

# The correlation of TNF alpha levels with the lipid profile of dengue patients

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Immunological factors, such as cytokines, have been proposed as a cause of changes in the lipid profile of dengue patients. We studied whether serum lipid levels and serum TNF- $\alpha$  levels are associated in a group of dengue patients from an endemic region in the Northwest of Mexico. We found statistically important differences in the serum lipid profile and the TNF- $\alpha$  levels of dengue patients compared with the control group, were observed. However, TNF- $\alpha$  levels did not correlate with the lipid profile of dengue patients.

## KEYWORDS

dengue fever, dengue hemorrhagic fever, lipid profile, tumor necrosis factor

## 1 | INTRODUCTION

Lipids play an important role in bacterial, parasitic, and viral infections.<sup>1</sup> Changes in the serum lipid profile have been observed during dengue virus (DENV) infections.<sup>2-4</sup> Furthermore, it has been reported that the lipid profile status may contribute to the pathogenesis of different manifestations of DENV infections in humans.<sup>2-5</sup> In addition, certain proinflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$  have been associated with changes in the clinical manifestations of dengue infections.<sup>2-4</sup> In 1991, it was suggested that TNF- $\alpha$  might play a role as a modulator of pathophysiological changes observed in patients with dengue hemorrhagic fever/dengue shock syndrome.<sup>6</sup> Several years later, measuring serum cytokine levels of TNF- $\alpha$  was proposed by

several authors as a laboratory tool for predicting severe dengue disease.<sup>7</sup> However, the interaction between metabolic and immunological host factors has not been demonstrated in dengue infection.

Thus, the aim of our study was to evaluate the correlation between serum levels of TNF- $\alpha$  and the lipid status of patients with dengue.

## 2 | SUBJECTS AND METHODS

### 2.1 | Subjects

All participants in the present study, which was a hospital-based case-control study, were part of a larger cohort of patients, and healthy blood donors (HBD).<sup>8</sup> The cases were treated at the emergency

department of the hospital, while healthy blood donors (controls) were interviewed in the blood bank of a hospital located in Northwestern Mexico over the period 2014-2016. The patients included 41 adults with a median age [IQR, 25-75th percentile] of 34 [22.5-42] years, diagnosed with dengue fever (DF;  $n = 25$ ; 28 [20.5-38.5] years), and dengue hemorrhagic fever (DHF;  $n = 16$ ; 37 [29.25-46.75] years), based on the WHO guidelines issued in 1997 for dengue disease.<sup>9</sup> DENV infection was confirmed using the Dengue Duo NS1 Antigen and IgM/IgG Rapid Strip Test (SD BIOLINE, Korea).

The HBD ( $n = 30$ ; 30.5 [24-42] years) were selected to match the patients with respect to gender, age, and ethnicity (all mestizos from Northwestern Mexico). The HBD were negative for both anti-dengue IgM and IgG antibodies, HIV, HCV, and had no previous history of hospitalization for a dengue-like illness.

At the hospital, a fasting blood sample was taken from each participant in order to determine the serum lipid profile of the 41 patients and the 30 HBD of the control group. Cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides levels were determined by enzymatic methods, while very-low-density lipoprotein (VLDL) cholesterol levels were measured using the method of Friedewald.<sup>10</sup> Serum levels of TNF- $\alpha$  were measured in 16 patients (13 DF and 3 DHF) and 30 HBD using an enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA).

To measure the TNF- $\alpha$  levels of dengue patients, all samples were taken during the acute phase of the disease (up to 5 days after the onset of symptoms); in the case of the HBD, we discarded those with inflammatory and chronic degenerative disorders or other severe medical conditions associated with changes in the lipid profile and in TNF- $\alpha$  levels.

All participants were fully informed of the study protocol before signing a written informed consent form. The study was approved by the Institutional Ethics and Research Committee of the Hospital General de Culiacan.

## 2.2 | Statistical analysis

The statistical tests were performed using the Graph Pad Prism software. The data were expressed as median and interquartile ranges [IQR, 25-75th percentile] to describe age and mean and standard

deviation (SD) were used to describe the lipid profile and the TNF- $\alpha$  levels. One-way ANOVA with Bonferroni's post hoc-test was used to determine the differences in lipid profile between the study groups (DF, DHF, and HBD). An unpaired *t*-test with Welch's correction was used to compare the TNF- $\alpha$  levels of dengue patients with those of the HBD, and a Pearson's correlation test was performed to determine the correlation between TNF- $\alpha$  levels, and the lipid profile of the participants. Statistical significance was set at  $P < 0.05$ .

## 3 | RESULTS

The comparison of the lipid profile of patients with that of the HBD showed lower cholesterol ( $126.7 \pm 38.56$  vs  $157.7 \pm 37.10$  mg/dL) and LDL levels ( $76 \pm 32.27$  vs  $100.6 \pm 27.64$  mg/dL) in patients compared with HBD, but similar levels of VLDL, HDL, and triglycerides in both groups. When the patients were categorized into DF and DHF, no statistical differences were detected between DF, and HBD. However, cholesterol, LDL, and HDL levels were significantly lower in DHF than in DF and/or HBD. The differences in VLDL and triglyceride levels between the three study groups were not significant (Table 1).

The TNF- $\alpha$  levels of dengue patients were significantly higher ( $21.94 \pm 7.50$  pg/mL) than those of HBD ( $14.16 \pm 10.40$  pg/mL) ( $P = 0.0058$ ), but there were significant differences ( $P = 0.017$ ) when comparing DF ( $23.26 \pm 7.67$  pg/mL), DHF ( $16.21 \pm 2.84$  pg/mL), and HBD.

Figure 1 shows the correlation analysis between the serum TNF- $\alpha$  levels and the serum lipids levels of patients with dengue. Cholesterol, LDL, VLDL, and triglyceride levels were correlated positively ( $r = 0.1388, 0.3016, 0.1064, \text{ and } 0.1064$ , respectively), whereas HDL was negatively correlated with TNF- $\alpha$  levels ( $r = -0.0776$ ). Nevertheless, these correlations did not show statistical significance ( $P = >0.05$ ), even when the patients were divided according to the clinical type of the disease.

## 4 | DISCUSSION

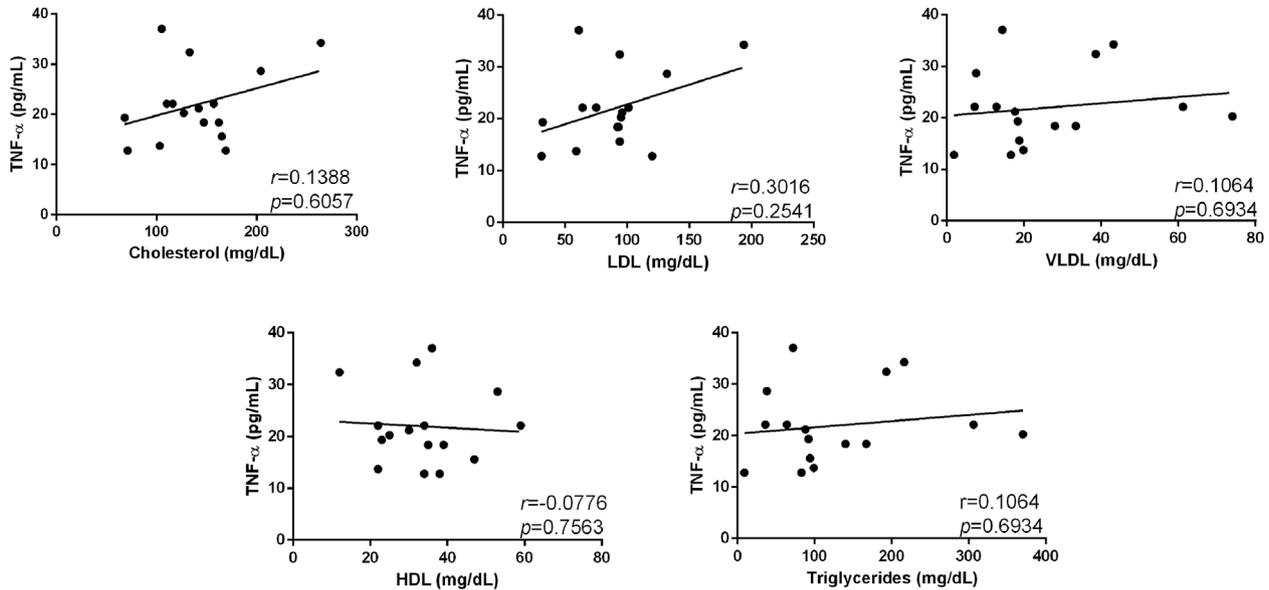
The last two decades have seen an increasing interest in the study of the possible relationships between the development of severe dengue

**TABLE 1** Values of lipid profile in healthy blood donors (HBD) and patients with dengue fever (DF) and dengue hemorrhagic fever (DHF) are showed

Lipid	HBD $n = 30$	DF $n = 25$	DHF $n = 16$	<i>P</i> -value
Cholesterol (mg/dL)	$157.7 \pm 37.10$	$140.1 \pm 39.75$	$106 \pm 26.42$	<0.0001*
LDL (mg/dL)	$100.6 \pm 27.64$	$87.28 \pm 34.43$	$58.38 \pm 18.37$	<0.0001*
VLDL (mg/dL)	$23 \pm 13.01$	$23.31 \pm 18.23$	$33.96 \pm 15.23$	0.0555
HDL (mg/dL)	$33.80 \pm 15.07$	$36.76 \pm 13.53$	$20.81 \pm 10.11$	0.0014*
Triglycerides (mg/dL)	$115 \pm 65.07$	$116.6 \pm 91.16$	$169 \pm 76.17$	0.0555

The Bonferroni's multiple post-hoc comparison test showed significant differences in: cholesterol between HBD versus DHF ( $P = <0.0001$ ) and DF versus DHF ( $P = 0.0142$ ), in LDL between HBD versus DHF ( $P = <0.0001$ ) and DF versus DHF ( $P = 0.0072$ ), while that HDL level between HBD versus DHF ( $P = 0.0086$ ), and DF versus DHF ( $P = 0.0014$ ).

*P* values were compared with one-way ANOVA and the asterisks shows differences statistically significant in between the three groups.



**FIGURE 1** Correlation between the lipid profile and TNF- $\alpha$  levels of dengue patients.  $P$  and  $r$  values were estimated by Pearson's correlation test

and changes in circulating lipids.<sup>2-4</sup> Lipid metabolism is extensively regulated by cytokines during the host response to infection.<sup>1</sup> TNF- $\alpha$  is a key pro-inflammatory cytokine that has been associated with many biological functions such as lipid metabolism<sup>11</sup>; it is well known that DF and DHF patients have significantly higher levels of pro-inflammatory cytokines.<sup>12,13</sup>

In the present study, we found increased levels of cholesterol and LDL in HBD compared with dengue patients, whereas cholesterol, LDL, and HDL levels were significantly lower in DHF than in DF and/or HBD. Furthermore, we found higher levels of TNF- $\alpha$  in DF patients compared to HBD. Finally, we found no correlation between circulating lipids and TNF- $\alpha$  levels.

The results of the present study agree with those of van Gorp et al<sup>4</sup> They found a statistically significant decrease in cholesterol, HDL, and LDL levels in patients with DHF compared with healthy subjects, which suggests that lipid levels may be a biomarker for dengue prognosis. In the same line of evidence, Survana and Rane<sup>3</sup> confirmed later that low levels of cholesterol, HDL, and LDL are associated with severe forms of dengue infection, supporting the hypothesis of van Gorp et al<sup>4</sup> They also observed that VLDL values were different between DF, DHF, and Dengue shock syndrome (DSS) patients, but triglycerides, HDL, and LDL showed no differences (both studies were performed on a pediatric population and they used 1997 WHO dengue guideline for the patient classification in their studies). In contrast Duran's study,<sup>2</sup> which included both adult and pediatric patients (based on 2009 WHO classification for dengue disease), observed a significant decrease in total cholesterol, HDL, and LDL levels, as well as an increase in triglyceride, and VLDL levels, in patients with severe forms of dengue infection.

Although our patients were reclassified according to the 2009 WHO guideline for dengue; the number of patients among the study groups was modified to 22 DWWS, 17 DWS, and two SD (data not

shown). However, the same pattern of dyslipidaemia was observed as originally classified according to the 1997 WHO criteria as low cholesterol, LDL, and HDL and high levels of VLDL and triglycerides in severe patients (FHD or DWS/SD) compared to HBD. This reduction in cholesterol levels in dengue patients has been explained by an accumulation of cholesterol in different organs, causing liver steatosis,<sup>14</sup> but it could also be related to an increase in endothelial permeability associated with severe dengue disease, which could potentially cause a leakage of cholesterol molecules, or to an increase in the uptake of cholesterol, and LDL particles by DENV-infected cells.<sup>15,16</sup>

In 1991, Vitarana et al<sup>17</sup> found high TNF- $\alpha$  levels in patients with severe dengue. In the same year, Yadav et al<sup>6</sup> proposed that TNF- $\alpha$  might modulate the pathophysiological changes observed in DHF/DSS, suggesting for the first time that there could be a link between innate immunity and inflammation processes in the pathogenesis of dengue.

In the present study, the comparison of the mean levels of serum TNF- $\alpha$  between DF (23.26 pg/mL) and DHF (16.21 pg/mL) patients showed significant differences ( $P = 0.026$ ). This result contrasts with the findings of other studies performed in different populations (eg, Thailand, India, and Colombia) that have found higher levels of TNF- $\alpha$  in DHF patients compared with DF patients.<sup>18-20</sup> A larger sample should be studied in order to explain these differences. Now, however, we want to highlight the significantly higher levels of TNF- $\alpha$  in the DF group compared to healthy blood donors. The time at which the sample was taken and/or the degree of severity of the illness might explain the observed variations in TNF- $\alpha$  levels.

The correlation between the circulating lipid profile and TNF- $\alpha$  levels was not statistically significant, suggesting that they are part of independent physiological processes, at least in Mexican patients with dengue. However, a larger sample would be necessary to confirm this.

Finally, we consider it necessary to study the possible role of other inflammation mediators in molecular mechanisms underlying the biological interaction between energy metabolism and the immune response.

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