

## Research Article

# Detection of Hepatitis C Virus Coinfection in Patients with Dengue Diagnosis

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Received 22 February 2014; Accepted 24 April 2014; Published 14 May 2014

Academic Editor: Masayuki Kurosaki

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Coinfection produced by dengue virus (DENV) and hepatitis C virus (HCV) is a serious problem of public health in Mexico, as they both circulate in tropical zones and may lead to masking or complicating symptoms. In this research, we detected active coinfecting patients by HCV residing in the endemic city of Mérida, Yucatán, Mexico, with positive diagnosis to dengue during the acute phase. We performed a retrospective analysis of 240 serum samples from dengue patients. The IgM-ELISA serological test was used for dengue diagnosis, as well as viral isolation to confirm infection. DENV and HCV were detected by RT-PCR. Thus, 31 (12.9%) samples showed DENV-HCV coinfection, but interestingly the highest frequency of coinfection cases was found in male patients presenting hemorrhagic dengue in 19/31 (61.29%), with a predominance of 12:7 in males. Firstly, coinfection of DENV-HCV in Mérida, Mexico, was detected in young dengue patients, between 11 and 20 years old (38.7%), followed by those between 21 and 30 years old (32%); only 16.13% were between 0 and 10 years of age. Diagnosis of HCV infection in patients with dengue is highly recommended in order to establish potential risk in clinical manifestations as well as dictate patients' special care.

## 1. Introduction

Worldwide, dengue virus (DENV) is one of the most important vector transmitted viruses. There are four DENV serotypes, classified as DENV 1, 2, 3, and 4, that cause similar clinical outcomes. Dengue is endemic in 112 countries, due in part to the increased geographical distribution of vectors in recent years [1].

Dengue fever (DF) and dengue with hemorrhagic manifestation (HD) are recognized as the world's second most prevalent infection in tropical and subtropical regions, after malaria, but the first in the American continent [2]. Infection with any of the serotypes can be accompanied by a fever, with increased vascular permeability and thrombocytopenia

among other signs, but also dengue infection could turn out as unapparent.

Higher risk clinical manifestations, or severe forms, are associated with DENV serotypes 2 and 3, as in some cases dengue fever with HD or dengue shock syndrome has been associated to those serotypes [3, 4]. Globally, it has been estimated that nearly 100 million people per year suffer DF, and 500,000 HD, having a mortality rate of 0.5 to 3.5% [5]. In Mexico, the states with higher incidence are Morelos, Quintana Roo, Tabasco, Veracruz, and Yucatán [6], where the prevalence is estimated in 80% [7].

The circulation of other pathogenic agents infecting dengue patients has been detected, exacerbating the dengue clinical symptoms or confusing the diagnosis [8–10]. In tropical

TABLE 1: Primers used for amplification in the molecular diagnosis for dengue and HCV.

Virus	Primer	Sequences	Fragment size (bp)	Reference
DENV	DV1 (+)	5'-GGRACKTCAGGWTCTCC-3'	—	[22]
	DV3 (-)	5'-AARTGIGCYTCRTCCAT-3'	470	
	DSP1 (-)	5'-AGTTTCTTTTCCTAAACACCTCG-3'	169	
	DSP2 (-)	5'-CCGGTGTGCTCRGCYCTGAT-3'	362	
	DSP3 (-)	5'-TTAGAGTYCTAAGCGTCTCTTG-3'	265	
	DSP4 (-)	5'-CCTGGTTGATGACAAAAGTCTTG-3'	426	
HCV	HCV 1 (+)	5'-ACTCCACCATAGATCACTCCC-3'	241	[23]
	HCV 2 (-)	5'-AACACTACTCGGCTAGCAGT-3'		
	HCV 3 (+)	5'-TTCACGCAGAAAGCGTCTAG-3'	144	
	HCV 4 (-)	5'-CTTTATCCAAGAAAGGACCC-3'		

areas, concurrent infections with two different dengue serotypes, in the same patient, have been reported [11], besides coinfections by other arboviruses [12]. Also, in tropical zones, simultaneous circulation of several arboviruses and hepatitis C viruses (HCV) has been detected [13].

HCV is classified in the *Flaviviridae* family as a unique member of *Hepacivirus* genus. 80 to 85% of the cases of infection by HCV are chronic and responsible for hepatic injury. It has been estimated that HCV infects 3 to 4 million people each year. This means that nearly 170 million are at risk of a hepatic cirrhosis and hepatocellular carcinoma [14, 15]. HCV infection can be acquired in several ways, most regularly by exposure to contaminated blood or its products, maternal-fetal transmission, and in a lower rate sexual contact with infected people [16]. In 2011, Mexico reported that seroprevalence was 1.5% in general population, although among seropositive subjects only 48.3% presented HCV RNA [17].

HCV chronic infection is frequently associated with a marked thrombocytopenia [18], with the probable contribution of antiplatelet antibodies and the consequent alteration of the coagulation system [19]. Also, HD produced by DENV infection is characterized by thrombocytopenia associated with hemorrhage [20]. Here, we establish frequency of coinfections and examined the correlation of chronic HCV infection with severe dengue. The importance of coinfections produced by both viruses in public health conducted to this research, so we determined the presence of HCV in serum from patients with dengue positive diagnosis, residing in Mérida city, Yucatán.

## 2. Material and Methods

**2.1. Patient Serum Samples.** Sera samples were obtained from positive dengue patients in the acute phase of infection during the dengue epidemical outbreaks of years 2006 to 2008. They were collected at the arbovirology laboratory from Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán (UADY). This protocol was approved by the UADY Bioethical Committee and every patient signed their authorization to participate in this investigation, as an informed consent. This was a retrospective,

observational, and descriptive study. In total, 240 positive serum samples for dengue infection were analyzed, 120 (50%) from patients with HD and 120 from DF patients, abiding to the World Health Organization [4]. The basic epidemiologic information of patients included gender, age, and the first sign or fever date. Patients were not asked about HCV infection status.

**2.2. Detection of DENV Infection.** Diagnosis of DENV infection was performed by IgM detection with the MAC-ELISA test, previously described [21]. Also, molecular diagnosis was done by RT-polymerase chain reaction (PCR) test, using specific primers for viral genome [22] as shown in Table 1.

Amplicons were detected in 2% agarose gels stained with ethidium bromide and visualized in a transilluminator with ultraviolet light. As confirmation method, viral isolation was practiced using patient serum samples directly in Vero cells (green monkey kidney cells).

**2.3. Detection of HCV Infection.** The presence of anti-HCV IgG antibodies was looked for in all of the 240 serum samples from dengue positive patients, using the commercial kit Advanced Quality Rapid ANTIHCV Test (Accutrack, Xiamen, China). Also, detection of HCV RNA was done by a two-stage PCR with two pairs of primers deduced from the 5'-noncoding region [23] (Table 1).

**2.4. Control Determination of VHC and DENV.** As both viruses belong to the same family, primers were tested for crossed amplification using RNA extracted from peripheral blood mononuclear cells from DENV infected cells and positive controls for HCV to avoid amplification of false positive during PCR. HCV positive samples were obtained from Laboratorio de Enfermedades Emergentes y Reemergentes del Centro de Investigaciones Regionales Dr. Hideyo Noguchi, UADY. Viral RNA was extracted by PCR amplification tests with specific primers.

**2.5. Statistical Analysis.** Data was analyzed using contingency tables of chi-squared and *t*-Student calculations. Risk analysis for groups were done, considering relative risk >1. This was

TABLE 2: Gender distribution of patients with dengue fever (DF) or hemorrhagic dengue (HD) and hepatitis C (HCV).

Gender	Dengue infected patients Total: 240 (100%)			Coinfected HCV-DENV patients Total: 31/240 (12.9%)		
	Total	DF	HD	Total	HCV-DF	HCV-HD
	<i>n</i> = 240 (100%)	<i>n</i> = 120 (50%)	<i>n</i> = 120 (50%)	31 (100%)	12/31 (38.71%)	19/31 (61.29%)
Male	131 (100%)	66 (50.4%)	65 (49.6%)	16 (100%)	4/16 (25%)	12/16 (75%)
Female	109 (100%)	54 (49.5%)	55 (50.5%)	15 (100%)	8/15 (53.3%)	7/15 (46.7%)

TABLE 3: Age and gender distribution of coinfection HCV-dengue cases.

Age (years)	Male		Female	
	<i>n</i>	%	<i>n</i>	%
0–10	2	12.5	3	20
11–20	7	43.7	5	33.4
21–30	5	31.3	5	33.4
31–40	1	6.25	1	6.6
41–50	1	6.25	1	6.6
51–60	0	0	0	0
61–70	0	0	0	0
71–80	0	0	0	0
Total	16	100	15	100
Median	23.5		23	
±SD	21 ± 9		20 ± 12	

a cross-sectional study. Calculations were done according to program PASW 18.

### 3. Results

We identified the presence of HCV in 12.9% (31/240) of the examined dengue patient samples. From the 31 DENV-HCV coinfecting patients analyzed here, 16 were males and 15 female patients, which indicate a similar distribution by gender, 15/109 (13.8%) in females and 16/131 (12.1%) in males; it does not show a significant statistical difference (Table 2).

Among the 16 coinfecting male patients, HD was predominant in 12 cases, while only 4 had DF (Table 2). Analyzing the most associated dengue form with HCV coinfection, we found that the bigger HCV infection percentage (61.29%, 19/31) was in HD patients, while only 12/31 (38.71%) was in DF cases (Table 2).

In this study, we found that the age group that presented greater coinfection frequency was that of young patients, between 11 and 20 years with 38.7% of cases, being 7/12 males and 5 females. Second, 10 out of 31 coinfecting patients (32%) were between 21 and 30 years with 5 cases from each sex. The group from 0 to 10 years of age included 16.13% of the total of coinfecting cases that were distributed 3:2 (girls:boys). The less affected patient group by coinfection dengue-HCV were adults from 30 years and older (Table 3). The relative risk of presenting HD was 1.60 bigger for males with HCV infection with respect to females. Although the dengue patients were between 2 months and 68 years of age, with a mean of 15 ± 14 years, coinfection DENV-HCV cases were mainly presented

in patients between 10 and 47 years of age (mean 21 ± 9) for males and 20 ± 12 (from 11 months to 46 years) for females (Table 3).

### 4. Discussion

The seroprevalence of HCV infection has been reported in 1.5 to 3.5% in Mexico and Latin America [14, 17]. Therefore, the frequency of HCV coinfection in dengue patients found here clearly indicates an increase over the current estimation, as it was almost 13%. This suggests that some conditions may contribute to an increased susceptibility of hepatic damage in coinfecting patients, complicating the symptoms of either dengue or hepatitis C infection.

Although a bigger frequency of active HCV infection cases in patients with HD was observed, there was no statistical significance indicating a relationship between HCV infection and hemorrhagic manifestations during DENV infection, probably due to the sample size. Serologically, only one patient out of the 240 (0.4%) showed IgG antibodies to HCV.

The distribution of DENV-HCV coinfections found here indicates a predominance in HD male patients (12:7 M:F), as our original sample included 50%, 120, cases of DF and 50% of HD (Table 2). Besides, in the group of 16 sera from male patients that presented coinfection of DENV-HCV, 75% (12/16) corresponded to HD cases (Table 2). Despite the reduced sample size used in this research, a bigger association to double viral infection was evident in HD patients among all dengue male patients. These results are relevant considering

that, worldwide, there are around 100 million of DF cases by 500 000 of HD [5].

Data indicates the necessity of identifying if DENV infection may mask the HCV infection or vice versa, resulting in difficulties of clinical diagnosis or furthermore complications during patients care due to analgesic toxicity. It has been considered that HCV can play a potential role in the hepatic disfunction related to the dengue infection [24]. Hepatic affectation related to chronic HCV infection can be a synergistic factor contributing to the increase of hemorrhagic cases during dengue infection, especially in adults [25].

Although there are no statistically significant differences, perinatal HCV infection is more common in girls than boys [26]. Other studies have suggested that sexual or drug abuse would be more associated with males [17].

It is known that, in endemic zones, patients come into adulthood with immunity to all the different DENV serotypes [27, 28]. HCV-DENV coinfections were presented mainly in young patients, so they are the ones who require more attention during the dengue outbreaks, since they may present severe complications.

It is well known that chronic infections affect platelet counting directly [18, 19] and HCV infection may lead to hemorrhagic problems [29]. In patients with HCV infection, where liver and the coagulation system are altered, clinical dengue manifestations could be grave.

Thus, HCV infection represents an important risk factor for complications in patients that also have dengue infection.

The HCV diagnosis must be substantially improved in the local health systems, primordially in dengue endemic areas, and the HCV testing for all those suspected acute dengue patients should be included for clinical management and prophylaxis.

## Conflict of Interests

The authors declare that they have no conflict of interests.

## Acknowledgments

The authors would like to thank Biologist Carlos Marcial Baak Baak for his assistance in the statistical calculations. This work was supported in part by FOMIX de Yucatán, Mexico. Agreement no. YUC-C06-2008-108310. MIS and MECM were COFAA, IPN fellows. Also, MIS was EDI-IPN program fellow and MECM was EDD-IPN program fellow.

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