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RESEARCH ARTICLE

Thrombocytopenia as a Diagnostic Marker for Identifying Patients with Malaria in Endemic Regions

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Abstract

Malaria can present in a myriad of hematological alterations with thrombocytopenia being one of those. It is typically diagnose by the microscopic examination of the blood using blood film. The aim of the present study was to screen all patients with thrombocytopenia for the prevalence of malaria during the seasonal malaria. The patients with thrombocytopenia were included in the study. A total of 389 cases having thrombocytopenia were identified; malaria was seen in 21.8%. Among the conformed malarial patients, 63.5% *Plasmodium falciparum* and 36.5% *Plasmodium vivax* was seen. Further it was observed that 91.7% of thrombocytopenic patients with malaria were also had some degree of anemia as well. Taken together, a total of 20 patients had pancytopenia with more 41.9% cases with *P. vivax* and 13% with *P. falciparum*. Based on our findings, we advocate the use of thrombocytopenia as an important marker in the diagnosis of malaria; and in endemic regions patients with thrombocytopenia should be screened for possible malaria.

Introduction:

Malaria is a life-threatening disease caused by parasites of genus plasmodium that are transmitted by Malaria vectors to man through the bites of infected female Anopheline mosquitoes. There are five plasmodium species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *plasmodium ovale* and *Plasmodium knowlesi*) causes human malaria, among which the *P. falciparum* and *P. vivax* are the most common and *P. falciparum* is the most deadly.

According to world health organization (WHO) report in 2013 it is estimated about 207 million cases of malaria in 2012 and accounts for an estimated 627000 deaths. (WHO, 2013) A vast majority, about 85% malarial cases were in

African Region, followed by the South-East Asian Region (10%) and 4% from East Mediterranean region. 89% of the death cases reported from African Region followed by East Mediterranean (6%) and 5 % from South-East Asia Region (Gupta et al., 2013).

Malaria parasite affects multiple human organs such as brain spleen, liver gastrointestinal tract (GIT), Gall bladder and blood vessels. Therefore the clinical picture may be of wide range that is from simple malaise to life threatening central nervous expression like coma. Blood abnormalities have been observed in patients with malaria; Anemia and thrombocytopenia is the most common (Wickramasinghe, 2000; Khan, 2008).

The frequency and severity of thrombocytopenia appears to vary with the severity of infection and the type of malarial parasites (Leowattana et al., 2010; Kochar et al., 2010). Thrombocytopenia could also be a marker for the presence of malaria in endemic regions (Maina et al., 2010). 961 children from Kenya, children with low platelet counts (<150,000/mm^3) were 13.8 times more likely to have malaria. Similarly in a systematic review on returning travelers to endemic regions, thrombocytopenia had a likelihood ratio of 5:6 (confidence interval 4.1-7.5) of predicting malaria (Taylor et al., 2010).

It has therefore, been suggested by several authors that it may be cost effective for patients with low platelet counts in malaria endemic regions to be systematically screened for the presence of malarial parasites (Mbanya et al., 2002). This is further corroborated by our literature review as outlined in Table 1 where thrombocytopenia was noted ranging from a quarter to all patients with Malaria; along with severe thrombocytopenia (< 50,000 /mm^3) being present in up to half of the patients. Our aim, therefore, was to screen all patients with thrombocytopenia presenting to lab for the presence of malaria during the malaria season and thus evaluate the efficacy of routine testing for malaria in patients presenting with thrombocytopenia.

Materials and Methods:

Ethical approval for the study was obtained from Provincial Reference laboratory (PRL) Malaria control program, Fatima Jinnah General & Chest Hospital Quetta and the research conducted was performed according to the Declaration of Helsinki.

The study was a prospective carried out at PRL Malaria control program Fatima Jinnah General & chest Hospital, tertiary care hospital in the city of Quetta, Baluchistan, the largest province in Pakistan. Quetta is a metropolitan city and the capital of the province. People belonging to different communities live here along with many refugees from the country Afghanistan migrated during the early 1980s and 1990s. With respect to Malaria, according to WHO EMRO, the estimated number of annual malaria episodes in Pakistan is 1.5 million; among which 40% were reported from province Baluchistan (WHO, 2011).

Automated complete blood counter was used to detect thrombocytopenia and all the positive patients were included in the study. Further, the presence of thrombocytopenia was confirmed manually; and thick and thin Giemsa smears were also prepared and evaluated for the presence of malarial parasites. Data on other hematological parameters was also recorded. A database was built in Microsoft Access for input of the data.

Results:

Among the studied a total of 389 cases were identified as having thrombocytopenia (Platelet count < 150,000/mm^3). Out of identified cases 85 (21.85%) cases and further their smears were found to be positive for Malaria; i.e. nearly 1 in every 5 patient with thrombocytopenia had underlying malaria. 63.5% cases were found positive for *P. falciparum* and 36.5% cases also for *P. vivax* infection.

Table I: Research Articles documenting the incidence of Thrombocytopenia in patients with malaria┼

S.NO	PAPER [REFERENCES]	TYPE OF MALARIAL PARASITES	THROMBOCYTOPENIA [NO OF CASES]	%age	SEVERE THROMBOCYTOPENIA [NO OF CASES]	%age
1.	Singh et al 2011 (25)	*P. vivax*	22	96	9 (< 50,000)	36
2.	Kochar et al (6)	All types	262	24.6		
		P. vivax	143	31.09	26 (< 20,000)	18.18
		P. falciparum	85	16.19	9 (< 20,000)	10.59
3.	Sharma et al (26)	*P. vivax*	213	96.3	13 (< 20,000)	6.1
4.	Ansari et al (27)	*P. falciparum*	256	69.18	180 (<50,000) 37 (<20,000)	48.6 10
5.	Rodriguez et al (28)	*P. vivax*	46	58.97	18 (<60,000)	24.36
6.	Jadhav (29)	Both types *P. falciparum* *P. vivax*	1242	79.4	 50 (<20,000) 15 (<20,000)	 8.5 1.5
7.	Rasheed et al (30)	Mixed	248	80		
8.	Maina et al 2010 (7)	*P. falciparum*	256	49		
9.	De Laval et al 2010 (31)	*P. Ovale*	31	50		
10.	Leowattana et al (5)	Uncomplicated and Complicated *P. falciparum*	81 100	73.6 90.9		
11.	Inan et al (32)	Both *P. falciparum* and *P. vivax*	15	75		
12.	Malik et al (33)	Both *P. falciparum* and *P. vivax*	274	70		
13.	Parakh et al (34)	Severe *P. vivax* Malaria	5	100		
14.	Shaikh et al (35)	Both types *P. falciparum* *P. vivax*	100 46 56	80.6 71.87 93.33		
15.	Daneshvar et al (36)	*P. Knowlesi*	107	100		
16.	Taylor et al (37)	Both *P. falciparum* and *P. vivax*	119	78.8		
17.	Lee et al (24)	*P. vivax*	185	95.4		
18.	Adedapo et al (38)	*P. falciparum*	412	59.3		
19.	Obaldia et al (39)	*P. vivax*	20 (an animal study)	77		
20.	Beg et al (40)	Mainly *P. falciparum* and *P. vivax*	189	36.4		
21.	Kumar et al (21)	*P. vivax*	24	88.9		

┼ Please note that 2 articles in pubmed by Mbanya et al and Inan et al were not in English; incidence was noted from the abstract in English

Table II: Frequency of severity of thrombocytopenia based on Malarial subtype

S.No	Thrombocytopenia	*P. falciparum* No of cases	Percentage	*P. vivax* No of cases	Percentage
1.	Mild (100,000 – 150,000 /mm^3)	21	38.9%	14	45.2%
2.	Moderate (50,000-99,000)	18	33.3%	08	25.8%
3.	Severe (20,000-49,000 /mm^3)	10	18.5%	08	25.8%
4.	Very severe (10,000-19,000 / mm^3)	05	9.3%	01	3.2%
	Total	54		31	

Table III: Frequency of changes in WBC count and severity of anemia based on malarial subtype

VARIABLE	*P. falciparum*		*P. vivax*	
WBC COUNT	**No**	**% age**	**No**	**% age**
Normal WBC Count	47	87	18	58
Leucopenia	7	13	11	35.5
Leukocytosis	0	0	2	6.5
Total	54		31	
SEVERITY OF ANEMIA				
Normal	3	5.6	3	9.7
Mild (10-13.8 g/dl for males and	28	51.8	14	45.2
10-12.1 g/dl for females)				
Moderate (8-9.9 g/dl)	15	27.8	8	25.8
Severe (< 8 g/dl)	8	14.8	6	19.3
Total	54		31	
PANCYTOPENIA				
Pancytopenia	7	13	13	41.9

Discussion:

Based on literature search, the degree of thrombocytopenia has been classified into various groups; with severe being defined as < 50,000 / mm^3 (Gupta et al., 2013). We classified thrombocytopenia into four groups as outlined in Table II. There were 41.2% cases mild thrombocytopenia, 30.6% moderate thrombocytopenia, 21.2% severe thrombocytopenia and 7% very severe degree of thrombocytopenia.

Based on literature search thrombocytopenia is commonly associated with *P. falciparum* and has been also reported to occur in patients co infected with *P. falciparum* and *P. vivax.* Its occurrence has less commonly reported in cases of *P. vivax* malaria (Morales et al., 2005). Table II outlines the severity of thrombocytopenia based on *P. falciparum* and *P. vivax*. Based on the definition of severe thrombocytopenia (<50,000 /mm^3) used by several authors Table 1, a total of 28.2% cases had severe degree of thrombocytopenia. Most of the cases in our study were caused by *P. falciparum*, along with it being the etiology in the very severe cases as mentioned in Table II. The mean of Mean Platelet Volume (MPV; normal 6-13 fl) was observed to be 7.83 fl (range 3.1-11.9fl). In a study of McKenzie et al, (2005) demonstrated the effect of malaria on a total leucocytes counts and found leucopenia in the *P. falciparum* infected patients than those in the *P. vivax*, which, in turn, were lower than those in the uninfected patients. In our study 21.2% patients found with leucopenia, 2.4% of the patients were with increased leucocytes count and 76.4% of the patients were with a normal White Blood Cell (WBC) count. Decrease in hemoglobin (Hb) concentration leading to anemia is frequently associated with malaria infection. Among the parasites species P. falciparum causes the most deadly and profound anemia with high risk of death (Menendez et al., 2000). In our research work we found about 91.7% of thrombocytopenic patients with positive malaria were also had some degree of anemia as well. Among them 49.4% cases had degree of anemia, 27.1% had moderate degree of anemia and 16.5% had severe degree of anemia. Taken together, a total of 20 patients had pancytopenia 41.9% patients with *P. vivax* and 13% patients with *P. falciparum*. Likewise in a study on pancytopenia in children, malaria was the cause in 8.69% of the cases (Memon et al., 2008). Although, both *P.*

falciparum and *P. vivax* have been reported in literature to cause pancytopenia, it is more often seen in association with *P. falciparum*. In our study, more cases of pancytopenia were noted within patients with *P. vivax* infection.

Although thrombocytopenia is a hematological disorder and develop early on in malarial patients, however the exact mechanisms behind its pathogenesis are not completely understood (De Mast et al., 2010). According to the findings of Coelho et al, (2013) indicated that platelet phagocytosis may contribute to thrombocytopenia found in vivax malaria. Identification of thrombocytopenia is important not only as a screening tool for identification of malaria in endemic regions but also has important prognostic significance. In a study from Zambia, thrombocytopenia had the strongest association with the presence of cerebral malaria (Thuma et al., 2011). Apparently, platelet activation by plasmodium can potentially lead to the formation of micro-aggregates of infected red blood cells and platelets which can occlude blood vessels and it also leads to binding to and activation of the endothelium (Cox et al., 2010). Increased Von Willebrand Factor concentrations along with decreased ADAMTS13 activity is also thought to play a role; along with oxidative stress, alterations in splenic function and immune mechanisms (Löwenberg et al., 2010; de Mast et al., 2009; Kumar et al., 2006).

Thrombocytopenia is not only seen as a feature of severe disease but also is frequently seen in asymptomatic cases of malaria in endemic regions and travelers bringing ‘imported malaria (Jeremiah et al., 2007; Chung et al., 2007). Based on our findings, we strongly advocate the use of thrombocytopenia as an important aid in the diagnosis of malaria; especially in areas within developing countries where hematologists may not be as readily available to diagnose malaria based on thick and thin smears. Scoring systems may be developed which could incorporate common signs and symptoms like fever, hepato-splenomegaly and thrombocytopenia and/or anemia; since the results of an automated complete blood count are readily available even in remote facilities. The usefulness of thrombocytopenia in helping aid the diagnosis is elucidated well by an interesting study by Lee et al, (2008) examined the sensitivity and specificity of a malarial antigen test in an area where *P. vivax* was endemic. Alone, the sensitivity and specificity of the antigen test for malaria was 96.4% and 98.9% respectively. When thrombocytopenia was added to the evaluation, the positive predictive value of the test rose to 100%; thereby aiding in the diagnosis.

Thrombocytopenia is therefore, not only an important diagnostic and prognostic marker of severe malarial illness in endemic regions; but also can aid in the diagnosis in travelers to regions where malaria is an epidemic. Scoring systems incorporating the use of thrombocytopenia should be developed.

Conclusions:

Based on our findings, we advocate the use of thrombocytopenia as an important aid in the diagnosis of malaria; and in endemic regions patients with thrombocytopenia should be screened for possible malaria. Scoring systems incorporating the use of thrombocytopenia should be developed. Table 1 summarizes the frequency of thrombocytopenia noted in different studies on thrombocytopenia and malaria; and reiterates the importance of screening these patients for presence of underlying malaria.

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Declaration of interest:
All authors declared that they have no conflict of interest.

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