Acta Tropica 176 (2017) 34–38

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Short communication

Detection of selected arboviral infections in patients with history of persistent fever in Pakistan

Tahir Yaqub[a,*], Muhammad Zubair Shabbir[a], Nadia Mukhtar[b], Zarfishan Tahir[b], Tariq Abbas[c], Ehab Amir[d], Gabriel Defang[d]

[a] University of Veterinary and Animal Sciences, Lahore, Pakistan
[b] Institute of Public Health, Lahore, Pakistan
[c] Islamia University, Bahawalpur, Pakistan
[d] Naval Medical Research Unit, Cairo, Egypt

ARTICLE INFO

Keywords:
Surveillance
Viral haemorrhagic fever
Chikungunya virus
West Nile virus
Pakistan

ABSTRACT

Surveillance is a valuable tool for understanding prevailing and previously undiagnosed infections in a geographic area. We examined 480 archived serum samples from patients with history of persistent fever (> 40 °C, 60–72 h) who were referred to hospitals in Rawalpindi/Islamabad, Lahore, and Faisalabad districts for dengue antibody detection in 2014-15. Each sample was processed for detection of antigens and seroconversion, using real-time polymerase chain reaction and enzyme linked immunosorbent assay, respectively, against dengue haemorrhagic fever (DHF) virus serotypes 1–4, West Nile virus fever (WNVF), Crimean-Congo haemorrhagic fever (CCHF), and Chikungunya virus (CGV). The presence of antigens and antibodies to at least one of the studied viral haemorrhagic fevers (VHFs) was detected in 465 (96.8%, 95% CI: 94.9–98.1) and 442 samples (92.1%, 95% CI: 89.3–94.2), respectively. No sera were found positive to CCHF. There was a significant association between gender and positivity to at least one of the VHFs ($\chi^2 = 8.12$, df = 1, $p < 0.005$). Except for DHF serotype 2 and 3 ($\tau\tau = 0.41$), Goodman and Kruskal's *Tau* statistic revealed no significant association for occurrence of different viruses within the studied population ($\tau\tau = 0$–0.06). Cosinor analysis confirmed significant seasonality, with a higher number of cases of persistent fever in August through November, peaking in October. The study suggests circulation of multiple arthropod-borne viral infections and, in addition to DHF, ascertain the needs for screening patients for CGV and WNVF too. It also demonstrates the necessity of well-integrated disease surveillance in several geographic regions and at-risk populations in Pakistan to develop appropriate disease and vector control strategies.

1. Introduction

Arboviruses represent an escalating threat to public health worldwide. More than 100 viruses of *Togaviridae*, *Flaviviridae*, and *Bunyaviridae* have been identified as infecting humans via various vectors (Gubler, 2001). The complex interaction among vector, virus, host, and the environment produces an unpredictable epidemiological pattern that varies from sporadic, to low-level continuous endemic transmission, to outbreaks with the potential to spread over a wide geographic area (Gan and Leo, 2014). Although it remains under-reported, occurrence of Crimean-Congo haemorrhagic fever (CCHF) (Jogezai, 2007; Alam et al., 2013), dengue haemorrhagic fever (DHF) (Akram et al., 1998; Khan et al., 2008), and West Nile virus fever (WNVF) (Hayes et al., 1982) has been recorded in Pakistan. Other arboviral infections have likely occurred, especially in remote areas, but may have remained unrecognized due to lack of infrastructure, research, and diagnostic resources, coupled with limited surveillance. Since surveillance of antigens, antibodies, or both could provide essential components in understanding the circulation of important, but unidentified, arboviral infections in a specific geographic region, particularly during a period in which no epidemic is ongoing, we assessed antigen- and antibody-based prevalence of CCHF, serotypes of DHF (1, 2, 3, and 4), WNVF, and Chikungunya virus (CGV) in patients with a history of idiopathic persistent fever (> 40 °C).

2. Methods

From August 2014 through July 2015, 480 blood serum samples were processed for detection of antigen and antibodies to CCHF, DHF serotypes 1–4, WNVF, and CGV through commercially available real-

* Corresponding author.
E-mail address: tahiryaqub@uvas.edu.pk (T. Yaqub).

http://dx.doi.org/10.1016/j.actatropica.2017.07.019
Received 28 April 2017; Received in revised form 18 July 2017; Accepted 19 July 2017
Available online 25 July 2017

time PCR and enzyme linked immunosorbent assay (ELISA). The study comprised patients were from dengue endemic areas exhibiting persistent idiopathic fever (> 40 °C) during the previous 60–72 h and were showing one or more clinical symptoms including haemorrhage on skin, severe headache, abdominal or chest pain, vomiting, diarrhoea, and low platelet count (1×10^8/mL). Patients had been admitted to government-designated dengue treatment wards in referring hospitals in Rawalpindi/Islamabad (n = 463), Lahore (n = 16), and Faisalabad (n = 1). Most advanced disease diagnostic and health facilities are located in these districts of Punjab Province, and both local residents and those from a broad area of the country access these facilities. Since further improved health units and facilities are available in designated hospitals in Rawalpindi/Islamabad, and the district is adjacent/near to areas of other provinces of the country where limited health facilities are available, a greater number of people access facilities at these hospitals in twin-city (Rawalpindi/Islamabad) than any other district in Punjab, providing a much higher number of study subjects from tertiary-care hospitals in Rawalpindi/Islamabad than from any other district. The samples were originally submitted for detection of DHF serotypes; however, given the potential involvement of other VHFs with similar clinical outcomes, screening for CCHF, CGV, and WNVF was included.

Approval for sample processing in compliance with IACUC guidelines was obtained from the institutional review board at the Institute of Public Health, Lahore vide letter no. 421 dated July 01, 2014. The labelled sera (approximately 2 mL) of each patient kept at −80 °C was processed for antigen and antibody detection. Seroconversion was determined through highly sensitive and specific (> 90%) commercially available ELISA kits against DHF serotypes 1–4 (AccuDiag™ Dengue IgG ELISA kit and AccuDiag™ Dengue IgM ELISA kit, Abcam®, MA, USA), CGV (Human Chikungunya Virus IgG ELISA Kit, Bioactiva Diagnostica GmbH, BAD Homburg, Germany), WNVF (Human West Nile Virus ELISA Kit, Glory Science Co., Ltd. USA), and CCHF (Human Anti-CCHF IgG ELISA Kit, ALPHA Diagnostic International). Each serum sample was processed and interpreted according to manufacturer guidelines.

For genomic detection of target antigens, RNA was extracted from serum using the QIAamp Viral RNA Mini Kit (Qiagen, USA) as per manufacturer's instructions. Commercially available validated real time PCR kits were used for detection of antigen of DHF serotypes 1–4, CGV, WNVF, and the CCHF virus (RealStar® Altona Diagnostics, Hamburg, Germany). The assays were specific to the corresponding target antigen, and, as determined by probit analysis, the analytical sensitivity (per μL of elute) was found to be 4.7 copies for DHF (CI: 0.309–1.452), 0.528 for CGV (CI: 0.309–1.452), 0.67 for WNVF (CI: 0.34–2.28), and 14.2 for CCHF (CI: 12.4–16.4).

The recorded sample information and its positive or negative reaction to antibody- or antigen-based assay was recorded in Excel. The data were analysed using R software v. 3.3.1. The Chi-square test was applied to assess association between seropositivity for any arbovirus (VHF + vs. VHF −) and gender. For age groups (≤19; 20–39; 40–59; ≥60), the assumption of expected count ≥5 was violated; therefore, we used Fisher's exact test as an alternative to chi-square. Goodman and Kruskal's *Tau* was employed to determine the association between antigen and antibody pairs (categorical variables) using the Goodman Kruskal Package v. 0.0.2. The magnitude of association between variables ranged from 0 to 1, while k represented the number of levels in each variable (VHF antigen/antibody detected vs. not-detected). To evaluate seasonality in VHF hospital admissions, we carried out a cosinor analysis: a statistical method that fits cosine function to a time series. Cosinor function combined with Poisson regression quantified mesor (mean level), amplitude (peak value), and phase (time to peak value) in rhythmic time-series data. For this analysis, we used the R package *Season*. $P < 0.05$ was considered significant.

3. Results

Serum samples from referred patients (n = 480) were analysed for the presence of antigens and antibodies against target arboviral agents. Antigens and antibodies to at least one of the target VHFs were detected in 465 (96.8%, 95% CI: 94.9–98.1) and 442 patients (92.1%, 95% CI: 89.3–94.2), respectively. No serum sample was found positive for CCHF. With respect to antigens, an overall higher prevalence of DHF serotype 2 was observed (298/480, 62.1%, 95% CI: 57.6–66.4), followed by DHF serotype 3 (96/480, 20%, 95% CI: 16.6–23.9), DHF serotype 4 (30/480, 6.3%, 95% CI: 4.33–8.90), CGV (17/480, 3.5%, 95% CI: 2.14–5.72), WNVF (15/480, 3.1%, 95% CI: 1.83–5.22), and DHF serotype 1 (1/480, 0.2%, 95% CI: 0.01–1.34). We found mixed infection of DHF serotype 2 and 3 in 1.7% of the studied population (8/480, 95% CI: 0.78–3.39). Antibody detection also revealed the highest prevalence of DHF serotype 2 (298/480, 62.1%, 95% CI: 57.6–66.4), followed by DHF serotype 3 (96/480, 20%, 95% CI: 16.6–23.9), DHF serotype 4 (30/480, 6.3%, 95% CI: 4.33–8.90), WNVF (6/480, 1.3%, 95% CI: 0.51–2.84), CGV (3/480, 0.6%, 95% CI: 0.16–1.98), and DHF serotype 1 (1/480, 0.2%, 95% CI: 0.0–1.34).

Since each serum sample was processed for detection of the viral genome, IgG, and IgM, against DHF serotypes 1–4, we found similar prevalence of antigens and antibodies (IgG and IgM) in studied patients (n = 433, 90.2%; 95% CI: 87.2–92.5, $\tau\tau = 1$). This was evident also in Goodman and Kruskal's *Tau* model analysis, which showed a positive association between antigens and antibodies against serotypes of DHF ($\tau\tau = 1$, within-patient antigens and antibodies). Seroconversion against the DHF serotypes revealed a higher incidence of acute infection (IgM) than past exposure (IgG). Among the seropositive, the percent prevalence of IgM and IgG was 85.9 (n = 256, 95% CI: 81.3–89.5) and 14.1 (n = 42, 95% CI: 10.4–18.7), respectively, for DHF serotype 2; 46.8 (n = 45, 95% CI: 36.7–57.3) and 42.7 (n = 41, 95% CI: 32.8–53.2), respectively, for DHF serotype 3; and 53.3 (n = 16, 95% CI: 34.6–71.2) and 46.7 (n = 14, 95% CI: 28.8–65.4) for DHF serotype 4. Seropositivity only to IgM was observed for DHF serotype 1. However, identifying only the genome and IgG-based seroconversion against CGV (n = 17, 95% CI: 2.14–5.72 and n = 3, 95% CI: 0.16–1.98) and WNVF (n = 15: 95% CI: 1.83–5.22 and n = 6, 95% CI: 0.5–2.84) showed a weak within-patient association between findings of antigens and antibodies for WNVF ($\tau\tau = 0.39$) and CGV ($\tau\tau = 0.17$). Since we were limited to identify only IgG-based seroconversion for WNVF and CGV, it is not surprising that we found a lower rate of seroconversion compared to identification of the viral genome. We found no significant association for occurrence of different viruses within the studied population ($\tau\tau$ = 0–0.06) except for DHF serotype 2 and 3 ($\tau\tau = 0.41$). As we found no patient positive for CCHF, it was not included in the matrix plot (Fig. 1).

The studied population varied according to sex (322 male, 158 female) and age group (≤19, n = 112; 20–39, n = 257; 40–59, n = 91; ≥60, n = 20). There was a significant association between gender and positivity to at least one of the VHFs ($\chi^2 = 8.12$, df = 1, $p < 0.005$). Of the 322 males, 298 were found positive (92.5%, 95% CI: 88.9–95.0) to at least one of the VHFs under study. It is noteworthy that all females (n = 158) were found positive to at least one of the studied VHFs (100%, 95% CI: 97–100). We did not find a significant association of VHF positivity (antigens and antibodies) with age group using Fisher's exact test ($p > 0.05$). The mean age of the patients was 30.25 (95% CI: 29.07–31.42). Genome-based prevalence of at least one of the VHF was 22.8%, 54.1%, 18.8%, and 4.3% in the ≤19, 20–39, 40–59, and ≥ 60 age groups, respectively. The prevalence with seroconversion was 23.1%, 53.7%, 18.8%, and 4.3%, respectively, in the corresponding age groups.

Cosinor analysis confirmed significant seasonality in patients, with persistent fever due to arbovirus infections, admitted to hospitals during the study period. The estimated peak was 397 in phase 9.9 (late September) and the low point 3.9 (late March). Fig. 2 shows the average