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MALARIA

A PRELIMINARY REPORT ON THE STUDIES ON THE ACTION OF 'ABN-61' (A PREPARATION OF DITA-BARK & QUININE) ON CASES OF HUMAN MALARIA

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General Consideration

Malarial fevers are caused by a protozoal parasite belonging to the class Sporozoa, suborder Hæmosporidia, genus plasmodium. There are three main species of malarial parasites which infect man. They include the following:—*Plasmodium vivax* (Grassi & Feletti, 1890) causing benign tertian malaria, *plasmodium falciparum* (Welch, 1897) causing malignant malaria, *plasmodium malariæ* (Laveran, 1881) causing quartan malaria. These species are widely distributed in the tropics. *Plasmodium ovale* (Stephens, 1922) is a rare species and was mainly reported from Africa. It should be noted that besides man, birds and monkeys are also parasitised by various species of plasmodia some of which include the following:—*plasmodium relictum* (præcox) in sparrows, *plasmodium gallinaceum* in fowls, *plasmodium knowlesi* in Simian monkeys.

Inoculation of *plasmodium knowlesi* in man can give rise to fever of mild type with a tendency to spontaneous cure. This species of parasite has recently been introduced for malaria therapy in dementia paralytica (general paralysis of insane). Further, the parasites of bird malaria and monkey (Simian) malaria are being extensively used in the experimental work on malaria.

So far as the human plasmodia are concerned, they pass their life cycle in two different hosts. The one called asexual cycle (Schizogonous) is passed in man (intermediate host) and the other called the sexual cycle (gametocytic) is passed in certain types of female anopheline mosquitoes.

When gametocytes are only found, they indicate that the man is a carrier.

The view that the parthenogenesis of gametocytes may explain the cause of relapse is not accepted. These gametocytes are infective to a female anopheline mosquito and if not taken by these insects they live only for a period of 20 to 40 days, after which they undergo spontaneous degeneration, even without any treatment.

Parasiticidal Action of Quinine

The parasiticidal action of any specific drug depends on the rate of parasitic multiplication and the rate of parasitic destruction. The schizogony cycle of various species of malarial parasites and the number of merozoites formed by each are shown below:—

Plasmodium vivax—16 to 24 merozoites every 48 hours; produces paroxysm every third day; not easily destroyed by quinine.

Plasmodium falciparum—28 to 32 merozoites every 48 hours or under; produces paroxysm every 36 or 48 hours; most influenced by quinine.

Plasmodium malariæ—6 to 12 merozoites events 72 hours; prduces paroxysm every fourth day; refractory to quinine.

The specific action of antimalarial drugs, like quinine and atebrin, brings down the parasitic count of all species of human plasmodia to a sub-clinical level which is unable to cause any febrile paroxysm. These specific drugs also have a destructive action on gametocytes of *pl. vivax* and *pl. malariæ* but are unable to kill the gametocytes of *pl. falciparum*. The only drug which has a destructive action on these gametocytes is 'Plasmochin'.

Relapses are frequent in malarial infections. This is due to the fact that after the fever subsides, the parasites disappear from the peripheral circulation and retire as it were, to some internal organs where they continue to exist. In such a case, the number is insufficient to cause symptoms or to be detected by ordinary laboratory methods of examination. The first relapse usually appears about 3 to 4 weeks after the initial attack has ceased, when the parasite reappears in the peripheral blood. Relapses tend to occur even with specific remedies like quinine and atebrin. Liability to relapse varies with different species of parasites. With *pl. vivax* it has been found to last upto 3 years from the time of the original infection, with *pl.* malariæ upto 6 years; and with *pl. falciparum* upto 9 to 18 months. Relapses occurring after a long interval should be differentiated from cases arising from re-infection, which, however, is not always possible to be verified, or eliminated.

History of Malaria Therapeutics

The parasite of malaria was discovered by the French military surgeon, Alphonse Laveran in 1880 and it took another 18 years when Sir Ronald Ross in 1898 established the mosquito transmission theory. But the treatment of malaria with cinchona bark was known long before the etiology of the disease was discovered. The story of a specific remedy for malaria with quinine starts from the year 1630 when the Countess del Chinchon, wife of a Spanish Viceroy of Peru was cured of 'ague' (malaria) by a native bark. Linnaeus in about the year 1740 named the tree as cinchona in honour of the Countess. The Peruvian bark was subsequently introduced in Europe by the Jesuits in the first half of the 17th century. Later in the first quarter of the 19th century, Pelletier and Cavantou (1820) isolated the principal alkaloid 'quinine' from the cinchona bark. The name 'quinine' is said to be derived from 'quina' the spanish way of spelling the Peruvian word 'kina', that is, bark. For a long time quinine and other allied alkaloids held the field of specific antimalarial therapy.

Researches proceeded on to find out a stronger and better substitute for quinine and it was the last war of 1914-1918 which gave an impetus to German chemists like Professor Schuleman and his colleagues to synthesise a product from methylene blue to supplement the natural alkaloid quinine. It was in September 1926 that they announced a product called 'Plasmochin' and its efficiency was tested by Rohel against bird malaria. 'Plasmochin' was an expensive drug and the hope that it would replace cinchona alkaloids as an antimalarial remedy was not met with great success, because of its feeble action on the

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schizogony cycle of *falciparum*. The only striking effect of this drug which superseded all other antimalarial remedies so far discovered, was its destructive action on the gametocytes of *pl. falciparum* and in this respect it is even superior to quinine. Six years after the discovery of 'Plasmochin' another new compound, also a derivative of methylene blue, 'Atebrin' was obtained by Mauss and Mietzsch. This synthetic product, first named 'Erion' was found effective against bird malaria by Kikuth in 1932. This drug acts powerfully on all species of malarial parasites and compares favourably with quinine, but like the latter drug it is ineffective against gametocytes of *pl. falciparum*. Recently other synthetic antimalarials like Certuna by Kikuth and Cilional by Schuleman have been prepared.

Quinine in Malaria

For over 300 years the alkaloids of cinchona bark were without any competition as specific in malaria and even today. quinine stands as the foremost remedy against malaria. The quinine problem of the province, as well as of India has assumed a great dimension during the present war. The Official Report of 1939 showed that the annual consumption of quinine in India was 210,000 pounds of which, 70,000 pounds were produced locally and for the balance, India had to depend on foreign import. But the annual requirements are really greater. It has been mentioned on a conservative estimate that about 100 million persons in India suffer from malaria in each year and therefore, the annual requirement of quinine for the purpose of effective mass treatment will amount to 1,200,000 pounds (calculating on the basis of 90 grains to each patient), that is, about six times the present yearly consumption. But even then one has to consider the question of relapses. Besides this, whenever there is a possibility of a widespread outbreak of malaria throughout the country (as happened recently during and following famine conditions in Bengal), the amount of quinine requirement will be increased still further.

With limitations of foreign import, the country has to depend mainly on her own production. Although the present production is somewhat over 90,000 pounds and even if the State desires to increase the production of cinchona plantation, it will take a number of years (8 to 10 years) to raise the quinine output so as to make the country self-sufficient. Under the present circumstances the continued demand on quinine as a specific antimalarial remedy when no other specifics like atebrin (mepacrine) and plasmochin (pamaquine) are available, will greatly reduce the quinine stock of India. Some firms have undertaken the manufacture of synthetic antimalarials in India and have achieved a certain amount of success but then again one should remember that there are limitations in this field also, as India has to depend on others for quite a large number of basic chemicals which are imported from foreign countries.. Attempts have, therefore, been made to search for a satisfactory substitute when there are possibilities of the quinine supplies being inadequate to meet the demands of the physician. Experiments were, therefore, conducted in order to get over the difficulty of procuring the requisite quantity of quinine for each case.

Search for an Indigenous Remedy

The possibility of obtaining some indigenous herb which could either replace quinine or even supplement it to a certain extent as an antimalarial remedy was always thought of. Our attention was first directed to *Alstonia scholaris*, popularly known as *chhatim* which enjoyed the reputation of having some antimalarial properties. People of Bengal and of many other parts of India had a great belief in *chhatim* as a febrifuge or antiperiodic and it is a common practice in the Ayurvedic treatment of medicine to prescribe this drug in the form of decoction (*pachan*) for malaria and other febrile conditions. After an extensive investigation with 'dita-bark' it was found that *chhatim* with quinine was as powerful an antimalarial drug as pure quinine itself and it was further observed that whereas a pound of quinine alone could serve only 70 persons (calculating on the basis of disbursement of 100 grains to each person) quinine when mixed with *chhatim* could treat about 200 patients. In this way the quinine requirement may be brought down to one-third of the total requirement of quinine. This preparation was named "ABN-61" and its efficacy as a specific antimalarial remedy was tried in cases of human malaria with great success. Control cases with quinine alone were also observed side by side to find out the amount of quinine required to bring about an effective cure. Although the minimum effective dose of quinine alone is 5 grains three times a day for 6 days, making a total of 90 grains, a greater amount is necessary in a large number of cases and still greater amount should be given to prevent relapses.

If the treatment of malaria is carried out with "ABN-61" the quinine requirement will be greatly reduced and individual cases will only require 12 to 18 grains to control the temperature and 36 to 42 grains of quinine to produce an effective cure. In quinine, the medical practitioners have found a genuine and specific remedy for the disease and have naturally pinned their faith so much as not to fall back upon any other drug when quinine can be procured. But we very well know that the present quinine stock will not be able to supply India's full requirements. From the report of cases (to be published in a later communication) it will be observed that the drug ABN-61 will go a very long way towards the solution of this problem.

Historical Note

The plant Astonia scholaris belongs to the natural order apocynaceæ. Its vernacular names, are Chhatim (Ben.), Saptaparna (Sans.), meaning seven leaf; Chhatim, Datyuni (Hindi).

Rheede (1678) noticed the medicinal use of the bark by the natives along with salt and pepper in febrile dyspepsia. Rumphius (1741) found that the bark was useful in catarrhal dyspepsia and in the febrile states consequent upon that affection and also for enlarged spleen. (Pharmacographia Indica, Vol. 2, 1891). Nimmo (1839) suggested the use of the bark as an antiperiodic. Gibson (1853) described it as antiperiodic.

In a report on the centennial exhibitions, presented to the American Pharmaceutical Associations (Transactions, 1877), it was stated that equal doses of ditain (obtained from dita-bark) and sulphate of quinine had the same medicinal effects while ditaine is free from any disagreeable secondary symptoms which were usual concomitants of large doses of quinine. The results arrived at with the alkaloid of dita-bark in Manila hospitals and also in private practice were simply marvellous. It was being employed with most satisfactory results in the Islands of Mindanao where malignant fevers were prevalent (quoted from Watt's 'A dictionary of economical products of India, Vol. I,

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1898). Cathcart (1879) in the *American Journal of Pharmacology* stated that 'the bitter bark of *Alstonia constricta* (the Australian species), sometimes called the native quinine, is in common use by the shepherds in the interior of Southern Australia as a domestic remedy for malarial fevers'. Stille and Maisch (1896) reported that the dita-bark and its alkaloid was used successfully in intermittent fever in the East Indies.

The first report of Proceedings of Central Indigenous Drugs Committee of India (published, Calcutta 1901) showed that *A. scholaris* had some febrifuge effect which was not lasting. Stewart used it in one drachm doses and reported that in mild cases of fever it was as effective as quinine. Nailer (1901) used the drug in 14 cases of "ague" in Tanjore (Madras) and found that in all cases it caused the temperature to fall steadily in a short time; no persipration was induced but the urine was observed to be increased and high coloured (quoted from Kirtikar and Basu's Indian Medicinal Plants, 1911).

Goodson, Henry and Macfi (1930) studied the activities of *A. scholaris* and *A. constricta* on bird malaria and found that echitamine (alkaloid of *A. scholaris*) had only a feeble action in doses of 5 mgm.

Buttle (as reported by Sharp, 1934) showed the inactivity of alstonian sulphate in bird malaria.

Mukherji, Ghosh and Siddons (1942) used a 1 to 2 per cent solution of the sulphate of total alkaloids of *Alstonia scholaris* as an intramuscular injection in doses of 20 mgm. per kilo body weight in cases of induced malaria (*plasmodium knowlesi*) in monkeys. They observed that the drug failed either to retard or to control the progress of infection. Later, they prepared a tincture(1 in 10) from the powdered bark containing 1·3 grs. of total alkaloid per ounce. This was administered in doses of one ounce thrice daily in a number of cases of human malaria of which one was of *pl. vivax* infection and three of *pl. falciparum* infections. They found that the drug had no demonstrable antimalarial action as it did not produce any remarkable febrifugal effect or alter in any significant way the course of infection.

From the above it will be found that the drug was found to be highly satisfactory by earlier workers while subsequent observations mostly applied on bird malaria and monkey malaria tend to prove the inefficacy of the drug in malaria infections. Such were the conflicting reports on the antimalarial property of *Alstonia scholaris*.

Present Observations with Aisotonia Scholaris

A series of preparations were made from dita-bark alone which were used in cases of human malaria. In the earlier works a very satisfactory result was not obtained as hoped for. In some cases, however, a beneficial effect by the administration of pure *chhatim* (dita-bark) was observed in that it either delayed the onset of the next paroxysm or reduced the duration of the next paroxysm. Daily examination of the blood showed that in earlier phases of infection the drug lowered the parasite count but could not retard the progress of infection. Later, a preparation was made in the form of tablet with *chhatim* (dita-bark) and quinine which was named 'ABN-61". This latter drug was then experimented on a series of cases of human malaria and found to be very effective. Case no. 1 shows that 14 tablets of AB-7 (preparation of dita-bark only) could not control the temperature while 10 tablets of ABN-61 (preparation of dita-bark and quinine) had a definite effect on the temperature and parasite count.

"ABN-61" as an Antimalarial Remedy—The therapeutic action of any antimalarial drug is usually first tested on either bird malaria or monkey malaria and then finally applied on human malria. It is quite resonable that before a drug is applied on human cases, a preliminary biological test on animals is necessary to select only those drugs which give indications for further study. The present observation was, however, directed mainly

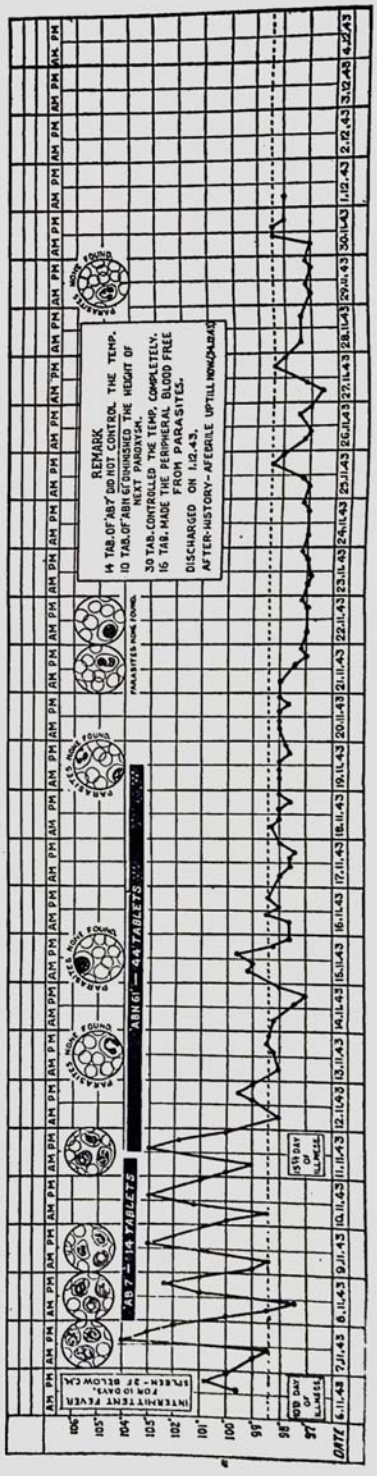

Chart of a case of benign tertian malaria (case no. 1) showing quotidian type of temperature. Two generations of *plasmodium vivax* multiplying on alternate days. Circular diagrams on the top show the forms of parasites as found in the peripheral blood examined by thin film method. AB 7—Tablets of *Chhatim* only. ABN-61—Tablets of *Chhatim* and Quinine.

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against human malaria and the investigation was conducted on patients admitted in the Carmichael Medical College Hospitals, Belegachia (Calcutta).

Dosage and Method of Administration—The general method followed was to administer 2 tablets of "ABN-61" three times a day to adult persons for a period of at least six days. The tablets were given orally and were swallowed with the aid of drinking water or the crushed tablets were dissolved in water before being taken. No other accessory treatment was introduced except the administration of alkali mixture and intravenous glucose in certain cases. The final dosage of "ABN-61" and the course of treatment was altered in some cases which were, however, guided by the nature of the effect observed on temperature and parasite count. In children the dose of "ABN-61" was modified accordingly. Each tablet of "ABN-61" contained only 1 grain of quinine and the total requirement to cure an attack was about 36 to 42 grains given in the course of 6 to 7 days.

Experimental Method—The methods of observation followed in the present series were to confirm all cases of clinically diagnosed malaria, *i.e.*, cases of sudden onset of fever with chill and rigor associated with or without enlargement of the spleen, by finding the malarial parasite from a microscopical examination of thin blood film. The drug was administered only in those cases which revealed the presence of malaria parasite. Further in every case where the parasite was detected in the peripheral blood, the patient was allowed to have the malarial paroxysm while in the hospital and then the treatment was commenced. During the period while the drug was withheld a simple alkaline mixture was only given. All the cases in which the drug "ABN-61" was tried had no history of taking quinine prior to admission in the hospital. After the drug was administered particular attention was paid regarding its effect in controlling the temperature, its destructive effect on asexual and sexual forms of the parasite, the time taken to make the peripheral blood from parasites, its effect on enlarged spleen and any untoward effect resulting from its administration. The parasiticidal action was observed by making a parasite count before the drug was commenced and then daily during the course of treatment. After the drug was discontinued, the blood was examined occasionally to check whether there was any reappearance of the parasite in the peripheral blood. Before the patient was discharged from the hospital, the blood was again thoroughly examined for parasites both by thin and thick film methods. Those cases who showed crescents (gametocytes of *pl. falciparum*) were not discharged from the hospital till the crescents disappeared. These forms of parasite are resistant even to quinine but can be destroyed by plasmochin. But in the present observation no further treatment was given and the crescents showed a spontaneous degeneration and gradual decrease in number till they finally disappeared. As the gametocytes are formed from the asexual forms, it is evident that the destruction of the latter will eventually lead to the disappearance of the former from the peripheral blood. To study the effect of the drug on the incidence of relapse, the patient was kept under observation in the hospital for a period of one fortnight to three weeks from the time of cessation of temperature. The cases were then followed up by instructing the patient to intimate any rise of temperature and further to submit a weekly report of the temperature.

Summary of the Results of Observation—The criterion of cure in malaria with any specific antimalarials is judged from its effect in controlling the temperature, causing the disappearance of the parasite from the peripheral blood and preventing the occurrence of relapses or lowering the incidence of relapse rate. Results obtained by treating cases of human malaria with the drug "ABN-61" so far fulfils all the above criteria. The present investigation on human malaria showed that 12 to 18 tablets of "ABN-61" administered in the course of 2 to 3 days were required to control the temperature and another 6 to 12 tablets were necessary to make the peripheral blood free from parasites. Excepting the gametocytes of *plasmodium falciparum*, the drug was found to have a powerful destructive effect on all species of malarial parasites which infect man. The drug had a definite inhibitory effect on the schizogony cycle. Daily examination of the blood during treatment showed a striking reduction in the number of parasites. In some cases a clear evidence of degeneration of parasites was obtained. The drug, therefore, exerted some physical effects of varying degrees and altered the morphological appearance of the parasite.

Conclusion

The present study justifies the recommendation of "ABN-61" as a safe antimalarial remedy as it was found to have marked parasiticidal action on all species of malarial parasites with the exception of the gametocytes of *plasmodium falciparum*. Further, while under the treatment of "ABN-61" the patient did not complain of any disagreeable symptoms (tinnitus and deafness) as are associated with cases treated with effective therapeutic doses of quinine (varying from 45 to 90 grains or more). This is, therefore, a distinct advantage over quinine. In the present series of cases no idiosyncrasy to the drug has yet been observed.

Acknowledgment

Our thanks are due to Messrs. Gluconate Ltd. who have helped us considerably in carrying out this investigation. The tablets ABN-61 and other tablets of *chhatim* only and also of *chhatim* and quinine as required for the present investigation were prepared by the same firm and were supplied to us free of cost.

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Comments from Expert : After 75 years

Malaria in India with Special Reference to Severe *Vivax* Malaria

Dhanpat Kumar Kochar[1], Abhishek Kochar[2]

P vivax **remains a substantial health problem and economic burden in India with proven difficulties to control it, particularly in urban areas. Although number of malaria cases in India has declined in the recent years, the relative proportions of *P. vivax* cases are increasing. *P. vivax* is transmitted by a variety of vectors across diverse ecological habitats and shows polymorphism in the pattern of relapse. It can also be overlooked as a pathogen when a mixed infection with *P. falciparum* is present. During last two decades, there is substantial evidence that *P. vivax* is associated with all sort of severe manifestations including cerebral malaria and death in India. This may be because of improved diagnostic facilities, reporting, investigation and/ or changes in *P. vivax* pathogenicity, which may be specific to individual parasite populations in different areas. As there is heterogeneity in transmission intensities of the *P. vivax*, there is tremendous scope for research in India for studying the parasite biology detection and treatment of hypnozoites to ensure radical cure.**

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Key words **: Anopheles mosquito, Severe Vivax Malaria, Chloroquin, Artesunate.**

Malaria is a vector-borne parasitic tropical disease found in 91 countries worldwide caused by *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale, and Plasmodium knowlesi*. It is transmitted by the bite of the female *Anopheles* mosquito. The disease incidence depends on environmental suitability for local vectors in terms of altitude, climate, vegetation, and implementation of control measures, and is linked to poverty, natural disasters, and war. *Plasmodium falciparum* and *P vivax* are the predominant species worldwide. The great majority of *falciparum* malaria occurs in sub-saharan Africa whereas *P. vivax* malaria is much less common because the population in this region is Duffy antigen negative. *P. vivax* malaria is common in Southeast Asia including India and America. *P malariae* and *P ovale* have a global distribution, however *P knowlesi* is predominantly seen in Malaysia, and adjacent southeast Asian countries[1].

Indian population is having a great burden of malaria cases because an estimated 95% population lives in areas where climatic conditions favour malaria transmission[2]. Whole India is endemic for malaria, except hilly areas of 2000 m above sea level where the mosquito is scarce due to unfavourable climatic condition. The presentation of malaria in India is very complex and the ratio of *P. vivax* and *P. falciparum* varies from place to place across the India. Few states like Orissa, U.P., Gujarat, West Bengal, Maharashtra, Madhya Pradesh, Rajasthan, Karnataka and Andhra Pradesh are highly endemic for malaria and contribute 90% of the total malaria cases in the country[3]. Malaria epidemiology in North-eastern states of India is complex due to high aboriginal population, varied terrain, rich forest cover and favourable climatic conditions for vector growth and malaria transmission[4]. However, alpine environment in Arunachal Pradesh and Nagaland, high proportions of *P. vivax* cases (60-80%) have been reported, while in Manipur, *P. vivax* contribute 42 – 67% and in Assam with subtropical to tropical climate it varies from 23-31 per cent. Meghalaya, Tripura and Mizoram are consistently having the lowest population of *P. vivax* cases over several years. Because of rapid construction, migration, and the

Editor's Comment :

- **Investigation of Malaria in any state any time in a case of Acute febrile illness is highly relevant.**
- **Malaria is changing its character - benign vivax malaria is not at all benign.**
- **There is upsurge of drug resistance Malaria.**
- **Early diagnosis and prompt therapy and ahereness is key to prevent resistance.**

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mushrooming of slums in the urban setting of India malaria particularly *P. vivax* control is very troublesome.

Mosquito Vectors :

Although multiple vector species may be present in any specific region, there is no single vector species which is found all over India. *Anopheles culicifacies* is responsible for an estimated 65% of malaria in India as this is the main malaria vector in rural, peri-urban areas, and in the plains. This species is mostly zoophagic and breeds in plain-land ecosystem. *A. stephensi* is also primarily zoophagic and an important vector for malaria in urban areas. It prefers human hosts in the absence of cattle. In the forest areas of North-eastern regions, *A. dirus* is an efficient vector which breeds in temporary water collections. It is exophagic and exophilic by nature. *A. minimus* is another vector in the forest areas of North-eastern regions, which is breeding in slow-flowing streams and exhibiting zoophilic and exophilic behavior. Another species responsible for perennial transmission in hill and foothill areas of central and Southern India is *A. fluviatilis*.

Biology :

The life cycle of parasite is completed in human and mosquito. The sporozoites are inoculated in humans by the bite of an infected female *Anopheles* mosquito. The parasite undergoes a pre-erythrocytic liver stage lasting for 1–2 weeks before the onset of the blood stage, where serial cycles of asexual replication produce increased number of parasite causing fever. A subpopulation of intraerythrocytic parasites switches to sexual development, producing female and male gametocytes, which are taken by mosquito via a blood meal. After a series of changes in mosquito the oocyst releases sporozoites which migrate to the mosquito salivary glands, completing the lifecycle. In *P. vivax* and *P. ovale* infections, a proportion of sporozoites become dormant hypnozoites, capable of producing relapses even after months or years of initial infection.[5]

Pathogenesis :

Usual incubation periods is around 10–18 days depending of different species, however, some strains of *P vivax* have a 3–6 month primary incubation period. The classical periodic fever with spikes occurs at fixed interval corresponding to the erythrocytic cycle length of the infecting species (24 h for *P. knowlesi*, 48 h for *P falciparum*, *vivax*, or *ovale* and 72 h for *P malariae*), but such patterns are rarely observed these days. In *P. falciparum* infection, the parasitized RBCs sequester inside small and medium sized vessels, avoiding parasite clearance in the spleen and causing host endothelial cell injury and microvascular obstruction. Cytoadherence is mediated by *P falciparum* erythrocyte membrane protein 1 (PfEMP1), which binds to different endothelial receptors; for example, intercellular adhesion molecule–1 and endothelial protein C receptor are associated with cerebral malaria. Parasitized RBC also binds to uninfected cells (rosetting), and they become rigid and less deformable, exacerbating microvascular obstruction. In the brain, it produces additional hypoxic injury through release of nitric oxide (NO) leading to coma and convulsion, in the lungs it predisposes to respiratory failure and ARDS. In pregnant women, sequestration in the intervillous space of the placenta leads to placental malaria causing of maternal anaemia, low birth weight, preterm labour, and increased risk of abortion and stillbirth. Placental cytoadherence is mediated by binding to chondroitin sulphate A (CSA), and the effects are most severe in primigravid women. Anaemia is a common feature of malaria and is typically multifactorial in origin including intravascular haemolysis, bone marrow suppression and dyserythropoiesis.[5,6]

Clinical presentation :

Malaria is described conveniently as uncomplicated and severe malaria. Fever is the cardinal symptom of malaria. It can be intermittent with or without periodicity or continuous and may be associated with chills and rigors. The fever is often accompanied by headache, myalgia, arthralgia, anorexia, nausea and vomiting. The symptoms of malaria can be non-specific and mimic other diseases like viral infections, enteric fever etc. Severe malaria is an emergency and manifestations can develop over a span of time as short as 12 – 24 hours and may lead to death. Severe malaria has specific diagnostic criteria which includes the most common manifestations of different organ dysfunction.

The important clinical and laboratory criteria for defining severe malaria are impaired consciousness or unrousable coma, prostration, i.e. generalized weakness so that the patient is unable walk or sit up without assistance, failure to feed, multiple convulsions – more than two episodes in 24 h, deep breathing, respiratory distress (acidotic breathing), circulatory

collapse or shock, systolic blood pressure < 70 mm Hg in adults, and < 50 mm Hg in children, clinical jaundice plus evidence of other vital organ dysfunction, haemoglobinuria, anuria or oliguria, abnormal spontaneous bleeding, pulmonary oedema (radiological). The laboratory parameters includes hypoglycaemia (blood glucose < 2.2 mmol/l or < 40 mg/dl), metabolic acidosis (plasma bicarbonate < 15 mmol/l), severe normocytic anaemia (Hb < 5 g/dl, packed cell volume < 15%), haemoglobinuria, hyperparasitaemia (> 2%/100 000/ìl in low intensity transmission areas or > 5% or 250 000/ìl in areas of high stable malaria transmission intensity), hyperlactataemia (lactate > 5 mmol/l), renal impairment (serum creatinine > 3mg%).

Characteristic fundoscopic findings in cerebral malaria include retinal whitening, changes in blood vessel contour, haemorrhages and papilloedema. These changes have direct correlation to the severity of disease. Neuroimaging typically shows some evidence of brain swelling but this is less prominent in adults than in children, in whom brain swelling is strongly associated with a fatal outcome. Foetal and maternal complications are more common in pregnancy with severe malaria; therefore, they need prompt attention.[7,8]

Complications of malaria :

The neurologic sequelae (Post malaria neurological syndrome) in the form of psychosis, cerebellar ataxia, cranial nerve involvement, hemiplegia and aphasia may be seen after recovery from coma but are usually self limiting. Other long term neurological complications following an episode of cerebral malaria may cause permanent visual, motor deficits, learning disorders and epilepsy. Haematological complications of malaria include hyper-reactive malarial splenomegaly (HMS), and rarely, splenic rupture. *P. malariae* can cause anaemia and nephrotic syndrome. Delayed haemolytic anaemia can occur after artemisinin treatment in some persons. The key event appears to be pitting, a splenic process whereby ring-stage parasites killed by artesunate are expelled from their host erythrocytes which return to the circulation, but with a reduced lifespan.[9,10]

Status of *vivax* malaria: Benign to Severe :

Until the beginning of this century, it was believed and preached that most serious and life- threatening complications of malaria are caused only by *P. falciparum* infection, whereas *P. vivax* infections are relatively mild, and runs a benign course and does not require hospitalization[11,12,13]. However, the dominant paradigm of *P. vivax* being a benign infection has been challenged recently from India. First authentic report of severe *vivax* malaria in world literature came in 2004 from Bikaner, India, when Kochar et al. reported that both sequestration and non–sequestration related complications like cerebral malaria, renal failure, circulatory collapse, convulsion, severe anemia, thrombocytopenia with or without bleeding, hemoglobinuria, abnormal bleeding, acute respiratory distress syndrome (ARDS), hepatic dysfunction, jaundice, and pregnancy-related complications including intrauterine growth restriction (IUGR) and miscarriage can be caused in patients suffering with *P. vivax* malaria. Two out of 11 patients died and one developed postmalarial psychosis. Polymerase chain reaction (PCR) test was used to confirm the diagnosis of *P. vivax* as well as to rule out *P. falciparum* coinfection.[14] Since then, similar reports are coming from all over India (Table 1). In many of these studies, the authors have used stringent test to exclude *falciparum* malaria and other coinfection. There is substantial variation in the reported geographic distribution and the incidence of severe manifestations of *vivax* malaria in different parts of India.

Recently, there are several reports of severe *vivax* malaria from other countries like: Thailand, Brazil, Indonesia, Papua New Guinea (PNG), and many other Asian and African countries including autopsy confirmation.[15,16] The occurrence of severe symptoms seems to be more frequent among females, pregnant women, individuals presenting with their first malarial infection, and those with other acute or chronic illnesses. In a joint collaborative multinational study, the overall case fatality was about 20-fold higher in India as compared to Brazil, and therefore highlighting the variability observed in different settings.[11] Recent evidences suggest that *vivax* malaria had almost similar risk of developing severe malaria, multiorgan dysfunction, and mortality as seen with *P. falciparum* infection.[17] The occurrence, relation, and magnitude of thrombocytopenia is also more in *P. vivax* of malaria.[18,19] There are several possible explanations for this observation which includes possibility of a longer liver stage of *P. vivax* allowing prolonged periods

for the parasite to remain in host environment, even if transmission is interrupted and the primary infection has been treated successfully.

Diagnosis :

The diagnosis of malaria is essentially clinical and is confirmed by the demonstration of presence of parasites in the peripheral smear or parasite-derived proteins by RDTs. Microscopy of stained thick and thin peripheral blood smear (PBF) remains the gold standard for confirmation of malaria. It is cost-effective, fairly sensitive and highly specific. It helps in knowing the exact species and quantification of parasites, and also in assessing response to antimalarial treatment. Microscopic evidence may be negative for asexual parasites in the some patients of severe malaria due to sequestration and partial treatment. Efforts should be made to confirm these cases by RDT or repeat microscopy. However, if the symptoms clearly point to severe malaria and there is no alternative explanation, such a case should be treated accordingly. RDTs are based on the detection of circulating parasite antigen in whole blood. These tests are fairly sensitive and specific and can be done in the field situation. These tests are good to study the species but not for quantification of parasites. The hidden sequestered biomass in severe malaria can be estimated from PfHRP2 concentrations in plasma. However, RDTs using monoclonal antibodies against parasite Lactate dehydrogenase (Optimal) may be very useful. Quantitative buffy coat (QBC) test using fluorescent dye and PCR can also be used for the diagnosis. The sensitivity of PCR assay is very high, and it can detect even 1 parasite/mL of the blood. Other diagnostic technique include loop- mediated isothermal amplification (LAMP), microarrays, automated flow cytometry (FCM), automated blood cell count (ACC), ultraviolet laser desorption mass spectrometry (LDMS), enzyme-linked immunosorbent assay (ELISA)/ enzyme immunoassay (EIA), latex agglutination assay, and cultivation of live malaria parasites.

Managent of Malaria :

The Early diagnosis and prompt treatment of malaria aims at complete cure, prevention of progression of uncomplicated malaria to severe disease, prevention of death, interruption of transmission, minimizing risk of selection and spread of drug resistant parasites.

The *P. vivax* is highly sensitive to chloroquine in India and resistance to it is not reported in general. Treatment of parasitologically, confirmed cases of uncomplicated cases of *P. vivax* malaria in India requires administration of 3 day course of oral chloroquine (25 mg/kg) for treatment of acute blood stage infection along with 14-day course of oral primaquine (0.25 mg/kg) to treat the dormant hypnozoites stage (radical cure). Primaquine should not be used in infants, pregnant women, and individuals with glucose- 6-phosphate dehydrogenase (G6PD) deficiency. As G6PD deficiency testing is not routinely available in the field, the patients are instructed to stop primaquine treatment and report back to the health care facility in case of dark urine or hematuria and cyanosis or blue coloration of the lips. As per recommendation of treatment of malaria in India, severe *P. vivax* malaria should be treated similar to severe *P. falciparum* malaria along with 14 days primaquine therapy.[7,8,20]

As an alternative of primaquine, recently United States Food and Drug Administration has approved, tafenoquine 300 mg single dose for the radical cure (prevention of relapse) of *P. vivax* malaria in patients aged 16 years and older who are receiving appropriate antimalarial therapy for acute *P. vivax* infection. This approval is an important step forward, as a new, single dose treatment for relapsing malaria[21].

Artemisinine combination therapy (ACT) is the treatment of choice for *P. falciparum* uncomplicated malaria. ACTs consist of a combination of an artemisinin derivative that rapidly reduces parasitaemia and a partner drug that removes residual parasites over a longer period. The leading ACTs in use are artemether–lumefantrine, artesunate–amodiaquine, dihydro-artemisinin–piperaquine, artesunate–mefloquine, and artesunate plus sulfadoxine–pyrimethamine. Artemether–lumefantrine should be given with milk or food containing fat to enhance lumefantrine absorption. In endemic areas, prescription of a single dose of primaquine (0·25 mg/kg) with an ACT is recommended to reduce the risk of onward transmission. This dose is considered safe in G6PD deficiency.

Severe malaria caused by *P. falciparum*, *P. vivax* and mixed infection is a medical emergency and should be treated urgently and preferably in the intensive care unit. The principles of management include specific antimalarial drug treatment, care of the unconscious patient, symptomatic treatment and

treatment of associated complications. Specific antimalarial therapy should be started immediately even on the basis of clinical diagnosis if parasitological confirmation is likely to be delayed.

Artesunate should be administered in the dose of 2.4 mg/kg bodyweight IV on admission, at 12 and 24 h, and then once a day for 7 days. Alternatively, artemether or artether can be used parenterally. Once patients can tolerate oral therapy, they should receive complete dosage of artemisinin-based combination therapy (ACT) for 3 days. Alternatively, quinine is given as loading dose of 20 mg/kg to be diluted in glucose or glucose normal saline followed by 10 mg/kg bodyweight every 8 h, and the infusion should take a minimum of 4 h. Later on, the treatment should be switched to oral therapy to complete the 7 days of treatment along with doxycycline (3 mg/kg once daily) or clindamycin (10 mg/kg twice daily), except for pregnant women and children younger than 8 years of age for whom doxycycline is contraindicated. The loading dose of quinine is not given if the patient has taken oral quinine or mefloquine in the previous 24 h. The dose of quinine is reduced to 5–7 mg/kg bodyweight if IV therapy is continued after 48 h. The dose of artemisinin does not require any adjustment.

Severe malaria often causes multiorgan dysfunction and the presence of these complications influences the patient's overall outcome and requires vigorous and meticulous treatment simultaneously. The hyperpyrexia is treated by tepid sponging and paracetamol. Convulsions are treated by intravenous lorazepam followed by loading dose of phenytoin or fosphenytoin. Convulsive status should be treated with usual protocols. Hypoglycaemia is very common in children and pregnant women and should be treated by IV 25–50% glucose. Usually, hypoglycaemia responds well to standard therapy, although hyperinsulinaemic hypoglycaemia in association with quinine therapy responds well to long-acting somatostatin analogues. Blood transfusion is generally recommended if the haemoglobin level is less than 5 g/100 mL (haematocrit less than 15%). In acute renal failure or severe metabolic acidosis, haemofiltration or haemodialysis should be started early. The fluid balance is critical in severe malaria because of narrow window between overhydration (pulmonary oedema) and under hydration (exacerbation of renal impairment and tissue hypoperfusion). Pulmonary oedema is treated by avoiding excessive rehydration and use of oxygen, whereas over hydration requires stopping IV fluids and use of a diuretic (furosemide: 40 mg IV) along with withdrawing 3 mL/kg of the blood by venesection into a donor bag. Circulatory collapse, shock and algid malaria are treated by parenteral antimicrobials and vasopressors, along with correction of haemodynamic disturbances. Bleeding and DIC requires transfusion of fresh blood or clotting factors, along with vitamin K (10 mg IV). Bleeding associated with marked thrombocytopenia may require platelet transfusion. Hyperparasitaemia (greater than 20%) should be treated by exchange transfusion. A close monitoring of pregnant women is essential because of high incidence of pulmonary oedema and hypoglycaemia. Parenteral artesunate is preferred in the second and third trimesters, whereas quinine is the drug of choice in the first trimester. Single dose of primaquine in *P. falciparum* infection and 14 days primaquine treatment in *P. vivax* and mixed infection should follow for treatment of gametocytes.[8,20]

Adjunctive Therapy :

Despite highly effective primary therapy against the parasite (quinine and artesunate), mortality and morbidity from cerebral malaria remains very high. Adjunctive therapies administered in the meantime might reduce the risk of mortality and neurocognitive sequelae in view of the fact that antimalarial drugs often take at least 12–18 h to kill the parasites. New therapies being considered as adjunctive therapy in cerebral malaria include reduction of iron burden (desferoxamine, deferiprone), anticoagulation (heparin), inhibition of cytoadherence (PfEMP-1 antagonists, levamisole, N -acetylcysteine and atorvastatin), Rho-kinase inhibition (fasudil), endothelium fixing drugs (NO and l -arginine), immune modulation (dexamethasone, immunoglobulins, anti–TNF monoclonal antibodies, pentoxifylline, rosiglitazone and curcumin), neuroprotection (erythropoietin) and correction of acidosis (dichloroacetate and N–acetylcystained). A number of agents have been or are being tested, but none has shown unequivocal evidence of improvement in clinical trials. Consequently, none of these agents can be recommended as part of the standard management strategy at present. Albumin is the only adjunctive therapy associated with reduced mortality in children. Among other agents, levamisole and arginine may be the most promising, based on

preliminary studies, but no large trials have yet been completed.[22,23]

Vaccine development :

A malaria vaccine, deployed in combination with current malaria control tools, could play an important role in future control and eventual elimination of malaria. Despite more than a century of extensive research, only one malaria vaccine candidate (RTS,S/AS01) has approval for use in countries where malaria is endemic. It provides significant protection against *falciparum* malaria infection over a 3–4 year period in older children. However, efficacy was relatively lower in very young children (6–12 weeks old). An overall reduction in long-term mortality remains to be demonstrated. A contrasting approach to producing sporozoite-based immunity is the *P falciparum* sporozoite (PfSPZ) vaccine, an intravenous injection of irradiation-attenuated sporozoites. PfSPZ has now entered clinical trials in Africa; Transmission-blocking vaccines against sexual-stage antigens have also been tried but till date no effective vaccine is available.[24,25]

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Severe *vivax* malaria in India

Sl. No	Author	No. of Patients	Major Complication in Adult / Child	PCR confirmed	doi / PMID
1	Kumar R et al - 2020 from Manipal	122	Jaundice, Renal failure, Anemia	–	PMID: 28367735 PMCID: DOI: 10.1080/20477724.2017.1309342
2	Anvikar et al – 2020 from Gujarat	30	Jaundice	PCR Confirmed	PMID: 32490754 DOI: 10.1080/21505594.2020.1773107
3	Methews et al – 2019 from Delhi	150	Thrombocytopenia, Jaundice, ARDS, Spontaneous bleeding, Metabolic acidosis, Shock, Renal Failure, Cerebral Malaria (Adult)	–	PMID: 31579662 DOI: 10.4103/tp.TP_2_19
4	Mukhtar et al – 2019 from Sudan	1	Cerebral Malaria (Adult)	–	PMID: 31533821 DOI: 10.1186/s12936-019-2961-1
5	Akhlaq et al – 2018 from Karachi	7	Cerebral Malaria	–	PMID: 30191906 DOI: 10.4997/JRCPE.2018.302
6	Kochar et al – 2017 from Bikaner	1	Cerebral Infarct (Adult)	PCR confirmed	PMID:28748845
7	Kumar R et al – 2017 from Manipal	511	Hyperbilirubinemia, ARDS, AKI, Cererbral malaria (Adult)	PCR confirmed	10.1080/20477724.2017.1309342 PMID:28367735
8	Kumar P et al – 2017 from New Delhi	1	Peripheral Gangrene (Adult)	-	10.4103/ijccm.IJCCM_424_16 PMID:28515614
9	Kochar et al – 2016 from Bikaner	150	Retinopathy (Adult)	PCR confirmed	doi: 10.1080/20477724.2016.1213948 PMCID: PMC5072115
10	Jain et al – 2016 from Dehradun	48	Hepatopathy (Adult)	-	10.1016/j.actatropica.2016.03.031.
11	Mallela et al – 2016 from Manipal	1	Subdural haemorrhage with severe thrombocytopenia (Adult)	-	10.7860/JCDR/2016/15418.7098
12	Gupta et al – 2016 from New Delhi	12	Anemia with thrombocytopenia (Adult)	-	10.1016/j.meegid.2016.02.014
13	Sequiera AM, Lacerda M, Kochar DK et al – 2015 from Bikaner	462	Anemia, jaundice, renal failure, cerebral malaria, death (Adult)	PCR Confirmed	10.1186/s12916-015-0302-y
14	Mitra et al – 2015 from Vellore	83	Thrombocytopenia and hyperbilirubinemia (Adult)	-	PMID: 26714506
15	Gupta et al – 2015 from Manipal	1	ARDS (Adult)	-	10.3855/jidc.6813
16	Kochar et al – 2014 from Bikaner	221	Hepatic dysfunction, thrombo-cytopenia, anemia, cerebral malaria, MODS, death (Adult)	PCR Confirmed	PMID: 25253213
17	Kumar et al – 2014 from Jaipur	1	Renal cortical necrosis (Adult)	-	10.4103/0971-4065.133789
18	Dev et al – 2014 from Faridabad	1	Myocarditis and heart failure (Adult)	-	PMID: 25467272
19	Muley et al – 2014 from Vadodara	66	Thrombocytopenia (Adult)	-	10.1155/2014/567469
20	Mittal et al – 2014 from Delhi	64	Severe anemia (Child)	-	PMID: 24947218
21	Nandwani et al – 2014 from Meerut	110	Acute kidney injury, jaundice, severe anemia (Adult)	-	10.1007/s12639-012-0208-y
22	Nayak et al – 2013 from Bikaner	5	Cardiovascular involvement (Adult)	PCR confirmed	PMID:24717201
23	Singh et al – 2013 from Chandigarh	19	Jaundice with hepatic dysfunction (Adult)	-	10.1155/2013/341862
24	Singh et al – 2013 from Dehradun	61	Thrombocytopenia (Adult & Child)	-	10.7860/JCDR/2013/6914.3479
25	Jain et al – 2013 from Jabalpur	22	Cerebral malaria, seizures, severe anaemia, and respiratory distress (Adult)	PCR confirmed	10.1179/204777213X13777615588180

Sl. No	Author	No. of Patients	Major Complication in Adult / Child	PCR confirmed	doi / PMID
26	Sharma et al – 2013 from New Delhi	54	Severe thrombocytopaenia jaundice with deranged LFT values (Child)	-	10.7860/JCDR/2013/5633.3370
27	Rizvi et al – 2013 from Alighar	62	Hepatic and renal dysfunction (Adult)	-	10.4103/1596-3519.117624
28	Sarkar et al – 2013 from Darjeeling	200	Jaundice (Adult)	-	10.4103/2229-5070.113912
29	Tanwar et al – 2012 from Bikaner	278	Thrombocytopenia (Child)	-	10.3109/09537104.2011.607520
30	Limaye et al – 2012 from Mumbai	50	Thrombocytopenia (Adult)	-	PMID: 23777019
31	Agarwal et al – 2012 from Rohtak	1	Multiple splenic infarct (Adult)	-	10.1016/S1995-7645(13)60051-6
32	Kaushik et al – 2012 from Dehradun	63	AKD (Adult)	-	10.1093/trstmh/trs092
33	Sharma et al – 2012 from New Delhi	46	Severe anemia and thrombocytopenia (Child)	-	10.1179/2046905512Y.0000000012
34	Kute et al – 2012 from Ahmedabad	1	Renal acute cortical necrosis and acute kidney injury (Adult)	-	10.1007/s00436-012-2975-x
35	Yadav et al – 2012 from New Delhi	131	Hepatic, renal and respiratory disease (Child)	-	10.1007/s12098-011-0603-x
36	Tanwar et al – 2011 from Bikaner	13	Cerebral malaria (Child)	PCR confirmed	10.1179/1465328111Y.0000000040
37	Kochar et al – 2010 from Bikaner	143	Thrombocytopenia (Adult)	PCR Confirmed	10.3109/09537104.2010.505308
38	Kochar et al – 2010 from Bikaner	65	Hepatic dysfunction, thrombocytopenia, anemia, cerebral malaria, renal failure, MODS, death (Adult)	PCR Confirmed	10.4269/ajtmh.2010.09-0633
39	Sarkar et al – 2010 from Kolkata	3	ARDS (Adult & Child)	-	10.4103/0970-2113.68323
40	Kochar et al – 2009 from Bikaner	40	Jaundice, renal failure, anemia, thrombocytopenia, MODS, death (Adult)	PCR confirmed	PMID: 19190212
41	Parakh et al – 2009 from Delhi	3	Cerebral malaria and severe anemia (Child)	-	10.1179/027249309X12547917868844
42	Thapa et al – 2009 from Kolkata	1	Severe thrombocytopenia with severe bleeding (Child)	-	10.1097/MPH.0b013e3181b7eb12
43	Harish et al – 2009 from Jammu	2	Thrombocytopenia and cerebral complication (Child)	-	10.1007/s12098-009-0087-0
44	Harish et al – 2009 from Jammu	2	Severe thrombocytopenia and cerebral malaria (Child)	-	10.1007/s12098-009-0087-0
45	Sarkar et al – 2008 from Varanasi	3	Cerebral malaria (Adult)	-	10.4103/0972-5229.45084
46	Kochar et al – 2007 from Bikaner	1	cerebral malaria in status epilepticus (Adult)	PCR confirmed	10.1016/S0140-6736(07)61417-2
47	Kaur et al al – 2007 from Delhi	1	Severe thrombocytopenia with acute renal failure (Child)	-	PMID: 17264156
48	Kochar et al – 2005 from Bikaner	11	cerebral malaria, jaundice, renal failure, pregnancy related complications death (Adult)	PCR Confirmed	PMID: 15705338
49	Makkar et al – 2002	1	Thrombocytopenia (Adult)	-	PMID: 12495609
50	Patial et al – 1998 from Shimla	1	Cerebral dysfunction (Adult)	-	PMID: 9770881
51	Kakar et al – 1999 from New Delhi	1	Thrombocytopenia (Adult)	-	PMID: 10626136
52	Mishra et al – 1989 from Raipur	1	Cerebral malaria (Adult)	-	PMID: 2687230
53	Sachdev et al – 1985 from New Delhi	1	Cerebral Malaria (Child)	-	PMID: 3900435
54	Verma et al – 1976 from Jammu	1	Cerebral malaria (Child)	-	PMID: 776824