

# Variations in key plasma microRNAs in *Plasmodium vivax* malaria patients in Iran

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Brown C. Variations in key plasma microRNAs in *Plasmodium vivax* malaria patients in Iran. *J Clin Microbiol Infect Dis*. 2022;5(2):13-14.

## ABSTRACT

Malaria is still a prevalent parasite illness throughout the globe, causing RBC infection and splenomegaly. To effectively diagnose malaria and elucidate its pathologic alterations, new specialized biomarkers such as MicroRNAs (miRNAs) are being created. The goal of this study was to look at changes in *Plasmodium vivax* plasma miRNA indicators in malaria patients in Chabahar, Iran. We took blood samples from 20 people for the current descriptive-analytical investigation, which took place in 2018. The plasma levels of miR-145, miR-155, miR-191, and miR-223-3p were measured using real-time

quantitative polymerase chain reaction (RT-qPCR). Patients with *P. vivax* had plasma levels of miR-223, miR-145, and miR-155 that were 5.6, 16.9, and 1.7 times greater than healthy people, according to the 2-CT technique of Real-time PCR. The expressions of all three miRNAs were substantially higher in malaria patients compared to controls (P<0.05). Although the difference was statistically insignificant, the expression of miR-191 was 1.405 times greater in malaria patients than in controls. *P. vivax* was discovered to alter host miRNAs such as miR-223, miR-145, and miR-155 in the current study. As a result, these tiny compounds proved to be biomarkers for detecting *P. vivax* malaria.

**Key Words:** MicroRNA; Malaria; *Plasmodium vivax*

## INTRODUCTION

Malaria is a serious parasite illness that affects people all over the world, with an estimated 219 million infected cases and 409000 fatalities in 2016. *Plasmodium* species such as *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, and *P. Knowlesi* cause human illnesses. *P. vivax* is a health issue since it is responsible for about 90% of malaria cases in Iran's south and southeast. The fact that *P. vivax* malaria might recur after weeks to months adds to its significance. Malaria is routinely diagnosed using Giemsa-stained thin and thick blood smears. The test's low sensitivity and specificity, as well as false-negative findings and incorrect *Plasmodium* species identification in patients with low-density malaria, delay therapy and increase the risk of complications if two *Plasmodium* species cause the illness. To detect malaria more efficiently, modern diagnostic technologies such as malaria antigen isolation, flow cytometry, antibody separation, and particular biomarkers such as MicroRNAs (miRNAs) were created. MirRNAs are also useful in determining the pathogenic alterations that occur in malaria. MiRNAs, which are short non-coding RNAs, regulate gene expression in cell development, differentiation, and death by blocking target genes on mRNA. The expression of miRNA is influenced by tissue type and pathological circumstances. Although miR-451 is expressed particularly in the circulating erythroid line, its expression in red blood cells is unrelated to *Plasmodium* growth in red blood cells.

In the endemic areas of India and Brazil, incidences of cerebral malaria or severe malaria with pathological sequelae caused by *Plasmodium vivax* mono-infection have been documented in recent years. A 60-year-old *Plasmodium vivax* patient was also diagnosed with acute respiratory distress syndrome. MicroRNAs can be utilized to distinguish between severe and mild *Plasmodium falciparum* malaria. Because of pathogenic alterations in malaria, the levels of several microRNAs have altered. In individuals with fatal cerebral malaria, *Plasmodium falciparum* raised plasma levels of hsa-miR-150-5p and hsa-miR-3158-3p compared to non-fatal malaria patients. When mice with cerebral malaria were compared to animals that were not infected with malaria, MiR-155-5p and MiR-223 were significantly dysregulated. *P. vivax*, like *P. falciparum*, may cause severe malaria and can be sequestered in the spleen, especially in silent patients; hence, microRNA can be utilized to differentiate between severe and asymptomatic cases. As a result, MicroRNA can be utilized to diagnose various malarial diseases. In plasma, extracellular miRNAs are quite stable. Many illnesses, including malaria, cause variations

in plasma miRNAs, which are used as non-invasive biomarkers to detect their detrimental consequences. During HIV/AIDS infection, the miRNAs miR-223 and miR-191 are downregulated in the peripheral blood mononuclear cells of HIV/AIDS patients. As a result, these miRNAs can be utilized to diagnose HIV infection as biomarkers. *Toxoplasma gondii* and *Leishmania* major infections upregulate miR-155 in host cells. MiR-145-5p was found to have a negative connection with the peak parasitemia in the hearts of mice 30 days after infection with *Trypanosoma cruzi*. Infections with *P. chabaudi* cause organ-specific changes in miRNA expression. In the spleen and liver of female C57BL/6 mice infected with *P. chabaudi*, MiR-145 is downregulated by 0.48-0.14-fold. In 2018, it was discovered that miRNAs have a regulatory function in the development of severe malaria. In malaria patients, plasma levels of miR-451 and miR-16 were shown to be lower. Furthermore, the involvement of miRNAs and gene expression in illnesses like malaria has received a lot of attention in the literature. Changes in miRNA plasma levels can aid in the diagnosis of severe malaria in *P. vivax* patients. The goal of this study was to look at variations in *P. vivax* plasma miRNAs in malaria patients in Chabahar, Iran.

Many illnesses, including viral, bacterial, and parasitic infections, have been linked to changes in miRNA expression. Infection with *P. vivax* causes alterations in the quantity of miRNAs in humans. *Plasmodium* infection altered various miRNAs in rat plasma, brain and hepatic cells, including miR-16, miR-27, miR-150, and miR-451. When comparing patients with complex *P. vivax* malaria to those with simple *P. vivax* malaria, a number of miRNAs, including hsa-miR-7977, were shown to be elevated. Anti-malaria drugs like chloroquine have been linked to alterations in miRNA levels in malaria patients. Furthermore, miRNAs can be used to track the pathophysiological condition of a patient and diagnose malaria. MicroRNAs like hsa-miR-4497, according to Gupta et al., can be employed for the early detection of severe malaria caused by *P. falciparum*, which can assist forecast and treat the disease more successfully. Furthermore, hsa-miR-3158-3p can be employed as a biomarker for the prognosis of *P. falciparum*-caused cerebral malaria in both children and adults. Biomarkers should have adequate accuracy, reliability, and detection capabilities in addition to high specificity and sensitivity. Given that miRNAs released in bodily fluids such as plasma may be identified after a long period and even after undergoing severe circumstances, these non-invasive biomarkers can be utilized to evaluate and monitor pathophysiological state. The connection of miRNAs in the serum, plasma, urine, and other bodily fluids with many disorders, including

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Received: 25-Feb-2022, Manuscript No. PULJCMID-22-4398; Editor assigned: 27-Feb-2022, Pre QC No. PULJCMID-22-4398 (PQ); Reviewed: 13-Mar-2022, QC No. PULJCMID-22-4398 (Q); Revised: 15-Mar-2022, Manuscript No. PULJCMID-22-4398 (R); Published: 18-Mar-2022, DOI: 10.37532/puljcmid.2022.5(2).13-14



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malaria, has been studied.

Mice infected with *Schistosoma japonicum* had high serum levels of miR-223, miR-122, and miR-34a, but low serum levels of miR-199a-3p, 199a-5p, and miR-146b. MiRNA levels in the blood can be used as a biomarker to diagnose parasite infections. The current study analyzed miR-191, miR-223, miR-145, and miR-155 as miRNAs with considerably elevated plasma levels in patients with *P. vivax* malaria, in line with prior infectious illness investigations. The levels of miR-191, miR-223, miR-145, and miR-155 were shown to differ between patients with *P. vivax* malaria and healthy people. In individuals with *P. vivax* malaria, mean plasma levels of miR-451 and miR-16 were shown to be significantly lower. There was also a modest negative connection between parasitemia levels and the four miRNAs studied. The strongest link between parasitemia and miR-233 ( $r = -0.23$ ) was discovered, although it was not statistically significant. Some studies have found a minor or substantial drop in plasma levels of miR-150 in patients with sepsis, miR-451 in patients with renal cell carcinoma (non-infectious), and miR-16 in patients with nasopharyngeal carcinoma (non-infectious) compared to a healthy control group. In the current investigation, there were no declines in miRNA expression in *P. vivax* patients. The current study's shortcomings

were its limited sample of malaria patients. The number of malaria patients in Iran has reduced considerably since a malaria eradication programme was implemented. In 2018, Pakistan and Afghanistan accounted for 98.4% of the 631 cases reported in the nation. In order to study miRNAs, it was necessary to isolate plasma immediately after sample and store it in a freezer. Another drawback of the current study was the one-year sampling period, which limited the volume of plasma obtained from malaria patients. More research is needed to find more accurate biomarkers and understand their mechanisms. The current work was the first to look at the expression levels of miR-223, miR-145, and miR-155 during malaria infection. These tiny compounds might be used as biomarkers for *P. vivax* infection.

## CONCLUSION

Despite the fact that miRNAs have been proven to be non-invasive biomarkers in acute human infectious disorders, their links to malaria pathogenesis are yet unknown. *P. vivax* was discovered to alter host miRNAs such as miR-223, miR-145, and miR-155 in the current study. As a result, these small molecules can be used as biomarkers for risk assessment, illness diagnosis, prognosis, and monitoring of *P. vivax* therapy.