Adiponectin Levels as a Marker of Inflammation in Pulmonary Tuberculosis

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ABSTRACT:

Background: tuberculosis (TB) is a multisystem disease with myriad presentations and manifestations that may be pulmonary or extra-pulmonary. It is the most common cause of infectious diseases related mortality worldwide. Mechanisms underlying host defense to TB infection are poorly understood. Adiponectin is a protein produced exclusively in adipose tissue; it is lower in obese subjects than their lean counterparts. Another adipocyte hormone is leptin which is higher with fat mass and body mass index (BMI). Wasting is a known feature of TB with a decrease in BMI. Patients with pulmonary TB disease often suffer from weight loss and BMI and leptin are known to be inversely correlated with adiponectin. Thus, increased adiponectin may be a promising marker for severity of the disease independent of the BMI and leptin.

Aims of the work: the work aimed to evaluate the levels of serum adiponectin in patients with pulmonary TB in both active and latent TB patients as a diagnostic marker for tuberculous inflammation. Also, levels of serum leptin and measured BMI were evaluated and compared to make a relationship among active TB, latent TB, and healthy control groups.

Subjects and Methods: the subjects were divided into 3 equal groups. The 1st group included 25 patients with active pulmonary TB. The 2nd group included 25 patients with latent TB after 6 months of diagnosis. The 3rd group included 25 healthy control volunteers. Serum levels of adiponectin and leptin were measured and BMI values were calculated to all groups.

Results: Serum adiponectin levels in patients with active pulmonary TB were very highly significantly increased compared to both latent TB patients and healthy subjects ($P<0.0001$), while serum adiponectin levels in patients with latent pulmonary TB were only significantly increased compared to the healthy subjects ($P<0.05$). On the other hand, serum leptin levels in patients with active pulmonary TB were very highly significantly decreased compared to both latent TB patients and healthy subjects ($P<0.0001$), while serum leptin levels in patients with latent pulmonary TB were only significantly decreased compared to the healthy subjects ($P<0.05$). Also, BMI in patients with active pulmonary TB were very highly significantly decreased compared to both latent TB patients and healthy subjects ($P<0.001$), while BMI in patients with latent pulmonary TB were only significantly decreased compared to the healthy subjects ($P<0.05$).

Conclusion: the higher levels of adiponectin with lower levels of leptin in association with lower BMI measurements might indicate activity and severity of the TB disease. On the other hand, the lower levels of adiponectin with higher levels of leptin in association with higher BMI measurements 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 independent of the BMI and leptin levels.

Key words: TB patients, leptin, adiponectin, body mass index.

Introduction:

Tuberculosis (TB) is a multisystem disease with myriad presentations and manifestations. It is the most common cause of infectious disease related mortality worldwide. (1) Despite the considerable progress made since discovery of TB, it remains one of the world's deadliest diseases. Tuberculosis was being preventable and curable for more than 100 years; it might also be a disease of our future. (2) Tuberculosis is likely to have affected humans for most of their history and remains a major cause of death worldwide despite the discovery of effective and affordable chemotherapy more than 50 years ago. There were an estimated 1.3 million TB

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deaths (including TB deaths in HIV-positive individuals) in 2012. [3]

Tuberculosis causes life-threatening pulmonary and extra-pulmonary manifestations. Mechanisms underlying host defense to TB infection are poorly understood. In response to infection, the host immune cells secrete a number of cytokine and chemokine signals, which play active roles in initiation and regulation of the immune response at various stages of the disease development. The deleterious effect of excessive pro-inflammatory cytokines is attenuated by secretion of anti-inflammatory cytokines such as IL-10, transforming growth factor beta (TGF-β), and IL-4. Excessive production of these anti-inflammatory cytokines may completely suppress the pro-inflammatory response resulting in failure of elimination of TB infection. [4]

Wasting is a known feature of TB. Negative energy balance and wasting in chronic inflammation has been recognized as a prominent feature of TB and is one of the major obstacles to manage tuberculous patients. To monitor the disease state, markers of metabolism and inflammation are potentially useful. Adiponectin is a protein produced exclusively in adipose tissue; it plays a critical role in mediating physiological effects such as insulin sensitivity, inflammatory response, and cell proliferation. Adiponectin is lower in obese subjects than their lean counterparts. [4] Increased adiponectin may be a promising marker for severity of the TB independent of the body mass index "BMI". [5]

Adipose tissue was long been identified as an energy storage organ but in recent times extensive studies revealed the role of adipose tissue as an important endocrine organ with a number of metabolic activities; thus its function as a storage organ is now far from reality. [6] Oxidative stress which is a major indicator of inflammation correlates significantly with adiponectin metabolic pathway and lower adiponectin level is significantly associated with higher inflammatory state. [7] Altered adipokine secretion may contribute to impaired regulation of appetite and satiety, fat distribution, insulin secretion and sensitivity, energy expenditure, endothelial function, inflammation, blood pressure, and hemostasis. [8]. The early diagnosis and adequate treatment of tuberculous patients is mandatory to reduce its transmission and elimination worldwide. In the mean time, proper therapy of latent TB infected patients, who are at high risk of developing active infection, is important for disease control. There is an urgent need for rapid and accurate diagnostic methods in order to achieve higher sensitivity and specificity compared to traditional methods i.e., microscopic examination and culture of sputum. [9]

Leptin, the first adipocyte hormone identified, influences food intake through a direct effect on the hypothalamus. In humans, plasma leptin concentrations are highly correlated with BMI. [10] Leptin has been considered as the prototype for all adipocyte secreted hormones. Leptin levels are pulsatile and are associated with fat mass/BMI as well as circulating inflammatory markers. Leptin in low leptin states plays an important role in satiety, appetite, food intake, reproductive function, puberty, and activity with energy expenditure regulation. Originally, leptin was viewed as a potential anti-obesity therapeutic agent and was originally developed mainly with the aim to reduce body fat mass in obesity. [9]

The visceral fatty cells occurred before subcutaneous adipocytes. The visceral fatty cells have no receptors to insulin and all subcutaneous adipocytes are insulin-dependent. It is supposed that the most common cause of obesity is a disorder of biologic reaction of depositing fatty acids in the form of triglycerides. It is considered as a basis of that dysfunction of visceral fatty cells. The fatty cells absorb fatty acids in the form of non-polar triglycerides, deposit them in lipid drops and free fatty acids into intercellular medium in the form of polar unesterified fatty acids. The visceral fatty cells and adipocytes are regulatory, functionally, and patho-physiologically different cells. Leptin is a specific mediator of visceral fatty cells and adiponectin is a mediator of subcutaneous adipocytes. [11]

Adiponectin levels are inversely associated with obesity and are thought to decrease in individuals with increased adiposity through down-regulation of adiponectin. [12] Patients with pulmonary TB disease often suffer from weight loss. Body mass index is known to be inversely correlated with adiponectin. [13] Thus, increased

adiponectin may be a promising marker for severity of the disease independent of the BMI.

Objectives:

We aimed to evaluate and compare serum levels of adiponectin and leptin and BMI among patients with active and latent pulmonary TB to a control group of healthy volunteers (to evaluate their role as markers for tuberculous inflammation). Also, we aimed to compare adiponectin and leptin levels and BMI between the active and latent TB groups to assess the severity of the disease.

Patients and Methods:

Consents were first obtained from all patients and controls to participate in the study. Three groups with a total number of 75 were subjected to this study (from July to December 2014). The 1st group included 25 patients with active pulmonary TB. We select our patients from the Chest Department, Al-Husain University Hospital in Cairo, Egypt. They were diagnosed clinically by positive symptoms of TB **(14)**, radiologically by chest X-Ray appearance suggestive of TB, microscopically with three successive positive sputum smears, and by positive cultures for acid fast bacilli. **(15)** The 2nd group included 25 patients with latent TB after 6 months of diagnosis with no symptoms of TB, no radiological manifestations, and negative sputum smears and cultures for TB. They were chosen from the outpatient clinics (patients who completed their course of therapy and coming for follow up). The 3rd group included 25 healthy control volunteers with negative tuberculin test.

Exclusion criteria included patients and controls with other chest diseases as bronchitis, bronchial asthma, and emphysema. Subjects with other chronic diseases as diabetes mellitus and hypertension were also excluded.

Body mass index (BMI) was calculated as weight in kilograms (kg) divided by the square of the height in meters (m^2). **(12)** Venous blood samples (3 milliliters each) were collected from every one in each group and left to clot, then serum samples were obtained by centrifugation at 3000 g within 30 minutes of blood drawing and immediately refrigerated. Within 24 hours, all specimens were frozen and stored at -70°C. Serum levels of adiponectin were measured using AviBion Human Adiponectin ELISA kits. **(16)** Serum levels of leptin were diagnosed by Leptin Human ELISA Kit (ab108879). **(17)**

Data of the three groups were compared and analyzed using SPSS program version 16. Results were expressed as mean ± SD. The mean values of the groups were compared using Student's unpaired t-test. Statistical significance was set at $P<0.05$.

Results (Tables 1-3):

Table (1) shows comparison between active TB and control groups. It revealed that; serum levels of adiponectin were very highly statistically significantly increased between the 2 groups ($P<0.0001$), while the serum levels of leptin and BMI were very highly significantly decreased and highly significantly decreased between the 2 groups ($P<0.0001$ and $P<0.001$, respectively).

Table (2) shows comparison between latent TB and control groups. It revealed that; serum levels of adiponectin were statistically significantly increased between the 2 groups ($P<0.05$), while the serum levels of leptin and BMI were significantly decreased between the 2 groups ($P<0.05$).

Table (3) shows comparison between the active and latent TB groups. It revealed that; serum levels of adiponectin were very highly statistically significantly increased between the 2 groups ($P<0.0001$), while the serum levels of leptin and BMI were very highly significantly decreased and highly significantly decreased between the 2 groups ($P<0.0001$ and $P<0.001$, respectively).

Discussion:

Major adipocyte derived hormones are; adiponectin, leptin, resistin, and visfatin. Adiponectin is concerned in regulating blood glucose levels and fatty acids breakdown; it is the most abundant adipokines in the blood. **(6)** Leptin circulates in the blood at lower levels in lean individuals and their levels may increase in obese individuals due to their higher adipose tissue mass and their levels are directly associated with adipose tissue mass, since a higher adipose tissue mass results in elevated leptin levels. **(18)**

In this study the highest serum levels of adiponectin were found in the active TB group (with the lowest leptin and BMI), followed by the latent TB group (with an average leptin and BMI), while the lowest

adiponectin levels were found in the control group (with the highest leptin and BMI).

These results were in accordance with the results obtained by **Keicho *et al.*** [4] in their study of circulating levels of adiponectin and leptin. They found, in the no-symptom group of tuberculous patients, adiponectin and leptin showed negative and positive correlation with BMI, respectively.

The results of **Aimin and Paul** [19] were also consistent to our results; they searched for adiponectin and adipocyte fatty acid binding protein in the pathogenesis of cardiovascular disease and deduced that the discordant production of adipokines in dysfunctional adipose tissue is a key contributor to obesity-related cardiovascular disease.

These results were explained by **Kakali and Maitree** [6] in their study of adiponectin and they mentioned that adiponectin acts by 2 mechanisms to inhibit obesity; the first one by increasing insulin sensitivity and the other mechanism by increasing fatty acid oxidation; it also correlates with oxidative stress and act as important inflammatory biomarker with regulation of many genes in different metabolic pathways.

Keicho *et al.* [4] also explained our results in their research. They suggested that the low fat store and underlying inflammation may regulate metabolic markers in TB in a different way and they stated that decreased leptin with increased adiponectin or this ratio may be a promising marker for severity of the disease independent of BMI.

The results of **Ghantous *et al.*** [18] were in agreement with our results. They concluded that obesity is associated with high levels of the circulating hormone leptin, and they thoughts that leptin is responsible for several cardiovascular diseases associated with obesity, which was in accordance with our results which reveals that serum leptin levels was very low in active tuberculous patients with minimal BMI (while leptin levels were higher in the latent TB and control groups as their BMI rises). **Adel *et al.*** [20] searched for leptin and adiponectin as valuable serum markers explaining obesity/bronchial asthma interrelationship. They had similar results that showed that; there was a higher leptin serum levels in obese controls compared to non-obese controls and concluded that there is a strong association between asthma and obesity regarding serum level of leptin and adiponectin.

This conclusion was also explained by **Ghantous *et al.*** [18] who deduced that glucocorticoids and insulin act on adipocytes to increase leptin expression, possibly explaining the reason for increased leptin levels observed in obesity. Another explanation was done by **Perna *et al.*** [21]; they measured plasma adiponectin and leptin in tuberculous patients with their BMI before and after anti-tuberculous drugs and said that active TB infection can affect leptin expression, additional to wasting that occur in TB patients, and concluded that effective TB treatment increases circulating leptin levels, probably restoring normal immunological competence.

On the contrary, **Reinout *et al.*** [22] don't support our results as they concluded that weight loss in TB is not caused by enhanced production of leptin but the loss of body fat leads to low plasma leptin concentrations, and prolonged inflammation may further suppress leptin production, because leptin is important for cell-mediated immunity, low leptin production during active TB may contribute to increased disease severity, especially in cachectic patients.

Achim *et al.* [23] supported the same results and deduced that the close correlation of leptin with body fat mass is similar to observations in healthy subjects and leptin does not appear to be a component of the immune response to human pulmonary TB, which cannot account for the weight loss and anorexia associated with TB.

Reinout *et al.* [22] explained that plasma leptin concentrations in TB may be the result of two antagonistic mechanisms, whereas TB-associated loss of body fat mass may lead to reduced production of leptin, the host inflammatory response may increase leptin production and if, as an overall result, plasma leptin concentrations are increased in TB patients, then this might theoretically suppress appetite and food intake and be one of the mechanisms underlying weight loss, but if plasma leptin concentrations are decreased in TB, then this might suppress cellular immunity and aggravate disease outcome.

Conclusion:

In conclusion, higher levels of adiponectin with lower levels of leptin in

association with lower BMI measurements indicates activity and severity of the TB disease. On the other hand, lower levels of adiponectin with higher levels of leptin in association with higher BMI measurements indicate stability and latency of the TB disease. Thus, increased adiponectin with decreased leptin levels in the serum of pulmonary TB patients may be promising markers for severity of the disease independent of the BMI.

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Table (1): Comparison of adiponectin, leptin, and BMI between active TB and control groups

Parameters	Active TB patients (No = 25)	Controls (No = 25)
Serum adiponectin (µg/mL), Mean ± SD P-values	13.89 ± 1.31	8.68 ± 0.52 P<0.0001***
Serum leptin (ng/mL), Mean ± SD P-values	3.29 ± 1.93	6.45 ± 0.42 P<0.0001***
BMI (kg/m2), Mean ± SD P-values	19.49 ± 0.59	22.01 ± 1.52 P<0. 001**

P<0.001**: is considered highly significant. P<0.0001***: is considered very highly significant.

Table (2): Comparison of adiponectin, leptin, and BMI between latent TB and control groups

Parameters	Latent TB patients (No = 25)	Controls (No = 25)
Serum adiponectin (µg/mL), Mean ± SD P-values	10.07 ± 1.13	8.68 ± 0.52 P<0.05*
Serum Leptin (ng/mL), Mean ± SD P-values	5.95 ± 0.65	6.45 ± 0.42 P<0.05*
BMI (kg/m2), Mean ± SD P-values	20.51 ± 0.81	22.01 ± 1.52 P<0.05*

P< 0.05*: is considered significant.

Table (3): Comparison of adiponectin, leptin, and BMI between active and latent TB groups

Parameters	Active TB patients (No = 25)	Latent TB (No = 25)
Serum adiponectin (µg/mL), Mean ± SD P-values	13.89 ± 1.31	10.07 ± 1.13 P<0.0001***
Serum leptin (ng/mL), Mean ± SD P-values	3.29 ± 1.93	5.95 ± 0.65 P<0.0001***
BMI (kg/m2), Mean ± SD P-values	19.49 ± 0.59	20.51 ± 0.81 P<0.001**

P<0.001**: is considered highly significant. P<0.0001***: is considered very highly significant.