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Evidence supporting correlation between aging and dengue severity: A retrospective analysis on the 2010 dengue outbreak in Brazil

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ABSTRACT

Objective: The aim of this study is to explore the role of aging and non-communicable diseases (NCDs) as potential risk factors for severe clinical manifestations of dengue. This investigation was conducted within a cohort of patients from the 2010 epidemic in Belo Horizonte, Brazil.

Methods: A total of 144 subjects diagnosed with dengue were categorized into two groups, representing the early and late (critical) stages of the disease. Further stratification was performed based on age, distinguishing between young and aged subjects. NCD subjects were also divided into young and aged groups. The symptomatology, previous clinical history, age, and presence of NCDs were inquired. Blood samples were collected for the analysis of platelet, soluble tumor necrosis factor receptor 1 (sTNFR1), and aspartate aminotransferase (AST) levels. The Mann-Whitney test was explored for the comparison of two groups, whereas the Kruskal-Wallis test was used for the analysis of differences among the multiple groups. Categorical variables were examined by *Chi*-square test. Correlation assessments were conducted using binary logic regression analysis with a 95% confidence interval. $P<0.05$ was considered statistically significant.

Results: Compared to the younger subjects with NCDs, aged subjects exhibited a more severe form of dengue during the critical phase. The presence of NCDs was not associated with a worse dengue outcome in younger individuals, suggesting that aging itself maybe an important contributor to the severity of dengue.

Conclusions: In the studied cohort, we emphasize that aging is a significant factor associated with severe manifestations of dengue. While we do not discard the potential role of NCDs in exacerbating disease progression, it may be more prominently impact the aged groups.

KEYWORDS: Dengue; Severity; Aging; Comorbidity; Non-communicable diseases

Significance

It is recognized that dengue often manifests with heightened severity, prolonged aftereffects, and increased mortality rates in elderly individuals. Non-communicable diseases (NCDs) are also acknowledged to exacerbate dengue severity. However, our research proposes that the correlation between age and the severity of dengue might be independent of the presence of NCDs, suggesting an intrinsic role of age in the severe manifestation of dengue.

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1. Introduction

Dengue is a mosquito-borne viral disease that currently lacks no specific pharmacological therapy. Infection can be caused by any of the four Dengue virus serotypes (DENV1-4), leading to a wide spectrum of clinical manifestations that range from asymptomatic to symptomatic dengue without warning signs (similar to other viral infections) or dengue with warning signs or severe dengue[1]. The severe dengue is characterized by plasma leakage and fluid accumulation with respiratory distress, severe bleeding and organ involvement, including the liver, central nervous system, heart and other organs resulting in the death of the affected individuals[2–4].

The severe of the disease is associated with virus characteristics, including serotype and genotype, as well as host factors such as immune and genetic status and age[5–7]. Previous findings have shown a significant correlation between age and mortality among infected patients, as compared to those who survived. Indeed, aged patients have a higher risk of hospitalization and development of signs such as coma, prostration, hepatitis (AST >100 U/L) and renal failure (serum creatinine >2 mg/dL)[8–10]. Although, advanced age was associated with an increased prevalence of comorbidities that can aggravate the disease; age itself has been shown as an independent risk factor for severe dengue[11–13].

Then we inquired whether aging and the presence of non-communicable diseases (NCDs) such as Systemic Arterial Hypertension (SAH), Diabetes Mellitus (DM), asthma, or risk factors such as smoking, which were highly prevalent in the aged population, might contribute to the severity of dengue disease in older individuals. We revisited the data from a cohort study of individuals diagnosed with dengue fever in Belo Horizonte in the 2010 epidemic from Ferraz and coworkers[14] and correlated the clinical manifestations presented by these individuals with their age and the presence of NCDs.

2. Methods

2.1. Ethical considerations

This study was approved by the Research Ethics Committees of the Municipal Health Department of Belo Horizonte, Brazil, under the number 0009.0.410.203-09A.

2.2. Study design

The primary objective of this study was to assess the impact of aging on the severity of dengue. Subsequently, we also evaluated the effects of NCDs [Systemic Arterial Hypertension (SAH), Diabetes Mellitus (DM) or Asthma] on dengue severity. The parameters used in this work as markers for more severe dengue included thrombocytopenia (150/mm^3 of blood) followed by increased levels of Aspartate aminotransferase (AST) exceeding 50 U/L (units per liter) or occurrence of bleeding.

Two Basic Health Units (BHUs) were selected in Belo Horizonte, Brazil, and subjects with acute clinical suspicion of dengue fever were invited to participate in this study. Volunteers were included in the study between January 18 and March 30, 2010 (during the period between the 3rd and 13th epidemiological weeks). The individuals included were those who were at least 18 years old and weighed 50 kg or more. Excluded from the study were pregnant women, patients taking immunosuppressive drugs, and those whose hemodynamic conditions that would not allow for the collection of the previously stipulated amount of blood, including those who are severe anemic or in severe shock requiring vasoactive amines or experiencing hypertensive crisis. After obtaining informed consent for participation in the research, volunteers were requested to fill up a questionnaire about their symptomatology, previous clinical history, and comorbidities.

Blood samples (12 mL) were collected for the diagnosis of dengue infection and platelets quantification, and serum was obtained for the analysis of soluble tumor necrosis factor receptor 1 (sTNFR1) and AST levels. Complete epidemiological data of the individuals enrolled in the study and laboratory methods used for the diagnosis of dengue infection [RT-PCR, capture-ELISA for IgM anti-dengue (MAC-ELISA-PanBio), and NS1-detection by Platelia TM Dengue NS1 AG (Biorad) immunoassay] have been described in a previous work[14].

The sampling consisted of 144 subjects with a confirmed diagnosis of dengue who were divided into two groups based on age: young (under 55 years old) and aged (55 years old or older). Each group was further caregorized early and late phases of dengue. In prior reaearch documented by the United Nations, individuals aged over 55 years (pre-elderly) and those over 60 years (elderly) exhibited analogous clinical presentations of dengue[15]. In this study, we classified individuals aged 55 years and above as part of the older age cohort. To investigate the effects of NCDs on the manifestation of dengue, all groups were further subdivided into individuals with NCDs (positive) and without NCDs (negative). Subjects with risk factor for NCDs (smoking) according to Forouzanfar *et al*.[16], were also included in NCD groups.

2.3. Extraction of viral RNA

Viral RNA was extracted from 140 μL of serum using a QIAGEN QIAamp Viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. cDNA was synthesized using 400 ng

of extracted viral RNA, 5U/μL of MMLV Reverse Transcriptase in standard buffer (Promega, Madison, Wis), and the reverse primer (5' GGGTCTCCTCTAACCTCTAGTCCT 3') at 42 °C for 2 h. Real-time PCR reactions were performed according to Ferraz and coworkers[14].

2.4. Measurement of sTNFR1 concentrations

sTNFR1 concentrations in serum was measured by ELISA using commercially available antibodies and according to the procedures supplied by the manufacturer (R&D Systems, Minneapolis). Serum samples were analyzed at a 1:2 dilution in Phosphate-Buffered Saline (PBS). The detection limit of the ELISA assays was in the range of 4-8 pg/mL.

2.5. Evaluation of platelet levels

Platelets were counted in a Neubauer chamber. Briefly, 10 μL of solution (blood and ammonium oxalate 1% at a 1:100 dilution) were placed in the chamber, and the platelets were visualized in a Nikon XP-1000 microscope, 400X magnification, using phase contrast. The results are presented as the number of platelets per μL of blood.

2.6. Quantification of AST levels

Transaminase AST activity was measured in individual serum samples, using a commercially available colorimetric kit (Bioclin, Quibasa, Belo Horizonte, Brazil). Results were expressed as mean ± standard error of the mean of transaminase concentration in U/L of serum.

2.7. Statistical analysis

Data analysis was performed utilizing GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). The data were expressed as the median and interquartile range (IQR). Certain results were depicted as percentages or as mean±standard error. The Mann-Whitney test was applied to determine the statistical significance of differences between the means of two independent groups. The Kruskal-Wallis test was applied to evaluate the differences across the median ranks of multiple independent cohorts. The analysis of categorical variables was conducted using the *Chi*-square test. Binary logistic regression was employed to evaluate the likelihood of severe dengue under different conditions, with the findings being reported as Odds Ratio (*OR)* value accompanied by 95% confidence interval (*CI)*. Results with $P<0.05$ were considered significant.

3. Results

To fulfill our primary aim of assessing the influence of aging on dengue severity, we investigated several parameters including the prevalence of severe dengue (as defined by the criteria established for this research), clinical manifestations, the presence of a positive tourniquet test, and biomarkers indicative of dengue injury/severity (such as platelet count, AST levels, and sTNFR1) in both younger and older cohorts across the early and critical stages of the disease.

The demographic and clinical characteristics observed during the dengue infection were summarized in Table 1. During the early symptom phase, the average age of individuals in the young group was 34 years, while it was 63 years in the old group, with both groups comprising 65% females. The mean duration of symptoms at this stage was approximately 2 days for both age groups. In the late phase of symptomatology, patients in the young group had an average age of 40 years, in contrast to 64 years in the older cohort, with a higher female representation of 88% and 90%, respectively. The duration of symptoms extended to about 6-7 days for both groups at this stage.

In the evaluation of early and late phases of dengue, no statistically significant differences were observed between the young and old groups regarding the incidence of exanthema (early phase: 39% *vs*. 25%, $P>0.05$; late phase: 50% in both groups, $P>0.999$), the incidence of bleeding (early phase: 10% *vs*. 8%, $P>0.05$; late phase: 12.5% *vs*. 30%, $P>0.05$), and tourniquet test positivity (early phase: 59% *vs*. 50%, $P>0.05$; late phase: 75% *vs*. 90%, $P>0.05$). However, a significant difference was observed in the frequency of severe dengue during the late phase, with a higher prevalence in the old group compared to the young group (50% *vs*. 6%, $P<0.05$).

Table 1 also showed the biomarkers indicative of disease injury/severity. During the early phase, both the young and old groups exhibited comparable thrombocytopenia, with platelet counts falling below the threshold of $150 \times 103/mm^3$ of blood (120×10^3 platelets/mm^3 *vs*. 115×10^3 platelets/mm^3, $P>0.05$). However, during the late phase, the young group presented higher platelet counts when compared to the old group (140×10^3 platelets/mm^3 *vs*. 112.5×10^3 platelets/mm^3, $P<0.05$). The levels of AST in serum did not show significant differences among all groups (between 23.0 and 36.5 U/L, $P>0.05$). Additionally, in both early and late phases of dengue, the older group exhibited higher sTNFR1 compared to the young group (early phase: 1 021.0 pg/mL *vs*. 839.3 pg/mL, $P<0.05$; late phase: 1 346.0 pg/mL *vs*. 814.9 pg/mL, $P<0.05$).

Table 1. Disease parameters in the young (<55) and old (≥55) groups during the early and late phases of dengue.

	Early				Late			
Variables	Young (*n*=78)	Old (*n*=40)	χ^2/U	*P*	Young (*n*=16)	Old (*n*=10)	χ^2/U	*P*
Exanthema [*n* (%)]	30 (38)	10 (25)	2.138	0.144	8 (50)	5 (50)	0	>0.999
Bleeding [*n* (%)]	8 (10)	3 (8)	0.238	0.626	2 (12.5)	3 (30)	1.213	0.271
Positive torniquet test [*n* (%)]	46 (59)	20 (50)	0.864	0.353	12 (75)	9 (90)	0.891	0.345
Severe disease [*n* (%)]	6 (8)	4 (10)	0.182	0.670	1 (6)	5 (50)	6.640	**0.010**
Platelet count (median, IQR)	120.0, 60.0	115.0, 57.5	1422	0.435	140.0, 75.0	112.5, 38.0	30.5	**0.008**
AST (median, IQR)	28.2, 26.3	26.9, 20.8	1471	0.615	36.5, 29.7	23.0, 22.2	48.0	0.095
sTNFR1 (median, IQR)	839.3, 401.1	1021.0, 588.7	1200	0.040*	814.9, 486.3	1346.0, 773.4	34.0	**0.014**

P<0.05 are indicated by bold; AST: Aspartate aminotransferase; sTNFR1: Soluble tumor necrosis factor receptor 1.

Table 2. Evaluattion of age-associated risk for severe dengue across early and late stages.

Phases of dengue	*n*	Odds Ratio (95% *CI*)	*P*
Old age *vs*. young age			
Early	118	1.01 (0.97-1.05)	0.752
Late	26	1.11 (1.03-1.23)	**0.016**

P<0.05 are indicated by bold.

As shown in Table 2, There is a correlation between age and severe dengue during the late phase of the disease, with a statistically significant (*OR* 1.11; 95% *CI* 1.03-1.23; *P*<0.05). Conversely, the correlation was not evident in the early stage (*OR* 1.01; 95% *CI* 0.97-1.05; *P*>0.05).

As NCDs were prevalent in the samples (59% of all participants), our secondary objective was to assess the effects of NCD on the severity of dengue. For that, we analyzed the same parameters in the cohort according to their positive (+) or negative (-) NCD status during both the early and late phases of dengue. As shown in Table 3, during the early phase, in the positive (+) NCD group, the mean age of the subjects was 49 years with a female majority 64%. In the negative (-) group, the mean age was 36 years with a female majority of 68%. The average duration of symptoms was about 2 days in both groups. During the late phase, the mean age in the positive (+) group inceased to 56 years, with a female majority of 88%. The negative (-) group had a mean age of 37 years and a female majority of 89%. The average duration of symptoms was about 7 days in both groups.

No significant differences were observed when comparing subjects from the (+) and (-) NCD groups regarding the prevalence of exanthema (early stage: 28% *vs*. 42%, *P*>0.05; late stage: 53% *vs*. 44%, *P*>0.05), incidence of bleeding (early stage: 7% *vs*. 12%, *P*>0.05; late stage: 18% *vs*. 22%, *P*>0.05), torniquet test positivity (early stage: 53% *vs*. 62%, *P*>0.05; late stage: 88% *vs*. 67%, *P*>0.05) and frequency of severe disease (early stage: 4% *vs*. 16%, *P*<0.05; late stage: 29% *vs*. 11%, *P*>0.05).

Concerning the biomarkers indicative of disease injury/severity. During early dengue, both the (+) and (-) NCD groups exhibited comparable platelet counts ($122.5 \times 10^3/mm^3$ *vs*.$115 \times 10^3/mm^3$, *P*>0.05). However, in the late stage, the (+) NCD group showed decreased platelet counts compared to the (-) NCD group ($115 \times 10^3/mm^3$ and $140 \times 10^3/mm^3$, *P*<0.05). It is important to consider that most subjects within the (+) NCD group in the late phase of dengue were classified as "old". There was no significant differences in AST (early stage: 26.5 U/mL *vs*. 28.6 U/mL, *P*>0.05; late stage: 39.1 U/mL *vs*. 50.0 U/mL, respectively, *P*>0.05) and sTNFR1 levels (early stage: 899.6 pg/mL *vs*. 837.4 pg/mL, *P*>0.05; late stage: 1060 pg/mL *vs*. 623.5 pg/mL, *P*>0.05) when comparing subjects from the (+) and (-) NCD groups in both the early and late stages of dengue.

The study findings indicate no significant correlation between the severity of dengue and the presence of NCDs during both early and late stages, as detailed in Table 4. These results underscore the predominance of age as a determinantof dengue severity within the cohort, especially in the critical stage of the illness.

Finally, to assess the independent effects of NCDs and age on dengue outcomes, we categorized the subjects based on their NCD status (+ or -) and age group (young or old) and the parameters of the disease were analyzed. The demographic characteristics followed a consistent pattern, as previously presented in Table 1, with further details provided in Table 5, which also included the clinical and laboratory features of the groups. No significant differences in all parameters analyzed were observed when comparing the young or old, (+) and (-) NCD groups during the early stage of dengue, except for the incidence of bleeding. It was higher in the young (-) NCD group compared to the young (+) NCD group (*P*<0.05), suggesting an anomaly potencially attributable to sample heterogenicity, as no other significant changes in parameters were observed in this group during this phase.

Table 3. Disease parameters in the (+) and (-) NCD groups during the early and late phases of dengue.

	Early				Late			
Variables	(+) NCDs (*n*=68)	(-) NCDs (*n*=50)	χ^2/U	*P*	(+) NCDs (*n*=17)	(-) NCDs (*n*=9)	χ^2/U	*P*
Exanthema [*n* (%)]	19 (28)	21 (42)	2.542	0.111	9 (53)	4 (44)	0.167	0.680
Bleeding [*n* (%)]	5 (7)	6 (12)	0.736	0.391	3 (18)	2 (22)	0.079	0.778
Positive torniquet test [*n* (%)]	36 (53)	31 (62)	0.964	0.326	15 (88)	6 (67)	1.762	0.184
Severe disease [*n* (%)]	3 (4)	8 (16)	4.577	**0.032**	5 (29)	1 (11)	1.110	0.292
Platelet count (median, IQR)	122.5, 68.8	115.0, 45.0	1598	0.579	115.0, 45.0	140.0, 73.5	33	**0.017**
AST (median, IQR)	26.5, 19.2	28.6, 31.9	1577	0.503	39.1, 32.4	50.0, 37.4	70	0.741
sTNFR1 (median, IQR)	899.6, 431.9	837.3, 460.5	1647	0.775	1060.0, 749.0	623.5, 610.7	45	0.093

*P<0.05 are indicated by bold; AST: Aspartate aminotransferase; sTNFR1: Soluble tumor necrosis factor receptor 1.

Table 4. Evaluation of the risk of severe dengue with or without NCDs across early and late stages.

Phases	*n*	Odds Ratio (95% *CI*)	*P*
Old age *vs*. young age			
Early (+) NCDs	68	0.94 (0.85-1.02)	0.173
Early (-) NCDs	50	1.03 (0.98-1.08)	0.281
Late (+) NCDs	17	1.15 (1.01-1.40)	0.080
Late (-) NCDs	9	1.01 (0.97-1.05)	0.752

However, during the late stage, severe dengue was absent in the young (+) NCD group, whereas 50% of old (+) NCD group experienced severe dengue, showing a significant difference between the groups (P<0.05). Regarding the biomarkers indicative of disease injury/severity, old (+) NCD group exhibited lower platelets counts when compared to the young (+) NCD group (130.0 x 10^3/mm^3 *vs*. 112.5 × 10^3/mm^3, P<0.05), as well as lower than young (-) NCD group (140.0 × 10^3/mm^3 *vs*. 112.5 × 10^3/mm^3, P<0.05). The findings implied that within our cohort, NCDs might not exect the same impact on exacerbation of dengue manifestation during the critical phase of illness as age does.

4. Discussion

A number of studies have pointed out that dengue has more severe manifestations[17, 18], prolonged symptomatology[13, 19–21], and higher mortality[18, 22, 23] in old individuals. It is known that NCDs are associated with the more severe manifestation of dengue. However, this study indicated that the findings might have greater significance in the old population. Our results showed that in the 2010 dengue epidemic in Belo Horizonte, Brazil, old individuals had a more severe disease manifestations, longer duration of symptoms, and worse disease parameters compared to young counterparts,

Table 5. Disease parameters in the young and old groups categorized by the presence or absence of NCDs during the early and late phases of dengue.

Variables	Early						Late				
	Young		Old				Young		Old		
	(+) NCDs (*n*=37)	(-) NCDs (*n*=41)	(+) NCDs (*n*=31)	(-) NCDs (*n*=9)	χ^2/H	*P*	(+) NCDs (*n*=7)	(-) NCDs (*n*=9))	(+) NCDs (*n*=10)	χ^2/H	*P*
Exanthema [*n* (%)]	11 (30)	19 (46)	8 (26)	2 (22)	4.573	0.206	4 (57)	4 (44)	5 (50)	0.254	0.081
Bleeding [*n* (%)]	3 (8)	17 (41)	2 (6)	1 (11)	19.430	**0.001**	0	2 (22)	3 (30)	2.465	0.292
Positive torniquet test [*n* (%)]	18 (49)	28 (68)	18 (58)	3 (33)	5.248	0.154	6 (86)	6 (67)	9 (90)	1.811	0.404
Severe disease [*n* (%)]	3 (8)	6 (15)	2 (6)	2 (22)	2.678	0.444	0	1 (11)	7 (50)	6.909	**0.032**
Platelet count (median, IQR)	130.0, 77.5	115.0, 45.0	115.0, 65.0	100.0, 37.5	1.448	0.694	130.0, 55.0	140.0, 73.5	112.5, 38.8	7.903	**0.019**
AST (median, IQR)	28.6, 22.6	26.9, 34.0	25.2, 13.7	32.1, 57.4	1.567	0.667	22.2, 20.2	50.4, 33.0	48.6, 42.8	4.372	0.112
sTNFR1 (median, IQR)	835.7, 381.5	842.8, 473.3	1060.0, 559.3	824.0, 887.0	4.550	0.208	814.9, 598.1	799.3, 773.5	1346.0, 773.4	4.244	0.120

P<0.05 are indicated by bold; AST: Aspartate aminotransferase; sTNFR1: Soluble tumor necrosis factor receptor 1.

with a persistent reduction in platelets and increased levels of an important inflammatory marker of disease severity (sTNRF1) during the late phase of illness. Our study has demonstrated that the likelihood of developing severe dengue escalates by 10.9% with each additional year of age, as reflected by *OR* value, specifically in the critical stage of dengue. These results underscore the importance for healthcare providers to recognize the cumulative impact of age on dengue severity, emphasizing the need for age-stratified clinical assessments and interventions.

It is important to note that the AST levels did not exceed 51 U/L in any of the groups in this study. Severe dengue forms are typically characterized by higher AST levels. It is important to point out that the subjects in this study came from outpatient clinics rather than hospitalization, with a maximum duration of 12 days for symptom manifestation. Furthermore, as previously reported by our group[14], the 2010 dengue epidemic outbreak in Belo Horizonte was predominantly due to the DENV-1 serotype. This serotype has been usually associated with milder dengue manifestations in contrast of DENV-2, 3, and 4 serotypes which have been linked to more severe dengue manifestations in several tropical outbreaks[24–29]. Therefore, in the context of this study, platelet counts below $150 \times 10^3/mm^3$ of blood, accompanied by AST levels ≥ 50 U/mL of serum, or the presence of bleeding, were considered as indicator of severe dengue.

When participants were categorized based on the presence or absence of NCDs regardless of age, the differences between the groups were no longer observed, except for the decreased platelet counts detected in the (+) NCD group during the late phase of dengue. However, it is important to consider that approximately 60% of the subjects in this group were aged over 55 years, underscoring the pivotal role of age for severe dengue manifestation. Therefore, most of the analyzed parameters showed no statistical difference when young individuals with and without NCDs were compared during the critical phase of dengue. Additionally, a higher prevalence of bleeding during the early phase and elevated AST levels during the late phase of dengue were observed in younger individuals without NCDs. However, we do not dismiss the possibility that additional conditions, such as alcoholism or the presence of other pathologies, might be contributing to the observed changes in certain parameters within this population.

Some reasons might explain the high incidence of severe dengue in older people in our data. First of all, it is known that the aging process negatively affects the function of the immune system[30]. High plasma concentrations of IL-6, IL-1β, and TNF have been described in older adults and postulated as predictive markers of functional disability, frailty, and mortality in this population[31–33]. It is believed that these factors contribute to continuous stimulation of the immune system throughout life, resulting in a subclinical inflammatory state defined as "inflammation-aging"[34]. Furthermore, production of cytokines by T-cells in response to DENV infection is impaired in older people, a phenomenon referred to as immunosenescence[30]. As individuals age, thymic involution occurs, leading to a drastic reduction in the number of naïve T-cells leaving the thymus, a process that begins early in life and is almost complete after the age of 50[35, 36]. Additionally, older adults' virgin T-cells have numerous functional defects, including significantly shorter telomeres, decreased number of T cell receptors, reduced interleukin IL-2 production, and impaired expansion and differentiation in effector cells when compared to young naïve cells[36]. Thus, their ability to mediate effective immune responses against new antigens is usually decreased.

NCDs such as diabetes and SAH have also been proposed to predict more severe manifestations of dengue[37–40]. In severe dengue infections, there is typically a upregulated synthesis of pro-inflammatory cytokines, which contributed to tissue injury and septic shock[40]. However, while it is reasonable to suggest this synergy between chronic conditions and immune system alterations caused by severe DENV infection, this etiological relationship has not yet been fully elucidated. In our study, we observed a direct correlation between the presence of NCDs and advanced age with severity of dengue during the late phase. This is the period when those who develop severe disease will become critically ill. However, NCDs were not associated with a worse evolution of DENV infection in young individuals during this phase of illness. It's important to consider that not only subjects with diabetes or SAH were included in the (+) NCD group, but also individuals with asthma and those with risk factors for several NCDs (such as smoking) were included in this group.

We noted that NCDs does not contributed to the risk of severe dengue. Older individuals, who commonly present with NCDs exhibited a worse evolution of dengue during the critical phase, but this is not strictly associated with DENV infection. A clear example of this is the higher morbidity and mortality rates observed in older adults affected by COVID-19[41, 42]. Respiratory syncytial virus (RSV) and influenza virus (IVs) infections cause more severe respiratory illness with complications and mortality in older adults[10, 43]. Corroborating these findings, our data consistently indicate that aging paly a more significant role than the presence of NCDs in contributing the severity of dengue manifestation in the studied cohort. Indeed, including a control group of elderly individuals without NCDs would be provide a valuable comparative baseline to further substantiate these findings. Additinally, creating subgroups of subjects each with a NCD, such as SAH or diabetes could offer more insights into how each condition indepently affects dengue severity. However, the absence of subjects who met these criteria constituted

a limitation in our study. Future research will aim to address this gap by recruiting a more diverse sample that includes the specific subpopulations.

5. Conclusions

The strategy of analyzing data from previous studies has greatly contributed to investigating host factors such as age and comorbidities. In this context, this study raised the hypothesis that the correlation between age and a more severe manifestation of dengue appears to be independent of the presence of NCDs by themselves (at least in a prevalent DENV-1 epidemic such as occurred in Belo Horizonte in 2010). Nonetheless, to substantiate the notion that age acts as an independent risk factor for severe dengue, further research necessitate a more expansive cohort and and enhanced statistical robustness.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Ávila TV, Teixeira MM and Souza DdG concepted and designed of the study. Ferraz FO acquired the data. Ávila TV, Ferraz FO and Nunes LV analyzed and interpretated the data. Ávila TV, Nunes LV, Alessandri AL, Souza Passos IB drafted the article. Ávila TV, Costa VV, Teixeira MM and Souza DdG contributed to the final version to be submitted. Teixeira MM and Souza DdG supervised the project. All the authors have read and approved the manuscript.

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