Journal Of Medical Science And Clinical Research

IGM Publication
An Official Publication Of IGM Publication

Epidemiology of Malaria in Kanchipuram District Tamilnadu

Authors
Sourav Das[1], J. Manonmoney*[2]
[1]SRM Medical College Hospital & Research Centre, Kattankulathur, Chennai, Tamilnadu, India
[2]Department of Microbiology, SRM Medical College Hospital & Research Centre, Kattankulathur, Chennai, Tamilnadu, India

Abstract
Introduction: *Mosquito-borne diseases are the major concern in public health problems. Malaria is a protozoal disease caused by the parasites of the Genus Plasmodium - which are transmitted by the bite of female Anopheles mosquitoes. Prevalence of malaria worldwide is about 35%. Malaria infection diagnosed by Peripheral smear, Rapid Immunochromatography, and Molecular technique.*
Aim and Objective: *To study the epidemiological distribution of Plasmodium infection in patients suspected for Pyrexia of Unknown Origin (PUO) attending a tertiary care hospital in Kanchipuram District, Tamilnadu.*
Methodology
Study Design: *This Study was carried out at SRM MCH & RC, Tamil Nadu, India, from January 2018 to January 2019. This was an observational study.*
Malarial parasites were identified in a peripheral blood smear by the conventional method (thick smear and thin smear), Rapid Immunochromatographic technique (a serological technique using whole blood) and Molecular technique. DNA extraction from whole blood done by Spine Column technique. Amplification and Gel Documentation done.
Result: *Total of 83 blood samples were collected from patients with a clinical diagnosis of PUO. Out of 83 samples, 5(6%) samples were positive for Plasmodium vivax species by conventional method, Rapid Immunochromatographic technique. The Molecular technique will help to diagnose the gene.*
Conclusion: *In this study, Plasmodium vivax is identified more by conventional peripheral blood smear method as well as in Rapid Immunochromatographic method and Molecular Method.*
Keywords: *Epidemiology, Malaria, Plasmodium, Mosquito-borne, Immunochromatography.*

Introduction

The *Plasmodium,* well known as malarial parasites are Sporozoans (Class) and belong to the order Haemosporida. It is under Phylum Apicomplexa of Subkingdom Protozoa under the Protista Kingdom.[1] Malaria, an infectious disease caused by bite of infected mosquito. Causative agent for this disease are from protozoan of the *Plasmodium* genus which is a very common infection. It has a high social and economic impression on affected countries. It is really a very significant public health challenge.

History

The name of the disease malaria was given as far back as 1753. It is interesting to note that the treatment of the disease became first established

(in the middle of the seventeenth century before anything was known about its etiology and how the disease was transmitted. Another curious fact is that before the discovery of the plasmodia, the presence of pigment (black malarial) in organs with malarial infections had been observed by Meckel (1847) and Virchow (1849). The pigmented appearance of spleen and brain in malaria post mortem was however noted also by Lancisi in 1716 and Bright in 1831.

Milestones

1880: Laveran discovered the malarial parasite in an unstained preparation of fresh blood.

1883: Marchiafava used methylene blue for the staining of malarial parasite.

1885: Golgi demonstrated the Erythrocytic schizogony (also known as golgi cycle) of quartan malarial parasite.

1886: Golgi demonstrated the Erythrocytic schizogony of benign tertian malarial parasite; also differentiated the benign and quartan species.

1891: Romanowasky introduced the staining method of malarial parasite.

1897: Ross in Secunderabad found oocyst on the stomach wall of an anopheline mosquito which had previously fed on a malaria patient.

1898: Ross in Calcutta worked out the mosquito cycle with the parasite of bird malaria, whereas Bignami et al demonstrated the same with the parasitic of human malaria.

1900: Patrick Manson proved the theory of mosquito transmission.

1934: Tissue phase of malarial parasite was demonstrated in avian malaria.

1948: Shortt and others demonstrated the pre Erythrocytic schizogony of p. falciparum.

1949: Shortt and others demonstrated the pre Erythrocytic schizogony of P. falciparum.

1952: Jeffery et al demonstrated three days old pre-erythrocytic schizont of p. falciparum in the human liver.

1954: Garnham et al discovered the pre Erythrocytic schizogony of P.ovale.

Malaria is a serious, sometimes fatal life-threatening disease caused by *Plasmodium* parasites that are transmitted topeople by the bites of infected female *Anopheles* mosquitoes called "malaria vectors". Mainly four Plasmodium species caused infection in human. The species are *Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale*. Out of these species P. *falciparum* and *P. vivax* are the most common species and *P. falciparum* is the deadliest.[2]

Malaria is preventable and curable. Transmission of malaria infection depends on climatic conditions that can affectthe number and existence of mosquitoes, such as rainfall patterns, temperature and moistness. Malaria is an acute state febrile illness. In an individual, clinical features appear seven days ormore (may takes 10–15 days) after the bite of infective mosquito. The symptoms include various problems such as fever, headache, chills and vomiting etc. If untreated within 24 hours, *P. falciparum* malarial infection canprogress to severe illness even death can also occur. Early diagnosis and treatment of malariareduces disease rate and prevents occurrence of deaths. It also contributes to reducing malaria transmission.[1]

The World Malaria Report 2017, World Health Organization (WHO) framed data from 91 countries and areas with ongoing malaria transmission. The statistics is supplemented by data from national household inspections and databases held by other organizations. Up to year 2016 report shows that after an exceptional period of success in global malaria control, progress has stalled. It was estimated 216 million cases of malaria in 2016, an increase of about 5 million cases over 2015. Deaths reached 445,000, a similar number to the previous year.[3]

Malaria is preventable and curable. Transmission of malaria infection depends on climatic conditions that can affectthe number and existence of mosquitoes, such as rainfall patterns, temperature and moistness. Malaria is an acute state febrile illness. In an individual, clinical features appear seven days ormore (may takes 10–15 days) after the bite of infective mosquito. The

symptoms include various problems such as fever, headache, chills and vomiting etc. If untreated within 24 hours, *P. falciparum* malarial infection canprogress to severe illness even death can also occur. Early diagnosis and treatment of malaria reduces disease rate and prevents occurrence of deaths. It also contributes to reducing malaria transmission.[1]

The analysis of malaria prevalence showed that there had been a tendency of declining in the total prevalence of malaria infection. Which is based on distinct seasonal summary during August, September and October. In this study it shows that the zone in Basin Bridge reflects the maximum number of malaria cases followed by Adyar zone. To understand the details of malaria prevalence in Chennai, the hotspot analysis was conducted which can give an idea that really is there any relationship existed spatially or not. It was recorded that all the wards of north eastern Chennai were malarial infection hotspots during 2005-2011. These hotspot wards bunch to form the Basin Bridge zone that was seen to be heavily malaria affected area. Another very important observation from the hotspot study analysis was that there was a sudden emergence of hotspots in southern parts of Chennai during 2011. A strong spatial relation of clustered occurrence to the prevalence of malaria in Chennai could be defined which has not previously been recorded.[3]
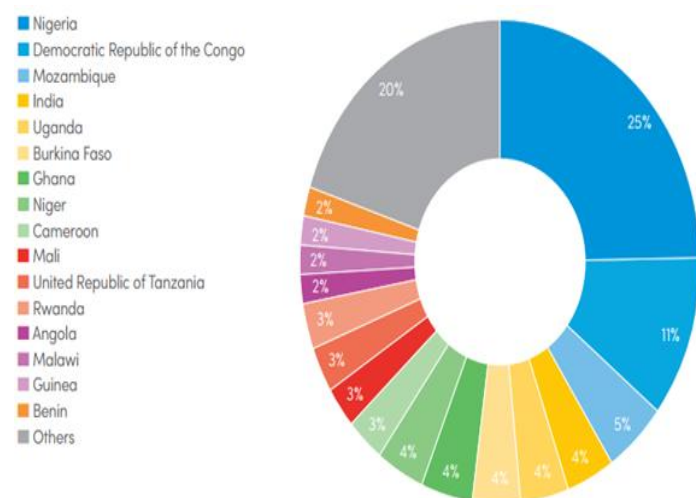
Figure 1 Estimated country share of total malaria cases, 2017
Source: World Malaria Report-2018, WHO

The "World Malaria Report 2017" published by World Health Organization (WHO) framed statistics from ninety-one countries and zonal areas where malaria transmission ongoing. The statistics are enriched by reports from national household inspections and databases produced by other organizations. Up to 2016 report implies that after an exceptional era of success in malaria control globally, progress has installed. It was estimated 216 million cases of malaria in 2016, an increase of about 5 million cases over 2015. A similar number of deaths reached 445,000 in contrast to the previous year.[3]

In Tamil Nadu, 56.6% of Malaria cases are stated from Chennai, 4.4% reported from the other urban malaria scheme towns and 39% were recorded from rural areas.[4]

The gold standard technique for the detection of malarial parasites in peripheral blood is thick smear and thin smear preparation and molecular diagnosis for species specific detection. In some mixed infection cases we can miss the other species but in molecular diagnosis we can detect all the infective species.

Aim and Objective
To study the epidemiological distribution of Plasmodium infection in Kattankulathur region, Kanchipuram district, Tamilnadu

Methods and Methodology
It's an observational study done in SRM Medical College Hospital and Research Centre, Kattankulathur region after Institutional ethical clearance. The study duration is one year (January 2018 to January 2019). Samples collected during the study period is 83.

Inclusion Criteria: Clinically suspected Malaria and all the PUO patients of all age groups referred for screening of Malaria infection and relapse cases (Jan2018-Jan2019) in SRM MCH & RC, kattankulathur.

Exclusion Criteria: Neonates and patients those who are already on Malarial treatment and responding to drugs.

Specimen Collection: Samples will be collected with the appropriate consent form; samples include:[5]

❖ Capillary blood (Peripheral blood smear preparation)

❖ Anticoagulated (EDTA) venous blood (Molecular study)

❖ Collection place: Sample collection room or specific wards, SRM Medical College Hospital & Research Centre, kattankulathur.

❖ Sample quantity: Adult: 2.5ml; Children: 1-2ml.

❖ Sample transport: Sample collection room attached to Central Laboratory so no need of special transportation.

Methods

1. Conventional (Peripheral blood smear, thick and thin smear): Thick smear and thin smear is prepared in slide. Smear fixed with ethanol and stained with Leishman stain. Observed under 100X in oil immersion field.

2. Immunochromatographic Rapid card test where Histidine Rich Protein (HRP 2) is impregnated on nitrocellulose membrane for detection of *P. falciparum* antigen and plasmodium Lactate Dehydrogenase (pLDH) impregnated to capture other three species antigen.

3. Molecular technique (Polymerase Chain Reaction) where genomic DNA extracted from the RBC infected with Plasmodium infection. Using Lyticase buffer and through spin column method DNA extracted and amplified by specific primers. Amplified product run on agarose gel and documentation done by Gel documentation.

PCR Amplification:[6]

Table 1: Primer Sequence for specific Plasmodium species

Species	Primer	Sequence (5'–3')	Size (bp) of PCR product
P.falciparum	FAL(F)	TTAAACTGGTTTGGG AAAACCAAATATATT	205
	FAL(R)	ACACAATGAACTCAA TCATGACTACCCGTC	
P. vivax	VIV(F)	CGCTTCTAGCTTAAT CCACATAACTGATAC	120
	VIV(R)	ACTTCCAAGCCGAA GCAAAGAAAGTCCT TA	
P. ovale	OVA(F)	ATCTCTTTTGCTATTT TTTAGTATTGGAGA	800
	OVA(R)	GGAAAAGGACACATT AATTGTATCCTAGTG	
P. malariae	MAL(F)	ATAACATAGTTGTAC GTTAAGAATAACCGC	144
	MAL(R)	AAAATTCCCATGCAT AAAAAATTATACAAA	

Table 2: PCR Program Timing Schedule [6]

PCR Program			
Steps	Temp(°C)	Time(min)	No. of cycles
Denaturation	94	1	30
Annealing	58	2	30
Elongation	72	2	30

Result

Table 4: Gender distribution in Plasmodium Positive Cases

Sl. No.	Category	No. of Patient
1.	Male	2
2.	Female	3

Table 5: Area wise distribution of Patients Samples

Sl. No.	Area Name	Sample Distribution
1.	Guduvanchery	11
2.	Maramalai Nagar	28
3.	Vigneshwarar Nagar	5
4.	Potheri	16
5.	Thailavaraam	9
6.	SRM Nagar	7
7.	Green Pearl Park	2
8.	K.K Nagar	3
9.	ALLI Nagar	2

Table 6: Zone wise sample positive case

Sl. No.	Area Name	Positive Sample Distribution
1.	Guduvanchery	-
2.	Maramalai Nagar	2
3.	Vigneshwarar Nagar	-
4.	Potheri	2
5.	Thailavaraam	-
6.	SRM Nagar	-
7.	Green Pearl Park	1
8.	K.K Nagar	-
9.	ALLI Nagar	-

Discussion

The present study is a hospital based prospective study done in SRM Medical College Hospital and Research Centre, (Kattankulathur region which comes under Chengalpattu Taluk, Kanchipuram District, Tamilnadu) 83 patients during the period of January 2018-January 2019.

In this study, *Plasmodium vivax* is identified more by conventional peripheral blood smear method as well as in Rapid Immunochromatographic method which shows same sensitivity and specificity in both methods. Mixed infection not observed in this study.[7] In the study of Nisha Siwal et al The proportion was found to be 49:51 *Plasmodium falciparum* and *Plasmodium vivax* respectively. Mixed species infections also found in 13% cases of total infections due to *Plasmodium falciparum* and *Plasmodium vivax.* In this study *Plasmodium vivax* only identified and confirmed by the appearance of erythrocytic stage in by conventional method and confirmed by PCR method. Mixed infection not seen in this study confirmed by PCR. As a justification of this study is that, Nisha Siwal et al, 2018 studied on 2,333 samples from malaria endemic regions in various geographic location in India whereas in this study, sample size is 83 which is very less in contrast with the above study.[6]

In the study of Rajan S. Bindu et al. out of 200 cases, 55 were positive by RDT. 30 of P. vivax, 24 of P. falciparum and 1 was a mixed infection. A peripheral blood smear showed 85.5% sensitive and 100% specific.[8] In my study out of 83 samples 5 samples are positive by conventional method. In Immunochromatographic rapid test kit shows 5 positive.

Samples collected from the PUO suspected patients attending SRM Medical College Hospital from various location of Kattankulathur region. Out of 83 cases 28 cases are from Maraimalai Nagar area following Potheri and Guduvancherry, 16 and 11 respectively. Out of the five positive cases 2 cases from Maramalai nagar, 2 cases from Vigneshwasrarnagar and 1 from Green pearl park. This gives the picture of occurrence rate of PUO

cases in Kattankulathur region near SRM Medical College Hospital. The specific zone wise and area wise distribution analysis will help in implementing malarial control activities.[10]

Conventional peripheral blood smear method a gold standard method recommended by WHO[9]. It is time consuming cumbersome method. As a comparison with Immunochromatographic rapid test kit method, it is very easy and by name itself rapid method. Observation in the result window showed the result of particular specimen. It's very fast like within 15-20 minutes' result will come.

As the principle based on Immunochromatography. In molecular test to diagnose in species level takes more time. The turnaround time depends based on the methods of diagnosis. As here we can see for Immunochromatography takes less time compared to all other methods.

Conclusion

In this study, Plasmodium vivax is identified by conventional peripheral blood smear method as well as in Rapid Immunochromatography method (ICT) which shows same sensitivity and specificity in both methods. Further analysis of other factors and specific area distribution will help in targeted malarial control.

References

1. Subhash Chandra Parija, Textbook of medical parasitology protozoology & Helminthology text and colour atlas. 4th edition. New Delhi. All India Publishers & Distributors.2016
2. Sweta M Murawala, Vyoma Chudasama, MahendraVegad. Prevalence of mosquito-borne *Plasmodium* infection in patients attending fever clinic in a tertiary-care teaching hospital, western India. International Journal of Medical Science and Public Health |2015|Vol 4 | Issue 11
3. The world Malaria Report 2017. © *World Health Organization 2017.* ISBN 978-92-4-156552-3

4. State Health Society Tamil Nadu. National Health Mission, Tamil Nadu.

5. WHO guidelines on drawing blood: best practices in phlebotomy, WHO Library Cataloguing-in-Publication Data, ISBN 9789241599221, World Health Organization 2010

6. Sri Krishna, Praveen K. Bharti, Himashu S. Chandel, Amreen Ahmad, Rajesh Kumar, Puspendra P. Singh, Mrigendra P. Singh, Neeru Singh, Detection of Mixed Infections with Plasmodium spp. by PCR, India, 2014, Emerging Infectious Diseases. www.cdc.gov/eid • Vol. 21, No. 10, Oct 2015

7. Nisha Siwal, UpasanaShyamsunder Singh, Manoswini Dash, Sonalika Kar,Swati Rani, Charu Rawal, Rajkumar Singh, Anupkumar R. Anvikar, Veena Pande,Aparup Das. Malaria diagnosis by PCR revealed differentialdistribution of mono and mixed speciesinfections by *Plasmodium falciparum* and *P.vivax* in India. Molecular epidemiology of Malaria in India. | March 2018| PLoS ONE 13(3): e0193046. |https://doi.org/10.1371/journal.pone.0193046.

8. Rajan S. Bindu, Bhavnaben A. Vora, Comparison of diagnostic methods of malaria by peripheral smear, centrifuged buffy coat smear and rapid antigen detection test, International Journal of Research in Medical Sciences,2017 Oct;5(10):4532-4537, DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20174591.

9. Malaria Microscopy Standard Operating Procedure – MM-SOP-08, WHO

10. Divya Subash Kumar, Ramachandran Andimuthu, Rupa Rajan and Mada Suresh Venkatesan.Kumar et al. Spatial trend, environmental and socioeconomic factors associated with malaria prevalence in Chennai. Malaria Journal 2014, 13:14. DOI:10.1186/1475-2875-13-14

http://www.malariajournal.com/content/13/1/14

11. Bhavnaben A. Vora, Rajan S. Bindu, Comparison of diagnostic methods of malaria by peripheral smear, centrifuged buffy coat smear and rapid antigen detection test, International Journal of Research in Medical Sciences,2017 Oct;5(10):4532-4537, DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20174591

12. Lalan Prasad Shah, N Krishnamoorthy, T Vijayakumar and P Basker, Risk of malaria transmission in stone quarry sites of Villupuram district in Tamil Nadu, India, International Journal of Mosquito Research 2018; 5(1): 33-40

13. B. Vishalakshi, J. Mariraj, S. Liba and S. Krishna, Cross Sectional Study Comparing Peripheral Blood Smear and Rapid Antigen Detection Test in Diagnosis of Malaria Conducted During Monsoon and Post Monsoon Period at Ballari, India. International Journal Current Microbiology and Applied Sciences; (2018) 7(2): 255-260

14. Jung-Yeon Kim., So-Young Ji1., Youn-Kyoung Goo1, Byoung-Kuk Na, Hyo-JooPyo, Han-Na Lee1, Juyoung Lee, Nam Hee Kim, Lorenz von Seidlein, Qin Cheng, Shin-Hyung Cho, Won-Ja Lee1., Comparison of Rapid Diagnostic Tests for the Detection of Plasmodium vivax Malaria in South Korea, PLoS ONE 8(5): e64353. doi: 10.1371/journal.pone.0064353

15. Sri Krishna, Praveen K. Bharti, Himashu S. Chandel, Amreen Ahmad, Rajesh Kumar, Puspendra P. Singh, Mrigendra P. Singh, Neeru Singh, Detection of Mixed Infections with Plasmodium spp. by PCR, India, 2014, Emerging Infectious Diseases. www.cdc.gov/eid • Vol. 21, No. 10, Oct 2015

16. Jonathan M Lauderdale, Cyril Caminade, Andrew E Heath, Anne E Jones, David A MacLeod, Krushna C Gouda, Prashant Goswami, Srinivasa R Mutheneni and Andrew P Morse, Lauderdale et al, Towards seasonal forecasting of malaria in India, Malaria Journal 2014,13:310, http://www.malariajournal.com/content/13/1/310.