VARIATION OF HAEMATOLOGICAL INDICES IN VARIOUS TYPES OF MALARIA
Samith Ahmed¹, Farhana Zakaria², Aravind P³

HOW TO CITE THIS ARTICLE:

ABSTRACT: BACKGROUND: Malaria is an endemic disease in coastal Karnataka especially in Mangalore. Malaria has signs and symptoms similar to other infectious diseases making it difficult to diagnose. Peripheral smear examination is a gold standard in detection of Malaria and hematological variations are considered a good index to detect complications arising from Malaria. Previously done studies globally have also revealed various hematological variations in Malaria parasite species. The present study intends to establish a relationship between the Malaria positive cases and its various hematological parameters. OBJECTIVES: The objectives of the study are: To record the Hb, PCV and morphology of RBCs in smears detected positive with Malaria. To make a Total count and Differential count of WBCs in the peripheral smears of Malaria patients. To establish a relationship of thrombocytopenia with Malaria. MATERIALS AND METHODS: 200 cases were undertaken in this prospective study held for a period of 1 year between September 2011 and August 2012. Patients who were detected positive for Malaria underwent extensive hematological parameters assessment. The peripheral smears of all Malarial positive cases were stained by Leishman’s stain and further a complete blood count including all hematological indices were recorded using automated Beckman coulter. The data obtained was extensively studied and statistical analysis was done by chi-square test, unpaired student t test and Mannwhaithey U test. RESULTS: P. vivax was the dominant species diagnosed (87.5%) in our study and the predominant clinical feature was fever with chills. Anemia was observed in 12.6% of P.vivax Malaria and 20% in P. falciparum cases. Leucopenia was observed in 29% (58) cases. The incidence of leucopenia was 29% in P. vivax and 32% in P. falciparum Malaria. Monocytosis was observed in 23% (46) cases of which P. vivax had 23% and P. falciparum had 20% cases. There was a significant co-relation between thrombocytopenia and Malaria. All (25) cases of P. falciparum were thrombocytopenic while 93% (163) of P. vivax had thrombocytopenia. CONCLUSION: The study establishes that Malaria is a true hematological disease since it affects almost all hematological parameters and this is significantly higher in P. falciparum when compared to P. vivax. KEYWORDS: Keywords: Malaria, Thrombocytopenia, Leukopenia, P. falciparum, P. vivax

INTRODUCTION: Malaria is a major cause of morbidity worldwide, with an estimated 250 million cases a year and between 1 and 2 million deaths.¹ There is an intimate relationship between the Malaria parasites and the blood. Gold standard of Malaria diagnosis is demonstration of parasite in the peripheral blood smear. However there seems to be a co-relation between platelet count, the WBC counts, Hb and morphology of RBC in Malaria. 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.
AIMS AND OBJECTIVES:
The present study is done with following aims and objectives:
1. To record the Hb, PCV and morphology of RBCs in smears detected positive with Malaria.
2. To make a Total count and Differential count of WBCs in the peripheral smears of Malaria patients.
3. To establish a relationship of thrombocytopenia with Malaria.

METHODOLOGY:
Source of Data: Cases subjected for Malarial parasite detection tested positive in Central Diagnostic Lab (CDL). 200 cases will be included in this study.

Method of Collection of Data: This is a Prospective study done from September 2011 to August 2012. 4 ml of blood is collected from the patient using sterile aseptic methods in an EDTA vacutainer. The blood drawn is fed into the automated cell counter where the Hb, PCV and Total counts of the WBCs were collected; with the remaining blood peripheral smears are made which are subjected to leishman's staining. The smears are evaluated and the RBC morphology, differential count of WBCs and platelet counts are done. A proforma containing the details of the patient and informed consent of the patient for the present study is maintained.

Inclusion Criteria: All slides of Malarial parasites tested positive after peripheral smear examination.

Exclusion Criteria: Slides not having Malarial parasites.

Statistical Analysis: A qualitative analysis of the data was done to find association between 2 factors. Chi square tests, student unpaired t test and Mannwhaithey U test methodology has been employed for statistical analysis.

Doughnut chart showing species distribution
### Species Numbers

<table>
<thead>
<tr>
<th>Species</th>
<th>Numbers</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em></td>
<td>175</td>
<td>12.81</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>25</td>
<td>12.39</td>
</tr>
</tbody>
</table>

**Mean Hb value**

### Mean Hb Value

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em></td>
<td>50</td>
<td>28.57</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>8</td>
<td>32</td>
</tr>
</tbody>
</table>

**Cases having Leukopenia**

### Cases having Leukopenia

<table>
<thead>
<tr>
<th>Species</th>
<th>Numbers</th>
<th>Mean (WBCs/cumm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em></td>
<td>175</td>
<td>5075</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>25</td>
<td>4900</td>
</tr>
</tbody>
</table>

**Mean Total Count of WBCs**

<table>
<thead>
<tr>
<th>Species</th>
<th>Within normal</th>
<th>Above normal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em></td>
<td>134</td>
<td>41</td>
<td>175</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>154</strong></td>
<td><strong>46</strong></td>
<td><strong>200</strong></td>
</tr>
</tbody>
</table>

**Cases having monocytosis**

<table>
<thead>
<tr>
<th>Thrombocytopenia</th>
<th>Normal</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em></td>
<td>163</td>
<td>93.14</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

**Cases having thrombocytopenia**
### Species Numbers Mean (plts/cu mm)

<table>
<thead>
<tr>
<th>Species</th>
<th>Numbers</th>
<th>Mean (plts/cu mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vivax</td>
<td>175</td>
<td>77233</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>25</td>
<td>70828</td>
</tr>
</tbody>
</table>

Mean value of platelets

**Rare Findings in our Study:** One of the rare findings in our study was phagocytosis of Malarial pigment by a monocyte, only 1 case (0.5%) was reported.

2 cases of Malarial infestations had an Hb level beyond 18 g/dl accounting for 1% of the cases. A PCV of 73.2% was seen in a 49 year old male.

None of the patients had severe sequelae of Malaria like CM, renal failure etc.

The mortality rate in our study was nil

Blood smear of a Malaria positive patient, RBCs show P. vivax trophozoites. Also phagocytosis of Malaria pigment in acute Malaria by monocytes is seen.

**DISCUSSION:** Malaria represents a huge burden for primary health care services accounting for around 30% of total outpatients and inpatients visiting the hospital. Hence this significant expenditure needs to be taken seriously and all aspects of Malaria need to be documented.2 Over the years numerous Malaria eradication programmes have been changed to Malaria control programmes underlying the healthcare problems of this parasitic disease.

<table>
<thead>
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</tr>
</thead>
<tbody>
<tr>
<td>P. vivax</td>
<td>19.2%</td>
<td>36.5%</td>
<td>66%</td>
<td>47.3%</td>
<td>87.5%</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>61.9%</td>
<td>52.7%</td>
<td>16%</td>
<td>50.9%</td>
<td>12.5%</td>
</tr>
</tbody>
</table>

Comparison of Malarial species affecting the patients

P. vivax was the dominant species encountered in our study amounting to 87.5%. This is in contrast to other studies done globally where P. falciparum was the dominant species diagnosed.
Whereas Shetty et al\(^5\) (Mangalore) who did a study in 2011 showed a predominance of *P. vivax* Malaria which can be compared to our study as it was done in the same region. In our study the Hemoglobin varied from as low as 6.9g/dl to as high as 19.3g/dl. The mean Hb being 12.81g/dl for *P. vivax* and 12.39g/dl for *P. falciparum*, owing to the awareness among patients who had fever with chills, they came to the laboratory for diagnosis of Malaria, therefore being detected at early stages and reducing the complications arising due to Malaria. Thus the findings were not significant. A comparison to previous studies done globally and is tabulated below.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em></td>
<td>13.7</td>
<td>12.5</td>
<td>12.9</td>
<td>12.81</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>12.43</td>
<td>11.6</td>
<td>12.62</td>
<td>12.39</td>
</tr>
</tbody>
</table>

Comparison of mean Hb of affected cases

The PCV was calculated and our mean PCV was 36.58% which was comparable to other previous studies though it was not a significant finding. The comparison is shown in the next table.

<table>
<thead>
<tr>
<th>Study</th>
<th>Okocha et al.(^9) (2005)</th>
<th>Taha et al.(^{10}) (2007)</th>
<th>Current study.(^{2012})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean PCV</td>
<td>39.54 %</td>
<td>36 %</td>
<td>36.58 %</td>
</tr>
</tbody>
</table>

Table 27: Comparison of mean PCV in Malarial positive patients

Anemia has frequently been associated with Malaria. The two common causes of anemia are increased hemolysis and decreased rate of erythrocyte production from bone marrow.\(^{11, 12}\) 13.5\% (27) of our cases had anemia and when compared to various studies done previously we found a variable data. Kassa et al\(^{13}\) in 2005 showed only 14.7\% anemic cases, whereas Aktar et al\(^4\) in 2011 showed 86.5\% anemic cases. We concluded that any morphological type of anemia is not a particular characteristic feature of Malaria and hence not a significant finding.

<table>
<thead>
<tr>
<th>Study</th>
<th>Aktar et al.(^4) ((2011))</th>
<th>Rasheed et al.(^3) ((2007))</th>
<th>Kassa et al.(^{13}) ((2005))</th>
<th>Current study. ((2012))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemic</td>
<td>86.5%</td>
<td>39.6%</td>
<td>14.7%</td>
<td>13.5%</td>
</tr>
</tbody>
</table>

Comparison of anemic cases in Malarial patients

Though the findings for Mean WBC counts were not significant they were however comparable to other studies done globally as shown in the table.

<table>
<thead>
<tr>
<th>Study</th>
<th>Kassa et al.(^{13}) ((2005))</th>
<th>Erhart et al.(^{8}) ((2004))</th>
<th>Taha et al.(^{10}) ((2007))</th>
<th>Rasheed et al.(^3) ((2007))</th>
<th>Current study. ((2012))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em></td>
<td>5500</td>
<td>6618</td>
<td>6080</td>
<td>5800</td>
<td>5075</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>4800</td>
<td>6232</td>
<td>6510</td>
<td>5900</td>
<td>4900</td>
</tr>
</tbody>
</table>

Comparison of Mean WBC counts in patients with Malaria

Leucopenia is a finding we have got in our study amounting to 29\% of the total studied cases and this reflects the state of hypersplenism. These findings are comparable to previous studies done abroad as shown in the next table.
Below 4000 cases showed leucopenia, with Kassa et al. (2005) reporting 23%, Erhart et al. (2004) finding 23.3%, Rasheed et al. (2007) at 20%, and the current study at 29%. This suggests that monocytosis may develop in more severe cases during convalescence, reflecting the elevated activity of the reticulo-endothelial system. Thrombocytopenia is a classic feature of malaria, with our study finding it in 94% of cases, attributed to enhanced splenic uptake, DIC, and immune-mediated causes. This finding is comparable to previous studies.

**REVIEW OF LITERATURE:** Hematologic abnormalities are common: Thrombocytopenia (platelet count < 150 × 10^9/L) occurs in up to 70% of patients and anemia in 25%. The leukocyte count is normal or low; leukocytosis is seen in less than 5% of cases and is a poor prognostic factor. Microscopy by Peripheral Smear is widely used for detection, identification, and quantification of Malaria parasites, and remains the gold standard. Blood should, if possible, be taken during or after pyrexia, and before the administration of anti-Malarial drugs.

Severe anemia is most commonly seen after P. falciparum infections, followed by that due to P. vivax. The nonparasitized red cells are usually normocytic and normochromic in patients with P. falciparum Malaria. Spherocytes and red cell fragmentation were not found on blood film. The anemia of Malaria can certainly not be explained by the hemolysis of parasitized red cells alone; it is frequently disproportional to the degree of parasitemia.

Most parasitized red cells are then destroyed, either by rupture at schizogony or by premature phagocytosis by monocytes and macrophages. The falling hemoglobin in these patients coincided with rising parasitemia and improvement after reduction of parasitemia. In pregnancy P. vivax was shown to be associated with anemia (hematocrit <30% at any stage) but not as severe as that of P. falciparum.

Leukocytes play a vital role in defense against Malaria. Initial nonspecific responses include phagocytosis, cytotoxicity and cytokine production. With repeated infections, there ensues specific
immunity which has a predominant antibody-dependent component. The total white cell count is usually within the normal range, or there may be a slight leucopenia in adults with acute P. falciparum Malaria. In a small proportion of children and adults with severe and complicated Malaria, leukocytosis may also occur.\textsuperscript{17}

The leukocyte count returned to normal within three days after anti-Malarial therapy.\textsuperscript{18} Phagocytosis by neutrophils, monocytes and tissue macrophages as a major mechanism in the host defense against Malaria has been noted from the earliest of studies.\textsuperscript{19} The particles that are ingested include merozoites, Malaria pigment (hemozoin), parasitized red cells, nonparasitized red cells, platelets, and occasionally other nucleated cells.

At the turn of the 19th century Malaria was thought to be associated with high platelet counts.\textsuperscript{20} However, in 1924 reduction in peripheral blood platelet concentration was described in man\textsuperscript{21} and has since been observed consistently during infection with all human Malaria parasites species.\textsuperscript{22,23,24}

Thrombocytopenia is a frequent finding in acute P. falciparum Malaria infection. It results from a combination of platelet activation, splenic pooling and reduced life-span due to antibody and cellular immune responses. Vivax' associated thrombocytopenia is common\textsuperscript{25} with multiple mechanisms resulting in peripheral destruction and splenic sequestration.\textsuperscript{8}

**RESULTS:** This prospective study was conducted from September 2011 to August 2012. The age group of patients included in this study ranged from an infant aged 1 year to a 69 year old man.

1. Male predominance was noted being 82%.
2. Mean age of presentation was 32.6 years for P. vivax and 33.2 years for P. falciparum Malaria.
3. Most common clinical features were fever with chills and rigor.
4. P. vivax was the dominant species of Malarial cases (87.5%) reported.
5. Mean Hb was 12.81g/dl for P. vivax and 12.39g/dl for P. falciparum cases. This was an insignificant finding in our study.
6. Mean PCV was 36.58%. Cases P. vivax reported 36.84% and P. falciparum reported 34.8%.
7. Anemia amounted to 13.5% (27) of cases. The incidence being 12.6% of the cases in P. vivax and 20% in P. falciparum Malaria.
8. Mean RBC indices of P. vivax cases were MCV-85fl, MCH-28.4pg/cell, MCHC-33.4g/dl and RDW-14.8%. Mean RBC indices of P. falciparum cases were MCV-77fl, MCH-25.1pg/cell, MCHC-29.2g/dl and RDW-12.7%.
9. Mean WBC counts was 5075 cells/cumm for P. vivax and 4900 cells/cumm for P. falciparum.
10. Leucopenia was observed in 29% (58) cases. In P. vivax it was 28.6% and in P. falciparum it was 32%.
11. Monocytosis was seen in 23% (46) cases and the incidence in P. vivax and P. falciparum cases was 23.4% and 20% respectively.
12. Thrombocytopenia was a significant finding amounting to 94% (188) cases with all (100%) P. falciparum cases and 93% of P. vivax cases showing thrombocytopenia.

To conclude our study, Malaria affects almost all blood components and is a true hematological infectious disease. The hematological parameters are more affected in P. falciparum Malaria when compared to P. vivax Malaria; thereby concluding that P. falciparum has more morbidity than P. vivax and if left untreated will lead to severe complications.
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