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A STUDY ON SEROPOSITIVITY OF *PLASMODIUM FALCIPARUM* AND PAN MALARIAL SPEICES AMONG FEBRILE PATIENTS ATTENDING MIMS GENERAL HOSPITAL, NELLIMARLA, VIZIANAGARAM BY USING PARAHIT TOTAL RAPID TEST KIT METHOD.

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Keywords:

ParaHit, Malaria, Histidine rich protein-2, Aldolase, Plasmodium falciparum, Pan Malarial species.

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ABSTRACT: Malaria, at one time a rural disease, diversified under the pressure of developments into various ecotypes. These ecotypes have been identified as forest malaria, urban malaria, rural malaria, industrial malaria, border malaria and migration malaria; the latter cutting across boundaries of various epidemiological types. In the present study, an attempt has been made to know the seropositivity of *Plasmodium falciparum* and Pan Malarial species among the febrile patients attending MIMS General Hospital, Nellimarla, Vizianagaram from January 2013 to December 2014. Out of total no. of patient samples(n=1021)collected, 36(3.52%)were seropositive. Out of total no. of seropositive patients(n=36), 20(55.55%) were males, 10(27.77%)were females and 06(16.66%) were children. In the present study, out of total no. of seropositive patients(n=36), 05(13.89%) were positive for *Plasmodium falciparum*, 19(52.78%) were positive for Pan Malarial species and 12(33.33%) were positive for mixed infection with *Plasmodium falciparum* and Pan Malarial species. Out of "20" seropositive males, 01(20%) patient was seropositive for *Plasmodium falciparum*, 14(73.68%) were positive for Pan malarial species and 05(41.67%) were positive for mixed infection with *Plasmodium falciparum* and Pan Malarial species. Out of "10" seropositive females, 04(80%) were positive for *Plasmodium falciparum*, 02(10.53%) were positive for Pan Malarial species and 04(33.33%) for mixed infection with *Plasmodium falciparum* and Pan Malarial species. Out of "06" seropositive children, 03(15.79%) were positive for Pan Malarial species, 03(25%) for mixed infection with Plasmodium *falciparum* and Pan Malarial species and there were no seropositives for single *Plasmodium falciparum* infection. The test is intended only for initial screening and reactive samples should be confirmed by a supplemental assay such as microscopic examination of blood. The present study reveals that rapid diagnostic tests are easy to use, reliable and simple to interpret. Rapid diagnostic tests are more suited to health workers in situations where health services are defined or absent. Therefore, these tests can be used as epidemiological tools for the rapid screening of malaria.

INTRODUCTION: Malaria is the most important parasitic disease of human beings transmitted by mosquitoes. Nearly 85% of malaria cases are caused by *Plasmodium falciparum*. It is the most dangerous life threatening species of malaria parasites. *Plasmodium falciparum* occurs mainly in the hotter and more humid regions of the world.

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It is the main species in Africa, Haiti and New Guinea. It also occurs along with *Plasmodium vivax* in South America, Oceania and South-East Asia. *Plasmodium falciparum* also occurs in parts of India, Pakistan, Bangladesh, Sri Lanka and Central America. Malaria is transmitted exclusively through the bites of *Anopheles* mosquitoes. The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment.

About 20 different Anopheles species are locally important around the world. All of the important vector species bite at night. Anopheles mosquitoes

breed in water and each species has its own breeding preference; for example some prefer shallow collections of fresh water, such as puddles, rice fields, and hoof prints. Transmission is more intense in places where the mosquito lifespan is longer (so that the parasite has time to complete its development inside the mosquito) and where it prefers to bite humans rather than other animals. For example, the long lifespan and strong human-biting habit of the African vector species is the main reason why about 90% of the world's malaria deaths are in Africa.

Transmission also depends on climatic conditions that may affect the number and survival of mosquitoes, such as rainfall patterns, temperature and humidity. In many places, transmission is seasonal, with the peak during and just after the rainy season. Malaria epidemics can occur when climate and other conditions suddenly favour transmission in areas where people have little or no immunity to malaria. They can also occur when people with low immunity move into areas with intense malaria transmission, for instance to find work, or as refugees.

Human immunity is another important factor, especially among adults in areas of moderate or intense transmission conditions. Partial immunity is developed over years of exposure, and while it never provides complete protection, it does reduce the risk that malaria infection will cause severe disease. For this reason, most malaria deaths in Africa occur in young children, whereas in areas with less transmission and low immunity, all age groups are at risk.

Symptoms:

Malaria is an acute febrile illness. In a non-immune individual, symptoms appear seven days or more (usually 10–15 days) after the infective mosquito bite. The first symptoms – fever, headache, chills and vomiting – may be mild and difficult to recognize as malaria. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness often leading to death. Children with severe malaria frequently develop one or more of the following symptoms: severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria. In adults, multi-organ involvement is also frequent. In malaria endemic areas, persons may develop partial immunity, allowing asymptomatic infections to occur.

For both *P. vivax* and *P. ovale*, clinical relapses may occur weeks to months after the first infection, even if the patient has left the malarious area. These new episodes arise from dormant liver forms known as hypnozoites (absent *in P. falciparum* and *P. malariae*); special treatment – targeted at these liver stages – is required for a complete cure.

Who is at risk?

Approximately half of the world's population is at risk of malaria. Most malaria cases and deaths occur in sub-Saharan Africa. However, Asia, Latin America, and to a lesser extent the Middle East and parts of Europe are also affected. In 2014, 97 countries and territories had ongoing malaria transmission.

Specific population risk groups include:

- Young children in stable transmission areas who have not yet developed protective immunity against the most severe forms of the disease;
- Non-immune pregnant women as malaria causes high rates of miscarriage and can lead to maternal death;
- Semi-immune pregnant women in areas of high transmission. Malaria can result in miscarriage and low birth weight, especially during first and second pregnancies;
- Semi-immune HIV-infected pregnant women in stable transmission areas, during all pregnancies. Women with malaria infection of the placenta also have a higher risk of passing HIV infection to their newborns;
- People with HIV/AIDS;
- International travellers from non-endemic areas because they lack immunity;
- Immigrants from endemic areas and their children living in non-endemic areas and

returning to their home countries to visit friends and relatives are similarly at risk because of waning or absent immunity.

Key facts:

- Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected mosquitoes.
- In 2013, malaria caused an estimated 584 000 deaths (with an uncertainty range of 367 000 to 755 000), mostly among African children.
- Malaria is preventable and curable.
- Increased malaria prevention and control measures are dramatically reducing the malaria burden in many places.
- Non-immune travellers from malaria-free areas are very vulnerable to the disease when they get infected.

According to the latest estimates, released in December 2014, there were about 198 million cases of malaria in 2013 (with an uncertainty range of 124 million to 283 million) and an estimated 584 000 deaths (with an uncertainty range of 367 000 to 755 000). Malaria mortality rates have fallen by 47% globally since 2000, and by 54% in the WHO African Region. Most deaths occur among children living in Africa where a child dies every minute from malaria. Malaria mortality rates among children in Africa have been reduced by an estimated 58% since 2000.

Diagnosis and treatment:

Early diagnosis and treatment of malaria reduces disease and prevents deaths. It also contributes to reducing malaria transmission. The best available treatment, particularly for *P. falciparum* malaria, is artemisinin-based combination therapy (ACT). WHO recommends that all cases of suspected malaria be confirmed using parasite-based diagnostic testing (either microscopy or rapid diagnostic test) before administering treatment. Results of parasitological confirmation can be available in 15 minutes or less. Treatment solely on the basis of symptoms should only be considered when a parasitological diagnosis is not possible.

Endemic malaria occurs every year and is seasonal. It is primarily seen in children. As naturally acquired immunity develops with increasing exposure, it occurs less frequently in the older population. Malaria parasites are found in the blood circulation all the time in 90%-100% of children less than 5years old in parts of Africa. Epidemic malaria occurs sporadically. Unlike in endemic malaria, people of all ages affected in epidemic malaria.

Aim of the present study:

To know the seropositivity for Plasmodium falciparum and Pan malarial species among the patients with fever attending MIMS General Hospital, Nellimarla, Vizianagaram from January 2013 to December 2014.

MATERIALS AND METHODS:

A total number of 1021 venous whole blood samples were included in the present study which were collected from febrile patients attending MIMS General Hospital, Nellimarla, Vizianagaram from January 2013 to December 2014.

All the collected samples were subjected to serological testing in Microbiology laboratory by using Para Hit Total device.

Kit contents and Description:

Reagent 1:

Test Device comprising of nitrocellulose membrane with Anti-HRP-II antibodies immobilized at “Pf” region, anti-aldolase antibody at “Pan” region and control reagent immobilized at “C” region. Conjugate releasing pad impregnated with Anti-HRP-II/Anti-aldolase antibodies.

Reagent 2:

Reaction buffer—Physiological buffer containing detergent and preservative.

Principal:

Para Hit Total is a rapid test for *P.falciparum* and Pan Malarial species based on the principal of immunochromatography in which nitrocellulose membrane is coated with Anti-HRP-II antibody (capture antibody)which is specific for *P.*

falciparum and anti-aldolase antibody which detects the presence of any Plasmodium species (*P.falciparum, P.vivax, P.ovale, P.malariae*).

When the test sample along with the reaction buffer flows through the nitrocellulose membrane the colloidal gold coupled with Anti-HRPII/Anti-aldolase(detection antibody) bind to Plasmodium antigens released from the lysed test sample. This antigen antibody complex moves through the nitrocellulose membrane and bind to the corresponding immobilized antibodies to HRP-II/Anti-aldolase(capture antibody) leading to the formation of magenta red colour band or bands which indicates reactive results. The control band will appear irrespective of reactive or non-reactive sample. The control band will serve to validate flow of the reaction mixture. Therefore the colour of control band should not be compared with colour of the test band for interpretation of results. Appearance of magenta red coloured band at test region in addition to magenta red coloured band at control region indicates positive result. Absence of magenta red coloured band at test region with the presence of magenta red coloured band at control region indicates negative result.

Procedure done:

1. A micropipette was used for taking blood and immediately transferred to the sample window "A" of the test device.
2. Four drops (200µl) of reaction buffer to buffer window "B" of the test device. After adding each drop 10 seconds were allowed for soaking.
3. The test results were read at the end of 25 minutes.
4. After reading the results the test device was disposed off as biohazard waste

RESULTS:

TABLE 1: TOTAL NO. OF SAMPLES AND SEROPOSITIVE PATIENTS.

Total no.samples collected	Total no.of seropositive patients	Percentage(%)
1021	36	3.52

TABLE 2: GENDER-WISE DISTRIBUTION IN TOTAL NO.OF SAMPLES (N=1021):

Males	Females	Children
354(34.67%)	370(36.24%)	297(29.09%)

TABLE 3: GENDER-WISE DISTRIBUTION IN TOTAL NO.OF SEROPOSITIVES (n=36)

S.no	Gender	No. of seropositives	Percentage (%)
1.	Males	20	55.55
2.	Females	10	27.77
3.	Children	06	16.66

TABLE 4: SEROPOSITIVITY FOR *PLASMODIUM FALCIPARUM* (n=05):

S.no	Gender	No.of seropositives
1.	Males	01(20%)
2.	Females	04(80%)
3.	Children	Nil.

TABLE 5: SEROPOSITIVITY FOR PANMALARIAL SPECIES (n=19):

S.no	Gender	No.of seropositives	Percentage (%)
1.	Males	14	73.68
2.	Females	02	10.53
3.	Children	03	15.79

TABLE 6: SEROPOSITIVITY FOR MIXED INFECTION OF *PLASMODIUM FALCIPARUM* AND *PANMALARIAL* SPECIES (n=12):

S.no	Gender	No.of seropositives	Percentage(%)
1.	Males	05	41.67
2.	Females	04	33.33
3.	Children	03	25

TABLE7: SPECIES AND GENDER WISE DISTRIBUTION IN TOTAL NO. OF SEROPOSITIVE PATIENTS (n=36):

S.no	Gender	*P.falciparum*	Pan Malarial species	*P.falciparum* + Pan Malarial species
1.	Males(n=20)	01(20%)	14(73.68%)	05(41.68%)
2.	Females(n=10)	04(80%)	02(10.53%)	04(33.33%)
3.	Children(n=06)	Nil	03(15.79%)	03(25%)

DISCUSSION: Malaria has been a problem in India for centuries. Details of this disease can be found even in the ancient Indian medical literature like the Atharva Veda and Charaka Samhita. In the 30's there was no aspect of life in the country that was not affected by malaria. During the latter parts of nineteenth and early twentieth century's, nearly one-fourth of India's population suffered from malaria, with the annual incidence estimated at around 75 million cases in 1953 and about 7-8

lakhs deaths annually, particularly in the states like Punjab and Bengal. The economic loss due to the loss of man-days due to malaria was estimated to be at Rs. 10,000 million per year in 1935. At the time of independence in 1947, of a population of 330 million, about 75 million people were estimated to be infected with malaria every year, and the direct mortality due to the disease was estimated at 0.8 million per annum. To combat this menace, the Govt. of India launched the National Malaria Control Programme in April 1953.

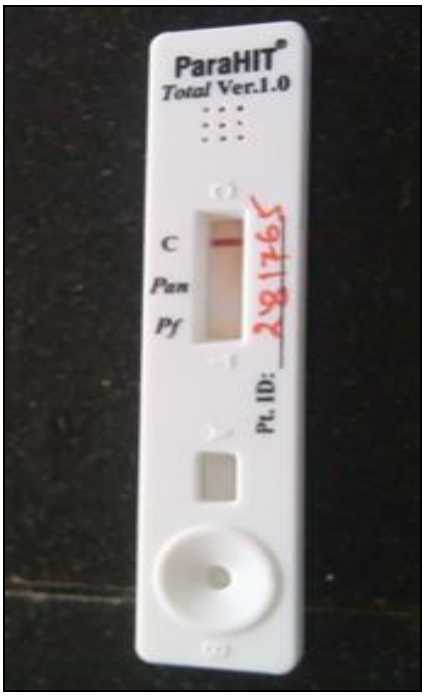

FIG.1: PARAHIT DEVICE SHOWING NEGATIVE RESULT FOR *P.FALCIPARUM* AND *PAN MALARIAL* SPECIES

Absence of magenta red coloured band at test region with the presence of magenta red coloured band at control region indicates negative result.

The programme proved highly successful and the number of malaria cases significantly declined to just 100,000 in 1964. Encouraged by this, the programme was changed to a more ambitious National Malaria Eradication Programme in 1958. By 1961 the incidence dropped to a mere 50,00 cases a year. But since then the programme suffered repeated set-backs due to technical, operational and administrative reasons and the cases started rising again. Early setbacks in malaria eradication coincided with DDT shortages. Later in the 1960s and 1970s malaria resurgence was the result of technical, financial and operational problems. In the late 1960s malaria cases in urban areas started to multiply, and upsurge of malaria was widespread. As a result in 1976, 6.45 million cases were recorded by the National Malaria Eradication Programme (NMEP), highest since resurgence.

FIG. 2: SEROPOSITIVITY FOR *PLASMODIUM FALCIPARUM*.

Appearance of magenta red coloured band at test region in addition to magenta red coloured band at control region indicates positive result.

The implementation of urban malaria scheme (UMS) in 1971-72 and the modified plan of operation (MPO) in 1977 improved the malaria situation for 5-6 years. Malaria cases were reduced to about 2 million. The impact was mainly on vivax malaria. Easy availability of drugs under the MPO prevented deaths due to malaria and reduced morbidity. The *P. falciparum* containment programme (PfCP) launched in 1977 helped reduce *falciparum malaria* in the areas where the containment programme was operated but its general spread could not be contained. *P. falciparum* showed a steady upward trend during the 1970s and thereafter. Rising trend of malaria was facilitated by developments in various sectors to improve the national economy under successive 5 year plans.

Malaria, at one time a rural disease, diversified under the pressure of developments into various ecotypes. These ecotypes have been identified as forest malaria, urban malaria, rural malaria, industrial malaria, border malaria and migration malaria; the latter cutting across boundaries of various epidemiological types. Further, malaria in the 1990s has returned with new features not witnessed during the pre-eradication days. These are the vector resistance to insecticide(s); pronounced exophilic vector behaviour; extensive vector breeding grounds created principally by the water resource development projects, urbanization and industrialization; change in parasite formula in favour of *P. falciparum*; resistance in *P. falciparum* to chloroquine and other anti-malarial drugs; and human resistance to chemical control of vectors.

According to the World Malaria Report 2014: 22% (275.5m) of India's population live in high transmission (> 1 case per 1000 population) areas, 67% (838.9m) live in low transmission (0–1 cases per 1000 population) areas and 11% (137.7m) live in malaria-free (0 cases) areas. In 2013, 0.88 million cases have been recorded, with 128 million tests being conducted on the suspected cases, with *P. falciparum* causing 53% and *P. vivax* causing 47% of the infections. The incidence of malaria in India accounted for 58% of cases in the South East Asia Region of WHO.

At present, official figures for malaria in India, available at NVBDCP, [7] indicate 0.7–1.6 million confirmed cases and 400-1,000 deaths annually.

Study Contradicts NVBDCP and WHO Data:

A study conducted by teams from the office of the Registrar General of India, Centre for Global Health Research at St Michael's Hospital and University of Toronto, Canada, published in The Lancet on 20 November 2010 has reported that malaria causes 205 000 malaria deaths per year in India before age 70 years (55 000 in early childhood, 30 000 at ages 5—14 years, 120 000 at ages 15—69 years) with a 1.8% cumulative probability of death from malaria before age 70 years. The report says that 90 per cent of the deaths were recorded in rural areas, of which 86 per cent occurred at home without any medical attention. It also found that Orissa reported the highest number of deaths — 50,000, followed by Chhattisgarh, Jharkhand and Assam. The study, which began in 2002, covered 6,671 areas, each with about 200 households. However, WHO has rebutted these estimates.

Yet other study on the global malaria mortality between 1980 and 2010 by Murray at al, published in The Lance*t* in Feb 2012, estimated the malaria mortality in India in 2010 at 46,800.According to the estimates of a 16-member committee set up by the National Vector Borne Disease Control Programme (NVBDCP) to assess India's actual malaria death burden, the total annual number of cases in India may be about 9.7 million, with about 30,014 – 48,660 deaths (40,297 on an average). Another paper estimates the malaria burden in India at India at 180 million, with as many as 90 million cases of *P. falciparum* malaria per year.

The biggest burden of malaria in India is borne by the most backward, poor and remote parts of the country, with >90-95% cases reported from rural areas and <5-10% from urban areas; however, the low malaria incidence in urban areas may be due to almost non-existing surveillance. The state of Orissa, with a population of 36.7 million (3.5%), contributes about 25% of the total annual malaria cases, more than 40% of *P. falciparum* malaria cases and nearly 20–30% of deaths caused by malaria in India, followed by Meghalaya, Mizoram, Maharashtra, Rajasthan, Gujarat, Karnataka, Goa, southern Madhya Pradesh, Chhattisgarh, and Jharkhand that also report significant number of malaria cases and deaths. The proportion of *P. vivax* and *P. falciparum* varies in different parts of India; P. falciparum accounts for 30–90% of the infections in the forested areas inhabited by ethnic tribes and <10% of malaria cases in mostly indogangetic plains and northern hilly states, northwestern India, and southern Tamil Nadu.

Unbridled urbanization, drought, migration of workers, and lax control efforts are all contributing to the resurgence of malaria in India and the problem is expected to exacerbate in the years to come. With increasing global warming, it is projected that in 2050s, malaria is likely to persist in Orissa, West Bengal and southern parts of

Assam, bordering north of West Bengal, but may shift from the central Indian region to the south western coastal states of Maharashtra, Karnataka and Kerala. Also the northern states, including Himachal Pradesh and Arunachal Pradesh, Nagaland, Manipur and Mizoram in the northeast may become malaria prone.

In the present study, out of total no. of patient samples (n=1021), 36(3.52%) were seropositive. Out of total no. of seropositive patients (n=36), 20(55.55%) were males, 10(27.77%) were females and 06(16.66%) were children.

In the present study, out of total no. of seropositive patients(n=36), 05(13.89%) were positive for *Plasmodium falciparum*, 19(52.78%) were positive for Pan Malarial species and 12(33.33%) were positive for mixed infection with *Plasmodium falciparum* and Pan Malarial species.

Out of "20" seropositive males, 01(20%) patient was seropositive for *Plasmodium falciparum*, 14(73.68%) were positive for Pan Malarial species and 05(41.67%) were positive for mixed infection with *Plasmodium falciparum* and Pan Malarial species.

Out of "10" seropositive females, 04(80%) were positive for *Plasmodium falciparum*, 02(10.53%) were positive for Pan Malarial species and 04(33.33%) for mixed infection with *Plasmodium falciparum* and Pan Malarial species.

Out of "06" seropositive children, 03(15.79%) were positive for Pan Malarial species, 03(25%) for mixed infection with *Plasmodium falciparum* and Pan Malarial species and there were no seropositives for single *Plasmodium falciparum* infection.

CONCLUSION: Although *Plasmodium vivax* is the major species responsible for malaria in most parts of India, *Plasmodium falciparum* infections have now increasingly been reported from some parts of India. In the present study, out of total no. of seropositive patients(n=36), 05(13.89%) were positive for *Plasmodium falciparum*, 19(52.78%) were positive for Pan Malarial species and 12(33.33%) were positive for mixed infection with *Plasmodium falciparum* and Pan Malarial species.

The Para Hit Total kit method used in the present study is a rapid test for *Plasmodium falciparum* and Pan Malarial species which provides a simple, rapid and in vitro qualitative screening test based on the detection of Plasmodium falciparum specific Histidine rich protein II (HRP-II) and a Pan Malarial species specific enzyme "Aldolase" produced by the respective parasites and released into the blood.

The test is intended only for initial screening and reactive samples should be confirmed by a supplemental assay such as microscopic examination of blood.

The present study reveals that rapid diagnostic tests are easy to use, reliable and simple to interpret. Rapid diagnostic tests are more suited to health workers in situations where health services are defined or absent. Therefore, these tests can be used as epidemiological tools for the rapid screening of malaria.

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