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Original Article

Microbiology Section

Evaluation of Concurrent Malaria and Dengue Infections among Febrile Patients- A Cross-sectional Study

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ABSTRACT

Introduction: There are several tropical mosquito borne infections, such as Malaria and Dengue, these are the two major and common arthropod borne infections that cause high morbidity and mortality in many patients and are major public health concern worldwide. Concurrent malaria and dengue infection is an important condition that is infrequently reported.

Aim: To find out the prevalence rate of co-infection for both dengue and malaria and also to associate the severity of such co-infections with symptoms and haematological parameters.

Materials and Methods: In the present observational cross-sectional study, samples were collected from 604 febrile patients clinically suspected for malaria and dengue attending the Medicine Out Patient Department (OPD) of Dhiraj Hospital, Gujarat, India. The samples were diagnosed for malaria by using rapid malaria antigen test kit and peripheral blood smear microscopy for the identification of *Plasmodium* spp. The dengue NS-1 antigen, (Immunoglobulin) IgM and IgG antibodies rapid kit were use to rule out dengue infection. The statistical analysis was done by software EPI info.

Results: In the present study, out of 604, 58 patients (9.6%) were positive for malaria and 80 patients (13.24%) were positive for dengue, while 21 patients (3.47%) were having concomitant infection with both dengue and malaria. The most affected age group was 31-60 years, 45.53%. The study revealed male preponderance (61.59%), hepatomegaly and jaundice were seen in 52.38% patients, haemorrhagic manifestations in 23.80%, kidney failure in 4.76% (1), and thrombocytopenia (platelet count <150,000/cubic millimeter (cmm)) were noted in 95.23% patients.

Conclusion: It is mandatory to test any febrile patient for both malaria and dengue so that the diagnosis of the patient is not held back by missing any one. The clinicians who treat febrile patients in or from endemic areas must thoroughly examine and diagnose for both malaria and dengue, still one or the other is positive. Vector control, health education and good hygiene are community based preventive measures that are needed to control both the diseases.

Keywords: Co-infection, *Plasmodium falciparum*, *Plasmodium vivax*, Serology

INTRODUCTION

In India, the most prevailing vector borne diseases are Malaria and Dengue which are the major concern for the public health too. According to Magalhaes BM et al., annually almost five lac people require hospitalisation for dengue and 2.5% have mortality with *P.vivax* malaria and dengue fever co-infection [1]. Malaria is a critical disease caused by *Plasmodium* species via bite of infected female Anopheles mosquitoes which is transmitted to people. It is preventable and curable. According to the latest World Health Organisation (WHO) malaria fact sheet, world malaria report, released on 30th November 2020, there were 229 million cases and deaths of 409 000 with malaria in 2019 [2]. In 108 countries including India, two species of malaria parasite which are *Plasmodium falciparum* and *Plasmodium vivax* are endemic [3,4]. Dengue is one of the most widespread arthropod-borne viral disease and is transmitted in humans by *Aedes aegypti* mosquitoes. The virus responsible for causing dengue, is called Dengue Virus (DENV). They have four serotypes, DENV-1, DENV-2, DENV-3 and DENV-4 so it is probable for a person to be infected four times. Dengue is found in tropical and subtropical areas because of favourable climates. Most common areas are urban and semi-urban. Dengue is endemic in India for over two centuries. Among 18 endemic states, the most affected regions are Delhi, West Bengal, Kerala, Tamil Nadu, Karnataka, Maharashtra, Rajasthan, Gujarat and Haryana [5]. In some tropical regions malaria and dengue both are endemic and therefore, may be an opportunity of co-infection of both. Increase in the concurrent infection has been seen in several areas like urban areas, deforestation, and agricultural area in peri-urban regions [6]. In 2006, the Amazon region was co-infected with dengue and malaria [7]. Considering the endemicity, it is practical to envision that the rate of concomitant infections would not be exceptional [8,9]. However, only a few cases of malaria and dengue co-infection have been reported due to non-systematic diagnosis of both diseases [10,11].

The non-specific symptoms and inappropriate use of antimalarial drugs leads to difficulty in clinical diagnosis and poor monitoring of the patients. Hence, it requires accurate species identification and exact laboratory investigations [12,13].

The two diseases malaria and dengue infections have common clinical features and therefore, impossible to differentiate. The differentiation is done by laboratory diagnosis, else it gives low outcome [14].

The present study was done to find out the prevalence rate of co-infection for both dengue and malaria and also to associate the severity of such co-infections with symptoms and haematological parameters.

MATERIALS AND METHODS

This observational cross-sectional study was conducted in the Department of Microbiology, Dhiraj General Hospital, from June 2015 to March 2020 at SBKS Medical Institute and Research Centre, Piparia, Dist- Vadodara, Gujarat, India. Approval was obtained from the Institutional Ethics Committee (IEC: no: SVIEC/ON/2015/15011) and informed consent was obtained from the patients.

Inclusion criteria: A total of 604 clinically suspected patients having fever, rigors, vomiting, nausea, abdominal pain, jaundice, headache, joint and muscle ache and other various symptoms like haemorrhagic manifestation, kidney failure, skin rash, etc were included.

Exclusion criteria: Patients who were suspected to have hospital acquired infections (in whom fever occurred after 48 hours of hospital admission) were excluded.

For detection of species, 4 mL of blood sample was collected in Ethylenediaminetetraacetic Acid (EDTA) bulb through venipuncture from suspected samples. All samples were tested with thick and thin peripheral blood smears and stained with Giemsa stain to detect *Plasmodium* species and simultaneously the rapid antigen detection test done by Malascan Pan/pf (Viola Diagnostic system) for co-confirmation of Malaria. Dengue infection was detected by using the Dengucheck combo rapid test system from serum/plasma to detect of NS-1 antigen and antibodies (IgM/IgG). The data including clinical diagnosis, haematological parameters were taken from the medical record department, and compared with the findings. The serological method were carried as per the manufacturer's instructions [15].

STATISTICAL ANALYSIS

Data for clinical diagnosis and haematological parameters were collected from hospital records; biochemical and pathological tests performed were registered. All the data were entered in excel sheet and analysis was done by software EPI info.

RESULTS

A total of 604 clinically suspected samples were analysed, The most affected patients were from the age group of 31-60 years (275, 45.53%) and less affected age group was 1-17 years (50, 8.28%). Male patients were more common 61.59% (372), than female 38.41% (232) [Table/Fig-1]. In 604 samples, 80 samples were found positive for dengue and 58 samples were positive for malaria. The concurrent infection of both dengue and malaria were 21 (3.47%) found and 445 samples were negative [Table/Fig-2].

Age (Years)	Total N (%)	Sex	
		Male N (%)	Female N (%)
1-17	50 (8.28)	31 (8.33)	19 (8.19)
18-30	219 (36.26)	117 (31.45)	102 (43.96)
31-60	275 (45.53)	180 (48.39)	95 (40.95)
>60	60 (9.93)	44 (11.83)	16 (6.90)
Total	604 (100%)	372 (61.59)	232 (38.41)

[Table/Fig-1]: Distribution of febrile patients according to age and sex.

Name of the Disease	Positive N (%)
Malaria	58 (9.60)
Dengue	80 (13.24)
Malaria+Dengue	21 (3.47)

[Table/Fig-2]: Prevalence of dengue and malaria infections.

In 58 malaria positive, 17 (29.31%) *Plasmodium vivax*, 25 (43.10%) *Plasmodium falciparum*, and 16 (27.59%) mixed infection with *Plasmodium vivax* and *Plasmodium falciparum* were detected. In 80 dengue positive cases, 38.75% (31) NS1, IgM 25% (20), NS1 and IgM 25% (20), IgG 5% (4), IgM and IgG 6.25% (5) were detected [Table/Fig-3].

Disease	Findings	Percentage N (%)
Dengue positive (n=80)	NS1 antigen	31 (38.75)
	IgM antibody	20 (25)
	IgG antibody	04 (5)
	NS1 Ag +IgM Ab	20 (25)
	IgM Ab +IgG Ab	05 (6.25)
Malaria positive (n=58)	PV Antigen	17 (29.31)
	PF Antigen	25 (43.10)
	Both PV and PF Antigen	16 (27.59)

[Table/Fig-3]: Distribution of dengue (N=80) and malaria positive (N=58) cases.

Of the 21 with co-infection, 7 (33.33%) were severe dengue cases, 11 (52.38%) showed warning signs of dengue and 3 (14.28%) showed dengue without warning signs with malaria as co-infection [Table/Fig-4].

Malaria co-infection in dengue cases	*Plasmodium falciparum*	*Plasmodium vivax*	*P.vivax* + *P.falciparum*	Total
Number of severe dengue	4 (19.04%)	2 (9.52)	1 (4.76)	7 (33.33)
Number of warning signs of dengue in percentage	2 (9.52%)	7 (33.33)	2 (9.52)	11 (52.38)
Number of dengue without warning signs	1 (4.76%)	2 (9.52)	0	3 (14.29)
Number of total	7 (33.33)	11 (52.38)	3 (14.29)	21 (100)

[Table/Fig-4]: Malaria co-infection in dengue cases.

Out of these 21, *Plasmodium falciparum* was positive in 33.33% and 52.38% of *Plasmodium vivax* were positive and mixed infection of both *Plasmodium vivax* and *Plasmodium falciparum* were detected in 14.29% cases [Table/Fig-4].

In malaria and dengue co-infection, 52.38% (11) had hepatomegaly and jaundice and 23.80% (5) had haemorrhagic manifestation, haemoglobin was <12 g/dL in all, kidney failure was found in 4.76% (1), thrombocytopenia (platelet count <150,000/cmm) in 95.23% (20) and condition also more common in *Plasmodium vivax* infections. No death was detected in dengue and malaria co-infection cases [Table/Fig-5,6].

Clinical diagnostic features	No. of *Plasmodium falciparum*	No. of *Plasmodium vivax*	No. of *Plasmodium vivax*+*Plasmodium falciparum*	No. of total (%)
Platelet count <150000/cumm	07	10	03	20 (95.23)
Hb<12 mg/dL	07	11	03	21 (100)
Serum bilirubin >1.2 mg/dL	02	04	02	08 (38.09)
SGPT>55 U/l	01	03	01	05 (23.80)
Serum creatinine >1.5 mg/dL	01	0	0	1 (4.76)
Blood urea >45 mg/dL	01	01	0	2 (9.52)

[Table/Fig-5]: Malaria-dengue co-infection in haematological parameters.
Hb:Haemoglobin; SGPT: Serum glutamic pyruvic transaminase

Clinical symptoms of dengue with malaria infection	Number of *Plasmodium vivax* (%)	Number of *Plasmodium falciparum* (%)	Number of mixed *Plasmodium vivax*+*Plasmodium falciparum* (%)	Number of cases (%)
Febrile patients with species of *Plasmodium*	11 (52.38)	07 (33.33)	3 (14.28)	21 (100)
Nausea	2 (9.52)	1 (4.76)	1 (4.76)	04 (19.04)
Jaundice and liver enlargement (Hepatomegaly)	8 (38.09)	2 (9.52)	1 (4.76)	11 (52.38)
Headache	5 (23.80)	2 (9.52)	2 (9.52)	09 (42.85)
Pain of joint	1 (4.76)	1 (4.76)	0	2 (9.52)
Muscle pain	2 (9.52)	1 (4.76)	1 (4.76)	04 (19.04)
Haemorrhagic manifestation	2 (9.52)	2 (9.52)	1 (4.76)	05 (23.80)
Kidney failure	0	1 (4.76)	0	1 (4.76)
Skin rash	0	1 (4.76)	0	1 (4.76)

[Table/Fig-6]: Clinical symptoms of dengue presentation concomitant with malaria infection.

DISCUSSION

Dengue and malaria are common vector-borne diseases but are preventable. Both diseases have clinically similar features and symptoms however, they are separate and given different treatment.

Consecutive presence of malaria and dengue in one individual can easily be missed as the detection of any one of them in an acute febrile patient can cover the diagnosis of other [16]. In this study, the occurrence of concurrent infections of 3.47% were found. Other studies have comparatively different prevalence of concurrent infection, they have found rate as of 6% in India, French Guiana had 1% and Pakistan had 27% [17-20]. In a study by Abrahamsen SK et al., from Karnataka, 100 patients of acute febrile illness were diagnosed with dengue (25%); malaria (8.0%), and enteric fever (14%) [21].

A study by Singh R et al., in a retrospective review of 160 patients reported 23% malaria and 2% dengue fever while up to 54% died because of unexplained fever due to acute febrile illness in Mumbai, India and 18% patients were noted to have mixed infection [22]. More than one aetiological agent with concurrent infections leads to illness with overlapping symptoms and hence, patients diagnosis and treatment could be challenging [23]. There was typical conception of malaria incidence in rural spot and in urban region dengue was found in various reports which were coming from different countries because of overlapping of mosquito biotypes [24]. In this study, only hospitalised patients were included and the co-infection incidence does not represent prevalence in the community or local population. The concurrent infection in a locality was determined but the vector load could not determined [25,26].

The diagnosis of dengue infection was positive by the IgM test, but the acute and past infection cannot be differentiated because IgM can persist for one week after the onset of the symptom, as reported in case reports and cross-reaction with other arboviruses [27,28]. A patient at one time can be diagnosed both with malaria and dengue after five days of fever, and can be positive when tested with dengue rapid kit and malaria rapid kit. Patients have also been infected with both concurrent DENV and *Plasmodium* parasite infections in the present study. Some other possibility for both infections was that patients were previously infected with the serum IgM DENV and then infection with the malaria parasite.

The patients who have dengue infection; have low immunity for the period of convalescence which makes it predisposed to other infections. If dengue infection shows no signs of improvement in conservative treatment beyond one week of fever, clinicians should consider other co-infection possibilities, prominently malaria. The present study diagnosed *Plasmodium vivax* in 52.38% which was different from other studies [25,26]. However, it attributed *Plasmodium* species prevailing in an exacting ecological region. Both malaria and dengue co-infection have clinical symptoms which are more possible to single dengue than only malaria infection. So, clinical examination of both concomitant dengue and malaria is complicated. The present study found complications in patients infected with *P.vivax* that were common in malaria and dengue co-infection with haemorrhagic manifestations of five cases, enlargement of liver and jaundice was in 11 cases. In *Plasmodium vivax* infections, the main investigations found haemoglobin <12 g/dL, and 95.23% had decreased platelet count. In *P.falciparum* infection, altered liver and renal function tests were observed in co-infections. Deranged liver function was also found in one study [26].

The condition is remarkable to significance that haemorrhagic manifestations are frequent in dengue cases and rare in *falciparum* malaria. The low levels of platelets can be caused by both malaria and dengue and hence, it can be complicating to choose which one is responsible for the haemorrhage. However, malaria with bleeding is considered as severe malaria and treated accordingly [27].

This study was hospital based and such work in community may give better idea of malaria /dengue dual infections so its results were supposed to be interpreted with warning. If the very low prevalence of dual infection was such questionable, prospective studies through similar investigation methods and patient groups must be tried to verify the superior cruelty of co-infection. The benign outcome has also been observed in the other two studies [25,26]. A positive outcome was attributed to the early medical treatment of co-infection cases [29,30].

There were three distinguished results showed in the study, both malaria and dengue co-infection which were mosquito vectors co-exist and they are common in a geographical region was the first result. Second, the clinical symptoms of dengue fever are predominant more than malaria in concurrent infection. Third, *P. falciparum* is concerned with severe dengue and the warning signs were more in patients infected with both dengue and malaria infections. WHO classified the dengue cases based on a study of 80 dengue positive patients, dengue without warning symptoms, dengue with warning symptoms and severe dengue were respectively 42, 28 and 10 cases were found. In this study, 11 showed dengue with symptoms, severe dengue cases were 7 and less cases of dengue without warning signs was 3 cases [30,31].

In the present study, concurrent infections of malaria and dengue must be suspected in febrile patients diagnosed with both malaria and dengue, whether one or the other is positive. The study indicated that both dengue and malaria prevalence rates of co-infection are correlated with symptoms and haematological parameters, as well as the severity of such co-infections.

Limitation(s)

This was a hospital based study and co-infection of malaria and dengue does not reflect the community prevalence.

CONCLUSION(S)

All the patients having fever must be diagnosed for both malaria and dengue. Moreover, when both co-infection occurs in one patient it can cause other complications as well. When patients is febrile or returning from endemic region the clinicians treat should treat thoroughly and order examinations for both malaria and dengue diagnoses. The vector control is an important preventive measure in the populations and also proper hygiene, public health education are the preventive measures that are necessary to control these both diseases.

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