



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Parasitological research doi: 10.1016/S2222-1808(16)61112-X

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Hematological alterations and parasitological studies among infected patients with *Plasmodium vivax* and *Plasmodium falciparum* in Hail, Kingdom of Saudi ArabiaNabil Hasona^{1,2*}, Omar Amer³, Azza Raef⁴¹Department of Biochemistry, College of Medicine, Hail University, Hail, Kingdom of Saudi Arabia²Faculty of Science, Chemistry Department, Beni-Suef University, Beni-Suef, Egypt³Department of Clinical Laboratory Science, College of Applied Medical Sciences, Hail University, Hail, Kingdom of Saudi Arabia⁴Department of Parasitology, Animal Health Research Institute, Zagazig, Egypt

ARTICLE INFO

Article history:

Received 27 May 2016

Received in revised form 1 Jun, 2nd revised form 27 Jun, 3th revised form 29 Jun 2016

Accepted 7 Jul 2016

Available online 15 Jul 2016

Keywords:

Parasite

Complete blood count

Predictor

Diagnostic

Malaria

Hematological

Smears

ABSTRACT

Objective: To investigate hematological alterations and parasitological studies among infected patients with *Plasmodium vivax* and *Plasmodium falciparum*.**Methods:** The present study was conducted from 1st September 2014 to 30th November 2015. A complete blood count, blood smears and malaria rapid diagnostic test were done for each patient.**Results:** There were 30 infected patients. These patients were infected *Plasmodium vivax* (20%) and *Plasmodium falciparum* (5%). Their age ranged between 20–60 years. The patients had severe malaria. There was a significant reduction in hemoglobin, platelet count, leucocyte and erythrocyte levels in infected patients caused by malaria compared with those of healthy control subjects. The percentage of neutrophil cells in the infected subjects was significantly higher than in the healthy group. The percentage of lymphocytes in the infected patients was significantly lower than in the healthy group.**Conclusions:** This study showed that results of complete blood count can provide a diagnostic predictor for increasing the prospect of malaria and enhancing quick treatment.

1. Introduction

Malaria is a foremost warning to global health. Malaria is among the most important of six diseases on the World Health Organization/Tropical Disease Research list[1]. Generally, a predictable 3.2 billion people are at risk of being infected with malaria and 1.2 billion are at high risk[1]. Malaria is a mosquito-borne parasitic infection caused by protozoa of the genus *Plasmodium*[2].

Plasmodium falciparum (*P. falciparum*) and *Plasmodium vivax* (*P. vivax*) are the two main types of malaria infecting beings. While significance on *P. falciparum* is apposite and the affliction of vivax malaria should be given due consideration as almost 40% of the world residents are at risk of vivax malaria[3]. *P. vivax* malaria is

predominant in many areas of the world, Asia and Latin America. It accounts for more than half of all the malaria cases[4].

World Health Organization commends that all publics of all ages in all epidemiological locations with doubted malaria should have a verification of diagnosis in every case of malaria either by microscopy or rapid antigen detection test. Microscopy is the gold standard for diagnosis of malaria and identification of species and also a standard to assess the degree of parasitemia.

Complicated malaria described as serious organ failures or anomalies in the patient's blood, usually arises in *P. falciparum* malaria. Signs of severe malaria comprise cerebral malaria, severe anemia, hemoglobinuria, thrombocytopenia, cardiovascular collapse and shock, acute kidney injury, metabolic acidosis and hypoglycemia[5]. In contradiction of falciparum malaria, vivax malaria is infrequently accompanying with serious complication[5]. Thrombocytopenia is a public signal found in patients with *P. falciparum* infection, but it has been recently stated in *P. vivax* or mixed infection cases in several widespread nations[6].

Hematopoiesis is one function of the spleen. The changes in the structure of the spleen during malaria infection can disturb hematopoiesis, which will decline the platelet count in the blood due to decreased production[7].

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The study protocol was performed according to the Helsinki Declaration and Institutional ethics committee approval was obtained from college of Medicine, University of Hail, Saudi Arabia.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

Prompt and early diagnoses are important for effective management in malaria. However, our knowledge of hematological profile of malaria endemic population of Hail and its relation to promising biochemical diagnostic potential and monitoring in malarial patients are limited. Thus, we investigated the hematological alterations in the persons infected with *P. falciparum* and *P. vivax* and compared with healthy subjects from Hail community. This study will provide a credential signs in understanding malaria pathogenesis and diagnosis.

2. Materials and methods

The study protocol was performed according to the Helsinki Declaration and institutional ethics committee approval was obtained from College of Medicine, University of Hail, Saudi Arabia.

The present study was a prospective observational study done in the Department of Clinical Laboratory Science, College of Applied Medical Science, Hail University, Kingdom of Saudi Arabia. The duration of the study was for a 2-year period from 1st September 2014 to 30th November 2015. Institutional ethics committee approval was obtained. The study population included for detection of malarial parasite was reported ill with fever, headache, vomiting and other clinical signs. The study included patients of age 20–60 years and both sexes with blood film in proved malaria and all the patients provided informed written permission before ingoing the investigational practice.

The study involved patients with definite diagnosis of *P. vivax* and *P. falciparum* with negative serology for hepatitis, HIV, syphilis and dengue. The healthy group was composed of healthy persons who were negative for malaria parasites as determined thick blood smear and had not been described any malaria episodes for at least 1 year. The blood smears were made and stained with Giemsa stain.

The intention of the diagnosis of malaria infection was to carry out treatment, therefore a rapid diagnostic test was made at the time of admission. Blood samples were collected from 90 individuals with negative for *P. vivax* or *P. falciparum* and 30 individuals with *P. vivax* or *P. falciparum*.

2.1. Hematological analysis

Whole blood samples were collected in ethylene diamine tetraacetic acid tube for determination of hematological parameters using automated (SYSMX.KX-21n) hematology analyzer.

2.2. Statistical analysis

The SPSS for Windows, version 18.0 (SPSS Inc, Chicago) was used for the statistical analyses. Results were expressed as mean \pm SE and values of $P < 0.01$ and $P < 0.001$ were considered highly and very highly significant, respectively.

3. Results

A total of 120 patients were involved in the study. Of these, 30 were diagnosed to have malaria confirmed by the appearance of malarial parasite on blood examination and by rapid diagnostic test. It was shown that 24 patients had *P. vivax* infection and 6 patients had *P. falciparum*, and the predominant species were *P. vivax* where the percentage of *P. vivax* was 20%, while the percentage of *P. falciparum* was 5%.

The diagnosis of the malaria parasite in the blood samples was confirmed by observing the various stages of the malaria parasite in the stained blood film under a binocular microscope.

3.1. Thick and thin blood film in a case of *P. falciparum*

P. falciparum rings had slight cytoplasm and one or two small chromatin spots. Red blood cells (RBCs) that were infected were not swollen, and numerous infections of RBCs were more common in *P. falciparum* than in other species. Infrequent appliqué forms were shown in Figure 1A, B.

P. falciparum gametocytes were falcate formed (Figure 1C, D). The chromatin was in a single mass or disseminates.

P. falciparum schizonts were rarely seen in peripheral blood. Mature schizonts had 8 to 24 small merozoites. Dark stain clumped in one form (Figure 1E).

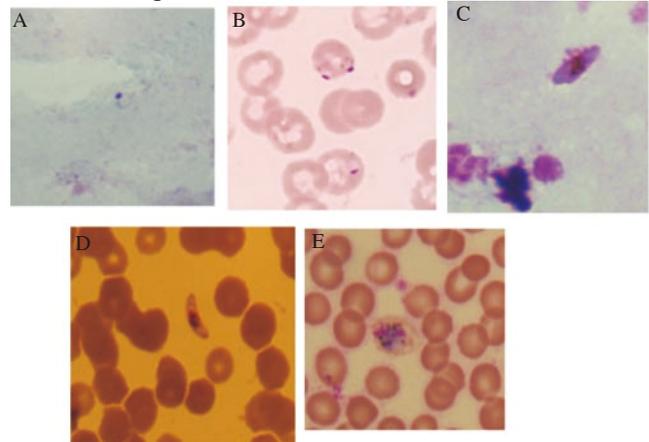


Figure 1. Thick and thin blood film in a case of *P. falciparum*.

3.2. Thin blood film in a case *P. vivax*

P. vivax rings had huge chromatin spots and cytoplasm became amoeboid as they grow. RBCs were normal to swollen in size and were be distorted (Figure 2A).

P. vivax gametocytes were circular with dispersed brown pigment and almost filled the RBCs. Schüffner's spots showed finer in Figure 2B.

P. vivax schizonts were large, had 12 to 24 merozoites, yellowish-brown, coalesced dye, and filled the RBCs as shown in Figure 2C.

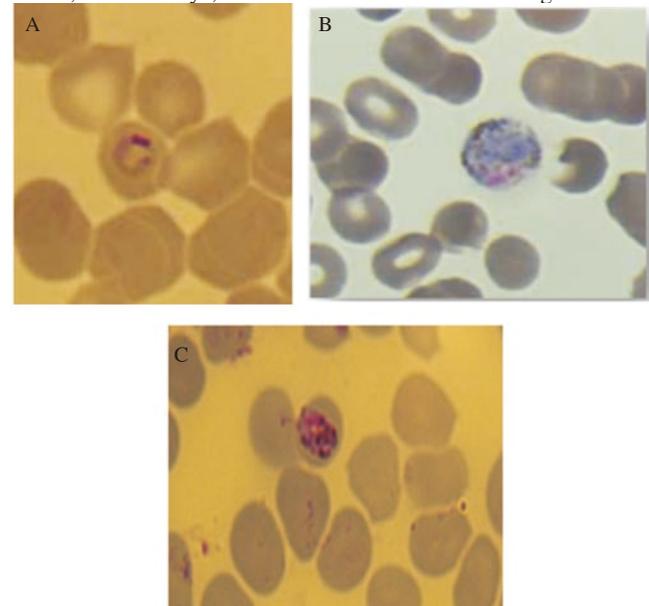


Figure 2. Thin blood film in a case of *P. vivax*.

3.3. Rapid diagnostic test

Figure 3A showed negative control (no malaria antigens were detected). Figure 3B showed positive result for *P. falciparum* (*P.*

falciparum antigens were detected). Figure 3C showed positive results for *P. vivax* (*P. vivax* antigens were detected).

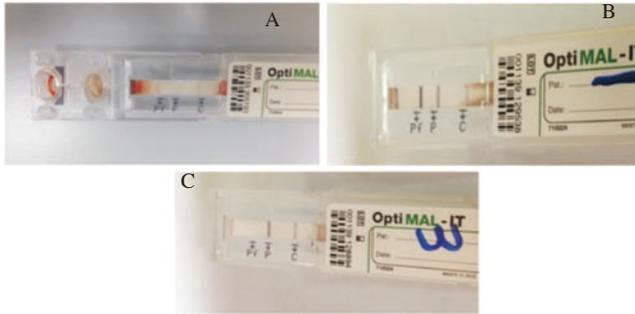


Figure 3. Rapid diagnostic test.

3.4. Complete blood count analysis

Hematological examinations were performed in healthy control and patients infected with *P. falciparum* and *P. vivax* (Table 1).

Table 1

Assessment of hematological markers between healthy and subjects infected with *P. falciparum* and *P. vivax*.

Parameters	Healthy control	Infected with <i>P. falciparum</i>	Infected with <i>P. vivax</i>
Hb (g/dL)	12.08 ± 0.10	9.65 ± 0.26***	9.47 ± 0.14***
WBC (10 ³ /μL)	6.94 ± 0.13	4.21 ± 0.35**	3.45 ± 0.12***
Lymphocytes (%)	40.00 ± 0.31	26.00 ± 0.58***	28.21 ± 0.54***
Neutrophils (%)	50.00 ± 0.41	69.83 ± 1.45***	68.00 ± 0.65***
Platelets (× 10 ³ /μL)	309.38 ± 2.64	104.43 ± 3.91***	163.83 ± 1.21***
RBC	4.72 ± 0.03	3.16 ± 0.25**	3.79 ± 0.04***

All values were expressed as mean ± SE. **: $P < 0.01$; ***: $P < 0.001$; WBC: White blood cell.

There was a significant decline in hemoglobin, platelet count and erythrocyte (reactive mesothelial cells) ($P < 0.001$) levels in infected patients compared to healthy control subjects. The percentage of neutrophil cells in infected subjects was significantly higher ($P < 0.001$) than in the healthy group. The percent of lymphocytes in the patients with malaria was significantly lower ($P < 0.001$) than in the healthy group.

There was a significant reduction in total leucocyte (WBC) in patients infected with *P. falciparum* and *P. vivax* respectively as compared to healthy ones.

4. Discussion

Malaria is a prime health issue in the tropical and temperate areas all over the world which affects a significant affliction on health cost. Universal efforts to enucleate malaria are being menaced on a matchless scale by quickly increasing resistance of *P. falciparum* to traditional antimalarial medications[8]. Prompt diagnosis and rapid treatment remain the mainstay for the control of the disease[8]. In Kingdom of Saudi Arabia, malaria is a non-endemic disease with exclusion of Gizan and Asir area. Though, some imported malaria cases were detected in non-malarious regions in Saudi Arabia among expatriates and imported malaria has become a significant clinical and communal health problem[9,10].

The universally conventional gold standard diagnostic technique for identifying malaria was microscopic evaluation of Giemsa-stained thick and thin blood smears. Microscopy constrained in malaria endemic regions are the need for skilled laboratory technicians, good quality reagents, and well-maintained microscopes as well as its time consumption[11].

Malaria rapid diagnostic tests are another diagnostic technique

for endemic areas, where microscopy has not been executed, as well as for non-endemic countries, where they are able to complement microscopy in screening febrile travelers[12].

P. falciparum blood smears are described by the manifestation of young trophozoites in the nonappearance of mature trophozoites and schizonts. The ring stages of *P. falciparum* tend to be smaller than the other species and are commonly more abundant. Multiply infected erythrocytes and appliqué forms are seen more often in *P. falciparum* than in the other species. The crescent-shaped gametocytes of *P. falciparum* are very distinguishing but tend to only appear late in the infection. Our results are in agreement with Josling and Llinás[13].

The characteristic features of *P. vivax* are the distended infected erythrocytes and the presence of granules called “Schüffner’s dots” over the erythrocyte cytoplasm. These granules are an indicator of caveola-vesicle complexes that form on the erythrocyte membrane. The developing trophozoite of *P. vivax* frequently has an ameboid appearance and the schizonts can have more than 20 merozoites. Our results are in accordance with Anderson *et al.*[14].

In malarial infection, erythrocytes are the principal target of the parasites leading to various changes in the infected RBCs after entering an erythrocyte. The growing malarial parasites change the RBC membrane and subsequent membrane protrusion help in the process of adhesion resetting and agglutination, which are central to the pathogenesis of falciparum malaria. The severity of malaria shows a variable degree of clinical manifestation and is mediated by transmission intensity[15].

Hematological anomalies are reflected a trademark of malaria and statistical investigation has revealed that hematological results may lead to an increased clinical doubt for malaria, consequently initiating a quick organization of specific remedy even in the nonexistence of a positive smear report for malaria.

Prediction of the hematological alterations assists the physician to begin an operative and prompt therapeutic intervention in order to inhibit the incidence of main complications. These investigations are assessable guides of blood that aid as an indicator for disease diagnosis.

Alterations in hematological markers are perspective to be influenced by any disease disorder which disturbs the physiology of hematopoiesis. Hematological deviations reflected the trademark of malaria infection are common and more pronounced in *P. falciparum* malaria infection, which may be due to the higher levels of parasitemia found[16].

The present study observes a significant reduction in the hemoglobin level in patients infected with *P. vivax*, *P. falciparum* as comparing with healthy subjects (Table 1). This observation is consistent with a previous report that *Plasmodium* infection is one of the commonest causes of hemoglobin degradation resulting in anemia and correlates with the severity of infection, particularly due to *P. falciparum*[17]. Further, the possible causes of this reduction may be due to increased erythrocyte breakdown or a declined level of erythrocyte assembly[18].

WBCs are responsible for body defense. In this study, the leucocytes count was significantly lower compared with that of the healthy controls. This is in agreement with the results of Jairajpuri *et al.* and van Wolfswinkel *et al.*[19, 20].

Leukopenia in malaria can be initiated by the collaboration of several events. It has been suggested that the sequestration of leukocytes causes the decline in WBC counts more than a decreased production or accelerated destruction[21]. Glycosylphosphatidylinositol, an immunogenic antigen common to all species of *Plasmodium*, stimulates the assembly of proinflammatory cytokines in monocytes and macrophages which

would increase phagocytosis producing cell debris, RBC and WBC phagocytosis[22].

The current study observes a significant decline in lymphocytes count in patients infected with *P. vivax*, *P. falciparum* as compared with healthy subjects (Table 1). This is in agreement with the results of Jairajpuri et al.[19].

Lymphocytopenia conveyed by an increase in the neutrophil is commonly seen in numerous infectious and noninfectious causes of systemic inflammation and stress[23].

The moderately large fall in peripheral lymphocyte level would propose this to be a non-definite effect, e.g. assembling in the inflamed spleens of patients instead of a response by malaria-specific lymphocytes. Others have jagged to the increased susceptibility of lymphocytes from malaria patients to undertake spontaneous apoptosis *in vitro*, maybe induced by soluble Fas ligand-Fas reaction[24].

The current study observes a significant decline in platelets counts in patients infected with *P. vivax* and *P. falciparum* as compared to healthy subjects (Table 1). This is in agreement with the results of Shaikh et al. and Khan et al.[25,26]. Previous studies have shown the relationship of thrombocytopenia to malaria but till date, the cause of thrombocytopenia is poorly understood.

Blood is the most simply manageable diagnostic tissue and hematological variations are the most commonest complications in malaria and they show a main role in malarial pathology. Prompt and early diagnoses are important for effective management of malaria. The hematological evaluation could help in prompt and accurate diagnosis and prevent disease progression by facilitating physicians in clinical correlation for better drug management. Most of the noticed malaria cases in Hail area were among expatriates while the predominant species were *P. vivax*.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are thankful to the Deanship of Scientific Research, Hail University, Kingdom of Saudi Arabia for supporting and providing all facilities to carry out this work.

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