MALARIA INFECTION: A CLINICOPATHOLOGICAL CORRELATION

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ABSTRACT:
Objectives:
The hematological changes associated with malaria are well recognized. This study was conducted to assess and compare the incidence and severity of hematological changes in various types of malaria in local population of Gadap city who visited the Fatima Diagnostic Laboratory of Fatima Hospital, Baqai Medical University between Jan. 2007 to Dec. 2008.

Methodology:
This observational study included 3,263 cases of fever who were suspected for malaria visited Fatima Hospital Laboratory of, Baqai Medical University during January 2007 to December 2008. The age, sex, place of referral and species of malarial parasites were recorded. The total number of male patients was 211 and female was 168 with a mean age group of 21 years. The diagnosis of malaria was confirmed by thick and thin film stained with Leishman’s staining for malarial parasite. Slides were reviewed by hematologist and Full blood counts were performed using automated Sysmex K-1000.

Result:
A total of 3,263 patients were subjected for malaria testing during the study period. Three hundred and seventy nine had a positive peripheral smear. There were two hundred and eleven (55.6%) male and one hundred and sixty eight (44.3%) female patients. One hundred and fifty eight (42%) were P falciparum positive, two hundred and three (53%) were P vivax positive and eighteen (5%) patients had mixed infection of both P. falciparum and P. vivax. Two hundred and eighteen (57.5%) patients had thrombocytopenia. Out of this, One hundred and ten (50.4%) patients with thrombocytopenia were P. falciparum positive and one hundred and eight (49.6%) patients with thrombocytopenia were P. vivax positive. One hundred and fifty seven (41%) patients had anemia. Out of this, Thirty seven (23.6%) patients with anemia were P. falciparum positive and one hundred and twenty (76.4%) patients with anemia were P. vivax positive. Sixty one (16%) patients had leukopenia. Out of this, Thirty three (55%) patients with leukopenia were P. falciparum positive and 27 (45%) with leukopenia were P. vivax positive.

Conclusions:
Malaria was found to be one of the most prevalent infections in the surveyed population and the frequency of vivax species was more as compared to falciparum malaria. The majority of falciparum malaria patients develop significant haematological complications with high frequency of thrombocytopenia and anemia as compared to vivax infection while changes in the white blood cells was less dramatic.

Keywords:
Malaria, Plasmodium vivax, Plasmodium falciparum, Thrombocytopenia, Leukopenia, Anemia
INTRODUCTION

Malaria is known to human beings since centuries. It is a disease of tropical and subtropical countries particularly Africa and Asia. Despite advances in knowledge, malaria continues to cause significant morbidity and mortality worldwide\(^1\). Malaria is one of the most prevalent human infections in the world. Over 40% of the world population lives in malaria-endemic area and it is estimated that 300-500 million cases and 1.5-2.7 million deaths occur each year\(^2\). Mortality rate is usually high (20%) in severe malaria (parasitemia >5%)\(^3\). Over 40% of world population lives in malaria endemic area including Southeast Asia, India, Pakistan, Bangladesh, Africa, areas of middle east, Central and South America\(^4\). Pakistan being a part of endemic belt has an incidence of one case per thousand populations. Severe malaria has been a major cause of mortality worldwide and Plasmodium falciparum is the main species for most of these deaths\(^5\). Although relatively uncommon in the developed countries, malaria remains one of the most prevalent infections in the developing and under developed world. It is a significant cause of morbidity and mortality in addition to creating an enormous social and economic burden. Today, the most important problem in the management of malaria is drug resistance of Plasmodium falciparum to the various anti-malarial drugs and occurrence of systemic complication\(^6\).

Malaria ranks among the major health problems in Pakistan. Endemic in ninety-one countries which consist of forty percent of the world population, malaria affects an estimated 300 million people per year worldwide causing more than a million deaths per year majority being children under five years of age\(^7,8\). Pregnant women and non-immune people are at particular risk. Problems have further been compounded by the emergence of drug resistance in anopheline mosquito which is the causative vector. Pakistan is a sub-tropical country having a vast system of irrigation and a lot of stagnant water after heavy rainfall in monsoon at Upper Punjab providing an ideal environment for mosquito breeding. Transmission of malaria remains throughout the year but becomes more intense after the rains in the months from July to November. It might become unstable to form an epidemic after every few years\(^9,10\).

Plasmodium falciparum infection is more dangerous and responsible for most of the deaths, which occur due to malaria. P. falciparum is common in Africa, Haiti, New Guinea, South America and Eastern Asia. In Pakistan Southern Punjab, Balochistan and Sindh are the areas where falciparum malaria is more common\(^11\). Malaria infection is the state in which a human host harbours actively multiplying malarial parasites. Malaria disease refers to the subset of malarial infections which have led to pathological disturbances and are manifested through specific symptoms and clinical signs. The disease population comprises the actual sick patients who need and often seek medical help, while the infected yet non-diseased population act as an asymptomatic reservoir feeding the transmission process and generating new infections as well as new patients with malaria disease\(^12\). Thus, the amount of malaria disease in a community is a measure of the actual impact of malaria on the health of the community, whereas the amount of malarial infection will reflect the level and stability of malarial transmission in the community. The finding of a high prevalence of infection associated with a low prevalence of disease is characteristic of epidemiologically stable malaria and shows that malaria transmission is more endemic than epidemic\(^13\).

Most of the systemic complications from malaria results from hyperparasitemia and hematologic changes are the most common complications encountered in malaria and play a major role in the fatality\(^14,15\).

Prediction of the hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications\(^16\). The aim of this study was to evaluate the hematological changes that may occur in different type of malaria in local population of Karachi city who visited Fatima Laboratory of Baqai Medical University between 2006 to 2007. A study was designed for including basic demographic data i.e. variables as name, age, sex, residence, education and occupation and hematological data i.e. recorded information about Hemoglobin level, complete blood counts (CBC), plasmodium species and the parasite level in some cases. CBC was performed using an automated
Coulter counter Symex KX model. All malaria-positive smears were reviewed by a hematologist for confirmation, identification of species, review of smear for platelet count, and in some cases, estimation of parasite level.

PATIENTS AND METHODS

This study was conducted from January 2006 to December 2007, at the Fatima Diagnostic Laboratory of Fatima Hospital, Baqai Medical University, Gadap Town, Karachi. The Population of Karachi is about 16 million and is a cosmopolitan city. Study population comprises of middle and lower middle class people. A total of 3,263 subjects were screened for the evidences of malaria presenting to the medical outdoor with a history suggestive of malaria. The symptomatology suggestive of malaria included a history of fever, rigors and chills, vomiting, headache, generalized body aches and diarrhoea. Patients having at least one of the six symptoms were included in the study. The Clinical diagnostic Criteria used to declare a patient suffering from severe and complicated malaria was based on the WHO Working Group Definition 18.

The study was randomized by enrolling every fifth patient with symptomatology of malaria. The age range was 1-70 years. After a detailed history and clinical examination, thick and thin peripheral blood smears who made to reach the diagnosis. All the patients with negative thin and thick peripheral blood smears were excluded from the study. The thick smear was stained by smear by Leishman’s stain. The slide was then studied under oil immersion lens (x100) of the microscope. The parasites were quantitated by independently counting asexual and sexual stages of both P. falciparum and P. vivax parasites against 300 white blood cells (WBCs) on the smear. The hematological parameters like hemoglobin, RBC count, total leukocyte count and platelet count were done on Sysmex KX-1000 coulter machine 19.

RESULTS

A total of 3,263 patients were screened during 2006 and 2007, of which there were two hundred and eleven (55.6%) male and one hundred and sixty eight (44.3%) female. Out of the total smears, the positive smears were three hundred seventy nine (11.6%). The most common type of malaria was P. vivax [two hundred and three (53%)] followed by P. falciparum [One hundred and fifty eight (42%)] and eighteen (5%) patients had mixed infection of P. falciparum and P. vivax as shown in table 1. Two hundred and eighteen (57.5%) patients had thrombocytopenia. Out of this, One hundred and ten (50.4%) patients with thrombocytopenia were P. falciparum positive and one hundred and eight (49.6%) patients with thrombocytopenia were P. vivax positive. One hundred and fifty seven (41%) patients had anemia. Out of this, Thirty seven (23.6%) patients with anemia were P. falciparum positive and one hundred and twenty (76.4%) patients with anemia were P. vivax positive. Sixty (16%) patients had leukopenia. Out of this, Thirty three (55%) patients with leukopenia were P. falciparum positive and 27 (45%) with leukopenia were P. vivax positive (table 2a and 2b). The mean platelet count was 94.7x10^3/ul in case of P. falciparum and 103. 7x 1 oJ lulu in case of P. vivax. The mean value of hemoglobin range was 11.0g/dl and 9.52g/dl in case of P. vivax. The mean value of total leukocyte count (TLC) was 8.6x1000/ul in case of P. falciparum and 5.88x1000/ul in case of P. vivax (table 3). When hematological values were compared with malaria species, there was no significant difference in the incidence of leukopenia in P. falciparum and P. viva but the cases of thrombocytopenia and anemia were more prominent in P. falciparum infection as compared to P. vivax infection. (table 3).
Table 1: SPECIES OF PARASITES FOUND IN 379 PATIENTS

<table>
<thead>
<tr>
<th>Species of Plasmodium</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vivax</td>
<td>203</td>
<td>53 %</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>158</td>
<td>42 %</td>
</tr>
<tr>
<td>Mixed infections</td>
<td>18</td>
<td>5 %</td>
</tr>
</tbody>
</table>

Table 2a: HAEMATOLOGICAL COMPLICATIONS IN PATIENTS WITH P. FALCIPARUM MALARIA

<table>
<thead>
<tr>
<th>Haematological Changes</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia</td>
<td>33</td>
<td>55 %</td>
</tr>
<tr>
<td>Anaemia</td>
<td>120</td>
<td>76.4 %</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>138</td>
<td>63.3 %</td>
</tr>
</tbody>
</table>

Table 2b: HAEMATOLOGICAL COMPLICATIONS IN PATIENTS WITH P. VIVAX MALARIA

<table>
<thead>
<tr>
<th>Haematological Changes</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukopenia</td>
<td>27</td>
<td>45 %</td>
</tr>
<tr>
<td>Anaemia</td>
<td>37</td>
<td>23.6 %</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>80</td>
<td>36.6 %</td>
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</table>

Table 3: COMPARATIVE RESULTS OF CASES WITH P. FALCIPARUM AND P. VIVAX INFECTION

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SPECIES OF PLASMODIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. falciparum</td>
</tr>
<tr>
<td>Total no. of cases</td>
<td>158</td>
</tr>
<tr>
<td>No. of cases in paeds. (0-15 yrs)</td>
<td>71 (41%)</td>
</tr>
<tr>
<td>No. of cases in adults (More than 15 yrs)</td>
<td>103 (59.19%)</td>
</tr>
<tr>
<td>Mean Hb</td>
<td>11.085 g/dl</td>
</tr>
<tr>
<td>Mean TLC</td>
<td>8.620 x 1000 ul</td>
</tr>
<tr>
<td>Mean platelet</td>
<td>94.705 x 1000 ul</td>
</tr>
</tbody>
</table>
DISCUSSION
This present study was conducted to assess and compare the incidence and severity of hematological changes associated with malaria due to P. falciparum and P. vivax infection. In our study, the most common type of malaria was P. vivax [two hundred and three (53%)] followed by P. falciparum [One hundred and fifty eight (42%)] and eighteen (5%) patients had mixed infection of P. falciparum and P. vivax. It has been reported previously that P. vivax is found to be the most common cause of malaria in Asia and Central and South America. Besides this, predominance of male patients was observed than female patients. Out of 379 cases, 211 were male and 168 were female. This shows significant predominance of male over female and this may be because of the more exposure of male to the bite of mosquito infected with malarial parasite.

Hematological changes associated with malaria infection are well recognized, but specific changes may vary with level of malaria, background hemoglobinopathy, nutritional status, demographic factors and malaria immunity. In this study, several significant changes involving hemoglobin (anemia), platelets count (thrombocytopenia) and white cells count (Leukopenia) were associated with different types of malaria. Hematological abnormalities are considered a hallmark of malaria, and reported to be most pronounced in P. falciparum infection, probably as a result of the higher levels of parasitemia found in these patients. In our study, Thrombocytopenia was observed in more than half of the patients, was a characteristic finding. Fifty Seven percent of patients with malaria developed thrombocytopenia and our results are in close agreement with our studies done by Bashwari and co-workers. More over there was no significant difference in the incidence of thrombocytopenia between P. falciparum (87%) and P. vivax (81%) but severe thrombocytopenia was more common in P. falciparum (63.3%) than P. vivax (33.6%) and this percentage is higher than as reported by other investigators.

Thrombocytopenia is a classical feature of malaria, and a low platelet count is usually seen in about 85% of patients with uncomplicated malaria and all patients with severe falciparum malaria. Although variable degree of reduction in circulating platelet count are consistently reported in the different types of malaria, severe thrombocytopenia is quite rare in P. vivax malaria. Similar observations were made by other workers as well.

In a study over two thousand patients with fever, Erhart et al reported platelet count of less than 150,000 increases the likelihood of malaria by 12-15 times. Various other studies have also found thrombocytopenia to be commonly associated with malaria, which resolves after therapy. The suggested mechanisms for thrombocytopenia include disseminated intravascular coagulation or excessive removal of platelets by reticuloendothelial system. Anti-Platelet IgG has also been implicated in the pathogenesis of thrombocytopenia. Thrombocytopenic malaria, in contrast to the non-thrombocytopenic variety correlates with a higher degree of parasitemia and increased cytokine production.

It was recently confirmed that increased production of tumour necrosis factor alpha (TNFa) was responsible for the changes in brain and haematological parameters of patients with P. falciparum malaria. TNFa causes rosetting and cytoadherence of infected red blood cells in brain capillaries. Moreover, it causes upregulation of receptors in the brain capillaries for malarial parasites. The pathogenesis of above findings is that P. falciparum produces a malarial toxin which is chemically confirmed to be glycosyl phosphatidyl inositol (GPI). GPI stimulates the production of high levels of TNFa and interleukin I by macrophages. These mediators are responsible for the production of changes in brain capillaries and morphological changes in red blood cells and platelets.

In this study, anemia was largely associated with P. falciparum infection (76.4%) as compared to anemia with P. vivax infection (23.6%). The present study demonstrates that low hemoglobin and low platelet count are the two hematological variables that increase the probability of malaria in case of P. falciparum infection. The pathogenesis of anemia in malaria is extremely complex, multi factorial and incompletely understood. It is thought to result from a combination of hemolysis of parasitized red blood
cells; accelerated removal of both parasitized an innocently unparasitized red blood cells, depressed as well as ineffective erythropoiesis with dyserythropoietic changes and anemia of chronic disease. Other factors contributing to anemia in malaria include decreased red blood cell deformability, splenic phagocytosis and/or pooling so they have an increased rate of clearance from the circulation. Tumour necrosis factor alpha (TNF-α) has been implicated, and may cause ineffective erythropoiesis. Other studies have also discussed the role of TNF-a in falciparum malaria. In another study done by Das and co-workers, the investigators' observations indicated that hemolysis is the prime cause of anemia seen in acute falciparum malaria, but destruction of parasitized erythrocytes is not the only cause of the hemolytic process, and bone marrow suppression appears to have an insignificant role. It should be born in mind also that red cell morphology in malaria patients may be influenced by their nutritional status i.e., patients could be iron deficient, folic acid or vitamin B12 deficient or they may have a concurrent thalassemia, which aggravates the severity of the anemia. P. falciparum malaria is one of the most common causes of anemia.

Tumour necrosis factor alpha (TNF-α) has also been implicated and may cause ineffective erythropoiesis. Several ultra structural changes have been reported in nonparasitized erythroblasts and erythrocytes in the blood of patients who are anaemic and suffer from acute falciparum malaria. These changes are suggested to be due to dyserythropoiesis and ineffective erythropoiesis. Numerous haemoglobin-like particles are liberated from erythrocyte plasma membrane indicating severe haemolysis which is considered to be one of the major factors producing anaemia during malarial infection.

The other plasmodia species rarely cause anemia because only selected red cell populations (reticulocytes in the case of P. vivax and P. ovale and older cells in P. malariae) are invaded. So the causes of anemia are multifunctioned, the most important being hemolysis as a result of direct invasion, where most of the erythrocytes hemoglobin is utilized. One should not forget the contribution of anemia of chronic disease, characterized by failure to utilize iron by the bone marrow related principally to increased levels of cytokines (TNF-α and interleukin 6). It has also been reported that anemia correlates with the severity of the infection.

In our study, the minority of patients showed leukopenia (16%) while majority of patients (84%) had a normal total WBC count, unlike some studies which showed that leukopenia appears to be a common finding in both non-immune patients with falciparum malaria. Their numbers may be reduced (leucopenia) by starvation, pernicious anemia, and certain infections, such as typhoid and malaria. WBC counts during malaria are generally characterized as being low to normal, a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation and to the spleen and other marginal pools, rather than actual depletion or stasis.

We concluded that P. falciparum as well as P. vivax can cause significant hematological changes with high frequency of thrombocytopenia, anemia and changes in the white blood cell are less dramatic and there has been conflicting reports regarding these changes. The hematological aspects of malaria infection constitute a very interesting area in various reports. The blood changes are so characteristic that the diagnosis of malaria should always be considered in the presence of above findings.
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