Resistance Pattern and Epidemiological Characteristics of Cases of Malarial Fever in Jharkhanand

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Abstract: Malaria is a protozoan disease transmitted by bite of infected Anopheles mosquitoes. Causative agents - Six species of the genus Plasmodium cause nearly all malarial infections in human. These are P. falciparum, P. vivax, P. ovale (two morphologically identical sympatric species), P. malariae and in Southeast Asia – the monkey malaria parasite P. knowlesi(2) Diagnosis- 1. Microscopy.

2. Clinical diagnosis 3. Antigen detection test using rapid immunochromatographic techniques. 4. Molecular test through PCR techniques. In this study we used the method microscopy for diagnosis of plasmodium falciparum malaria and parasite count, after that we started treatment artesunate, after complete course of treatment, again we used microscopy method for parasite count to know the affect of artesunate in parasite reduction. Results: Male were 59.7% and female were 40.3% in total cases of malarial fever in Jharkhand. Under age 20 years were 15.3%, between age 20–40 years were 43%, between 40–60 years were 33 % and above 60 years were 08.3% in total cases of malarial fever in Jharkhand. Malarial parasite count/ microlit on day 1 before artesunate were Less than 1 lakhs – 34.7%, 1 lakhs to 2 lakhs – 34.7%, More than 2 lakhs – 30.6%. Malarial parasite count/microlit on day 14 and day 28 after artesunate was zero, so artesunate is an effective drug for treatment of malarial fever in Jharkhand. Most of malarial patients were from rural 59.7% and urban patients were 40.2% in total cases of malarial fever in Jharkhand Blood Urea abnormally were normal in 41.6%, mild elevated in 40.8%, moderate elevated in 4.2% and severe elevated in 14.1% in total cases of malarial fever in Jharkhand Serum creatinine were normal in 33.3%, mild elevated in 36.1%, moderate elevated in 27.8% and severe elevated in 2.7% in total cases of malarial fever in Jharkhand. Haemoglobin were normal in 20.8%, mild anaemia in 23.6%, moderate anaemia in 47.2% and severe anaemia in 08.3% in total cases of malarial fever in Jharkhand.

Keywords: malarial fever, drug resistance in Jharkhand

1. Introduction

Malaria is a protozoan disease transmitted by bite of infected Anopheles mosquitoes. The most important of the parasitic diseases of human, is present in 106 countries containing 3 billion people and causes approximately 2000 deaths each day; mortality rates are decreasing as a highly effective control programs in several countries. Malaria has been eliminated from the United States, Canada, Europe and Russia. Its prevalence rose in many parts of tropics. Increase in the drug resistance of the parasite, the insecticide resistance of its vectors and human travel and migration have contributed to this resurgence.

Causative agents - Six species of the genus Plasmodium cause nearly all malarial infections in human. These are P. falciparum, P. vivax, P. ovale (two morphologically identical sympatric species), P. malariae and in Southeast Asia – the monkey malaria parasite P. knowlesi(2)

2. Diagnosis

1. Microscopy- simple light microscopic examination of Giemsa stained blood films is most widely practiced. Advantages include differentiation between species, quantification of the parasite density, and ability to distinguish clinically important asexual parasite density and ability to distinguish clinically important asexual parasite stages from gametocytes which may persist without causing symptoms. These advantages can critical for proper case management and evaluating parasite parasitological response to treatment. Second method is modification of light microscopy called quantitative buffy coat method, uses microhaemotocrit tubes precoated with fluorescent acridine orange stain.

Clinical diagnosis- Although reliable diagnosis cannot be made on the basis of signs and symptoms alone because of the non specific nature of clinical malaria, clinical diagnosis of malaria is common in many malarious areas. In much of the malaria endemic world, resources and trained health personnel are so scarce that presumptive clinical diagnosis is the only realistic option. Clinical diagnosis offers the advantages of ease, speed, and low cost. In area where malaria is prevalent, clinical diagnosis usually results in all patients with fever and no apparent other cause being treated for malaria.

Antigen detection test using rapid immunochromatographic techniques. A number of commercially available kits are based on the detection of the histidine rich protein 2 of P. falciparum, compared to light microscopy and QBC, this test yielded rapid and highly sensitive diagnosis of P. falciparum infection. Advantages to this technology are that no special equipment is required, minimal training is needed, the test and reagents are stable at ambient temperature and no electricity is needed. Additionally, a test based on detection of a specific parasite enzyme (lactate dehydrogenase) has been developed reportedly only detects viable parasite, which if true, eliminates prolonged periods of false positivity post treatment.
Molecular test through PCR techniques.-detection of parasite genetic material through polymerase chain reaction (PCR) techniques is becoming a more frequently used tool in the diagnosis of malaria, as well as the diagnosis and surveillance of drug resistance in malaria. Specific primers have been developed for each of the four species of human malaria. One important use of this new technology is in detecting mixed infection or differentiating between infecting species when microscopic examination is inconclusive. In addition, improved PCR techniques could prove useful for conducting molecular epidemiological investigations of malaria clusters and epidemic. Serology for detecting antimalarial antibodies in serum specimens. Specific serological marker have been identified for each of four species of human malaria. A positive test indicates past infection.

Treatment 1. Quinine and related compounds. 2. Antifolate combination drugs. 3. Antibiotics. 4. Artemisinin compound. Causes of resistance- antimalarial drug resistance has been defined as the ability of a parasite strain to survive and or to multiply despite the administration and absorption of a drug given in doses equal to higher than those usually recommended but within tolerance of the subject. This definition was later modified specify that the drug in question must gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action. Most researchers interpret this as referring only to persistence of parasites after treatment doses of antimalarial rather than prophylaxis failure, although the later is useful tool for early warning of the presence of drug resistance. In general resistance appear to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance while for others drugs, multiple mutations appear to be required. Provided the mutation are not deleterious to the survival or reproduction of the parasites while resistant parasites survive. Single malaria isolates have have been found to be made up of heterogeneous population of parasites that can have widely varying drug response characteristics, from highly resistance to complete sensitive.

1. Detection of drug resistance- in general four basic methods have been routinely used to study or measure drug resistance : in vitro, in vivo, animal model studies and molecular characterization. Additionally less rigorous methods have been used such as case report, case series or passive surveillance.

An in vivo test consists of the treatment of a group of symptomatic and parasitaemic individuals with known doses of drug and the subsequent monitoring of the parasitological and/or clinical response over time. One of the key characteristics of in vivo tests is the interplay between host and parasite. Diminished therapeutic efficacy of a drug can be masked by immune clearance of parasites among patients with a high degree of acquired immunity. Of the available tests, in vivo tests most closely reflect actual clinical or epidemiological situations (i.e. the therapeutic response of currently circulating parasites infecting the actual population in which the drug will be used). Because of the influence of external factors (host immunity, variations of drug absorption and metabolism, and potential misclassification of reinfections as recrudescences), the results of in vivo tests do not necessarily reflect the true level of pure antimalarial drug resistance. However, this test offers the best information on the efficacy of antimalarial treatment under close to actual operational conditions—what can be expected to occur among clinic patients if provider and patient compliance is high. The original methods for in vivo tests required prolonged periods of follow-up (minimum of 28 days) and seclusion of patients in screened rooms to prevent the possibility of reinfection. These methods have since been modified extensively and the most widely used methods now involve shorter periods of follow-up (7 to 14 days) without seclusion under the assumption that reappearance of parasites within 14 days of treatment is more likely due to recrudescence than reinfection. Additional modifications reflect the increased emphasis on clinical response in addition to parasitological response. Traditionally, response to treatment was categorized purely on parasitological grounds as Sensitive, RI, RII, and III. Later modifications have combined, to varying extent, parasitological and clinical indicators. Because anaemia can be a major component of malaria illness, in vivo methodologies allow investigation of haematological recovery after malaria therapy. This is obviously not possible with in vitro or molecular techniques. Failure of complete parasitological clearance, even in situations where recurrence of fever is rare, can be associated with lack of optimal haematological recovery among anaemic patients. Unfortunately, these methodologies, while termed “standardized” are, in practice, not standardized. Major differences in sample size, enrolment criteria, exclusion criteria, length and intensity of follow-up, loss-to-follow-up rates, and interpretation and reporting of results are apparent in published papers on in vivo trials. These differences are at times so dramatic, that it is difficult, if not impossible, to compare results from one study to another with any level of confidence (CDC, unpublished data, 1999). The methodology currently being used and promoted, especially in sub-Saharan Africa, is a system that emphasizes clinical response over parasitological response. Close adherence to this protocol does provide comparable data; however, these data are not readily comparable to data collected using other in vivo methods. Although not called for in the protocol, categorization of the parasitological response using the standard WHO definitions would allow some ability to compare to historical levels and provide useful parasitological results that would aid in interpreting the clinical results. From the point of view of a researcher interested in pure drug resistance, in vitro tests avoid many of the confounding factors which influence in vivo tests by removing parasites from the host and placing them into a controlled experimental environment. In the most frequently used procedure, the micro-technique, parasites obtained from a finger-prick blood sample are exposed in microtitre plates to precisely known quantities of drug and observed for inhibition of maturation into schizonts. This test more accurately reflects “pure” antimalarial drug resistance. Multiple tests can be performed on isolates, several drugs can be assessed simultaneously, and experimental drugs can be tested. However, the test has certain significant disadvantages.
The correlation of *in vitro* response with clinical response in patients is neither clear nor consistent, and the correlation appears to depend on the level of acquired immunity within the population being tested. Prodrugs, such as proguanil, which require host conversion into active metabolites cannot be tested. Neither can drugs that require some level of synergism with the host’s immune system. Although adaptation of erythrocytic forms of *P. vivax* to continuous culture has been achieved, non-falciparum erythrocytic parasites generally cannot be evaluated *in vitro*. In addition, because parasites must be cultured differential die-off of parasites can occur. If, for instance, resistant strains are less likely to adapt, the results would be biased towards sensitive responses. Because venous blood is typically needed, resistance in the more vulnerable younger age groups is often not studied. Finally, these tests are technologically more demanding and relatively expensive, which makes them potentially more difficult to adapt successfully to routine work in the field.

Animal model studies- This type of test is, in essence, an *in vivo* test conducted in a non-human animal model and, therefore, is influenced by many of the same extrinsic factors as *in vivo* tests. The influence of host immunity is minimized by using lab-reared animals or animal-parasite combinations unlikely to occur in nature, although other host factors would still be present. These tests allow for the testing of parasites which cannot be adapted to *in vitro* environments (provided a suitable animal host is available) and the testing of experimental drugs not yet approved for use in humans. A significant disadvantage is that only parasites that can grow in, or are adaptable to, non-human primates can be investigated.

Molecular techniques—These tests are in the process of being developed and validated, but offer promising advantages to the methods described above. Molecular tests use polymerase chain reaction (PCR) to indicate the presence of mutations encoding biological resistance to antimalarial drugs. Theoretically, the frequency of occurrence of specific gene mutations within a sample of parasites obtained from patients from a given area could provide an indication of the frequency of drug resistance in that area analogous to information derived from *in vitro* methods. Advantages include the need for only small amounts of genetic material as opposed to live parasites, independence from host and environmental factors, and the ability to conduct large numbers of tests in a relatively short period of time. Disadvantages include the obvious need for sophisticated equipment and training, and the fact that gene mutations that confer antimalarial drug resistance are currently known or debated for only a limited number of drugs (primarily for dihydrofolate reductase inhibitors [pyrimethamine], dihydropteroate synthase inhibitors [sulfadoxine], and chloroquine). Confirmation of the association between given mutations and actual drug resistance is difficult, especially when resistance involves more than one gene locus and multiple mutations. If these complexities can be resolved, molecular techniques may become an extremely valuable surveillance tool for monitoring the occurrence, spread, or intensification of drug resistance.

### Aim
Correct interpretation of resistance pattern by *in vivo* tests and epidemiological characteristic of cases of malarial fever in Jharkhand

### Objectives
1. To study the resistance pattern of malarial cases to artemesunate by *in vivo* tests
2. To study the epidemiological characteristic of malarial fever in Jharkhand

### Methodology
- **Type of study:** It is prospective type of study.
- **Source of data:** Patients of malaria admitted in Department of Medicine of Rajendra institute of medical sciences, Ranchi.

#### Methods of collection of data
Study subjects: The present study will be conducted on hospitalised patients at RIMS whose fever suggestive of malaria.

Duration of study: September 2016 to October 2017.

#### Informed consent will be obtained from all the patients.

**Inclusion criteria:**
1. Patients older than 16 year of age
2. Patients whose illness suggestive of malaria.
3. Patients whose stained peripheral-blood smear demonstrates asexual forms of the *Plasmodium*.
4. About to receive antimalarial drug.

**Exclusion criteria:**
1. Patients less than 16 years of age
2. Recent surgery or Trauma
3. Outdoor patients
4. Patients allergic to antimalariais
5. Patients already taken antimalarial in recent past
6. Concurrent other serious nonmalarial infection
7. Patients undergo death or LAMA (leave against medical advice) during the study.

3. Blood Microscopy
The capillary blood samples drawn from finger/heel pricks of the subjects would used to perform thick smear microscopy. Preparation of these blood samples (three thick blood smear slides per participant) for microscopy would be in accordance with WHO standard microscopy technique, and read with ×1, 000 magnification (with oil immersion lens). Two trained and experienced microscopists at the laboratory would do the slides independently. Microscopy would be considered positive only when asexual parasite forms – trophozoites and schizonts (not gametocytes alone) – were detected, since asexual forms are indicative of active infection. Parasite densities would be determined by counting the number of parasite seen per 200 white blood cells and the parasite density per microlitre would be calculated based on putative mean count of 8000 leucocyte per microlitre.
No. Parasites × (8000 ÷ No. WBCs counted) = No. parasites per µL of blood

Thin smears: The percent of infected RBCs is determined by enumerating the number of infected RBCs in relation to the number of uninfected RBCs. A minimum of 500 RBCs total should be counted. (No. infected RBCs ÷ Total No. RBCs counted) × 100 = Percent Infected RBCs

In this study, since most of the patients are severe anaemic, so a constant number of RBA cannot be taken to calculate parasite count of the patients. Therefore, in this study, for estimation of parasite count, we have used the formulae:

No. of parasite/microL = no. of infected RBC x patients RBC count/Total No. of RBC counted

A blood film assumed negative when the examination of 100 thick film fields did not show the presence of asexual forms of P. falciparum. The same technique would employed for establishing the parasite density on each of the subsequent blood film examinations.

Antimalarial chemotherapy
Patient whose smear for malarial parasite becomes negative would be excluded from the study.
Patient having smear positive for malarial parasite would be given standard antimalarial treatment (artesunate 2.4mg/kg stat iv followed by 2.4mg/kg at 12 and 24 h and then daily for 3 days

Follow up
The follow up blood smear examination of malarial smear positive patient taken antimalarial drug in day 14 and day 28.

Sex distribution of cases of malarial fever

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>percent</th>
</tr>
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<tbody>
<tr>
<td>Female</td>
<td>29</td>
<td>40.3</td>
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<tr>
<td>Male</td>
<td>43</td>
<td>59.7</td>
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</table>

Male were 59.7% and female were 40.3% in total cases of malarial fever in Jharkhand.

Age Distribution of cases of malarial fever in Jharkhand

<table>
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<tr>
<th>Age in years</th>
<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>&lt;20</td>
<td>11</td>
<td>15.3</td>
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<tr>
<td>20-40</td>
<td>31</td>
<td>43</td>
</tr>
<tr>
<td>40-60</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>&gt;60</td>
<td>06</td>
<td>08.3</td>
</tr>
</tbody>
</table>

Under age 20 years were 15.3%, between age 20-40 years were 43%, between 40-60 years were 33% and above 60 years were 08.3% in total cases of malarial fever in Jharkhand.

Malarial Parasite/ microlit in day 1 before artesunate

<table>
<thead>
<tr>
<th>Parasete count / microlit</th>
<th>Frequency</th>
<th>percent</th>
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<tbody>
<tr>
<td>&lt;100000</td>
<td>25</td>
<td>34.7</td>
</tr>
<tr>
<td>100000-200000</td>
<td>25</td>
<td>34.7</td>
</tr>
<tr>
<td>&gt;200000</td>
<td>22</td>
<td>30.6</td>
</tr>
</tbody>
</table>

Malarial parasite count / microlit on day 1 before artesunate were
Less than 1 lakhs – 34.7%
1 lakhs to 2 lakhs – 34.7%
More than 2 lakhs – 30.6%

Malarial Parasite Count/ Microlit on Day 14 and Day 28 after Artesunate Was Zero

<table>
<thead>
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<th>Rural</th>
<th>Frequency</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Rural</td>
<td>43</td>
<td>59.7</td>
</tr>
<tr>
<td>Urban</td>
<td>29</td>
<td>40.2</td>
</tr>
</tbody>
</table>

Most of malarial patients were from rural 59.7% and urban patients were 40.2% in total cases of malarial fever in Jharkhand

Blood urea abnormality in cases of malarial fever

<table>
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<tr>
<th>Normal</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30</td>
<td>41.6</td>
</tr>
<tr>
<td>Mild</td>
<td>45 - 80</td>
<td>40.8</td>
</tr>
<tr>
<td>Moderate</td>
<td>80 - 120</td>
<td>4.2</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt;120</td>
<td>14.1</td>
</tr>
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Blood Urea abnormality were normal in 41.6%, mild elevated in 40.8%, moderate elevated in 4.2% and severe elevated in 14.1% in total cases of malarial fever in Jharkhand.

Serum creatinine of cases of malarial fever

<table>
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<th>Normal</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;1.2</td>
<td>33.3</td>
</tr>
<tr>
<td>Mild</td>
<td>1.2 - 3</td>
<td>36.1</td>
</tr>
<tr>
<td>Moderate</td>
<td>3 - 6</td>
<td>27.8</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt;6</td>
<td>02.7</td>
</tr>
</tbody>
</table>

Serum creatinine were normal in 33.3 %, mild elevated in 36.1%, moderate elevated in 27.8% and severe elevated in 2.7% in total cases of malarial fever in Jharkhand.

Haemoglobin level in cases of malarial fever

<table>
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<th>Normal</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&gt;12</td>
<td>20.8</td>
</tr>
<tr>
<td>Mild</td>
<td>10 - 12</td>
<td>23.6</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 - 10</td>
<td>47.2</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;7</td>
<td>08.3</td>
</tr>
</tbody>
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Haemoglobin were normal in 20.8%, mild anaemia in 23.6%, moderate anaemia in 47.2% and severe anaemia in 08.3% in total cases of malarial fever in Jharkhand

4. Discussion

A total of 72 patients of P. Falciparum positive cases were selected from the patients admitted in the department of medicine RIMS, Ranchi. They were evaluated for their demography, clinical features, blood parameters and malarial parasite count at the time of admission to find out malarial parasite load and its value in assessing the affect of artesunate on malarial parasite.

Regarding age distribution, maximum numbers of falciparum malaria cases were seen in age group of 21-40 years i.e.43.1% in the young adults. This group represents the most active group of the society. Young people of this age group frequently engage in outdoor activities like

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working in agriculture farms, close to areas having stagnant water like ditches, pond etc. which are breeding places of mosquitoes. This increases the chance of mosquito bite. Regarding sex distribution in different age groups, below 40 years of age, the incidence of falciparum malaria was almost equal in both sexes. In the age group 41 and above, the disease was found more common in males. Overall the incidence in males (59.7%) are higher than females(40.2%).

According to Pasrol, G. et al (1976)53, malaria is more common in males because they are more frequently exposed to acquiring malaria due to their outdoor activities and female are better clothed.

Bruneel et al (2003)32 found mean age of malaria was 38 years and there was predominance of males. The present study also collaborates with above study.

Regarding rural and urban distribution of falciparum cases under study, majority of the patients suffering from falciparum malaria were from rural areas of Jharkhand (59.7) Maximum numbers of cases were from certain pockets namely silli, bundu, sonahatu, tamar sikiyari and tatisilwai. This is due to differences in control measures and health care services in rural and urban population.

According to WHO (1979 Tech Rep. Ser. No. 640)54 malaria is more common in ill ventilated and ill lighted house, found in mainly rural areas.

Regarding clinical features of P Falciparum, almost all patients are presented with fever with chills and headache in emergency, having positive kit test (optimal test) for malaria.

Anaemia is an inevitable consequence of severe malaria. On admission, 79.2% of patients had reduced haemoglobin level Approximately 08.3% of patients were severely anaemic (Hb<7 g/dl) and 23.6% mild anaemia (Hb 10 -12g/dl), 47.2% moderate anaemia (Hb 10 – 7g/dl).

In a study in Thailand (by Phillips et al.1986a)33, haematocrits fell below 20% in approximately 30% of adult patients. Our study have much higher percentage of moderate anaemic patients. Several large trials (Hien et al.1996; Dondorp et al.2005b)34, 35 have therefore set the anaemia component of the severe malaria definition more strictly as a haematocrit<20% together with a parasite count>100000/microL.

Jaundice is common in adult patients with malaria. Hyperbilirubinaemia is predominantly unconjugated (a combination of haemolysis and hepatic dysfunction). Tendon enlargement of the liver and spleen is, however, a common finding in all human malarials, especially in young children and non-immune adults.

Regarding assessment of liver function, majority of the patients (40.3%) suffering from falciparum malaria admitted had jaundice (having bilirubin level greater than 3 mg per dl).

In a study in Thailand (by Wilairatana et al.1994)36, of 390 patients hospitalised with acute falciparum malaria, one-third (124) were clinically jaundiced (total serum bilirubin>3 mg/dl ). Our study have much higher percentage(40.3%) of jaundiced patients.

Acute kidny injury is a common manifestation of severe falciparum malaria and in adults is often lethal. Plasma creatinine has the best prognostic value in terms of the subsequent need for renal replacement therapy and outcome (Hanson et al.2011a, 2013)37, 38. In our study, majority of the patients have elevated blood urea(58.4%) and serum creatinine(66.4%). Out of which 30.5% of patients have elevations in serum creatinine (value >3 mg per dl). Blood urea is elevated in more number of patients as compared to serum creatinine.

Regarding parasite count, in our study, the number of cases was divided into 3 groups based on malarial parasite count(<100000, 100000–200000 and >200000 parasites per microL). About 65.3% of the patients had parasite count above one lakh per microL, out of which 30.6% of patients had parasite count greater than 2 lakh per microL.

5. Summary

The present study “resistance pattern and epidemiological characteristics of cases of malarial fever in jharkhand” was carried out in 72 patients of department of medicine, RIMS, Ranchi. The salient finding of this study are :

1) Maximum numbers of falciparum malaria cases were seen in age group of 21–40 years.
2) Below 40 years of age, the incidence of falciparum malaria was almost equal in both sexes. In the age group 41 and above, the disease was found more common in males. Overall the incidence in males (59.7%) are higher than females(40.2%).
3) Majority of the patients suffering were from rural areas of Jharkhand (59.7%). Maximum numbers of cases were from certain pockets namely silli, bundu, sonahatu, tamar sikiyari and tatisilwai.
4) Almost all patients are presented with fever with chills and headache in emergency, having positive kit test (optimal test) for malaria.
5) 79.2% of patients had reduced haemoglobin level. Of which mild anaemia 23.6%, moderate anaemia is 47.2% and severe anaemia are 08.3%
6) 66.7% of the patients had jaundice
7) Majority of the patients have elevated blood urea(58.4%) and serum creatinine(66.4%). Out of which 30.5%of patients have
elevations in serum creatinine (value >3 mg per dL). Blood urea is elevated in more number of patients as
compared to serum creatinine.
9) About 65.3% of the patients had parasite count above one lakh per microL, out of which 30.6% of patients
had parasite count greater than 2 lakh per microL.
10) Parasite count /microlit after artesunate on day 14 and
day 28 are zero.

6. Conclusion

1) Parasite count after treatment with artesunate on day 14
and day 28 was zero. So there is no resistance to
artesunate for malarial parasite.
2) Artesunate is an effective drug for malarial parasite in
Jharkhand.

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