STATUS OF DENGUE AND MALARIA CO-INFECTION AMONG PATIENTS ATTENDING MARDAN MEDICAL COMPLEX, PAKISTAN

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ABSTRACT

BACKGROUND: Dengue fever and Malaria are the most common mosquito borne diseases in the world and have emerged as a global public health problem. Both of these diseases can effect a person alone or as a co-infection. These diseases can cause death and as a co-infection are more severe than single infection. This study was conducted to determine the frequency of patients with Dengue and Malaria co-infection and the proportion of confirmed cases of Dengue and Malaria in the same period.

METHODS: A descriptive cross sectional study on primary data was conducted at Mardan Medical Complex Mardan, from 1st September 2017 to 31 Jan 2018. Data was collected from 481 subjects and screened for dengue and malaria infections. A structured Proforma was used for data collection and all patients who are advised both malaria and dengue tests were included. Those who were not willing to contribute were excluded. Non probability convenient sampling technique was used and a sample size of 481 was obtained. For dengue infection, ICT strip method of IgM, IgG antibodies test and NS1 antigen test were performed. For malaria diagnosis , thick and thin slides were made and examined through light microscope. Age with gender and district of domicile was also noted after the informed consent.

RESULTS: Majority of patients were from district Mardan and second highest were from district Nowshera. Male infected parentage was higher than that of females. Mean age of all patients was 29.48 years. In 481 patients 122 patients were co-infected with malaria and dengue, 164 patients were only dengue positive and 205 patients were only malaria positive.

CONCLUSION: Malaria infected patients were more than dengue patients and also malaria contribute as a co-infection. The severity of disease is more if co-infection exists. Patients must be investigating both for dengue and malaria at the same time. This will help in initiatingr early response if co-infection exists.

KEY WORDS: Dengue Fever, IgM, IgG, MP, Aedese aegypti, P.vivax, P.falciparum.

INTRODUCTION

Dengue and malaria both are the most common mosquito borne diseases of mankind and emerged as a global public health problem.

In 1906, transmission by the Aedes mosquitoes was affirmed, and in 1907 dengue was the second infection (after yellow fever) that was appeared to be caused by a virus[1]. High endemic transmission is reported in Vietnam, Indonesia, Thailand, India, Pakistan, Malaysia and Philippines. Dengue is still continuous extending its range[2]. Dengue is transmitted by Aedes mosquitoes, mainly Aedes aegypti and also Aedes alpopictus. There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3 and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue[3].

The first confirmed outbreak of Dengue Hemorrhagic Fever (DHF) in Pakistan was reported in 1994 and the serotype was confirmed and reported DEN-2[4]. Infection of dengue can result in a broad spectrum of disease syndromes which ranges from asymptomatic or mild infection, classical dengue fever (DF), to the fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)[5]. The genome of single strand RNA virus is composed of three structural protein genes that encode the nucleocapsid or core protein (C), a membrane-associated protein (M), an envelope protein (E), and seven nonstructural (NS) protein genes: NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5[6]. Among the NS proteins, NS1 is a highly conserved glycoprotein, which is essential for virus replication. During the acute phase of DENV infection, NS1 protein is found associated with intracellular organelles and can be transported via cellular secretion pathway to the infected cell surface.

NS1 antigen test (nonstructural protein 1) for dengue is introduced in 2006. It allows rapid detection on the first day of fever, before antibodies appear some 5 or more days later. It has been adopted for use in some 40 nations[7].

Primary dengue virus infection is characterized by elevations in specific immunoglobulin M (IgM) levels 3 to 5 days after the onset of symptoms; this generally persists for 30 to 60 days[8,9], whereas IgG appear by the fourteenth day and persist for life.

Malaria is another mosquito borne life threatening disease which is trans-

mitted through the bite of amosquito. Charles Louis Alphonse Laveran was the French army surgeon, on 5th of November 1880 he was the first who notice the malaria parasites in the blood of a patient effected from the malaria[10].

In most cases, malaria is transmitted through the bites of female Anopheles mosquitoes. There are more than 400 different species of Anopheles mosquito; around 30 are malaria vectors of major importance[11].

4 major types of malaria species infecting human are, Plasmodium Falciparum, P. Vivax, P. Malariae and P. Ovale. Other than these species of malaria there are number of plasmodium species able to infect humans but these are very rare. In these other species of plasmodium, the most reported is P. Knowlesi which is found in SE Asia and usually infect Macaque monkeys but also have ability to infect human and causing sever malaria even can cause death[12].

Plasmodium Falciparum is causing majority of malaria deaths worldwide. P. Vivax which is second most important specie and commonly present in Latin America and Southeast Asia. Plasmodium vivax and P. Ovale have the major complication of dormant liver stage in which it can be reactive without the mosquito bite. Plasmodium Ovale and P. Malariae both species have small contribution of infection. Fifth specie of human infection is plasmodium knowlesi which normally infect primates but can cause human infection cases are very rare, the mode of transmission is still unclear[13].

According to WHO estimates, released in December 2016, there were 212 million cases of malaria in 2015 and 429 000 deaths[14].

Pakistan is almost in the middle of the malarial belt around the globe encompassing tropical and subtropical countries. Some 270 million new cases of malaria occur every year of which 95% are reported from these areas[15].

Despite a well-established malaria control programme, 500,000 malaria infections and 50,000 malaria-attributable deaths occur each year in Pakistan[16].

Concurrent infection of malaria and dengue are when both the diseases occur simultaneously in an individual. Since there are similarities in the clinical characteristics between these two infections, diagnosis of malaria and dengue co-infections might be either misdiagnosed or misinterpreted as mono-infections[17].

The first 2 patients with concurrent malaria (Plasmodium falciparum) and dengue were identified in July 2005 18 and November 2006 (P. vivax)[19].

The biological and clinical characteristics of dengue and malaria are very similar, all clinicians treating patients in or returning from endemic areas should systematically order examinations for both diagnoses, even if one of the disease is positive[20].

The diagnosis of dengue infection is usually made based on positive dengue IgM; however, this cannot confirm recent dengue, because IgM can persist for months and cross-react with other arboviruses[21].

Anti-dengue IgM false negative reactions are observed in secondary infections and there are a small percentage of secondary infection patients without detectable IgM antibodies 22. A study conducted (2006) in Pakistan, 9 out of 11 patients with dengue specific IgM were also found positive for malarial parasites on the peripheral smear 23. The clinical significance of the concurrent infection has gained added significance as concurrent dengue-malaria infection has higher morbidity than either of the infections alone 24. The objective of this study was to determine the frequency of patients with dengue and malaria co-infection and to determine the proportion of confirmed cases of dengue and malaria in the same period.

MATERIAL & METHODS

The study design was descriptive cross sectional study on primary data. The study was conducted in Pathology of department of Mardan Medical Complex Teaching Hospital Mardan during 1st September 2017 to 31 January 2018.

All patients who are clinically checked and advised for dengue antibodies and antigen test with MP test are included. These patients are referred from OPD and A & E department. Sampling technique in this study was Non probability purposive sampling. Data was collected all referred patients from OPD and A&E department of MMCTH Mardan were on daily basis for a period of 2.5 months. Between this duration I have collected data from 481 patients.

The data collection tool was used a Structured Performa. The variable of interest wasage of patient, gender, dengue antibodies (IgM, IgG) test, dengue antigen (NS1) test, MP test, and district from where patient belongs. The patients who are advised individually for MP or Dengue test are not included in this study. The tests report and patient data was entered in Performa. For dengue test blood is collected in Gel tube properly labeled and send to serology section. Blood sample tube was first centrifuged and serum was separated. ICT strip method was used to diagnose dengue Antibodies (IgM, IgG) and Antigen(NS1). Serum was collected in a dropper or Adjuster which is automatic micropipette and two drops of serum was placed in a ICT strip. ICT strip have one positive control line which shows validity of test or control and result line which shows positive or negative result of the patient. For Malaria parasite blood is collected in EDTA tube and after labeling properly sample tube was send to hematology section of the Lab. Slides are prepared in staining room with Giemsa stain. After the preparation of slide visualized under the microscope which is a gold standard for diagnosing malaria. After the end of study duration all data was entered in SPSS Version 20 systematically.

RESULTS

A sample of 481 patients was selected through non probability convenient technique. Out of those383(79.6%) were from district Mardan,56 (11.6 %) werefrom district Nowshera, 26(5.4 %) were from district Swabi and 16 (3.3 %) werefrom other districts.

TABLE 1: DESCRIPTION OF PATIENTS AGE IN YEARS

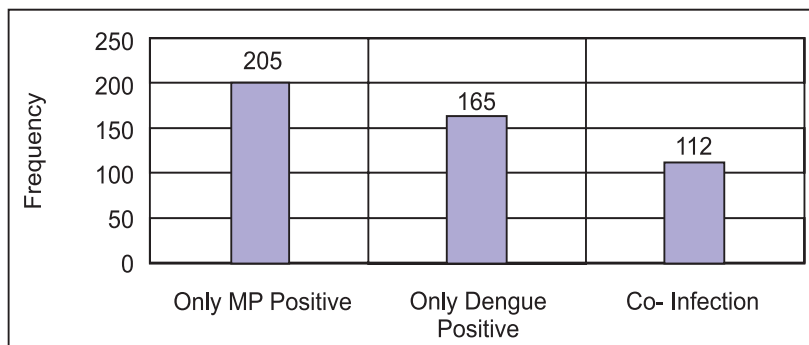| Mean	29.48
Median	27.00
Mode	25
Std. Deviation	16.718
Range	84
Minimum	1
Maximum	85

FIG 1: LAB FINIDINGS
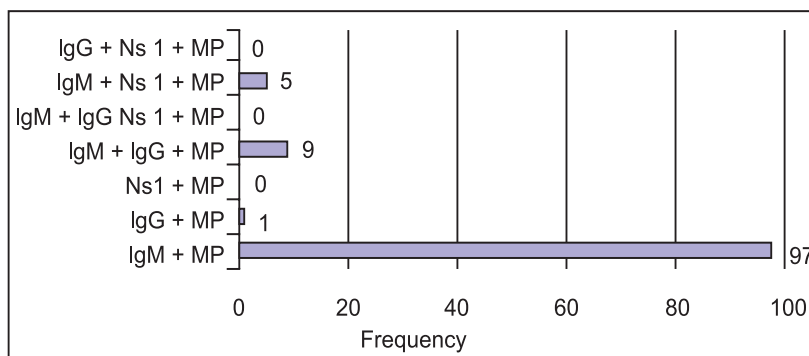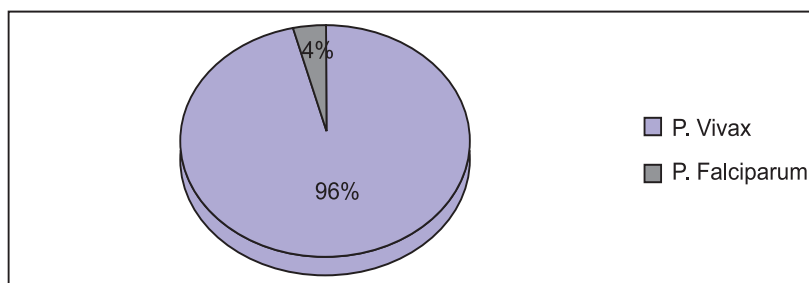

FIG 2: CO-INFECTION STATUS

FIG 3: MP POSTIVE PATIENTS

DISCUSSION

Mardan district have only one big complex teaching hospital and according to facilities the only hospital in the surrounding cities. Other district patients are also received at this hospital with a large number of patients. In 2017 there is large discussion was on dengue fever and people was also too much scary from this disease because many people died suffering from this disease. Government was also focusing too much to control this mosquito transmitted disease which affect thousands of people in different districts or cities of Khyber Pakhtunkhwa (KPK).

The malaria is also mosquito transmitted disease and can cause death but government was mostly focusing on dengue in previous few years. So that's why I selected this study to check the frequency of co-infected patients which is more on risk as compared to individually with one infection and also need to confirm the proportion of dengue effected patient's vs malaria effected patients.

Malaria control program is working from last few years but still malaria is more effecting people than dengue as in this study shows.

It is usually assumed that P. falciparum is the most likely Plasmodium specie to cause severe malaria, there is growing evidence in the literature that P. Vivax can also cause severe complications[25]. 112 patients are diagnosed as a co-infection patient of malaria and dengue in this study all co-infection patientswere P. Vivax positive. The presence of co-infection and disease could lead to severe complications[26]. In this study positive patients are considered on the basis of IgM, IgG antibodies and NS1 antigen positive test individually one of this or more than one of this antibody positive test with MP positive. Co-infected patients presented deep thrombocytopenia more frequently than patients with single infections. Low platelets are common in dengue and malaria. In febrile patients living or returning from endemic areas, it is a good predictive factor of malaria[27, 28] and in case of negative malaria diagnosis it is a good predictive factor of dengue 27. 97 patients out of 112 patients were IgM antibody positive with MP test positive which are 87 % of these total patients. almost all previous studies on dengue and malaria co-infections relied on IgM diagnosis[29,30]. 9 patients with IgM and IgG antibodies positive with malaria positive which contributes 8 % of total. 4% patients which are 5 patients in numbers are IgM antibody positive and NS1 antigen test positive with malaria positive. Only 1 patient with total of 1 % contributes was IgG antibody positive and NS1 antigen test positive with malaria positive test. Only NS1 antigen test positive with MP positive is not noted which means

clinically sign and symptoms may bejust because of dengue. There is also no case is noted in co-infection which is IgG positive and NS1 positive with MP positive, IgM and IgG positive and NS1 positive with MP positive. A study conducted (2006) in Pakistan, 9 out of 11 patients with dengue specific IgM were also found positive for malarial parasites on the peripheral smear[31].

There are 164 patients out of 481 patients which are only positive for dengue test. On the basis of only IgM positive patients were 88 (54%) which is the highest numbers in 164 patients. Second highest percentage was the NS1 antigen positive patients are 25 % which includes 41 patients. NS1 antigen test positive with antibodies test contributes 8 % of patients in which IgM with NS1 positive are 6 % and both of antibodies (IgM, IgG) with NS1 positive are 2 % and there is no case of NS1 with IgG positive. Secondary infection shows that IgG rises within 1 to 2 days after onset of symptoms, simultaneously with IgM antibodies. Therefore, patients with secondary infections will have a positive IgG result, usually, but not always with a positive IgM result[17, 32]. IgG antibody test positive patients in this study was 10 only which was 6 % of total only dengue positive patients. The Infection of primary dengue immunoglobulin G titers are mostly detectable at low levels at the end of first week of illness and after that slowly increasing, IgG in serum can be detectable after several months and also possible for lifelong. In the secondary infection IgG antibodies are detectable at high level in the acute phase. The greater increase in IgG antibodies in acute phase and convalescent paired sera can used to report recent infection of dengue[23]. Both of antibodies which are IgM, IgG positive patients was 11 (7 %).

Clinical presentation, laboratory diagnosis and management of dengue in Pakistan has been quite complex due to concurrent or super infection with malaria, typhoid and hepatitis[32,33].

Differentiating malaria from dengue, based on purely clinical grounds is difficult. Compared with those with malaria, patients with dengue are

more likely to develop abrupt onset of fever, with severe headache, myalgias and arthralgias (severe pain gives it the name of break bone fever), and rashes[34].

Aedes aegypti and Aedes albopictus species of mosquito also transmit yellow fever, chikungunya and zika infections[3]. So there is need of precaution to avoid from mosquito bites.

To reduce mosquito bites such as wearing full-sleeve clothes and long dresses to cover the limbs, use of repellents, coils and electric vapor mats, impregnated bed nets, trap lights, magnetic repellents and curtains cannot be overemphasized. Vector reduction is the most important measure in control of dengue but strategies differ from the ones which are employed for malaria control. Aedes. Aegyptiis a day biting mosquito. That means that the mosquito is most active during daylight, for approximately two hours after sunrise and several hours before sunset[35].

Overall percentage of all these received patients in this study at MMCTH Mardan are 66% (43 % only MP positive + 23 % co-infected patients) malaria positive,34 % (164) patients was only dengue positive patients. Individually P. Falciparum is more life threatening than any other of malaria species 4 in this study 8 patients of total malaria patients are P. Falciparum positive.

CONCLUSION

Malaria infected patients were more than dengue patients and also malaria contribute as a co-infection. The severity of disease is more if co-infection exists. Co-infection needs to be properly managed on priority basis. Government should focus on both diseases equally because both are mosquito borne disease so need to devise strong policies to control mosquitoes borne diseases. Patients must be investigating both for dengue and malaria at the same time. This will help in initiating early response if co-infection exists.

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