophil count observed group I was 58.53% ± 0.95. In group II and group III neutrophil percentage determined was 62.95% ± 0.97 and 68.53% ± 1.59 respectively. A significant difference ($p \leq 0.05$) was found in neutrophils percentage in both group II and III as compared to group I **(Fig. 2c).**

The number of platelets in control subjects (group I) was 262.95 ± 9.26 and in diabetic patients (group II) it was slightly increased i.e. 268.32 ± 10.42. There was a significant elevation in the number of platelets in group III, the value observed was 355.03 ± 24.34 **(Fig. 2d)**

3.3. IL-10 measurement:

Mean baseline levels of IL-10 were higher among group II (31.5 ± 0.45) and lower in group III (7.96 ± 0.31) when compared with control subjects (15.54 ± 0.15) as shown in **(Fig. 3)**. The mean difference found was significant at p value of 0.05.

4. DISCUSSION

People having diabetes become more susceptible to infections, including tuberculosis, which is one of the chief causes of morbidity and death in diabetic patients. Chronic hyperglycemia impairs initiation of adaptive immunity, as a result a broad range of pathogens including *Mycobacterium tuberculosis (M.tb)* make their way into the bodies of patients. The present work was aimed to predict a biomarker to check risk of tuberculosis in type 2 diabetes mellitus based on levels of IL-10 (anti-inflammatory cytokine).

Demographic data showed that aging contributes toward disease progression as in our study the individuals of group I with mean age 34.3 years ± 1.17 were more younger than group II with mean age 47.9 years ± 1.26 and group III individuals with mean age 52.2 years ± 1.25. The results are in agreement with previous study conducted in India and Korea by Dye *et al.*, 2011**;** Reed *et al.*, 2013 that aging increases the incidence of tuberculosis. In India similar results have been reported that older TB patients had significantly higher prevalence of diabetes (Raghuraman *et el*., 2014; Alisjahbana *et al.*, 2007; Guptan and Shah, 2000). It has also been reported by Magee *et al.*, 2013 in Peru that TB-T2DM patients were significantly more likely to be older than patients with TB only. As immune system turns down with age and might be this fact makes the older diabetic people more prone towards getting

infections such as tuberculosis than younger ones. High BMI in group II might be that weight gain in diabetic patients is counterproductive and increases insulin resistance. Al-Goblan *et al*., (2014) reported that BMI and obesity has strong association with type 2 diabetes. Badawi *et al*., 2010, Bays *et al.,* 2007 and Miller *et al*., (2014) had similar findings.Whereas group III had lower BMI, as compared to group I and II. In accordance with the present research, a follow up study conducted in India, also represented that fall in BMI was the strongest unfavorable effect on TB incidence per capita (Dye *et al.,* 2011). Previously it has been shown by Magee *et al.,* (2013) that low body mass index (>18.5kg/m^2) was more pronounced among individuals with both type 2 diabetes and tuberculosis in Lima, Peru, a result consistent with present research. Immunity plays an important role in disease progression. The main parameters recruited in the present study were total WBCs, lymphocytes, neutrophils, and platelets. The statistically significant differences for the recorded hematological parameters of control subjects and group II and III were observed. In our finding, small increase in total WBCs was observed between healthy control and in group II (8.12×10^3/µL ± 0.24) and group III (9.20×10^3/µL ± 0.36). Our data confirms Veronelli *et al.,* 2004 findings that obese patients possessed elevated WBCs count. Another study by Veenstra *et al.,* 2006 indicated the augmented level of WBCs in TB patients.

Moreover the mean lymphocyte count of both group II (T2DM) and group III (T2DM-TB**)** was significantly lower when compared with healthy control. Our results are in agreement with previous study by Villacian *et al.,* (2005). Sen *et al.,* 2009 reported lymphocyte bactericidal activity was also reduced in patients with diabetes which in turn make them vulnerable towards infections like tuberculosis. As lymphocytes involved in cell-mediated and humoral immunity so this might be a possible reason that decrease in number of lymphocytes in T2DM patients make them more susceptible towards tuberculosis. Andrade *et al.,* 2014 reported that increased number of neutrophils in patients with diabetes and in both diabetic and TB patients in India. In another study in Tanzania by Faurholt-Jepsen *et al.,* 2012 also reported high neutrophils count in diabetic patients with tuberculosis. When compared with controls neutrophils of diabetic patients had reduced oxidative killing ability and chemotaxis this might be the possible reason that DM weakens the innate and adaptive immune responses which are required to fight against the TB proliferation (Delamaire *et al*., 1997). Consistent with the previous studies we found significantly higher neutrophils count in patients with T2DM and with bothT2DM and TB.

The platelet counts of T2DM-TB patients were higher than that of respective healthy controls. The fact behind that was, at the beginning of the TB process, pro-inflammatory cytokines (TNF-α & IFN-γ) released as a result expression of acute-phase proteins and thrombocytosis stimulated. The results of our study are comparable to a study performed in Sao Paulo State University, Brazil, by Oliva *et al*., (2008) on 80 pulmonary TB patients revealed that patients with less clinical disease duration showed higher platelet count values.

Mycobacterial infections can be determined by the level of cytokines; however, unbalanced cytokine responses are more likely to be associated with promotion of pathology in TB. In current study the plasma levels of IL-10 were significantly increased in group II and significantly decreased in group III in comparison to healthy controls. Our data clearly revealed that the T2DM-TB nexus is characterized by decreased circulating levels of IL-10 the anti-inflammatory cytokine. Similar results were recorded by Dooley and Chaisson, 2009 that DM might leads to TB and the proposed underlying mechanism was that DM impairs the chemotaxis of monocytes, and it affects the phagocytosis, and antigen presentation. Boussiotis *et al*., 2000 reported that increased IL-10 levels were linked with survival of *M.tb* and severe clinical phenotype of the disease. Progressive TB infection and increased anti-mycobacterial immunity was observed in IL-10 transgenic mice and in IL-10 deficient mice respectively. This is very similar to our findings in T2DM, where we had observed elevated levels of anti-inflammatory cytokine IL-10 in plasma.As IL-10 involved in deactivating macrophages, and decreased the secretion of pro-inflammatory cytokines such as INF-γ and TNF- α, which contributes to the granuloma formation, capable of controlling the disease progression and limited Antigen presentation which may have far-reaching consequences on both innate and acquired immunity in vivo (Beamer *et al.,* 2008), so this might be a possible reason that increase in level of IL-10 in T2DM patients make them more susceptible towards tuberculosis.

In conclusion, we showed that patients with type 2 diabetes over expressed anti-inflammatory cytokine IL-10 which make them more susceptible towards tuberculosis and other than that its normal value showed protective response during tuberculosis. Therefore, a delicate balance between suppressing and activating host responses to pathogens is maintained by IL-10.

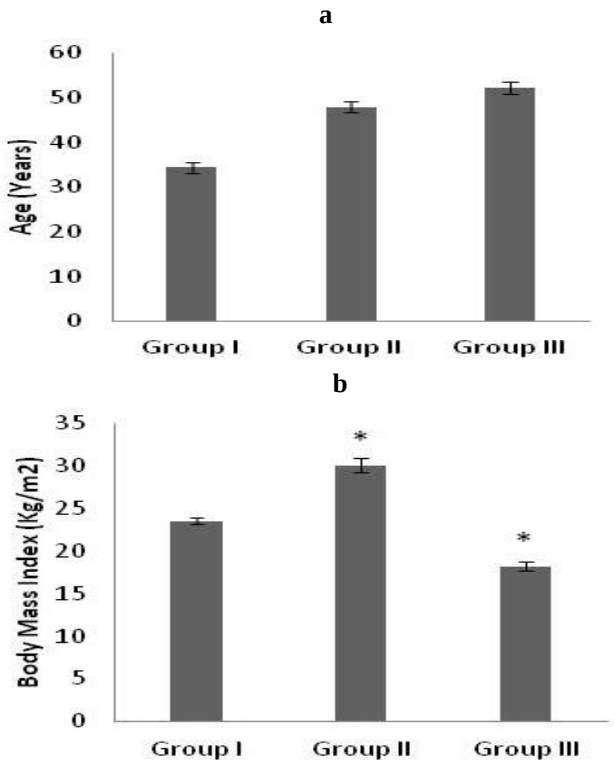
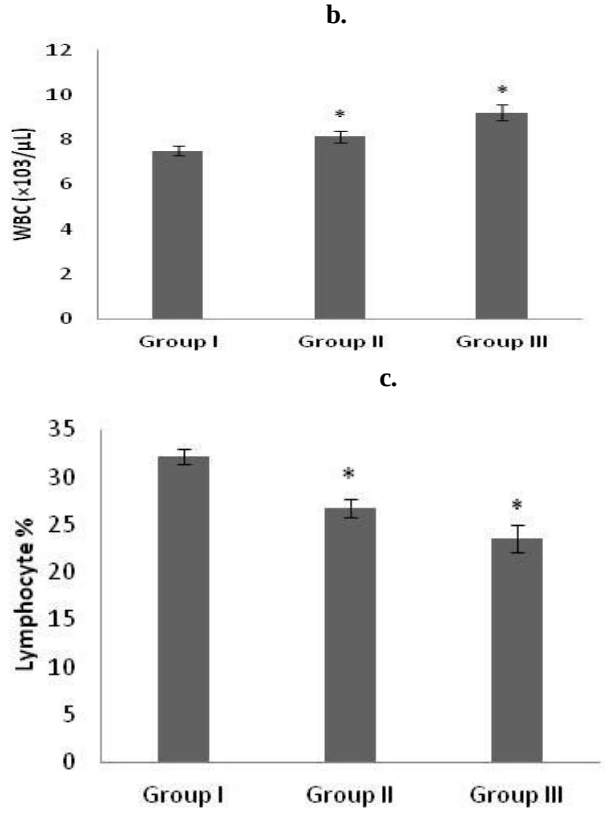


Fig. 1. Demographic parameters showing age (a) and BMI (b) variation in study Groups; Group I (Control), Group II (Type 2 Diabetes Mellitus) and Group III (Type 2 Diabetes Mellitus and Tuberculosis) (* Represents $p \leq 0.05$).



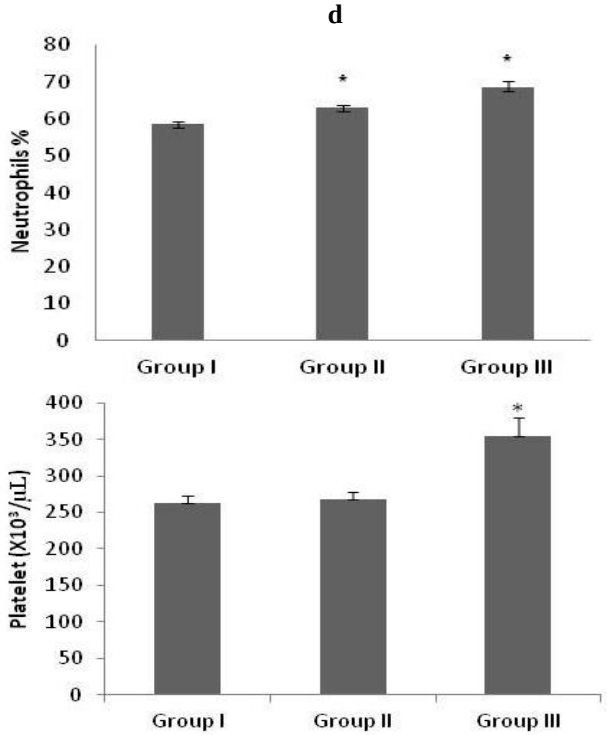


Fig. 2. Mean Value of total White blood cell count (a), Lymphocyte Percentage (b), Neutrophils percentage (c) and Platelet count (d) in study Groups; Group I (Control), Group II (Type 2 Diabetes Mellitus) and Group III (Type 2 Diabetes Mellitus and Tuberculosis) (* Represents $p \leq 0.05$).

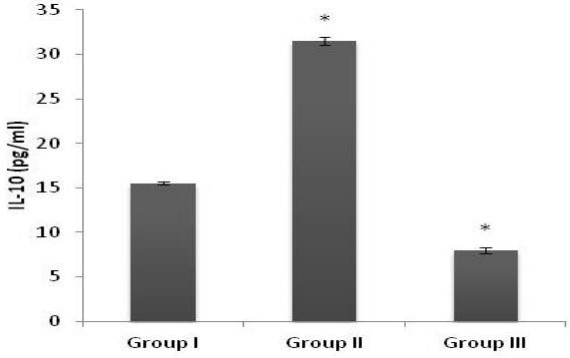


Fig. 3. Mean Value of Interleukin (IL)-10 in Study Groups; Group I (Control), Group II (Type 2 Diabetes Mellitus) and Group III (Type 2 Diabetes Mellitus and Tuberculosis) (* Represents $p \leq 0.05$).

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