

Malaria is still one of the most dangerous infectious diseases. It is a mosquito-borne parasitic disease, usually caused by *Plasmodium falciparum* or *Plasmodium vivax*. It is endemic in tropical regions. In Poland it is currently a disease brought by travelers and people working in endemic regions and it is the most common disease that they may face after returning.

The aim of the study was to evaluate the possibility of using Raman spectroscopy in the study of structural changes in erythrocytes depending on the type of parasite attack. Electron paramagnetic resonance (EPR) spectroscopy was also used. Previously, spectroscopic studies were performed by other researchers on cell cultures of erythrocytes infected with *Plasmodium* and were promising.

Patients hospitalized for malaria in the Department of Infectious Diseases of the University Hospital in Krakow in 2012-2023 were included in the study and their results were compared with a control group of healthy volunteers. Routine laboratory diagnostics were performed in patients with malaria, including capillary blood smears to assess the degree of parasitemia and to confirm the species of parasite. In addition, blood samples were taken from patients (at the beginning, during the treatment and at the end of treatment) and healthy volunteers for Raman spectroscopy and EPR studies. These studies were carried out as part of interdisciplinary cooperation with the Faculty of Chemistry of the Jagiellonian University in Krakow during the Cross-institutional PhD Studies "Interdisciplinarity for Innovation Medicine" InterDokMed. The studies were in line with good medical practice, in accordance with the Helsinki Declaration, approval of the Bioethics Committee of the Jagiellonian University was obtained.

Differences in erythrocytes in terms of changes in heme structure and protein enzymes involved in hemoglobin degradation were observed, both between malaria patients and healthy

volunteers, as well as between patients infected with different *Plasmodium* species. The bands of aspartic proteinases, which lead to the lysis of hemoglobin into oligopeptides, and whose origin can be attributed to the activity of parasites, were also indicated. Marker bands indicating lipid disorder/order in the erythrocyte membrane as an increase in the *gauche* to *trans* conformation ratio were determined. In order to better compare changes in the erythrocytes of patients diagnosed with malaria caused by *P. falciparum* or *P. vivax*, a statistical method of data processing was used - principal component analysis (PCA) and two-dimensional correlation (2D-COS). They made it possible to highlight the differences. Therefore, Raman spectroscopy can be used to assess structural changes in erythrocytes depending on the species infecting *Plasmodium*, which gives future hopes for new diagnostic methods. However, this would require much further research and validation.

Whole blood studies using EPR spectroscopy, on the other hand, show forms of iron Fe(III), which had reduced symmetry in patients diagnosed with malaria. Hemozoin signals were also visible, indicating the activity of parasites. The use of the 2D two-dimensional correlation method made it possible to indicate the changes were more visible.