Evaluation of Serum Circulating Levels of ICAM-1 as Tuberculosis Risk-assessment Factor in Type 2 Diabetes Patients

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Objective: Type 2 diabetes mellitus, due to its deteriorating effect on the immune system, makes a person susceptible to various other diseases, such as tuberculosis. The alarming increase in the number of diabetes mellitus cases in Pakistan may be a contributing factor to the increased tuberculosis incidence. The expression of cell adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) is important in determining cell permeability, and the latter's altered expression may ease the entry of infectious agents into the cell. Therefore, the present study evaluated the role of ICAM-1 in type 2 diabetes and tuberculosis patients so that a potential link between these 2 epidemics could be found.

Methods: To explore this hypothesis, the expression of ICAM-1 was measured tested in 3 groups of subjects: group I consisted of 100 healthy individuals (control), group II consisted of 100 type 2 diabetics, and group III consisted of 100 individuals with both type 2 diabetes and tuberculosis. Demographic information was obtained from all the participants and compared by group and ICAM-1 levels in the blood were determined by ELISA.

Results: The results revealed that, in comparison to group I, the individuals of group II had significantly ($p \leq 0.05$) increased levels of ICAM-1, making them more prone to infection (by promoting the increased invasion of mycobacterium) and hence at increased risk of contracting tuberculosis.

Conclusion: In conclusion, the present study demonstrated that elevated levels of ICAM-1 in patients with type 2 diabetes mellitus are likely associated with the development of tuberculosis. [*P R Health Sci J 2019;38:22-26*]

Key words: Tuberculosis, Type 2 diabetes mellitus, ICAM-I, Serum levels, Risk factors

Tuberculosis, an infectious bacterial disease, is caused by *Mycobacterium tuberculosis* (M.tb). In Pakistan tuberculosis is one of the chief health problems. According to the WHO, Pakistan ranks fifth among countries with high burdens of tuberculosis. It has been estimated that approximately 420,000 new cases of tuberculosis arise every year, globally (1). Tuberculosis is also associated with smoking, malnutrition, lung disease, and certain cancers (2). There exists a positive association between tuberculosis and diabetes mellitus, and this association is stronger among the people living in tuberculosis-endemic areas with high incidence rates of diabetes mellitus (2). This investigation has led to the re-emerging significance of type 2 diabetes mellitus as a risk factor for tuberculosis (3). The connection between tuberculosis and diabetes mellitus is well recognized, and studies have suggested that the risk of tuberculosis is twofold higher in diabetic patients than in individuals without diabetes mellitus (4).

At many stages both diabetes mellitus and tuberculosis complicate each other. Diabetes mellitus adversely affects the treatment outcome of tuberculosis by causing a delay in the immunological response against M.tb. It has been assumed that diabetes mellitus promotes the growth of tubercle bacilli by increasing the availability of nitrogenous substances and glycerol, which aids in the growth of bacteria (5); furthermore, reduced oxidative killing potential and neutrophils chemotaxis, and leukocyte bactericidal activity (6) have also been investigated in diabetic patients. These findings suggest that diabetes mellitus is involved in the impairment of those immune responses necessary to counter the propagation of M.tb (7).

Intercellular adhesion molecule-1 (ICAM-1) serves as an adhesion molecule which plays a crucial role in the cellular interactions involved in the immune responses as well as in the recruitment of cells. ICAM-1 basal expression has been reported

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on the surface of leukocytes, macrophages, and endothelial cells (8). Various cytokines, such as interferon gamma (IFN-γ) and tumor necrosis factor (TNF-α), are involved in the upregulation of surface ICAM-1 expression (9). During an inflammatory response, high levels of ICAM-1 have been detected in serum and extracellular fluids (10). Elevated levels of ICAM-1 may indicate the presence of a pathological condition associated with the inflammation of the vascular wall and have also been detected in insulin-dependent diabetes, reflecting ongoing immune processes (11). In diabetic patients, endothelial cell dysfunction has been observed (12). Endothelial cell dysfunction is mediated by free radicals caused by elevated glucose. Findings also suggest that glucose plays a role in generating increased ICAM-1 levels in diabetes (13), which can provide a site for M.tb entry into the cell.

This study was designed to investigate if type 2 diabetes affects ICAM-1 levels, which could impair the immune response against M.tb.

Materials and methods

The ethical approval for the study was provided by the Board of Studies, Department of Zoology, GC University, Lahore, Pakistan. Three groups were defined. Group I consisted of 100 healthy individuals who were free from any medical condition that could cause the elevation of any of the study parameters and was called the "control group." Group II also had 100 individuals, which individuals had been clinically diagnosed with type 2 diabetes mellitus, only, while group III had 100 subjects who had been clinically diagnosed with diabetes and tuberculosis and were undergoing treatment for both ailments. Patients having HIV or HCV and receiving some form of immunosuppressive therapy were excluded from participating in this study. These groups were compared in terms of their age, gender, BMI (body mass index), disease history, total leucocyte count, neutrophil and lymphocyte percentage, and serum circulating levels of ICAM-1.

From each individual who participated in the study, 2.5 ml of blood were collected in serum-separating tubes, for the isolation of serum to evaluate ICAM-1 levels, and 2.5 ml was collected in EDTA-coated tubes, for the investigation of other blood parameters. ELISA was conducted to determine ICAM-1 levels, using a commercial kit (R& D Systems, DY720-05).

The data were collected and analyzed using the Statistical Package for the Social Sciences software (SPSS). One-way ANOVA was applied to the data to compare the differences between various groups. A p-value lower than or equal to 0.05 was considered to be significant.

Results

The various parameters analyzed showed that there were significant differences between the 3 groups. The mean ages, gender distribution, and the mean BMIs of all the 3 groups are shown in Table I.

Analyses of the blood samples collected from the participants were done for all 3 groups. The mean white blood cell count in group I (the control group) was $7.49 \times 10^3/\mu L$ (±0.20), while in group II and III, the counts increased significantly, as can be seen in Table II.

Table 1. The mean values of age and BMI and the gender distributions in group I (control), group II (type 2 diabetes mellitus), and group III (type 2 diabetes mellitus and tuberculosis).

	Age (years)	BMI	Gender
Group I	34.3 ± 1.17	23.5 ± 0.38	32% (F) 68% (M)
Group II	47.9 ± 1.26* (p = 6.1E-13)	30.11 ± 0.80* (p = 5.7E-15)	66% (F) 34% (M)
Group III	52.2 ± 1.25* (p = 2.2E-17)	18.2 ± 0.5* (p = 4.9E-18)	46% (F) 54% (M)

*p ≤ 0.05

The neutrophil percentages varied in all 3 groups; when compared with that of the control group, significant differences in neutrophil percentage were found in both of the other groups (p<0.05). Similarly the percentages of lymphocytes observed in groups II and III were significantly different from that of group I (Table II).

The levels of ICAM-1 were calculated in all 3 groups using ELISA. The level of ICAM-1 found in group I was 343.3 ng/ml (±40.2); in group II, it was significantly higher (p<0.05), at 541.8 ng/ml (±5.2), while in group III, it was significantly lower, at 127.7 ng/ml (±4.1; p<0.05), than that of the control group (Figure I).

Table 2. Mean values of total white blood cells (WBC), neutrophil percentages, and lymphocyte percentages in group I (control), group II (type 2 diabetes mellitus), and group III (type 2 diabetes mellitus and tuberculosis).

	Total WBC	Neutrophils	Lymphocytes
Group I	$7.49\times10^3/\mu L$ ± 0.20	58.5% ± 0.95	32.14% ± 0.84
Group II	$8.12\times10^3/\mu L$ ± 0.24* (p = 0.022)	62.9% ± 1.59* (p = 0.001)	26.78% ± 0.97* (p = 5E-07)
Group III	$9.20\times10^3/\mu L$ ± 0.36* (p = 1.5E-08)	68.5% ± 1.5* (p = 8.4E-08)	23.54% ± 1.50* (p = 4.5E-05)

*p ≤ 0.05

Discussion

Type 2 diabetes mellitus is a major health threat worldwide (14). Its incidences are increasing, with the passage of time, throughout the world (15). Many clinical studies have confirmed that patients with type 2 diabetes mellitus are prone to a variety of infectious diseases, including pulmonary tuberculosis. Our study emphasizes the potential of ICAM-1 as a risk factor of tuberculosis in patients with type 2 diabetes mellitus.

The demographic data collected showed a trend towards higher rates of tuberculosis in older diabetic Pakistani patients with a mean age of 52.2 years (±1.25). In accordance with the present research, a follow-up study conducted in Peru also found that older individuals were more prone to having comorbid tuberculosis and diabetes than were their younger counterparts (16). In India higher incidences of type 2 diabetes mellitus and tuberculosis were recorded in the 52 years old and older age group (7). A study conducted in southern Mexico also described a higher proportion of type 2 diabetes mellitus and tuberculosis in an older age group (45 to 64 years old) (17). It is a well-known fact that immune function declines with age, in particular, cellular immune function; therefore, the possible cause of the development of tuberculosis in older diabetic patients might be the impaired immune systems of those patients, which impairment makes them more vulnerable to developing tuberculosis than younger patients are.

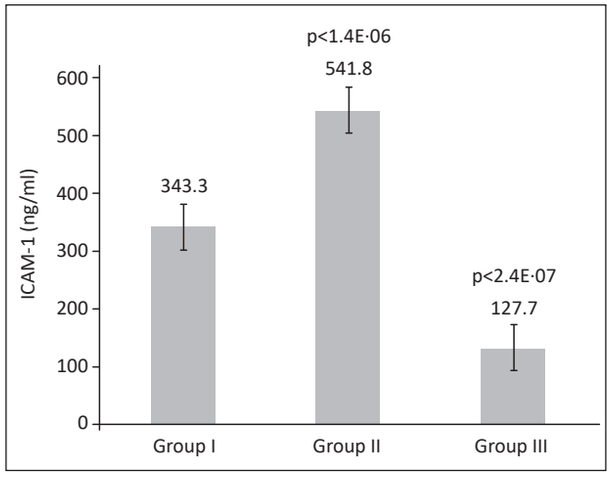

Figure I. Levels of ICAM-1 in group I (control), group II (type 2 diabetes mellitus), and group III (type 2 diabetes mellitus and tuberculosis).

In the context of gender-wise incidences of disease, the current study reported that the incidence of type 2 diabetes mellitus was higher in females than males, and the percentages of type 2 diabetes mellitus and tuberculosis were higher in males than females in the Pakistani population. A study conducted in European populations supports the findings of this current investigation by suggesting that type 2 diabetes mellitus was more likely to be found in women than in men in the population studied (18). Obesity has been associated with type 2 diabetes risk, and is higher for women than it is for men (19). The results of the present investigation are also similar to the results of a study conducted in the south west of Iran, in that both studies showed that tuberculosis was more pronounced in diabetic males than females (69.4% vs. 30.6%) (2). Besides age and gender, the interaction of diabetes and BMI has an important effect on tuberculosis risk. In the present study, an analysis of BMI data suggested that low and underweight type 2 diabetes mellitus individuals (mean value: 18.2 ± 0.5) were at higher risk than their normal- and overweigh counterparts of tuberculosis development in Pakistan. However, patients with type 2 diabetes mellitus only were found to have higher BMIs (mean value: 30.11 ± 0.80). Several studies have reported that BMI and obesity have strong associations with type 2 diabetes mellitus (20–21). As reported by several studies, the increased risk of metabolic disorder is associated with increased body fats (22), which might be a possible reason for the finding of higher BMIs in patients with type 2 diabetes mellitus. The work of Magee et al. and Dye et al. (16, 23) also showed higher BMI in the diabetic patients.

Type 2 diabetes mellitus causes extensive complications in various organs and tissues (24). It is also found to be involved in the impairment of the immune response, which is required to counter the proliferation of M.tb (7). Elevated white blood cell counts in group II and group III may reflect the underlying activation of the immune system as a result of the inflammatory response against M.tb (25). Similarly decreased lymphocyte counts (compared to that of the control group) may have increased the chances of the people in groups II and III to get tuberculosis, as poor glucose control in diabetics tends to reduce the bactericidal activity of leukocytes, which in turn increases the susceptibility of such patients to tuberculosis (7). The decrease in lymphocyte percentage in group II was statistically significant, but clinically, it was in a permissible range, whereas, in group III, the decrease was both statistically and clinically significant. A slightly higher percentage of neutrophils was observed in group II and III, which higher percentage is one of the critical components of the innate immune response against tuberculosis. It has been reported that microorganisms have increased adherence to diabetic cells—compared to such adherence to non-diabetic cells—which might be a possible reason for the increased rate of infection in diabetic patients (26). It has also been reported that in diabetic patients, chemotaxis and the oxidative killing potential of neutrophils are reduced (27), which tends to impair the innate and adaptive immune responses necessary to fight against tuberculosis proliferation.

ICAM-1 serves as an adhesion molecule that plays a key role in cell–cell interactions and is involved in the immune response as well as in the recruitment of cells (9). Evidence suggests that soluble ICAM-1 might be involved in the progression of various diseases. In the current study, elevated serum ICAM-1 levels in diabetic patients are presented as a possible indicator of tuberculosis development. Significantly increased levels of ICAM-1 were observed in group II, which increases might have been the result of oxidative stress, obesity, and other diabetes complications. In accordance with our current findings, the findings of a study performed on diabetic rats also reported the increased expression of ICAM-1 (28). Studies have reported that elevated levels of ICAM-1 in several pathological conditions including obesity, which might be responsible for such elevated levels in patients with type 2 diabetes mellitus (29) may be due to its increased expression by adipocytes (30). Levels of ICAM-1

also correlate with disease severity, and elevated levels of soluble ICAM-1 may indicate the existence of a pathological condition associated with the inflammation of the vascular wall (31). Studies have also reported on the immunosuppressant effects of ICAM-1 as well as its inhibitory effects on the extravasation of monocytes and neutrophils. It also interferes with the activation of the T cells involved in the formation of the immunological synapse (30).

A functioning actin cytoskeleton is necessary for the expression of ICAM-1 (32). It has also been reported that elevated levels of ICAM-1 are associated with the disturbance of the actin cytoskeleton, thereby affecting paracellular permeability and producing gaps in the cell junctions (33), which might facilitate the invasion of bacteria. Elevated ICAM-1 levels are also responsible for the reduced function of endothelial cell barriers. Thus, the increased expression of ICAM-1 makes diabetic patients prone to infectious diseases (e.g., tuberculosis), by promoting the increased invasion of the mycobacterium through loose junctions. Moreover decreased cellular immunity in diabetic patients further worsens the condition. All these dysfunctional processes put diabetic patients at increased risk of tuberculosis. The significant decrease of serum ICAM-1 levels observed in group III can be attributed to the fact that these individuals were undergoing therapies that were themselves slowing the inflammatory processes. The supplements provided along with the tuberculosis drugs to improve metabolic control and total antioxidant status (13, 34) might have lowered ICAM-1 values. The variations of the ages and BMIs in the control and other groups could not have impacted the ICAM-1 levels significantly, as endothelial cell function is not significantly affected by these parameters unless accompanied by a medical condition (35).

In conclusion, the present study demonstrates that the presence of elevated levels of ICAM-1 can be used to predict tuberculosis infection in patients with type 2 diabetes mellitus. Further studies should be carried out to determine its actual immune-suppressant effects so that susceptible individuals can be targeted.

Resumen

Objetivo: La diabetes mellitus tipo 2, debido a su deterioro en el sistema inmunológico, hace que una persona sea susceptible a otras enfermedades como la tuberculosis. El aumento alarmante de los casos de diabetes mellitus en Pakistán puede ser un factor que contribuye al aumento de las incidencias de tuberculosis. La expresión de moléculas de adhesión celular como la molécula de adhesión intercelular-1 (ICAM-1, por sus siglas en inglés) es importante para determinar la permeabilidad celular y la expresión alterada puede facilitar la entrada de agentes infecciosos en la célula. Por lo tanto, el presente estudio evaluó el papel de la ICAM-1 en pacientes con diabetes tipo 2 y tuberculosis para poder determinar un posible vínculo entre estas dos epidemias. Métodos: Para explorar esta hipótesis ICAM-1 se probó en tres grupos de sujetos: el grupo I consistió en 100 individuos sanos como control, el grupo II tenía 100 diabéticos tipo 2 y el grupo III tenía 100 individuos con diabetes tipo 2 y tuberculosis. Estos grupos se compararon por factores demográficos y los niveles de ICAM-1 se determinaron a partir de sangre mediante ELISA. Resultados: Los resultados revelaron que, en comparación con el grupo I, los individuos del grupo II tenían niveles significativamente mayores ($p \leq 0.05$) de ICAM-1 haciéndolos más propensos a la infección al promover una mayor invasión de micobacterias y por lo tanto un mayor riesgo de tuberculosis. Conclusión: En conclusión, el presente estudio demostró que los niveles elevados de ICAM-1 en pacientes con diabetes mellitus tipo 2 pueden estar asociados con el desarrollo de la tuberculosis.

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