Supportive presumptive diagnosis of *Plasmodium vivax* malaria

Thrombocytopenia and red cell distribution width

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ABSTRACT

Objectives: To evaluate hemoglobin, leukocyte, platelet counts and red cell distribution width values during acute vivax malaria.

Methods: This study, which comprises 90 symptomatic vivax malaria patients compared with 52 healthy controls, investigated hemoglobin, leukocyte, platelet counts and red cell distribution width values during acute disease prior to the treatment in vivax malaria, from May 2002 to December 2004 in Adana, Cukurova region, located in the southern part of Turkey, along the Mediterranean coast.

Results: Mean values for hemoglobin, leukocyte and platelet counts in the vivax malaria group in our study were found to be significantly lower in comparison to the control group. Anemia and thrombocytopenia were also observed in the malaria group while not in the control group ($p<0.05$, $p<0.0001$). Mean red cell distribution width values were found to be significantly higher in the malaria group ($p<0.0001$).

Conclusion: Our findings indicated that routinely used laboratory findings such as low hemoglobin, leukocyte or platelet counts and especially high red cell distribution width values could present a more supportive clue in the diagnosis of vivax malaria in endemic areas.

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Malaria, being a cause of high mortality and morbidity all over the world, is still an important public health concern with emphasis in both primary and secondary prevention. *Plasmodium vivax* (*P. vivax*) malaria is an endemic infection in southern part of Turkey and is commonly considered a benign condition.[1] The diagnostic gold standard in malaria has been peripheral blood smear,[2] but it carries the disadvantage of requiring experienced staff and being time-consuming.[3] Other objective diagnostic methods include immuno-fluorescent antibody (IFA),[4] polymerase chain reaction (PCR),[5,6] Western blot[7] and immunoblot tests.[8] Interests have been consequently directed toward more rapid and objective tests like dipstick tests,[9,10] as these recently introduced, immuno-assay based tests are easily performed and interpreted without need for complex equipment or technical support. But in spite of their rapidness, the routine use of these tests is not cost-effective in all patients with suggesting symptoms like fever and chills, especially in endemic countries, which are not economically developed. Additionally, while dipstick tests are comparable with a microscopic observation of a thin blood smear using a 100x immersion objective for a period of approximately 30 minutes, a parasite density of over 88/mm^3 has been reported to be required for the diagnosis of malaria.[10] As in cases of vivax malaria, which can present with a low parasitemia, their sensitivities tend to decrease. Hence, clues from routine blood testing can be very valuable, as these are not only routinely performed in all patients applying to a health center, but they do not require an additional finance, either; taking into account that the financial evaluation is one of the main determinants of health planning in the World of today. As the hematological system is one of the main targets of malaria, thrombocytopenia, anemia and leukopenia are commonly encountered in malaria, but not solely; raising need for more sensitivity and specifity.[11,12] While one approach could be the evaluation of these hematological parameters- not only separately, but also in combination; another one could be the introduction of a newer and more specific indicator,

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which we postulated as Red cell Distribution Width (RDW). It describes population dispersion of red cell volume;[13] or in other words, the range of changes in size of red blood cells,[14] which mostly present enlarged after malarial invasion.[15,16] Vivax malaria, which has a widespread prevalence and predominance in some parts of the world, like Turkey, remains to be less investigated when compared to falciparum malaria.

Therefore, we aimed to determine a cost-effective diagnosis of vivax malaria among untreated primary care patients in Cukurova region, Turkey, evaluating the hematological parameters from routine blood testing such as red cell distribution width, platelet counts, hemoglobin level and white blood cell counts.

Methods. The study population consisted of patients with *P. vivax* malaria, who were diagnosed between May 2002 and December 2004 and followed up at the Malaria Research Institute of Adana province, Turkey for a period of 3 years. In the study, 90 patients with *P. vivax* malaria infection and 52 healthy participants were investigated for hematological parameters. The mean age of the patient group (33.8 ± 18.6 years) was not statistically different from that of the control group (39.0 ± 15.0 years) (p>0.05), with a male to female ratio of 1.6 to 1. The inclusion criteria were: (i) the presence of recurrent fever, chills and perspire, (ii) the age to be 15 years old and older, (iii) the diagnosis of malaria proved by microscopic examination of intracellular parasites on thick, stained blood films, which is the current standard for definitive diagnosis in nearly all settings.[2] The exclusion criteria were: (i) the presence of an infectious disease other than malaria (*Leishmania, Salmonella* and *Brucella* species and HIV, which were all detected serologically or hematological abnormalities detected by thin smear), (ii) the presence of insufficient nutrition, verified by body weight and height (body mass index) or symptoms like geophagia, and others (iii) not being a local inhabitant in Adana (such as, being a visitor or migrant farm-workers, and others, as these would not represent epidemiologically the status in Adana). The diagnosis of malaria was made when the characteristic parasites of *P. vivax* were identified on peripheral thick and thin blood smears. All the peripheral blood films were examined by an experienced technician and confirmed by a parasitologist under the microscope at 100x magnification for the presence of malaria parasites. Peripheral blood films contain schuffner dots for *P. vivax*. The results were additionally verified using dipstick tests (OptiMAL® Rapid Malaria Test [Cat.No.710000, DiaMed AG, Switzerland] and serologically using immunoblot test for *P. vivax* merozoit surface protein (MSP) and circumsporozoit protein (CSP) [Malaria-Profile Euroline®, DN 2260-1601-1 G kit, Euroimmun Medizinische Labordiagnostica AG, Germany]. Five milliliters of blood were drawn from each participant for platelet and leukocyte counts, hemoglobin and red cell distribution width determinations using automated counter. Reference values for hematological parameters used in the study can be summarized as follows: platelet count below 150 x 10^9/l is defined as thrombocytopenia, hemoglobin levels below 13.5 g/dl for men; and 11.5 g/dl for women as anemia, total leukocyte counts less than 4000 cells/l as leukopenia, red cell distribution width values higher than 15% as abnormal distribution of red cells, such as anisocytosis.[17,18] The study protocol was reviewed and approved by the Faculty of Medicine Ethics Committee of the University of Cukurova, Adana, Turkey and informed consent was obtained for each participant. The parametric data were statistically compared using student's t-test.

All the participants who were diagnosed with malaria were prescribed with standard anti-malarial treatment regimen.

Results. In the malaria group the distribution of clinical manifestations at admission were fever (91.5%), chills (39%), headache (23.1%), and cold sore (6.7%).

Table 1 - Hemoglobin values, leukocyte counts, platelet counts and red cell distribution width values (mean ± SD) in vivax malaria patient group compared to the control group.

Parameter	Malaria group (mean ± SD)	Control group (mean ± SD)*	t-test	Significance*
Hemoglobin (g/dl)				
Males	12.5 ± 2.0	13.8 ± 2.0	2.29	p<0.05
Females	11.4 ± 1.7	12.5 ± 1.4	3.04	p<0.01
Leukocyte count (x10^9 cells/l)	6.2 ± 1.9	7.6 ± 2.2	4.07	p<0.0001
Platelet count (x10^9 cells/l)	133.2 ± 77.0	265.0 ± 80.4	9.63	p<0.0001
Red cell distribution width (%)	16.3 ± 3.7	14.1 ± 1.8	-3.85	p<0.0001

*statistically significant

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Hemoglobin values, leukocyte and platelet counts, and red cell distribution width values were determined in vivax malaria patient group and compared with the values of the control group. While hemoglobin values (separately for men and women), leukocyte and platelet counts were found to be lower in the malaria group than those of the control group, red cell distribution width values were found to be higher in the malaria group compared to the control group. All the differences were statistically significant (**Table 1**) (**Figures 1a to 1d**). Mean corpuscular volume (MCV) values were not found to be different between 2 groups; with 83.6 ± 11.7 fl and 84.5 ± 6.5 fl in the malaria and control groups, respectively (t=0.481, df=142, p=0.481). Taking into account the mean values of participants, patients in the malaria group were categorized to be in the anemia, leukopenia, thrombocytopenia and abnormal red cell distribution width groups, while control group members were not.

Discussion. Vivax malaria has been endemic in Turkey for a long time with *Anopheles sacharovi* identified as the vector. The prevalence of malaria in Turkey followed a decreased pattern between 1950 and 1975, compared to values exceeding 140,000 cases per year despite the lower population of the country before 1950. A sudden increase was observed at the end of 1970s, with values exceeding 100,000 cases per year. In 1957, a malarial eradication project with active and passive case detection combined with chloroquine-primaquine treatment of identified patients with malaria was initiated by the Turkish government in collaboration with the World Health Organization and

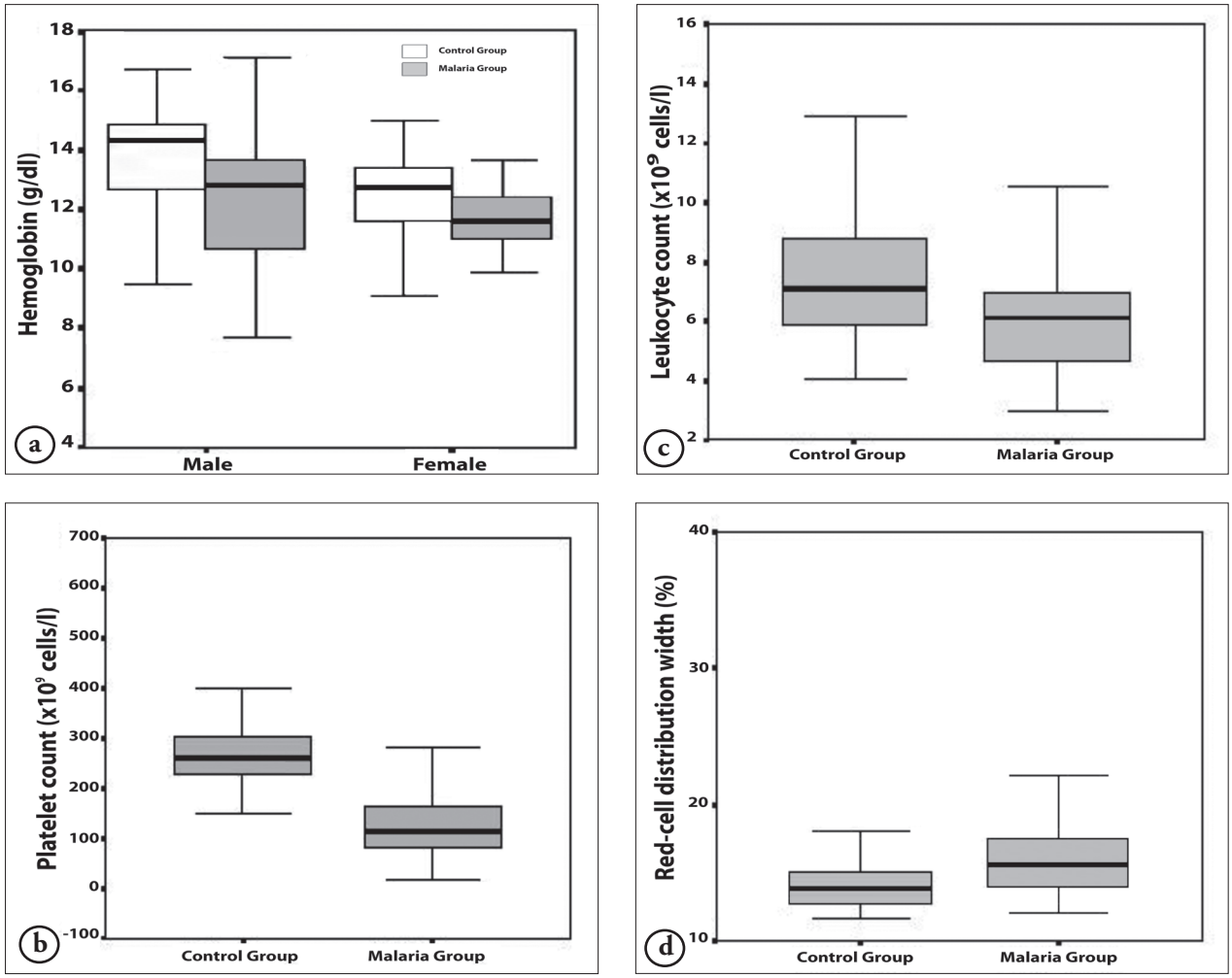

Figure 1- **a)** Hemoglobin values, **b)** platelet counts, **c)** leukocyte counts and **d)** red cell distribution width values in vivax malaria patient group compared to the control group.

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United Nations Children's Fund (UNICEF). Improved socioeconomic conditions and governmental malaria control activities succeeded to control the disease, although 2 peaks were observed in mid 1980s and mid 1990s with a resulting 10,224 cases reported at the end of 2002.[19] Duffy antigen prevalence in Turkey has not known entirely but duffy negativity rate in European population was 0%.[20]

A variety of hematological alterations like progressively increasing anemia, thrombocytopenia, leukocytosis or leukopenia have been reported in *Plasmodium falciparum (P. falciparum)* malaria and *P. vivax* malaria.[21-23] Besides the finding of lower mean values for hemoglobin, leukocyte count and platelet count in the vivax malaria group in our study, compared to the control group, anemia and thrombocytopenia were also observed in the malaria group while not in the control group. Lathia and Joshi[21]studied falciparum (64) and vivax (6) malaria patients and reported significantly lower platelet counts and lower hemoglobin levels compared to non-malaria patients with fever. Oh et al[12] reported anemia (51.5%), leukopenia (19.9%) and thrombocytopenia (85.1%) in 101 vivax patients. Erhart et al[24] reported a likelihood of malaria by 12-15 times in thrombocytopenic falciparum (414) and vivax (646) malaria patients with fever compared to febrile non-malaria patients, defining thrombocytopenia as the key indicator of malaria and they found significantly lower white blood cell counts and hemoglobin levels than those with no malaria infection. The mechanism of thrombocytopenia in acute malaria is not clear. While it was described by non-immunological mechanisms like Disseminated Intravascular Coagulation (DIC),[25] which is not supported anymore,[26] or direct interaction between plasmodium and platelets as *P. vivax* had been demonstrated by electron microscopy inside the platelets,[27] immune mechanisms were also reported. The peripheral destruction of platelets as well as red cells was explained by immune complexes like platelet-associated IgG antibodies (PAIgG) directly binding to malaria antigen on platelets[28] or by CD4T-cells as demonstrated in a mouse malaria model.[29]. Elevated recombinant macrophage colony-stimulating factor (M-CSF) in malaria, by enhancing macrophage activity, was also reported to be responsible for the increased macrophage-mediated platelet destruction.[30] Red cell distribution width appeared as a new parameter to be studied in vivax malaria. In contrast to our study, Lathia and Joshi[21] reported high red cell distribution width to have a poor sensitivity and specifity in the diagnosis of malaria. But this fact can be attributed to the fact that patients involved in that study were mostly (91.4%) affected by falciparum malaria. It has been demonstrated that in a period of 15 to 20 hours of infection of red cells by vivax malaria parasites the cells become noticeably enlarged and pale as the trophozoites grow to approximately half the size of the infected cells, accompanied by the appearance of brownish pigment granules. The increase in size continues during a following 24 hours and the parasites come very nearly to fill the infected cells. At the end of an approximate 48 hours the infected red cells rupture releasing merozoites capable of infecting new red cells. But as the cycle is never entirely synchronous, parasites at more than one stage of development will usually be seen in the blood smear, hence, red cells of different sizes. While infected red cells are enlarged in vivax (and also ovale) malaria in *P. malaria* and *P. falciparum* malaria they retain their original size.[15] This explains the role of increased red cell distribution width in vivax malaria in comparison to falciparum malaria.

As in much of the malaria-endemic world, resources and trained health personnel are so scarce that presumptive clinical diagnosis is the only realistic option. Our approach comprising routinely used laboratory findings such as hemoglobin, leukocyte or platelet counts and red cell distribution width values can present a more supportive clue in the diagnosis of vivax malaria.

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