Original Article

A survey of clinical and laboratory characteristics of dengue fever epidemic from 2014 to 2018 in Guangzhou, China

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Background: In 2014, a serious dengue outbreak occurred in Guangzhou, South China. In this study, the clinical and laboratory characteristics of dengue fever (DF) group and other febrile illnesses (OFI) group in Guangzhou were described.

Methods: Clinical and laboratory data collected by studying 1,792 patients from Nanfang Hospital, Southern Medical University during 2014 and 2018. Laboratory data was analyzed by SPSS 22.0 statistical software including Full blood counts (SYSMEX XE-5000), Laboratory Biochemical tests (Roche Cobas 8000), Dengue virus RNA (RT-PCR-Fluorescence Probing) and Dengue IgG/IgM Antibody (Colloidal Gold), Dengue Virus NS1 (ELISA).

Results: In the DF group and OFI group, gender ratios were 1.08:1 (male/female, P>0.05) and 1.45:1 (male/female, P<0.05). Adults aged 25–34 years old (29.4%) with the main peak appeared in the DF group, and the same main peak appeared in the OFI group: 25–34 years old (25.13%). Patients were from Medical emergency (41.2% DF group, 29.4% OFI group). The distribution of fever days before treatment was mainly focused within 5 days, with a main peak in the 2 fever days before treatment (24.6%) in the DF group and the main peak in 1 fever day before treatment (46.9%) in OFI group. The major symptoms of the DF group were presented with were fever (100%), myalgia (34.77%), pharyngeal hyperemia (31.33%), headache (25.65%), adenoids (19.62%), and rash (13.25%). In the OFI group, Pharyngeal hyperemia was the most common clinical symptom, accounting for 27.24%, and the next symptom was adenoids (21.26%). The sensitivity and specificity of DV RNA were 61.54%, 100%, respectively, compared to the DF Nonstructural protein 1 (NS1). Dengue virus (DENV) Immunoglobulin M (IgM) IgM in both groups was statistically significant, with DENV-IgM in the DF group were stronger (Z=–7.863, P<0.001), and DENV immunoglobulin G (IgG) were no statistically significant (Z=–1.212, P=0.226). In DF group, 37.14% of serum samples had elevated Alanine transaminase (ALT) levels, 76.85% of them had elevated aspartate aminotransferase (AST) levels, 32.08% of them had elevated creatine kinase (CK) levels, and 2.67% of them had elevated C-reaction protein (CRP) levels, compared with 13.51% of them had elevated ALT levels, 30.65% of them had elevated AST levels, 6.06% of them had elevated CK levels and 69.35% of them had elevated CRP levels of the OFI patients. The prominent manifestations were thrombocytopenia (occurring in 28.07% of the DF group, compared to 5.18% of OFI group) and leucopenia (occurring in 43.27% of DF group and 3.63% of OFI group). The DF incidence of all fever cases was 49.0% within three months in 2014, compared with 1.4% in 2015, 0% in 2016, 0.9% in 2017 and 6.4% in 2018 (P<0.001). DF and OFI

can occur in any age and sex. DF occurred in the young and the old, OFI occurred in children and youth. The clinical symptoms of myalgia, headache, rash, weak, Chills, follicular hyperplasia in both groups were statistically significant (P<0.001).

Conclusions: IgM can be easily recognized for early diagnoses, DENV-RNA had lower sensitivity and higher specificity, and DF NS1 enzyme-linked immunosorbent assay (ELISA) has a higher sensitive and specificity. DF is a serious public health problem and an emerging continuous threat in Guangzhou. In high-prevalence areas, effective epidemic monitoring and prevention measures need to be undertaken. After the unprecedented outbreak in 2014, on account of the government and citizen paying more attention to the DF epidemic, the cases of DF were decreased significantly from 2015 to 2018.

Keywords: Dengue fever (DF); other febrile illnesses (OFI); diagnostics; enzyme-linked immunosorbent assay (ELISA); RNA; Nonstructural protein 1 (NS1)

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Introduction

Dengue, mosquito-borne disease and primarily transmitted to humans by the female Aedes mosquitos (1), is the second-most globally prevalent vector-borne disease in the world, only behind malaria in terms of morbidity and mortality (2). It is estimated that dengue fever (DF) incidence has increased 30-fold over the last 50 years (3). The rapid increase of DF incidence has become severe public health, threating approximately half of the world's population, especially in Southeast Asia, the west Pacific Ocean regions, and southern Africa, which due to geographical environment, population density, and other factors (4). DENV is a member of the genus Flavivirus, family Flaviviridae, positive-strand RNA viruses, which is a single-stranded, positive-sense, RNA virus with a genome of about 11 kb (5,6). The causative agents of dengue disease are antigenically divided into four distinct serotypes, DENV1 through DENV4 (7). Infection with DENV results in varying degrees of pathological conditions, ranging from mild asymptomatic DF to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which may turn fatal (8). The first exposure of an individual to any of the four dengue virus serotypes is known as primary dengue infection, with high titers of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies appearing in 3–5 and 6–10 days respectively, after the onset of infection (1). A secondary infection, with a previously unencountered DENV serotype, usually results in classical DF, while 23% of cases develop into DHF or progress to DSS and death (1).

A key role in dengue therapy and control of the dengue epidemic is an early diagnosis of DF. Until now, the routine diagnostic methods to confirm DF include a combination of clinical dengue symptoms, viral isolation, viral RNA detection, and serological assays (9). However, none of these methods are sufficiently sensitive and specific to be used as a stand-alone diagnostic tool (10). Although Virus isolation was the "gold standard" for the diagnosis of DF, it was time-consuming, required cell facilities, and yielded low detection rates (11). RNA and other molecular tests have been widely applied for the rapid detection and identification of dengue virus serotypes in clinical diagnosis. However, in many poorly equipped laboratories of China, molecular diagnostics is still not possible. Other serological assays, including detection of IgM/IgG, are the most commonly used methods because of smooth operation, inexpensive, and saving time, with poor sensitivity and specificity. Nonstructural protein 1 (NS1) is a glycoprotein secreted by DENV infected mammalian cells and is essential for viral replication and viability (12). It has been found that the utility of the NS-1 antigen has underlined its importance in the early stages of DENV infection because the NS1 antigen is detectable in blood from the first day after the onset of fever up to Day 9 (13), is found earlier than IgM (14). So NS1 tests enzyme-linked immunosorbent assay (ELISA) may be a better choice for DENV detection (15).

In mainland China, DF is a severe infectious disease by the Ministry of Health of China, and sporadic dengue cases have been reported in Guangdong, Zhejiang, and Fujian, which are in Southeast China (16). Guangzhou, the capital of Guangdong Province, with the highest population density of more than 1,800 persons per square kilometer, is a typical city that has experienced annual DENV transmission,

Table 1 Epidemiological characteristics of the enrolled patients (n=1,792)

Characteristics	DF	Other febrile illnesses (OFI)	Total
No. of patients	602	1,190	1,792
Median age (range) in years	32 (1 day–81 years)	27 (5 months–89 years)	29 (1 days–89 years)
Gender ratio (male/female)	1.08/1 (312/290)	1.45/1 (705/485)	1.31/1

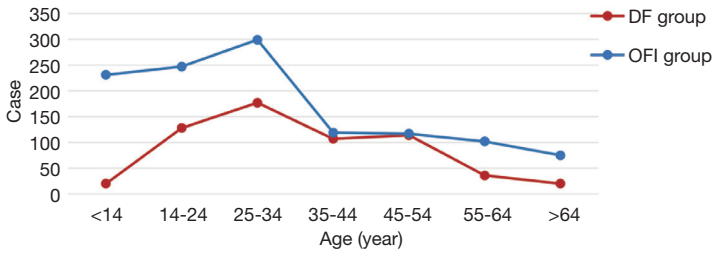

Figure 1 Age composition of the cases reported in the dengue outbreak in Guangdong from 2014 to 2018.

accounting for more than 50% of the DENV cases in China (6,17). In July 2014, one of the most significant outbreaks of dengue occurred in Guangzhou since 1986, providing great concern of the society. The reasons for the dengue epidemic are complex. In the study, we will investigate the probable origin of dengue in Guangzhou, China.

Methods

Clinical information included age, gender, Fever days before treatment, other epidemiological characteristics, and samples were collected from patients at Nanfang Hospital, Southern Medical University, from 2014 to 2018 in Guangzhou, China. Full blood counts were completed by automatic blood cell analyzer (SYSMEX XE-5000) for all patients. Detection Kit analyzed laboratory diagnostic tests including DENV-RNA, DENV-IgM/IgG, NS1 antigen detection for Dengue virus RNA (RT-PCR-Fluorescence Probing, Hua yin Medical Technology Co Ltd, Guangzhou, China), Diagnostic Kit for Dengue IgG/IgM Antibody (Colloidal Gold, Wondfo Biotech, Guangzhou, China) and Diagnostic Kit for Dengue VirusNS1 (ELISA, WANTAI BioPharm, Beijing, China). Laboratory Biochemical tests were analyzed by Cobas 8000 (Roche Diagnostics GmbH).

Results

Description of the outbreak

A total number of 1,792 of patients were included in our study, from which 602 cases were classified as DF group and 1,190 cases as other febrile illnesses (OFI) group. In the DF group, it consisted of 51.8% male patients which gender ratio was 1.08:1 (male/female, P>0.05), with the median age, was 32 years old (range, 1 day to 81 years). In the OFI group, it consisted 59.2% of male patients in which gender ratio was 1.45:1 (male/female, P<0.05), with the median age 27 years old (range, 5 months to 89 years). 87.38% of patients were in the 14–54 years age group of DF group, the main peak appeared in the 25–34 years age group (29.40%). 65.3% of patients were in 0–34 age group of OFI group, with three main peak: 0–14 years old (19.41%), 15–24 years old (20.76%) and 25–34 years old (25.13%) (*Table 1*, *Figure 1*).

Cases from different departments

In the DF group, patients were from fever outpatient 215 cases (35.7%) and medical emergency 248 cases (41.2%). In the OFI group, suffers were from fever outpatient 189 cases (21.1%), medical emergency 263 cases (29.4%), and 189 cases of pediatric outpatient (21.1%, P<0.001), shown in *Figure 2*.

Fever days before treatment

The distribution of fever days before treatment was shown in *Figure 3*, mainly focused within 5 days of fever days, which accounted for 89.10% in the DF group and accounted for 91.70% in the OFI group. In the DF group,

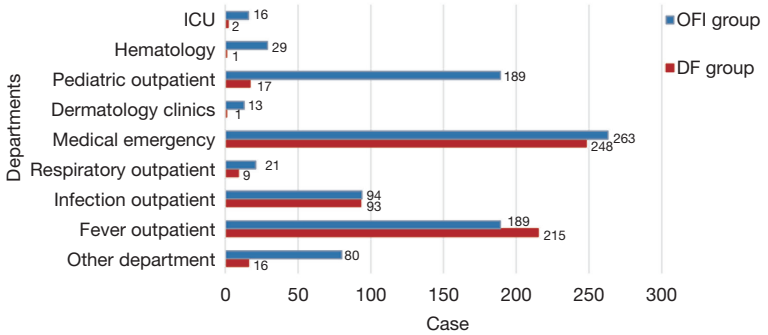

Figure 2 Cases of DF group and OFI group from different departments in Nanfang Hospital, Southern Medical University, from 2014 to 2018. DF, dengue fever; OFI, other febrile illnesses.

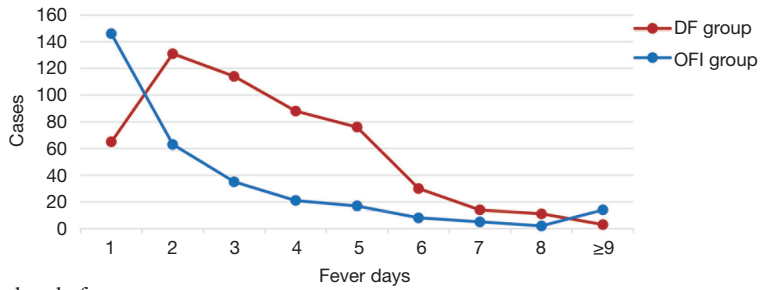

Figure 3 Distribution of fever days before treatment.

the main peak appeared in the 2 fever days before treatment (24.60%), and in the OFI group, patients with the main peak in 1 fever day before treatment (46.90%).

Clinical symptoms

The clinical symptoms in both of DF group and OFI group mainly were typical or mild manifestations with upper respiratory tract infection, as pharyngeal hyperemia and adenoids. The primary symptoms of the DF group were presented with were fever (100%), myalgia (34.77%), Pharyngeal hyperemia (31.33%), headache (25.65%), adenoids (19.62%), and rash (13.25%). The results of clinical symptoms were presented in *Table 2*, and the clinical symptoms of myalgia, headache, rash, weak, chills, follicular hyperplasia between the DF group, and OFI group were with P value less than 0.001. In the DF group, myalgia was the most common clinical symptom, accounting for 34.77%, with 7.14% in the OFI group. In the OFI group, Pharyngeal hyperemia was the most common clinical symptom, accounting for 27.24%, and the next symptom was adenoids (21.26%) (*Table 2*).

Laboratory diagnostic tests

In the experiment, 42 paired serum samples were collected to detect both DF-NS1 and RNA, and 356 paired serum samples were collected to detect both DF-NS1 and DENV-IgM/IgG during the acute stage of illness. Among the 39 cases with DF-NS1 positive, DF-RNA, with 24 positive cases and 15 negative cases. The other 4 cases detected by DF-NS1 and DF-RNV were both negative. The sensitivity and specificity of DF-RNA were 61.54% and 100%, respectively, compared to the DF-NS1.

DENV-IgM in both groups was statistically significant, with DENV-IgM in the DF group were stronger ($Z=-7.863$, $P<0.001$), and DENV-IgG were no statistically significant ($Z=-1.212$, $P=0.226$). Among the 284 DF-NS1 positive cases, IgM antibodies were detected from 162 cases, and IgG antibodies were detected from 24 cases. Among 72 cases with DF-NS1 antigen-negative, IgM and IgG antibodies were detected from 3 cases, which had lower sensitivity (IgM 57.04%, IgG 8.45%) and high rate of omission diagnostic (IgM 42.96%, IgG 91.55%), compared to the DF-NS1 (*Tables 3-5*).

Table 2 Clinical symptoms of DF group and OFI group

Symptoms	DF group, N=581 (%)	OFI group, N=602 (%)	P
Chills	61 (10.50)	35 (5.81)	0.001*
Rhinobyon	9 (1.55)	20 (3.32)	0.071
Rash	77 (13.25)	20 (3.32)	<0.001*
Pharyngeal hyperemia	182 (31.33)	164 (27.24)	0.025
Headache	149 (25.65)	46 (7.64)	<0.001*
Myalgia	202 (34.77)	43 (7.14)	<0.001*
Sore throat	57 (9.81)	43 (7.14)	0.048
Follicular hyperplasia	20 (3.44)	4 (0.66)	<0.001*
Dizzy	38 (6.54)	18 (2.99)	0.002
Weak	68 (11.70)	15 (2.49)	<0.001*
Vomiting	25 (4.30)	12 (1.99)	0.013
Lumbago	10 (1.72)	1 (0.17)	0.004
Hemorrhage (bleeding spots under the skin)	10 (1.72)	2 (0.33)	0.013
Nausea	21 (3.61)	10 (1.66)	0.023
Running nose	29 (4.99)	33 (5.48)	0.899
Bleeding gums	2 (0.34)	0 (0.00)	0.226
Conjunctival congestion	2 (0.34)	0 (0.00)	0.226
Pruritus	1 (0.17)	1 (0.17)	1.000
Arthralgia	1 (0.17)	5 (0.83)	0.259
Adenoids	114 (19.62)	128 (21.26)	0.874
Poor appetite	5 (0.86)	2 (0.33)	0.374
Diarrhea	14 (2.41)	10 (1.66)	0.286
Convulsion	1 (0.17)	0 (0.00)	0.476
Shiver	4 (0.69)	3 (0.50)	0.714

*, P value of these clinical symptoms less than 0.001. DF, dengue fever; OFI, other febrile illnesses.

Table 3 Comparison of dengue virus NS1 antigen detection by ELISA and viral RNA detection by PCR

DF-NS1 detection (n)	DF-RNA detection (n)		Total	P
	Positive	Negative		
Positive	24	15	39	0.018
Negative	0	4	4	–
Total	24	19	43	–

NS1, Nonstructural protein 1; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction.

Table 4 Comparison of DF-NS1 antigen detection by ELISA and DENV-IgG detection by Colloidal Gold

DF-NS1 detection (n)	DENV-IgG detection (n)		Total	P
	Positive	Negative		
Positive	24	260	284	0.026
Negative	3	69	72	–
Total	27	329	356	–

DF, dengue fever; NS1, Nonstructural protein 1; ELISA, enzyme-linked immunosorbent assay; DENV, dengue virus; IgG, immunoglobulin G.

Table 5 Comparison of DF-NS1 antigen detection by ELISA and DENV-IgM detection by Colloidal Gold

DF-NS1 detection (n)	DENV-IgM detection (n)		Total	P
	Positive	Negative		
Positive	162	122	284	<0.001
Negative	3	69	72	–
Total	165	191	356	–

DF, dengue fever; NS1, Nonstructural protein 1; ELISA, enzyme-linked immunosorbent assay; DENV, dengue virus; IgM, immunoglobulin M.

Laboratory biochemical tests

In DF group, 39 (37.14%) of 105 cases had elevated ALT levels, 83 (76.85%) of 108 cases had elevated AST levels, 17 (32.08%) of 53 cases had elevated CK levels and 32 (42.67%) of 75 cases had elevated CRP levels, compared with 5 (13.51%) of 37 cases had elevated ALT levels, 19 (30.65%) of 62 had elevated AST levels, 2 (6.06%) of 33 cases had elevated CK levels and 43 (69.35%) of 62 cases had elevated CRP levels of the OFI patients. AST, ALT, CK, and CRP levels were significantly higher in the DF group than the OFI patients (P<0.01). In both groups, no significant difference in other cardiac function tests, such as LDH, α-HBDH, CK-MB, and other biochemical tests, including TP, ALB, PCT. In the DF group, AST levels are higher than ALT levels (*Table 6*).

Full blood count

The prominent manifestations were thrombocytopenia (occurring in 28.07% of the DF group, compared to 5.18% of OFI group) and leucopenia (occurring in 43.27% of the DF group and 3.63% of OFI group). From the data in our stud, 44.44% had decreased LYM%, 30.21% had elevated NEU% levels, 54.58% had elevated MONO%, and 7.41%had decreased Hgb had decreased PLT in DF group, compared with 54.40% decreased LYM%, 45.60% elevated NEU%, 17.10% elevated MONO%, and 19.17% decreased Hgb in OFI group. In all indicators of full blood count, WBC, LYM%, NEU%, MONO%, Hgb, PLT were statistically significant between the DF group and OFI group (P<0.001) (*Table 7*).

Annual incidence

From October to December 2014, DF incidence of all fever cases was 49.0% (584/1,193), compared with 1.4% (3/219) in 2015, 0% (0/50) in 2016, 0.9% (1/110) in 2017 and 6.4% (14/220) in 2018 (P<0.001) (*Figure 4*).

Discussion

During 2014, the largest dengue epidemic occurred in Guangdong province in the past 25 years, China (18). Especially in Guangzhou, accounting for 90% of all of the reported dengue cases occurred in this outbreak (statistics from Guangdong province Health and Family Planning Commission) (17).

On the one hand, the causes of the massive dengue outbreak were unknown, but there are some factors associated with it, such as climate change, globalization, travel, and trade (19). Firstly, Guangzhou is the most densely populated city in southern China, surrounded by many dengue-endemic countries. It has a humid subtropical climate influenced by the Asian monsoon (20). Except

Table 6 Biochemical tests of DF group and OFI group

Biochemical tests	Range	Total	DF group	OFI group	Statistics	
					Z	P
ALT	↑	44	39	5	–3.000	0.003
	Normal	96	66	30		
	↓	2	0	2		
AST	↑	102	83	19	5.938	<0.001
	Normal	67	25	42		
	↓	1	0	1		
TP	↑	0	0	0	0.364	0.716
	Normal	62	42	20		
	↓	42	27	15		
ALB	↑	0	0	0	0.418	0.676
	Normal	53	36	17		
	↓	50	32	18		
LDH	↑	52	31	21	–0.472	0.637
	Normal	34	22	12		
	↓	0	0	0		
αHBDH	↑	55	32	23	–0.921	0.357
	Normal	30	20	10		
	↓	1	1	0		
CK	↑	19	17	2	–2.625	0.009
	Normal	61	33	28		
	↓	6	3	3		
CK-MB	↑	16	6	10	–2.187	0.029
	Normal	70	47	23		
	↓	0	0	0		
PCT	↑	33	28	5	–2.160	0.551
	Normal	1	0	1		
	↓	0	0	0		
CRP	↑	75	32	43	–3.112	0.002
	Normal	62	43	19		
	↓	0	0	0		

↑, indicates the results with a higher upper limit of the reference range; ↓, indicates the results below the lower limit of the reference range. DF, dengue fever; OFI, other febrile illnesses.

Table 7 Full blood count of DF group and OFI group

Full blood count	Range	Total	DF group	OFI group	Statistics	
					Z	P
WBC	↑	97	13	84	−13.993	<0.001
	Normal	554	452	102		
	↓	229	222	7		
LYM%	↑	32	22	10	−4.729	<0.001
	Normal	515	437	78		
	↓	333	228	105		
NEU%	↑	243	155	88	−5.520	<0.001
	Normal	583	490	93		
	↓	54	42	12		
MONO%	↑	313	280	33	−6.057	<0.001
	Normal	555	399	156		
	↓	12	8	4		
EOS%	↑	1	1	0	−0.778	0.436
	Normal	492	379	113		
	↓	387	307	80		
BASO%	↑	39	35	4	−1.709	0.087
	Normal	871	682	189		
	↓	0	0	0		
RBC	↑	48	32	16	−0.475	0.635
	Normal	809	641	168		
	↓	23	14	9		
HgB	↑	20	15	5	−5.117	<0.001
	Normal	785	634	151		
	↓	75	38	37		
PLT	↑	13	1	12	−6.421	<0.001
	Normal	713	542	171		
	↓	154	144	10		

↑, indicates the results with a higher upper limit of the reference range; ↓, indicates the results below the lower limit of the reference range. DF, dengue fever; OFI, other febrile illnesses.

for the influence of temperature, which was be found positively associated with dengue incidence, there are other atmospheric factors, such as relative humidity and wind velocity may also increase the risk of DF infection (21). Secondly, Guangzhou is one of the most important international financial centers in Southeast Asia because of globalization. Increasingly overseas commercial investors, business people, laborers, and foreign tourists have led to more international exchanges in this region, which could increase the risk of imported DF cases from endemic areas (6,22). It was reported that the main reasons for the pattern of the serve outbreak in 2014 were related to the

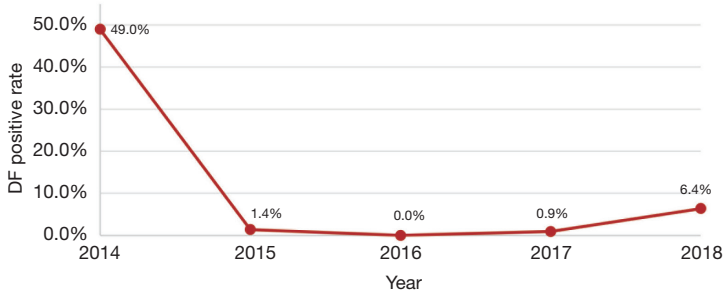

Figure 4 DF positive rate from 2014 to 2018. DF, dengue fever.

date of the first imported case, unusually high precipitation in 2014, interventions, and vertical transmission (23). Thirdly, Guangzhou has a current population of more than 10 million, which has a vast mobile population city in China. The migration increased in the susceptible population promotes the transmission of infectious diseases and creates significant challenges to prevent and control dengue endemic (2,24). Finally, the rapid development of urbanization and construction in this region may have also contributed to the transmission of dengue. On the other hand, human factors contribute to the risks of morbidity and the spread of dengue, including both urban and rural settlement patterns (20). Recent data suggests that dengue vaccines are at a crossroads even with modest efficacy (25,26), so the only effective way to prevent DF is controlling mosquito vector.

This study showed that DF could occur in any age and any sexes. Males were found to be affected by DF slightly more than females in our study, but this difference was not statistically significant (P>0.05). Initially, dengue infection was thought to be a disease of children, but it has been reported that the elderly had a comparatively higher incidence of acquiring dengue infection (27). But in this study, adults aged 25–34 (29.40%) years with the main peak, and youngster aged 14–24 (21.26%) with a secondary peak while the elderly aged 45–54 (18.94%) with a third peak appeared in DF group; This finding is in general agreement with other studies (17,28,29). It may be the youngsters and adults have a larger crowd size, like to spend more time outdoors sports and ignore the protection of mosquito biting. And the aged 45–54 years like planting flowers in household courtyards, which provides many containers with water around the house, helping mosquito breeding (22). All of these factors may result in the middle-aged and elderly more susceptible to dengue infection. It was reported that the elderly infected with the dengue virus appears to be more likely to develop severe illness (30). In the OFI group, adult aged 25–34 (25.13%) years are the highest incidence, and the aged 14–24 (20.76%) years with a secondary peak while child aged less than 14 (19.41%) years with a third peak, who are prone to upper respiratory tract infection because of low immunity, while adults will pay more attention to the prevention of child mosquito bites which may reduce the incidence of DF infection.

From the distribution of specimens for inspection departments, patients are from Fever outpatient, and Medical emergency indicated that the symptoms in the DF group and the OFI group are characterized by acute onset. In the OFI group, the proportion of pediatric outpatients 189 cases (21.10%) was significantly higher than the DF group, which guided clinical diagnosis: when the child has a fever may be a non-dengue infection. According to our data statistics, we found that approximately 90% of all fever patients had gone to the hospital within 5 fever days before treatment, but in the OFI group fever days before treatment was significantly earlier than the DF group, with 46.90% patients mainly focused within 1 day of fever days. The patients of the OFI group were primarily for children whose parents paid more attention to their illness, for they usually with poor tolerance to fever.

The clinical symptoms in all fever patients mainly were typical or mild manifestations with upper respiratory tract infection, as pharyngeal hyperemia and adenoids. However, the clinical symptoms, including myalgia, headache, rash, weak, chills, follicular hyperplasia, were with P<0.001 between the DF group and OFI group. It was thought that myalgia could be a specific sign of clinical symptoms for DF, which was the most common clinical symptom in the DF group.

In our study, we used three methods for the diagnosis of DF, including DF-RNA, DENV-IgM, and IgG, DF-NS1. The test of DENV-IgM and IgG was the most rapid and convenient serological technique, which had lower

sensitivity (IgM 57.04%, IgG 8.45%) and a high rate of omission diagnostic (IgM 42.96%, IgG 91.55%), compared to the DF-NS1 test. Therefore, only the detection of IgM and IgG antibodies may miss a lot of DF diagnoses. IgM and IgG antibodies persisted in the serum for more than 3 months, which could be a limiting factor in confirmatory diagnosis. The detection of DENV-IgG was not considered authentic, which cross-reactivity with other closely related members of flaviviruses (9). Some studies founded that DENV-IgM lever increased highly in primary infections 3–5 days after the onset of fever, which levels peaked in the serum about two weeks after the onset of symptoms, and DENV-IgG increased 1–2 days rapidly after the onset of fever (31). It was thought that DENV-IgM tests were more suitable for early diagnosis of DF, and the level of DENV-IgG was used to find a secondary infection by IgG needing a >fourfold rise in titer in paired acute and convalescent sera. The sensitivity and specificity of DF-RNA were 61.54% and 100%, respectively, compared to the DF-NS1 test. In all specimens, 38.5% cases (15/39) were DF-NS1 positive but negative for DF-RNA, and this rate was like a previous report (37%) (32). It may be half-life period of NS1 protein was longer; the NS1 circulating in a patient's blood is longer periods than viral RNA (33). So NS1 antigen remained positive which affords a valuable diagnostic test after DENV-RNA amplicon disappeared (34). Besides, there may be false-positive results of the detection of DF-NS1. Compared to virus isolation and RT-PCR methods, they offer a cheaper and more convenient option that only requires basic technical training (35). Particularly during the early stage of infection before the induction of a humoral immune response to the virus (34), NS1 antigen immunoassays is a rapid diagnostic test and is also effective and its ability to detect the dengue virus infection early in the course of disease helps in opting for appropriate treatment and management planning (13). And now, NS1 antigen detection kits are commercially available, although it cannot differentiate DENV serotypes. Some studies suggested that different diagnostic methods should be selected according to patient's onset time: detections combined DF-RNA with NS1 will be more suitable for acute phase (days 1–4), and detections combined DF-NS1 with IgM could increase the detection rate for early convalescence (days ≥5) (11).

According to Biochemical data, liver injury was a significant risk factor for DENV infections (36). In the DF group, AST, ALT, CK, and CRP increased significantly ($P<0.01$), and AST levels are higher than ALT levels, compared with the OFI group. During dengue virus infections, the pathogenesis of liver involvement is still poorly understood (37). Several mechanisms may be involved in dengue virus inducing liver cell apoptosis. These include direct cytopathic effects of virus or host immune response on liver cells, circulatory compromise, and/or hypoxia caused by hypotension or localized leakage inside the liver capsule and the influence of cellular and humoral immune factors in the liver (37,38). The prominent manifestations were thrombocytopenia (occurring in 28.07% of DF group, compared to 5.18% of OFI group) and leucopenia (occurring in 43.27% of DF group and 3.63% of OFI group). Dengue virus caused DF and DHF, and the major pathophysiological hallmark was increased vascular permeability. In DHF, following plasma leakage caused by increased vascular permeability, hypovolemic shock occurred. Constant hematological abnormalities occurring in DHF and often include bone marrow suppression, leucopenia, and thrombocytopenia (39).

After the severe outbreak in 2014, the government paid more attention to the early detection of imported cases, early mosquito control, and the quarantine of every suspicious case (23). Our data showed that the morbidity of the DF from 2015 to 2018 significantly decreased compared to 2014. Up to date, dengue infection occurred in 2016 and 2017 were less, but an uptrend appeared in 2018 (40). However, because of the subtropical zone, substantial floating population, terrible community environmental conditions, we need to pay more attention to the preventive measures of the DF. On the one hand, there is not an effective anti-DENV vaccine formulation approved for use in humans due to the variety of serotypes and complicated pathogenesis (41). Relevantly, Sanofi Pasteur dengue vaccine Dengvaxia has now been licensed in a few countries, but it recorded poor efficacy in dengue naïve individuals during phase III evaluation (1), which lacks the critical dengue T cell epitopes of the nonstructural region and dengue NS1 that both play vital roles in providing protection against dengue (42-44).

On the other hand, treatment is usually based on symptoms and is performed through medical support since there is no antiviral drug available for dengue. Therefore, the primary way to avoid DF is that community residents, including the individual, need to take protective measures, including clothing, repellents, insecticide-treated mosquito nets, and household fixtures, to avoid biting by mosquitoes. Simultaneously, maintenance of environmental hygiene is considered to be an effective way of dengue prevention and control, including "cleanup" campaigns, regular container

emptying and cleaning, installation of water supply systems, solid waste management, and so on (12). Elimination of the breeding places of the mosquitoes, the use of larvicides, and the use of ultralow-volume aerosolized adulticides are the critical point for preventing and controlling DFs (45). The government needs to publicize the knowledge of DF in the community. The participation by the community can be of immense importance in winning the battle against vector mosquitoes (2,3).

Meanwhile, the detection and diagnosis of dengue infection should be strengthened with the identification of viral genomic RNA, antigens, or antibodies. Additionally, the communication and cooperation between the quarantine authorities and the tourism authorities should be enhanced to guarantee no more imported cases. Last but not least, it is significant to develop an alternative dengue vaccine candidate who would enable higher efficacy and applicability to a broader group of subjects, including infants and naïve populations.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by Medical Ethics Committee of Nanfang Hospital, Southern Medical University.

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