KINETICS OF PLATELET COUNT IN P. VIVAX MALARIA; A STUDY IN YOUNG SOLDIERS

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ABSTRACT

Objective: To estimate the time of recovery from moderate to severe thrombocytopenia in young soldiers with *P. vivax malaria*.

Study Design: A cross-sectional analytical study.

Place and Duration of Study: Combined Military Hospital Pano-Aqil, Mangla and Kohat, from Sep 2012 to Sep 2017.

Methodology: Young adult patients suffering from *P. vivax malaria* with platelet count of less than 100 x 10^9/L and no co-morbidity were included in this study. All included patients were treated with standard dose of tablet Chloroquine as per WHO protocol. Peripheral blood film was examined daily till eradication of parasite and complete blood counts were carried out daily till the platelet count was decreasing and then on alternate days after the count started to rise. Data was grouped on basis of day of fever. It was collected prospectively on pre-designed proformas on Microsoft Access 2013 and was analysed using SPSS 21.

Results: A total of 390 patients were studied out of which 319 patients finally met the inclusion criteria for data analysis. Average age of patients was 28.25 + 6.97 years. Treatment was started on 3.08 ± 1.33 days from fever onset. The maximum decline in platelet count was seen on day 3 to 5 of fever onset (62.49 x 10^9/L ± 19.13 on day 4). Recovery of platelet count (more than 100 x 10^9/L) was seen by day 9 to 11 without any complication. All patients had platelet count more than 100 x 10^9/L by day 13.

Conclusion: Moderate to severe thrombocytopenia seen in *P. vivax malaria* is benign and almost all patients recover by two weeks.

Keywords: Hospital stay, *P. vivax malaria*, Thrombocytopenia.

INTRODUCTION

Malaria is one of the important diseases resulting in significant morbidity and mortality in Pakistan[1]. *P.vivax* is the most common cause of malaria worldwide[2]. 79% of all malaria cases in Pakistan are caused by *P. vivax*[3]. It is mostly a benign condition, as named "benign tertian" in past, but recently a change in its clinical outcome with occasional severe complications, like episodes of severe bleeding, have been increasingly documented[4-7]. One of the reason for this change in trend is increased incidence of thrombocytopenia in patients of *P. vivax malaria*. Studies have shown that platelets have protective role in infections especially malaria[8]. Thrombocytopenia in *P. vivax malaria* cases has been well documented in local and international studies[9-12]. It is considered to be secondary to increased platelet phagocytosis in malaria[13]. Although association of thrombocytopenia with severity of malaria had been frequently documented in past, this was considered to be more of a problem with *P. falciparum malaria* rather than *P. vivax*. Recent data suggests that thrombocytopenia should also be considered in *P. vivax malaria* as indicator of its severity[14].

In recent years cases of concurrent infection with certain life threatening diseases and malaria have also been reported. These include infections like Dengue, Chikungunya, Crimean Congo haemorrhagic fever, Zika[15-18]. Thrombocytopenia is a common feature in all. This has led to prolong hospital stays and frequent testing for platelet counts as clinicians feel unsafe to discharge

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patients from hospital till counts are fully recovered, especially in military hospitals. Most studies in past have quantified the degree of thrombocytopenia but very few have documented the rate of rise or the time taken by platelet counts to recover.

This study was done to estimate the time it takes for platelets to recover to safe limits (more than 100 x10^9/L)[19], so that patients can be discharged and followed up in outdoor departments till full recovery (>150 x 10^9/L). The results of this study can help reduce the hospital stay thus bring down the related cost and complications.

METHODOLOGY

A cross-sectional analytical study, conducted on young males (soldiers) with *P. vivax malaria* and moderate to severe thrombocytopenia. The study was set in three military hospitals over span of 5 years from September 2012 to September 2017 after approval by ethics committees of respective hospitals. Thrombocytopenia was defined as mild (101 to 150 x 10^9/L), moderate (50 to 100 x 10^9/L) and severe (<50 x 10^9/L)[20].

Estimated sample size was 384 patients (based on Krejcie and Morgan formula with confidence interval at 95%, margin of error 5% and population proportion 50%)[21]. Patients were selected prospectively on basis of inclusion/exclusion criteria and after consent. Previously healthy adult males with *P. vivax malaria* and platelet count of 100 x 10^9/L or below were included in the study. Patients were excluded from study if they had any co morbidity. Patients were also excluded from the study if they had splenomegaly on examination.

Malaria was confirmed by microscopic examination of thick and thin blood films. Complete blood count (CBC) was done by automated hematology analyser (XR-100). Other tests were done to exclude co morbidities and complications. These tests included liver function tests, serum creatinine, plasma glucose, urinalysis, Dengue serology, Hepatitis screen, ultrasound abdomen and chest radiograph.

Patients admitted with malaria were evaluated as per inclusion/exclusion criteria. Day of fever onset was noted at the time admission and blood samples were grouped accordingly. Day of starting treatment was also recorded with reference from day of fever onset. Patients were treated with Tablet Chloroquine only (dose as per WHO guidelines; 4 tablets stat, 2 after 6, 24, 48 hours)[22]. Tablet Paracetamol was used for fever as and when required. Patients were reviewed daily for any complications. Any patient requiring additional drug during admission, like proton pump inhibitors or metoclopramide etc, was excluded from the study as these drugs can cause hematological changes. Treatment was continued till completion of dose and patients were monitored till platelet count was >100 x 10^9/L. After discharge, patients were followed up in outdoor clinics on weekly basis till recovery of platelets to >150 x 10^9/L. Peripheral blood film microscopy for malarial parasites was done daily to monitor eradication of parasites and to rule out coexisting *P. falciparum malaria*. Complete blood count was done daily as long as platelet counts were decreasing. It was done on alternate days once the platelets started to increase and continued till recovery of platelets to >100 x 10^9/L. Since the patients were included in study after onset of illness, a limitation to this study was that baseline complete blood counts could not be recorded.

Data was collected on Microsoft Access database 2013. It was analysed on Microsoft Access 2013 for means and standard deviation, Microsoft Excel 2013 for graphs and SPSS 21 for tests of significance (ANOVA). For comparison and analysis of data, complete blood counts results were grouped on basis of day of fever. For each day mean, standard deviation and range of hemoglobin (Hb), total leukocytes count (TLC) and platelets were calculated respectively. Graphs were plotted: histograms to check for normal distribution and; trend line with marker graphs to highlight changes seen in mean values over days. *p*-value was calculated by analysis of variance, as there were more than two groups, and a value of ≤0.05 was considered significant.

RESULTS

A total of 390 adult soldiers were studied out of which 319 patients finally met the inclusion criteria for data analysis. Main reason for patients' exclusion from the study was requirement for intravenous fluids, antiemetics and proton pump inhibitors. Six patients were excluded due to presence of splenomegaly.

The average age of the patients was 28.25 ± 6.97 years. Presentation to hospital and start of treatment was on average at 3.08 ± 1.33 days of fever onset. The mean of lowest platelet count recorded was 49.5 ± 29.12 x 10^9/L, hemoglobin 9.58 ± 1.02 g/dl and total leucocyte count 2.83 ± 0.86 x 10^9/L. Mean hospital stay of patients was 7.25 ± 4.26 days.

Table summarises the mean of blood counts seen in 1672 samples of 319 patients. The data is grouped according to the day sample was taken from onset of fever (number of samples for each day is also shown). The maximum decline in platelet count (62.49 ± 19.13 x 10^9/L) was seen on 4th day of onset of fever (p<0.01). Recovery of platelet count (more than 100 x 10^9/L) was seen by day 9 to 11 without any complication (p<0.01). All patients had platelet count more than 100 x 10^9/L (124.29 ± 13.63/L) by day 13 (table, figure, p<0.01). The platelet count curve in figure also shows that after initial drop, the rise of platelets is linear showing a steady rise. Hemoglobin reduced to lowest on day 6-7 of fever but then gradually recovered and anaemia was not severe. The mean of lowest hemoglobin recorded in each patient was 9.58 ± 1.02 g/dl. The observed difference in the mean total leucocyte count was significant but no specific trend of rise and fall was noted over this period of 2 weeks (table). No serious complications were observed in any case.

Table: Mean Hemoglobin, TLC and Platelet count of patients. (no of patients 319, no of samples 1672). The *p*-value calculated by analysis of variance.

Day of Fever	Hemoglobin g/dl Mean ± SD	TLC x 10^9/L Mean ± SD	Platelet x 10^9/L Mean ± SD	No. of Samples Per Day	*p*-value
0	12.84 ± 0.69	4.71 ± 1.17	100.80 ± 24.08	10	<0.01
1	12.92 ± 1.15	4.96 ± 1.73	97.09 ± 29.19	32	<0.01
2	12.63 ± 1.27	4.81 ± 1.25	82.92 ± 24.14	99	<0.01
3	12.75 ± 1.37	4.70 ± 1.42	74.18 ± 22.52	207	<0.01
4	12.43 ± 1.44	4.73 ± 1.38	62.49 ± 19.13	243	<0.01
5	12.31 ± 1.31	4.85 ± 1.35	67.41 ± 22.79	267	<0.01
6	12.19 ± 1.42	5.06 ± 1.31	76.19 ± 22.90	196	<0.01
7	12.18 ± 1.21	5.28 ± 1.10	86.80 ± 21.48	187	<0.01
8	12.32 ± 1.30	5.56 ± 1.14	95.42 ± 27.37	107	<0.01
9	12.40 ± 1.25	5.73 ± 1.11	98.49 ± 16.96	150	<0.01
10	12.66 ± 1.24	5.97 ± 1.23	107.19 ± 28.12	43	<0.01
11	12.62 ± 1.15	6.13 ± 0.77	112.26 ± 19.49	85	<0.01
12	13.15 ± 0.79	6.30 ± 0.71	119.68 ± 25.05	25	<0.01
13	12.76 ± 1.26	6.19 ± 0.68	124.29 ± 13.63	21	<0.01

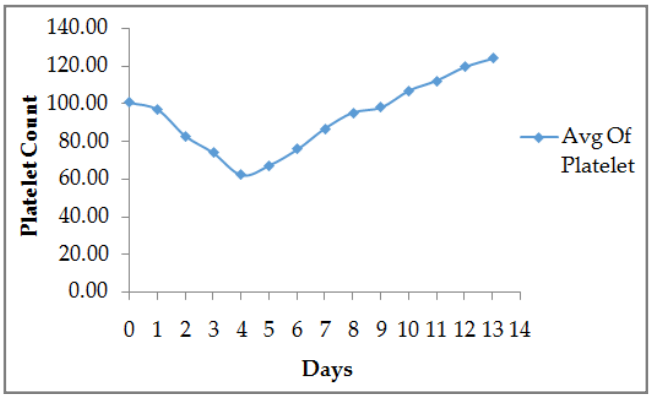

Figure: Trend of mean platelet count plotted against day of fever (p<0.01).

DISCUSSION

The findings of our study are in line with the observations made by other researchers in terms of degree of hematological changes seen in cases of *P. vivax malaria*. This makes our additional obs-

ervation on time taken to recover from moderate to severe thrombocytopenia significant. Although this parameter has been measured in some studies, as discussed below, none has recorded it precisely with the objective to estimate safe hospital discharge time.

The mean age of our patients was 28.25 ± 6.97 years as compared to other studies from Pakistan, 35 ± 20 years in Hafeez *et al*[23], and; 31 ± 17.4 years Burhan *et al*[24]. The mean of lowest platelet counts recorded in each patient of our study was 49.5 ± 29.12 x 10^9/L with a range of 14 to 100 x 10^9/ L (n 319, and 100 x 10^9/L being the maximum count for inclusion in study). A local study documented a mean lowest count of 79 ± 27.4 x 10^9/L with a range of 11 to 146 x 10^9/L (n 150, and 150 x 10^9/L being maximum count for inclusion in this study) 23; while in an Indian study documented a mean lowest count of 67.99 ± 53.37 x 10^9/ L in its severe malaria group (n 40)[25].

For platelets kinetic, an experimental study done on monkeys observed severe thrombocytopenia at 14 days after induction of malaria infection. It was noted that thrombocytopenia was one of the earliest clinical manifestations of the infection and returned to normal levels after another 14 days after peak parasitaemia. Thrombocytopenia in our study was observed to be lowest between 3rd to 5th days of fever. If the incubation period is added (more than 7 days in *P. vivax malaria*), it is then roughly the same as observed in the study on monkeys. Similarly time taken by thrombocytopenia to recover was 13 days in our study as compared to 14 days in the experiment.

In another study Tylor *et al*, compared the platelet kinetics in immune and non-immune populations in Papuan[20]. Like our study they also used only Chloroquine for treatment. Along with other measurements they also recorded the time taken for platelets to recover but time intervals between measurements were long. Blood samples were taken on day 0, 7 and 28 in contrast to our study where the complete blood count was done more frequently, daily initially and then on alternate days till recovery above 100 x 10^9/L, thus making it more reliable for commenting on minimum length of hospital stay required. In this study platelet count for all patients (severe and non-severe malaria cases) ranged from 8 to 313 × 10^9/L (14 to 100 x 10^9/L in our study). Most patients' count recovered on day 7 and by day 28 mean counts had increased but thrombocytopenia remained in 20% of patients. Recovery in this study was measured at 150 x 10^9/L as compared to 100 x 10^9/L in our study. Since there was no intervening platelet counts check between day 7 and 28, approximate time of platelet count recovery to more than 100 x 10^9/L could not be determined. They suggested that patients needed a follow up of longer than four weeks for full platelet recovery. As per our results once the upward trend of platelet count is established and platelet count is in a safe range (more than 50 x 10^9/L), the patient can be discharged and followed up in outdoor after two weeks.

Mean hospital stay in our study was 7.25 ± 4.26 days. It was comparable to 7.78 ± 3.58 days recorded in the severe malaria group (mean platelet count 67.99 x 10^9/L) of study by Gupta BK *et al*[25], Hospital stay in non-severe malaria group (mean platelet count 123 x 10^9/L) was 5.10 ± 1.55 days in this study.

The results of our study can help reduce the hospital stay in asymptomatic malaria cases with severe thrombocytopenia. As observed, these patients do not develop any complication in the recovery phase and can be safely discharged from hospital when their platelets begin to rise. They can be followed up at two weeks to confirm full recovery of platelets.

Other hematological parameters like hemoglobin and TLC, though not an objective of our study, were also measured and the results were comparable with the studies above. Hemoglobin reduced to lowest on day 6-7 of fever but then gradually recovered and anaemia was not severe (mean lowest hemoglobin being 9.58 ± 1.02 g/dl). Mean total leucocyte count did not show any specific trend as in other studies discussed above

but in contrast to study by Taylor *et al*, no leucocytosis was recorded in our study[20].

CONCLUSION

Two important inferences are drawn from our study. First, thrombocytopenia seen in *P. vivax malaria* cases is benign and resolves in all patients by 2 weeks. Second, an asymptomatic patient with upward platelet count trend can be safely discharged with follow up at two weeks, thus reducing hospital stay.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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