

## RESEARCH ARTICLE

# Inflammatory status and severity of disease in dengue patients are associated with lipoprotein alterations

Damariz Marin-Palma<sup>1,2</sup>, Cherilyn M. Sirois<sup>3</sup>, Silvio Urcuqui-Inchima<sup>2</sup>, Juan C. Hernandez<sup>1\*</sup>

**1** Infettare, Facultad de Medicina, Universidad Cooperativa de Colombia, Medellin, Colombia, **2** Grupo Immunovirologia, Facultad de Medicina, Universidad de Antioquia, UdeA, Medellín, Colombia, **3** Department of Biology & Chemistry, Springfield College, Springfield, MA, United States of America

\* [juankhernandez@gmail.com](mailto:juankhernandez@gmail.com)



## Abstract

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**Citation:** Marin-Palma D, Sirois CM, Urcuqui-Inchima S, Hernandez JC (2019) Inflammatory status and severity of disease in dengue patients are associated with lipoprotein alterations. PLoS ONE 14(3): e0214245. <https://doi.org/10.1371/journal.pone.0214245>

**Editor:** Dong-Yan Jin, University of Hong Kong, HONG KONG

**Received:** November 28, 2018

**Accepted:** March 9, 2019

**Published:** March 22, 2019

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**Data Availability Statement:** All relevant data are within the manuscript and its Supporting Information files. data set is available at Figshare: [10.6084/m9.figshare.7675643](https://doi.org/10.6084/m9.figshare.7675643).

**Funding:** The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by Colciencias Grant 141565741029, Universidad Cooperativa de Colombia and Universidad de Antioquia UdeA. The funder had no role in the study design, data

## Introduction

The triggering of severe dengue has been associated with an exacerbated inflammatory process characterized by the production of pro-inflammatory cytokines such as IL-1 $\beta$ /IL-18, which are the product of inflammasome activation. Furthermore, alteration in the levels of high-density (HDL) and low-density lipoproteins (LDL) has been observed; and HDL are known to have immunomodulatory properties, including the regulation of inflammasomes. While HDL would be expected to counteract hyperactivation of the inflammasome, the relationship between HDL and dengue severity, has not previously been explored.

## Methodology

We conducted a cross-sectional study of 30 patients with dengue and 39 healthy controls matched by sex and age. Lipid profile and levels of C-reactive protein were quantified. Serum levels of IL-1 $\beta$ , IL-6, IL-10, IL-18, and TNF- $\alpha$ , were assessed by ELISA. Expression of inflammasome-related genes in PBMC was quantified by qPCR.

## Results

Dengue patients presented an alteration in the parameters of the lipid profile, with a significant decrease in HDL levels, which was more pronounced in dengue patients with warning signs. Moreover, a decrease in the expression of the inflammasome-related genes NLRP1, NLRC4, caspase-1, IL-1 $\beta$  and IL-18 was observed, as well as an increase in serum levels of C-reactive protein and IL-10 in dengue patients versus healthy donors. Significant positive correlations between LDL levels and the relative expression of NLRP3, NLRC4, IL-1 $\beta$  and IL-18, were found.

collection and analysis, decision to publish or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

## Conclusion

The results suggest that there is a relationship between the alteration of LDL and HDL with the imbalance in the inflammatory response, which could be associated with the severity of dengue.

## Introduction

Dengue virus (DENV) is responsible for one of the most prevalent infections in the world transmitted by arthropods, with nearly 100 million symptomatic infections annually. Dengue presents a wide spectrum of symptoms ranging from a feverish illness with headache, joint and muscle pain, up to severe manifestations such as multi-organ failure, hemorrhages and death [1]. Several studies have been conducted to identify the mechanisms involved in variations in the severity of the disease; antibody-dependent enhancement during a second infection [2, 3] and a cytokine storm [4], appear to be key factors. However, there are primary infections that also develop into severe dengue disease, which suggests the existence of additional mechanisms involved in the development of severe dengue symptoms. In this sense, it has been found that multiple baseline metabolic/immunologic parameters are related to the clinical state of the patients [5–8], including the characteristics of their serum lipid profile and a predisposition for an exacerbated inflammatory response [9]. Contradictory results have been reported for the lipid profile in dengue patients. It has been reported that patients with severe dengue present increased levels of triglycerides, very-low-density lipoprotein (VLDL) and high-density lipoproteins (HDL), as well as lower levels of total cholesterol and low-density lipoprotein (LDL), compared with other clinical presentations and healthy donors [10]. Other studies have described that triglycerides, total cholesterol and LDL have similar behavior to the healthy subjects, while HDL levels are lower in patients with severe dengue [11]. HDL are heterogeneous macromolecular complexes that transport cholesterol from peripheral tissues to the liver, for elimination [12, 13]. Of interest, here is that HDL exert pleiotropic effects on the immune system, such as reduction of inflammation and apoptosis [13–16]. Recently, it was reported that HDL regulate inflammasome activity [17]. The inflammasomes are cytosolic complexes involved in the maturation of the pro-forms of inflammatory cytokines of the IL-1 family, including IL-1 $\beta$  and IL-18 [18, 19]. Thacker *et al.* have reported that primary macrophages and human monocyte-like THP-1 cells produce lower levels of IL-1 $\beta$  in response to cholesterol crystals when pre-treated with HDL [17].

The involvement of inflammasome activation during viral infection [20], including DENV infection, is well established. Dengue patients have shown high levels of pro-inflammatory cytokines, especially those with severe clinical forms [21, 22]. Furthermore, it has been described that *in vitro* DENV-infection of macrophages induces an inflammatory response through activation of the NLRP3 inflammasome, leading to the release of IL-1 $\beta$  [23]. Likewise, it has been shown that platelets contribute to endothelial permeability through the activation of inflammasomes, specifically NLRP3, and the release of IL-1 $\beta$ , which seems to alter endothelial function [24].

Although there is evidence that both HDL levels and pro-inflammatory cytokines are altered during DENV infection [25], their specific relationship remains unknown. As lifestyle-based and pharmacological modulation of lipid profiles is commonly integrated into medical care, understanding a possible anti-inflammatory function of HDL or lipid profile changes during DENV infection could be helpful for managing dengue virus disease. Therefore, the

aim of this work is to describe the characteristics of the lipid profile in dengue patients and to explore the association between HDL levels and inflammatory markers, particularly the inflammasome components, during dengue pathogenesis.

## Materials and methods

### Study population

Thirty dengue patients were included and classified as having dengue without warning signs (DNWS), dengue with warning signs (DWWS) or severe dengue (SD), based on the WHO classification, 2009 [26]. Patients with co-infections, history of statin treatment, cancer, pregnancy or mental health problems, were excluded. Likewise, 39 healthy individuals (controls) with a negative serum test result for dengue (*SD BIOLINE Dengue Duo rapid test*) were included, matched by age and sex, and from the geographical area to dengue-positive subjects. All subjects were adults, read and signed an informed consent approved by the ethics committee of the Universidad Cooperativa de Colombia, who also approved the whole study (0800–005). In addition, all research protocols were made according to the principles of the Declaration of Helsinki.

### Blood samples and processing

Plasma and serum were separate by centrifugation at 3500 rpm from approximately 20 mL of peripheral blood, which was obtained by venipuncture and collected in Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ 07417). The samples were stored at  $-70^{\circ}\text{C}$  until use. For dengue diagnosis, the detection of NS1 antigen and IgM and IgG specific antibodies in serum were achieved by using the *SD BIOLINE Dengue Duo rapid test* (Dengue NS1 Ag and IgG/IgM; Alere, Australia). Peripheral blood mononuclear cells (PBMCs) were isolated using the gradient of density method with Ficoll-Histopaque (Sigma-Aldrich Chemical Co., St. Louis, MO) and were used for total RNA extraction, as described previously [27].

### Lipid profile and C-reactive protein (CRP) quantification

Triglycerides, total cholesterol and HDL levels were quantified in serum through colorimetric assays, using the Triglycerides, Total Cholesterol and Cholesterol HDL Direct kits (Biosystems Ref. 11828, 11506 and 11648, respectively; Costa Brava 30, Barcelona, Spain); the results were reported in mg/dL. Serum CRP was quantified by immunoturbidimetry in a certified clinical laboratory in Medellin- Colombia.

### Quantification of cytokines by ELISA

Serum levels of IL-1 $\beta$ , IL-6, IL-10, IL-18 y TNF- $\alpha$  were quantified by ELISA using commercial kits from BD Biosciences (Franklin Lakes, NJ) and eBioscience (Vienna, Austria), following the manufacturer's instructions and in duplicate, as previously described in detail [27].

### mRNA quantification of the inflammasome-related genes

As previously reported in detail [27, 28], total RNA was isolated from PBMCs using the RNeasy Mini Kit (QIAGEN, Inc., Valencia, CA, USA), following the manufacturer's recommendations. RNA concentration/purity was determined by spectrophotometry at 260/280 nm. cDNA synthesis was conducted with 230 ng of total RNA and using the Revertaid H Minus First Strand cDNA Synthesis Kit (Fermentas, Glen Burnie, MD, USA), following the manufacturer's recommendations.

The mRNA of NLRP1, NLRP3, NLRC4, AIM2, ASC, caspase-1, IL-1 $\beta$ , and IL-18 was quantified by real time RT-PCR using the Maxima SYBR Green qPCR master mix (Fermentas). The specific amplification of  $\beta$ -actin (housekeeping gene) was used as a control. Amplification protocols were 39 cycles and standardized for each gene [28, 29], S1 Table. Real time RT-PCR analysis was conducted with the software CFX Manager, version: 1.5.534.0511 (Bio-Rad, Hercules, CA, USA).

### Statistical analysis

The statistical package GraphPad Prism 5.0 (San Diego, CA, USA) was used. Data were analyzed for normality and homoscedasticity using the Shapiro-Wilk and Levene tests. For the comparison between groups, the t-Student or Mann-Whitney test were applied for parametric and non-parametric data, respectively. For comparison between three or more groups, parametric ANOVA or Kruskal-Wallis tests were used; in case of a statistical association, post hoc tests (or multiple benchmarks) HDS of Tuckey or Dunn respectively, were carried out. Finally, for correlation analysis, Pearson or Spearman tests were applied to parametric or non-parametric data, respectively.

## Results

### Demographic data

Thirty dengue patients were included and classified according to their clinical presentation as DNWS, DWWS or SD and compared to 39 healthy controls, in a period between October 2015 and April 2017. Clinical and laboratory criteria used to classify the patients according to the severity of the disease included clinical symptoms such as fever, myalgia/arthralgia, headache, retro-ocular pain, asthenia, anorexia and presence of rash and pruritus. Additional symptoms like vomiting, abdominal pain, diarrhea, cough, sore throat, dizziness, somnolence, lethargy, irritability, and convulsions were also considered. Hemorrhagic signs, including petechiae, purpura/ecchymosis, epistaxis, hematemesis/melena, and hematuria were included. As well as, plasma leakage and organ dysfunction manifestations. Finally, the laboratory parameters electrolytes, leukocytes and platelets counts, hematocrit, and transaminases were assessed. Major demographic and clinical data are presented in Table 1. It is noteworthy that the

Table 1. Clinical and demographic information.

	Group			
	Controls (n = 39)	Dengue patients		
		DNWS (n = 8)	DWWS (n = 18)	SD (n = 4)
Women : Men	16 : 23	1 : 7	8 : 10	3 : 1
Age in years: Median (IQR)	35 (26)	42 (22.7)	29.5 (32.5)	46.5 (44.0)
Days of evolution: Median (IQR)		5.0 (2.8)	6.0 (1.5)	6.5 (2.5)
% Hematocrit: Median (IQR)		46.2 (11.0)	42.9 (4.7)	41.6 (5.0)
Platelets/ $\mu$ L: Median (IQR)		124,000 (126,000)	73,000 (57,000)	44,500 (112,750)
AST in IU/mL: Median (IQR)	RR (15–37)	74 (138.5)	137 (140)	161.5 (745.3)
ALT en IU/mL: Median (IQR)	RR (30–65)	43 (72.5)	122 (124.5)	93 (583)
1° infection: 2° infection		4:4	8:10	1:3
Hepatomegaly / Splenomegaly (%)		0	5.6	50
Petechiae (%)		37.8	44.4	75

RR: Reference range, IQR: Interquartile range, IU: International unit, AST: Aspartate transaminase, ALT: Alanine transaminase, DNWS: Dengue with no warning signs, DWWS: Dengue with warning signs and SD: Severe dengue.

<https://doi.org/10.1371/journal.pone.0214245.t001>

majority of patients (92%) presented thrombocytopenia as characterized by a platelet count below the reference value of 150.000 platelets/ $\mu$ L. Days of evolution of dengue disease had a median of around 6 days for all the groups; and the number of patients presenting with primary ( $n = 13$ ) and secondary ( $n = 17$ ) DENV infections was comparable.

### Dengue patients have an altered standard lipid profile

During dengue infection, patients have previously been shown to have multiple metabolic alterations associated with the acute inflammatory response, including the lipid profile [10]. In our study population, dengue patients had significantly lower HDL levels ( $p < 0.0001$ , Fig 1A) compared to healthy controls. Similarly, lower LDL ( $p = 0.0002$ ; Fig 1B) and total cholesterol ( $p < 0.0001$ ; Fig 1C) levels and higher triglycerides and VLDL levels ( $p < 0.0001$ ; Fig 1D and 1E, respectively) were observed. The difference in HDL levels in dengue patients versus controls was maintained even when patients were distinguished by clinical disease severity (DNWS, DWWS and SD; Fig 2A). Moreover, when HDL levels were compared between the groups of dengue patients, those with more severe clinical disease (DWWS group) had lower HDL levels than those with no clinical warning signs (DNWS group;  $p < 0.05$ ; Fig 2B), but no differences were found when comparing with SD group. Similar results were observed with respect to the LDL levels in dengue patients according to their clinical stages (Fig 2C and 2D).

### Dengue patients show decreased expression of inflammasomes-related genes

The NLRP3 inflammasome has been shown to be activated during DENV infection [23]. Here we found that the mRNA levels of NLRP1 ( $p = 0.0032$ ), NLRC4 ( $p = 0.0026$ ), caspase-1 ( $p = 0.0005$ ), IL-18 ( $p < 0.0001$ ) and IL-1 $\beta$  ( $p = 0.0179$ ), were lower in dengue patients compared with healthy controls (Fig 3), however differences between NLRP3, AIM2 and ASC were not found. When inflammasome-related genes expression was evaluated according to the clinical stages of dengue, a statistically significant decrease was found in the mRNA of NLRP1 ( $p < 0.05$ ), NLRC4 ( $p < 0.01$ ), caspase-1 ( $p < 0.01$ ) IL-18 ( $p < 0.0001$ ) and IL-1 $\beta$  ( $p < 0.05$ ) in DWWS patients, compared with healthy controls (Fig 4).

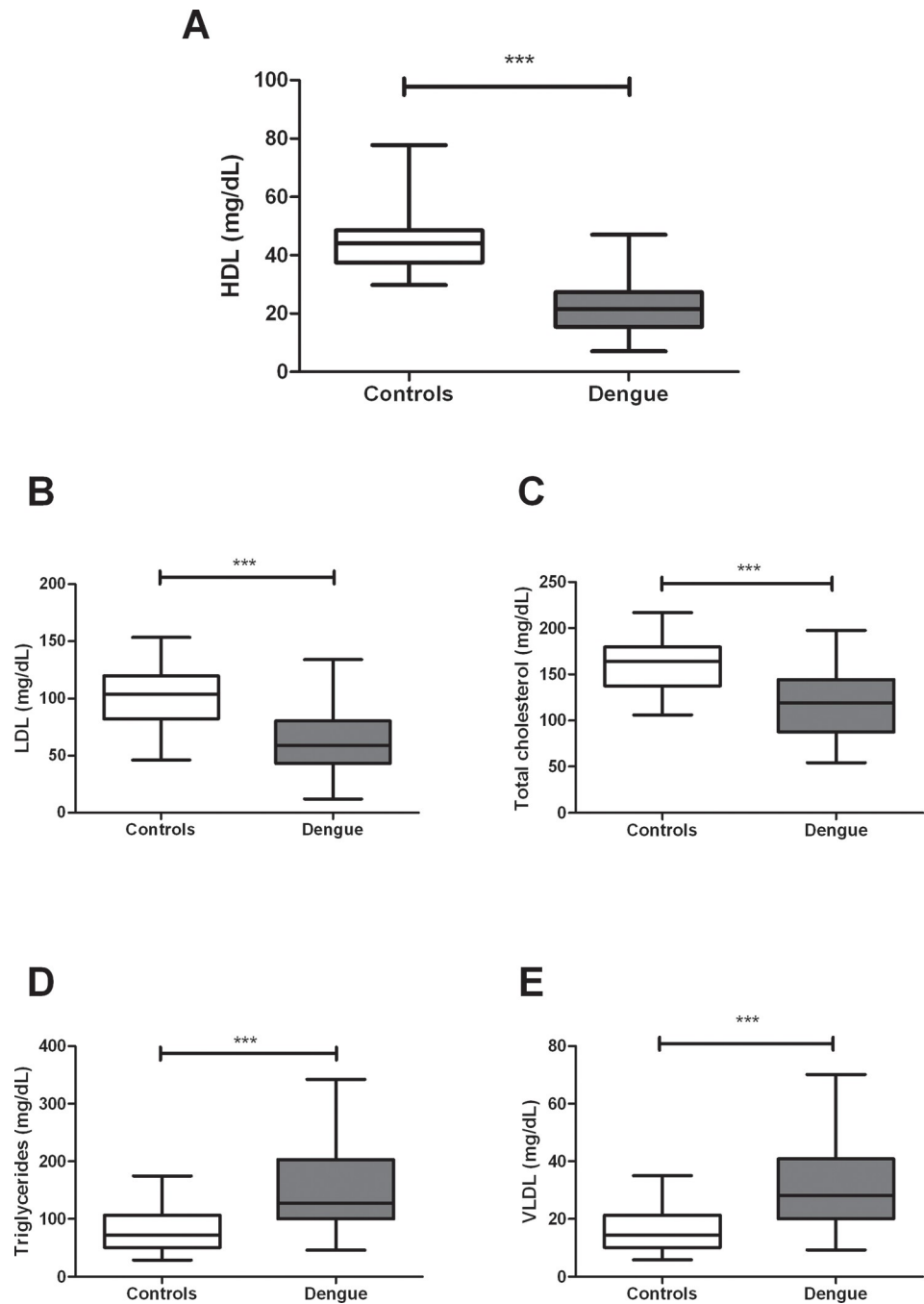
### Dengue patients express higher levels of IL-10 and C-reactive protein

The next step was to quantify the serum levels of IL-1 $\beta$ , IL-6, IL-18, IL-10, TNF- $\alpha$  and CRP, as indicators of the inflammatory status in the dengue patients. A significant increase in IL-10 levels from dengue patients was observed in comparison to healthy controls ( $p < 0.0001$ ; Fig 5A). However, the IL-18 levels were similar in individuals with dengue versus healthy controls (Fig 5B). No detectable serum levels of IL-1 $\beta$ , IL-6 and TNF- $\alpha$ , were observed.

Likewise, an increase in serum CRP levels was found in dengue patients compared to healthy controls ( $p < 0.0001$ , Fig 6A), which was more evident for DWWS and SD patients ( $p < 0.0001$  and  $p < 0.05$  respectively; Fig 6B).

### LDL levels are positively correlated with inflammasomes

Previously it was reported that LDL have the ability to induce the production of IL-1 $\beta$ , through the NF- $\kappa$ B and caspase-1 pathways in macrophages [30]. Since our results showed an alteration in lipoproteins levels in dengue patients compared to controls, we proceeded to analyze whether HDL and LDL levels might statistically correlate with inflammasome-related genes expression. While no significant correlations were observed for HDL (S1 Fig); positive correlations were found between LDL and NLRP3, NLRP1, NLRC4, IL-18 and IL-1 $\beta$  (Fig 7).

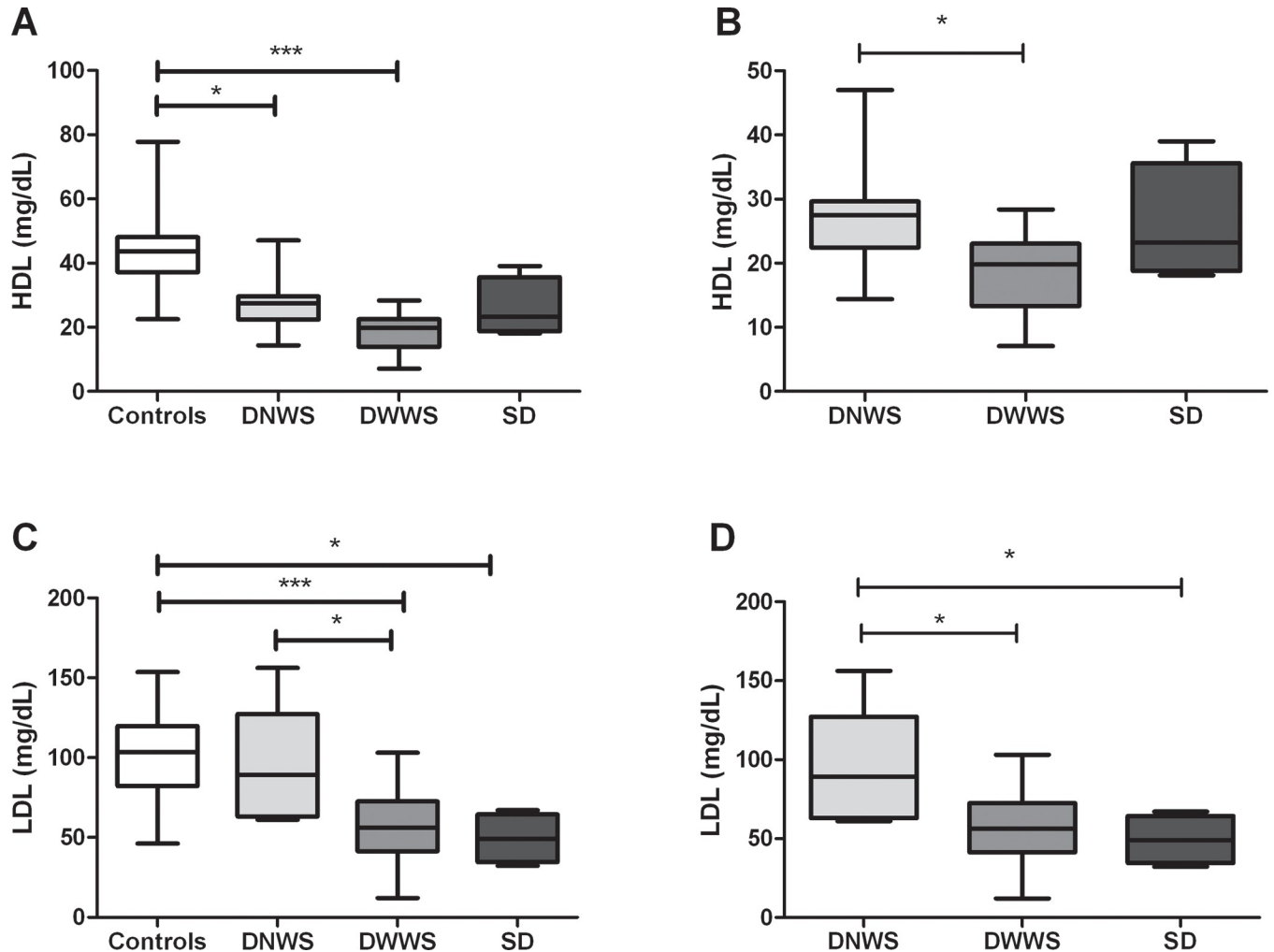


**Fig 1. Serum lipid profile in individuals with dengue differs from that of healthy controls.** (A) HDL, (B) LDL, (C) Total cholesterol, (D) Triglycerides and (E) VLDL were quantified by duplicated, using a colorimetric assay. The results are presented as medians with maximum and minimum values (box and whisker plot). Statistical comparison was made using the Mann-Whitney U test with a confidence level of 95%. Significant differences are represented at the top of the figure (\*\* $p < 0.001$ ).

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## Discussion

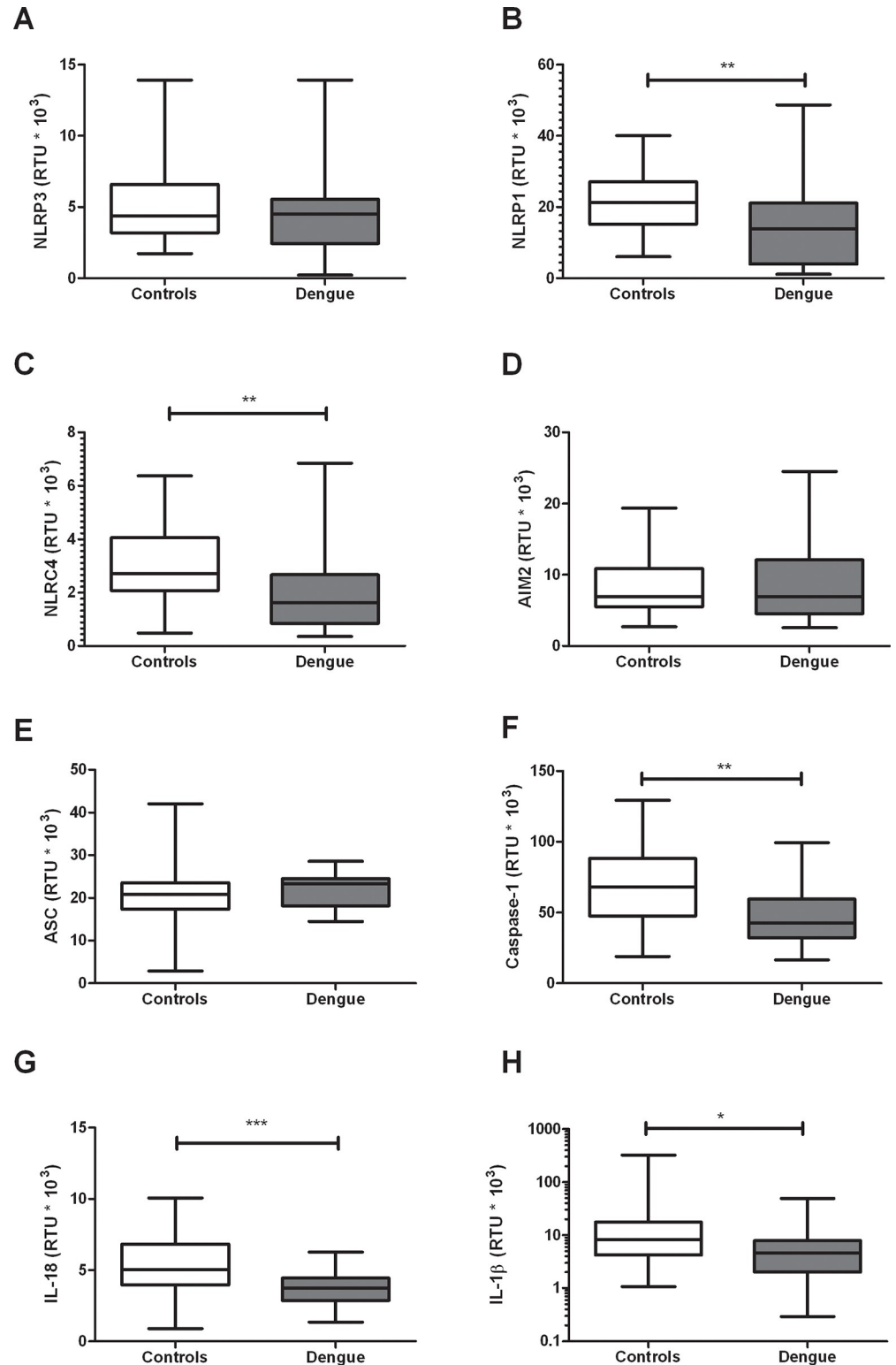
At present, several strategies are employed to modulate blood lipoprotein levels aiming to reduce the risk of cardiovascular disease. Strategies based on a healthy lifestyle, such as regular



**Fig 2. Serum HDL and LDL levels from dengue patients according to clinical classification.** HDL (A and B) and LDL (C and D) levels were assessed by duplicated; in the serum of individuals classified as having dengue with no warning signs (DNWS), dengue with warning signs (DWWS) or severe dengue (SD). The results are presented as medians with maximum and minimum values (box and whisker plot). Statistical comparison was made using the Kruskal-Wallis test with a confidence level of 95% and post *hoc* tests (or multiple benchmarks) HDS of Dunn were applied. Significant differences are represented at the top of the figure (\*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ).

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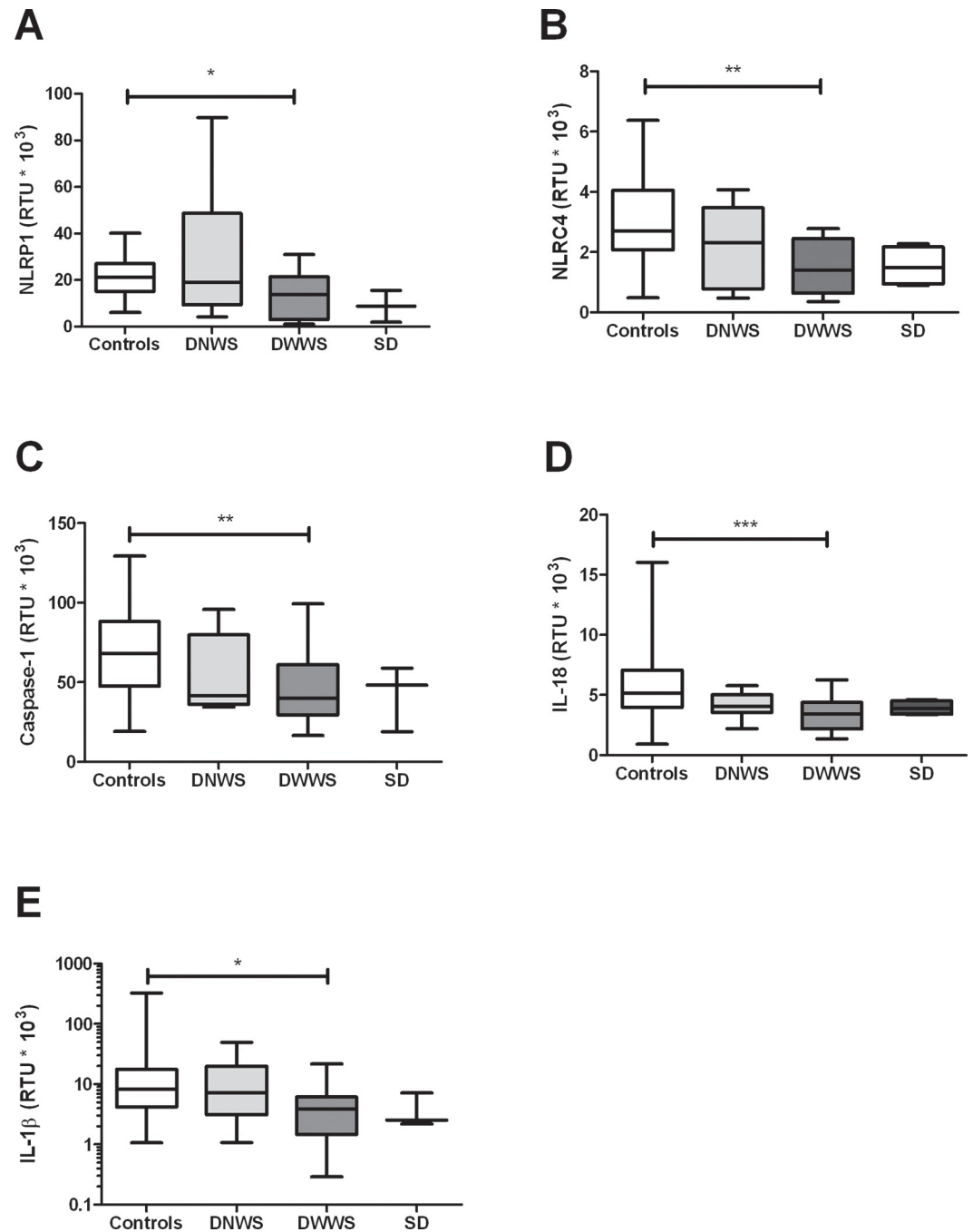
exercise and a low-fat diet, and the use of statins, are the most common. However, the impact that HDL may have on other diseases, such as viral infections, is just beginning to be studied as evidence of its immunomodulatory effects emerges. Here we present evidence supporting the hypothesis that concurrent changes in the serum lipid profile and inflammasome-mediated cytokine production may be mechanistically relevant in the progression of DENV infection to clinically severe forms of dengue. While previous reports regarding the HDL levels of dengue patients are contrasting [9, 10], we found that patients with dengue had lower levels of HDL, LDL and total cholesterol compared to the healthy controls, similar to that reported by Biswas *et al.* [9]. When the comparison was made according to the clinical stages, the DWWS patients had lower levels of HDL and LDL than those DNWS patients. These results suggest that patients with lower HDL and LDL levels may be progressing to the life-threatening forms of the disease. However, it is noteworthy that our severe dengue (SD) patients did not show a clear behavior as the DWWS patients. The discrepancies observed regarding the relationship



**Fig 3. Inflammasome-related genes expression in dengue patients.** The expression of (A) NLRP3, (B) NLRP1, (C) NLRC4, (D) AIM2, (E) ASC, (F) caspase-1, (G) IL-18 and (H) IL-1β was evaluated by real time PCR in PBMCs (by duplicates) from dengue patients and healthy controls. The β-actin gene was used as a constituent gene to normalize the RNA content. The results are presented as medians with maximum and minimum values (box and whisker plot). Statistical comparison was made using the Mann-Whitney U tests with a confidence level of 95%. Significant differences are represented at the top of the figure (\**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001).

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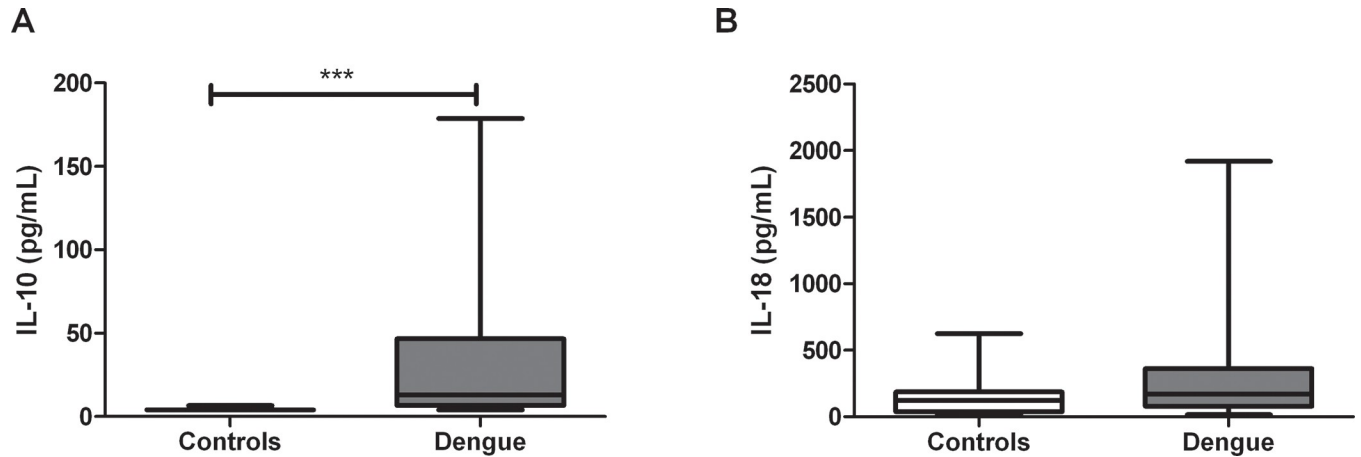




**Fig 4. Inflammasome-related genes expression in dengue patients, according to clinical classification.** The expression of (A) NLRP1, (B) NLRC4, (C) caspase-1, (D) IL-18 and (E) IL-1 $\beta$  in PBMCs was quantified by real time PCR by duplicated. The  $\beta$ -actin gene was used as a constitutive gene to normalize the mRNA content. The results are presented as medians with maximum and minimum values (box and whisker plot). Statistical comparison was made using the Kruskal-Wallis test with a confidence level of 95% and post *hoc* tests (or multiple benchmarks) HDS of Dunn, were applied. Significant differences are represented at the top of the figure (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

<https://doi.org/10.1371/journal.pone.0214245.g004>

between HDL and the severe forms of dengue may be due to differences in the study design and the population included. While the study carried out by Duran et al. [10] had a cross-sectional design in a population between 2 and 66 years and sampling at the acute phase, the

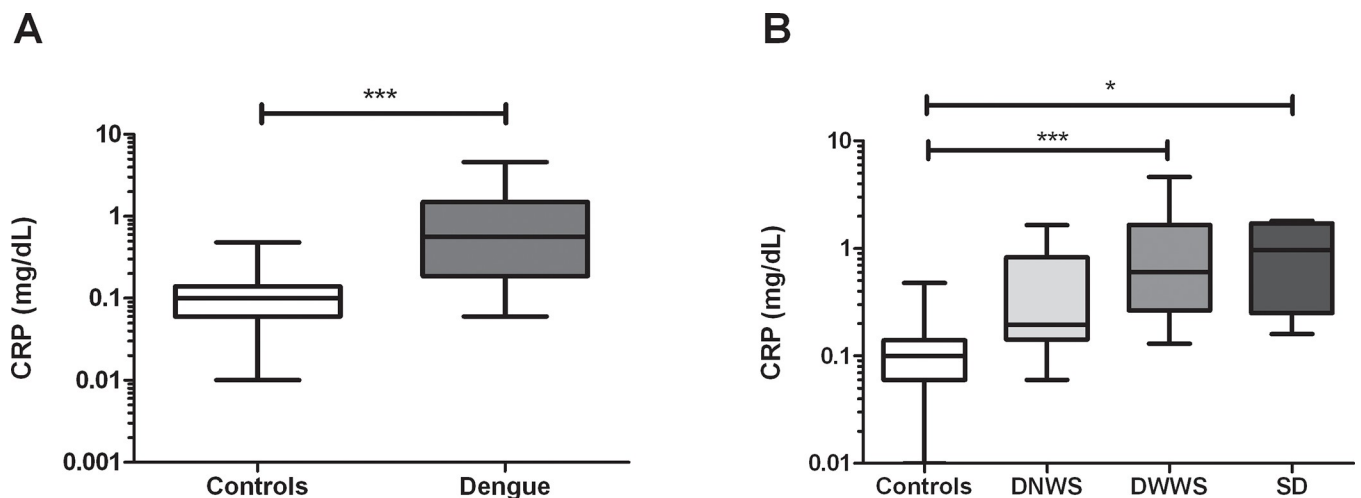


**Fig 5. Serum levels of IL-10 and IL-18 in dengue patients.** Serum levels of (A) IL-10 and (B) IL-18 were quantified by ELISA (in duplicates). The results are presented as medians with maximum and minimum values (box and whisker plot). The statistical comparison was made using the Mann Whitney U test with a confidence level of 95%. Significant differences are represented at the top of the figure (\*\* $p < 0.001$ ).

<https://doi.org/10.1371/journal.pone.0214245.g005>

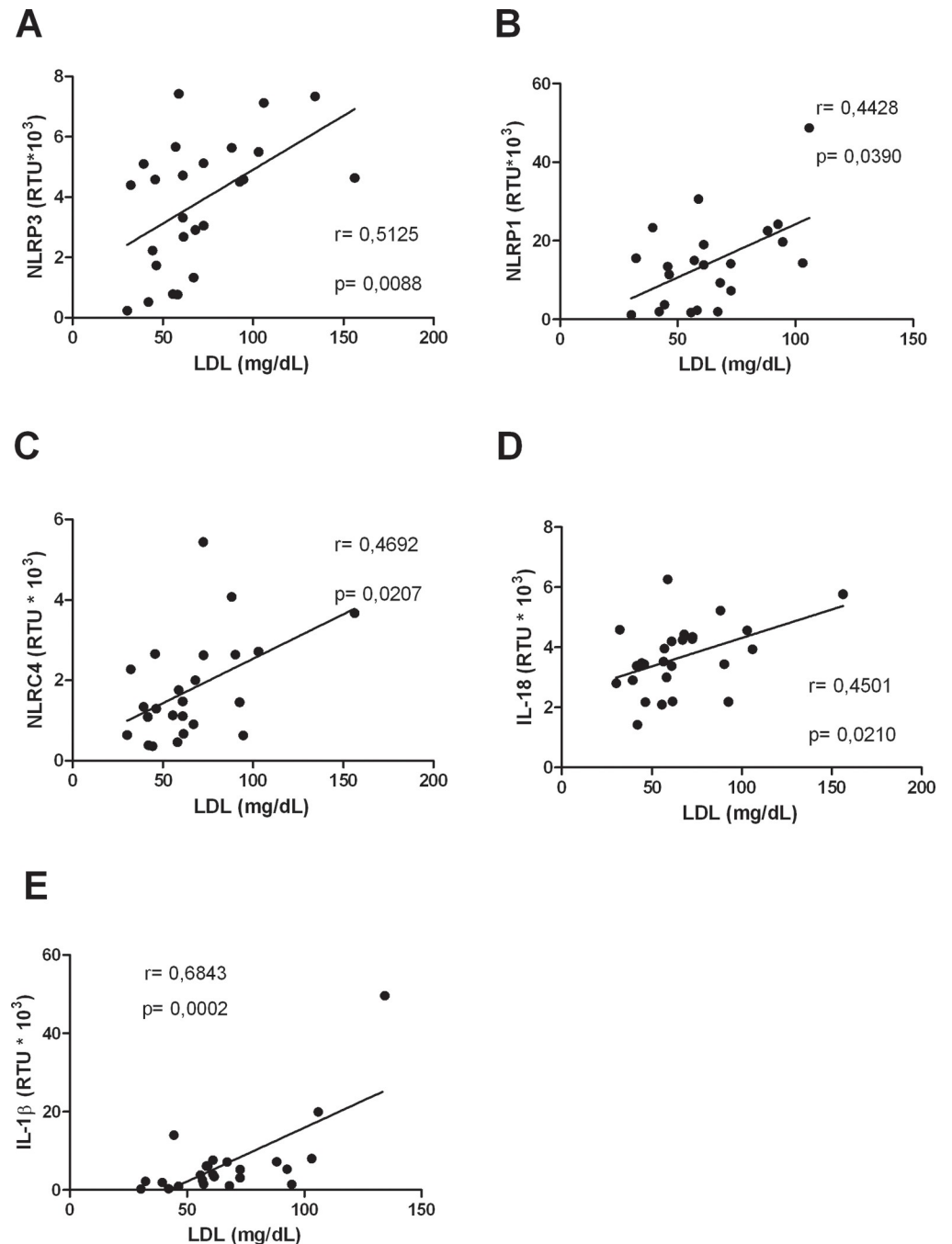
study by Biswas et al. [9] was longitudinal, on pediatric patients. In this sense, the difference in age could influence the lipid profile, and the behavior of the studied markers in response to dengue infection may be also different. Moreover, it is important to mention that although we did a cross sectional study, the lipoprotein levels appear to correlate with the clinical phase of the infection. Taken together these observations and the previous report of increased HDL in patients with SD [10, 25], this suggests that the relationship between lipid levels and clinical severity is not strictly linear, and that further studies (especially with longitudinal designs) are needed to clearly define the mechanistic relationship between serum lipids and dengue pathology, according to age and clinical phase.

Several studies provide context for understanding the participation of cholesterol-containing lipoproteins like HDL and LDL in DENV infection. Puerta-Guardo *et al.* report altering



**Fig 6. Serum C-reactive protein levels in dengue patients.** Serum levels of CRP were quantified in dengue patients (A) total dengue patients or (B) dengue patients according to the clinical stages and healthy controls, using a colorimetric assay. The results are presented as medians with maximum and minimum values (box and whisker plot). The statistical comparison was made using the Mann Whitney U and Kruskal-Wallis tests with a confidence level of 95% and post hoc tests (or multiple benchmarks) HDS of Dunn, were applied. Significant differences are represented at the top of the figure (\* $p < 0.05$  and \*\* $p < 0.001$ ).

<https://doi.org/10.1371/journal.pone.0214245.g006>



**Fig 7. LDL levels are correlated with inflammasome-related genes.** Correlation between LDL levels and (A) NLRP3, (B) NLRP1, (C) NLRP4, (D) IL-18 and (E) IL-1 $\beta$ . Correlations between variables were assessed using the Spearman test. The  $r$ -value and  $p$ -value are indicated in each figure. A  $p$ -value of less than 0.05 was considered a significant correlation.

<https://doi.org/10.1371/journal.pone.0214245.g007>

the formation of plasma membrane lipid rafts by eliminating cholesterol reduces DENV infection of host cells [31]. In addition, it has been reported that there is less production of dengue viral particles in cells treated with lovastatin, a drug that decreases the production of cholesterol [32]. Likewise, the elimination of cholesterol from the viral particles reduces their

infectivity by affecting the fusion with the endosomal membranes, thus blocking the replication cycle [33]. In light of these findings, it is interesting to consider that HDL has also been shown to modulate the cholesterol content of the lipid rafts, mainly through the ABCA1 and ABCG1 transporter proteins [34]. Considering that DWWS patients showed lower levels of HDL, it is interesting to speculate that the decrease in this lipoprotein during DENV infection could lead to reduced cholesterol flow in the lipid rafts; thus favoring a better production of DENV particles due to a higher level of cholesterol in the cells, and that this contributes to greater severity of the disease.

While serum lipids may be able to directly affect DENV virology, the interrelation of lipids with the activation and maintenance of inflammation is likely to also play a role in dengue pathogenesis. DENV infection induces temporal changes in the lipidome and serum metabolome, thus modulating the development and maintenance of inflammatory responses [35]. Previous *in vitro* studies have reported that DENV can promote the expression of IL-1 $\beta$  and IL-18 in macrophages through the activation of the NLRP3 inflammasome [23]. In addition, infection of platelets with DENV leads to the assembly of the NLRP3 inflammasome, activation of caspase-1, and the production of IL-1 $\beta$ , which is secreted in microparticles and can promote increased vascular permeability [24], a phenomenon associated with the severity of the disease. It has also been found that other arboviruses, such as Chikungunya and West Nile virus, have the ability to activate inflammasomes and induce the expression of AIM2 [36]. However, the present study found a decrease in some inflammasome components in patients with dengue, especially in DWWS patients. This may be due to the clinical stages of the patients (critical and convalescent), in which the inflammatory response could be negatively regulated. In this concern, it is important to note that an increase in IL-10 was found in dengue patients; this anti-inflammatory cytokine is produced late in infection [37], has immunosuppressive functions, and is associated with greater severity of infection [38, 39]. So, it is possible that inflammasome activation is established at the beginning of viral infection and strong production of pro-inflammatory cytokines contributes to immune-pathogenesis and clinical disease outcome. However, in the later phase of the infection, regulatory mechanisms (evidenced by IL-10) are activated and downregulate expression of inflammasome components, as observed in this study.

In corroboration of this logic, previous studies have reported that dengue patients show a decrease in IL-1 $\beta$  levels, which return to baseline levels after the recovery phase [40]. Moreover, this decrease in IL-1 $\beta$  is more significant in patients with hospitalization requirements compared to those who do not require it [41], suggesting that patients with lower IL-1 $\beta$  production present greater severity of the disease. It could be important to consider the clinical classification algorithm used in this study, in comparison with other reports using the previous classification strategies. The hypothesis that patients with DWWS have decreased levels of IL-1 $\beta$  could be considered, but given the lack of infection control, the levels increase again. This could explain our results showing that DWWS patients exhibited less expression of some inflammasome components. And also, the results reported by other authors were found increased expression of IL-1 $\beta$  in patients with severe dengue [21], as well as other pro-inflammatory cytokines [42, 43]. However, is necessary considering other differences in the study design, including the disease stage when the samples were taken. For this reason, it would be important to carry out longitudinal studies to evaluate the inflammasome activation and the production of cytokines during the different phases of the disease, in order to elucidate the production dynamics of these molecules and their association with the clinical outcomes.

While we found a decrease in the expression of inflammasome-related genes in dengue patients, we also found increased levels of the inflammatory marker CRP, and this was higher in DWWS patients, suggesting a residual inflammatory process. This is in accord with the

previously reported observation that patients with dengue who had plasma loss (a characteristic of severe disease) presented higher levels of CRP [44]. Taken together, these data suggest that the inflammatory response during dengue infection is complex, and that the balance and dynamics between pro-inflammatory molecules can promote the serious forms of the disease.

Although HDL can negatively modulate inflammasome activation [17], in the present study no correlations were found between HDL and the expression of inflammasome-related genes in dengue patients. However, a decrease in HDL levels may lead to an alteration in HDL's known anti-inflammatory activities, thus inducing an imbalance in the inflammatory response and consequently greater pathogenesis of the disease.

On the other hand, a positive correlation was found between LDL and some inflammasome components. Our findings indicated that dengue patients with higher levels of LDL also showed higher content of mRNA from inflammasome-related genes. This is noteworthy because oxidized LDL can promote the activation of the NLRP3 inflammasome [45]. In addition, DENV infection favors the cellular uptake of LDL [46], leading to an increase in intracellular cholesterol and subsequent formation of cholesterol crystals [47]; this, in turn, could be promoting the activation of the inflammasomes [48] and contributing to the inflammatory response involved in the resolution of the infection. It is possible that this relationship proceeds during the initial stage of the disease; nevertheless, continuous LDL uptake leads to decrease of this lipoprotein, as observed in the present study.

## Conclusion

The development of an effective immune response against infection by DENV is necessary for a favorable clinical outcome. However, the regulation of this response is equally fundamental to prevent tissue damage and severe disease symptoms. The results presented here indicate a relationship between serum lipoproteins and the inflammatory response during DENV infection. Specifically, the presented data support a relationship between LDL and inflammasome-related genes, which could modulate the dengue disease outcome. The present findings must be interpreted in the context of the time of disease progression in the studied patients, and suggest the need for longitudinal studies that will be capable of more completely assessing the dynamics of individual lipids and inflammation markers, as well as their correlations over the course of disease progression.

## Supporting information

**S1 Fig. Correlations between HDL and inflammasome-related genes.** Correlation between HDL levels and (A) NLRP3, (B) NLRP1, (C) NLRC4, (D) AIM2, (E) Caspase-1, (F) ASC, (G) IL-18 and (H) IL-1 $\beta$  were assessed by the Spearman test. The *r*-value and *p*-value are indicated in the figure. A *p*-value lower than 0.05 was considered a significant correlation. (TIF)

**S1 Table. Sequence of the primers.**  
(DOCX)

## Acknowledgments

The authors thank the donors who participated in this study and the collaboration of the personnel from the institutions where the patients were recruited: IPS -Universitaria (Clínica León XIII, Medellín, Colombia), IPS Virrey Solís and Dr. Carlos Julio Montes from Secretaria de Salud de Medellín and Gladys Osorio Pavas. We thank the technical research committee of the IPS Universitaria for its support for carrying out the research. We acknowledge the help of

Dr. Paula A. Velilla for her contributions to the design and analysis of the results for the research.

## Author Contributions

**Conceptualization:** Cherilyn M. Sirois, Juan C. Hernandez.

**Formal analysis:** Damariz Marin-Palma, Juan C. Hernandez.

**Funding acquisition:** Juan C. Hernandez.

**Investigation:** Damariz Marin-Palma, Juan C. Hernandez.

**Methodology:** Damariz Marin-Palma, Silvio Urcuqui-Inchima, Juan C. Hernandez.

**Project administration:** Juan C. Hernandez.

**Writing – original draft:** Damariz Marin-Palma, Cherilyn M. Sirois, Silvio Urcuqui-Inchima, Juan C. Hernandez.

**Writing – review & editing:** Cherilyn M. Sirois, Silvio Urcuqui-Inchima, Juan C. Hernandez.

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