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Table Of Contents

Journal Cover 1
Author[s] Statement 3
Editorial Team 4
Article information 5
Check this article update (crossmark) 5
Check this article impact 5
Cite this article 5
Title page 6
Article Title 6
Author information 6
Abstract 6
Article content 7

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Evaluation of IL-18 and TNF-? in patients with Plasmodium vivax Malaria infection in Diyala Province/Iraq: Evaluasi IL-18 dan TNF-? pada pasien dengan infeksi Malaria Plasmodium vivax di Provinsi Diyala/Irak

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Abstract

Malaria, caused by *Plasmodium vivax*, remains a significant health issue in northeastern Iraq. This study collected 5 ml of blood samples from 50 malaria patients and 50 healthy controls at Jalawlaa Hospital in Diyala Province. The infection rates were 13% in the 10-24 age group, 22% in the 25-39 group, and 15% in the 40-64 group, with no significant differences in gender or residency. Patients showed elevated levels of *P. vivax* IgG antibodies (2.14±0.17) compared to the control group (0.09±0.02), with significant increases in IL-18 (16.97±1.04 vs. 1.73±1.73) and TNF-α (18.45±0.81 vs. 1.31±0.31) levels (P≤0.0001). A strong correlation was found between *P. vivax* IgG antibodies and IL-18 and TNF-α levels (r=0.666, 0.730). ROC analysis showed excellent diagnostic accuracy for *P. vivax* IgG antibodies, with 98% sensitivity and 100% specificity at a cutoff value of >1.02. Additionally, a mutation in the TNF gene (SNP rs12934561) was identified, with wild TT and CC variations changing to CC, TT, and TC in patients compared to controls. These findings suggest that *P. vivax* IgG, IL-18, and TNF-α are valuable biomarkers for malaria diagnosis and highlight genetic variations linked to immune responses.

Highlights:

1. Malaria caused by *Plasmodium vivax* identified in northeastern Iraq, affecting various ages.
2. Patients showed high *P. vivax* IgG, IL-18, and TNF-α levels (P≤0.0001).
3. TNF gene mutation (SNP rs12934561) found, indicating genetic variation in immune response.

Keywords: IL-18, TNF-α, Plasmodium vivax, Malaria, Diyala Province/Iraq.

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Introduction

Malaria continues to be a major problem of global health in spite of different strategies performed by the governments and the World Health Organization (WHO). Malaria is still a main reason for death in several countries, such as Bangladesh [1]. Despite the difficult situation in Iraq, great development is performed in the malaria field. The past two indigenous malaria cases in Iraq were reported in 2008. Currently, Iraq is developing a national strategy of malaria for 2016–2020 [2]. The imported malaria is the infection which is acquired in malaria-endemic areas but diagnosed in non-endemic countries following clinical sign and symptom development [3]. Malaria parasite's movement through human's migrations or through population's movement from one area to another can lead to a spread of the disease to non-endemic countries or areas, in which malaria was formerly eliminated [4]. As anopheles mosquito vectors still exist in several non-endemic areas, thus secondary transmissions can be caused by imported cases [5]**.**In non-endemic countries,the death rate by malaria was 60 times higher than death rates in endemic countries, mostly due to the delayed diagnosis and treatments of patients, since in non-endemic countries, health professionals have little experience and orientation to deal with the cases [6]. Infection with malaria by Plasmodium vivax is more commonly prevalent than infection with malaria by Plasmodium falciparum, however, the complications and severity of malaria are more observed in P. falciparum infection [7]. As once believed, P. vivax malaria is not made a benign infection by the low blood-stage parasitemia 5–7. P. vivax causes significant morbidities and can be related to serious malaria and deaths, although low levels of the parasite are detected [5,8–20]. Remarkably, the primary treatments against these infections, chloroquine & primquine, are used continuously since 1952, in spite of worsening resistances to chloroquine & the operational insufficiency of primquine as the majority of patients with malaria are living and seeking for treatments [8]. Both the acquired and innate immune responses are stimulated by IL-18. IL-18 acts on macrophages, T-helper 1 (Th-1) cells, natural killer cells (NKCs), natural killer T cells (NKTCs), B-cells, dendritic cells (DCs) as well as non-polarized T-cells forproducing interferon gamma (IFN-γ) in the existence of IL-12 [9]. Receptors of TNF-α sTNFRI and sTNFRII, which are bound to the circulating TNF-α and regulate its activities, are positively correlated with parasitemia and malaria severity in the African P. falciparum disease [10]. It has been proposed that P. vivax provokes higher host inflammations per a parasitized red cell than P. falciparum which recently showed a bias towards regulatory cytokines in the Brazilian uncomplicated P. vivax malaria [11]. It is still uncertain whether this bias is translated into milder clinical signs and reduce the severe disease risks [11]. The study aimed to evaluate IL-18 and TNF-α in Plasmodium vivax Malaria patients.

M ethods

In this study, (5) ml of blood samples were taken from patients with malaria who were diagnosed with Plasmodium vivax and 50 samples were taken from healthy individuals as a control group, in Jalawlaa Hospital/ Diyala Province. By using ELISA technique, the anti- malaria IgG antibody's, IL-18, and TNF-α were determined. Molecular diagnosis for detection ofd TNF-α gene was performed by PCR technique, and the primers used in sequencing rs2430561 SNP gene were:

TNF-F: TGTAAAACGACGGCCAGTCGTTGCTCACTGGGATTT

TNF-R: CAGGAAACAGCTATGACCATGTCTTCCTTGATGGTCTC

Statistical analysis

For data analysis, the SPSS-20 program (Faculty version) was used including Mean ± SD with t-tests. The ($P < 0.05$) value was considered significant.

Results and Discussion

The demographical picture in table (1) showed that the infection rate with malaria in the age group (10-24) years was 13 (13.0%) compared to the healthy control 6 (6.0%) and in the age group (25-39) years was 22 (22.0%) in comparison with the control group 18 (18.0%), and in the age group (40-64) years was 15 (15.0%) compared to the control group 26 (26.0%) with no significant differences (P= 0.06).

Test		Case	Control	Total	P-value
Age (M±SE)		34.58±2.07	39.94±1.86	-	0.06
Age (Years) range	(10-24)	13 (13.0%)	6 (6.0%)	19 (19.0%)	0.06
	(25-39)	22 (22.0%)	18 (18.0%)	40 (40.0%)	
	(40-64)	15 (15.0%)	26 (26.0%)	41 (41.0%)	
Total		50 (50.0%)	50 (50.0%)	100 (100.0%)	
Sex	Male	26 (26.0%)	25 (25.0%)	51 (51.0%)	

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