Leukopenia in malaria

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ABSTRACT

Aim: To study the frequency of Leukopenia in patients with malaria.
Study design: Descriptive cross-sectional study
Study duration: January 2019 to, December 2019.
Methods: Patients who had a fever of 101ºF or higher in the previous 72 hours and were suspicious of having malaria were tested for the occurrence and species recognition of the parasite using thin and thick films of peripheral blood taken by light microscopy. Non-probability consecutive sampling was used to choose 500 individuals over the age of 12 who had a confirmed malarial parasite (MP) on a peripheral blood film. Strict inclusion and exclusion criteria were followed. Complete Blood Count (CBC) was performed on all patients to look for the total white blood cells (WBC) count. Leukopenia was defined as WBC counts less than 4000 cells/mm^3.
Results: All the 500 included patients were malarial parasite positive on smear. Out of these 318 (63.6%) were male and 182(36.4%) were female. Among all 482 (96%) were P. vivax positive showing that it is the most common plasmodium species in the Northwestern regions of Pakistan. P. falciparum infection was found in a significantly lower population with only 14(2.8%) cases in our study and 4 cases of mixed infection of both P.vivax and P.falciparum were reported. The Total White Blood Cells (WBC) count was found to be ≤4000/mm^3 in 108 (21.6%) cases. This showed that malaria can cause leukopenia in a considerable amount of individuals.
Conclusions: The present study indicates that the decrease in total white blood cells count occur in patients having malaria and is more frequently seen in plasmodium Vivax.
Key Words: Leukopenia, Malaria

INTRODUCTION

Malaria is one of the world's most serious health concerns, especially in undeveloped states like Pakistan [1]. Malaria is a significant public health issue that is the world's fifth largest cause of mortality from infectious disease[2], accounting for annual death of one million[3]. Plasmodium causes malaria, which is spread by mosquitos. Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi are the five species of plasmodium causing malaria. In Pakistan, the most prevalent malaria causing species of plasmodium are P. vivax and P. falciparum[1, 4].The financial costs of malaria management is worrying, since the yearly expense of economic aid to malaria endemic nations has risen from over $100 million to nearly $1.8 billion between 2003 and 2010 [2].Moreover, malaria prevention will need a worldwide funding of US$ 4.75 billion between 2020 and 2025 [5].

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The problem of malaria is mostly found in subtropical and tropical areas, where the rainfall and temperature are conducive to the persistence of malaria-causing Plasmodium parasites[6].
It has been shown that 7% of the Pakistani population is at danger of acquiring malaria, with an anticipated 1.6 million infections each year across the country[7]. Plasmodium falciparum species was responsible for roughly 30% of all reported cases, majority of the cases (42%) were reported from Balochistan province [7]. According to current study both Plasmodium vivax and Plasmodium falciparum [8, 9] are prevalent in Pakistan, responsible for 75 percent and 25 percent of malaria infections, respectively[10]. Unchecked immigration of Refugees from Afghanistan, urbanization, an extensive agricultural system, earth quakes, floods, and projects for development of water are the various factors that contribute to a suitable ecosystem for persistence of malaria in Pakistan. Malnourishment, a lack of individual immunity, or exposure to high-transmission regions, overburdened healthcare system, and unsanitary shelters that serve as breeding grounds for mosquitoes contribute to increasing transmission of malaria in Pakistan, particularly in camps of Afghan refugee [8, 11]. Malaria has seasonal fluctuations with the majority of cases occurring between September and November, following the rainy season[10]. In addition, epidemic breakouts in certain geographic locations, particularly in Balochistan, Sindh, Khyber Pakhtunkhwa, and federally managed tribal territories, have the greatest malaria load [10]. But, because of malaria control initiatives run by a variety of organizations across the globe, the projected cases of

malaria declined from 244 million to 225 million from 2005-2009, resulting in a 21% drop in expected worldwide fatalities caused by malaria from 2000 to 2009 [2, 7].

Malaria may damage every organ or system in the body, with the hematological system being the most vulnerable. Anemia and thrombocytopenia are caused by the majority of the aforementioned changes to thrombocytes and erythrocytes [12-14]. Changes in leucocytes counts are less common, but they've been linked to variables including seriousness, Plasmodium species, co-infections, and response of treatment [15-18]. There have been reports of leukocytosis, leukopenia, neutrophilia, and neutropenia in these individuals, as well as the existence of premature neutrophils and alterations in lymphocyte, monocyte, and eosinophils [19, 20], however no research have found a particular profile of leukocyte modification in patients having malaria. In malaria patients leucocyte counts are lower than in healthy individuals, either infected with P. falciparum or P. vivax [21]. Furthermore, these alterations have been shown to be flexible throughout disease, with lower lymphocyte and eosinophil counts at beginning and rising as symptoms diminish [21, 22]. In serious instances of malaria caused by P. falciparum, various reports have been reported about the mortality-associated with leukocytosis, co-infections, and bacteremia caused by gram negative bacteria [23] Numerous theories have been proposed to clarify these hematologic pathologies, including (I) suppression of bone marrow due to imbalance response of immune system, (ii) shorter average life of cell, and (iii) Induced immune response or microvasculature sequestration and redistribution of leukocyte to lymphoid organs and tissues [17, 20, 24–26]. Unfortunately, unlike so many other infections common in tropical locations, like Dengue fever, these studies are rarely discussed, and their clinical usefulness has yet to be proven [27]. The whole Blood Count evaluates the cellular components of the blood, such as erythrocytes, leucocytes, and thrombocytes, both quantitatively and qualitatively [28], and is an important tool for determining haematological alterations. The total WBC as well as subgroup counts such as eosinophils, neutrophils, basophils, monocytes, and lymphocytes is included in the leukogram, which is part of the CBC that examines leucocytes. Age, race, physiological state (pregnancy), drug usage, and time of day all influence their reference levels [29]. The purpose of this research is to describe variation in the level of leucocyte count in patients presenting to tertiary hospitals in Khyber Pakhtunkhwa endemic areas. Our goal is to promote the usage of simple diagnostic techniques like the CBC, which allow for rapid diagnosis of patients at risk for clinical problems and the implementation of suitable treatment interventions, thus enhancing patient outcomes.

MATERIALS AND METHODS

This was a cross sectional study carried out in the Department of medicine, Hayat Abad Medical Complex Peshawar over a period of one year from January 2019 to December 2019. Patients who presented to the healthcare Outpatient department with a fever greater than 101oF in the preceding 72 hours and were accused of having malaria were diagnosed for the presence and identification of species of the parasite using thick and thin films of peripheral blood under light microscope at the hospital's malaria diagnostic laboratory, in accordance with WHO recommendations [30]. Non-probability purposive sampling was used to choose 500 patients of both sexes between the ages of 13 and 88 years with a positive malarial parasite (MP) on peripheral blood film. After obtaining informed consent from these participants, they were enrolled into the research. A complete clinical examination was done with special reference to the presence of fever, jaundice, bleeding spots and hepatosplenomegaly and to exclude fever with localizing signs such as meningitis, pneumonia, upper respiratory tract infection, skin and subcutaneous tissue infection etc. Patients who were already receiving antimalarial therapy, or were on long-term antibiotics, or were immuno-compromised were excluded from the research. All cases in which clinical or investigational evidence of any other infection or co infection with malaria were excluded from the study after all workup of acute febrile illness including baseline investigations, NS1 antigen, dengue IGM antibody, typhidot IgM and urinalysis. Additionally, individuals with co-morbid diseases such as hemolytic disease or other hematological disease affecting the blood profile, such as primary aplastic anemia, leukemia, lymphoma, or autoimmune diseases, were excluded from the research.

For laboratory studies, 2cc blood was taken from research participants having high grade fever for more than 3 days in CBC bottles comprising anticoagulant (EDTA) and immediately delivered to the hospital's main laboratory for analysis of the full blood picture using a 3rd generation analyzer (Sysmex KX-21N). Leukopenia was defined as a count of total white blood cells (WBCs) fewer than 4000 cells/mm^3. Each source of knowledge was recorded into a proforma.

Statistical Analysis

SPSS statistics software, 22nd edition, was used to input and analyze data. Categorical variables like gender, white blood cell and distribution of plasmodium were expressed as frequencies along with percentages. Numerical variables of the study like age and leucocyte counts were expressed as mean and standard deviation. Fisher’s exact test was applied to determine the association of white blood cell count in various plasmodium species. A p value of less than 0.05 was considered as significant.

Ethical aspects: After obtaining informed consent, all of the patients were enrolled in the study, which was approved by the Institution's Medical Research Ethics Committee.

RESULTS

A total of 500 out patients were included in this study. All of them were malarial parasite positive on smear. Out of these 500 patients, 318 (63.6%) were male and 182 (36.4%) were female.

Out of these 500 patients, 482 (96%) were P.*vivax* positive showing that it is the most common plasmodium species in the North western regions of Pakistan. P. *falciparum* infection was found in a lower population with only 14 (2.8%) cases in our study and 4 (0.8%) cases of mixed infection of both P.*vivax* and P.*falciparum* were reported (Table 1). P.ovale, P.malaria and P.knowlesi- infected patients were not found in our study as they are not endemic in our regions of the Asian subcontinent.

The Total WBC count was found to be less than 4000 cells/mm^3 in 108 (21.6%) cases and greater than 4000 cells/mm^3 in 392 (78.4%) cases. This showed that malaria can cause leukopenia in a considerable amount of individuals. (Table 1)

Table 1: Characteristics of patients enrolled in study (N=500).

Parameters	N
Age*	28.56 ± 14.91
Gender	
Male	318 (63.6%)
Female	182 (36.4%)
Plasmodium Species	
Plasmodium Vivax	482 (96.4%)
Plasmodium Falciparum	14 (2.8%)
Mixed (Plasmodium Vivax + Plasmodium Faciparum)	4 (0.8%)
White blood cell count	
≤ 4000 cell / mm^3	108 (21.6%)
> 4000 cell mm^3	392 (78.4%)

*Continuous variable was presented as Mean ± Standard deviation
Categorical variables were presented as Frequencies and Percentages

We compared the distribution of total WBC count in the various plasmodium species reported and found that out of the 108 patients with leukopenia, 102 (21.16 %) were P.*Vivax* positive, and 6 (42.86 %) cases were P.*Falciparum* positive. This shows that Leukopenia is significantly more frequent in P. falciparum malaria than P.vivax malaria ($p < 0.001$), while out of the 392 cases of normal TLC counts, 380 (78.84%) cases were P.*Vivax* positive and 8 (57.14%) cases were P.*Falciparum* positive (Table 2)

Table 2: Comparison of total WBC count with Plasmodium species.

Total WBC	Plasmodium Species			p-value
	P. Vivax (n = 482)	P. Falciparum (n = 14)	Mixed : P. Vivax + P. Faciparum (n = 4)	
≤ 4000 cell / mm^3	102 (21.16%)	6 (42.86%)	0 (0%)	< 0.001*
> 4000 cell mm^3	380 (78.84%)	8 (57.14%)	4 (100%)	

WBC: White blood Cell, P: Plasmodium; Categorical variables were presented as Frequencies and Percentages.

*Significant p-value calculated by Fisher's Exact test.

DISCUSSION

In both falciparum and vivax malaria, a variation called leukopenia is present [21, 31]. Malaria-related leukopenia can be induced by a combination of factors. It has been hypothesized that leukocyte sequestration, rather than reduced production or faster destruction, explains the drop in leucocyte numbers [17]. The glycosyl-phosphatidyl-inositol (GPI) is observed in all species of Plasmodium triggers the growth of pro-inflammatory cytokines in monocytes and macrophages [21, 32]; it could boost phagocytic activity, producing cellular debris and increasing the phagocytosis of erythrocytes and leucocytes, maintaining higher concentrations of pro-inflammatory immune cells, especially TNF, and cause hematopoiesis to be disrupted [21, 33, 34].In our study there was significant (21.6% of patients) Leukopenia in malaria, as demonstrated by the highly significant p value (<0.001) as studied by the single's t test, which is similar to the reports of Latif I, Jamal Awhich showed Leukopenia in 39% of the studied subjects. Similarly, Leukopenia was also reported in a study by Anwar A et al [36] conducted in different hospitals of Lahore in 2016, showing a significant reduction (p< 0.0001) in WBC counts.[35]

Asghar AS et al conducted a study in Lahore in 2013 with 133 confirmed male patients of malaria, and reported Leukopenia in 39% of cases.[37]They also reported that significant numbers of cases of malaria were associated with thrombocytopenia and Leukopenia and Thrombocytopenia [38, 39, 40, 41] emerged as strongest indicators of malaria.

The results of our study are also consistent with the study of Reddy et al conducted in India in 2016 d which showed Leukopenia in 44% of the studied patients and also showed that hematological parameters can be used as predictors of malaria[38, 39, 42, 43, 44, 45]. Another study from India by Ekhart LM et al showed that patients with malaria tended to have significantly lower WBC counts than those who were malaria negative[45]. Similarly a good number of confirmed cases of malaria showed significant Leukopenia in a study by Darkley and Cook in 2009[46]. As in our research, leukopenia was defined using a threshold value of 4,000 cells/mm^3. Other research found Leukopenia in 22.1 percent of P. vivax infections and 18.4 percent of mixed infections [47]. Levels under 3,000 cells/L were found in 83 (12 percent) of experimental P. falciparum infections, while counts under 1,000 cells/L were found in 79. (9 percent) [48]. In the United Kingdom, leukopenia was reported in 7% of P. falciparum malaria patients[49].Tobon-Castano et al. found that the leukogram status of malaria patients unveiled that leukocyte subgroups were not uniformly allocated (KS, 0.001); leukocyte counts at diagnostic testing were normal in 79 percent (n= 698) of patients, while 18 percent (n= 157) had leukopenia; the median value for these cells was 6,100/L [50].

Several authors' studies, including those from Kenya, [19] sub-Saharan African countries [51], and Peru [52] indicated that Leukopenia was present in a substantial proportion of malaria patients.

Even though leukopenia has been reported in individuals with simple malaria, neutrophil leukocytosis is a common anomaly in patients with severe falciparum malaria who have a poor prognosis. Senthilkumaar P and Sarojini S [53] showed an increase in the total WBC count. This might be due to the different geographical area of study and the nutritional factors by which the infected patients develop their immunity towards the infection with high production of WBCs.

Different studies have different frequency of leukocytosis[17, 49, 50]. It is mostly reported in falciparum malaria. No such alterations were identified in our research study. A previous study done by Tobon-Castano A et al reported it in about 4% cases they were statistically non-significant (p > 0.05); and leukocytosis due co-infections was also not observed.[50]

Even though both Plasmodium falciparum and Plasmodium vivax infections have been documented to induce alterations in leucocyte counts, the magnitude of these alterations varies across authors for both species. The significantly greater connection of Leukopenia with P. falciparum malaria than with P. vivax malaria in the current study is coherent with some of the world's literature[35], whereas others observed no significant variation in leucocyte counts between P. falciparum and P. vivax infections over

the study period [52]. P. falciparum infected patients had a lower leucocyte count than P. vivax-infected patients, and both of these types of patients had lower leucocyte counting than non-infected patients, according to a previous study conducted in individuals visiting Thai and Peruvian outpatient malaria clinics in 1988 and 1999[17].

In a study assessing WBC counts in P. falciparum and P. vivax infected people of an endemic region at a health center in India, the average WBC levels were not significantly different [54]. In this study, WBC counts of 4000 cells/mm^3 were found in 10.7% of 112 P. falciparum infected people and 15.2% of 118 P. vivax infected people; but, contrary to our findings, WBC counts of 4000 cells/L were found to be more common in P. falciparum infected patients than in P. vivax infected patients. Another study showed that leukopenia was more common with P. falciparum infection than P. vivax infection among 81 US troops in Vietnam [55]. In two case studies from industrialized countries that included comparative analysis, WBC levels during P. falciparum and P. vivax infections[56] and during "non-falciparum" infections [57] did not differ substantially.

Due to the large number of factors, a broad range of results is to be expected. Even within relatively small sample studied here, variation may have reflected the timing of observations and the clinical stage of infections; the statics found in a cross-sectional survey may not fully reflect basic features [58]. Additionally, the disparities in the findings cannot be attributed to a single cause. As observed by Hacket, "everything there is about malaria is so moulded by local conditions that it becomes a thousand epochs." Differences in ethnic background, gender, and immunological response of studied patients, as well as complex, multiple, and incompletely understood pathophysiologic procedures causing hematological alterations in malaria, may be accountable for this heterogeneity. Future research will hopefully shed more light on the causes of WBC count fluctuation.

To conclude, our research reveals changes in WBC counts, i.e. leukopenia, and therefore suggests careful monitoring throughout therapy to restore normal levels. After a malaria infection has progressed to the convalescent phase, there have been some alterations in the dissemination of white blood cell lines (WBC) [59], and there is a concise normalizing trend in WBC counts after disease resolution [21], suggesting that WBC numbers can be used as an indicator for the disease progression and its management.

CONCLUSIONS

The present study indicates that decrease in total white blood cells occurs in patients having malaria and is more frequently seen with plasmodium falciparum malaria. The appearance of Leukopenia in acute febrile patients with a clinical suspicion of malaria raises the chances of malaria and should timely a more thorough exploration for the malarial parasite. Our goal is to promote the use of simple diagnostic techniques like the CBC, which allow for early detection of individuals at risk for clinical problems and the implementation of suitable treatment interventions, thereby enhancing patient outcomes.

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Complete Collecte data. Dr.Salman Khan, Dr. Zia Ullah and Dr. Muhammad Yousaf analyze results. They also helped in writing up various sections of the article.

Conflict of interest: The author declares no conflict of Interest

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