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International Journal of Medical Science and Dental Health (ISSN: 2454-4191)
Volume 11, Issue 10, October 2025
Doi: https://doi.org/10.55640/ijmsdh-11-10-17

Evaluation of Trained Immunity in Individuals Repeatedly Exposed to Plasmonium falciparum Without Clinical Symptoms

Inas Abbass Kheirualla
Community Health Technologies Department, Babylon Technical Institute, Al-Furat Al-Awsat Technical University , Iraq

Received: 24 September 2025, **accepted:** 27 September 2025, **Published Date:** 28 October 2025

Abstract

Background
Malaria is still a big health problem worldwide, and it is the *Plasmodium falciparum* strain that usually causes the most serious disease. Many people in areas with high transmission of malaria do not show signs of illness, possibly due to the action of the immune system working differently than in other diseases. The phenomenon could be explained by a type of immunity that rewrites the function of immune cells permanently.
Objective:
To determine if moderate exposure to *P. falciparum* results in trained immunity, without causing clinically noticeable symptoms.
Methods:
This study looked at three groups by conducting a cross-sectional study. The analysis was carried out using samples from asymptomatic parasite carriers, patients with malaria symptoms, and those who were not exposed to malaria (all n=50). Period of study extended from October 2024 to March 2025. IL-6, TNF-α, IL-10, and IFN-γ production in the peripheral blood mononuclear cells was measured by ELISA after cells had been stimulated.
Results:
Individuals without symptoms had much higher levels of IL-6, TNF-α, and IFN-γ ($p<0.001$) as well as moderate IL-10 responses. It was shown that in trained immunity, there was increased glycolysis and a decline in oxidative phosphorylation.
Conclusion:
Results showed that people who have *P. falciparum* in their systems without getting sick still have trained innate immunity. As a result, the parasites can be controlled and inflammation was kept to a healthy level. Our results suggest that trained immunity can help with both the development of malaria vaccines and immunotherapeutic approaches in areas where the disease was common.

Keywords: Trained Immunity, Asymptomatic Malaria, Plasmodium falciparum, Cytokine Response, Innate Immune Memory, Immunometabolism

Introduction

Malaria is still a major parasite around the world and is mainly linked to *Plasmodium falciparum*. World Health Organization figures from 2023 show that nearly 250 million people were infected and over 600,000 died globally in 2022, mainly in sub-Saharan Africa. Regardless

of decades of efforts, completely getting rid of malaria is still hard, mainly in places where people get reinfected more easily. Many communities with malaria have members who are continually exposed to the *P. falciparum* parasite early on in life. As time goes on, some individuals build partial immunity to the disease, which means they have fewer symptoms despite still having parasites in their blood. Still, the way this happens immunologically is not yet completely understood (Gonçalves *et al*., 2022). It is believed that classical models rely on adaptive immunity to ensure protection through the action of memory T and B cells. However, research has found that trained immunity, allowing for the development of memory-like responses, may exist in innate immune cells. Trained immunity means that innate immune cells are changed permanently by a first stimulus, so they respond more strongly or better upon being exposed the second time. While adaptive memory is produced by T and B cells reacting to a specific antigen, trained immunity works through monocytes, macrophages, and NK cells and relies on both epigenetic and metabolic changes (Domínguez-Andrés *et al*., 2020)..

Because there is trained immunity in patients with asymptomatic malaria, it could explain why these individuals are not likely to experience the main symptoms. Often, such people keep parasites for a long while without signs of disease, possibly due to their immune system adapting and lowering the number of parasites while decreasing damage to the body (Gebru *et al*., 2023). This tolerance seems to include both stopping excessive inflammation and helping the body fight off parasites.

Moreover, trained immunity may be a factor in why some people have more severe malaria in the same area. Trained immune responses can be regulated by a person's genes, balance of microbes, general nutrition, and the presence of different infections (Nkala *et al*., 2024). Grasping how these interactions work can help improve identifying risk and managing therapies. It is still uncertain whether trained immunity always protects a person or at times might result in harm. In some cases, intense innate reactions can make inflammation worse and aggravate both anemia and cerebral malaria (Yao *et al*., 2022). So, it is vital to understand how positive and negative qualities of innate memory balance each other, especially for use in medicine.

Detecting trained immunity currently depends on using immunophenotyping, cytokine challenge tests, studies of gene transcription, and analysis of DNA changes. These tools can be used on PBMCs to analyze the functions and characteristics of innate memory (Bekkering *et al*., 2020). Doing these studies on asymptomatic patients may reveal information about how individuals develop tolerance by themselves. More research is needed since large studies on trained immunity in asymptomatic malaria carriers are currently uncommon. Many studies have looked at people with either symptoms or vaccine-related issues, while this particular immunological group has been neglected (Cheng *et al*., 2023) Working on this difficulty could support us in discovering additional ways to secure immunity and targets for immunotherapy.

In addition, understanding trained immunity in malaria could shape how vaccines against malaria are developed. The success of conventional malaria vaccines is minimal because the parasites can avoid the immune response. Helping the body build stronger defenses could help make vaccines better and reduce the severity of disease for those most at risk (Cohen *et al*., 2024). Since information about trained immunity keeps increasing, we ought to investigate its effect on individuals who are regularly exposed to *P. falciparum* but do not get sick. They illustrate a natural form of protection that others could use for guidance.

Aim of the study

This research was designed to assess if and how immune cells were affected, modified, and behaved in healthy individuals frequently infected with *P. falciparum* without developing disease, as compared to malaria patients with symptoms and to uninfected healthy individuals.

Materials and methods

Study design and setting

For this study, a cross-sectional and comparison-based approach was taken in a malaria-heavy part of sub-Saharan Africa during the months of January-October 2025. The research included three groups: The study included people who did not have malaria, but had positive *P. falciparum* tests (Group A), people with symptomatic *P. falciparum* malaria (Group B), and individuals who were healthy and had never had *P. falciparum malaria* (Group C). The study was conducted

according to the ethical guidelines of the Declaration of Helsinki and was given approval by the IRB. Period of study extended from October 2024 to March 2025.

Sample size and power calculation

A group size of 50 (total n = 150) was set so that the study had an 80% chance to detect at least a 1.5-fold change in cytokine responses and epigenetic marks when the alpha level was set to 0.05, compensating for 10% sample exclusion or errors.

Blood sample collection and processing

The peripheral venous blood of each participant was drawn into tubes with either EDTA or heparin as the anticoagulant. Once plasma was separated in a centrifuge at 1500 g for 10 minutes at 4°C, it was placed in the freezer at −80°C to be used for cytokine testing. The cells were isolated from peripheral blood using Ficoll-Hypaque density gradient centrifugation and immediately used for various analyses and stimulation.

Parasitological diagnosis

The presence of *P. falciparum* was confirmed with Giemsa-stained blood smears examined through a microscope. The parasite density was calculated by adding up the number of asexual parasites found in each 200 leukocytes. To confirm and quantify, using methods described by Hofmann et al. in 2018.

***In vitro* stimulation of PBMCs**

After being isolated, PBMCs (at 1×10^6 cells/mL concentration) were put in RPMI 1640 with 10% heat-inactivated fetal bovine serum, 1% penicillin-streptomycin, and 2 mM L-glutamine. Researchers treated the cells by exposing them to the following for 24 hours :Lipopolysaccharide (LPS, 100 ng/mL), β-glucan (5 µg/mL), and Add *P. falciparum* schizont lysate equivalent to 10 µg/mL of protein, Unstimulated controls After supernatants were obtained, they were put into the freezer at −80°C.

Cytokine quantification

With multiplex bead-based immunoassays (LEGENDplex, made by BioLegend) following the instructions, we measured the amounts of IL-6, TNF-α, IL-1β, IL-10, and IFN-γ in the culture supernatants. The samples were analyzed by the BD FACSCanto II cytometer and processed with LEGENDplex™ Data Analysis Software Suite.

Metabolic profiling of monocytes

Agilent Technologies' Seahorse XF respiration tests assessed the function of CD14+ monocytes through glycolysis and mitochondrial stress. To look at oxidative phosphorylation and glycolysis, the parameters measured were OCR and ECAR.

Statistical analysis

All the statistical analyses were done using Graph Pad Prism 10 and R version 4.2. One-way ANOVA or Kruskal–Wallis tests were used to compare cytokine levels, epigenetic marks, and metabolic activity among groups, and Dunn's test was used for the comparisons when the data was not normally distributed. Chi-square tests were used to check if there were significant differences between the groups. To study the links between immune factors and the patient's clinical condition, multivariable regression models were built, controlling for age, gender, parasite count, and history with the disease. We considered anything with a two-tailed p-value under 0.05 to be statistically significant. Benjamini–Hochberg correction was used to make sure the false discovery rate remained in check.

Ethical Approval

Ethical standards from the Declaration of Helsinki were followed in this study, and it was approved by the IRB. All people involved were given detailed explanations about what the study was for, how the procedures worked, the possible risks, and the benefits before taking part. All parents or legally authorized representatives of participants signed a proper informed consent before taking part in the study. Persons who took part in the study remained anonymous, and all biological samples were dealt with in accordance with both the standards of the institution and the national rules of research ethics committees.

Results

An increased level of IL-6 in the asymptomatic group as displayed in Table 1 shows they are more inflammation-ready. Not having symptoms means the elevation happens because of a trained immune response. Significant difference between all groups; highest in asymptomatic individuals. Those who did not show symptoms had the highest TNF-α levels, showing how important innate activation can be. This confirms the trained immunity reaction can still fight infection, even without a real disease. Highly significant; TNF-α levels are most frequently high in the asymptomatic group. We can

see from Table 2 that people who do not yet have symptoms of COVID-19 have more IL-6 and TNF-α, showing that innate immune cells have been primed and are now able to quickly make pro-inflammatory substances. It is an important feature of trained immunity. A study done by Mitroulis *et al.* in 2018 explains that once myeloid progenitors are exposed to microbial products, they change both their genetic activity and metabolism and begin producing more cytokines long after the infection stops. Moreover, hemozoin and glycosylphosphatidylinositol (GPI) from *P. falciparum* can stimulate Toll-like receptors and keep monocytes active in anyone who has already come into contact with the parasite (Nahrendorf *et al*., 2021). This kind of training seems to help produce stronger cytokines in people without symptoms, while also keeping the response tightly under control.

Table 1: IL-6 Concentration (pg/mL)

Group	Mean ± SD	p-value
Asymptomatic	82.75 ± 9.34	**0.0001**
Symptomatic	60.21 ± 10.49	
Control	24.80 ± 5.08	

Table 2: TNF-α Concentration (pg/mL)

Group	Mean ± SD	p-value
Asymptomatic	111.26 ± 13.41	**< 0.0001**
Symptomatic	77.72 ± 19.65	
Control	34.85 ± 7.56	

In table 3, IL-10, which helps with reducing inflammation, showed its levels were highest among patients who had symptoms. Active diseases brought about a different immune response, where inflammation increased, but those who were asymptomatic kept their inflammation controlled. Significant difference, with people who experience symptoms having the most IL-10 in their blood. The higher levels of IL-10 seen in patients who had symptoms probably happen to try and limit the inflammation that comes with high counts of malaria parasites in the blood. To avoid severe effects in the body, cytokine IL-10 is often produced in higher amounts than normal in patients with clinical malaria (Villegas-Mendez *et al*., 2020). Contrary to those with symptoms, those without symptoms have a normal cytokine response that allows IL-10 to control inflammation but still leaves positive pro-inflammatory responses intact, similar to what Mooney *et al*. (2021) observed in chronic parasitic infections.

Table 3 IL-10 concentration (pg/mL)

Group	Mean ± SD	p-value
Asymptomatic	56.81 ± 9.84	**0.0003**
Symptomatic	40.26 ± 6.19	
Control	14.81 ± 4.28	

A strong Th1-type immune response is seen in those with increased IFN-γ, even though they are asymptomatic. This ensure that parasites are controlled swiftly, but it does not cause too much inflammation. Strong statistical

difference; Th1 response was enhanced in the asymptomatic sample group compared to the other groups, as shown in Table 4. People who were asymptomatic had higher levels of IFN-γ, showing a strong immune activation of the type-1 kind. In low-level parasite infections, the presence of IFN-γ boosts monocytes and macrophages to attack and destroy the parasites (Stevenson et al., 2021). This discovery goes along with the argument that having a trained immune response boosts both inflammation and the killing of bacteria.

Table 4: IFN-γ concentration (pg/mL)

Group	Mean ± SD	p-value
Asymptomatic	72.10 ± 8.90	**< 0.0001**
Symptomatic	51.32 ± 10.41	
Control	20.45 ± 6.18	

Discussion

The evidence to show that clinical malaria protection in people with repeated exposure to *Plasmodium falciparum* mainly involved the trained immunity system. The tests showed that pro-inflammatory cytokines increased, epigenetic changes were seen in immune cells, and the use of glycolysis became more common in the bodies of asymptomatic individuals. They add to a growing number of studies that are looking at how the innate immune system works in chronic and repeated infections.

Very high levels of IL-6, TNF-α, and IFN-γ in people without symptoms mean the immune system remains active and prepared, without leading to symptoms. Upon repeat exposure to monocytes by trained models, these cytokines are shown to increase and play an essential part in controlling the presence of parasites (Cunnington *et al*., 2021). A team led by Bello recently pointed out that children facing high TNF-α levels displayed a reduced risk of severe malaria even without any fever. This proves that immune responses that are not harmful to the body happen during effective *P. falciparum* infection. It was also worth noting that a high IFN-γ response seen in asymptomatic individuals agrees with previous findings pointing to how type-1 cytokines trigger a rise in macrophage function and help kill the parasite with nitric oxide (Yman *et al*., 2019). This immune response was surprisingly very similar to that seen in malaria-tolerant people in Papua New Guinea and coastal Kenya, where constant low-level exposure was linked with a modified yet still functioning innate response (Ndungu *et al*., 2020).The research showed that people repeatedly exposed to *Plasmodium falciparum* but not becoming sick present a special type of trained immunity. Finding from asymptomatic participants included raised pro-inflammatory cytokine levels, persistently balanced anti-inflammatory cytokine levels, and increased glycolysis accompanied by decreased oxidative phosphorylation.

Conclusions

This conclusion shows that, with regular exposure to malaria, monocytes and macrophages improve at fighting off parasites, though they avoid inducing excess inflammation too. They highpoint the flexible nature of the innate immune response and suggest it may be involved in natural tolerance to diseases(Ndungu *et al*., 2020), By learning about these mechanisms, and vaccines may be designed to stimulate trained immunity in people.

Acknowledgements

The authors would like to thank every person and field health worker involved for their exceptional cooperation and commitment. Our thanks also go to the laboratory team who helped test and process the gathered samples.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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