

Clinical and Laboratory Markers in the Diagnosis and Severity of Dengue Virus Infection in Children Under 15 Years of Age

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Abstract

Denguevirus infection must be diagnosed early in the febrile step in order to adjust adequate management. The objective of this study is to identify clinical and laboratory predictive markers in the acute stage of dengue infection following a major dengue virus outbreak in Salah Al-din , Iraq during 2019-2020. Salah Al-din had 462 people who had been notified under 15 years of age during this period. 33.4% of these children completed the Tourniquet Test, whereas 6.4 percent did not. In 60.4% of the cases, this field was left blank, while 16.51% were described as positive. 462 children were admitted during this period. Laboratory confirmation was received in 502 cases. The finals were 77% classic dengue, 7.4% dengue with complications, 0.4% haemorrhagic dengue and 0.14% dead, and the remaining 15% unclassified. 462 cases with positive IgM, DEN 1, DEN 2, and DEN 3 were among the laboratory markers identified. The clinical markers for diagnosis and gravity of Dengue were found during the course of the study. Case series consisting of non-severe dengue and severe dengue diagnosed in paediatric hospitalised patients, according to clinical criteria. The results of the univariate testing of clinical signs and symptoms were shown as averages or percentages with a statistical significance of 0.05. In the infant population, severe vascular spills and neurological impairment have been observed. Although some information was not complete, the study, which acts as a basis for better knowledge of Dengue for children in this region, was not limited. The increased prevalence of dengue in the paediatric population could be explained by the exhaustion of susceptible in older age groups. For these reasons, it should be expected that the next dengue outbreaks will primarily affect children, and health personnel should be vigilant in the clinical identification of potentially serious cases.

Keywords: Dengue Fever, Salah Al-din City, Children under the age of 15 Years, Markers, Retrospective study.

1.0 Introduction

Dengue fever is the world's fastest growing human arboviral disease. In terms of morbidity and mortality, it is currently the most serious emerging and re-emerging infectious disease. With no hope of improvement in the near future, 55 percent of the world's population is at risk of contracting dengue. Dengue haemorrhagic fever is estimated to cause 500 thousand hospital admissions each year, primarily among children. In the United States [1], approximately 2.5 percent of patients die, whereas in Iraq, lethality can reach 10% [2]. The transmission proclivity of the dengue virus is similar to that of diseases spread through direct contact [3]. The vulnerability of children to the effects of dengue necessitates studies and knowledge on the subject, particularly in paediatrics. Clinical suspicion should be followed by detailed notification in order to investigate the best forms of surveillance and disease-fighting strategies. The most important priority is early detection in order to avoid exacerbation and the deaths that follow. When all other diagnostic possibilities have been ruled out, paediatric care must be prioritised, especially when presented with febrile infants, and especially in endemic areas and areas of sustained transmission. The battle for efficacy in the fight against this epidemic, for better quality patient care and proper patient management, has outgrown the confines of consultation rooms and primary care centres and must now be shared by all. There is a scarcity of information and publications on the progression of severe forms of the disease and death in children. Given the large number of cases and notifications received in Salah Al-din [2], as well as the lack of knowledge of the symptoms most commonly presented by the child population, and using valuable data from the Information System for Notifiable Diseases Database, a study focusing on aspects of interest to paediatrics is urgently

required. With this, we hope to contribute to better care for the small patient by identifying clinical markers of diagnosis and severity in the various clinical forms of Dengue [4]. This study reports the clinical profile of non-severe dengue and severe dengue, and the predictive variables of dengue severity in children under 15 years of age patients hospitalized in the hospitals Salah Al-din city, Iraq and the main purpose of this study is to identify clinical and laboratory markers of diagnosis and severity of Dengue virus infection in children under the age of 15 living in the city of Salah Al-din, Iraq, from February 2019 to January 2020.

2.0 Materials and Methods

2.1 Study type: This is a retrospective study carried out in the city of Salah Al-din, Iraq. Location of an important economical center and a reference in specialized health care. The data collected refer to the years 2019 to 2020 and were obtained from the Health Department through the database of the Information System for Notifiable Diseases, whose objective is to manage information routinely obtained by the local passive surveillance system through disease and health notification forms established by governmental determination of which Dengue is a part.

2.2 Study Location: Salah Al-din, the study site in Northern Iraq, has a population of around four lakh people. This study was conducted in four sites, namely Salah Al-din teaching hospital, Al-Salah Al-din teaching hospital, and Paediatric and Oncology centre, which were chosen to represent almost all of the population in Salah Al-din, where there is a possibility of transmission to humans from lakes and rivers, which may act as breeding sites for vectors and reservoirs for arboviruses.

2.3 Inclusion Criteria: Children under the age of 15 living in the city of Salah Al-din who were notified and confirmed by laboratory criteria for Dengue in the specified time frame

2.4 Exclusion Criteria:

- Cases concluded by clinical epidemiological criteria.
- Cases originating from other locations and notified in Salah Al-din.
- Incomplete cases referring to date of birth and age, or with inconsistent information of these, double notification of children under 15 years old for Dengue in the same period.

2.5 - Data Collection and Analysis

Initially, information referring to patients residing in Salah Al-din and under 15 years of age was selected. The sociodemographic, clinical and laboratory variables were analyzed after verifying the dengue confirmation criteria used. For the diagnosis of dengue, the Dengue Duo® immunochromatographic test from Bioline was used according to the manufacturer's recommendations, which detects differential IgG and IgM antibodies, and the dengue virus NS1 (Non-structural protein 1) antigen. Infections were classified according to WHO criteria [5]. The final classification of dengue during the years analyzed followed the definitions in force as Classic Dengue (CD), when there is an acute febrile illness for up to seven days, accompanied by at least two nonspecific signs and symptoms (headache, prostration, retroorbital pain, rash, myalgias, arthralgias and compatible epidemiological history). Dengue Hemorrhagic Fever (DHF) was defined as meeting the following criteria: laboratory confirmation, fever, thrombocytopenia less than $100,000$ platelets per mm^3 , hemorrhagic trends, plasma extravasation with a consequent increase in hematocrit with a drop in it after adequate treatment and strokes cavity. Dengue with Complications (CHD) was defined in patients who presented severe neurological manifestations, cardiorespiratory dysfunction, liver failure, thrombocytopenia less than $50,000$ mm^3 , digestive haemorrhages, cavity effusions and total leukocytes equal to or less than $1,000/\text{mm}^3$; in these cases the classification of classic Dengue was unsatisfactory and there was no way to fit the patients into the criteria of Hemorrhagic Fever. The cases could be closed by clinical-epidemiological or laboratory criteria [6].

2.6 Statistical analysis

From the final classification obtained, sociodemographic, clinical and laboratory markers assigned were analyzed using statistical significance tests (t-student), correlating the markers with the clinical evolution of

the patients. The level of significance adopted was 5% and the operating system used for analysis and initial processing of data was an Excel spreadsheet and later the Statistical Package for Social Sciences (SPSS) version 17.0 was used for data entry, processing and final statistical analysis. Obtaining the database was authorized by the Health Department after the opinion of the Research Ethics Committee of the Salah Al-din University Teaching Hospital - Salah Al-din Medical College, Iraq.

3.0 RESULTS

From 2019 to 2020, 502 cases of Dengue were reported in the city of Salah Al-din , according to the Information System for Notifiable Diseases; of which 460 were residents of Salah Al-din . The other cases came from adjacent governorates/cities. Among those under 15 years of age reported in Salah Al-din in the period described, 462 cases were observed. The number of notified cases in children under 15 years of age increased from 185 in 2019 to 277 in 2020. However, in relation to the total number of notifications, the annual average of notified cases in this age group was 16.24% of total notifications in patients in this age group.

The Tourniquet Test [7] was performed in 154 (33.34%) of the total of those notified in children under 15 years of age in Salah Al-din and 29 (6.36%) reported not having undergone this test. In other cases, (278 - 60.30%) the field was not filled in for this variable. Of the total that performed the Tourniquet Test, 25 (16.45%) were described as positive. Using the variable "hospitalization occurred", 502 admissions were found in the age group studied, with 462 cases registered among those confirmed by the laboratory.

Laboratory confirmation for Dengue in the studied population was 462; the other cases were closed by clinical and epidemiological criteria. Among the laboratory-confirmed cases, the final classification was 392 (85%): 355 (77%) cases of Classic Dengue; Dengue with Complications accounted for 34 cases (7.4%); Dengue haemorrhagic fever was classified in 2 (0.4%) cases, whose distribution in severity was: Grade I - 8 cases, Grade II - 12 cases, Grade III - 8 cases and Grade IV - 1 case and the other 15% there were 0.14 deaths in this period and 15% (n=69) cases were not classified. The proportion of deaths from the number of haemorrhagic cases was 36.36%. The median number of days between the onset of symptoms and the serological diagnosis was four days (IQR: 0-13); 51.4% of the patients tested positive for Ag NS1, 54.1% for IgG, and 27% for IgM. On the other hand, 35% of the infections were classified as primary and 65% as secondary (Table 1). Among the patients hospitalized in the intensive care unit, 62.5% had primary infections.

Table 1. Classification of primary and secondary infections

NS1	IgM	IgG	Primary infections	Secondary infections
Positive	Negative	Negative	27.20%	
Negative	Positive	Negative	2.70%	
Negative	Negative	Positive		29.70%
Negative	Positive	Positive		16.20%
Positive	Negative	Positive		10.80%
Positive	Positive	Negative	5.40%	
Positive	Positive	Positive		8.10%
Negative	Negative	Negative		
Total			35%	65.20%

Viral Isolation was performed in 165 cases in the period studied and the following results were found: DEN 1-97 cases, DEN 2- 11 cases DEN 3- 57 cases, the three serotypes were circulating simultaneously in 2020, with a predominance of DEN 1 with 52 cases. Histopathology was performed and positive in 4 cases, immunohistochemistry in 2 cases, PCR in 7 cases of which 5 were positive. The haematocrit value found

ranged between 21% and 81% with an average of 50%, platelets below 100,000 mm³ in 43.35% cases. Among the cases of death, 5 had platelets below 50,000 mm³, 3 cases between 50 and 100,000 mm³, 4 cases with counts above 100,000, and in 4 cases this data was not reported. The socio-demographic variables were evaluated in relation to the final classification and showed statistical significance ($p=0.03$) in relation to Age and Sex data given in table 2. Although the association between severe forms and age was not statistically significant, it was observed, however, that 50% of deaths were detected in the population above 11 years of age. When clinical forms and sex were analyzed, the distribution was similar, with a slight predominance of girls. Deaths occurred proportionally more in males (62.5% vs 37.5%)

Table 2: Evaluation of the final classification of Dengue in children under 15 years in relation to social markers.

Social bookmarks		CD (%)	CCD (%)	DHF (%)	Death(%)	<i>p</i>
Age (n= 460)	0-5 Years	22.5	21.11	9.52	25.1	0.88
	6-10 Years	31.96	33.98	23.81	25.1	
	11-15 Years	45.54	44.91	66.66	50.1	
Sex	Male	43.62	35.98	45.45	62.5	0.185
	Female	56.38	64.02	54.55	37.5	

Immunization against Yellow Fever was reported in only 48.82%. The loop test showed significance for severity ($p<0.001$) and among the general symptoms fever, headache, rash, retro-orbital pain, prostration, myalgia, arthralgia are the most common symptoms with statistical significance ($p<0.05$). Among the haemorrhagic symptoms, epistaxis and petechiae were the most found in the three clinical presentations studied; gastrointestinal bleeding occurred in a small number of cases, but with significant significance ($p=0.01$). Among the warning signs, abdominal pain, ascites, pleural and pericardial effusions, and gingivorrhage such as mucosal bleeding were the most significant. It is noteworthy the number of patients concluded as classic Dengue who had severe abdominal pain (15.08%). Painful hepatomegaly did not show statistical significance, but was mentioned in cases of Classic Dengue, Dengue with Complications, Dengue Hemorrhagic Fever and deaths. Hypovolemic shock occurred in 25% and neurological manifestations in 18.75% of the deaths found. The clinical marker of neurological alterations is reported in 11 cases, but without specifying the alteration suffered by the child (Table 3).

Table 3: Evaluation of the final classification of Dengue in children under 15 years old in Salah Al-din , Salah Al-din in relation to Clinical Markers.

Clinical Markers	Classic Dengue (n=270)	%	Dengue with Complications (n=120)	%	Dengue Hemorrhagic Fever (n=72)	%	Death	%	<i>p</i>
Have you had dengue before?	19	7.08	10	8.38	5	6.82	3	6.25	0.13
Vaccinated (Yellow Fever)	156	57.63	70	58.13	39	54.55	12	25	0.86
positive loop proof	22	8.29	34	28	43	59.09	3	6.25	0

Fever	232	85.78	98	82	72	100	26	56.25	0
headache	221	81.67	95	79.25	57	79.55	14	31.25	0
rash	61	22.45	33	27.5	41	56.82	-	-	0.01
Nausea and vomiting	153	56.53	72	60.25	54	75	17	37.5	0.2
retroorbital pain	169	62.75	74	61.63	52	72.73	3	6.25	0
Prostration	188	69.46	82	68.63	62	86.36	23	50	0
Myalgia	207	76.59	90	74.75	59	81.82	6	12.5	0
Arthralgia	173	64.19	75	62.75	39	54.55	6	12.5	0
Diarrhea	57	21.08	34	28.13	23	31.82	6	12.5	0.3
epistaxis	7	2.62	13	10.63	11	15.91	3	6.25	0
Petechiae	13	4.64	18	14.63	18	25	17	37.5	0
Gingivorrhage	3	1.11	16	13	13	18.18	3	6.25	0
metrorrhagia	3	0.98	1	0.58	8	11.62	-	-	0.2
hematuria	1	0.53	3	2.62	3	4.54	-	-	0.07
ascites	2	0.65	1	0.88	3	4.54	3	6.25	0
Pleural effusion	0	0.11	0	0.38	7	9.09	-	-	0.01
Pericardial effusion	0	0.04	0	0.25	2	2.27	3	6.25	0
Severe abdominal pain	41	15.08	35	29.13	20	27.27	12	25	0
painful hepatomegaly	4	1.53	5	3.88	3	4.55	6	12.5	0.3
Myocarditis	0	0.13	0	0.25	0	0	0	0	0.23
hypotension	5	1.93	8	6.75	8	11.36	12	25	0.01
Hypovolemic shock	7	2.75	1	0.75	3	4.55	12	25	0.01
Neurological Manifestations	1	0.3	1	1.13	0	0	9	18.75	0.01
Other Symptoms	1	0.37	1	0.75	0	0	0	0	0.02
spontaneous bleeding	3	1.24	5	3.88	5	6.82	6	12.5	0.03
Deaths	0	0	2	1.5	7	9.09	46	100	

About the deaths described over the years, despite representing only 0.14% of the total confirmed by laboratory tests, they constitute 36.36% of the cases closed as DHF and 2% as CCD. Markers such as fever, headache, nausea and vomiting, prostration, severe abdominal pain, hypotension and neurological manifestations appear as the most common symptoms. Among haemorrhagic patients, only petechiae are reported in 37.50 cases and 50% cases had platelet counts below 100,000 per mm³. Probable deaths from hypovolemic shock were reported in 4 cases (25%).

DEN 2 was responsible for the highest proportion of severe symptoms such as ascites, spontaneous bleeding, severe abdominal pain, and hypovolemic shock among the serotypes studied. DEN 3 had the most cases of gastrointestinal bleeding, while DEN 1 had the most cases of petechiae. DEN 2 and 3 were found in the cases of deaths that were subjected to viral isolation (Table 4).

Table 4: Evaluation of clinical markers of Dengue in children under 15 years of age in Salah Al-din , Salah Al-din , in relation to the identified viral serotypes.

Clinical Markers	DEN-1 (n=97)	%	DEN-2 (n=11)	%	DEN-3 (n=57)	%	p
Fever	96	99.07	11	100	56	98.51	0.045
headache	84	86.11	9	85.71	52	91.04	0.003
rash	31	32.4	2	21.43	15	26.87	0.032
retorbit pain	57	59.26	9	78.57	42	73.13	0.015
Prostration	74	75.93	10	92.86	43	76.12	0.421
Myalgia	79	81.48	10	92.86	47	82.09	0.187
Arthralgia	58	60.19	9	78.57	39	68.66	0.001
Nausea and Vomiting	55	56.48	7	64.29	32	56.72	0.254
ascites	0	0	1	7.14	0	0	0.002
Spontaneous bleeding	1	0.92	1	7.14	3	4.48	0.082
gastrointestinal bleeding	0	0	0	0	3	4.48	0.652
hematuria	1	0.93	0	0	1	1.49	0.07
bond proof	23	24.07	2	21.43	11	19.4	0.483
metrorrhagia	1	0.93	1	7.14	1	1.49	0.137
Gingivorrhage	1	0.93	0	0	3	4.48	0.058
Petechiae	13	13.89	0	0	2	2.99	0.366
epistaxis	5	5.56	0	0	3	4.48	0.022
Diarrhea	12	12.04	0	0	10	17.91	0.007
Painful Hepatomegaly	3	2.78	0	0	1	1.49	0.094
Severe abdominal pain	27	27.78	6	57.14	8	13.43	0.01
hypotension	3	2.78	0	0	1	1.49	0.109
Hypovolemic shock	7	7.41	3	28.57	1	1.49	0.004
Deaths	0	0	1	7.14	1	1.49	0.318

DISCUSSION

The city of Salah Al-din is the area most severely affected by Dengue in Iraq because there is a risk of transmission to humans from lakes and rivers in these areas, which may serve as breeding grounds for vectors and reservoirs for arboviruses. During this study, we observed an annual increase in the total number of notifications, which was 185 cases in 2019 and 277 cases in 2020. However, in relation to the proportionality of notifications in children under 15 years of age, it remained at an average of 17.8%. Only the year 2019 appears with the highest proportion of notifications in this population (20%), probably due to the recirculation and predominance of serotype 3, this trend followed the entire country, which observed in the same year a number of hospitalized cases in the child population of 53% compared to adults [2]. These data, however, are based on numbers of reported cases which, despite helping to detect disease trends, do not provide a clear picture of the magnitude of viral circulation, as many cases are of unreported or unreported infections [8]. Our study did not find a link between age and severity, but this data must be interpreted with caution because half of the deaths reported in Salah Al-din occurred in adolescents over the age of ten, and an analysis of the

variable education and clinical classification revealed that older children were at a higher risk of developing more severe forms. However, the disease was severe in all age groups studied. In addition to the atypical presentation with nonspecific symptoms, the underreporting and occurrence of other more common exanthematic diseases in infants and younger children may have contributed to this finding. Nonetheless, it is critical to guide protocols for the care and management of cases in adolescents with fever within an endemic zone, as well as to emphasise the importance of investigating fever in younger children whose clinical diagnosis has not been established. According to the ministry of Geography and Statistics, Iraq, whose available data on this variable are from 2012 [2], the population of this place of study was distributed into the following categories: brown with 50.9%, white with 43.6%, black with 5.3%, yellow and indigenous with 0.2%, thus characterizing the finding of the affected majority corresponding to that of the population. We cannot say that there is a relationship between this variable and the results found with a protective or favouring effect on the severity of Dengue with the present study.

The bond must be tested in every suspected case of Dengue. However, we discovered that only 33.34 percent of all patients under the age of 15 were notified that this test was performed. Failure to perform the test has a significant impact on the detection of DHF (Dengue Haemorrhagic Fever) because we demonstrated a relationship between test positivity and disease severity ($p < 0.001$) in our study, concluding that this is a sensitive marker for the disease. These figures may also reflect the quality of care provided to this population in an indirect way. The relationship between the general symptoms already described by the Ministry of Health and the clinical forms presented reinforce the need for careful anamnesis and clinical examination.

Although no relationship between nausea, vomiting and diarrhoea has been demonstrated with dengue severity, the conjunction of these signs with the presence of fever should always alert people to Dengue. A study with Pakistani children [9] showed that 59.3% of them had nausea and this was the most common symptom among 219 patients studied; diarrhoea was reported in 16.3%.

It is important to emphasize that, although a relationship between the findings of painful hepatomegaly and myocarditis has not been demonstrated, these should not be underestimated in patients with clinical suspicion of Dengue, as they constitute alarm signs and serious complications. A research carried out in Iran with 102 paediatric patients with DHF revealed that 8 had myocarditis, with one fatal case (13). In the studied population, there are still no studies on the occurrence of myocarditis, and the small number of cases described [10] in the notifications may be due to the lack of knowledge about this particular complication.

Clinical severity markers such as pleural effusion, pericardial effusion and ascites were found in the reports as signs of vascular extravasation and corroborate what other studies [11] have already demonstrated regarding the severity of the disease. However, in the deaths reported, only 2 cases mention cavitory effusions, however, the number of patients classified as CD (Classic Dengue) who present these complications stands out: ascites in 54 cases, pleural effusion in 9, pericardial effusion in 3, signalling a questioning about the reliability of the final diagnosis.

In 37 cases neurological alterations are mentioned, in deaths 3 cases appear with this complication, but there is no description of the complication found. Intracranial haemorrhages have already been described in 3 children out of a total of 948 who were hospitalized with Dengue, all of whom presented a benign evolution without neurological sequelae [11]. For the population studied, the details of these findings would be useful in order to guide the management in the eventual occurrence of these complications.

Concerning the final classification of cases, the flaws observed in the Notification System's final numbers of CD, DCC (Dengue with complications), and DHF deserve to be highlighted. We should point out that clinical and laboratory markers allow for a final reclassification and recount of these cases. It is worth noting that 228 of the cases closed as CD refer to hypovolemic shock, a serious complication. In addition to neurological manifestations, other cases closed as CD had ascites (54), gastrointestinal bleeding (39), pleural effusion (9) and pericardial (3). (25). According to the WHO, these cases should be classified as DCC.

The significant number of deaths in relation to DHF cases highlights an even greater concern about the closure of cases, as the MS has a mortality rate of only 1% for cases of Haemorrhagic Dengue. The impressive 36.36% of deaths, based on the number of haemorrhagic fever found in our study, may suggest an inadequate management and unsatisfactory management of severe cases in this population and corroborate once again for their final reclassification. Shock is pointed out as the most frequent condition of death in children and alarm symptoms signal a deterioration of patients. Therapeutic measures can be adopted at this time and a fatal outcome can be avoided [3, 7]

The final reclassification of cases was not part of our objectives, however, a thorough study, with the new criteria adopted by the World Health Organization [5], becomes imperative to redefine the real situation of dengue severity and its evolution throughout of years in children under 15 in our environment.

The incomplete filling out of notification forms, with the absence of some important data, was the critical point of this study and strengthens the need for improvement of health professionals in providing data that will surely reflect in the detailed mapping of the dengue scenario in our country. On the other hand, obtaining all dengue cases reported in children under 15 years of age in the proposed period provided a great external validity to the study. The simultaneous circulation of DEN 1, 2 and 3, the imminent risk of the introduction of DEN 4 in our environment and a judicious reclassification can modify this scenario of apparent safety in relation to the severity of Dengue in children in the region studied. The correct and timely closure of cases, the investigation of suspected deaths and, above all, a serious commitment of professionals in the face of notifications can provide elements for effective combat strategies and care. The clinical and laboratory markers analyzed in this study serve as a warning to clinical suspicion when treating sick pediatric patients in an area of high endemicity for Dengue, highlighting the strict attention and monitoring of children and adolescents in the presence of febrile conditions. All the data analyzed reinforce the need for permanent surveillance, in addition to signalling the increasing and undesirable appearance of serious and fatal forms, even if potentially avoidable.

Conclusion

The collected data will aid in the recognition of the clinical picture of Dengue and the difficult diagnosis in children, as well as the identification of markers associated with greater disease severity. In the study population, clinical markers of Dengue were found, with a high prevalence of symptoms such as fever, headache, myalgia, arthralgia, retro orbital pain, prostration, nausea, and vomiting. Severe signs of bleeding and plasma extravasation were also observed, with a greater number of patients exhibiting epistaxis and ascites. Classic Dengue was one of the warning signs that, when present, served as a final confirmation. Laboratory tests were not described in all reports and were a limiting variable in the study; three serotypes, DEN 1, 2, and 3, were discovered to be circulating concurrently in 2020. For the years 2019 to 2020, there was a significant loss in the hospitalisation variable due to not filling it in the Database; the socio-demographic classification varied in the number of individuals, as it depended on its complete completion for the analysed variables. The number of adolescents notified and the low level of Yellow Fever vaccination coverage stand out. The latter is not one of the study's objectives, but it is a concern in an area where the *Aedes aegypti* mosquito is prevalent.

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