Association of Adipocytokines and Vitamin D Status in Tuberculosis
Hazhar M. Balaky*, Akam Jasim Mustafa1, Parween Abdulsamad Ismail3
1Mergasor Technical Institute, Erbil Polytechnic University, Erbil, Kurdistan Region, Iraq
2Department of Chemistry, Faculty of Science, Soran University, Kurdistan Region, Iraq
3Department of Chemistry, College of Education, Salahaddin University Erbil, Kurdistan Region, Iraq

**Keywords:** Leptin, Adiponectin, Vitamin D, Tuberculosis, Adipocytokines.

**ABSTRACT**
Leptin and Adiponectin are considered principal indicators of tuberculosis (TB). They are critical parts of the pathophysiological processes to which treatment can be applied. Thus, this research aimed at finding out the role of Adipocytokines and 25-dihydroxy vitamin D in the aetiology of tuberculosis. The research sample included (90) participants divided into (50) tuberculosis patients and (40) healthy subjects representing the control group who were age and sex-matched. Circulating levels of adipocytokines and 25-dihydroxy vitamin D were analysed by using ELISA techniques. Compared to the subjects of control group, tuberculosis subjects had significantly (P<0.0001) lower levels of serum leptin (5.84±1.83 pg/mL) and vitamin D (2.77±0.52 ng/mL). However, compared to their control counterparts, tuberculosis patients had significantly (P<0.0001) higher levels of adiponectin (16.30±1.23 ng/mL). The ROC curve analysis of Leptin, Adiponectin, and 25-dihydroxy vitamin D indicated a high diagnostic value in predicting the risk of tuberculosis. The present study findings revealed that elevated serum adiponectin with decreased serum leptin levels and hypovitaminosis D are strongly correlated with the pathogenesis and state of tuberculosis.

© THIS IS AN OPEN ACCESS ARTICLE UNDER THE CC BY LICENSE
http://creativecommons.org/licenses/by/4.0/
Introduction

A report on global tuberculosis has been published on behalf of the World Health Organization (WHO) annually since 1997, which supplies the latest evaluation of the global tuberculosis status and outlines the advancement and endeavour in avoidance, prognosis, and treatment of the illnesses [1].

*Mycobacterium tuberculosis* is the etiologic agent of tuberculosis in humans. Tuberculosis most commonly affects the lungs (Pulmonary TB). TB can also occur outside the lungs (Extrapulmonary), most commonly in central nervous, lymphatic or genitourinary systems, or in the bones and joints and even the skin [2].

One major contributor to tuberculosis is morbidity and fatality among all individuals in the world. The bacillus *Mycobacterium tuberculosis* (Mtbc), which causes TB, is transmitted by droplets in the air [3]. Although *Mycobacterium tuberculosis* complex (MTBC) organism typically causes extrapulmonary tuberculosis (EPTB), it can potentially infect other organs. Clinical manifestations of extrapulmonary tuberculosis are frequently meningitis, lymphadenitis, ocular, oral, pleuritis, pericarditis, peritonitis, musculoskeletal, abdominal, genitourinary, and military forms [4]. *Mycobacterium tuberculosis* infection can remain dormant or develop into active disease forms, although one in four persons globally show an immune response to it. Before the latest modification, patients with active tuberculosis infection, but no visible signs or symptoms of the disease, were thought to have latent tuberculosis. While those who
have an active illness are said to have TB disease. Tuberculosis patients have a 5- to 10-percent chance of getting TB disease over their lifetime, which rises in various immunodeficiency states to a 16–% annual risk in HIV patients for activating TB infection into TB disease [3]. According to the most recent estimations, *Mycobacterium tuberculosis* infects most of the people on earth. The World Health Organization claims that the incidence of tuberculosis peaked globally about 2003 and appeared to be progressively dropping until 2019. In 2019, 1.6 million people died from tuberculosis, according to WHO estimates [5]. Adipose tissue had previously been thought of as an organ for storing energy, but recently it has been recognized as a crucial endocrine organ with a number of metabolic roles. Because of this, its historical function as a storage organ is no longer relevant [6]. Poor nutrition, demonstrated by wasting and anorexia, is a significant feature of TB since it is a condition with a high rate of catabolism, an accelerated rate of protein breakdown, and muscle atrophy, which results in weight loss, deteriorating clinical functions and a poor prognosis.

The studies demonstrated that cytokines are secreted by white adipose tissue (WAT) called adipokines, representing a hormone-like factor such as leptin and adiponectin [7]. Leptin, an appetite-related hormone, maybe a novel candidate for the causation of malnutrition linked to TB [8]. As one of the most significant adipose-derived hormones, leptin influences the brain's ability to control food intake and body weight. Leptin promotes fat deposition, reproduction, bone formation, and neuroendocrine function physiologically by binding to the receptor and decreasing appetite, controlling energy metabolism, and all of these processes [9]. However, leptin levels in TB patients have been the subject of contradicting prior research. One study found a substantial positive association between the healthy individual group and the TB group, as well as between circulating level of leptin and body mass. It also found that TB patients had lower pre-treatment plasma leptin levels than the subjects of control group [10]. Diet, adiposity, energy balance, hormonal variables, as well as numerous intrinsic adiposity factors and cytokines, all have an impact on leptin release and its circulating levels. The evidence is still ambiguous and incomplete for the correlation between the inflammatory response and starvation. It is still unknown whether weight loss in tuberculosis is likely caused by an excessive generation of cytokines [8]. An adipokine called adiponectin affects the immune system in a pro- and anti-inflammatory manner. It can program pro-inflammatory behaviors in macrophages and act as an enhancer of inflammation in
addition to inhibiting adhesion molecules and the generation of specific pro-inflammatory cytokines. In pulmonary tuberculosis, wasting and inflammation are correlated with low plasma leptin and high adiponectin levels \(^{[11]}\). Adiponectin levels are hypothesized to fall in people with increased adiposity through down-regulation of adiponectin, which is why they are inversely correlated with obesity sufferers of pulmonary TB. Adiponectin and body mass index are known to be negatively associated. Increased adiponectin may therefore be a promising indicator of disease severity that is independent of BMI \(^{[12]}\). A vital hormone called vitamin D can support human mineral balance. According to several studies, the lack of vitamin D considerably raises the likelihood of progression of active TB. The active form of vitamin D, 1,25-dihydroxy vitamin D \((1,25(OH)D3)\), has been shown in studies to alter human antimycobacterial responses and enhance the innate immune system by modifying antigen presentation. According to several studies, TB patients’ low serum vitamin D was present than in the healthy subjects of control group \(^{[13]}\).

The study aimed to measure Adipocytokines and Vitamin D in the serum and determine if there is a possibility to use these Adipocytokines as predictor for the tuberculosis

2. Materials and Methods

A Study population and design

The current study comprised 90 persons whose age ranged between (38-69) years, 50 of them were clinically diagnosed as having Tuberculosis. The samples were collected at the Erbil TB center. They were compared to the control group of 40 subjects as being healthy persons who were matched with TB patients in terms of age and gender.

Collection of Blood Samples

An aliquot of 5 millilitres of venous blood specimens was collected from each subject, placed in Gel and clot activator tubes (yellow cap), left to stand at room temperature for 10 minutes, and subsequently centrifuged at (3500 rpm) for 15 minutes. The serum samples were immediately transferred to Eppendorf tubes that had been pre-labeled and coded. These samples were preserved at \(-20^\circ\text{C}\) for later examination. Hemolyzed serum specimens were discarded.

Determination of the Biochemical parameters

The circulatory levels of Human Leptin, Adiponectin, and Vitamin D were assayed using the enzyme-linked immunosorbent assay (ELISA) technique via Monobind Inc.’s AccuBind® ELISA kits (USA).

Statistical Analysis

With the use of the computer program GraphPad Prism version 9, the study’s data
was statistically evaluated. The results of the statistical analysis were expressed as Mean±SE. The research biochemical parameter means were evaluated using an unpaired student's t-test between TB patients and healthy subjects of control group. Because the confidence interval (CI) of choice was 95%, P-values were all two-sided, and significance was defined as a value below 0.05.

3. Results and Discussion

Table (1) shows the levels of studied parameters (Leptin, Adiponectin, and Vitamin D), whose mean values were (5.84±1.83 pg/mL, 16.30±1.23 ng/mL, and 2.77±0.52 ng/mL), respectively, for the patients group and (53.84±14.31 pg/mL, 2.05±0.22 ng/mL and 24.49±2.53 ng/mL), respectively, for the healthy subjects of control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Patients</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (pg/mL)</td>
<td>53.84±14.31</td>
<td>5.84±1.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>2.05±0.22</td>
<td>16.30±1.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin D (ng/mL)</td>
<td>24.49±2.53</td>
<td>2.77±0.52</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

If the P-value is <0.05, it's considered statistically significant.

Table1. Levels of Leptin, Adiponectin, and Vitamin D in TB patients and control

Figure 1. Leptin in sera of the two studied groups

Leptin plasma concentrations in patients with tuberculosis (TB) may be influenced by two conflicting mechanisms, particularly persistent inflammation, which reduces leptin synthesis and causes a loss of body fat mass and the acute inflammatory response of the host, which raises leptin levels and could result in anorexia, appetite
suppression, and a loss of body mass. Leptin has a crucial part in cellular immunity, the mechanism through which the body fights Tuberculosis-causing mycobacterium, hence low levels can impair the prognosis of TB \([14]\). In contrast, when fat mass decreases, the concentration of leptin released by fatty tissue is increased, giving the appearance of starvation and boosting food consumption and appetite until weight gain \([15]\). This leads to a satiety response, which suppresses appetite and decreases energy intake until weight loss occurs. Leptin may play important roles in the regulation of the immunological response, which may be closely associated with the development and incidence of PTB, as has been forcefully emphasized \([16]\). It has been shown that inflammation and the circulating level of leptin, as expressed in PTB, may change irrespective of weight reduction. In general, a decrease in leptin production may be significantly impacted by the host's inflammatory response, which promotes hematopoietic cell proliferation, differentiation, and activation and results in PTB \([17]\). Clinical studies have shown that the PTB's circulating level of leptin was alarmingly low due to weight loss, suggesting that protracted inflammation may further decrease leptin synthesis \([18]\). Additionally, experimental data suggested that a lower circulating level of leptin in tuberculosis could inhibit cellular immunity, which is necessary to combat Tuberculosis-causing mycobacterium, and worsen the course of the disease \([19]\). Given the facts discussed above, it is possible to make a valid assumption that changes in the circulating level of leptin are related to PTB's fast progression and bad prognosis. However, contrary results suggested that a changed serum leptin level may not be related to PTB infection and may not be responsible for anorexia and weight loss \([9]\). Leptin is required for cell-mediated immunity, especially in individuals with cachexia, hence lower leptin synthesis during active TB may increase the severity of the condition \([12]\). This was in line with the results of Ghantous et al. \([20]\), who came to the conclusion that obesity is linked to high concentrations of the circulating level of leptin. They hypothesized that leptin is to blame for many cardiovascular diseases that are linked to obesity. This is because circulating levels of leptin were very low in active Tuberculosis patients with low BMI (as opposed to higher levels in the latent TB and control groups, whose BMI was elevated). They elaborated this by stating that insulin and glucocorticoids operate on adipocytes to promote leptin expression in obese people, which may account for the elevated leptin levels seen in obesity. According to \([21]\), patients with active pulmonary TB may experience less appetite.
and signs of weight loss when their leptin levels are raised. Consistent with \cite{12}, two competing mechanisms could be responsible for the circulating level of leptin in TB patients: Leptin production may be decreased by the reduction of body fat mass associated with TB, but it may be increased by the inflammatory response of the host. Theoretically, if plasma leptin concentrations are higher in TB patients, thus this might reduce hunger and food consumption and be one of the mechanisms causing weight gain \cite{22}. For cellular immunity to \textit{M. tuberculosis}, leptin is essential, and hence suppressing its levels may contribute to poorer TB outcomes, particularly in patients with cachexia. A type of leptin known as metreleptin significantly influences the metabolism of food intake, body weight, energy expenditure, glucose and lipid metabolism, immunity, and the structure and function of the brain. Theoretically, treating TB patients with meterleptin may be useful, although this has not yet been done in many countries \cite{14}.

PTB is a condition that is quite prevalent throughout the world, and patients who have it frequently struggle with anorexia and malnutrition. According to reports, leptin plays a significant part in the hormonal controlling of energy balance in human bodies \cite{23}. In the current meta-analysis, we sought to examine the relationships between the circulating level of leptin and PTB pathogenesis. The current research demonstrated that PTB patients' circulating concentrations of leptin were considerably lower than those of healthy subjects in the control group. This indicates that down-regulated levels of serum leptin may be involved in the etiology of PTB. Circulating concentrations of leptin in adipocytes may strongly correlate with body weight, as leptin is a key satiety element that controls body weight by reducing appetite and promoting energy metabolism. Hence, the amount of body fat stores may be strongly correlated with serum leptin levels in adipocytes \cite{24}. Circulating concentrations of leptin were altered by an individual's nutritional state, declining with famine and rising with obesity. White adipose tissue produced the majority of the serum leptin, indicating a strong correlation between this marker and adipocyte size and body fat mass \cite{25}. Additionally, a down-regulated blood leptin level may be a contributing factor to the underlying process of weight loss caused by acute inflammatory diseases. Prolonged inflammation may also further inhibit leptin synthesis. We all know that individuals with PTB frequently have considerable weight loss, which is likely a result of the inflammatory mediators that are produced during the development of PTB. Moreover, as PTB progresses, there may be significant
inhibition of leptin synthesis due to the generation of inflammatory mediators \[9\]. In this regard, it is conceivable that people with tuberculosis infections may have lower-than-normal leptin serum concentrations. As stated by \[26\], there was a positive link between body mass index and circulating levels of leptin in patients with PTB, and the serum leptin level declined as PTB disease severity increased. According to research by Herlina and his colleagues, PTB patients had lower body mass indices and lower levels of serum leptin than healthy adults did. They also suggested that down-regulated leptin levels brought on by weight loss may reduce leptin generation, which may be related to the progression of PTB \[19\]. Furthermore, Yurt \textit{et al.} demonstrated that PTB patients had decreased leptin levels and suggested that the down-regulation of leptin concentrations may be linked with an imbalance between catabolic and anabolic processes, which may contribute to the development of PTB \[27\].

**Serum level of Adiponectin**
Statistically significant (p<0.0001) higher level of serum adiponectin was observed in tuberculosis patients when compared with the control group (see Figure 2).

![Figure 2. Adiponectin in sera of the two studied groups](image)

The amazing ability of \textit{Mycobacterium TB} to survive in the infected host in a non- or semi-replicating latent state has been demonstrated \[28\]. According to earlier studies, latent bacteria are most likely present in host cells in both the pulmonary and extra-pulmonary regions. A nutrient-rich organ called adipose tissue offers a favourable environment for latent \textit{Mycobacterium TB} \[29, 30\]. Fatty tissue acts as a storage area for a variety of infections, including \textit{Mycobacterium TB}, \textit{Trypanosoma cruzi}, \textit{Rickettsia}, \textit{HIV}, and species \textit{Simian immunodeficiency virus} (SIV). In addition to acting as a storage area of triglyceride, adipose tissue functions as an endocrine organ that supports immune defense against infection, inflammation, and energy balance. Infection and persistence of \textit{Mycobacterium tuberculosis} (Mtb) may have a dynamic effect on the pathology and physiology of adipose tissue, which controls metabolic and energy
homeostasis [31]. Due to its crucial involvement in the pathophysiology of numerous viral and metabolic illnesses, adipose tissue has attracted greater attention [32]. Adipose tissue performs metabolic and immune tasks as well as acts as a reservoir for a variety of diseases, such as bacteria and parasites [33]. Previous research has shown that Mtb survives in adipose tissue in TB patients and infects adipocytes. It has been demonstrated that adipocytes with Mtb infection undergo an immunological response [31].

According to a previous study, ablating adipose tissue has a detrimental effect on adipokine and cytokine levels, immune cell infiltrations, inflammatory signaling, and bacterial burden. Additionally, it has been shown that Adipose tissue proinflammatory signaling is increased and TB persists in adipose tissue. In addition to infecting and persisting in adipose tissue, tuberculosis also controls lung pathology, which significantly affects how severe the disease is. Adipocyte/fat cell physiology is impacted both directly and indirectly by M. tuberculosis infection. Infected Mice with M. tuberculosis progress to severe lung disease following acute fat (adipocyte) loss. Pulmonary lipid build-up is increased by acute fat cell loss in adipose tissue brought on by induced fat cell death [34]. According to clinical investigations [12,25], adiponectin levels in serum have a favourable correlation with the severity of tuberculosis. Adipose tissue loss during TB is presumably caused by a decline in the nutritional intake but an increase in nutrient use during the disease. However, newly discovered processes are to blame for the elevated risk of tuberculosis (TB) linked to the loss of fatty tissue. Studies in the lab shed light on the relationship between adipose tissue and Mtb. In 2006, Neyrolles et al. [35] found that M. tuberculosis could be found in the fatty tissue of kidneys, stomachs, lymph nodes, hearts, and skin in (6/19) Brazilians and (6/20) French people who had died from causes other than tuberculosis. Additionally, they found that M. tuberculosis could penetrate adipocytes with ease and persist in a non-replicating form, evading the effects of anti-TB medications. The idea that adipose tissue might represent one of the Mtb reservoirs has been supported by several animal studies. Researchers in Switzerland conducted tests to determine how Mtb bacilli spread from the lungs to adipose tissue and then return to the lungs in immunocompetent mice. In this work, mice were given different intra-nasal dosages of Mtb bacilli, and by week 7, Mtb could be found in different fatty depots distant from the lungs [29]. In the same study, mice lacking Mtb had subcutaneous implantation of pre-adipocytes harboring Mtb. Lung Mtb
infection was discovered 5 weeks after insertion. This shows that Mtb can spread easily from the lungs to adipose tissue and back to the lungs. Intriguingly, a different study found that the infiltration of mononuclear phagocytes, Mtb-specific CD8+ T cells, and activated NK cells during Mtb infection in adipocytes have all been linked to the infection. This suggests that adipocytes undergo remarkable mild inflammatory alterations during Mtb infection to help keep the infection in a dormant form \[36\]. In this study, the active TB group had the highest serum levels of adiponectin, whereas the control group had the lowest levels. These outcomes were consistent with those attained by Keicho et al \[25\]. Adiponectin levels were discovered to be increased in the blood of tuberculous patients. They proposed that metabolic indicators in TB may be regulated by the low-fat store and underlying inflammation in many ways \[12\].

**Serum levels of vitamin D**

Comparing patients with tuberculosis to the control group, a statistically remarkable (p<0.0001) reduced level of serum vitamin D was found in these individuals (see Figure 3).

**Figure 3.** Vitamin D in sera of the two studied groups

On the circulating level of vitamin D in patients with TB and the general population, several studies have produced contradictory findings. Between-population variations in serum vitamin D levels are significant, and they are impacted by a variety of geographic and cultural factors \[37\]. Immune dysregulation and systemic inflammation are caused by vitamin D deficiency (VDD), which is extremely common worldwide. The following could be the underlying causes of the link between VDD and TB. VDR is a ligand-activated transcription factor that controls gene expression through vitamin D elements (VDRE), which are known to be vital for health and regulate cellular processes found in myocytes, endothelial cells, and a variety of immune cell types, including T and B lymphocytes, macrophages, dendritic cells, and neutrophils \[38\]. To regulate the immune system, vitamin D regulates both innate and
adaptive immunity. After macrophages are treated with 25(OH)D, several cytokines are generated. Additionally, the antimicrobial peptides cathelicidin and β-defensin, which encourage autophagy in Mycobacterium TB, are expressed following vitamin D exposure \[39\]. The pathogenesis of pulmonary cavitation does not include matrix metalloproteinases (MMPs), and the process is brought on by reactive oxygen and nitrogen species via vitamin D. VDD may compromise immunity, increasing the likelihood of occurrence of TB and sepsis \[40\].

Malnutrition and TB have been linked in several studies, suggesting that TB is caused by a variety of conditions \[41\]. Malnutrition also has an impact on the immune system, which raises the chance of contracting TB. Studies from earlier years \[42, 43\] investigating the association between TB and VDD did meta-analyses. Children were only present in 2/15 of the studies that made up's meta-analysis \[44\]. Most previous meta-analyses that included patients of all ages did not disclose results for the subgroup of children. TB in children may be caused by VDD in a number of ways. A growing body of research suggests that VDD influences immunity; a deficiency in vitamin D may probably increase the generation of chemokines, activate dendritic cells, and change T cell activation, which may all contribute to M tuberculosis infection. Children with VDD in underdeveloped nations are more likely to contract TB \[45\]. We discovered a strong correlation between VDD and the emergence of TB in the current investigation, indicating their association with higher risk. VDR polymorphisms or inadequate diet may be responsible for the lower level of 25(OH) D in patients with active TB compared to the subjects of control group. Through malnutrition, insufficient vitamin D absorption, and insufficient sun exposure, TB raises the incidence of VDD, immune dysregulation, and VDR polymorphism \[46\].

Additionally, studies used different definitions of VDD, which may have added to the observed heterogeneity. Hence, VDD needs to be defined consistently. Through pathogenetic pathways, additional variables may interact with TB to cause VDD in TB patients. Lack of vitamin D has been liked to a higher risk of tuberculosis in some populations. Nnoahem and colleagues determined in a meta-analysis that vitamin D insufficiency is linked to a high risk of tuberculosis. Studies on Indian populations have shown that Asian populations' lower levels of vitamin D may contribute to the high frequency of tuberculosis in this area \[47\]. Another study in Karachi, Pakistan, discovered that contacts with TB had low circulating levels of vitamin D \[48\]. Other trials confirmed the effectiveness of vitamin
Doi: https://doi.org/10.25130/tjps.v28i5.492

D as a TB preventative [49]. It has long been recognized that a vitamin D deficiency is linked to weakened immunity and an increased likelihood of tuberculosis. Numerous immune cell types, such as monocytes, macrophages, and T-lymphocytes, have been shown to support resistance to *Mycobacterium TB* (MTB) [13].

**ROC Curve Analysis**

The areas under the curves (AUC) of serum leptin, adiponectin, and vitamin D were (0.9643), (1.000), and (1.000), respectively, according to the Receiver Operating Characteristic (ROC) curve (see Fig. 4).

![ROC curves of (a) Leptin, (b) Adiponectin and (c) Vitamin D](image)

In the current work, the diagnostic utility of Leptin, Adiponectin, and Vitamin D was determined by the measures of ROC curve analysis. Results indicate a high diagnostic value in predicting the risk of Tuberculosis. We demonstrated that elevation of Adiponectin and decrease in leptin and Vitamin D levels were useful markers to differentiate between Tuberculosis and non-Tuberculosis subjects due to their high value of area under the curve (AUC).

**Correlations among the studied biochemical parameters**

The relationship between Vitamin D level and measured biochemical parameters is presented in Fig. 5. The results demonstrated that there was none remarkable positive association between serum Vitamin D level with Leptin, which are (r=0.282; *P*=0.286); while there was none remarkable negative association between serum Vitamin D level with Adiponectin, which are (r= -0.069; *P*=0.797).
4. Conclusions
According to our findings, the pathogenesis of tuberculosis may be correlated with lower serum leptin and elevated serum adiponectin levels. As a result, the level of serum leptin and adiponectin may be an important and useful biomarker for determining the onset and progression of tuberculosis. Our study's findings suggest that hypovitaminosis D was linked to the development of tuberculosis.

Conflict of Interest: All authors declare no conflicts of interest.

References
with and without type 2 diabetes mellitus. PloS One, 8(11), e80122.


risk of tuberculosis: a meta-analysis. Drug design, development and therapy, 11, 91.