

Assessment of metabolic (Adiponectin, Leptin) and inflammatory markers (TNF- α , hs-CRP & IFN- γ) in Pulmonary Tuberculosis: Prospective Study

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Abstract

Introduction: Tuberculosis (TB) remains one of the world's deadliest communicable diseases. In 2013, an estimated 9.0 million people developed TB and 1.5 million died from the disease. TB is slowly declining each year and it is estimated that 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment.

Objectives: The present study enrolled Pulmonary TB subjects with the objectives to estimate the levels of serum Adiponectin, Leptin, Tumour Necrosis Factor (TNF- α), high sensitivity-C Reactive Protein (hs-CRP) & Interferon- γ (IFN- γ) and to find correlation between Adiponectin, Leptin, Adiponectin/Leptin ratio, TNF- α , hs-CRP & IFN- γ in patients before and after treatment.

Materials and Method: The study involved all the patients who were newly diagnosed as pulmonary tuberculosis in the OPD of Pulmonary Medicine of SGT Hospital. Venous blood samples of 50 patients of TB were collected before and after treatment with Anti TB drugs to estimate the levels of Adiponectin, Leptin, hs-CRP, INF- γ and TNF- α by commercially available kits in the Laboratory of Department of Biochemistry.

Results: The levels of Adiponectin were higher in subjects diagnosed as new case sputum positive Pulmonary TB whereas the levels of Leptin and BMI measurements were low. Inflammatory markers like TNF- α , hs-CRP & IFN- γ were high in subjects before treatment and decreased after 6 months of Anti-Tubercular Treatment under DOTS Category 1.

Conclusion: Adiponectin, Leptin, TNF- α , hs-CRP & IFN- γ can be labeled as potential biomarkers in the pathogenesis of Tuberculosis.

Keywords: Tuberculosis, Inflammatory Biomarkers, Metabolic Biomarkers Anti-tubercular treatment

Introduction

Tuberculosis (TB), an airborne infectious disease caused by *Mycobacterium tuberculosis* complex, continues to be a major public health problem primarily in low-income and middle-income countries.⁽¹⁾ According to the WHO, in 2014, an estimated 9.6 million people developed active TB disease, of which 1.5 million died.⁽¹⁾ Patients with Pulmonary Tuberculosis disease suffer from chronic constitutional symptoms including weight loss and thus metabolic markers may be affected in this disease. Thus, early detection of this disease needs a top priority in developing countries like India. Hence, markers that help in early detection of tuberculosis as well as parameters, which can assess nutritional status in this debilitating disease, are of utmost importance.⁽²⁾ Adiponectin is a secretory protein synthesized by adipocytes and plays an important role such as insulin sensitivity, inflammatory response and cell proliferation. It serves as important marker for severity of tuberculosis and is independent of body mass index. Leptin is another adipokine secreted by adipose tissues. It is one of the mediators of energy metabolism and is induced by even mild weight loss. The Leptin levels are decreased in tuberculosis, which is associated with weight loss. Hence, change in Leptin and Adiponectin and their ratio are important markers for the severity of tuberculosis and as markers of nutritional status independent of body mass index.⁽³⁾ It is generally

believed that undernourishment diminishes protective immunity against *Mycobacterium tuberculosis*.

TNF- α , hs-CRP & IFN- γ are important markers of inflammation in tuberculosis. TNF- α act synergistically with IFN- γ for the formation of granuloma. This occurs by activation of macrophages and cytokines production. Serum CRP is an acute phase reactant proteins synthesized by hepatocytes under the influence of interleukin-1 released at the site of inflammation, infection and trauma. The level of CRP is raised in active tuberculosis and values start returning towards normal once tuberculosis is controlled by treatment.^(4,5)

Hence, in our study we estimated the values of inflammatory markers such as TNF- α , hs-CRP & IFN- γ along with metabolic markers such as Adiponectin and Leptin in Tuberculosis patients. We also determined ratio of Adiponectin and Leptin as metabolic marker of severity of Tuberculosis, as these adipokine levels are affected independent of body mass index in Tuberculosis patients.

Materials and Method

The present study was conducted in the Departments of Biochemistry and Pulmonary Medicine of SGT Hospital associated with SGT Medical College & Research Institute, Budhera, Gurgaon from November 2015 to October 2016. The study involved all the patients who were newly diagnosed for Pulmonary Tuberculosis on the basis of history, clinical examination, sputum

microscopy and radiological findings (plain chest X-ray) attending the OPD of Department of Pulmonary Medicine of SGT Hospital. Fifty (50) newly diagnosed patients of Tuberculosis were studied during the study period. It was non-randomized, prospective cohort and hospital based study design.

Inclusion Criteria:

1. Age more than 15 years.
2. Newly diagnosed cases of Pulmonary Tuberculosis on the basis of history, clinical examination, sputum microscopy and radiological findings (chest X-ray) before the start of treatment.
3. Those who were willing to give consent for participation

Exclusion Criteria:

1. Age less than 15 years.
2. Patients positive for HIV and HBs Ag.
3. Patients with chronic inflammatory disorders and chronic septic illnesses.
4. Prior history of Tuberculosis or patients already on Anti-tubercular treatment.

Patients were enrolled twice in the study for sampling, first after the confirmation of the diagnosis of New Case Sputum Positive Pulmonary Tuberculosis and secondly after completion of 6 months of Anti-Tubercular Treatment under DOTS Category 1, four drugs (Isoniazid 300 mg, Rifampicin 450 mg, Pyrazinamide 1500 mg, Ethambutol 1200 mg) at thrice-weekly schedule for 2 months Intensive phase and two drugs (Isoniazid 300 mg, Rifampicin 450 mg) at thrice-weekly schedule for remaining 4 months continuation phase. 5 ml venous blood samples were collected from the subjects after overnight fasting. Adiponectin, Leptin, hs-CRP, INF-γ and TNF-α were estimated by commercially available kits in the Laboratory of Department of Biochemistry, SGT Hospital, Gurgaon.

Written informed consent was obtained from each participant at enrollment time and the study was approved by the Institutional Ethics Committee.

Statistical Analysis: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0, for Windows (SPSS, Inc., Chicago). The data were expressed as mean ± standard deviation (SD). Paired Student’s t-test was used to compare the values between pre and post-treated study

subjects. Pearson’s correlation coefficient was used to find the correlations among the variables. $P < 0.05$ was considered statistically significant.

Results

Out of total study subjects, 41 were males and 9 were females (Table 1). The mean age of the study population was found to be 47.060 ± 17.269 . The mean Body mass index (BMI) before and after anti-tubercular treatment of the study subjects was (20.302 ± 1.817) and (22.407 ± 1.710) respectively. It was observed that the BMI of the study subjects increased after treatment with anti-tubercular drugs. The levels of Adiponectin, hs-CRP, TNF-α, INF-γ decreased after treatment whereas the levels of Leptin increased after treatment (Table 2).

Table 1: Demographic characteristics of patients study Population

Parameters	(N=50) (Mean ± SD)
Sex	Male-41 Female-9
Age	(47.060 ± 17.269)
BMI (kg/m ²) before Treatment	(20.302 ± 1.817)
BMI (kg/m ²) after Treatment	(22.407 ± 1.710)

Table 2: Levels of Adiponectin, Leptin, hs-CRP, TNF-α, INF-γ

Parameters	Before Treatment (mean ± SD)	After Treatment (mean ± SD)
Adiponectin (µg/ml)	12.868 ± 2.084	10.428 ± 1.789
Leptin (ng/ml)	6.124 ± 0.889	8.270 ± 0.770
Adiponectin/Leptin Ratio	2.176 ± 0.615	1.284 ± 0.325
hs-CRP (µg/ml)	26.446 ± 4.027	17.052 ± 2.713
TNF-α (pg/ml)	15.280 ± 1.216	10.202 ± 1.015
INF-γ (IU/ml)	7.244 ± 0.661	5.658 ± 0.567

The Adiponectin/Leptin ratio also decreased after anti-tubercular treatment in the study subjects. As depicted, the association between Adiponectin, Leptin, Adiponectin/Leptin ratio and hs-CRP in Pre and Post treated study subjects were found to be statistically significant (Table 3).

Table 3: Correlation of Adiponectin, Leptin, Adiponectin/Leptin ration with hsCRP, TNF-α, INF-γ, BMI in Pre and Post treated study subjects

	Subjects before treatment (N=50)				Subjects after treatment (N=50)			
	Pearson's r (p values)				Pearson's r (p values)			
	BMI (Kg/m ²)	hs-CRP (µg/ml)	TNF-α (pg/ml)	INF-γ (IU/ml)	BMI (Kg/m ²)	hsCRP (µg/ml)	TNF-α (pg/ml)	INF-γ (IU/ml)
Adiponectin (µg/ml)	-0.033 (0.821)	0.906 (0.000)	-0.026 (0.892)	0.173 (0.231)	0.035 (0.810)	0.754 (0.000)	0.017 (0.906)	0.025 (0.861)
Leptin (ng/ml)	-0.030 (0.835)	-0.607 (0.000)	-0.045 (0.758)	-0.016 (0.910)	-0.075 (0.607)	-0.513 (0.000)	-0.011 (0.939)	0.034 (0.812)

Adiponectin /Leptin Ratio	0.002 (0.987)	0.808 (0.000)	0.010 (0.945)	0.103 (0.477)	0.038 (0.796)	0.711 (0.000)	0.034 (0.815)	0.004 (0.978)
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Discussion

In the present study, the serum Adiponectin levels were high in study subjects before treatment. On the other hand, the serum Leptin levels and BMI measurement were low in study subjects before treatment. Elmargoushy NM, et al.⁽⁶⁾ studied the assessment of Adiponectin as a marker for severity of pulmonary tuberculosis where they observed higher levels of Adiponectin and Lower measurements of BMI in cases of Tuberculosis when compared with healthy controls. Therefore, Adiponectin was inversely related to BMI and it was concluded that serum Adiponectin levels might be a promising marker for severity of the disease independent of BMI. Perna V, et al.⁽⁷⁾ studied that the effective treatment of pulmonary tuberculosis restores plasma leptin levels and observed overall increase in Leptin levels after treatment with anti-tubercular drugs. Higher levels of Adiponectin with lower levels of Leptin in association with lower BMI measurement correlates well with disease activity and severity of Tubercular disease. On the other hand, the lower levels of Adiponectin with higher levels of Leptin in association with higher BMI measurement might indicate stability and latency of the TB disease. Thus, increased Adiponectin in the serum of pulmonary TB patients may be promising markers for severity of the disease. It can also be proposed from the present study that since tubercular patients have suppressed levels, so low Leptin concentrations might correlate with increased susceptibility to infection.

It was observed in the present study that the levels of hs-CRP, TNF- α and INF- γ were high in study subjects before treatment. Petruccioli E, et al.⁽⁸⁾ also observed higher levels of INF- γ and TNF- α in-patient with active Tuberculosis and similar results were observed in the present study as well. Kannapiran M, et al.⁽⁹⁾ studied C-reactive protein levels in patients with pulmonary tuberculosis and observed a sharp decrease in CRP levels following anti-tubercular treatment. It was also observed in our study that there was decline in the CRP levels in Post treated patients with anti-tubercular therapy.

In the present study, correlation of Leptin with other inflammatory markers (BMI, hs-CRP, TNF- α and INF- γ) was negative in pre and post treated subjects whereas a positive correlation was observed for Adiponectin, Adiponectin/Leptin ratio. A similar result was observed in a study conducted by Elnemr GM⁽¹⁰⁾.

The strengths of our study include: a) hospital-based pulmonary TB patients were assessed simultaneously with the values of inflammatory markers such as TNF- α , hs-CRP & INF- γ along with metabolic markers such as Adiponectin and Leptin in Pre and Post treated tuberculosis patients, b) our study population was followed up very closely to assure treatment adherence

during the study period and received anti-TB drugs under directly observed manner, c) the personnel performing the laboratory and chest radiographs analysis were unaware of the treatment outcomes, d) scarcity of north Indian data measuring these markers both in Pre and Post anti-tubercular treated cases.

The limitations of our present study include the fact that we did not measure all other cytokines that play a relevant role in the immune response to active tuberculosis, and the fact that we did not monitor the clinical and bacteriological response of the patients throughout the anti-tuberculosis treatment period. A future interest in research lies in comparing the change in the level of these inflammatory markers in other granulomatous diseases involving Th1 (T-Helper lymphocyte 1) inflammatory cells. In addition, there is scarcity of data of cross-sectional studies done on these markers in retreatment, defaulter and resistant cases of tuberculosis.

Summary and Conclusion

Adiponectin and Leptin are adipokines secreted by the adipose tissues. Change in Leptin and Adiponectin and their ratio are important markers of the severity of tuberculosis and also as markers of nutritional status independent of body mass index. It is generally believed that malnourished subjects have diminished protective immunity against Mycobacterium Tuberculosis. TNF- α , hs-CRP & INF- γ are important markers of inflammation in Tuberculosis. TNF- α and INF- γ act synergistically to produce granuloma and activate macrophages further leading to production of inflammatory cytokines. Serum CRP is acute phase reactant proteins synthesized by hepatocytes under the influence of interleukin-1 released at the site of inflammation, infection and trauma. The level of CRP is raised in active tuberculosis and values start returning towards normal once tuberculosis is controlled by treatment. Hence, Adiponectin, Leptin, TNF- α , hs-CRP & INF- γ can be labeled as potential biomarkers in the pathogenesis of Tuberculosis.

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The role of each authors: Neeraj Indora; performed the study and wrote the manuscript, Kapil Sharma; wrote the manuscript, Busi Karunanand; Supervised the study and wrote the manuscript; DPS Sudan; Co-supervised the study and wrote the manuscript; Ashok Kumar Shah; help with the statistics & revised the manuscript.

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Conflict of interests

The authors declare that they have no conflict of interests.

References

1. World Health Organization. Global Tuberculosis Report 2015 (WHO, 2015).
2. Mohamed NA, Fawzy MA, Elgamry R, Gad DM, Ibraheem HA. Role of adiponectin and other inflammatory biomarkers in COPD patients. *Egyptian Journal of Chest Diseases and Tuberculosis* 2013;62(1):45–50.
3. Keicho N, Matsushita I, Tanaka T, Shimbo T, Hang NTL, et al. Circulating Levels of Adiponectin, Leptin, Fetuin-A and Retinol-Binding Protein in Patients with Tuberculosis: Markers of Metabolism and Inflammation. *PLoS one* 2012;7(6):e38703.
4. Beglinger C, Dudler J, Mottet C, Nicod L, Seibold F et al. Zellwegerg Screening for tuberculosis infection before initiation of anti-TNF- α therapy. *Swiss Med Wkly* 2007;137:621–622.
5. Khuder HS, Norrei AY, Khuder YS. High sensitive C - reactive protein in Patients with Pulmonary Tuberculosis in Tikrit City. *IJAPBC* 2013;2(4).
6. Elmargoushy NM, Elnashar NA, Mohammad SM, Elnashar MA, Mohammad HA. Assessment of adiponectin as a marker for severity of pulmonary tuberculosis. *The Egyptian Journal of Hospital Medicine*. 2014 Oct 1;57:418-22.
7. Perna V, Pérez-Pérez A, Fernández-Riejos P, Polo-Padillo J, Batista N, Domínguez- Castellano A, Sánchez-Margalet V. Effective treatment of pulmonary tuberculosis restores plasmaleptin levels. *European cytokine network*. 2013 Oct 1;24(4):157-61.
8. Petruccioli E, Petrone L, Vanini V, Sampaolesi A, Gualano G, Girardi E, Palmieri F, Goletti D. IFN γ /TNF α specific-cells and effector memory phenotype associate with active tuberculosis. *Journal of Infection*. 2013 Jun 30;66(6):475-86.
9. Kannapiran M, Immanuel C, Krishnamurthy PV, Sarma GR. C-reactive protein levels in patients with pulmonary tuberculosis. *Lung India*. 1989 Feb 1;7(1):34.
10. Elnemr GM, Elnashar MA, Elmargoushy NM, Elnashar NA, Elnashar MA. Adiponectin levels as a marker of inflammation in pulmonary tuberculosis. *The Egyptian Journal of Hospital Medicine*. 2015 Apr 1;59:208-14.