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Original Article

Microbiology Section

Co-existence of Malaria and Dengue: An Incidental Observation

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ABSTRACT

Introduction: Malaria and dengue are two most important arthropod borne diseases responsible for high morbidity and mortality across the globe. Both these communicable diseases have been a major threat to the public health not only in India but also in other tropical and sub-tropical regions of the world.

Aim: To study the prevalence of Dengue and Malaria along with the cases of co-infection among the patients visiting a tertiary care hospital located in central India.

Materials and Methods: The present prospective study was conducted for a period of two years from January 2019 to December 2020, in the serology section of the Department of Microbiology of a teaching tertiary care hospital. Three to five millilitres (mL) of venous blood samples from 1519 patients were tested for both dengue (NS1 antigen, IgM and IgG antibodies) by Enzyme-linked Immunosorbent Assay (ELISA) method and malaria peripheral smear and antigen by immunochromatographic method. All demographic parameters were simultaneously analysed. Statistical analysis was performed with the help of Chi-square test.

Results: Out of 1519 blood samples tested, 267 (17.5%) samples were positive for dengue and 6 (0.39%) samples were positive for malaria. No case of co-infection was detected. Maximum dengue cases were detected during post monsoon period while malaria cases were detected in monsoon and post monsoon period. Among the various dengue positive cases, 185 (69.2%) patients were diagnosed with recent primary infection while 20 (7.49%) patients had primary infection.

Conclusion: The present study concluded that seroprevalence of dengue was high in our geographical region with malaria being negligible. Present study incidentally recorded the fact that the two diseases may coexist in an individual but both the vectors rarely share the same geographical site.

Keywords: Co-infection, Dengue virus, Enzyme linked immunosorbent assay, Malaria parasite, Seroprevalence

INTRODUCTION

Malaria and dengue are two most common communicable diseases causing threat to public health in India and other tropical and sub-tropical regions of the globe [1,2]. Both are known to be vector borne diseases causing febrile illness and playing a significant role in terms of morbidity and mortality on account of its ease in globalised travel [1,3,4]. Among these two infections, malaria can become chronic in contrast to dengue [5].

Malaria is a parasitic infection caused by a protozoan parasite *Plasmodium* while dengue is known to be caused by a single-stranded Ribonucleic Acid (ssRNA) arbovirus called as Dengue Virus (DENV). *Plasmodium* is known to exist in form of five different species i.e *Plasmodium vivax (P. vivax), P. falciparum, P. malaria, P. ovale* and *P. knowlesi*. While, dengue virus exists in the form of four different serotypes i. e DEN-1, 2, 3 and 4. Malaria is known to be transmitted through female *Anopheles* mosquito while DENV is known to be transmitted through a female *Aedes aegpti* mosquito [4,6,7]. The typical transmission cycle of both malaria and dengue follows the human-vector-human cycle with rare chances of DENV shifting from an animal transmission cycle to human transmission cycle. The cumulative burden of these diseases have increased recently especially due to frequent outbreaks of dengue in several parts of the world including India [4].

Malaria parasitic infection has not been documented in the Indore city of Central India since several years however a dengue outbreak was first known to be documented in the year 2009 [8]. Infections from each of these is quite infuriating even if present independently. Now the question arises, can these two infections be present in a certain geographical area simultaneously? There are several published reports from different parts of the world indicating synchronous infection of both malaria and dengue in the same individual [1-5,9]. The severity of co-infection is always known to be greater than the monoinfection [9]. Also, according to the World Health Organisation (WHO), such co-infections in an individual is regarded as a 'severe malaria case' [10]. Superimposed infection may complicate the diagnosis and result into more severe form of combined disease since either of the pathogen may induce severe manifestations in form of thrombocytopenia, central nervous symptoms and cytokine storm [11]. Therefore, a more specific diagnosis is advised in either of the cases.

The present study was conducted to find out the seroprevalence of dengue and malaria along with the cases of co-infection among the patients visiting a tertiary care hospital in Indore, Madhya Pradesh, India.

MATERIALS AND METHODS

The present prospective study was conducted for a period of two years from January 2019 to December 2020 in the Serology section of the Department of Microbiology of a teaching tertiary care hospital located in central India. All the blood samples received in the serology laboratory for the investigation of dengue and malaria parasite, during the period of two years were considered for study. The study was orally approved by the Institutional Ethical Committee (IEC).

Inclusion criteria: All the blood samples that were tested for both malaria parasite and dengue were considered for the study.

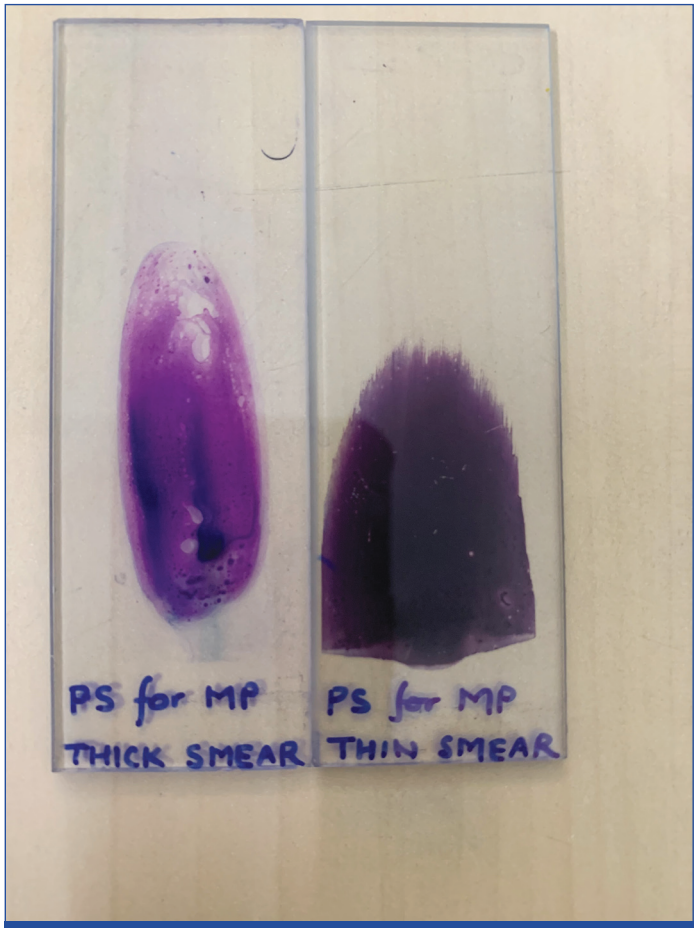
Exclusion criteria: The lipemic, haemolysed and icteric samples were rejected and excluded from the study.

Study Procedure

A 3-5 mL of venous blood was collected from 1519 patients in each of the two tubes (Ethylenediaminetetraacetic acid i.e EDTA tube and a plane tube). The patients were, clinically suspected cases of febrile illness compatible with dengue and/or malaria. The blood in the plane tube was further subjected to centrifugation at 1000 rpm

(rotation per minute) for five minutes. The serum obtained was then used to identify Dengue NS1 antigen (Qualisa NS1 Antigen, Tulip Diagnostics, Goa, India) and Dengue IgM and IgG antibodies (Qualisa Dengue IgM & IgG, Tulip Diagnostics, Goa, India) by solid phase ELISA. Optical Density was measured at 450 nm by automated ELISA machine, Erba Manheim Elan 30 (Transasia Bio-Medicals ltd., Mumbai, MS, India). The test was done according to the manufacturer's instructions.

Also, the whole blood from the same patient was used to perform Malaria antigen test (BeneSphera Malaria PAN/Pf kit, Avantor Performance Materials India Ltd., Gurgaon, Haryana, India) by using rapid diagnostic immunochromatographic test kit, following the prescribed protocol. Also, Field stained thick and thin Peripheral Smears (PS) were simultaneously prepared to identify the malaria parasites [4,5] [Table/Fig-1]. All demographic parameters were simultaneously analysed.



[Table/Fig-1]: Think and thin peripheral blood smears.

STATISTICAL ANALYSIS

The collected data was transferred to the computer and Microsoft Excel 2000 (version 9). Analysis Tool Pack was used for analysis of data. Chi-square test was performed and p≤0.05 was considered statistically significant.

RESULTS

A total of 1519 blood samples were tested for both Dengue (NS 1 antigen and IgM and IgG antibodies) and Malaria PS and antigen. Among them, 718 samples were from male patients and 801 were from females. Out of 1519 samples, 267 (17.5%) samples were found to be positive for dengue (any one or multiple parameters) and 6 (0.39%) samples were positive for malaria. Out of 267 dengue positive samples, 169 (63.3%) were from male patients while 98 (36.7%) were from females. The difference was not found to be statistically significant (p=1.8291). Similarly, out of six malaria positive samples, 4 (66.7%) were from males and 2 (33.3%) were from females [Table/Fig-2]. Among these, five samples had *P. vivax* and one sample had *P. falciparum* as the malaria parasite. Highest number of patients suffering from dengue belonged to the age group of 11-20 years followed by 21-30 years of age. While those suffering from malaria belonged to the age groups 21-30 and 31-40 years of age with more patients in the age group of 21-30 years. Whereas, more male patients suffering from dengue belonged to the age group of 11-20 years while more females belonged to the age group of 21-30 years. The difference was not found to be statistically significant (p=1.83289) [Table/Fig-3].

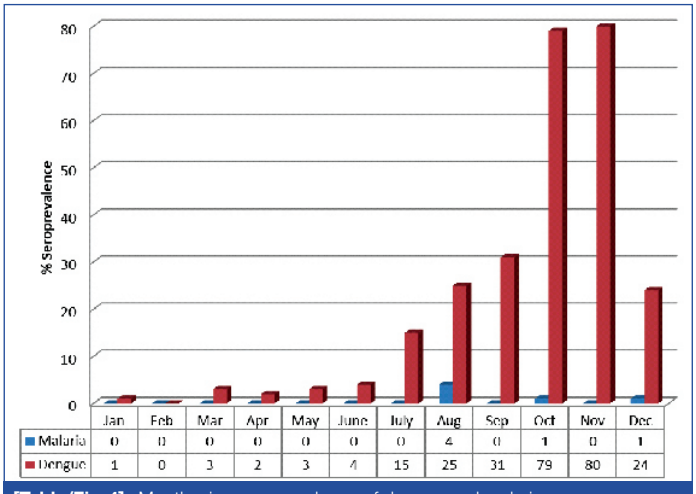
Name of the disease	Positive samples		Sample from Male patients		Sample from Female patients		p-value
	No.	%	No.	%	No.	%	
Dengue	267	17.5	169	63.3	98	36.7	1.8291
Malaria	06	0.39	04	66.7	02	33.3	

[Table/Fig-2]: Gender wise distribution of malaria and dengue cases.

Gender / Age group (Years)	Dengue		Malaria		p-value
	Male (63.3%)	Female (36.7%)	Male (66.6%)	Female (33.3%)	
0-10	07	03	0	0	
11-20	92	31	0	0	
21-30	47	50	02	02	
31-40	14	07	02	0	
41-50	3	01	0	0	
51-60	1	04	0	0	1.83289
61-70	05	0	0	0	
71-80	0	02	0	0	
81-90	0	0	0	0	
More than 91	0	0	0	0	
Total	169	98	04	02	

[Table/Fig-3]: Age wise distribution of patients tested positive for dengue and malaria.

Highest numbers of dengue positive cases were detected during post monsoon period (October-November). Positive malaria cases were also detected during monsoon and post monsoon period [Table/Fig-4]. Among the various dengue positive cases, 185 (69.2%) patients were diagnosed with recent primary infection while 20 (7.49%) patients had primary infection. A total of 185 (69.2%) patients tested positive for NS1 antigen, 53 (19.8%) tested positive for IgM while 76 (28.4%) tested positive for IgG immunoglobulins [Table/Fig-5].



[Table/Fig-4]: Month wise seroprevalence of dengue and malaria.

S. No.	Dengue diagnostic parameters (Seropositivity)			Interpretation of results	No. of cases (1519)	Positivity (%)
	NS1	IgM	IgG			
1.	+	-	-	Recent primary infection	149	55.8
2.	-	+	-	Primary infection	20	7.49
3.	-	-	+	Secondary infection	55	20.5
4.	+	+	-	Recent primary infection	22	8.23
5.	+	-	+	Recent primary and secondary infection	10	3.74
6.	-	+	+	Recent Secondary infection	07	2.62
7.	+	+	+	Second exposure of infection or recent secondary infection	04	1.49
8.	-	-	-	Unknown febrile infection	1252	82.4

[Table/Fig-5]: Interpretation of various dengue diagnostic parameters.

DISCUSSION

The present prospective study was conducted with an aim to determine the seroprevalence of dengue and malaria among the patients visiting the hospital with febrile illness along with the complains of myalgia, headache and nausea. Each of the patients was tested for both malaria and dengue apart from other haematological and clinical parameters. Initially, the study was set up for 12 months period. But later on, in order to confirm the observations of last one year, the study period we extended for one more year.

During the study period of 24 months, authors observed that only dengue was prevalent in the area. Less than one percent (0.39%) cases of malaria was detected. It was surprising because during early years of this decade, there were enormous number of cases of malaria. Dengue used to be detected sporadically among very few numbers of patients before and after a dengue outbreak in 2009 [8].

When dengue and malaria coexists in the same patient then it is called as a concurrent infection of dengue and malaria. Due to similarities in the clinical characteristics exhibited by the two infections, it is often misinterpreted or misdiagnosed as a mono infection. However, the biological picture and treatment protocols of both the infections may vary greatly. Dual infection sometimes drastically changes the spectrum of clinical manifestations by posing a diagnostic challenge. However, the cases of dual infection have been rarely documented across the globe [1-5,9,12].

In the present study, no case of co-infection in the period of 24 months was found. However, it was astounding to observe that 17.5% cases of dengue were observed over a period of 24 months while there were only 0.39% cases of malaria. On collecting the travel history of patients who were detected as malaria positive, it was observed that all of the six patients had travelled to malaria endemic areas like Zabua, Jabalpur and Ratlam districts of Madhya Pradesh, India, a few days prior to the appearance of symptoms. Therefore, to confirm the endemicity of dengue and total absence of malaria parasite in our area, literature search was done [1-7,9]. Basically, it is all about the availability of mosquito vectors in the geographical region [7].

As it is well known that each infection has specific mosquito vector and each of these vectors have a different habitat. Malaria mosquito vector i.e Anopheles prefers forest, while dengue mosquito vector i.e Aedes prefers urban areas. Therefore, overlapping of habitats is not easily available [4,13,14]. The typical, human-vector-human transmission cycle is commonly exhibited in both malaria and dengue. However, there is a great potentiality of dengue virus to shift from animal transmission cycle (sylvictic jungle cycle in monkeys) to human transmission cycle [4]. Dengue is assumed to be an urban disease but the Aedes mosquito has been located in forested areas when it is not circulating in humans. It is known to spread in cities especially where there are abundant stocks of fresh water available along with an unplanned urbanisation that creates an environment that support the breeding of vectors [1,5,15-18].

Since, long time malaria had been endemic in our area and hence population must have developed immunity against malaria parasite [19]. Even interspecies cross protection can be one of the possible reasons for elimination of one and emergence of other mono infection in a certain geographical area [11]. So, co-infection in most of the geographical areas would have been occurring by chance and that is why there are scarce published cases as far as concurrent infections are concerned with respect to malaria and dengue [2-5,7]. Whenever, there are cases of co-infection, there might be history of travel to a certain vector endemic regions. The reasons for disappearance of malaria vector in our region can also be attributed to establishment of 'cleanest city' since last four years. Vectors might not be getting enough establishing sites for propagation. Instead, the day biting dengue vectors prefer to reproduce in sites where fresh water is stored in flower vase and planters inside residential areas. Also, the primary vectors for dengue are diurnal and therefore renders the use of insecticide treated mosquito nets useless in dengue control [11]. Thus, it can be said that the same individual might carry the two organisms but the two vectors may not coexist in the same geographical sites there by reducing the chances of co infection. The chances of an individual carrying a co-infection may be imputed to their travel history in malaria/dengue endemic region [7]. However, in geographical areas where both the vectors-we cannot rule out the simultaneous occurrence of both the diseases in a single individual. Since both the diseases share some clinically indistinguishable characteristics it become very essential to differentiate the two on the basis of laboratory diagnosis in order to rule out poor patient outcomes.

Though, it was an incidental finding in present study, it was quite sure that there might be a wide overlap between the endemic areas of both these vectors in some parts of the world. The worldwide documentation of co-infection are scarce, either both the cases are not being laboratory confirmed simultaneously or the reason of under diagnosis of one of the infection may be because of self limitation turning into recovery in case of dengue or carrier status in case of malaria when patients do not visit clinicians during acute febrile illness [7]. Even sometimes if one underlying disease is laboratory diagnosed in a patient with acute febrile illness, there is always a very low index and suspicion for co-infection. However, severity could prompt the testing for dual infections [4,11]. Sometimes, co-infection in an individual is missed during cases when laboratory detection of one of them in an acute febrile patient masks the diagnosis of another [3].

Still, worldwide studies have detected co-infection in the range of 1-27% thereby challenging the classical concept that dengue occur in urban and malaria in rural areas [1,3,5]. Authors feel that collection of travel history would help in understanding the vector spread and overlapping of mosquito biotypes.

In this study, maximum number of cases was diagnosed in the post monsoon period and present study results resembled those published by other authors in the previous studies. Just like present study, different authors have also noticed a gradual increase in cases following August with a peak reaching in November (post monsoon period) and soon tapering down to zero after the month of December [20-23]. This may be because the post monsoon cooler months favor the breeding of vectors thereby extending the risks of viral transmission.

Even if the study did not come across any cases of concurrent infection of dengue and malaria, it was suggested the laboratory confirmation of both the vector borne diseases in patients visiting a hospital with febrile illness because even if the vector may not be prevalent in the patients' residential locality there may be chances that the individual would have travelled in the areas endemic for either of these infections.

Initially, 'Anopheles' was the 'domestic' vector. Urbanisation and globalised travel was responsible for proliferation of '*Aedes aegyptii*' and establishment of dengue virus in the city. Now, 'Aedes' has become endemic in our region and sporadic cases of malaria in our city are attributed to the travel history of patients in malaria endemic zones.

Limitation(s)

Though, this study covered most of the aspects related to both the diseases, there were few limitations. Firstly, the study only referred haematological and clinical parameters for the purpose of correlation. However, those parameters in the manuscript was not tabulated. Secondly, since authors did not mark the day when the particular patient's sample was tested after the onset of illness, hence the data would have underestimated the overall prevalence. Thirdly, because antibodies to other pathogens including SARS-CoV-2 virus (as the study was being conducted during the COVID-19 pandemic months) is known to cross react with dengue ELISA, there might be possibilities of false positivity in dengue IgM antibody results.

CONCLUSION(S)

The present prospective study incidentally detected that only dengue cases prevailed in the past two years with the cases of malaria in a very insignificant number. No case of co-infection was detected. Therefore, it can be concluded that though the two diseases may coexist in an individual, but they may rarely co-exist in the same geographical area. Co-existence in an individual may be ascribed to his travel history in the dengue/malaria endemic area. However, the threat of dengue is prevailing in our region.

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REFERENCES

[1] Faruque LI, Zaman RU, Alamgir ASM, Gurley ES. Hospital-based prevalence of malaria and dengue in febrile patients in Bangladesh. Am J Trop Med Hyg. 2012;86(1):58-64.
[2] Kotepui M, Kotepui KU. Prevalence and laboratory analysis of malaria and dengue co-infection: A systemic review and meta-analysis. BMC Public Health. 2019;19:1148.
[3] Shah PD, Mehta TK. Evaluation of concurrent malaria and dengue infections among febrile patients. In J Med Microbiol. 2017;35:402-05.
[4] Selvaretnam A, Sahu PS, Sahu M, Ambu S. A review on concurrent infections of malaria and dengue in Asia. Asian Pac J Trop Biomed. 2016;6:633-38.
[5] Rao MRK, Padhy RN, Das MK. Prevalence of dengue viral and malaria parasitic co-infections in an epidemic district, Angul of Odisha, India: An eco-epidemiological and cross-sectional study for the prospective aspects of public health. J Inf. Public Health. 2016;9:421-28.
[6] Salam N, Mustafa S, Hafiz A, Chaudhary AA, Deeba F. Parveen S. Global prevalence and distribution of coinfection of malaria, dengue and chikungunya: A systemic review. BMC Public Health. 2018;18(1):710.
[7] Wiwanitkit V. Concurrent malaria and dengue infection: A brief summary and comment. Asian Pac J Trop Biom. 2011;1:326-27.
[8] Jain S, Bajpai T, Bhatambare GS, Deshmukh AB. Serodiagnostic and clinical presentation of dengue virus infection in a tertiary care hospital. Journal of Pure and Applied Microbiology. 2012;6 (spl.ed.):33-37.
[9] Arsalan Q, Qadar LT, Ochani RK, Tahir F, Majid Z. Collateral presentation of malaria and dengue viral hemorrhagicfever: A rare case. Cureus. 2019;11(2):e4050.
[10] World Health Organization. Guidelines for the treatment of Malaria. 3rd ed. Geneva: WHO; 2015. (Online): http://www.cdc.gov/malaria/about/biology.
[11] Bygbjerg Lb C, Simonsen L, Schioler KL. Elimination of falciparum malaria and emergence of severe dengue: An independent or interdependent phenomenon? Hypothesis and Theory. 2018;9:1120.
[12] Epelboin L, Hanf M, Dussart P, Epelboin OS, Djossou F, Nacher M, et al. Is dengue and malaria co-infection more severe than single infections? A retrospective matched-pair study in French Guiana. Malar J. 2012;11:142.
[13] Obsomer V, Defourny P, Coosemans M. The Anopheles dirus complex: Spatial distribution and environmental drivers. Malar J. 2007;6:26.
[14] Cox J, Grillet ME, Ramos OM, Amandor M, Barrera R. Habitat segregation of dengue vectors along an urban environmental gradient. Am J Trop Med Hyg. 2007;76(5):820-26.
[15] Gubler DJ, Suharyono W, Sumarmo, Wulur H, Jahja E, Saroso JS. Virological surveillance for dengue haemorrhagic fever in Indonesia using the mosquito inoculation technique. Bull World Health Organ. 1979;57(6):931-36.
[16] Mondini A, Neto FC. Socioeconomic variables and dengue transmission. Rev Saude Publica. 2007;41:923-30.
[17] Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 2002;10:100-03.
[18] Gubler DJ. Cities spawn epidemic dengue viruses. Nat Med. 2004;10:129-30.
[19] Jordan Ws. Jr New approaches to vaccine development. Proceedings of a meeting organized by the World Health Organization. Book review. Int Health News. 1985;6(6):06-08.
[20] Patel P, Bhatnagar R. Seroprevalence of dengue infection: A hospital based study from Udaipur, Rajasthan. The Journal of Community Health Management. 2018;5(1):10-12.
[21] Garg A, Garg J, Rao YK, Upadhyay GC, Sakhuja S. Prevalence of dengue among clinically suspected febrile episodes at a teaching hospital in North India. J Infectious Diis Immu. 2011;3(5):85-89.
[22] Ukey PM, Bondade SA, Paunipagar PV, Powar RM, Akulwar SL. Study of seroprevalence of dengue fever in Central India. Indian J Community Med. 2010;35(4):517-19.
[23] Bhat SK, Sastry AS, Senthamarai S, Sivasankari S. Seroprevalence of dengue virus infection in patients attending to a tertiary care hospital in Kanchipuram, Tamil Nadu, India. Int J Res Health Sci. 2014;2(3):818-22.

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