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Concurrent dengue and malaria infection in Lahore, Pakistan during the 2012 dengue outbreak

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SUMMARY

Introduction: We conducted this study to determine the frequency of malaria and dengue–malaria co-infection in patients admitted to our hospital as 'probable' cases of dengue fever during the 2012 outbreak of dengue, and to ascertain whether dengue–malaria co-infection was more severe than either infection alone.

Methods: This cross-sectional observational study was conducted at Jinnah Hospital Lahore, Pakistan between August and November 2012. Patients with 2–10 days of fever and with two or more of the following: myalgia, arthralgia, retro-orbital pain, headache, skin rash, and hemorrhagic manifestations plus thrombocytopenia and leukopenia, were classified as probable cases of dengue fever and were subjected to reverse transcriptase (RT)-PCR and/or dengue-specific IgM by ELISA. The diagnosis of malaria was established on thick and thin blood film microscopy. Severe disease was defined by the presence of an altered level of consciousness, World Health Organization grade ≥2 bleeding, jaundice, circulatory shock, hemoglobin <50 g/l, platelet count $<50 \times 10^9$/l, serum creatinine >265 μmol/l, or death.

Results: There were 85 probable cases of dengue fever. Sixty-four (75%) were male and the median age was 22 years (range 12–90 years). Of 52 patients for whom results of diagnostic tests for both dengue and malaria were available, five (10%) had isolated dengue infection, 18 (35%) isolated Plasmodium infection, and 17 (33%) dengue–malaria co-infection. Thirty-five out of 52 (67%) probable cases had malaria and 17 out of 22 (77%) dengue-specific IgM reactive patients had concurrent malaria. Patients with isolated malaria had significantly lower median hemoglobin concentrations (124.5 g/l vs. 144.0 g/l, $p = 0.04$) and median hematocrit (36.0 vs. 41.7, $p = 0.02$) at presentation than cases of isolated dengue. Patients with dengue–malaria co-infection had a significantly lower rate of jaundice than those with isolated dengue (0% vs. 40%, $p = 0.04$). The frequency of severe disease was comparable amongst the three groups; this was seen in five (100%) cases of isolated dengue, 17 (94%) cases of isolated malaria, and 16 (94%) cases of dengue–malaria co-infection.

1. Introduction

Dengue and malaria are two of the most important mosquito-borne diseases worldwide. The true burden of these two diseases is not known. A recent study estimated the global burden of dengue to be 390 million infections per year, an infection total more than three times the estimates of the World Health Organization (WHO).[1] Many tropical and sub-tropical countries are endemic for both dengue and malaria. The two diseases share many clinical features and may be clinically indistinguishable. It is important, however, to differentiate between the two conditions, as malaria is treatable and any delay in treatment may result in a poor outcome. There are few published reports on concurrent dengue and malaria infection. The reported frequencies of concurrent infection in febrile patients presenting to outpatient departments of hospitals

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in various studies have been quite variable and range from 1% in French Guiana[2] to 6% in India[3] and 27% in Pakistan.[4] However, these studies differ greatly in terms of patient selection criteria and methods used for the diagnosis of dengue infection. There are also conflicting reports on whether dengue–malaria co-infection is more severe than either infection alone.[3,5]

Pakistan is endemic for malaria, whereas dengue has established itself in an epidemic transmission cycle. The largest outbreak of dengue in Pakistan was observed in 2011 in Punjab. In the setting of outbreaks in a resource-limited country, it is not cost-effective to subject all febrile patients to specific diagnostic testing for dengue. The Dengue Expert Advisory Group (DEAG) Punjab, a committee of expert clinicians, pathologists, and public health professionals, was established to develop recommendations and guidelines for the prevention and management of dengue fever at the provincial level. The DEAG has issued guidelines wherein stringent clinical and laboratory criteria are used to select 'probable' cases of dengue fever who may then be subjected to specific tests for dengue infection. Although this stringent case definition may give a sense of high specificity for selecting cases of dengue infection, the clinical and laboratory parameters are non-specific and may also be observed in cases of malaria. In malaria endemic settings it is, therefore, important to ascertain the frequency of malaria infection in patients categorized as probable cases of dengue fever. There is a risk of misdiagnosing cases of malaria as cases of dengue infection in the setting of a dengue outbreak, as the two diseases share many clinical and laboratory characteristics. The late recognition and treatment of malaria is associated with a poor outcome. This holds especially true for patients who have a dengue–malaria co-infection and in whom the confirmation of dengue may lead clinicians to overlook a concurrent malaria infection.

We analyzed the data of patients admitted to the dengue ward of our hospital during the 2012 outbreak of dengue fever to determine the frequency of isolated malaria and dengue–malaria co-infection in patients categorized as probable cases of dengue fever, and to ascertain whether dengue–malaria co-infection was more severe than either infection alone.

2. Methods

In this cross-sectional observational study, we analyzed the data of probable cases of dengue fever admitted to the dengue ward of Jinnah Hospital Lahore between August and November 2012. Jinnah Hospital Lahore is a 1200-bed tertiary care hospital that has a fever clinic and a dedicated dengue ward established during dengue outbreaks for the screening and admission of cases of dengue fever. The guidelines for classification, diagnosis, admission, and management are issued by the DEAG Punjab. These guidelines are a modification of the 2011 WHO Regional Office for South-East Asia (WHO SEARO) guidelines for the management of dengue fever.[6] The process of evaluation at the fever clinic and admission to the dengue ward is described below and summarized in Figure 1.

Patients with fever of 2–10 days of duration with two or more of the following are classified as 'suspected' cases of dengue fever: myalgia, arthralgia, retro-orbital pain, headache, skin rash, and hemorrhagic manifestations. All suspected case patients are subjected to a complete blood count analysis using an automated analyzer that is available in the fever clinic; results are available in less than an hour. Patients with thrombocytopenia (platelet count $<100 \times 10^9$/l, or a declining trend on serial blood count analysis) and leukopenia (leukocyte count $<4 \times 10^9$/l, or a declining trend on serial blood count analysis) are classified as 'probable' cases of dengue fever. All probable cases are admitted to the dengue ward and are subjected to specific diagnostic testing for dengue

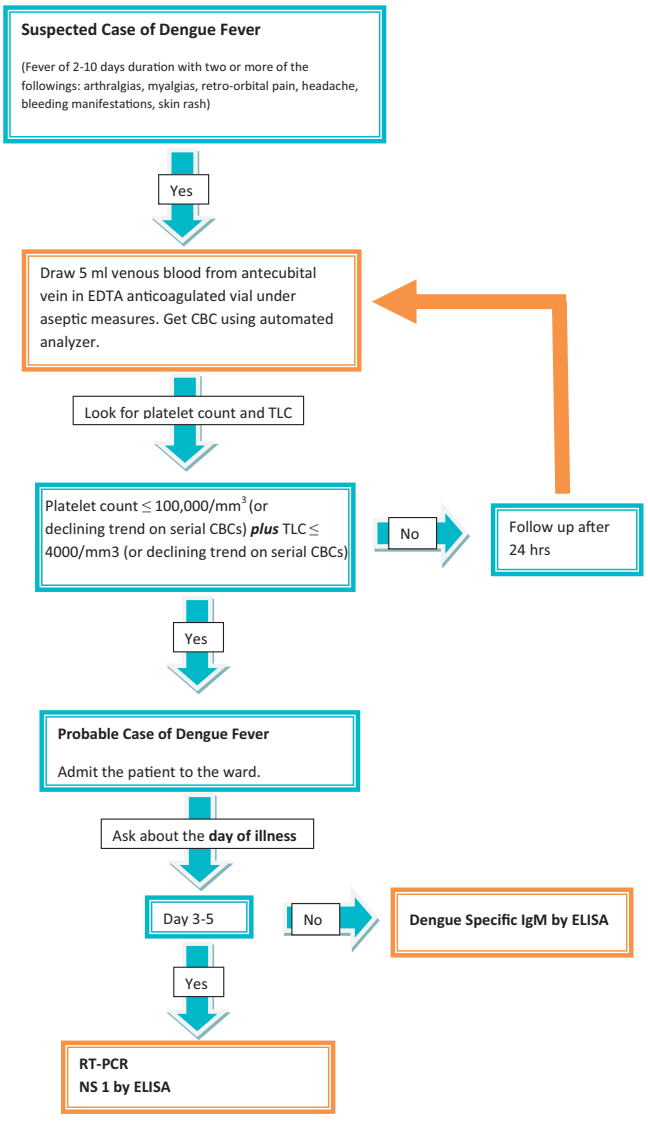
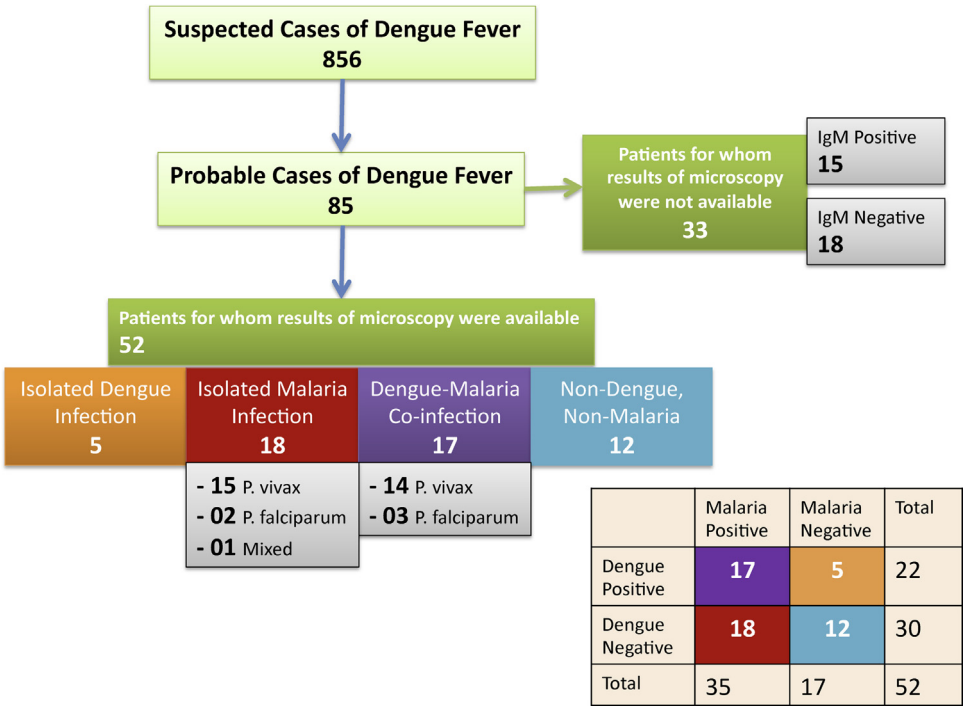


Figure 1. Algorithm for selection and admission of probable cases of dengue fever. On presentation to the outpatient department, a diagnosis of suspected dengue infection is made on the basis of clinical features. All suspected cases undergo a complete blood count analysis. Patients with thrombocytopenia and leukopenia are classified as probable cases of dengue fever and admitted to the dedicated dengue ward. Patients who do not fulfill the criteria for probable cases are followed in the outpatient department after 24 h. All probable cases of dengue fever undergo specific diagnostic testing depending on the day of illness.

infection. Patients presenting within 5 days of the onset of illness undergo reverse transcriptase (RT)-PCR for dengue virus (DENV) RNA detection, or ELISA for non-structural protein 1 (NS1) antigen detection. Patients presenting more than 5 days after the onset of illness undergo ELISA for dengue-specific IgM. Anti-dengue IgM antibodies were measured by indirect IgM ELISA using a commercially available kit (Human GmbH, Wiesbaden, Germany). This method uses dengue-specific antigens (DEN-Ag) coated on microtiter wells. (RT-PCR was also performed on a few patients after day 5 if they were febrile at the time of admission.) Patients who test positive for any of these specific tests are reported as 'confirmed' cases of dengue fever. All suspected, probable, and confirmed cases are reported on an online dashboard established by the Punjab Information Technology Board (PITB) and data are available for monitoring the outbreak.

During 2012, we also evaluated all adult probable cases of dengue fever who were febrile at the time of admission for



	Malaria Positive	Malaria Negative	Total
Dengue Positive	17	5	22
Dengue Negative	18	12	30
Total	35	17	52

Figure 2. Results of specific diagnostic testing for dengue and malaria. Out of 856 suspected cases, 85 were probable cases of dengue fever and were hospitalized. ELISA for dengue-specific IgM was performed in all 85 probable cases. The results of diagnostic tests for both dengue and malaria were available for 52 (61%) probable cases. Of these 52 patients, five (10%) had isolated dengue infection, 18 (35%) isolated Plasmodium infection, and 17 (33%) dengue–malaria co-infection.

Plasmodium infection by thick and thin blood smear microscopy. A senior hematologist examined 200 fields of Giemsa-stained thick blood smears at ×1000 magnification under immersion oil. The Plasmodium species were identified. However, data on asexual parasitemia were lacking. At least two blood smears were examined from blood collected 12–24 h apart before reporting a result negative for malaria.

Patients with isolated dengue infection were treated with acetaminophen and symptomatic therapy. Patients with an uncomplicated *Plasmodium vivax* infection were given the standard dose of oral chloroquine, followed by the administration of primaquine after ruling out the contraindications. Patients with a *Plasmodium falciparum* infection and/or complicated malaria were treated with the standard dose of artemether–lumefantrine. Patients received intravenous fluids and blood transfusions when clinically indicated.

Severe disease was defined by the presence of any of the following: altered level of consciousness, multiple convulsions (more than two episodes in 24 h), WHO grade 2 or higher spontaneous bleeding, pulmonary edema, circulatory collapse or shock (pulse pressure ≤20 mmHg, systolic blood pressure <90 mmHg), hypoglycemia (blood glucose <2.2 mmol/l, or <40 mg/dl), metabolic acidosis (plasma bicarbonate <15 mmol/l), severe normocytic anemia (hemoglobin <50 g/l, packed cell volume <15%), platelet count $<50 \times 10^9$/l, jaundice (serum total bilirubin >2.5 mg/dl, or >43 μmol/l), renal impairment (serum creatinine >3 mg/dl, or >265 μmol/l), and death.

The data of all adult probable cases of dengue fever admitted to the dengue ward were evaluated retrospectively. Patients were included in the analysis if the results of diagnostic tests for both dengue and malaria were available. The clinical, laboratory, and treatment data were abstracted on a standardized form. Data were analyzed using SPSS software v. 17 (SPSS Inc., Chicago, IL, USA). Frequencies for isolated dengue, isolated malaria, and dengue–malaria co-infection were calculated. A comparison was made between three groups: isolated dengue, isolated malaria, and dengue–malaria co-infection. Patient characteristics were compared using Fisher's exact test for categorical variables and non-parametric tests (Kruskal–Wallis and Mann–Whitney *U*-test) for continuous variables. A *p*-value of <0.05 was considered significant at a confidence interval of 95%.

3. Results

Out of 856 suspected cases, 85 were probable cases of dengue fever and were hospitalized. Sixty-four (75%) were male and the median age was 22 years (range 12–90 years). RT-PCR was performed on febrile phase serum for 15 patients and was positive in three patients (the serotype in these cases was DENV-2), while ELISA for NS1 was performed for an additional two patients and was reactive in one patient. ELISA for dengue-specific IgM was performed for all 85 probable cases and was positive in 37 (43%). The results of diagnostic tests for both dengue and malaria were available for 52 (61%) probable cases. Of these 52 patients, five (10%) had isolated dengue infection, 18 (35%) isolated Plasmodium infection, and 17 (33%) dengue–malaria co-infection (Figure 2). Thirty-five out of 52 (67%) probable cases had malaria and 17 out of 22 (77%) dengue-specific IgM reactive patients had a concurrent infection with Plasmodium. The characteristics of patients for whom the results of microscopy were available or not available were comparable.

The results of both direct (RT-PCR, NS1) and indirect methods (IgM by ELISA) for the diagnosis of dengue infection were available for 17 patients. Four patients were RT-PCR/NS1- as well as IgM-positive and Plasmodium-negative (isolated dengue infection). Two patients tested negative for RT-PCR/NS1, IgM, and Plasmodium (non-dengue, non-malaria). Eleven patients were RT-PCR-negative, IgM-positive, and Plasmodium-positive.

A comparison of demographics and clinical features, is shown in Table 1. Patients with dengue–malaria co-infection had a significantly lower rate of jaundice than those with isolated dengue (0% vs. 40%, $p = 0.04$). Patients with dengue–malaria

Table 1
Comparison of the clinical profile of cases of isolated dengue, isolated malaria, and dengue–malaria co-infection[a]

	Isolated dengue (D) (n = 5)	p-Value D vs. M	Isolated malaria (M) (n = 18)	p-Value M vs. DM	Dengue–malaria co-infection (DM) (n = 17)	p-Value DM vs. D
Gender male	4 (80)	0.6	13 (72)	0.45	11 (65)	0.48
Age, years						
Median	18	0.08	23.5	0.76	22	0.12
Range	16–22		12–48		12–32	
Intermittent fever, yes	4 (80)	0.41	11 (61)	0.55	11 (65)	0.48
Rigors and chills	5 (100)	0.46	15 (83)	0.12	17 (100)	1.0
Myalgia	4 (80)	0.65	15 (83)	0.46	13 (76.5)	0.69
Headache	4 (80)	0.61	13 (72)	0.60	12 (71)	0.58
Retro-orbital pain	2 (40)	0.67	7 (39)	0.27	4 (23)	0.42
Vomiting	3 (60)	0.49	13 (72)	0.32	10 (59)	0.68
Petechiae	0 (0)	0.78	1 (6)	0.28	3 (18)	0.44
Gum bleeding	0 (0)	0.78	1 (6)	0.74	1 (6)	0.77
Epistaxis	1 (20)	0.39	1 (6)	0.74	1 (6)	0.41
Hematemesis	0 (0)	0.46	3 (17)	0.32	1 (6)	0.77
Melena	1 (20)	0.54	2 (11)	0.47	3 (18)	0.67
Macroscopic hematuria	1 (20)	0.39	1 (6)	0.28	3 (18)	0.67
Altered mental status	0 (0)	0.78	1 (6)	0.51	0 (0)	1.00
Abdominal pain	1 (20)	0.32	8 (44)	0.56	7 (41)	0.38
Clinical hepatomegaly	1 (20)	0.39	1 (6)	0.15	4 (23)	0.69
Clinical splenomegaly	0 (0)	1.00	0 (0)	0.10	3 (18)	0.44
Pallor	3 (60)	0.37	7 (40)	0.50	8 (47)	0.45
Jaundice	2 (40)	0.39	4 (22)	0.06	0 (0)	0.04
Hypotension	0 (0)	0.78	1 (6)	0.48	2 (12)	0.59

[a] Results are n (%) unless stated otherwise.

co-infection also had a lower rate of jaundice than the isolated malaria group, however this difference was not statistically significant (0% vs. 22%, $p = 0.06$). The other clinical manifestations were comparable.

Patients with isolated malaria had a significantly lower median hemoglobin concentration (124.5 g/l vs. 144.0 g/l, $p = 0.04$) and median hematocrit (36.0 vs. 41.7, $p = 0.02$) at presentation than cases of isolated dengue (Table 2). Patients with dengue–malaria co-infection also had a lower median hemoglobin concentration (130.0 g/l vs. 144.0 g/l) and hematocrit (39.3 vs. 41.7) at presentation than patients with isolated dengue, however the difference was not statistically significant ($p = 0.09$ and $p = 0.07$, respectively).

The frequency of severe disease was comparable amongst the three groups; this was seen in five (100%) cases of isolated dengue, 17 (94%) cases of isolated malaria, and 16 (94%) cases of dengue–malaria co-infection. The comparison of individual determinants of severe disease is presented in Table 3. All patients recovered and were discharged from the hospital.

4. Discussion

Our study showed that 35 out of 52 (67%) probable cases of dengue fever had malaria and 17 out of 22 (77%) dengue-specific IgM reactive patients had concurrent malaria. DENV-2 was the serotype in three patients who tested positive on RT-PCR. The percentages of cases of isolated dengue, isolated malaria, and dengue–malaria co-infection in probable cases of dengue fever were 10%, 35%, and 33%, respectively. Patients with isolated malaria had a significantly lower hemoglobin concentration and hematocrit at presentation than cases of isolated dengue. Patients with dengue–malaria co-infection had a significantly lower rate of jaundice than those with isolated dengue. The remaining clinical and laboratory parameters were comparable. There was no between-group difference in terms of severity of the disease.

The first case of concurrent DENV and *P. falciparum* was reported in 2005,[7] and this was followed by reports of mixed infections with DENV and *P. vivax*,[8] *P. falciparum*, or both. Abbasi et al. from Pakistan reported concurrent dengue and malaria infection in 26 of 112 (23%) febrile patients evaluated for dengue at a hospital in Karachi.[4] Carme et al. evaluated 1723 consecutive febrile patients in Cayenne Hospital, French Guiana, and found dengue in 238 (13.8%), malaria in 393 (22.8%), and mixed dengue and malaria infection in 17 (1%) patients.[2] *P. vivax* was the predominant Plasmodium species. Mohapatra et al. found dengue–malaria co-infection in 27 of 469 (5.7%) febrile patients tested for dengue and malaria in Odisha, India.[3] Isolated dengue and malaria were seen in 340 (72.5%) and 102 (21.7%) patients, respectively. *P. falciparum* was the predominant Plasmodium species found. Rapid diagnostic tests were used for the detection of IgM and NS1 for

Table 2
Comparison of the laboratory profile of cases of isolated dengue, isolated malaria, and dengue–malaria co-infection[a]

	Isolated dengue (D) (n = 5)	p-Value D vs. M	Isolated malaria (M) (n = 18)	p-Value M vs. DM	Dengue–malaria co-infection (DM) (n = 17)	p-Value DM vs. D
Hemoglobin at presentation, g/l	144.0 (132.0–147.0)	0.04	124.5 (72.0–160.0)	0.95	130.0 (83.0–158.0)	0.09
Lowest hemoglobin g/l	108.0 (96.0–119.0)	0.93	109.0 (64.0–136.0)	0.99	114.0 (68.0–130.0)	0.93
Hematocrit at presentation	41.7 (41.4–42.8)	0.02	36.0 (28.1–43.7)	0.69	39.3 (26.0–46.4)	0.07
Lowest hematocrit	33.1 (27.3–35.3)	0.46	31.0 (23.7–38.0)	0.50	31.4 (19.6–38.4)	0.95
WBC ($\times 10^9$/l) at presentation	4.65 (1.20–9.30)	0.93	4.25 (1.30–8.60)	0.46	3.70 (1.60–9.50)	0.79
Lowest WBC ($\times 10^9$/l)	2.50 (1.10–6.70)	0.56	3.10 (1.30–4.50)	0.59	2.80 (1.40–6.60)	0.93
Platelet count at presentation ($\times 10^9$/l)	25.5 (19.0–54.0)	0.29	46.0 (9.0–179.0)	0.35	54.0 (15.0–173.0)	0.12
Lowest platelet count ($\times 10^9$/l)	16.5 (13.0–21.0)	0.53	19.0 (5.0–64.0)	0.99	22.0 (2.0–57.0)	0.45

WBC, white blood cell count.
[a] Results are the median (range).

Table 3
Comparison of individual parameters of severity in cases of isolated dengue, isolated malaria, and dengue–malaria co-infection[a]

	Isolated dengue (D) (n = 5)	p-Value D vs. M	Isolated malaria (M) (n = 18)	p-Value M vs. DM	Dengue–malaria co-infection (DM) (n = 17)	p-Value DM vs. D
Altered level of consciousness	0 (0)	0.78	1 (6)	0.51	0 (0)	1.00
Hypotension	0 (0)	0.78	1 (6)	0.48	2 (12)	0.59
WHO grade ≥2 bleeding	0 (0)	0.46	3 (17)	0.31	5 (29)	0.23
Platelet count $<50\,000 \times 10^9$/l	4 (80)	0.54	16 (89)	0.52	16 (94)	0.41
Serum creatinine >265 μmol/l	0 (0)	0.78	1 (6)	0.51	0 (0)	1.00
Serum bilirubin >43 μmol/l	2 (40)	0.39	4 (22)	0.06	0 (0)	0.04
Death	0 (0)	1.00	0 (0)	1.00	0 (0)	1.00

WHO, World Health Organization.
[a] Results are n (%).

diagnosing dengue infection. Due to the high false-positivity and low specificity of rapid diagnostic tests for IgM,[9] there may have been an over-estimation of the dengue–malaria co-infection rate. The results of these and other studies may not be comparable due to the variable patient selection criteria and diagnostic methods used.

Our study shows that the rate of malaria infection was higher than the rate of dengue infection in patients admitted as probable cases of dengue fever. Even amongst the confirmed cases of dengue infection, there were more cases of dengue–malaria co-infection than isolated dengue infection. The results of this study cannot be generalized as we only analyzed a sample of febrile patients selected through application of a strict operational case definition. However, our findings underscore the importance of testing for malaria infection even when the likelihood of a dengue infection is high or a dengue infection is confirmed. Whereas the management of dengue infection is symptomatic, malaria is a treatable disease and the early institution of treatment can decrease the risk of complications. The DEAG algorithm was developed to select cases more likely to have a dengue infection. Our findings suggest that this algorithm for the selection of probable cases of dengue fever did not help differentiate between dengue and malaria infection. Dengue and malaria share many clinical and laboratory characteristics, and specific diagnostic testing is required to establish the diagnosis.

One of the limitations of our study is that RT-PCR and NS1 testing as direct evidence of dengue infection was available for only 17 patients, while dengue-specific IgM as indirect evidence of dengue infection was used in all 85 patients. Unlike the immunochromatographic method for the detection of IgM, which has a low specificity and high false-positivity,[9,10] ELISA for the detection of IgM has been reported to have a high specificity (approximately 98%).[10] RT-PCR has the highest diagnostic yield during the early phase of illness and its sensitivity decreases over time during the course of illness. A serological diagnosis is helpful in patients who present later in the course of their illness. Out of 17 patients for whom the results of both direct and indirect diagnostic tests for dengue were available, there were 11 RT-PCR-negative IgM-positive patients who tested positive for Plasmodium. There are three possible explanations for these cases: (1) Patients had a current dengue infection but they were tested by RT-PCR later in the course of their illness when only IgM antibodies were detectable. (2) Patients had had a dengue infection in the recent past (last 2–3 months) and were not currently infected with dengue. Despite a high specificity of IgM by ELISA for dengue,[10] once detectable, IgM antibodies may persist in the serum of infected patients for 2–3 months. Therefore, although a reliable marker of recent infection with dengue, a single positive IgM does not necessarily imply a concurrent dengue infection. This limits its utility in confirming a dengue–malaria co-infection. (3) Patients had a false-positive IgM due to malaria. This latter point is less likely given the high specificity of ELISA for dengue-specific IgM.[10]

Except for jaundice being more common in cases of isolated dengue and malaria than in dengue–malaria co-infection, we did not find a statistically significant between-group difference in terms of severity of the disease using the predefined criteria. A potential confounder could be the pooling of Plasmodium species while analyzing the severity indices across the groups, as *P. falciparum* is known to cause more severe disease than *P. vivax*. Since the frequency of *P. falciparum* infection was comparable between the isolated malaria and dengue–malaria co-infection groups in our study, it is less likely to have confounded our results. Mohapatra et al. reported significantly less severe disease in cases of dengue–malaria co-infection, presumably due to early presentation to the health facility.[3] Epelboin et al., on the other hand, found significantly more severe disease in cases of dengue–malaria co-infection in a retrospective matched-pair study in French Guiana.[5] In that study, the malaria was caused predominantly by *P. vivax* as compared to *P. falciparum* in the study by Mohapatra et al. in India. Patients with the co-infection had a greater risk of deep thrombocytopenia and anemia. These differences were not observed in our study. However, there is the risk of a type II error in our study due to the small sample size. Another limitation of our study is that the asexual parasitemic load was not measured. Further larger, preferably multicenter, prospective studies are needed to confirm whether co-infection is more severe than the single infections alone. These studies should take into consideration the confounding effects of different Plasmodium species, DENV serotypes, and primary versus secondary dengue infection.

In conclusion, our study highlights the importance of testing for malaria in patients suspected of having dengue infection in malaria endemic settings. The two infections are clinically indistinguishable and specific diagnostic testing is needed to confirm the diagnosis. The confirmation of one infection should not preclude the possibility of co-infection. The DEAG diagnostic algorithm did not differentiate dengue from malaria infection. We did not find a statistically significant between-group difference in terms of severity of the disease using predefined criteria. Further well-designed prospective studies are needed to understand the effect of co-infection on the severity of the disease.

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Ethical approval: The study is in compliance with the Declaration of Helsinki and the study protocol was approved by the Ethics Review Committee of Allama Iqbal Medical College/Jinnah Hospital Lahore.

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