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"TROPICAL AETIOLOGY OF FEBRILE ILLNESS IN A TERTIARY CARE HOSPITAL OF NORTHERN INDIA"

Microbiology

Dr. Vinita Choudhary* Assistant Professor, Department of Microbiology, MGUMST, Jaipur. *Corresponding Author

Dr. Chetan Choudhary Assistant Professor, Department of Emergency Medicine, MGUMST, Jaipur.

Ms. Ayushi Sharma Demonstrator, Department of Microbiology, MGUMST, Jaipur.

Mr. Vinod Kumar Sharma Demonstrator, Department of Microbiology, MGUMST, Jaipur.

Dr. Pushpendra Saraswat Director of laboratory; Central Research Laboratory, MGUMST, Jaipur.

ABSTRACT

Background: During rainy season, majority of the febrile illness present as undifferentiated pyrexia of varying etiology. The purpose of this study is to identify the etiology and prevalence of tropical pathogens in tropical and sub-tropical regions. **Method:** Serum specimen of 150 patients were collected aseptically in Vacutainers. Nucleic Acid was extracted manually and was processed in Multiplex Real-Time PCR for identification of 8 tropical pathogen. **Result:** In this study, 150 patients presented with signs and symptoms of febrile illness patients and were tested by Multiplex PCR. Out of total 40 (26.66%) positive specimens, 19(47.5%) were positive for Dengue, 12(30) were positive for Rickettsia (scrub typhus),7(17.5) were positive for Chikungunya, 2(5%) were positive for Plasmodium. 110 (73.33%) were negative for all these tests hence the aetiology was not known. **Conclusion:** The prevalence pattern of tropical aetiology presented during the rainy season was found to be of dengue (47.5%), followed by Scrub Typhus (30%) and Chikungunya (17.5%)

KEYWORDS

febrile illness, Multiplex Real-Time PCR, tropical fever

INTRODUCTION

In tropical and subtropical regions, many infectious diseases are more prevalent due to ecological, geographical, and socioeconomic factors that facilitate their spread. This high diversity of tropical pathogens mirrors the rich biodiversity in the tropics and subtropics as they include bacteria, fungi, parasites, helminths, and viruses.[1,2] A large number of vector-borne pathogens are spread through insects or invertebrates [3,4] and the extrinsic incubation period for these pathogens is affected by climate [5] , which can impact behaviour and physiology of insects or invertebrates.[6] The growth of the human population, habitat encroachment, migration, trade, and pollution all create new opportunities for the spread of microbes due to global changes such as climate change and climate variability.[7]

There are many tropical pathogens around the world. **Table 1** There are 14 tropical diseases categorized into protozoa, bacterial, and viral infections that are globally important. There is often a lack of local and global estimates of the burden of these tropical diseases, poverty, geographical isolation, and a lack of coordinated control measures for some of these diseases.[8] Protozoan infections are the first to be considered: malaria, one of the most deadly and difficult parasitic diseases to control, and further threatened by the emergence and spread of resistance to antimalarials. [9] Next, we have bacterial infections, including leptospirosis, a common zoonotic disease with outbreaks appearing in rural and urban environments, [10,11] and recently known to strike adventure travellers melioidosis, a disease reported worldwide. [12,13] and salmonellosis. which causes enteric fever and has a high global incidence[14] Furthermore, most infectious diseases are primarily caused by viruses: Chikungunya fever in the Indian Ocean islands, on the Indian subcontinent, in southeast Asia, Africa and Europe, along with its emergence in the Americas .[15,16,17]

Dengue fever including dengue haemorrhagic fever, [18,19,20] and West Nile fever which is becoming more widespread in Africa, the Middle East, Europe and Asia, [21] Japanese encephalitis has spread to Australasia [22] and Asia [23] yellow fever is prevalent in West and Central Africa ,[24] hand, food, and mouth disease has been reported in Southeast Asia, [25,26] and Rift valley fever has spread to Yemen, Saudi Arabia, northern Egypt, and the French island of Mayotte [27] and Hantavirus haemorrhagic fever, which can cause serious diseases in humans with mortality rates as high as 12% (Hantavirus renal syndrome) and 60% (Hantavirus pulmonary syndrome) in some outbreaks. [3,28] Despite being medically significant, some of these diseases have grossly underestimated incidence rates because of a lack of access to rapid diagnostic tests. [10,1]

As tropical diseases spread around the world, it is essential that we are prepared to deal with them. Fast and accurate diagnosis of medically important diseases is the first goal of this preparedness. Differential diagnosis is determined primarily by clinical examination, taking into consideration local disease prevalence, exposure, and travel history. It is difficult to make a correct diagnosis based on traditional clinical observations due to the similarity and non-specific nature of symptoms induced by many tropical pathogens [10,29,30,31] The correct diagnosis is necessary for implementing effective control measures, from timely therapeutic intervention to effective treatment and effective clinical management based on community-wide measures to improve the patients' clinical outcome, disease mapping, impact monitoring, and post-elimination surveillance. A correct diagnosis can only be made through laboratory confirmation of the presence of tropical pathogens in clinical specimens. As a highly specific and sensitive method of molecular detection, multiplex real-time PCR has been used to diagnose numerous infectious diseases. [32]

Table 1: Tropical Diseases, Categorized By Protozoal, Bacterial, And Viral Infections, Including Causative Agents And Endemic Areas. [8]

Tropical disease	Causative agent	Endemic areas
Bacterial interactions		
Leptospirosis	Pathogenic *Leptospira* group	Global distribution
Salmonellosis	Enteric fever: *S. enterica* serovars *S. Typhi*, *S. Para typhi* (*S. enterica*)	Global distribution
Melioidosis	*Burkholderia pseudo mallei*	Global distribution
Viral infections		
Chikungunya fever	*Chikungunya virus* (CHIKV)	Reunion island, South-East Asia
Dengue fever	*Dengue virus* (DENV) (serotype 1 to 4)	Global distribution

Japanese Encephalitis	*Japanese Encephalitis virus* (JEV)	South-East Asia, Indian subcontinent, sporadically in Northern Australia
West Nile fever	*West Nile virus* (WNV)	West and Central Africa, America
Hand, foot and mouth disease	Human *enterovirus A EV-71* (EV71)	Global distribution
Viral haemorrhagic fevers	*Bunyaviridae*: *Hantaviruses* including *Dobrava-Belgradevirus* (DOBV), *Hantaanvirus* (HTNV), *Seoulvirus* (SEOV), *Puumala virus* (PUUV), *Tula virus* (TULV), *Andes virus* (ANDV)	Global distribution
Rift Valley fever	*Rift Valley virus* (RVV)	Arabian Peninsula and Africa
Protozoan infections		
Human African trypanosomiasis	*Trypanosoma brucei gambiense*, *T. brucei rhodesiense* (*T. brucei*)	Africa
Chagas disease	*Trypanosoma cruzi* (*T. cruzi*)	Latin America
Malaria	*Plasmodium falciparum* (*P. falciparum*), *P. knowlesi P. malariae*, *P. ovale*, *P. vivax*)	Global distribution

MATERIALS AND METHODS

The present study is laboratory based descriptive type of observational study. The study was carried out in the Molecular Biology Laboratory, Mahatma Gandhi Medical College &Hospital, Jaipur (Rajasthan) during the period of January 2021 to March 2022. A total of 150 serum samples were processed for this study. Demographic data (such as age, sex, in-patient, out-patient status) of the patients were recorded.

TRUPCR® TROPICAL FEVER PANEL KIT (MULTIPLEX REAL-TIME PCR BASED DETECTION KIT) [33]

Tropical Fever Panel Kit is designed for the qualitative detection of Dengue virus (DENV), Chikungunya virus (CHIKV), Salmonella spp., West Nile virus (WNV), Plasmodium spp., Rickettsia spp., Leptospira spp and ZIKA virus, along with Internal control from clinical samples using Real Time PCR technique. The results from the Tropical Fever Panel Kit must be interpreted within the context of all relevant clinical and laboratory findings.

Tropical Fever Panel Kit is a one-step real time reverse transcription PCR assay in which RNA templates are first reverse-transcribed to generate complementary cDNA strands followed by DNA polymerase-mediated cDNA amplification. During cDNA replication in real-time PCR, the fluorescent signal is generated from the presence of an oligonucleotide probe specific for target DNA sequence. The probe contains a fluorescent dye molecule on its 5' end and a quencher molecule on its 3' end. The probe hybridizes with one of the chains of the amplified fragment. During synthesis of a complementary chain, Taq DNA polymerase which possesses 5' - 3' exonuclease activity cleaves the probe. As a result, the fluorescent dye and quencher dye are separated, and the total fluorescence of reaction volume increases in direct proportion to the number of amplicon copies synthesized during PCR. The fluorescent signal is measured in each cycle of reaction.

Tropical Fever Panel Kit is an in vitro test for the qualitative detection of bacterial, parasitic and viral nucleic acid from clinical samples as an aid to the evaluation of infections with Dengue virus, Chikungunya virus, Salmonella spp., West Nile virus, Plasmodium spp., Rickettsia spp., Leptospira spp. and ZIKA virus. In this kit there are three independent reactions running parallel in 3 tubes: the first detects 3 target organisms CHIKV (FAM), Plasmodium spp (HEX); second detects DENV (FAM), ZIKA (HEX), and IC (TEXAS RED) and third detects Salmonella spp. (FAM), Leptospira spp. (HEX) and WNV (TEXAS RED).

QUALITATIVE RESULT ANALYSIS

1. The negative control reactions for probe/primer sets should not exhibit fluorescence growth curves that cross the threshold line. If a false positive occurs with one or more of the primers and probe NTC reactions, sample contamination may have occurred.
2. The positive control reactions for each probe/primer reactions should give Ct values.
3. All clinical samples should exhibit Internal control reaction curves that cross the threshold line at or before 30 cycles, thus indicating the specimen is of acceptable quality. However, it is possible that some samples may fail to give Internal control amplification due to:
   (a) Presence of ethanol or salt in the extracted sample due to improper extraction of nucleic acid from clinical materials
   (b) Presence of high pathogen load in the sample
   (c) Improper assay set up and execution
   (d) Reagent or equipment malfunction. [33]

OBSERVATIONS AND RESULTS

The present study is laboratory based descriptive type of observational study. the study was carried out in the Department of Molecular Biology, Mahatma Gandhi Medical College & Hospital, Jaipur (Rajasthan) during the period of January 2021 to March 2022.

150 Serum sample received in Department of Molecular biology, Mahatma Gandhi Medical College & Hospital, Jaipur, (Rajasthan) were included in the study.

Table 2: Distribution Of Positive And Negative Sample In IPD And OPD.

Result	Number (Percentage)	IPD	OPD
Positive	40 (26.66%)	32 (21.33%)	8 (5.33%)
Negative	110 (73.33%)	100 (66.66%)	10 (6.66%)
Total	150 (100%)	132 (87.99%)	18 (11.99%)

Table 3: Distributions Of Tropical Pathogens

Total Positive	RNA Detected		DNA Detected	
	Dengue	19(47.5%)	Rickettsia (scrub typhus)	12(30%)
	Chikungunya	7(17.5%)	Plasmodium	2 (5%)
			Salmonella	0
			Leptospira	0
			West Nile	0
40 (100%)		26(65%)		14(35%)

Table 4: Age Group Distributions

Age group	Total Positive	RNA Detected	DNA Detected
1-20 years	8 (20%)	5 (12.5%)	3 (7.5%)
20-40 years	16 (40%)	11 (27.5%)	5 (12.5%)
40-60 years	10 (25%)	6 (15%)	4 (10%)
60-80 years	6 (15%)	4 (10%)	2 (5%)
Total positive	40 (100%)	26 (65%)	14 (35%)

DISCUSSION

While every disease presents specific diagnostic challenges, clinical needs associated with specificity, sensitivity, total analysis time, and implementation would eventually impact the design and development of the diagnostic method. Infectious diseases of the tropical region are characterized by fever as their main clinical symptom. In India, the etiologies of human febrile illness can vary by region suggesting that diagnosis, treatment, and control programs must be developed based on etiological data specific to the respective region. Common causes of Tropical fever include dengue, malaria, leptospirosis, enteric fever, chikungunya and rickettsia.

A correct diagnosis can only be made through laboratory confirmation of the presence of tropical pathogens in clinical specimens. As a highly specific and sensitive method of molecular detection, multiplex real-time PCR has been used to diagnose numerous infectious diseases.

This prospective sectional study was conducted in the Molecular biology laboratory, Mahatma Gandhi Medical College & Hospital, Jaipur during the period of January 2021– March 2022. The present study includes 150 patient specimens presenting with fever.

In the present study, among 150 patient specimens with acute febrile

illness, 40 sample were Positive (26.66%) and110 were Negative (73.33%). 32(21.33%) Sample were Positive from IPD and 8(5.33%) samples Positive from OPD.

In this study many cases were reported in July and September which is the rainy and premonsoon period. This is similar to studies done by Jhansi Charles et al (2015) [34] and Priyadarshini Shanmugam et al. (2016) [35] which showed had peak incidence of cases during rainy and premonsoon seasons. Since large number of cases occur during rainy and monsoon period, preventive measures have to initiated promptly like control of mosquitoes and rodents. Public education and awareness campaigns must be launched to spread information on good hygienic practices, vector control measures, and protective measures.

Distribution of DNA and RNA Detection: Out of 40 positive samples 26 (65%) RNA & 14 (35%) DNA were detected.

In this study blood samples from 150 patients were subjected to Nucleic acid detection by multiplex real-time PCR according to the duration of fever. 19(47.5%) were positive for Dengue, this is similar to studies done by Sharma Shashi et al (2018). [36]

The number of dengue cases reported to WHO increased over 8fold over the last two decades, from 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 increased from 960 to 4032, affecting mostly younger age group. The total number of cases seemingly decreased during years 2020 and 2021, as well as for reported deaths. However, the data is not yet complete and COVID-19 pandemic might have also hampered case reporting in several countries. [37]

12(30) were positive for Rickettsia (scrub typhus), This is similar to studies done by Trung vu Nguyen et al (2019). [38]

7(17.5) were positive for Chikungunya, In the present study, the detection of CHIK virus RNA in 48.6% of samples by RT-PCR. [39]

According to the National Vector Borne Disease Control Programme (NVBDCP), India reported 27,553 suspected cases and 3342 laboratory-confirmed cases of CHIKV infection in 2015. Most (75%) of the suspected cases of CHIKV infection were in Karnataka. The number of chikungunya cases continued to grow into 2016, when there were 64,057 suspected cases, 41.2% of which were diagnosed with CHIKV infection. A total of 55.4% of all the suspected cases and 53.9% of all the confirmed cases originated from three states in India, namely, New Delhi, Maharashtra, and Karnataka. In 2017, a total of 67,769 suspected chikungunya cases were reported from states throughout the country. During 2018–2020, India recorded more than 170,000 suspected and 27,120 confirmed cases in various states of the country. [40]

2(5%) were positive for Plasmodium. This is similar to studies done by Rao N. Pavitra et al (2019) .[41]

Overall, our finding that 54 out of 954 patients (5.7%) According to the World Malaria Report 2020, there were 241 million cases of malaria globally in 2020 (uncertainty range 218–269 million) and 627 000 malaria deaths (uncertainty range 583–765 thousand). Malaria case incidence reduced from 81 in 2000 to 59 in 2015 and 56 in 2019, before increasing again to 59 in 2020. Globally, malaria deaths reduced steadily over the period 2000–2019, from 896 000 in 2000 to 562 000 in 2015 and to 558 000 in 2019. In 2020, malaria deaths increased by 12% compared with 2019. The increases in malaria cases are deaths were associated with disruption to services during the COVID-19 pandemic. [42]

The age group most commonly affected was 20-40 years (40%) followed by 40-60 years (25%) and 1-20 years (20 %). 60-80 (15%) years. This was similar to study conducted by Das et al 2015. [39]

CONCLUSION

Majority of them showed peak seasonal incidence of cases during rainy and post monsoon seasons, preventive measures have to initiated like control of mosquitoes, rodents and the general public have to be educated and awareness should be created on good hygienic practices, vector control measures and protective measures. Dengue was the commonest cause of acute febrile illness, other different causes prevailing in tropical countries should be suspected and correct diagnosis is crucial to prevent the delay in starting appropriate therapy. Diagnostic tests with good correlation of clinical findings, relevant laboratory parameters and epidemiology of disease is essential to prevent complications and to reduce morbidity and mortality in patients with acute febrile illness.

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