"We support the need for continuous growth and development. We recognise that there is need for science in the service of the social sectors to be supported far more extensively, in putting it to work for all mankind”

World Declaration on the Control of Malaria
27.10.1992
DISCUSSION

This study consists data of various aspects of malarial parasites which includes 440 admitted-patients, 1052 thick-thin smear examination of fever cases came to out-patient department, 226 serological tests of 200 fever patients by ParaSight-F test, cultivation of Plasmodium falciparum in tissue culture medium in 10 patients, asymptomatic parasitaemia in 229 blood donors and prevalence of malaria in 70 HIV positive cases. This data includes work done between 1st October '94 to 30th November'97.

1. Which populations were affected by malarial parasites?

As shown in table no.1, 440 patients were admitted during the study period. These admitted patients’ general condition may be such that it would warrant indoor admission, intensive treatment and parental drugs. There were 226 males and 214 females. There was no statistical significant difference in the sex preference for malarial parasites. As shown in table, malaria affects young individuals. Warrell et al (93) found that mean age of their 100 patients was 23.5 years but their group included all patients above 6 year of age.

Population of pregnant/post-partum patients having malaria (table no.5)

Out of 440 patients, 214 were female of which 46 were pregnant/post-partum patients (i.e. 10.45% of study group and 21.5% of female group) had malarial parasite in their smear with clinical manifestations of malaria. 40 out of 46 patients (86.96%) had falciparum malaria. 14 out of 46 (30.43%) patients died. The predisposition to severe malaria during pregnancy is a well established fact but it is not clear whether a natural immune depression in pregnancy or
factors associated with the placenta encourage parasite multiplication and clinical manifestations. The placenta appears to be a site of preferential parasite sequestration and development. The literature on pregnancy and malaria suggests that high parasitaemias and complications are frequent in primigravidae and during 2nd trimester. There is loss of immunity in early pregnancy. Prevalence of parasitaemia as well as parasite densities decrease in last trimester as the immunoresponsiveness is regained. Sholapurkar et al (1988) demonstrated that in this region, parasites densities are higher in pregnant as well as puerperal patients and that there was no significant difference between parasite densities in 1st, 2nd, 3rd trimester and puerperium or those between primigravidae and multigravidae.

Out of our 46 patients, 38 were primigravidae and 8 were multigravidae. Thus increase prevalence of malaria was found in primi as reported in literature. Though much literature is not available on malaria during postpartum period. Our 22 patients developed malaria during immediate postpartum period. Following delivery, a large amount of blood is auto-transfused from uterine vasculature into systemic vasculature. In patients, whose uterine and placental blood is packed with mature forms of parasites there would be sudden flooding of systemic circulation by these mature parasites. These would then get sequestered in other organs, brain being the preferred site. This may explain the deterioration noticed in many patients immediately after labour.
2. Malaria prevalence during various months of the year. Its relation with transmission of malarial parasite.

Dutta P. et al observed more cases of falciparum malaria during months of Sept., Oct., & Nov. This would depend on transmission of malarial parasites by the vector. The vector density depends upon various geographical factors, one of which is rain fall. Gupta R. tried to correlate rainfall with upsurge of malaria in Rajasthan. He concluded that overall malaria incidence showed a moderate correlation with annual rainfall while the incidence of falciparum malaria showed a significant correlation. Rainfall in the months of June, July and August did not show any significant correlation with annual malaria incidence. Rainfall in the month of September showed significant correlation with the incidence of overall malaria as well as falciparum malaria. Tewari S.C. et al in their study of epidemiological aspects of persistent malaria along the river Thenpennai(Tamilnadu) found that P. vivax was the dominant species of parasite and transmission was perennial. Two main peaks which were observed were March-April and October-November.

Table no.2 and table no. 17 shows that prevalence of both vivax and falciparum malaria varied from month to month. Maximum cases occurred during the month Sept. and Oct. Vivax malaria was common in month of July i.e. early transmission month while cases of falciparum were more in Sept. & Oct. It seems that this month wise prevalence has relation with the rainfall and climatic conditions of our region.
3. Does prevalence of malaria vary in different areas of Kheda districts?

This study analysed the information given by the patients of their place of location. Maximum number of patients came from Anand Taluka, this may be because our institution is most suitable and near for Anand Taluka patients. Patients also came from nearby talukas. One of fact that emerges is that malaria is quite common in Kheda district and patients came from almost all talukas of Kheda.

Sharma et al had also carried out work in Nadiad taluka in a village-wise analysis of receptivity and vulnerability to malaria. Malaria annual parasite incidence (API) showed relationship with water table followed by soil type, irrigation and water quantity. Thus village wise prevalence varied. [2] Though malaria is endemic in Kheda district, prevalence in different talukas may vary due to geographical variation.
4. The malarial parasites.

Amongst admitted patients the prevalence of *P. falciparum* were higher. Out of 440 patients, 272 i.e. 61.82% were of falciparum malaria while 166 i.e. 37.72% were of vivax malaria. One of the explanation for higher prevalence of falciparum malaria cases in admitted patients may be due to the fact that falciparum malaria produces more severe and complicated malaria which requires indoor admission in more no. of cases. However the OPD case analysis also showed around 50% of falciparum cases. More number of falciparum cases in relation to vivax malaria is been noted all over India.

Resurgence of *P. falciparum* malaria is becoming a major problem in north-east India.[98]. ‘National Malaria Control Strategy document’ describes that *P. falciparum* percent is increasing steadily i.e. 21.8% in 1981 has doubled to 43.9% in 1991[49]. Sharma V.P. Director of Malaria Research Centre of India also documents the resurgence of *P.falciparum* in India.[47]

490 cases of malaria admitted during one year, in a service hospital were analysed by Maj Upadhyaya P.K.and Col Bhalla J.S.(1987).They found 342(69.8%) cases had *P.vivax*, 138(28.2%) had *P.falciparum* and 10 had mixed infections.[99]

5. What causes death due to malarial parasite?

Sanchetee P.C.and Varma P.P. in their editorial article mentions “Despite aggressive treatment available, mortality of 10% to 40 % encountered in cerebral malaria.”[100] Bajiya H.N. and Kochar D.K. did a hospital based study of 185 adult patients, 62 (33.5%) died and 123(66.5%) survived.[101]
In our study out of 440 patients, 64 (14.5%) patients died. Out of 166 vivax patients, 2 patients died. Out of 272 falciparum patients, 62 (22.7%) patients died. It is a well-known fact that *P. falciparum* species leads to various complications and death.

The various causes which lead to death were analysed in this study. Cerebral malaria and other cerebral symptoms like convulsion, neuropsychiatric manifestations were important cause of death in falciparum malaria in this study. All patients who died had cerebral malaria.

Macpherson G.G. et al (1985) in their post-mortem brain biopsy study found parasitised erythrocytes in cerebral and other vessels, higher in cerebral malaria than without cerebral malaria. [102] This may be the cause of more mortality in cerebral malaria cases. Other factors which were associated in patients who died were heavy parasitaemia, anaemia, hypoglycaemia, pregnancy\post-partum, renal failure, septicaemia and respiratory failure due to adult respiratory distress syndrome, in our study. In most cases these factors operated in combination. Daroff R.B. et al studied 1200 cases of *P. falciparum* malaria. He analysed the previous mortality statistic of cerebral malaria which is presented below for comparison with our study. [103]
Mortality of cerebral malaria due to *P. falciparum* in previously reported series.[103]

<table>
<thead>
<tr>
<th>Authors</th>
<th>Sample</th>
<th>No. of cases</th>
<th>Deaths</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arbuse</td>
<td>US troops</td>
<td>7</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td>Fitzhugh et al</td>
<td>Allied troops, US and Allied troops</td>
<td>68</td>
<td>28</td>
<td>41</td>
</tr>
<tr>
<td>US Army</td>
<td>US and Allied troops</td>
<td>57</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>Ewing</td>
<td>New York city</td>
<td>34</td>
<td>13</td>
<td>38</td>
</tr>
<tr>
<td>Rothe</td>
<td>Children in Kenya (below age 14)</td>
<td>97</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

Cerebral manifestation was the most common cause of death. In all 62 patients of *falciparum* malaria who died had cerebral symptoms. It has also been found in present study that along with neurological features, patients of cerebral malaria also develop serious multiple organ system involvement. Severe manifestations are also been reported by other workers and have correlated with the poor outcome.[104] Arya (1987) found that average period between admission and death was 40 hours.[105]

6. Clinical spectrum of the malarial parasite species:

Various typical and atypical clinical features were been noted due to malarial parasite affecting the various systems. Atypical clinical features, severe manifestations, cerebral manifestation, neuro-psychiatric manifestations, atypical fever were more commonly associated with *falciparum* malaria. Jaundice with direct and indirect bilirubinaemia may be mistaken with viral hepatitis. One of the differential diagnosis in a case of jaundice in India should
be malaria, especially due to falciparum species. One of the fact which was been observed that splenomegaly was more common with vivax species.

Various unusual acute and chronic manifestations and complications of malaria is described by various workers.[106] Some of the misleading manifestation described in falciparum malaria are multi organ dysfunction syndrome (MODS), septic shock, adult respiratory distress syndrome and viral hepatitis like picture.[107] Jaundice is an important complication of malaria is described by various workers.[108]

7. Staining characteristics of malarial parasites.

Smear examination for malarial parasites was one of the most important aspects of this study. It has been repeatedly emphasised by many workers that most important cause of smear negative malaria is not the absence of parasites in the blood but fallacies in examination of blood.

Thick smear preparation is a must, so that scanty parasitaemia is not missed. This also proves adequate for species diagnosis of malarial parasites. In our study, in most cases species of malarial parasite could be identified by thick smear. Although JSB stain has been used in national programme and Field’s stain has been recommended sometimes. Giemsa stain gives better and more consistent results.

As described in table 10, various observations were made of Giemsa staining in this study. It was concluded that it is the most rewarding and life saving investigation. Though it is considered to be easy and simple investigation, it requires great expertise, dedication and meticulous approach.
Even with all the serological and immunological techniques currently available and under development, it is probably true to say that the only certain means of diagnosing all four of the human malaria is by microscopic examination of the blood. This examination should be a routine procedure in medical practice not only in all malarious areas, but also in non malarious countries whatever may be the symptoms of primary diagnosis, if the patient has been travelling abroad within a year. The main reason for this is that the clinical picture of malaria may be of infinite variety; this infection may also occur as a result of blood transfusion from an infected donor or it may be a complicating factor of other diseases. One should remember that the presence of malaria parasites in the blood is a sign of infection but not necessarily a cause of the disease; persons who have resided for many years in malarious areas may have scanty malaria parasites in their blood, but the symptoms which made them see the doctor may be due to a different cause.

8. What favours growth of malarial parasites?

Associated diseases with malaria: Diabetes is known to predispose growth of any microorganisms. We found that 30 patients had diabetes associated with malaria. Malarial parasites utilise glucose for their growth. This explains accompaniment of diabetes with malaria. Krajden S. et al (1991) describes a case who had insulin dependent diabetes mellitus presented with diabetic ketoacidosis and having presence of *P.falciparum* with 1% parasitaemia.[109]
Whether tuberculosis predisposes malaria or not is difficult to conclude. Our 20 patients had associated tuberculosis. This could be due to high prevalence of tuberculosis in our country. T. B. and malaria both affects cell mediated immunity and prevalence is higher in poor people, thus association could be related to such factors.


334 patients received chloroquine for therapy. Chloroquine and Artimisinin cleared parasitaemia very fast in sensitive patients. In 22(5%) patients, chloroquine did not clear the parasitaemia. Chloroquine resistance was thought to be present in these 5% patients.

In 154 patients quinine was given. This was given in patients who did not respond to chloroquine, in patients who had already taken chloroquine from general-practitioner and also in patients who came with severe complications. This data on chloroquine resistance may be underestimated. Barat L M and Bloland P B (1997) in their recent article on drug resistance among malarial parasites, believe that 'one must draw distinction between documentation of cases of drug resistance and clinically and epidemiologically significant drug resistance.'[110] Thus our area needs a dedicated study on antimalarial drug resistance. Seventeen states in the country have reported R-III resistant to chloroquine, with more than 10 foci being reported from the states of Assam, Gujarat, Orissa and Rajasthan.[48].28 days in vivo test is suggested by WHO however this may be impractical because 1 to 10 blood examinations are required [66].
The other drugs which were used by clinicians were tetracycline with quinine, sulfadoxine-pyrimethamine combination, mefloquine and artimisinin. Because of problem of chloroquine resistant malaria, mefloquine is suggested for prophylaxis to nonimmune person coming to malarious area. Croft and Garner P (1997) did a review of trials of mefloquine to prevent malaria. They concluded that mefloquine may be effective in preventing malaria (very few studies have proved this) and side effects were found to be higher which resulted into higher withdrawal rates. [111] Primaquine was given as gametocytocidal in falciparum malaria and to eliminate hypnozoites in vivax malaria. However this was given, not as routine and it very much depended upon treating physician.

10. Association between malarial parasite and septicaemia. Analysis of blood culture results.

Septicaemia is known to exist with malarial parasite infection. It may complicate the picture of malaria, may cause fever, hypotension and death. A. Kharazmi et al described Pseudomonas aeruginosa septicaemia in a Danish patient with severe Plasmodium falciparum. [112] Metha et al described 1 case of E.coli septicaemia and 2 cases of Salmonella septicaemia out of 32 unusual cases of malaria. [106]

Clinically septicaemia was suspected in 25 cases. Blood culture was done in these 25 cases of which 15, were positive. Pneumococci was isolated in 2 cases who also had pneumococcal pneumonia. E.coli was isolated in 2 cases, while Salmonella in 4 cases. Out of these 4 cases who had salmonellosis, widal
came positive in 2 cases. *Pseudomonas aeruginosa* was isolated in 2 cases and unidentified gram negative bacilli were isolated in 5 cases.

These cases of septicaemia were due to nosocomial infection or was due to malaria is difficult to comment. 6 patients died out of 15 who had blood culture positive.


One of the way by which falciparum species is differentiated from vivax is through their stages of development. As mentioned by other workers, presence of schizonts in peripheral smear can be seen in falciparum infections and it signifies the bad prognosis. In 4 of our patients, schizonts were seen in the peripheral blood. All these 4 patients died.

In 120 patients of falciparum malaria, gametocytes were seen. One of the observation which was made that patient who developed renal failure had increased number of falciparum gametocytes. This finding was not described earlier, thus requires more confirmation.
12. Importance of family history and history of stress.

Family history of malaria was obtained in 44(10%) patients. This may be because they had the same environment and same genetic make-up. This history helps to suspect diagnosis of malaria. It may be possible that the same mosquito might have injected sporozoites in more than one individual.

Immunity has a definite relation with mental stress. This is one of the recent concepts in immunology. Malarial immunology may be altered due to stress. We cater services to medical students and university students of V.V. Nagar. We noticed that malaria occurred more commonly in students at time of examination. The other stress factors were also identified.

One of the recommended intervention could be to give prophylaxis against malaria during such stressful periods.

13. Degree of parasitaemia.

In this study, we found a significant correlation between hyper parasitaemia and mortality but this association was not so strong with lesser degree of parasitaemia. The reports in literature about lack of correlation between degree of parasitaemia and severity of disease is in the form that many patients with a severe manifestation like cerebral malaria have scanty parasitaemia [113] and many patients with hyper parasitaemia are successfully treated on out patient basis without ever developing any complication. In other words, high parasite counts are more likely to lead to severe disease but all patients with severe disease do not necessarily have high parasite counts. In
general in falciparum malaria, following correlation has been found between parasite density and severity of malaria.[114].

Less than 10,000\text{c/mm} -- 1\% mortality

More than 500,000\text{c/mm} -- 50\% mortality

Fluctuating parasitaemia in peripheral blood because of sequestration of mature forms of parasites in internal organs may be responsible for some of the discrepancies observed between parasite count and severity of disease, but the role of protective host immunity and immunological mechanisms in pathogenesis of severe malaria also have to be considered.


To estimate endemicity of malaria, various methods is adapted. One of the method is to find out blood smear positivity in asymptomatic individual. This study selected blood donors of our institution and also Anand Red Cross blood bank to obtain better result. It was done in August '95, August being the month for transmission of malarial parasites. Out of 229-blood smear examined, none was found positive.

This finding appears to be paradoxical in the sense that malaria is endemic in our region, eventhough we did not get any positive smear. The possible explanations are as follows:

(I) The history of fever is been asked in all volunteer blood donors.

(II) Most of these blood donors were young healthy students. The source of blood of both these blood banks is university students.
Thick smear (Giemsa, 1000x) showing developing schizonts (DS) and rings at 24 hrs in RPMI 1640 culture medium.

Thick smear (Giemsa, 1000x) showing developing schizonts at 28 hrs in vitro culture.
(III) More number of blood donors should have been examined to get positive results.

15. Analysis of Parasight-F test for diagnosing *P. falciparum*.

The test was found to be good additional support for diagnosing falciparum malaria. It was found to be highly specific and a very sensitive test. Except in one patient, who had Coomb's positive autoimmune haemolytic anaemia, where it gave false positive result? it was matching with the microscopic result. The test remain positive also in urine. Valle M.R.et al(1991) suggested that variety of *P. falciparum* antigens are released into urine during infection. They concluded that detection of malarial antigens and antibodies in urine may lead to a approach for the diagnosis of malaria. [115] The visual impression of the positive colour change was equated with the parasite count. Observation was made that fainter the positive line, fewer were the parasite, while thicker and brighter the colour the parasite count was higher. Mohapatra P.K. et al (1996) in their study found a weak qualitative association between parasite density and intensity of colour development of the test strip. [116] The test was positive and was very important as a diagnostic tool when patient received anti-malarial. Intra- personal disagreement about positive and negative result was almost nil. This test was very simple and was considered to be a good supporting evidence. Drawback was its cost and that it cannot detect other plasmodial species. In 5 patients who had already received chloroquine, the peripheral smear was negative, while the test gave positive result. This is one of the advantages of this test because in clinical set-up such situation
Thin smear (Giemsa, 1000x) showing developing mature schizonts (MS) at 30 hrs in RPMI 1640 culture medium.
C.J. Shiff et al found that this test had 88.9% sensitivity and 87.5% specificity. They compared these test results with 272 thick blood films microscopically and confirmed by the QBC malaria test. [5].

Christine Beadle et al found this test to be 96.5-100% sensitive for detection of greater than 60 \( P. falciparum \) asexual parasites/microL blood. [6]

This test is developed on the basis that infected erythrocytes synthesises histidine rich proteins. HRP-I or the knob associated HRP, HRP-II & HRP-III are found of which HRP-II is identified in all \( P. falciparum \) parasites regardless of knob phenotype. [117] On basis of this, HRP-II based ParaSight-F test is developed.

**16. Cultivation of \( P. falciparum \):**

Cultivation of erythrocytic stages of Plasmodium species in vitro can be divided into two eras- that of the short term cultivation of human and numerous animal species and that of the continuous cultivation of \( Plasmodium. falciparum \) initiated by Trager&Jensen (1976), expanded by the successful adaptation of their procedure by other investigators for continuous cultivation. After screening several newly developed culture media, it was discovered that medium RPMI 1640 (Moore et al1967) was superior for continuous cultivation of \( P. falciparum \).[92]

In this study, cultivation was done like continuous method. However each time it was terminated after 48 hours. The cultivation method used in this study was like microtest recommended by WHO. The observation and utility of this cultivation is described in table 22.
Thin smear (Giemsa, 1000x) showing (MS) developing mature schizonts and rings mature schizonts at 32 hrs.

Thick smear (Giemsa, 1000x) showing rupturing schizonts at 36 hrs in RPMI 1640 culture medium.