FASTING SERUM RESISTIN AND BLOOD GLUCOSE LEVELS IN NEWLY DIAGNOSED MALE PATIENTS OF PULMONARY TUBERCULOSIS WITH AND WITHOUT DIABETES MELLITUS TYPE 2.

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ABSTRACT

The aim of this cross-sectional comparative study was to determine and compare fasting serum resistin and fasting blood glucose (FBG) level in the newly diagnosed pulmonary tuberculosis (TB) patients having and not having diabetes mellitus type 2 (DM). Eighty four male subjects were divided equally into groups. Group 1, healthy controls (n=28), group 2, newly diagnosed patients of pulmonary tuberculosis without DM (n=28) and group 3 included new pulmonary TB patients with newly diagnosed DM type 2 (n=28). Serum resistin and fasting blood glucose levels were determined before starting anti-TB and hyperglycemia treatment. Severity of TB was determined by analysis of sputum for bacterial load. Fasting serum resistin was done by ELISA and fasting blood Glucose by a glucose oxidase kit. Fasting blood glucose showed a significant difference between the three study groups (p= 0.000). Fasting serum resistin levels did not show a statistically significant (p= 0.098) difference between the groups. However a significant difference in serum resistin levels (p= 0.029) was observed when the TB patients were divided according to sputum smear analysis reports. It is concluded that although non-significant difference was found between serum resistin levels of diabetic and non-diabetic TB patients, disease severity can influence serum resistin levels. Hyperglycemia present in the TB non-diabetic patients before starting ATT should be taken into consideration.

Keywords: Resistin, Fasting blood glucose, Pulmonary TB, Diabetes Mellitus type 2

INTRODUCTION

Tuberculosis is a chronic disease caused by Mycobacterium tuberculosis (M. TB)1. It is estimated that 1.5 million people died from TB in 20132. Pakistan is one of the five countries that carry almost half the world’s TB burden. Prevalence of DM in Pakistan’s urban areas is 6% in males and 3.5% in females3.

Studies suggest that DM and TB are linked with each other and coexist in many patients5,6. Resistin is a 12.5kilo Dalton, 108 amino acid polypeptide, cysteine rich adipocytokine, that antagonizes insulin action by inducing severe hepatic and peripheral insulin resistance7. It is also suggested that resistin employs pro-inflammatory signaling pathways to alter immune mechanisms. Fasting serum resistin level is higher in type 2 diabetics when compared to non-diabetics9.

Despite the importance of resistin, relatively conflicting information is available about the regulation of its production and its actions. It is suggested that resistin has an ability to induce Th-1 immune response10. In humans, monocytes and macrophages are the main secretors of resistin11. When blood monocytes convert into tissue macrophages the expression of resistin mRNA increases four folds12. The serum level of TNF-α is raised in pulmonary TB and the production was more in advanced TB cases as than the healthy controls13. It was demonstrated in an in-vitro study that IL-1 and TNF-α are potent stimulators of resitin’s mRNA14. The purpose of this study was to determine and compare the fasting resistin and blood glucose levels in Pakistani pulmonary TB patients with and without coexisting DM type 2.

MATERIALS AND METHODS

This was across sectional comparative study. Subjects were selected from Lahore General Hospital and Gulab Devi Chest Hospital, Lahore for...
nine consecutive months in 2013-2014. The sample size was determined using power of the study 90% and α level of 5% using mean values for resistin from Ehtesham et al, 20118. The Study population consisted of 84 male subjects between 30-55 years of age and with a body mass index (BMI) ≤ 25. The study population was divided equally into three groups. Group 1: Healthy controls (28), Group 2: TB non-diabetics (28) and Group 3: TB diabetics (28). Inclusion criteria for group: 1 was no history of TB and DM, for Group 2: newly diagnosed patients of pulmonary TB with no history of DM, Group 3: newly diagnosed patients of pulmonary TB and DM type 2 or known diabetic not getting treatment for past 1 month. Diagnosis was based on WHO criteria: Fasting blood glucose ≥ 126 mg/dl or random blood glucose ≥ 200 mg/dl. The Exclusion criteria for all the groups was history of current steroid intake, hypertension, major surgical intervention in past 3 months, endocrine disorders, extra-pulmonary TB. The Sampling technique was non-probability convenience sampling. Samples were collected after taking permission from hospitals and all the subjects included in our study. Following formula was used to calculate Body mass index (BMI) of the patients: BMI = Weight in kilograms / Height in meter^2

The patients were instructed to come after an overnight fast of 12 hours for blood sample collection. All samples were drawn in sitting position after a rest of at least 10-15 minutes. Five milliliter (ml) of venous blood was collected in yellow topped vacutainers. The blood was centrifuged for 20 minutes and resulting serum was divided into aliquots and kept at -80°C.

Glucose in serum was determined by a glucose oxidase kit of Pointe Scientific, Inc. USA using photoelectric colorimeter, AE-11, Tokyo Erma Optical works, LTD. Japan.

Fasting serum resistin was quantitatively measured by ELISA test kit of Glory Science Co., Ltd., U.S.A and ELISA analyzer Rayto RT 2100 C, USA.

STATISTICAL ANALYSIS
IBM SPSS 20 was used to do statistical analysis. The tests of normality (Shapiro-Wilk’s test) were applied on the data and results presented as median and interquartile ratio (IQR). Kruskal Wallis test was used for comparison of medians of the three study groups. Mann Whitney U test was used for the pair wise comparison of the variables. A P-value ≤ 0.05 was taken as significant.

RESULTS

Table 1: Median (IQR) of BMI of the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Median (IQR)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 28)</td>
<td>21.91(19.72-23.79)</td>
<td>0.974</td>
</tr>
<tr>
<td>2 (n = 28)</td>
<td>18.01(15.35-19.55)</td>
<td>0.144</td>
</tr>
<tr>
<td>3 (n = 28)</td>
<td>20.03(18.38-22.56)</td>
<td>0.001**</td>
</tr>
</tbody>
</table>

*P-value obtained by applying Pearson Chi-Square test

Comparison of serum resistin and FBG between the study groups using Kruskal-Wallis test showed a P-value of 0.098 and 0.000 respectively.

Table 2: Comparison of serum resistin and FBG between study groups using Mann-Whitney U test

<table>
<thead>
<tr>
<th>Group</th>
<th>Median (IQR)</th>
<th>Resistin (ng/ml)</th>
<th>FBG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 28)</td>
<td>5.05(4.915-5.52)</td>
<td>68(63.25-76.50)</td>
<td></td>
</tr>
<tr>
<td>2 (n = 28)</td>
<td>5.3(4.93-5.875)</td>
<td>75(69.25-81.75)</td>
<td></td>
</tr>
<tr>
<td>3 (n = 28)</td>
<td>5.05(4.915-5.52)</td>
<td>68(63.25-76.50)</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.144</td>
<td>0.035*</td>
<td></td>
</tr>
</tbody>
</table>

When TB patients were separated according to bacterial load in sputum smear analysis then a statistically significant difference was observed between fasting serum resistin levels of patients with mild (n=45) and moderate disease (n=5) patients (p= 0.023) and between moderate and severe disease patients (p= 0.028).
**DISCUSSION**

A statistically non-significant difference was observed between fasting serum resistin levels of the three study groups. Resistin is a cytokine that activates pro-inflammatory pathways during various chronic inflammatory diseases. Macrophages are the main inflammatory cells involved in immune reaction against M.TB infection. The up-regulated expression of resistin in macrophages is due to inflammatory stimuli (TNF-α, IL-6), implicating this adipokine in inflammation and inflammation-related diseases. This knowledge prompted to expect that the serum level of resistin would be higher in pulmonary TB patients when compared to controls but a statistically significant difference was not found between the two groups.

The findings of our study are consistent with the findings of Chao et al who have reported a non-significant difference between levels of fasting serum resistin in healthy and mild TB cases. Majority of the TB cases in their study had mild disease and they suggested that the systemic inflammatory response in mild TB infection is not sufficient enough to produce high levels of resistin.

The difference in the findings of this study from the present study can be as a result of variations in the study population. Both males and females were included in their study while only males were included in this study. Resistin exhibits sexual dimorphism with females having approximately 20% higher level than males. Females tended to have a higher body fat mass than males. It is still not clear whether difference in distribution of body fat or in sex steroids is the reason for a higher resistin level in female than males.

Only weight of the subjects was measured by Ehtesham et al and BMI was not calculated, while in the present study BMI was calculated to exclude obese individuals. Although the mechanisms at molecular level are not yet well understood, obesity is one of the most important factors leading to DM. The causal relation between adiposity and serum resistin remains uncertain. The present study included only non-obese subjects since the part played by resistin in obese and diabetic patients is still being investigated in humans. Burnett al suggested that variations in data regarding resistin levels may show influence of genetic factors or environmental factors on resistin expression and be subject to differences in ethnicity.

All the patients included in this study had a low socioeconomic status. The newly diagnosed cases of TB were found to have a significantly lower BMI (18.01Kg/m²) as compared to control group (21.91 Kg/m²) which is consistent with the finding that during TB infection changes in appetite-regulatory hormones such as resistin, leptin and ghrelin leads to appetite suppression, cachexia and wasting that improves with treatment.

Pulmonary TB is a progressive disease and its severity can be classified on the basis of load of bacterium in the sputum smear analysis. Majority of the TB cases included in the present study had mild pulmonary TB (78%) and we found a non-significant difference between fasting serum resistin levels of mild and severe cases. In this aspect, result of the present study is consistent with Ehtesham et al., they also reported a non-significant difference between mild and severe TB cases in their study. Chao et al on the contrary found a significant difference between the severe and mild cases. They further reported a trend of increased resistin levels in DM only cases and in severe TB with DM as compared to non-diabetics but it did not reach statistical significance.

Our study showed a significant difference between fasting blood glucose levels of group 1 and 2. Higher rates of glucose intolerance have been observed in TB patients as compared to the controls. Studies have suggested that TB can lead to impaired glucose intolerance. Generally, IGT normalizes after the TB has been successfully treated, but it remains a significant risk factor for developing type 2 diabetes in the future.

**CONCLUSION**

It is concluded that although non-significant difference was found between serum resistin levels of diabetic and non-diabetic TB patients, disease severity can influence serum resistin levels.

**Table 3:** Median (IQR) of serum resistin in TB population (Group 2 and 3) according to bacterial load in sputum smear analysis

<table>
<thead>
<tr>
<th>Bacterial load</th>
<th>Resistin (ng/ml)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>mild(n=45)</td>
<td>5.25(5.025-5.545)</td>
<td>0.023*</td>
</tr>
<tr>
<td>moderate(n=5)</td>
<td>4.915(4.775-5.28)</td>
<td></td>
</tr>
<tr>
<td>severe(n=6)</td>
<td>5.65(5.275-5.955)</td>
<td></td>
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</tbody>
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patients, before starting ATT, should be taken into consideration.

RECOMMENDATIONS
A diabetes only group was not present in our study and future studies including such a group would be beneficial.

REFERENCES


