



Analysis of hematological and biochemical parameters of *Plasmodium falciparum* infected patient from Hail region, Saudi Arabia: A case report

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ABSTRACT

Falciparum malaria is represented a great threat to public health globally as well as it associated with high morbidity and mortality. The study aims to evaluate the hematological alterations and parasitological studies of infected patient with suspected malaria signs. This prospective study was carried out on a case report at Hail General Hospital, and Faculty of Applied Medical Sciences, University of Hail, Saudi Arabia. Thin and thick blood films were used to identify *Plasmodium* species.

Results showed that the patient was infected with *Plasmodium falciparum*. Hematological changes associated with malaria include severe anemia and hyperbilirubinemia; however, white blood cells and platelets were within normal values. Based on the study, low RBC counts and haemoglobin level combined with malaria microscopy examination being most useful for predicting and diagnose of uncomplicated malaria but we cannot considered hematological parameters were an indicator of our case, therefore further evaluation of those parameters within large sample size should be undertaken.

Keywords: Hematology, Anemia, *Plasmodium falciparum*, Hail, Saudi Arabia

INTRODUCTION

Malaria is considered as the most widespread vector-borne parasitic infectious disease in the tropical and sub-tropical countries which incite the mortality and morbidity as well as it caused mainly by five *Plasmodium* species that included *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*,

and *P. knowlesi* (Osaro et al., 2014; Al-Awadhi et al., 2021). As well, *Plasmodium* mainly transmitted by blood-feeding female anopheline which injects infective sporozoites into skin of human, as their complex life cycle alternating between human hosts and the vector of mosquitoes (Bremar, 2009; Kuehn and Pradel, 2010).

However, infection with *P. falciparum* represented more than 90% of the global malaria mortality and thus it is still a relevant global threat to public health (Snow, 2015). Furthermore, an estimated of 781,000 deaths and 225 million cases of falciparum malaria were recorded annually (WHO, 2010). Moreover, *P. falciparum* infection is frequent associated with cerebral malaria and mortality that promote neurological sequelae (Schiess et al., 2020). Although clinical presentations are common in malaria diagnosis particularly in poor countries, however, those symptoms of fever are reasonable for malaria diagnosis due to lack of specificity as it is common with other illnesses making clinical diagnosis is more confront (Erhart et al., 2004). In addition, this is leads to over use of of antimalarial drugs in terms of reducing specificity which lowering patients healthcare quality (Reyburn et al., 2007). While, microscopic diagnosis continues to be the gold standard, most economical technique for malaria diagnosis, but its sensitivity and specificity for parasite detection are still weak particularly in endemic areas in Africa, as this technique needs a qualified technical expertise, consumes more time and deficient for a satisfactory health care issues (Reyburn et

al., 2007; WHO, 2009; Maina et al., 2010). Meanwhile, *P. falciparum* invades RBCs to develop intra-erythrocytic trophozoite stages which consumes more than 70% of red cell hemoglobin producing a crystalline of haemozoin (Tilley et al., 2011). Consequently, in patients infected with *P. falciparum* accompanied with a drop in hemoglobin (Hgb) level causing anemia especially in severe cases (Sakzabre et al., 2020). Hence, the malaria infections disturb haematopoietic physiology, thereafter altering hematological parameters that may associated with malaria parasitemia, hemoglobinopathy, nutritional status, demographic factors and malaria immunity (Price et al., 2001; Awoke and Arota, 2019). Global hematological abnormalities during malaria infections were recorded, whereas (Khan et al., 2012) reported that thrombocytopenia was more significant and common in *P. falciparum* malaria of Pakistan patients. Furthermore, (Awoke and Arota, 2019) recorded that the mean values of Hgb, Hct, platelets, WBCs, RBCs, and lymphocytes were significantly decreased in patients of malaria than negative individuals. Malaria mainly impacts the tropical regions as a consequence of adequate breeding conditions as high humidity, high temperatures, and large rainfall levels with

many stagnant waters, that promotes the life cycle of mosquitoes which spread the infections (Eze Evelyn et al., 2012). However, malaria outbreaks in Saudi Arabia occurred in 1998, but only 82 local cases were recorded by 2012 in terms of the elimination strategies (Coleman et al., 2014). Nonetheless, the southwestern parts of Saudi Arabia as Aseer and Jazan areas near the Yemen border are still have malaria cases (Hawash et al., 2019). In addition, (Memish et al., 2014) reported Saudi cases in Makkah were infected with *P falciparum* (69%) and *P vivax* (25%). Extensive research has shown that the hematological changes anticipate the clinician to build an appropriate and early therapeutic interference to avoid serious complications (Jairajpuri et al., 2014). Whilst, there are several studies have investigated the association between malaria and hematological parameters worldwide, only one study was carried out in Saudi Arabia by Hasona et al. (2016), thus more knowledge is necessary required to understand and to manage malaria diagnosis in that region. Therefore, the purpose of the current work was to investigate the hematological parameters of *P. falciparum* infected patient in Hail, Saudi Arabia as *Plasmodium* spp.

were identified using microscopy of blood films.

MATERIALS AND METHODS

Study design and Area

Case-control multicenter-based study was conducted among patient participants selected prospectively in January, 2018. Primary Care Centers were used to minimize the spectrum bias of referral Hail General Hospital contributing more severe cases with discrepant proportion of hematological alterations. An active case-finding network was organized with visits to participating centers to identify and interview the cases before any treatment was applied.

Inclusion and Exclusion Criteria

Physician-identified patient presenting with defining features consistent with malaria (history of fever with chills (axillary temperature 40.5 °C, sweating and headache) and referred to the laboratory for a malaria test. The tests served as an inclusion part of the study; however, informed consents were obtained from parents or guardians of the patients. The inclusion criteria of the study involved patients having symptoms of malaria with blood films were positive for *P. falciparum* to examine the hematological parameters changes among such patients as well as those recruiting patients who were treated in

Hail General Hospital of Saudi Arabia. Whereas, patients suffering from diseases other than malaria were excluded from the study. The included patients were then assessed by physicians, who documented the findings of clinical examinations, using the national guidelines for case management of malaria in Hail, updated to reflect standard WHO recommendations (WHO, 2001). The study included incident cases with *P. falciparum* confirmed infections; while healthy malaria negative controls of similar age, without parasitemia, assigned from the general population that selected the cases.

Case presentation

Suspected malaria in adult 42 years old male patient attending the hospital complaining from headaches, chills and abdominal pain during the study period. Furthermore, he was fully conscious and had an axillary temperature of 40.5°C. While, spleen and liver were palpable, there was sign of anemia. However, all other physical examinations were normal. Patient's history of recent travel to Sudan (his home country) for 2 weeks without antimalarial regime, was recorded.

Laboratory investigations

Sample Collection

For parasitemia, about 2–3 mL venous blood was drawn into potassium salt of EDTA tubes, then placed and transported

immediately on ice tank. Routine use of thick and thin films is recommended for diagnosis of malaria (Bailey et al., 2013). Therefore, two thin and thick films were prepared from whole-blood specimens and stained with Giemsa as outlined by (Petithory et al., 2005).

Parasite Density Estimations

To calculate malaria parasite density which depends mainly on White blood cells (WBCs) counts in thick films. Parasite densities were determined with absolute WBC counts as a ratio of *P. falciparum* counts relative to 200 WBC in thick films per slide. Five hundred WBC were counted where less than nine counted asexual stage *Plasmodium* parasites were counted after counting against 200 WBC. For *P. falciparum* counts ≥ 100 parasites per thick smear high power field, parasite counts were confirmed in thin films (against 2,000 red blood cells) and recalculated with 200 WBC (Adu-Gyasi et al., 2015). Parasites per μL of blood were calculated by using the formula: $\text{Parasite density}/\mu\text{L} = (\text{Number of parasites counted}/\text{WBC counted}) \times \text{WBC count}/\mu\text{L}$ of participant's whole blood.

Full blood counts

Complete blood counts (CBC) of patient participants were performed within an hour of sample collection using an automated

hematology analyzer (Sysmex KX-21N, Europe GmbH) according to manufacturer's instructions (Hasona et al., 2016). This Analyzer provided data on WBCs, RBCs and Hb. Commercial controls and quality assurance checks were performed on daily basis in accordance with the manufacturer's recommendations. Routinely, separate operators blinded to the results of the other assays performed all full blood counts (FBCs) in parallel with thin and thick blood films microscopy. On the other hand, other measurements including, liver function tests, blood glucose, blood chemistry were conducted as well as malarial peripheral smears, and reticulocyte count were withdrawn from the patient.

Validation

Parasitologist assessing blood slides, and data analysts remained unaware of case-control allocation until the end of the study. Whole blood samples were re-examined and crosschecked at Parasitology Laboratory at Department of Clinical Laboratory Sciences, Faculty of Applied Medical sciences for *P. falciparum* infections by expert parasitologist without reference to results of previous microscopy.

Ethical Consideration

This study was approved by the Ethical and Protocol Review Committee of University

of Hail, Applied Medical Sciences as well as signed informed consents were obtained from patients who agreed to be enrolled in the study. In addition, the current study was carried out at the Hematology Department of Hail General Hospital and parasitology laboratory at the Faculty of Applied Medical Sciences.

Data Analysis

Data were analyzed using IBM SPSS Statistics for Windows software version 21 with showing results as mean \pm SE. P-values <0.05 were considered statistically significant. The mean (\pm SD) values of the total WBC count, lymphocytes, neutrophils, eosinophils, monocytes, RBC count, hematocrit, hemoglobin, RDW, platelets, in malaria parasitemic patient of three samples versus normal values

RESULTS

Parasitological Studies

The current study revealed that the samples from patients with parasitemia were tested by microscopy where were suggested as *Plasmodium falciparum*. Based on their morphology, our findings showed that the majority of developmental stages were observed in periphery of Giemsa stained blood films. Early trophozoite of *P. falciparum* was appeared as rings that have delicate cytoplasm and one or two small

chromatin dots projecting from cytoplasm (Fig.1A). Moreover, multiple infected single erythrocytes was more common in *P. falciparum* without erythrocytes enlargement with little stippling. Infrequent appliqué forms (rings appearing on the periphery of the RBCs) were observed (Fig. 1B). Our results indicated that schizonts occupied about two third of the infected erythrocyte as well as a maximum number of 8 - 25 merozoites in mature schizonts was seen in the blood film with aggregates or clumps of dark pigments (Fig. 1C). Although schizonts are rarely seen in peripheral blood, however, presence of schizont in peripheral blood addressed

significant infections. The result, as shown in Fig. 1D, the detected gametocytes were crescentic or sausage in shape which is the usual form of mature gametocytes. Furthermore, with lysis of host cells, those gametocytes appeared as free with remnants of RBCs. While male microgametocytes tends to be thicker with pale blue cytoplasm and scattered pigments, however, female macrogametocytes appeared as elongate with blue stained cytoplasm and diffuse pigments. Besides this, various RBCs and WBCs were also deformed because of the given effect caused by malaria (Fig. 2A & B).

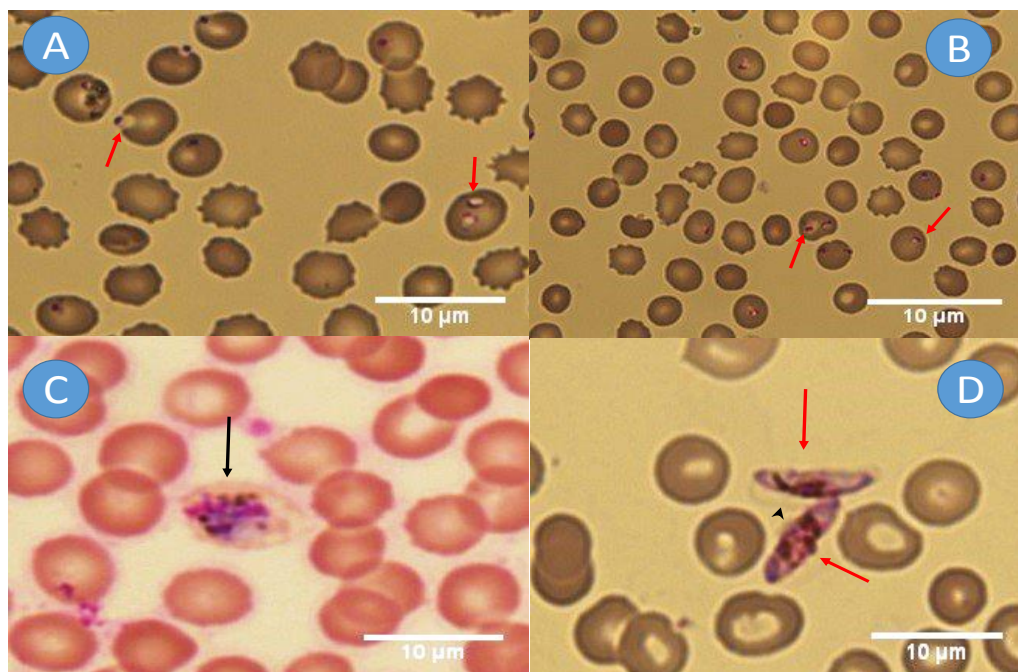


Figure 1: Morphological characteristics of *P. falciparum* parasites.

A. Ring form double chromatin dots (multiple infections) (Early trophozoite (arrows)). (X1000).
B. Trophozoites (X1000). **C.** Schizont (arrow) (X1000). **D.** Gametocytes (arrows); remnants of host cell (arrow head). (X1000). (Bar scale = 10 µm).

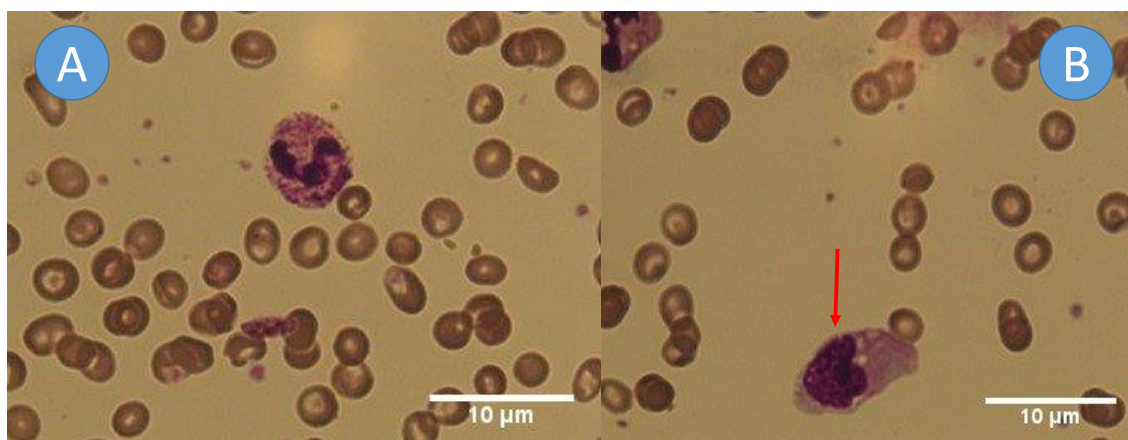


Figure 2: Deformed RBCs and WBCs due to *P. falciparum* disease. A. RBCs showing vacuolation. (X1000). **B.** WBCs Parasitized showing impair killing function of polymorphonuclear leukocytes (arrow). (X1000). (Scale bar= 10 µm).

Hematological Parameters

Our results revealed significant decline in RBC counts and hemoglobin level (Hb), while other hematological parameters were within normal (**Table 1**). Moreover, *P. falciparum* infection in our case was associated with a rise of bilirubin (Total and direct) and lactate dehydrogenase.

Table 1: Hematological laboratory findings of *P. falciparum* infected patient

Test	Patient' result	Normal values (Reference range) ¹
Hematology* (Means (±SD))		
RBC count (x10 ⁶ /µL)**	2.42±0.04	5.13±0.65
Hemoglobin (g/dL)**	6.23±0.11	14.1±1.51
MCV (fL)	79.07±0.13	87.4±8.44
MCH (pg/cell)	25.73±0.17	28.72±2.29
MCHC (%)	32.60±0.27	33.5±1.43
RDW (%)	15.60±0.05	12.3±1.13
Platelet count(x10 ³ /µL)	212±22.24	289±86.68
WBC count (x10 ³ /µL)	6.21±0.29	7.6±2.64
Differential cell count* (Means (±SD))		
Neutrophil %	59.14±0.97	54±13.57
Lymphocyte %	29.07±1.60	35.5±11.68
Monocyte %	10.20±0.49	7.5±2.64
Eosinophil %	1.44±0.19	4±2.26
Basophil %	0.16±0.01	0.5±0.38
Chemistry^a		
Glucose mg/dL	24.19	65-110
Creatinine mg/dL	1.24	0.6-1.2
Blood urea nitrogen mg/dL	10.1	7-21
Bilirubin, total mg/dL	21.3	0.2-1.3
Bilirubin, direct mg/dL	7.2	0.0-0.3
Lactate dehydrogenase (LDH) U/L	259	100-190
Alanine Aminotransferase U/L	30	10-40
Alkaline phosphatase U/L	61	38-126

*Mean values of three complete blood count (CBC) consecutive samples as well as mean values for reference normal range, which expressed as as mean ± SE., ** P < 0.05. ^aOnly one value for chemistry data.

¹Normal reference ranges (Cimo et al., 2005).

MCV: mean corpuscular volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red blood cell distribution width.

DISCUSSION

Malaria is a complicated disease whereas it is endemic in more than 90 countries including about 40% of the world's population as well as it infects both children and adults with a variety of clinical features between patients that range from asymptomatic uncomplicated to severe fatal malaria (Garcia, 2010; Wassmer et al., 2015). Although malaria is non-endemic in Saudi Arabia, therefore, the main purpose of the paper was to draw attention to update information about *Plasmodium falciparum* in infected patients especially in Hail province. To our knowledge this was a recent study evaluated the hematological changes during *P. falciparum* infections in Hail General Hospital of Saudi Arabia as only one study was conducted since few years ago by (Hasona et al., 2016).

The results of the current study indicated that the identified protozoan was *P. falciparum* relied upon microscopic analysis of blood film smears. This diagnosis was also supported by the patient's symptoms in terms of clinical investigation. Similar morphological identification was also reported by Field and Le Fleming (1940); and clinical features was similar as recorded by Albashir (2020). While malaria mainly associated with distinctive features of

hematological changes which concede a suggestive specific therapy, even in absence of positive blood smear for malaria (Jairajpuri et al., 2014), the current study revealed that RBC counts and Hb level were significantly lowered in the infected patient with falciparum malaria. These results were consistent with previous studies indicating that anemia were more frequent in patients with malaria (Muwonge et al., 2013; Tsoka-Gwegweni and Okafor, 2014). Although the mechanism of anemia is highly complex in regard of malaria as well as multifactorial and poorly explicated. However, this finding could be explained by insufficient erythropoiesis and increased elimination of both parasitized and nonparasitized erythrocytes during *P. falciparum* infections together with decreased erythrocytes deformability that removed from circulation by splenic phagocytosis (Dondorp et al., 1999). Further, Clark and Chaudhri (1988) added that tumor necrosis factor alpha (TNF) plays a significant contribution towards erythrophagocytosis and dyserythropoiesis during malaria.

The results of this study indicated that indices of red blood cell (MCV, MCH, MCHC, and RDW) were within normal

values. This finding was also reported by **Muwonge et al. (2013)** who suggested that uncomplicated malaria might correlated with low biochemical changes as less producing of cytokines and mild changes in coagulation profile. On the other hand, our study observed that platelets counts were within the normal range value. This finding is contrary to previous studies, which have advocated that thrombocytopenia and low platelets counts was an indicative finding of malaria in particular to acute infection (**Abro et al., 2008; Akinosoglou et al., 2012; Khan et al., 2012; Jairajpuri et al., 2014**).

This observation probably due to increased platelets depletion because of parasite multiplication than decreased production as well as reduced platelet life span and immune-mediated platelets complexes, however, the mechanism is still unclear (**Akinosoglou et al., 2012; Morrell, 2014**). Meanwhile, white blood cells play an essential aspect in terms of the defense against malaria infection (**Sakzabre et al., 2020**). The data of the present study showed that WBC counts, neutrophil, lymphocyte, monocyte, eosinophil and basophil were normal. These results seem to be consistent with other previous research as **Abro et al. (2008)** who found normal WBC counts,

neutrophil, eosinophil and basophil in 86%, 93%, 98% and 98% of the examined patients respectively. In addition, **Bashawri et al. (2002)** declared that majority of examined patients with malaria demonstrated normal values of WBC counts, neutrophil, eosinophil and basophil. Nonetheless, those findings don't support previous investigations by **Kotepui et al. (2014)** who found leukopenia with low neutrophil and lymphocyte counts in malaria infected, However, **Maina et al. (2010)** demonstrated significant higher neutrophil and monocyte counts in the malaria-infected patients compared to the non-malaria individuals. Whilst, it is difficult to explain such results, but neutropenia associated with malaria in several previous results probably attributed to changes in intravascular granulocyte distribution and margination of neutrophil (**Dale and Wolff, 1973**). However, with low sample size, results should interpreted with caution, which our analysis demonstrated poor sensitivities with some hematological parameters that related to normal data in parasitemic individual which recorded mainly with uncomplicated malaria case as in our study than severe cases in other studies (**Muwonge et al., 2013**).

With respect to blood chemistry analysis, our study showed that the examined patient has suffered from severe hypoglycaemia. This observation agreed with findings obtained by **Thien et al. (2006)** who described a hypoglycaemia as a complicated defining feature of severe *P. falciparum*. Further, the mechanism of hypoglycaemia during malaria infection is still not fully indiscernible (**Dekker et al., 1997**). Nevertheless, other researchers claimed that hypoglycaemia probably associated with increased glucose utilization and consumption by *Plasmodium* parasite, which reached to 20% in uncomplicated malaria cases (**Binh et al., 1997**); impaired in glucose production and inhibition of hepatic gluconeogenesis as well as fasting and starvation probably a main risk factor (**Thien et al., 2006**).

On the other hand, the present study recorded a rise in serum lactate dehydrogenase (LDH) than normal values. These results reflect those of **Garba and Ubom (2005)** who also observed increased in serum LDH above normal levels with patients with uncomplicated acute *P. falciparum*. Nevertheless, elevated of LDH is associated with diseases of liver, kidney, myocardial as well as red blood cells destruction, therefore our observed increase

of LDH could be attributed to hepatic damage due to invasion of sporozoites as well as destruction of red blood cells and subsequently releasing of LDH into circulation (**Garba and Ubom, 2005**). Further, the current study indicated hyperbilirubinemia with *P. falciparum*. In accordance with the present results, previous studies done by **Anand and Puri (2005)** and **Abro et al. (2009)** have demonstrated that malaria associated with jaundice and in an endemic areas, about 2.5% of patients with *P. falciparum* suffered from hemolytic jaundice. While, **Harris et al. (2001)** added that jaundice with direct hyperbilirubinemia and increased liver enzymes was recorded in 72% of examined patients with falciparum malaria indicating hepatocellular damage. It may be thus jaundice due to intravascular hemolysis of parasitized and non-parasitized red blood cells with unusual malarial hepatitis (**Anand and Puri, 2005; Al-Salahy et al., 2016**).

Effective control strategies have eliminated local malaria transmission in most Gulf Cooperation Council (GCC) countries of Arabian Peninsula, which are free from indigenous cases at present except some regions within Saudi Arabia that have locally and imported malaria cases (**Al-Awadhi et al., 2021**). Therefore, the present

study case offers a good model to gain better understanding the dynamics of eliminations programs in terms of growing numbers of people travel to and emigrate from endemic malaria areas to Saudi Arabia particularly of poor countries of Southeast Asia and Africa. While malaria is still represented as a diagnostic and treatment problem for clinicians in Saudi Arabia, our outcome did not significantly predicting changes of all the hematological parameters during malaria except for anemia. However, regarding to the current investigation, we assumed that the obtained hematological results mainly related to uncomplicated malaria case in non-endemic areas. Wherefore, further hematological and molecular studies are necessary required to investigate *Plasmodium* spp. in other areas in Saudi Arabia with large sample size to evaluate different diagnostic tools that would helping for better drug choice.

CONCLUSION

The study highlighted changes in hematological parameters caused by *P. falciparum* in a case study in the Hail of Saudi Arabia, which demonstrated anemia with normal values of other parameters as platelets, and WBC. This finding probably provide a diagnostic predictor for uncomplicated malaria. Therefore,

physicians should be attention of reported persons recently returned from endemic areas as well as communication of public health authorities in Saudi Arabia is essential to forcible control of possible transmission.

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الملخص العربي

تحليل المعلمات الدموية والكيميائية الحيوية لمريض مصاب بالمتصورة المنجلية في منطقة حائل ، المملكة العربية السعودية

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تمثل الملاريا المنجلية تهديداً كبيراً للصحة العامة على مستوى العالم فضلاً عن ارتباطها بارتفاع معدلات الوفيات. حيث تهدف هذه الدراسة إلى تقييم التغيرات الدموية والدراسات الطفيلية لمريض مشتبه بإصابته بأعراض الملاريا. أجريت هذه الدراسة الاستباقية على تقرير حالة في مستشفى حائل العام ، وكلية العلوم الطبية التطبيقية ، جامعة حائل ، المملكة العربية السعودية. حيث تم استخدام المسحة الدموية الرقيقة والسميكة لإجراء التشخيص الذي يؤكد وجود الطفيل بالإضافة إلى تحديد أنواع المتصورة.

وقد أظهرت النتائج إصابة المريض بالمتصورة المنجلية. حيث شملت التغيرات الدموية المرتبطة بالملاريا على فقر الدم الوخيم وفرط بيليروبين الدم. ومع ذلك ، كانت خلايا الدم البيضاء والصفائح الدموية ضمن القيم الطبيعية. بناءً على هذه الدراسة ، كان انخفاض عدد كرات الدم الحمراء ومستوى الهيموجلوبين جنباً إلى جنب مع الفحص المجهرى للملاريا أكثر فائدة للتنبؤ بالملاريا غير المعقدة وتشخيصها. ولكن على الرغم من ذلك ، لا يمكننا اعتبار هذه المعلمات الدموية دليلاً قاطعاً لتشخيص الإصابة في هذه الحالة ، لذلك يجب إجراء مزيد من التقييم لتلك المعايير من خلال إختيار حجم عينة كبيرة وجمع البيانات عنها.