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Evaluation of IL-18 and TNF-α in patients with Plasmodium vivax Malaria infection in Diyala Province/Iraq

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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..

Highlight :

- Malaria caused by Plasmodium vivax identified in northeastern Iraq, affecting various ages.
- Patients showed high P. vivax IgG, IL-18, and TNF-α levels (P≤0.0001).
- 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.

Materials and Methods

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

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).

Table (1): Demographical picture of the studied groups

Test		Case	Control	Total	P-value	
Age (M±SE)		34.58±2.07	39.94±1.86	-	0.06	N.S
Age range (Years)	(10-24)	13 (13.0%)	6 (6.0%)	19 (19.0%)	0.06	N.S
	(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%)	0.84	N.S
	Female	24 (24.0%)	25 (25.0%)	49 (49.0%)		
Total		50 (50.0%)	50 (50.0%)	100 (100.0%)		
Rural		25 (25.0%)	24 (24.0%)	24 (24.0%)	0.84	N.S

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Urban	25 (25.0%)	26 (26.0%)	26 (26.0%)		
Total	50 (50.0%)	50 (50.0%)	50 (50.0%)		

Accrding to the results, the ages of the infected patients ranged between 10 to 64 years, so there was no significant difference among them. While there are cases in children under the age of five who have very severe infections. Ranjha, et al, (2023) reported that the children, particularly under 5 years are highly vulnerable to malaria and account for 76 % of malarial mortality in the world in accordance with the World Malaria Report for the year 2022, since the younger people are susceptible to serious malaria type because of their immature immune systems. The infection risks and clinical diseases are increased following the weakening of maternal immunity. In the first years of human's life, the developing brains are more vulnerable to malarial infections and their consequences. Children may function as reservoirs for malarial transmission owing to the density of parasite and asymptomatic diseases [12]. But these results disagreed with (Okiring, et al, 2022) who proved the a female disproportionately participates in the malarial burden diagnosed in the facilities of public health in Uganda, particularly once this female reaches to the age of childbearing. Factors that contribute to this include more repeated visits to such facilities independent of malaria and higher reported risks of seeks for care at those facilities for febrile diseases [13]. Also there was no difference between rural and urban areas in the distribution of malaria cases in Jalawlaa district, and the reason is that the area is small and the rural area is close to the urban area and is almost integrated. Konate, et al, (2023) demonstrated that there are very high moral differences between infections in rural areas and urban areas in Mali [14].

The mean levels of P.vivax IgG antibodies in the patients (2.14±0.17) is higher than controls (0.09±0.02) with high significant differences P=≤0.0001. And the mean levels of IL18 in the patients (16.97±1.04) is higher than control group (1.73±1.73) with high significant differences P=≤0.0001. Also the mean levels of TNF-α in the patients (18.45±0.81) is higher than control group (1.31±0.31) with high significant differences P=≤0.0001, as shown in table (2).

Table (2): The mean levels of IgG, IL-18 and TNF-α between cases and control group

Test	Study	Mean	Std. Error Mean	T-test	P-value
P.vivax IgG	Case	2.14	0.17	11.58	≤0.0001 H.S
	Control	0.09	0.02		
IL18	Case	16.97	1.04	13.85	≤0.0001 H.S
	Control	1.73	1.73		
TNF-α	Case	18.45	0.81	19.66	≤0.0001 H.S
	Control	1.31	0.31		

H.S: Highly significant

According to the findings in our study, there was elevated levels of malaria IgG in patients in Jalawlaa/Diyala Province-Iraq, when compared to the control group. These results explained the difference between infected people and healthy people who have high levels of antibodies due to infection. Sandoval, (2021) reported that antibody levels are higher in people with malaria than in healthy individuals. This is due to the body's immune response against the invasion of this parasite [15]. However, the levels of IL-18 was increased the results matched with (AlRuwaisan et al, 2021) who demonstrated that many cells express the IL-18, such as Kupffer cell, macrophage, keratinocyte, osteoblast, astrocyte as well as dendritic cell (DC) [15]. IL-18 is a cytokine synthesized in the form of 24 kD pro-IL-18, which is biologically-inactive precursor peptide, then cleaved by caspase-1 into the biologically-active IL-18 [15]. The major salient biological characteristic of IL-18 which differentiates it from IL-1 is its capability for induction of high level of (IFN)–γ secretion in presence of IL-12 [16]. In addition, a considerable pool of intracellular cytoplasmic IL-18 exists and is regulated chiefly by the processing of caspase-1-mediated pro-IL-18 [16]. Moreover the tumor necrosis factor alpha levels are elevated below normal in malaria, This is what came Ademola, et al, (2023) who reported that the production of TNF is often increased by parasitic antigens when the schizonts rupture and merozoite are released from infected red blood cells. Malarial toxins are among some of the strongest TNF synthesis release inducers and release such as malaria pigments and glycosylphosphatidylinositol moieties. Due to the well-known cytotoxic activity of TNF's, it is initially assumed that it is the only cytokine responsible for direct parasite killing or within the cells of the host [17]. Also Hameed, et al, (2024) stated that TNF-α is important in the case of parasitic infection combined with viral infection [18]. Also, Mohammed et al., (2022) emphasized on the importance of cytokines in parasitic infections combined with viral infections [19].

Table 3 showed that the Mean±SE of P.vivax IgG antibodies in the rural areas was (2.20±0.23) in comparison with the urban areas (2.07±0.26) P=0.73, and the Mean±SE of IL-18 in the rural areas was (16.22±1.52) compared to the urban areas (17.72±1.44), P= 0.47. Moreover, the Mean±SE of TNF-α in the rural areas was (17.83±1.38) compared to the urban areas (19.08±0.86) p=0.44 with no significant differences.

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Table (3): The mean levels of IgG, IL-18 and TNF-α between cases and control according to residency

Test	Residency	Cases	P-value	Control	P-value
		Mean±SE		Mean±SE	
P.vivax IgG	Rural	2.20±0.23	0.73	0.03±0.006	0.07
	Urban	2.07±0.26		0.1±0.04	
IL-18	Rural	16.22±1.52	0.47	1.29±0.47	0.21
	Urban	17.72±1.44		2.14±0.48	
TNF-α	Rural	17.83±1.38	0.44	1.23±0.41	0.8
	Urban	19.08±0.86		1.39±0.47	

The results showed that there is no significant difference between rural and urban areas in terms of incidence. These findings does not match the incidence in some countries where malaria is more prevalent in rural areas than in urban areas (Thanh,. et al, 2021), [20]

The results of the current study showed that there was a direct correlation between the levels of P. vivax IgG antibodies with the levels of IL-18 and TNF-α, (r=.666,.730) respectively, These correlations were statistically highly significant (P=0.0001), whereas the results also indicated there was a negative correlation between the levles of P. vivax IgG antibodies with age (Years) with (r=-.028) and P=.786 as illustrated in table (4).

Table (4): Correlation analysis of IgG levels with the levels of IL-18 and TNF-α

		Age (Years)	P.vivax IgG	IL-18	TNF-α
P.vivax IgG	Pearson Correlation	-.028	1	.666**	.730**
	P-value	.786		.000	.000
	N	100	100	100	100
*. Correlation was significant at 0.05 level (2-tailed)					
**. Correlation was significant at 0.01 level (2-tailed)					

There was a direct correlation between IgG antibody levels to P. vivax with IL-18 and TNF-α levels, (r = .666, .730) respectively, and these correlations were highly significant. This is due to the occurrence of infection in patients with vivax malaria. Correlation indicates the high concentrations of both IL-18 and TNF-alpha, which are required to determine their levels in malaria infection and the extent of their influence on the severity of infection. These results agreed with (Hameed et al, 2024) who found a high correlation in levels of interleukins as well as TNF-alpha with parasitic infections [21].

The receiver operating characteristic curve (ROC) analysis was done to assess the diagnostic value of P. vivax IgG antibodies among malarial patients. The results of ROC analysis of P. vivax IgG antibodies in table (5) and figure (1) showed an excellent prediction of AUC value result for of P. vivax IgG antibodies with (P =.000) at cutoff value 1.0. The sensitivity was 98 % and specificity was 100% at optimal cutoff value more than 1.02 in patients compared to the control group.

Table (5): ROC analysis of P.vivax IgG between cases and control

Area	Cutoff	SE	P-value	95% C.I		Sensitivity	Specificity
				Lower Bound	Upper Bound		
1.000	1.02	.000	.000	1.000	1.000	98	100

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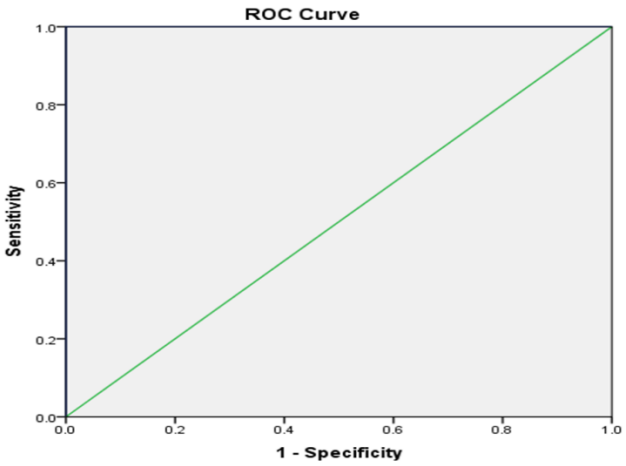
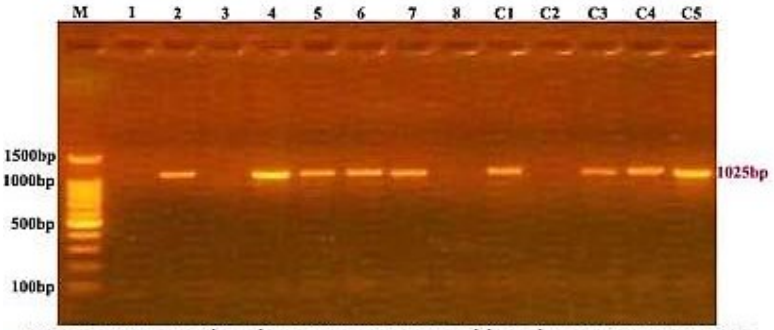


Figure (1): ROC analysis of P.vivax IgG between cases and control

ROC analysis is a strong tool to assess the diagnostic performances of an index test used for diagnosis of diseases or conditions. The value of AUC is a summary metric of ROC curves which reflect the test's capability to distinguish between diseased and nondiseased individuals. AUC values range from 0.5 to 1.0, with a value of 0.5 indicating that the test is no better than chance at distinguishing between diseased and nondiseased individuals. A value of 1.0 indicates perfect discrimination. AUC values above 0.80 are generally considered clinically useful, while values below 0.80 are considered of limited clinical utility. When interpreting AUC values, it is important to consider the 95% confidence interval and this confirms that the infection has occurred and that the levels of antibodies to Plasmodium vivax confirm the infection. (Çorbacıoğlu, et al, 2023), [22].

Figure (2) showed the amplification of specefic region of SNP, rs 12934569 for TNF-α gene in patients with Plasmodium vivax Malara.



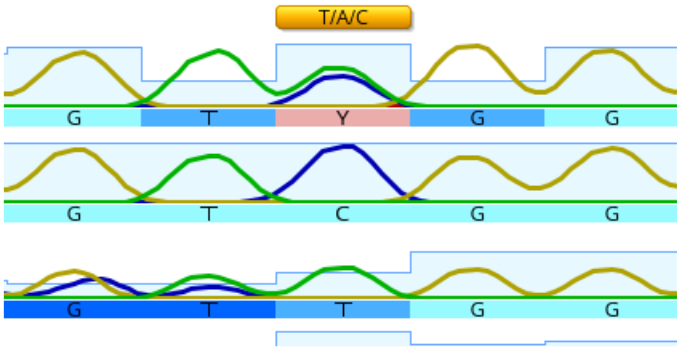
Results of the amplification of rs12934569 specific region of Human samples species were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-8 patients samples and C1-C5 as a control group resemble 1025bp PCR products

Table (6) and figure (3) showed a mutation occurred with TNF gene ID 9265 in SNPs, rs12934569. The variation of wild TT was changed to CC, and TC respectively compared to the control group.

Variation of wild SNPs of TNF-α gene ID 9265

TNF-α gene ID 9265	
SNPS	**rs12934569**
Wild	**TT**
Variation	**T>C**
Samples	**TC**
1	TC
2	TC
3	TC
4	CC

5	TC
6	TC
7	CC
8	TC
C1	TT
C2	TC
C3	TC
C4	TT
C5	TT



Analysis of rs12934569 SNP of TNF-α gene using Sanger sequencing. Single "T" peak indicative of a T homozygous allele. Single "C" peak indicative of a C homozygous allele. Presence of the "T" and "C" peak indicative of T/C heterozygous allele.

Kongjam, et al, (2023) reported that 8 eligible articles where TNF-α -308G >A polymorphisms were detected in uncomplicated malaria (UM) and severe malaria (SM) of P. falciparum as characterized in the case-control groups. Pooled odd ratios (OR) and 95% confidence intervals (95% CI) have been estimated in standard homozygous and recessive dominants [23]. Also, the current results is in a harmony with (Gichohi-Wainaina, et al, 2015) who showed the genotyping of 94.9% (581/612) children for TNF −1031 (TNF −1031T>C); allele frequency is 0.39. Also, the corresponding value for rs1800629 (TNF −308G>A) was 95.4% (584/612) and 0.17. in comparison with wild type genotype (TT), malaria rate elevated in TNF −1031CC genotype [24].

Conclusion

The results showed a highly concentrations in the levels of IL-18 and TNF-α with Malaria infected patients. However, there was a significant mutilation occurred in TNF-α gene sequence

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