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Observational study of clinical, epidemiological, and laboratory characteristics of pediatric patients with dengue in the city of Córdoba

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ABSTRACT

Introduction. Dengue has been the most widespread mosquito-borne disease worldwide in recent years. It develops with a broad spectrum of clinical manifestations and sometimes progresses to a critical condition known as severe dengue. It is managed with supportive treatment.

Available information about its clinical, epidemiological, and laboratory characteristics in the pediatric population is limited.

Objective. To describe the clinical, epidemiological, and laboratory characteristics of dengue.

Population and methods. Descriptive, observational, and retrospective study. It included patients aged 1 to 180 months seen due to probable or confirmed dengue at a children's hospital between 1/1/2020 and 5/31/2020.

Results. A total of 85 patients with positive microbiological or clinical-epidemiological criteria were included. Of these, 25 (29%) were confirmed by RT-PCR; all corresponded to DENV-1 serotype. Patients' median age was 108 months (interquartile range: 84–144). The main clinical manifestations were fever, headache, and myalgia. The most important laboratory findings were leukopenia, thrombocytopenia, and high transaminase levels.

Conclusion. The recognition and understanding of clinical and laboratory alterations that occur during dengue disease may allow an effective approach and help to reduce the more severe clinical form in children.

Keywords: *dengue; pediatrics; vector-borne diseases.*

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INTRODUCTION

Dengue has been the most widespread mosquito-borne viral disease worldwide in the past decades.[1,2] Each year, between 100 and 400 million infections occur, and almost half of the worldwide population is at risk of having dengue.[1] In Argentina, dengue is an epidemic disease, with cases occurring in the warmer months, closely related to outbreaks in neighboring countries.[3]

Initially, the symptoms of infection may manifest after an incubation period of 5–7 days; sometimes dengue progresses to a critical condition known as severe dengue.[1–3] The clinical suspicion of dengue is based on a non-specific febrile syndrome for less than 7 days, absence of upper airway involvement, and 2 or more of the following signs: headache and/or retro-orbital pain, malaise, myalgia, arthralgia, diarrhea, vomiting, loss of appetite and nausea, rash, petechiae or a positive tourniquet test, leukopenia, thrombocytopenia, and high transaminase levels.[3–12] The diagnosis is confirmed by virological and serological tests or based on clinical manifestations and epidemiological link in a proven outbreak of dengue.[3]

Since there is no specific treatment, supportive therapies are used to improve symptoms.[11] Therefore, it is important to closely monitor the disease to establish the correct treatment and prevent progression to more severe clinical forms.[1–3,11]

Unfortunately, available information about its clinical, epidemiological, and laboratory characteristics in the pediatric population is limited. For this reason, the objective of this study was to provide statistical data to extend the knowledge base.

OBJECTIVES

- To describe the clinical, epidemiological, and laboratory characteristics of dengue in pediatric patients.
- To analyze the course of biochemical parameters during the different phases of dengue.

POPULATION AND METHODS

Design

Descriptive, observational, and retrospective study.

Population

All patients aged 1 to 180 months with probable or confirmed dengue seen at Hospital de Niños de la Santísima Trinidad of Córdoba between 1/1/2020 and 5/31/2020 were included.

Probable cases were patients positive for NS1 antigen and/or immunoglobulin M specific for dengue (anti-DENV IgM), while confirmed cases were those with a positive reverse transcription-polymerase chain reaction (RT-PCR) for DENV or based on clinical signs and epidemiological link in the setting of a dengue outbreak.

Study procedures

Data were collected retrospectively from the non-specific febrile syndrome notification form and the laboratory database. Patients received follow-up until discharge, and the information about patients' clinical course was collected from the hospital's outpatient medicine records.

Variables

The following data were obtained: age, sex, days from symptom onset until medical visit, epidemiological week (EW), geographic location, clinical manifestations (fever, headache, myalgia, arthralgia, retro-orbital pain, rash, vomiting, diarrhea, abdominal pain, pruritus, conjunctival injection, and other symptoms), and laboratory parameters such as hematocrit, total leukocyte count (TLC), differential leukocyte count, platelets, erythrocyte sedimentation rate (ESR), prothrombin time (PT), activated partial thromboplastin time (aPTT), urea, creatinine, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), and alkaline phosphatase (AP).

Techniques used for sample analysis

Cell counts were obtained using Sysmex XT-1800, XT-2000i, and XS-1000 hematology analyzers; the differential leukocyte count was obtained by light microscopy.

PT and aPTT were measured using STA Compact Max2 and STart4 analyzers with a coagulometric method.

Urea, creatinine, CRP, AST, ALT, GGT, and AP levels were measured using the Roche Diagnostics Cobas 6000 autoanalyzer, which uses enzymatic, kinetic, and colorimetric methods to quantify the analytes.

An enzyme-linked immunosorbent assay (ELISA) and immunochromatography were done to detect the NS1 antigen and anti-DENV

IgM. Virus isolation and/or viral genome detection was performed by RT-PCR. These measurements were performed by the Central Laboratory of the province of Córdoba.

Ethical considerations

This study was approved by the Scientific Commission of Hospital de Niños in accordance with Provision 40 of the Health Research Ethics Review Board of the Ministry of Health of the province of Córdoba.

Statistical analysis

The median and interquartile range (IQR) were estimated to describe quantitative variables, whereas percentage and frequency of cases were used for qualitative variables.

Data corresponding to variations in laboratory parameters were grouped into the 3 phases of dengue: febrile, critical, and convalescent.

The results obtained between days 0 and 3 after the onset of symptoms correspond to the febrile phase; those obtained between days 4 and 7, to the critical phase; and those obtained from days 8 to 10, to the convalescent phase. Parametric tests (ANOVA) and non-parametric tests (Kruskal-Wallis) were used for analysis based on data distribution. The level of significance was established at 0.05.

Analysis was done using the InfoStat version 2020 statistical software.

RESULTS

During the study period, 85 patients met positive microbiological or clinical-epidemiological criteria for dengue. Of these, 25 cases (29%) were confirmed by RT-PCR, all DENV-1 serotype; 39 (46%), by clinical signs and epidemiological link; and 21 (25%) were considered probable cases.

Patients' median age was 108 months (IQR: 84–144). Male cases prevailed (n = 46, 54%).

Between EWs 11 and 15, 72 cases (85%) were diagnosed, with peaks between EWs 11 and 13, and a reduction as of EW 16 (*Figure 1*).

In relation to geographic location, all patients lived in the capital city of Córdoba; with 43 (51%) living in the southwest and west areas of the city.

Out of the 85 patients, 46 (54%) sought medical care in the 2 days after symptom onset. The main clinical manifestations were fever, headache, and myalgia, followed by abdominal pain and rash (*Figure 2*).

The most common laboratory finding was leukopenia (white blood cells < 4 × 10^9/L), which was observed in 29/78 cases (37%). Alterations in the differential leukocyte count included relative monocytosis in 54/77 (70%), presence of reactive lymphocytes (RL) in 24/77 (31%), and relative lymphocytosis in 19/77 (25%).

Among patients with neutropenia (n = 18/77, 23%), 9/18 developed moderate neutropenia (neutrophils < 1 × 10^9/L); 7/18, mild neutropenia (neutrophils < 1.5 × 10^9/L); and 2/18, severe neutropenia (neutrophils < 0.5 × 10^9/L).

Thrombocytopenia was observed in 19/78 patients (24%) and was the second most common finding. Of these, 16/19 had mild thrombocytopenia (platelets < 150 × 10^9/L) and 3/19, moderate thrombocytopenia (platelets < 100 × 10^9/L).

In relation to transaminases, increased AST

Figure 1. Distribution of dengue cases by epidemiological week (n = 85)

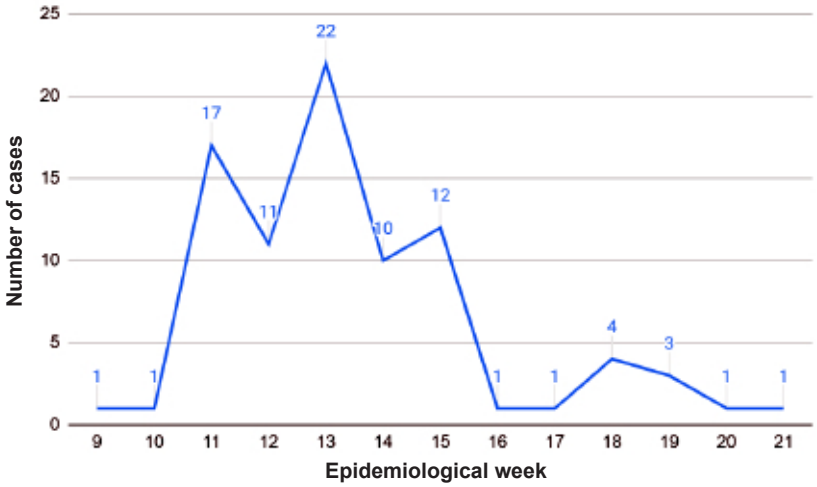
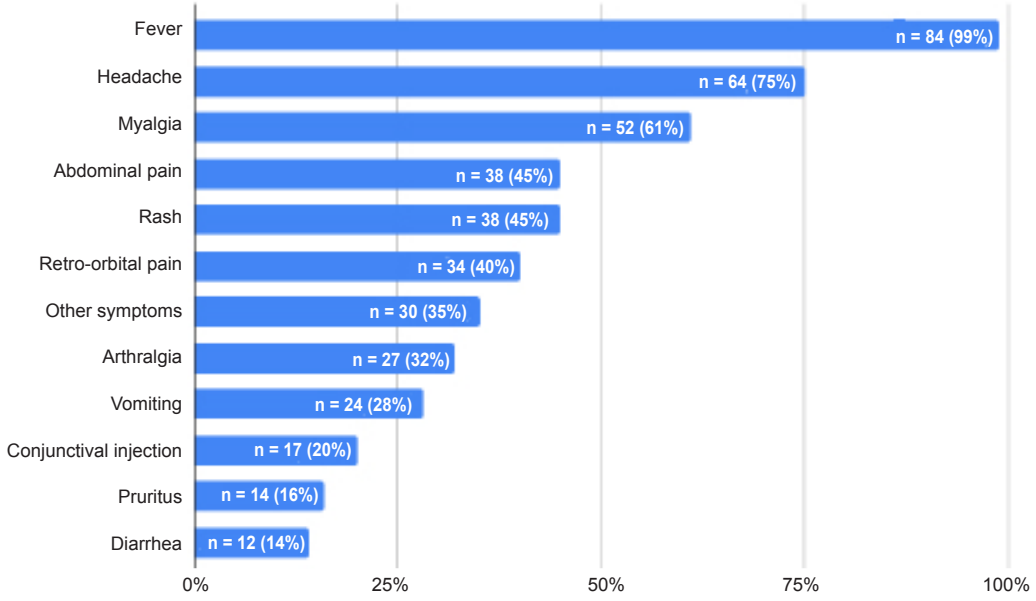


Figure 2. Clinical manifestations of patients with dengue (n = 85)



levels were observed in 16/69 cases (23%) and increased ALT levels, in 13/73 cases (18%), which was the third most important finding.

Only 3/78 study patients (4%) showed high hematocrit levels. No patient had an increase above 10% from baseline.

Supplementary tests found that 5/9 patients had a prolonged aPTT and 4/9, a prolonged PT. In addition, 32/60 cases (53%) in whom CRP was measured had values above 5 mg/dL, and 8/38 (21%) had ESR values above 15 mm/h.

Both AP and GGT levels were high in 4/46 cases (9%) and 2/21 cases (10%), respectively.

Only 3/60 patients (5%) had high creatinine levels. No patient had alterations in urea levels.

Lower leukocyte and platelet counts were recorded during the critical phase of dengue, and values returned to normal during the convalescent phase.

In addition, the increase in RL and the decrease in neutrophil count was observed between days 4 and 10.

Both AST and GGT were higher during the critical phase and remained elevated during the convalescent phase. However, the significant increase in ALT was recorded during the convalescent phase. AP was high during the febrile phase and decreased during the critical phase.

The other biochemical parameters did not show significant or clinically relevant changes (*Table 1*).

All patients had a favorable clinical course. Follow-up was conducted in an outpatient setting, with no need for hospitalization or other more complex tests. No severe dengue cases were observed during the study period.

DISCUSSION

In the 2019–2020 period, the largest outbreak of dengue in the history of Argentina was recorded, with a total number of 56 492 cases, which exceeded the cumulative number of the 2015–2016 period by 35.3%. At this stage, the increase in the curve of cases occurred late and abruptly, compared to the previous period.[13] Environmental and socioeconomic factors, such as climate change, unplanned urbanization, and increased population travel and migration, are related to such increase.[3,4]

In this regard, a vaccine would be a key element in the prevention of dengue.[1,5] The Dengvaxia vaccine has been recently approved in Argentina. Although it has been shown to be highly effective in seropositive individuals, the vaccine increases the risk for severe dengue in children under 9 years of age and in seronegative patients. Therefore, its use is restricted to a part of the population.[14,15]

During this period, 3 circulating virus serotypes were identified: DENV-1: 72%, DENV-4: 26%, and

Table 1. Laboratory parameters during the course of the disease

Variables	Febrile phase		Critical phase		Convalescent phase		*p* value
	N	Median (IQR)	N	Median (IQR)	N	Median (IQR)	
TLC (10^9/L)	79	4.28 (3.17–5.61)	120	3.66 (2.69–4.75)	27	5.63 (4.26–7.50)	< 0.0001
Neutrophils (10^9/L)	75	2.19 (1.62–3.46)	113	1.28 (0.92–1.89)	26	1.37 (0.96–2.15)	< 0.0001
RL (%)	75	0 (0–1)	113	3 (1–8)	26	3 (2–6)	< 0.0001
Platelets (10^9/L)	78	200 (164–240)	122	140 (112–177)	28	210 (148–266)	< 0.0001
Hematocrit (%)	80	40.2 (37.2–42.5)	122	39.2 (37.2–41.9)	28	37.5 (35.9–40.6)	0.0565
ESR (mm/h)	26	5 (3–12)	17	6 (5–13)	4	11 (8–13)	0.2137
AST (U/L)	57	34 (25–45)	77	47 (34–74)	14	48 (39–101)	0.0002
ALT (U/L)	59	19 (13–37)	81	22 (17–39)	14	45 (23–73)	0.0023
AP (U/L)	37	466 (420–551)	40	329 (215–415)	7	379 (221–386)	< 0.0001
GGT (U/L)	22	15 (11–23)	26	23 (16–60)	2	124 (9–238)	0.0463
CRP (mg/dL)	43	7.99 (4.97–16.18)	38	2.56 (1.46–4.18)	2	2.83 (0.45–5.20)	< 0.0001*
Urea (mg/dL)	51	22 (19–28)	54	19 (14–22)	7	18 (13–25)	0.0004
Creatinine (mg/dL)	50	0.58 (0.44–0.65)	53	0.54 (0.40–0.61)	7	0.54 (0.45–0.63)	0.4583
PT (%)	7	73 (64–82)	12	85 (69–97)	1	104	0.1645
aPTT (seconds)	7	46.2 (39.9–51.2)	12	40.8 (36.4–43.4)	1	35.0	0.1378

Values correspond to the median and interquartile range (IQR).
TLC: total leucocyte count.
RL: reactive lymphocytes.
AST: aspartate aminotransferase.
ALT: alanine aminotransferase.
CRP: C-reactive protein.
AP: alkaline phosphatase.
ESR: erythrocyte sedimentation rate.
PT: prothrombin time.
aPTT: activated partial thromboplastin time.
GGT: gamma-glutamyltransferase.
**No differences among the phases of dengue were detected with the Kruskall-Wallis test because the distribution of the variable values in each phase is not similar.*

DENV-2: 2%. In our study population, only the DENV-1 serotype was identified. The seasonal distribution was consistent with what has been described by the National Ministry of Health for the Central Region of Argentina.[13]

Almost half of patients sought medical care in the 2 days after symptom onset. This allowed to provide a close monitoring of dengue disease and prevent its progression to more severe clinical forms.[1–3,11] The most common clinical manifestations were similar to those published in other studies on dengue,[6,8,16–18] except for diarrhea (14% versus 30%), conjunctival injection (20% versus 20–50%),[16] and retro-orbital pain (40% versus 82.9%).[6]

In line with the findings of another study on dengue in the pediatric population, the most common laboratory alteration was leukopenia. However, in our population, it was recorded in 37% of cases, compared to 85% reported by Cazes et al.[6] In contrast, the time series study by Berberian et al. reported 57%.[16]

Among patients who developed neutropenia, 11% had severe neutropenia. This finding was very similar to that published by Thein et al., which concluded that these patients did not have an increased susceptibility to developing infections, despite having a low neutrophil count.[19]

RL were observed in 31% of cases. Some investigators even reported higher percentages.[7,17,20] Although the exact role of these cells in the pathophysiology of dengue is unknown, it is thought that their increase may be associated with a non-specific cellular response to the virus.[17,20]

No reports of monocytosis have been found in the bibliography. However, these cells would have a very relevant role in controlling viremia and progression to more severe clinical forms in some patients.[21]

In adition, thrombocytopenia was the second most common observed alteration, and was mild in most cases. The study conducted by Cazes et al. reported a similar percentage,[6] whereas

Berberian et al. found a higher percentage, reaching 46%,[18] Other studies found that it was the most common biochemical alteration.[7,8,17]

High transaminase levels were the third most frequent alteration found in these patients. DENV has the ability to directly infect hepatocytes and Kupffer cells.[7] Its replication inside the cells and the immune response of the body against the etiologic agent would be the cause of liver damage and the increase in the levels of these enzymes,[7] together with AP and GGT.[22]

In relation to coagulation tests, a previous study (n = 166) reported altered PT and aPTT values in 24% and 25% of cases, respectively.[17] In our study, the low number of patients with these measurements did not allow us to make inferences in that regard.

In addition, in the patients that were monitored, a lower TLC in the critical phase and a return to normal values in the convalescent phase were observed, which is consistent with another study on dengue in the pediatric population and the guidelines published by the Ministry of Health for infectious diseases.[3,6]

In parallel with the decrease in the TLC in the critical phase, an increase in RL and a decrease in platelet count were observed. These events have been consistently reported in the bibliography and, together with hemoconcentration, are associated with progression of dengue to more severe forms.[2,20] Even so, this phenomenon was not observed in our study population, which could be due to the absence of severe cases.

Finally, unlike other authors who described that neutropenia manifested in the critical phase of the disease and lasted 1 day,[19] in our study, it was present both in the critical and the convalescent phases, without returning to normal values during the time the patients were followed-up.

It is worth noting that the lack of information about the patients' comorbidities and some laboratory data were limitations of this study.

CONCLUSION

The recognition and understanding of clinical and laboratory alterations that occur during dengue disease may allow an effective approach and help to reduce the more severe clinical forms in children. ■

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