Research article

Clinical features and transmission risk analysis of dengue virus infections in Shenzhen, During 2014–2019

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ABSTRACT

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are among the most common tropical diseases affecting humans. To analyze the risk of clinical and transmission of DF/DHF in Shenzhen, the surveillance on patients of all-age patients with dengue virus (DENV) infections was conducted. Our findings revealed that the majority of DENV-infected patients are young to middle-aged males, and the development of the disease is accompanied by abnormal changes in the percentages of neutrophils, lymphocytes, and basophils. Demographic analysis revealed that these patients is concentrated in areas such as Futian District, which may be due to the higher mosquito density and temperature than that in other area. Subsequent, mosquito infection experiments confirmed that the effect of temperature shift on DENV proliferation and transmission. Not only that, constant temperatures can enhance the spread of DENV, even increase the risk of epidemic. Thus, the role of innate immune response should be highlighted in the prediction of severe severity of DENV-infected patients, and temperature should be taken into account in the prevention and control of DENV.

Introduction: Dengue fever (DF) and dengue hemorrhagic fever (DHF) are among the most common tropical diseases affecting humans, and which caused by the four dengue virus serotypes (DENV 1–4).

Objectives: To analyze the risk of clinical and transmission of DF/DHF in Shenzhen.

Methods: The surveillance on patients of all-age patients with dengue virus (DENV) infections was conducted.

Results: Our findings revealed that the majority of DENV-infected patients are young to middle-aged males, and the development of the disease is accompanied by abnormal changes in the percentages of neutrophils, lymphocytes, and basophils. Demographic analysis revealed that these patients is concentrated in areas such as Futian District, which may be due to the higher mosquito density and temperature than that in other area. Subsequent, mosquito infection experiments confirmed that the effect of temperature shift on DENV proliferation and transmission. Not only that, constant temperatures can enhance the spread of DENV, even increase the risk of epidemic.

Conclusion: 1. Elevated levels of neutrophils, lymphocytes, basophils, and temperature are all significant risk factors for dengue transmission and pathogenesis; 2. Temperature increasing is associated with a higher risk of dengue transmission; 3. Fluctuations in temperature around 28 °C (28 ± 5 °C) would increase dengue transmission.

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1. Introduction

DF/DHF is an arthropod-borne viral disease caused by the four DENV serotypes (DENV 1–4), which are transmitted by *Aedes mosquitoes*. Over the past 50 years, its incidence has increased by a staggering 30-fold [1]. Annually, tens of millions of cases are reported worldwide, and the rate of DENV infections is rapidly rising [2]. With the ongoing processes of globalization and global warming, DF/DHF is becoming increasingly prevalent across the globe [3]. Moreover, the risk of DENV infection is significantly underestimated, particularly in countries such as India, Indonesia, Brazil, and Africa [2], and approximately half of the global population is exposed to DENV endemic areas [4]. Severe cases of DENV infection are characterized by plasma leakage, severe hemorrhage, and organ failure, potentially leading to fatality [5]. Unfortunately, the development of a dengue vaccine faces significant challenges due to the antibody-dependent enhancement effect of DENV infection [6,7]. Currently, there are no specific drugs available for DENV-infected patients. Although, the FDA has approved the tetravalent dengue vaccine DENGVAXIA in 2019, it was only efficacy in individuals with prior DENV infection. Consequently, predicting the severity of DF/DHF cases and implementing effective control measures for DENV transmission are crucial for and treatment prevention strategies.

The key of preventing severe illness is to predict early risk factor in patients. Patients developing complications have the common underlying feature of increased capillary permeability leading to plasma leakage into the interstitial space. This typically occurs in days 5–7 of fever and lasts for 48–72 h known as the "critical phase" [8]. Proper fluid management during this phase is crucial to prevent complications. In clinical settings with limited resources, the onset of the critical phase is estimated by monitoring serial changes in hematocrit [9]. In more well-equipped larger hospitals, serial ultrasound scans are employed to detect fluid leakage into the body cavities. Unfortunately, both these methods detect plasma leakage only after it has already occurred, rather than predicting it beforehand. The innate immune response, including macrophages, dendritic cells, granulocytes, and mast cells are the first line for resisting the infectious agents. These cells are capable of recognizing and differentiating between physicochemical motifs expressed on microbes, and quickly adapting their antimicrobial defenses accordingly [10]. Therefore, identifying the status of the innate immune response during dengue infection is of great significance for early diagnosis and disease progression analysis in patients.

It's main measures for preventing and controlling infectious diseases that protecting susceptible populations and blocking the route of transmission. The primary mode of transmission for DENV is through the bite of *Aedes aegypti* or *Aedes albopictus* mosquitoes, which are considered the major risk factors [11]. Specifically, *Ae. albopictus* is the most significant DENV vector in China and is widely distributed in the southern region, particularly in Guangdong province [12]. The vector competence and transmission capacity of mosquitoes are known to be influenced by various factors [13]. And we already know in the previous work that temperature is one of the most crucial environmental factors affecting mosquito vector competence for DENV [14], but we don't yet know how temperature affects.

To assess the clinical risks associated with dengue fever and factors affecting the ability of mosquitoes to transmit DENV. We conducted a comprehensive prospective cohort study involving patients clinically diagnosed with dengue fever. Additionally, we performed in vivo and in vitro infection experiments with the DENV. Through rigorous analysis, we identified key factors influencing disease progression in patients with DENV infection, and confirmed the important role of temperature in the transmission of DENV by mosquitoes. This study not only elucidates the risk factors associated with severe cases of DENV infection and the spread of the virus, but also provides a valuable theoretical foundation for the treatment of dengue syndrome and the control of DENV transmission.

2. Materials and methods

2.1. Study design and patients

This study involved a total of 396 patients with dengue fever (DF) or dengue hemorrhagic fever (DHF) who were admitted to The Third People's Hospital of Shenzhen from January 11, 2014 to April 26, 2019. DF/DSF patients were diagnosed based on the guidelines for the diagnosis and treatment of dengue(2nd edition, 2014) [15]. The enrolled patients were categorized into two groups based on the presence or absence of bleeding symptoms. Clinical characteristics of these patients were monitored throughout their hospitalization or after discharge. The study received ethical approval from the Ethics Committee of The Third People's Hospital of Shenzhen (2018010), and written informed consent was obtained from all subjects prior to their enrollment.

2.2. Mosquito rearing

Ae. albopictus mosquitoes (obtained from Chinese center for disease control and prevention, Beijing, China) were kept under controlled conditions at a temperature of 27 $\pm$ 1 °C, with a 12:12 light-dark cycle and a relative humidity of 70%. Adult mosquitoes were provided with a 10% glucose solution ad libitum.

2.3. Virus strain

The DENV-2 strain utilized in this study was initially isolated from a patient sample infected with DENV-2 and cultured in C6/36 cells. The C6/36 cells (ATCC, No.CRL-1660) were cultivated as monolayers in T75 flasks at a temperature of 28 °C in RPMI medium (Gibco), supplemented with 10% FBS and 2% tryptose phosphate broth (Gibco). The DENV-2 was propagated in C6/36 cells to produce viral material for further experimentation.

2.4. Mosquito infection by blood meal

Prior to a blood meal, female mosquitoes aged 5–7 days were starved for at least 16 h. For feeding, the mosquitoes were provided with defibrillated horse blood (obtained from Shanghai Yuanye Biological Technology Co., Ltd., Shanghai, China) mixed with DENV-2 using Parafilm and the Hemotek® membrane blood feeding system (Hemotek, Lancaster, UK). The feeding duration was 1 h under light conditions at a temperature of 24 °C and a relative humidity of 70%. After feeding, the mosquitoes were anesthetized using ice, and fully engorged females were selected and maintained at 28 °C. The glucose solution provided to the mosquitoes was refreshed every 1–2 days until 14 days post-infection (dpi).

The evaluation of Ae. albopictus vector competence for DENV-2 involved assessing the infection rate and population transmission rate, as described below [16]:

1. Infection rate: The percentage of mosquitoes that tested positive for the virus in their bodies (number of positive mosquitoes/total number of mosquitoes tested).
2. Transmission rate: The percentage of mosquitoes that tested positive for the virus in their heads (number of positive mosquitoes/total number of mosquitoes tested).

2.5. Viral infection in C6/36

DENV-2 (at a multiplicity of infection (MOI) of 1) was incubated with C6/36 cells for 1 h at a temperature of 28 °C with 5% CO_2. After the incubation period, the cells were rinsed once with PBS, and 1 mL of complete media was added. The cells were further incubated for 4 days at 28 °C with 5% CO_2. At specific time points, both the supernatant and the cells were collected for the plaque assay to determine the presence of

Table 1

The baseline characteristics of participants with DENV infection.

	Hemorrhage	Non-hemorrhage	p value ()
White blood cell count (10^9/L)	3.574 ± 2.523	3.356 ± 2.009	0.3395
Neutrophil count (10^9/L)	2.159 ± 2.12	1.793 ± 1.609	0.0513
Neutrophil ratio(%)	56.78 ± 17.14	51.77 ± 16.42	0.0032
Monocytes ratio(%)	12.11 ± 5.389	11.02 ± 4.624	0.0318
Monocytes count (10^9/L)	0.4119 ± 0.2769	0.3572 ± 0.2328	0.0333
Lymphocyte ratio(%)	29.7 ± 14.23	34.78 ± 13.25	0.0003
Lymphocyte count (10^9/L)	0.9572 ± 0.6698	1.123 ± 0.755	0.0232
Eosinophil count (10^9/L)	0.02432 ± 0.05082	0.04727 ± 0.08693	0.002
Eosinophil ratio (%)	0.742 ± 1.919	1.371 ± 2.357	0.0044
Basophil ratio(%)	0.6665 ± 1.198	1.065 ± 1.507	0.0045
Basophil count (10^9/L)	0.02205 ± 0.04085	0.03609 ± 0.05818	0.007
Red blood cell count (10^{12}/L)	4.786 ± 0.5859	4.828 ± 0.5411	0.4609
Hematocrit(fl)	40.97 ± 4.764	41.73 ± 3.883	0.0812
Hemoglobin concentration(g/L)	140.2 ± 18.13	142.8 ± 14.79	0.128
Mean red blood cell volume(fl)	85.88 ± 6.085	86.73 ± 4.824	0.123
Mean erythrocyte hemoglobin(pg)	29.38 ± 2.504	29.66 ± 1.897	0.2112
Mean corpuscular hemoglobin concentration(g/L)	341.9 ± 12.92	342 ± 12.48	0.9477
Standard deviation in red blood cell distribution width(fl)	40.11 ± 3.729	39.79 ± 2.708	0.3235
Coefficient variation of red blood cell volume distribution width (%)	12.85 ± 1.162	12.62 ± 0.9233	0.033
Platelet (10^9/L)	11.34 ± 5.543	10.48 ± 5.677	0.1315
Mean platelet volume(fl)	11.28 ± 1.111	11.45 ± 1.11	0.1332
Thrombocytocrit	0.1312 ± 0.06264	0.1185 ± 0.05118	0.0321
Platelet larger cell ratio (%)	35.19 ± 9.161	36.42 ± 8.845	0.1894
Immature granulocytes ratio(%)	0.5337 ± 0.5884	0.5065 ± 0.6284	0.662
Immature granulocytes count (10^9/L)	0.02097 ± 0.04369	0.01879 ± 0.03667	0.5923

Table 2

The hazard ratios for hemorrhage in participants with DENV infection.
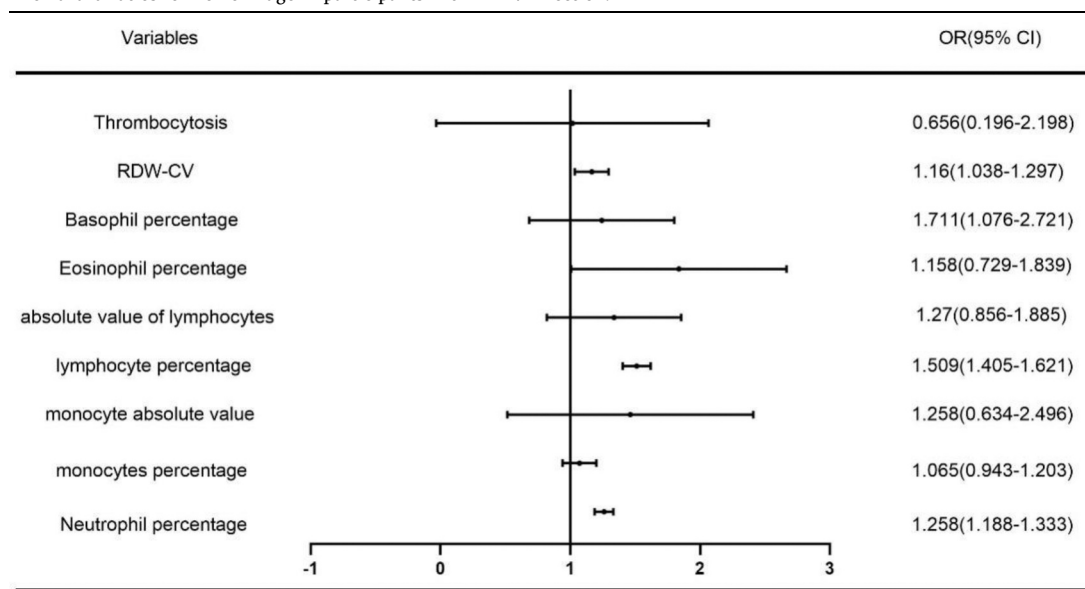
2.6. Viral RNA detection in mosquito

Total RNA was isolated using RNAiso Plus reagent (Takara, Dalian, Japan) according to the manufacturer's protocol and dissolved in 60 μL RNase-free water. Real-time PCR was performed using the One Step SYBR Prime ScriptTM PLUS RT-PCR Kit (TaKaRa, Japan) on a MyiQTM2 Optics Module (Bio-Rad, USA). DENV-2 detection as follows previous work [13]. DENV-2 detection primers are DENV-2 Forward-2:5′-tcccttacaaatcgcagcaac-3′and DENV-2 Reverse-2: 5′-tggtctttcccagcgtcaat-3′. AalRPS17 was used as the internal control for qRT-PCR. The primer sequences for AalRPS17 primers were AalRPS17 forward: 5′-acgtagttgtctctctgcgctc-3′ and AalRPS17 reverse: 5′-cgcttggtttcgtgacacatc-3′[17].

2.7. Statistical analysis

Statistical analysis (Student's t-test) was performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA). In all tests, the data represent the mean ± SEM. The results are analyzed using the unpaired t-test. A P value of < 0.05 indicates statistical significance. $P < 0.05$, *; $P < 0.001$, ** and $P < 0.001$, ***.

3. Result

3.1. The baseline characteristics of enrolled DF/DHF patients and establishment of risk prediction model of the plasma leakage

The baseline characteristics of all 396 patients shown that patients with bleeding had less neutrophils percentage (56.78 ± 17.14 vs 51.77 ± 16.42, p = 0.0032), monocytes count (0.4119 ± 0.2769 vs 0.3572 ± 0.2328, p = 0.0333), monocytes percentage (12.11 ± 5.389 vs 11.02 ± 4.624, p = 0.0318), red cell volume distribution width-coefficient of variation (RDW-CV) (12.85 ± 1.162 vs 12.62 ± 0.9233, p = 0.033) and thrombocytocrit (0.1312 ± 0.06264 vs 0.1185 ± 0.05118, p = 0.0321) than non-bleeding patient (Table 1). It is interesting to note that neutrophils percentage of bleeding patient is higher than patients without these outcomes, yet neutrophil count isn't significantly different between the two group. However, bleeding patient had higher of lymphocytes count (0.9572 ± 0.6698 vs 1.123 ± 0.755, p = 0.0232),

Table 3

Demographic characteristics of patients with DENV infection.

	Hemorrhage	Non-hemorrhage	p value ()
Age – median (95% CI)	34(32,36)	34(32,37)	0.3967
Age – no./total no. (%)			0.13
≤ 30 yr	57.51%(88)	42.48%(65)	
31–60 yr	55.95%(127)	44.05%(100)	
≥ 61 yr	5(31.25%)	11(68.75%)	
Sex – no./total no. (%)			
Female	61.02%(72)	38.98%(46)	0.0011
Male	53.24%(148)	46.78%(130)	0.1493
Onset to hospitaliazation –median days (95% CI)	5(4,5)	4(3,4)	<0.0001
Hospitalization median (95% CI)	4(4,4)	5(4,5)	0.1424

lymphocytes percentage (29.7 ± 14.23 vs 34.78 ± 13.25, p = 0.0003), eosinophils count (0.02432 ± 0.05082 vs 0.04727 ± 0.08693, p = 0.002), eosinophils percentage (0.742 ± 1.919 vs 1.371 ± 2.357, p = 0.0044), basophils count (0.02205 ± 0.04085 vs 0.03609 ± 0.05818, p = 0.007), and basophils percentage (0.6665 ± 1.198 vs 1.065 ± 1.507, p = 0.0045) than non-bleeding patients. Meanwhile, erythrocyte (RBC) and hemoglobin (HB) did not show statistically significant differences among the two groups. In relative risk analysis, patients with abnormal basophils percentage (OR: 1.711, 95% CI: 1.076–2.721), lymphocytes percentage (OR: 1.509, 95% CI: 1.405–1.621), neutrophils percentage (OR: 1.258, 95% CI: 1.188–1.333), and RDW-CV (OR: 1.16, 95% CI: 1.038–1.297) were more likely to have plasma leakage compared to

other patients (Table 2).

3.2. The epidemic virology investigation and demographic analysis of enrolled DF/DHF patients

A total of 396 DF/DHF patients were admitted to the Third People's Hospital of Shenzhen from 2014 to 2019. Among them, young to middle aged (median age: 34 years) males (278/396, 70.2%) are the predominantly population of DENV infection. However, age and gender were not contributed to plasma leakage (Table 3). In addition, we observed that the time from onset to hospitalization (4.827 ± 3.133 vs 3.506 ± 2.345, p < 0.001) or hospitalization days (4.764 ± 2.406 vs 5.182 ± 3.252, p = 0.1424) were not significantly affected to bleeding or not (Table 3).

The geographical distribution and temporal dynamics of these 396 patients are described in Fig. 1. Futian District, Longgang district, Nanshan district, Longhua district and Bao'an districtis are the main area where occur patients with DENV infection (Fig. 1). At the same time, we also noticed that in 2014, Futian district had the most dengue fever cases compared to other areas, while Longgang District had the most cases in 2019. Further, we analyzed the temperature changes of them in recent years, and found that the temperature fluctuations in these areas are smaller than those in other areas, the temperature fluctuation range is 18–33 °C for a long time, which is suitable for mosquito growth (Fig. S1, Table S1, Table S2).

3.3. Temperature affects the replication of DENV-2 in C6/36 and transmission by Ae. albopictus

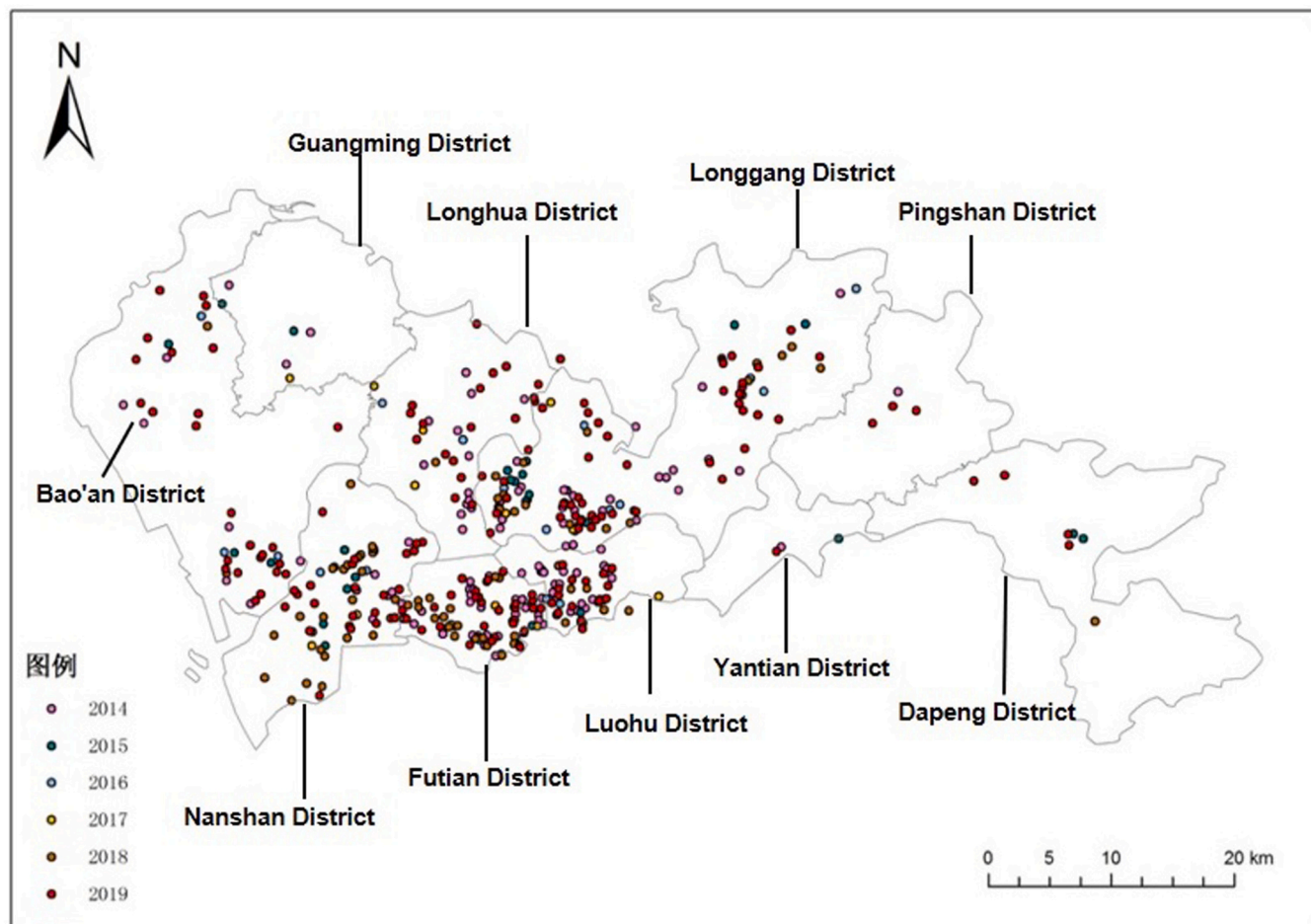In order to understand the geographical distribution characteristics,

Fig. 1. Geographical distribution of patients with DENV infection in Shenzhen from 2014 to 1019.
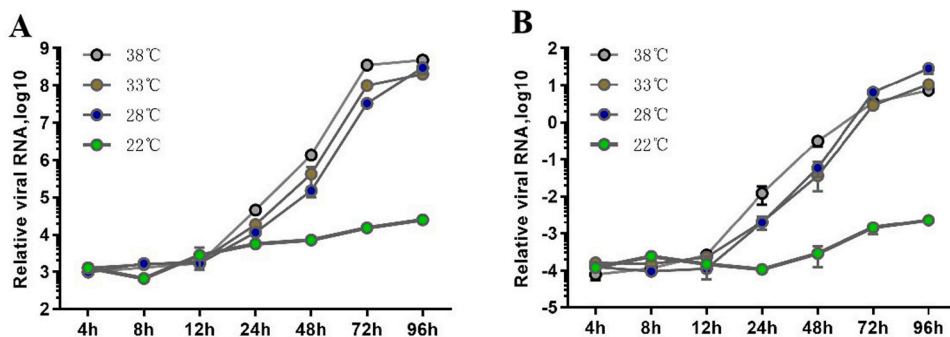
Fig. 2. C6/36 cells showed different susceptibility to DENV-2 infection with different temperature (22, 28, 33 and 38 ℃). Growth kinetics of DENV-2 in C6/36 cell lines. The RNA copies of DENV-2 in the cell (A) supernatants (B) were determined by qRT-PCR. The experiment was triplicated, and the standard deviations are also indicated. The results are expressed as the mean ± SEM. The results were analyzed using the unpaired t-test. A P value of < 0.05 indicates statistical significance. P < 0.05, * ; P < 0.001, ** and P < 0.001, *** .
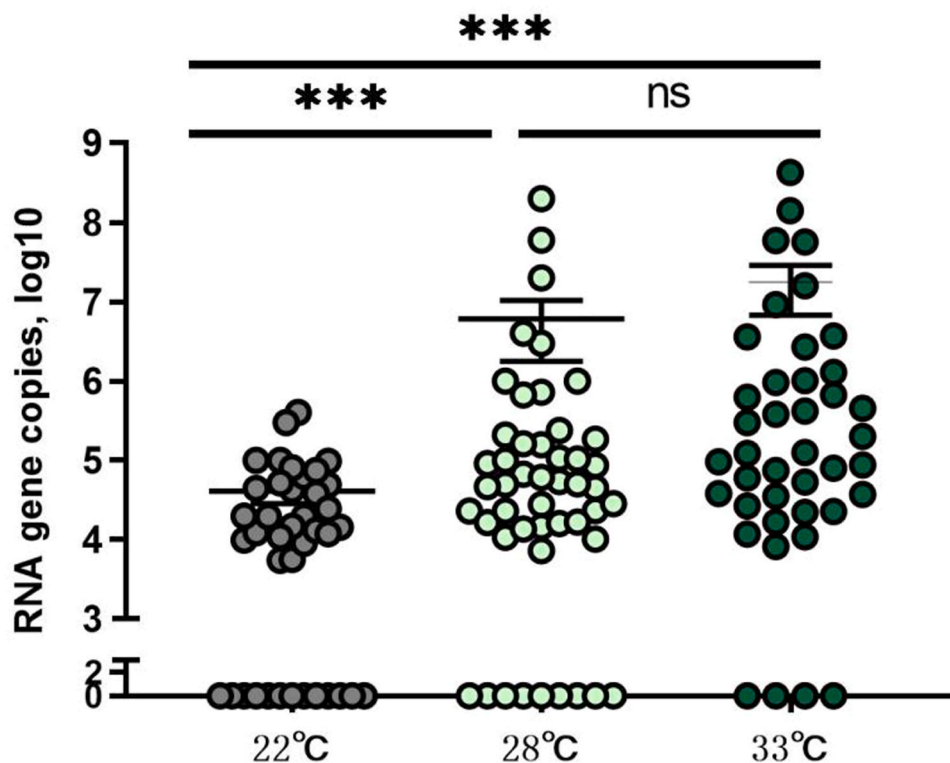

Fig. 3. Vector competence of *Ae. albopictus* orally infected with DENV-2 at different temperatures. *Ae. albopictus* orally infected with DENV-2 were reared at 22, 28 and 33 ℃. The head, and body of mosquitoes reared at each temperature were dissected at 14 days post-infection and detected by qRT-PCR. One dot represents one mosquito. The results are expressed as the mean ± SEM. The results were analyzed using the unpaired t-test. A P value of < 0.05 indicates statistical significance. P < 0.05, *; P < 0.001, ** and P < 0.001, ***.

we simulate the main temperature in summer in Shenzhen, C6/36 cell lines cultured in 22, 28, 33, and 38℃were used to measure the replication of DENV-2. Temperature had a significant effect on DENV-2 replication in C6/36 (Fig. 2). The growth kinetics of DENV-2 in 22 ℃ was significantly lower than that in 28, 33 or 38 ℃. Under 22 ℃ cultivation conditions, DENV-2 load showed almost no increase in cells and supernatant, while under other temperature cultivation conditions (28, 33, and 38 ℃), the DENV-2 load rapidly increased in supernatant and cells at 12 h post infection.

Further, *Ae. albopictus* females reared at 22, 28, and 33 ℃ were used to measure the rates of infection and transmission of DENV-2. The result of qRT-PCR showed that DENV-2 can infect *Ae. albopictus* at 22, 28, and 33 ℃ (Fig. 3). Similar with the above cell results, temperature has effects on infectivity of DENV-2 to *Ae. albopictus*. The average copy number (log10) of DENV-2 in *Ae. albopictus* arranged in ascending order was occurred at 22, 28, and 33 ℃ (4.56, 6.78 and 7.25, respectively). The average copy number of DENV-2 at culture temperature 22 ℃ is significantly lower than that at 28 and 33 ℃ ($P < 0.001$), while no significant difference occurred between 28 and 33 ℃ ($P > 0.05$). Similarly, DENV-2 the infection rate at 28 and 33 ℃ (75.6% and 90%) showed no difference ($P > 0.05$), and both higher than the infection rate

Table 4
Infection rate and transmission rate of DENV-2 at different temperatures.

	Temperature	22 ℃	28 ℃	33 ℃
DENV-2	Infection rate	19/40 (47.5%)	34/45 (75.6%)	36/40 (90%)
	Transmission rate	19/40 (27.5%)	26/45 (57.8%)	31/40 (77.5%)

at 22 ℃ (47.5%, $P < 0.001$). The infection status in the head was similar to that in the body. Detection of DENV-2 in the head showed that the transmission efficiency increased gradually in the order of 22, 28 and 33 ℃. Raising the culture temperature from 22 to 33 ℃ significantly increased DENV-2 transmission efficiency from 27.5% to 77.5% ($P < 0.001$). Interestingly, the transmission efficiency at 28 ℃ was obviously lower than that at 33 ℃ (57.8% and 77.5%, respectively, $P < 0.001$), whereas we had not found significant change in transmission efficiency at 28 and 33 ℃ ($P > 0.05$) (Table 4).
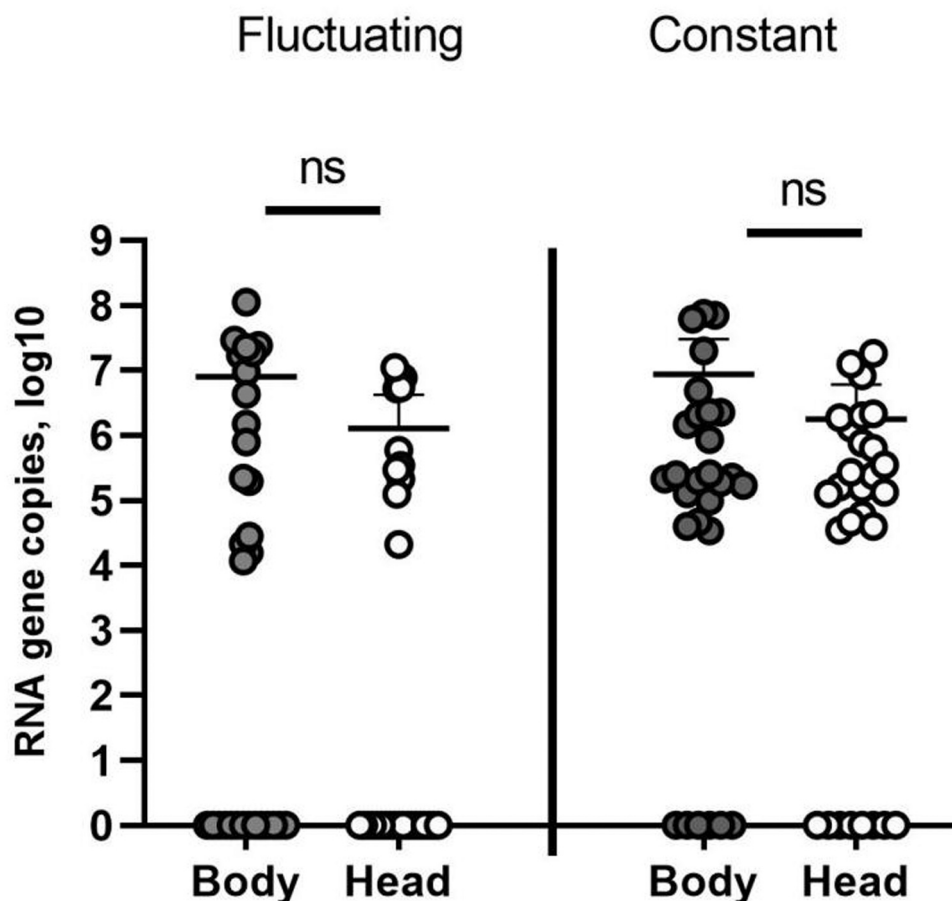
Fig. 4. DENV-2 RNA copies in head or body of *Ae. albopictus* between constant and fluctuating temperatures. The amount of DENV-2 in tissues of *Ae. albopictus* reared at constant temperature (A) and fluctuating temperature (B) was detected at14 dpi. One dot represents one mosquito. The results are expressed as the mean ± SEM. The results were analyzed using the unpaired t-test. A P value of < 0.05 indicates statistical significance. P < 0.05, *; P < 0.001, ** and P < 0.001, ***.

Table 5
Infection rate and transmission rate of DENV-2 at constant temperatures and fluctuating temperatures.

Temperature	Rate	DENV-2
Constant temperatures	Infection rate	22/28 (78.6%)
	Transmission rate	20/28 (71.4%)
Fluctuating temperatures	Infection rate	16/30 (53.3%)
	Transmission rate	11/30 (36.7%)

3.4. Temperature fluctuation suppression Vector Competence of Ae. albopictus for DENV-2

To determine the effect of fluctuation temperature on vector competence of *Ae. albopictus* for DENV-2, we measured vector competence and replication of DENV-2 in mosquitoes under fluctuating and constant temperature culture conditions, respectively. Among them, we use the DENV-2 load of the mosquito's body and head to indication the mosquito vector capacity and transmission capacity, respectively. *Ae. albopictus* reared at a constant temperature of 28 °C had a higher transmission rate (71.4%) as compared with those reared at a fluctuation temperature (36.7%) around a temperature of 28 °C (28 ± 5 °C) (Fig. 4, Table 5). Temperature also had a significant effect on DENV-2 infection rate, consistent with the trend in transmission rate, *Ae. albopictus* reared at a constant temperature of 28 °C are more likely to be infectious (78.6%) compared with those reared at a fluctuation temperature (53.3%).

4. Discussion

Our data confirmed that the certain clinical features of monocytes, neutrophils, lymphocyte, eosinophils, basophils , RDW-CV and thrombocytocrit are associate with bleeding due to DENV infection, and the percentage of basophil, lymphocyte and neutrophil have been identified as important risk factor for the severity of DENV infection. It has been well-established that monocytes are the main target cells for DENV [18, 19], and DENV infection leads to remodeling of monocyte transcriptome, even apoptosis [20]. Therefore, a reduction in monocyte count is commonly observed in DENV-infected patients. Interestingly, our data also suggest that DENV infection is characterized by a rare occurrence of an inflammatory cytokine storm, and it can even lead to reduced expression of immune response genes (data not shown). Monocytes and Neutrophils play crucial roles in initiating the inflammatory response by releasing host inflammatory factors [21–23], the intercellular regulatory networks within these mononuclear cell lines mediate the transition between immunosuppression and immune activation in response to pathogenic infections [24,25]. With a reduction in monocytes and neutrophils, lymphocytes increase in number, and the immune response gradually strengthens. However, the disrupted balance between monocytes and lymphocytes, coupled with the over-stress state of lymphocytes, can lead to impaired repair mechanisms and ultimately result in severe vascular permeability [26]. Similarly, in this study, we also observed that the percentage of basophil, lymphocyte and neutrophil are important hazard factor for the severity of DENV infection. This is not surprising, DENV infection remodels the metabolic processes of lymphocytes, such as lipid metabolism [27] and amino acid

metabolism, which lead to altered immune response. Along with the decline of the body's immune function, additional risks can arise, such as co-infection. Co-infection of DENV and COVID-19 has been observed, with overlapping clinical phenotypes : such as lymphopenia, leukopenia, thrombocytopenia, and elevated transaminase level, resulting a great risk for the disease development [28].

Furthermore, our study has also provided insights into the impact of temperature on DENV proliferation and transmission. Consistent with previous research [29–31], we observed significantly higher DENV load at higher temperatures compared to lower temperatures. Additionally, our results herein further confirm that replication, infection and the transmission rates of DENV increase with rising temperatures from 22 ℃ to 33 ℃. However, as temperatures continue to rise, mosquito survival rates decline rapidly. It has been confirmed that raising the constant temperature from 18 °C to 32 °C enhances the efficiency of DENV infection and transmission [32], and shortened the extrinsic incubation period (EIP) of DENV in *Ae. Albopictus* mosquitoes [33]. Therefore, more hosts would be infected by *Ae. albopictus* bites in the limited reproductive cycle. Temperature also plays a crucial role in the development, survival, and feeding behavior of mosquitoes, thereby promoting viral replication within the vector [32,34,35]. The ideal temperature range for mosquito survival throughout all phases of development (88–93%) falls between 20 ℃ and 30 ℃ [36]. However, both higher and lower temperatures can have detrimental effects on mosquito survival [34, 36–38]. Higher temperatures lead to a shortened gonotrophic cycle and EIP [32,35], while extreme temperatures negatively impact the vector population by increasing egg and adult mosquito mortality and reducing the rate of eclosion [37].

In this study, we also note that there are obvious geographical characteristics in the occurrence of DENV, so we hypothesize that this is related to the alter pattern of temperature, in addition to the temperature level. We simulate the warmer months when DENV outbreaks occur in Shenzhen [12], and select a diurnal temperature regime around a mean temperature of 28 ℃. Our findings indicate that the temperature fluctuations is important factors for DENV transmission. Furthermore, we observed that compared to constant temperature conditions, diurnal temperature fluctuations around the mean temperature of 28 ℃ reduce the vector competence and transmission capacity of DENV.

When conducting such studies, it is crucial to screen for risk factors associated with disease progression and to consider temperature fluctuations to better assess DENV transmission. Additionally, further research is needed to analyze the immune regulation mechanisms of mononuclear in patients with DENV infection. Parallel studies in natural settings that take into account various environmental factors affecting the spread of DENV would provide valuable insights.

5. Conclusions

Based on the combined analysis of clinical data and mosquito infection experiments, we have confirmed the significant roles of higher levels of neutrophils, lymphocytes, basophils and temperature in the progression of DENV transmission and pathogenesis. Simultaneously, we also confirmed that higher temperature rises DENV transmission hazard, and fluctuations temperature around a temperature of 28 °C (28 ± 5 °C) would increase DENV transmission by mosquito infection experiments. Those findings emphasize the importance of considering both clinical factors and environmental conditions, such as temperature, in understanding and addressing the transmission dynamics and pathogenesis of dengue fever.

CRediT authorship contribution statement

GY designed and performed the experiments, analyzed the data and wrote the manuscript; GY,XX, HY, WJ, ZY, HL collected demographic information from the study population and helped with the experiments; XL, YY, HX designed the research; XL principally designed the

experiments and directed the project. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Author Contributions

GY designed and performed the experiments, analyzed the data and wrote the manuscript; GY,XX, HY, WJ, ZY, HL collected demographic information from the study population and helped with the experiments; XL, YY, HX designed the research; XL principally designed the experiments and directed the project. All authors read and approved the final manuscript.

Animal and Human Rights Statement

This work was approved by the Ethical Committee of Shenzhen Third People's Hospital ((2018010)). This study adhered to the tenets of the Declaration of Helsinki. The purpose and procedures used in the study were explained to all participants, and written consent was obtained from all participants before the conduct of the study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.csbj.2023.07.001.

References

[1] C.J.L. M, R.M. B, K.J. F, A.A. O, F. A-A, S.F. A, et al. Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: quantifying the epidemiological transition. Lancet 2015:386.

[2] Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013:496. https://doi.org/10.1038/nature12060.

[3] Patz JA, Epstein PR, Burke TA, Balbus JM. Global climate change and emerging infectious diseases. J Am Med Assoc 1996:275. https://doi.org/10.1001/jama.275.3.217.

[4] Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus. PLoS Negl Trop Dis 2012:6. https://doi.org/10.1371/journal.pntd.0001760.

[5] Jacqueline D, Lucy L, Eric M, Lian HT. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization: New Edition; 2009.

[6] Halstead SB. Dengue antibody-dependent enhancement: knowns and unknowns. Microbiol Spectr 2014:2. https://doi.org/10.1128/microbiolspec.aid-0022-2014.

[7] Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Arch Virol 2013:158. https://doi.org/10.1007/s00705-013-1645-3.

[8] Rajapakse S, Rodrigo C, Rajapakse A. Treatment of dengue fever. Infect Drug Resist 2012:5. https://doi.org/10.2147/idr.s22613.

[9] Dutta AK, Biswas A, Baruah K, Dhariwal AC. National guidelines for diagnosis and management of dengue fever / dengue haemorrhagic fever and dengue shock syndrome. J Indian Med Assoc 2011:109.

[10] Dai H, Fan Q, Wang C. Recent applications of immunomodulatory biomaterials for disease immunotherapy. Exploration 2022:2. https://doi.org/10.1002/EXP.20210157.

[11] Carneiro LAM, Travassos LH. Autophagy and viral diseases transmitted by Aedes aegypti and Aedes albopictus. Microbes Infect 2016:18. https://doi.org/10.1016/j.micinf.2015.12.006.

[12] Zhang Z, Jing Q, Chen Z, Li T, Jiang L, Li Y, et al. The increasing menace of dengue in Guangzhou, 2001-2016: the most important epicenter in mainland China. BMC Infect Dis 2019:19. https://doi.org/10.1186/s12879-019-4504-3.

[13] Hernández-Gaytán SI, Díaz-Vásquez FJ, Duran-Arenas LG, López Cervantes M, Rothenberg SJ. 20 years spatial-temporal analysis of dengue fever and hemorrhagic fever in Mexico. Arch Med Res 2017:48. https://doi.org/10.1016/j.arcmed.2018.01.003.

[14] Ye G, Wang Y, Liu X, Dong Q, Cai Q, Yuan Z, et al. Transmission competence of a new mesonivirus, Yichang virus, in mosquitoes and its interference with representative flaviviruses. PLoS Negl Trop Dis 2020;14. https://doi.org/10.1371/journal.pntd.0008920.

[15] National Health Commission of the People's Republic of China. Guidelines for the treatment of dengue. ⟨www.nhc.gov.cn/cms-search/xxgk/getManuscriptXxgk.htm?id=d417aa2e783949e48f8a7366d7fdfacc⟩.

[16] di Luca M, Severini F, Toma L, Boccolini D, Romi R, Remoli ME, et al. Experimental studies of susceptibility of Italian Aedes albopictus to Zika virus. Eurosurveillance 2016:21. https://doi.org/10.2807/1560-7917.ES.2016.21.18.30223.

[17] Parry R, Asgari S. Aedes Anphevirus: an Insect-Specific Virus Distributed Worldwide in Aedes aegypti Mosquitoes That Has Complex Interplays with Wolbachia and Dengue Virus Infection in Cells. J Virol 2018:92. https://doi.org/10.1128/jvi.00224-18.

[18] Wan SW, Wu-Hsieh BA, Lin YS, Chen WY, Huang Y, Anderson R. The monocyte-macrophage-mast cell axis in dengue pathogenesis. J Biomed Sci 2018:25. https://doi.org/10.1186/s12929-018-0482-9.

[19] Kurane I, Kontny U, Janus J, Ennis FA. Dengue-2 virus infection of human mononuclear cell lines and establishment of persistent infections. Arch Virol 1990: 110. https://doi.org/10.1007/BF01310705.

[20] Castillo JA, Urcuqui-Inchima S. Mechanisms of monocyte cell death triggered by dengue virus infection. Apoptosis 2018:23. https://doi.org/10.1007/s10495-018-1488-1.

[21] Yang L, Xie X, Tu Z, Fu J, Xu D, Zhou Y. The signal pathways and treatment of cytokine storm in COVID-19. Signal Transduct Target Ther 2021:6. https://doi.org/10.1038/s41392-021-00679-0.

[22] Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. Nat Med 2018:24. https://doi.org/10.1038/s41591-018-0036-4.

[23] O'Gorman WE, Hsieh EWY, Savig ES, Gherardini PF, Hernandez JD, Hansmann L, et al. Single-cell systems-level analysis of human Toll-like receptor activation defines a chemokine signature in patients with systemic lupus erythematosus. J Allergy Clin Immunol 2015:136. https://doi.org/10.1016/j.jaci.2015.04.008.

[24] Uno N, Ross TM. Dengue virus and the host innate immune response. Emerg Microbes Infect 2018:7. https://doi.org/10.1038/s41426-018-0168-0.

[25] Rathore APS, St John AL. Immune responses to dengue virus in the skin. Open Biol 2018:8. https://doi.org/10.1098/rsob.180087.

[26] Malavige GN, Ogg GS. Pathogenesis of vascular leak in dengue virus infection. Immunology 2017:151. https://doi.org/10.1111/imm.12748.

[27] Reyes-Ruiz JM, Campuzano-Vences R, Osuna-Ramos JF, De Jesús-González LA, Pérez-Méndez MJ, González-González C, et al. Case report: extrapulmonary manifestations of COVID-19 and dengue coinfection. Am J Trop Med Hyg 2021: 105. https://doi.org/10.4269/ajtmh.21-0177.

[28] Osuna-Ramos JF, Rendón-Aguilar H, Reyes-Ruiz JM, Del Ángel RM, Romero-Utrilla A, Ríos-Burgueño ER, et al. The correlation of TNF alpha levels with the lipid profile of dengue patients. J Med Virol 2018:90. https://doi.org/10.1002/jmv.25056.

[29] Gale P. Using thermodynamic parameters to calibrate a mechanistic dose-response for infection of a host by a virus. Microb Risk Anal 2018:8. https://doi.org/10.1016/j.mran.2018.01.002.

[30] Gale P. How virus size and attachment parameters affect the temperature sensitivity of virus binding to host cells: predictions of a thermodynamic model for arboviruses and HIV. Microb Risk Anal 2020:15. https://doi.org/10.1016/j.mran.2020.100104.

[31] Gale P. Towards a thermodynamic mechanistic model for the effect of temperature on arthropod vector competence for transmission of arboviruses. Microb Risk Anal 2019:12. https://doi.org/10.1016/j.mran.2019.03.001.

[32] Liu Z, Zhang Z, Lai Z, Zhou T, Jia Z, Gu J, et al. Temperature increase enhances Aedes albopictus competence to transmit dengue virus. Front Microbiol 2017:8. https://doi.org/10.3389/fmicb.2017.02337.

[33] Chuang TW, Chaves LF, Chen PJ. Effects of local and regional climatic fluctuations on dengue outbreaks in southern Taiwan. PLoS ONE 2017:12. https://doi.org/10.1371/journal.pone.0178698.

[34] Morin CW, Comrie AC, Ernst K. Climate and dengue transmission: evidence and implications. Environ Health Perspect 2013;121. https://doi.org/10.1289/ehp.1306556.

[35] Tjaden NB, Thomas SM, Fischer D, Beierkuhnlein C. Extrinsic incubation period of dengue: knowledge, backlog, and applications of temperature dependence. PLoS Negl Trop Dis 2013:7. https://doi.org/10.1371/journal.pntd.0002207.

[36] Tun-Lin W, Burkot TR, Kay BH. Effects of temperature and larval diet on development rates and survival of the dengue vector Aedes aegypti in north Queensland, Australia. Med Vet Entomol 2000:14. https://doi.org/10.1046/j.1365-2915.2000.00207.x.

[37] Alto BW, Juliano SA. Precipitation and temperature effects on populations of Aedes albopictus (Diptera: Culicidae): implications for range expansion. J Med Entomol 2001:38. https://doi.org/10.1603/0022-2585-38.5.646.

[38] Rueda LM, Patel KJ, Axtell RC, Stinner RE. Temperature-dependent development and survival rates of Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae. J Med Entomol 1990:27. https://doi.org/10.1093/jmedent/27.5.892.