

ORIGINAL ARTICLE

SERUM RESISTIN LEVELS AS PROGNOSTIC MARKER IN PULMONARY TUBERCULOSIS

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Background: Resistin targets various human cells and induces inflammation and autoimmune processes by promoting the release of multiple proinflammatory cytokines. The objective of this study was to determine the importance of serum resistin levels as prognostic biomarker in patients on antituberculous therapy. **Methodology:** This prospective cohort study was conducted in Military Hospital, and Army Medical College, Rawalpindi. The study recruited 90 patients by convenience sampling, divided into 2 equal groups comprising of active tuberculous patients and healthy controls. After written informed consent their blood samples were taken at various events (0, 2 and 4 months) and were assessed for serum resistin levels measured via ELISA. **Results:** The serum resistin levels were raised (43 ± 16 ng/mL) significantly in cases at time of diagnosis, when compared with healthy controls (10 ± 2 ng/mL). After 2nd and 4th month of treatment the levels were 15 ± 10 ng/mL and 22 ± 10 ng/mL respectively. The serum resistin level declined significantly over both events ($p < 0.01$). **Conclusion:** Serum Resistin levels were increased at time of diagnosis in tuberculous patients and declined significantly during therapy.

Keywords: Tuberculosis, serum resistin, pulmonary, prognostic marker

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INTRODUCTION

Resistin known as adipose tissue-specific secretory factor (ADSF) is a 12.5 kDa cysteine-rich secretory protein (XCP1) composed of 108 amino acids. It is produced by adipocytes in rodents. However in humans it is mainly secreted by macrophages and monocytes, whereas secretion from adipocytes is controversial.¹ Resistin targets various human cells and induces inflammation and autoimmune processes by promoting the release of multiple proinflammatory cytokines like TNF- α , IL-1, IL-6, IL-12 and lipopolysaccharide (LPS) which further increases the expression of resistin gene.²

Resistin belongs to a family of cysteine rich proteins that encodes by *Retn* gene that is encrypted on 1.3-kb segment on chromosome 19¹, whereas in mice, it is on chromosome 8 and 476-nucleotide transcript is present within three exons.³ Normally, the serum concentration of resistin in human is 7–22 ng/mL.⁴ Women have higher resistin levels compared with men, in contrast to rats which show high resistin levels in males.¹ Various chronic inflammations demonstrated its presence in blood like in coronary artery disease, rheumatoid arthritis and atherosclerosis.⁵

Tuberculosis is a chronic inflammatory disease caused by *Mycobacterium tuberculosis* affecting 9 million people globally and causing 1.5 million deaths each year. Serum resistin levels are associated with impairment of reactive oxygen species (ROS) and production of interleukin (IL)-1 β . Various prognostic markers for tuberculosis have been identified including ESR, CRP, interleukin (IL)-1, IL-8, Tumour Necrosis Factor (TNF)- α and many others. The aim of this study

was to determine the effectiveness of antituberculous therapy (ATT) in patients with pulmonary tuberculosis by measuring serum resistin levels. It is assumed that due to the interplay of resistin with a number of cytokines and chemokines, serum resistin may be an excellent biomarker for assessing prognosis of ATT.

MATERIAL AND METHODS

Ninety participants comprising of equal number of controls and cases with age 18–65 years were recruited. Cases were newly diagnosed patients of TB and controls were age and sex matched healthy volunteers. Controls with normal BMI were included in the study. They were studied through prospective cohort design, for 4 months and blood samples were collected at different time points, i.e., TB0 (at the start of the treatment), TB2 (2 months after commencement of treatment) and TB4 (4 months after commencement of treatment).

Diagnosis of TB was made on the basis of early clinical diagnosis supported by positive sputum smears or X-ray chest findings or Gene Xpert studies. Individuals with diabetes mellitus, hypertension, obesity and chronic infections were excluded from the study. The study was approved by the Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College and written informed consent was taken from all subjects.

For measurement of serum resistin level, 2.5 mL of blood was drawn from antecubital vein in EDTA treated tubes to prevent clotting. Samples were stored at -80 °C till analysis. Serum resistin levels were

measured by Human Resistin ELISA Kit (Invitrogen) for controls once only (at the start of study) and thrice for cases during the study.

Statistical analysis was carried out using SPSS-22. Continuous variables like age, BMI and serum resistin levels were presented as Mean±SD. Categorical variables like diagnostic criteria, symptoms, chest findings and pleural effusion were expressed as frequency and percentages. Repeated measures analysis of variance (ANOVA) followed by Post hoc Tukey's was applied between various groups to find statistical difference in serum resistin levels, and $p < 0.05$ was considered significant.

RESULTS

All measurement and levels of resistin were determined as per preceding section. The mean ages and BMI of cases and controls and other characteristics of cases are shown in Table-1. It was determined during analysis that serum resistin levels were significantly influenced by variation in age of the controls ($p < 0.01$) (Table-2).

Serum resistin levels were estimated in subgroups of active tuberculous cases at and in healthy controls. Mean values of serum resistin levels in TB0 were 43.27 ± 15.2 ng/mL, TB2 15.27 ± 9.55 ng/mL and TB4 22.0 ± 10.4 ng/mL respectively which were significantly different from control ($p < 0.01$) having mean value of 10.47 ± 1.5 ng/mL. The difference of serum resistin between the subgroups was also statistically significant.

Table-1: Characteristics of tuberculous patients

Characteristics	Cases	Controls
Total (n)	45	45
Age	38.42 ± 16.11	38.27 ± 15.83
BMI	19.95 ± 2.90	20.76 ± 3.21
History of contact	5 (11.11%)	
Fever	34 (75.5%)	
Cough	40 (88.9%)	
Productive cough	20 (44.4%)	
Weight loss	33 (73.3%)	
Anorexia	32 (71.1%)	
Night sweats	23 (51.1%)	
Tiredness	35 (77.7%)	
Pleural effusion	15 (33.3%)	

Table-2: Serum resistin levels between different age groups among healthy individuals

Age (Years)	16–35	36–50	>50	<i>p</i>
N	23	13	9	<0.01*
Serum resistin (ng/mL)	10 ± 1	11 ± 2	10 ± 2	

*Significant

DISCUSSION

Our key finding was a significant decrease in resistin levels with treatment in active tuberculous patients. The mean resistin levels at time of diagnosis were higher as compared to controls and gradually reduced

with treatment at 2nd and 4th month of treatment. Post treatment levels were still higher than controls. Previously, the serial measurement of serum resistin was also done in patient of acute lymphoblastic leukaemia at time of diagnosis and during maintenance phase to validate its role in evaluating the prognosis of disease. A significant decline was observed with successful treatment.⁶

Ehtesham *et al*⁷ conducted a similar study in active TB patients, healthy contacts and controls and compared resistin levels with CRP levels at various consecutive intervals of treatment. They also showed raised resistin levels in healthy contacts suggesting protective immune process in individual from frequently exposed microorganism.⁷ Serum resistin levels were not affected by BMI in our study supporting results of previous studies that showed no link between obesity and resistin.

In rats and mice, resistin is regulated by fasting feeding cycle but in humans its secretion and regulation is intensely dependent upon the production of monocytes and macrophages, thus in humans hyperresistinemia is not related to adipose tissue mass.⁸ Resistin levels alter with increasing age⁹ coinciding with the findings of our study. In rats the resistin levels increased with age initially and changes were directly related to the fat stores of body.⁹ No significant difference in resistin levels between genders was observed in our study coinciding with the findings of Gerber *et al*, showing that estradiol has no effect on resistin levels.⁸ In another study it was found that during prepubertal age resistin levels were significantly raised in both genders, more so in females, compared with males followed by a decrease in adulthood. It also had been reported that high resistin levels were seen in pregnant females due to expression of resistin mRNA on placental tissue.¹⁰ The resistin levels were markedly raised in cases at TB0 due to marked inflammation.

Resistin is suggested to play a key role in infections and chronic diseases, i.e., resistin levels increase in chronic inflammatory process.¹¹ Tuberculosis is a chronic infection characterized by increased proinflammatory cytokines that leads to rise in serum resistin levels. In our study TB patients showed high resistin levels at time of diagnosis which progressively decreased with anti-tuberculous therapy. Supporting our findings a study conducted in patients with inflammatory bowel disease presented with upsurge of serum resistin levels along with raised adiponectin levels whereas serum leptin levels were decreased.¹² Aliefendioglu¹³ and Senolt¹⁴ conducted studies in patients with rheumatoid arthritis and neonatal sepsis that show increase in serum resistin with chronic inflammatory response of the body.

The regulation of resistin release from macrophages is controlled by the action of cytokines and other inflammatory products. Serum resistin level drops as the inflammatory response settles and the inflammation resolves leading to decrease in release of TNF- α , IL-6 and IL-1 β . These cytokines exacerbates the release of resistin along with the positive feedback from the resistin itself. The results of Gonzalez-Gay¹⁵ also support our study showing that there was decrease in resistin levels with resolution of inflammation. They blocked the release of TNF- α by Infliximab (anti TNF- α drug) administration in arthritic patients and showed a decrease in serum resistin levels along with reduction in CRP levels. Thus explaining the cascade of resistin release and role of proinflammatory cytokines. The role of resistin as a biomarker of inflammation was established by determining the risk of cardiac diseases in type II diabetics by assigning various exercises to them that lowers the inflammatory response in atherosclerosis resulting in subsequent decrease in serum resistin levels along with fall in IL-18 levels.¹⁶

Our key finding was a significant decrease in resistin levels with treatment but remained higher even at 4th month with continuing therapy when compared with controls. Relative rise in serum resistin levels at TB4 indicates the hepatotoxicity, as ATT (Rifampicin and Isoniazid) is notorious in causing hepatitis.

The limitations of the study were, for establishment of serum resistin level as an authentic biomarker, the follow-up of patients must have been carried out till the end of treatment. Various other inflammatory markers like high-sensitivity CRP, Interferons, TNF- α and interleukins should have been determined for comparison.

CONCLUSION

Our study showed a substantial rise in serum resistin levels at time diagnosis that decrease with therapy thus reflect the affectivity of treatment by reduction in leucocyte count and symptomatic improvement. The study provided an indication of therapeutic effect, i.e., with successful treatment, the bacterial load decreases.

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