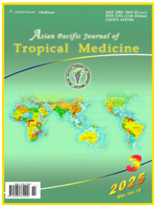

apjtm.org

Review Article

Asian Pacific Journal of Tropical Medicine

doi: 10.4103/apjtm.apjtm_84_24

Serological markers for enhanced malaria surveillance from an elimination point of view: A narrative review

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ABSTRACT

Malaria continues to pose a significant global health challenge despite a significant achievement in control and elimination in certain areas. Accurate and timely diagnosis is crucial for effective disease management and control, and finally leading to elimination. However, microscopy and rapid diagnostic tests (RDTs) have traditionally been the primary malaria diagnostic tools used globally, with certain shortcomings, including their limited sensitivity, specificity, and inability to identify asymptomatic infections. Serological markers have emerged as promising alternatives in malaria serosurveillance, particularly in countries where targets have already been set for elimination. This review highlights the advantages of serological markers over conventional diagnostic techniques and discusses some of the most promising serological markers against *Plasmodium* species-specific antigens. The implementation of serosurveillance, coupled with the utilization of these serological markers represents a transformative shift in malaria surveillance. By capitalizing on the immune memory of individuals, serosurveillance also enables the identification of recent and past infections. This approach is particularly valuable in low-transmission settings and for tracking changes in malaria prevalence over time. While recognizing the use of serological markers across various global contexts, this review predominantly emphasizes their significance within the framework of India.

KEYWORDS: Microscopy; Rapid diagnostic tests; Serosurveillance; Serological markers; Sensitivity; Specificity

1. Introduction

According to the World Health Organization (WHO) Malaria Report 2024, malaria remains a major global health concern, with 263 million cases reported worldwide in 2023. The WHO South-East Asia Region accounted for 1.5% of the global malaria burden, with India contributing to half of the region's estimated malaria cases[1]. In 2023, the National Centre for Vector Borne Disease Control (NCVBDC) documented 94 042 cases of *Plasmodium (P.) falciparum* (Pf) and 50 485 cases of *P. vivax* (Pv) in the country[2]. Malaria, a parasitic disease caused by various *Plasmodium* species and spread primarily by female *Anopheles* mosquitoes, poses substantial public health challenges. While Pf and Pv are predominant, other species like *P. malariae*, *P. ovale*, and *P. knowlesi* also present threats, but to a lesser extent owing to less number of reported cases[3]. Moreover, Pf is known for its ability to induce severe and potentially fatal malaria, with complications including cerebral malaria[4], severe anaemia, and multi-organ failure[5], particularly pronounced in non-immune individuals. The emergence of drug resistance against frequently employed antimalarial drugs hinders both treatment and control efforts[6]. On the other hand, the Pv also contributes to severe malaria cases, and poses challenges due to its unique ability to form hypnozoites in the liver, leading to frequent relapses of the infection[7]. Low-density asymptomatic infections pose challenges for both Pf and Pv, significantly complicating surveillance efforts in areas with low transmission rates[8,9]. Effective diagnostics plays an important role in malaria elimination efforts, facilitating timely case identification and treatment. Accurate diagnostics enable

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How to cite this article: Mamnani V, Verma K, Bharti PK, Nitika N. Serological markers for enhanced malaria surveillance from an elimination point of view: A narrative review. Asian Pac J Trop Med 2025; 18(3): 102-112.

Article history: Received 2 February2024 Revision 22 February 2025 Accepted 3 March 2025 Available online 6 March 2025

healthcare providers to differentiate between *Plasmodium* species, guiding appropriate treatment and mitigating the transmission of the disease[10]. Additionally, surveillance through diagnostics allows for targeted interventions, ultimately reducing transmission rates and advancing towards malaria elimination[11].

India continues to show a significant progress in malaria elimination journey. However, challenges persists, as India still accounts for the majority of malaria cases in the South-East Asia region. While initiatives like early case detection, vector control, and community engagement are crucial, it is imperative to address barriers hindering malaria elimination[12].

The review aims to describe key proteins for malaria serosurveillance, highlighting its role in life cycle of malaria parasite, its sensitivity, and specificity in recognizing previous infection.

2. Literature search

The study involved a PubMed search utilizing the following (search strategy: serology OR seroepidemiology OR serosurveillance OR antibody kinetics OR antibody response AND malaria) from January 1, 2000, and December 31, 2023. This yielded 2 442 papers in PubMed, and 26 relevant papers published were selected for the review (Figure 1). The selected papers specifically focused on potential and promising antigens targeting for both Pf and Pv species. The exclusion criteria employed in this paper eliminated studies primarily centered on vaccine development and design, those simultaneously addressing multiple infectious diseases alongside malaria, and those including special cases such as pregnant women, infants, and other particular groups deemed unrepresentative of the general population of interest. Furthermore, studies exclusively focusing on gametocyte antigen and *Anopheles* spp. antigen were excluded. Studies primarily investigating antibody responses on other *Plasmodium* species other than Pf and Pv have also been excluded.

3. Diagnostic challenges

Microscopy, rapid diagnostic test (RDT), and polymerase chain reaction (PCR) are well-recognized standard methods[13], yet limitations exist. Microscopy requires skilled staff and parasitemia potentially detectable by microscopy (*i.e.*, 5 to 200 parasites/μL)[14]. RDT is a quick and simple method. Still, it may not be able to detect low-level parasitemia (less than 200 parasites/μL)[15] especially in low-transmission settings. Additionally, it can also lead to false negative results due to deletion of Histidine rich protein-2 (HRP-2). Furthermore, the RDTs may yield false positives due to the cross-reactivity between antigens associated with other parasites and different pathogenic infections[16]. Moreover, the diagnostic efficiency of RDTs depends upon many other factors, such as temperature, storage conditions, *etc*[17]. Further, PCR requires a well-equipped laboratory, and its cost and technical demands limit its use in low-resource settings[18]. Overcoming the hurdles of malaria elimination in India, including diverse parasite strains[19] treatment resistance patterns, and a high prevalence of asymptomatic cases[20],

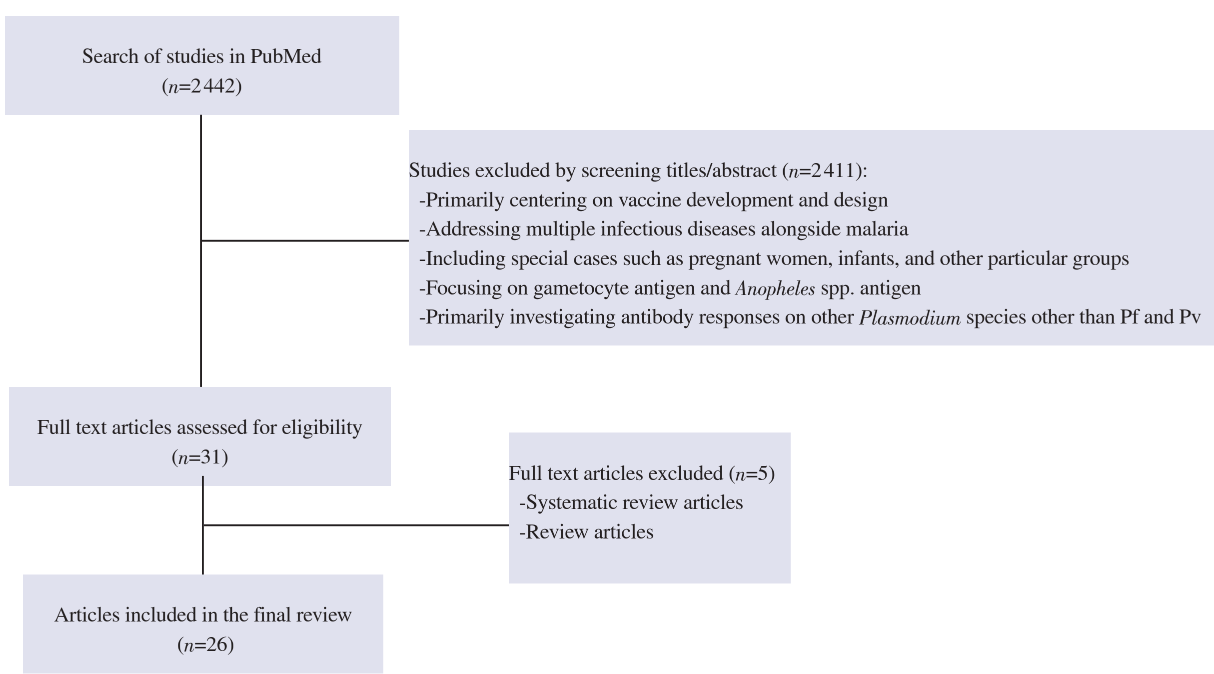

Figure 1. Flow chart of selection process. Search strategy: serology OR seroepidemiology OR serosurveillance OR antibody kinetics OR antibody response AND malaria.

demands adopting new strategies for accelerating the malaria elimination, thus it is imperative to develop and deploy efficient, sensitive and specific diagnostic and screening tools.

4. Significance of serosurveillance in bridging the gap between tracking and controlling malaria

Serosurveillance emerges as a promising tool for malaria surveillance, leveraging *Plasmodium*-specific antibodies to estimate disease prevalence and transmission dynamics[21]. This approach not only facilitates the monitoring of current infections and past exposures but also plays a crucial role in identifying transmission hotspots[22], enabling a more nuanced understanding of transmission dynamics. This timely and comprehensive information will help public health authorities pinpoint high-risk areas, efficiently allocate resources, and then implement targeted interventions.

4.1. Role of antibody–based serosurveillance in pre–elimination and post–elimination settings

Serosurveillance is essential for tracking malaria in both the pre- and post-elimination settings, especially as nations working towards the elimination of malaria witness a significant decline in transmission. It is a screening tool for finding asymptomatic carriers that traditional diagnostics may miss. Following elimination, it is essential to identify any infection reservoirs that might bring the disease into the community again and confirm the effectiveness of elimination, thus supplementing ongoing efforts[21].

Antibody-based serosurveillance could significantly contributes to malaria control and elimination strategies by monitoring transmission patterns and evaluating the effectiveness of interventions. In border regions of Myanmar, a study assessing prevention and control measures against Pv revealed low seroconversion rates for Pv merozoite surface protein 1-19 (PvMSP1-19) antibodies, indicating effective Pv control while emphasising on the need for continuous surveillance in low transmission areas[23].

Similarly, in China's post-elimination phase, maintaining a malaria-free status requires vigilant surveillance. Low seropositive conversion rates to PvMSP1-19 highlight the necessity of ongoing serological surveillance to prevent malaria resurgence[24].

Van den Hoogen *et al*. emphasized the significance of serological markers in tracking historical transmission, noting a decline in PfMSP1-19 antibody responses and seroconversion rates among children aged 1-5 years in Gambia from 1988 to 2011[25].

In Malaysia, despite no reported malaria cases, serosurveillance has provided invaluable insights. By assessing antibody responses to multiple antigens (PfAMA-1, PfMSP1-19, PvAMA-1, and PvMSP1-19), it has provided insights into transmission levels, variability, and the factors related to malaria exposure within indigenous communities[26].

Similarly, Sri Lanka utilized a similar set of serological markers (PfAMA-1, PfMSP1-19, PvAMA-1, and PvMSP1-19). Despite achieving successful elimination, challenges persist with historical susceptibility, as indicated by 60% of the population testing seropositive for antibodies to various malaria antigens. Moreover, the low or zero seroconversion rates observed in the surveyed districts confirm the state of elimination, signifying the absence of ongoing or concealed transmission[27].

These findings underscore the pivotal role of serosurveillance in assessing malaria control and elimination strategies, emphasizing the importance of sustained efforts in surveillance and intervention to prevent malaria resurgence and maintain progress towards elimination goals.

4.2. Criteria for selecting serological markers

Selecting antigens for serosurveillance assays involves a critical consideration, including factors like immunogenicity, persistence of antibodies, polymorphism, and cross-reactivity among different *Plasmodium* species[21,28]. The choice of antigens should align with specific surveillance objectives and contexts. In low-endemic areas, monitoring changes over time may be best served with highly immunogenic antigens like apical membrane antigen 1 (AMA1)[21,29], whereas moderate-to-high endemic areas might benefit from antigens such as erythrocyte-binding antigen (EBA)[21] to reflect recent transmission changes. Antigens eliciting sustained antibody responses are selected for evaluating cumulative exposure over prolonged durations, while those provoking short-lived responses are valuable for detecting recent exposure. The selection of antigens is also influenced by the target population and the intended purpose, prioritizing highly immunogenic antigens for young children, particularly in scenarios confirming elimination. Conversely, antigens provoking short-lived antibody responses are chosen to identify adult subpopulations with recent occupational exposure[21].

In elimination initiatives, ensuring both sensitivity and specificity in surveillance assays is crucial for accurately identifying high-risk subpopulations and geographical hotspots[30]. Polymorphism in numerous malaria antigens, potentially impacting sensitivity, can be addressed by incorporating various allelic variants into the assay to mitigate strain-specificity issues[21]. Cross-reactivity of antibodies between different malaria species can impact test specificity, especially in regions where multiple species coexist, such as Pf and Pv[31,32]. Hence, it is crucial to choose antigens that are either species-specific or exhibit significant sequence diversity and minimal cross-reactivity. While widely studied antigens like circumsporozoite protein (CSP), merozoite surface protein-1 (MSP-1), and apical membrane antigen 1 (AMA-1)[33–35] are conserved across Pf and Pv, the extent of antibody cross-reactivity requires further validation, particularly in regions with coexisting parasite species[36]. Furthermore, the utilization of antigens present in malaria vaccines, such as the RTS, S and R21 vaccine based on PfCSP, currently in various stages of research and field implementation, might pose challenges in distinguishing vaccine-induced antibodies from naturally acquired ones, potentially limiting their suitability for serosurveillance[37,38].

Therefore, serological markers serve as vital tools for improving our understanding of the epidemiology of malaria, offering important information about the frequency, distribution, and dynamics of the infection within communities[39]. Notably, a higher seroprevalence in young children frequently indicates that the infection is still being transmitted. Still, a similar pattern in older age groups may suggest that the disease has already been exposed[27]. These indicators assess antibody responses within certain populations, making it easier to distinguish between high and low endemic areas[40,41]. Additionally, serological markers provide essential information about an individual's immune status[42], which aids in assessing the efficacy of malaria prevention programs. Public health professionals can develop focused measures for malaria prevention, track the effectiveness of programs, and make accurate choices that decrease the disease's impact through the analysis of complex serological data[43]. Short-lived antibody responses play a crucial role in actionable surveillance, especially for Pv. These antibodies can serve as indicators of recent exposure to the malaria parasite, providing valuable insights for guiding interventions. By monitoring short-lived antibody responses, public health authorities can identify areas of high transmission and deploy targeted interventions such as vector control measures and chemoprevention strategies. This proactive approach enables timely and effective responses to prevent malaria outbreaks and reduce the burden of Pv infection among at-risk populations[44,45].

5. Role of essential proteins in life cycle of *Plasmodium*

The malaria parasite *Plasmodium*, possesses a diverse array of over 5000 proteins expressed throughout its complex life cycle[46]. This complexity initiates with the transmission phase, where infected female *Anopheles* mosquitoes inject sporozoites into the bloodstream during a blood meal. Notably, specific proteins like the CSP are predominantly expressed during the sporozoite stage. CSP plays a crucial role in sporozoite formation, oocyst egress, salivary gland invasion, and hepatocyte attachment[47]. These sporozoites then migrate to the liver, infect hepatocytes, multiply, and transform into merozoites. After exiting hepatocytes, merozoites invade red blood cells, marking the onset of symptomatic malaria[3]. MSPs, on the other hand, are expressed during the merozoite stage and are essential for red blood cell invasion and the establishment of the erythrocytic cycle[48]. Their presence in the bloodstream during infection makes them potential serological markers for identifying ongoing or past malaria exposure. Another key protein, the AMA-1, is expressed in both the pre-erythrocytic and asexual blood stages, where it facilitates merozoite reorientation before erythrocyte invasion[49]. This widespread expression across different stages enhances its potential as a serological marker for malaria surveillance. Additionally, early transcribed membrane proteins (ETRAMP) are localized to the parasitophorous vacuole membrane (PVM), playing a critical role in regulating interactions between the parasite and host cytosol. Their stage-specific expression and involvement in host-parasite interactions make them potential candidates for serological markers[50]. By targeting these proteins, serological assays can provide valuable insights into malaria exposure, transmission dynamics, and immune responses in endemic regions.

Here are some essential proteins crucial to both Pf and Pv along with their defining characteristics based on available literature which are arranged in alphabetical order below

5.1. AMA-1

The 62 kDa AMA-1 is crucial in the pre-erythrocytic and asexual blood-stages of *Plasmodium*. PfAMA1 plays a central role in facilitating the reorientation of merozoites before they invade erythrocytes, which is vital for the invasion process of *Plasmodium* species[51]. The presence of antibodies against PfAMA-1 hinders the invasion of both sporozoites and merozoites, highlighting its importance in the life cycle of the parasite[49,52,53]. More than 10% of amino acids within the ectodomains of PfAMA-1 and PvAMA-1 display polymorphism[21]. One study effectively identified transmission hotspots using a dual approach with PfAMA-1 and PfMSP1-19, demonstrating 95.5% sensitivity and 82% specificity in samples from individuals seeking healthcare[34].

5.2. CSP

CSP, the primary sporozoite-coating antigen for Pv[54], exhibited the highest seropositivity overall, with CSP210 leading, followed by CSP247 in individuals who are both uninfected and exposed to the parasite with consistent percentages observed one year later. IgG positivity to both PvCSP210 and PvCSP247 remained well maintained over a year, even without qPCR-detected blood-stage infections[55]. Due to sporozoite count fluctuations and short half-lives, CSP antibodies, present in all *Plasmodium* species, may be less reliable in low transmission regions but highly sensitive indicators in high transmission areas[56]. When it came to recognizing Pv cases, VK210 CSP antibodies demonstrated 61.42% sensitivity and 97.14% specificity[57]. They were especially helpful in differentiating between infected and uninfected individuals. Another study detected antibodies to the sporozoite CSP repeats of Pv, Pf and *P. malariae* (Pm). The findings indicated IgG antibody response to PfCSP (49%), PvCSP (62%) and PmCSP (46%). However, the findings suggest that in low transmission settings, these naturally acquired antibody responses may not serve as reliable markers for monitoring recent malaria exposure, particularly in regions with a high prevalence of Pv. Thus, relying solely on the CSP marker might not effectively gauge recent malaria exposure in such contexts[58].

5.3. ETRAMP

ETRAMPs, crucial components of malaria parasites, are predominantly associated with the parasitophorous vacuole membrane (PVM), acting as a vital barrier that shields the parasite from the host cell's cytosol and facilitates its evasion of the immune system. The research introduces positively charged proteins from Pf termed ETRAMPs, identifying thirteen with similar structures. Notably, six are uniquely present during ring stages, confirmed through Northern and Western analyses. Moreover, certain members exhibit stage-specific expression patterns[59]. Furthermore, PfETRAMP5 has emerged as an important short-term indicator of recent malaria exposure (in previous 3 months) in Haiti's elimination setting, antibody responses diminishing between two and six weeks after the intervention, which makes it especially helpful for assessing the efficacy of intervention strategies[60].

In the recent study in Palawan, the combination of four serological markers, including Pf glutamate-rich protein (PfGLURP) R2, PfEtramp5.Ag1, Pf gametocyte exported protein 18 (PfGEXP18), and PfMSP1-19, outperformed individual antigen seropositivity assessments, with high AUC of 0.959 1 in predicting recent Pf infection. This integrated approach underscored PfETRAMP's importance, alongside PfGLURP and PfGEXP18, as robust indicators of recent exposure to Pf, emphasizing their potential in malaria surveillance and control strategies[61]. Another study conducted *in silico* analysis identified nine genes encoding the ETRAMP family in Pv using a protein microarray method, serum positivity was evaluated in both Pv malaria patients and healthy individuals. Notably, among the PvETRAMPs, ETRAMP4 displayed a 62% positivity rate, similar to the positive control PvETRAMP11.2. The strong antibody responses to PvETRAMP4, coupled with observed IgG subclasses in mice, indicate the ETRAMP family's pathogen immunogenicity, underscoring its utility as a protein marker and for vaccine development[50].

5.4. Merozoite surface protein 1-19 (MSP1-19)

MSP1-19 is an essential malarial merozoite surface protein that differs in Pf and Pv and holds potential as a malaria vaccine candidate[62]. MSP1-19 serves as a specific serological marker for estimating malaria prevalence, exhibiting limited polymorphism with four residues[21]. It plays a role in evaluating endemicity changes, particularly in low transmission regions, and acts as a specific marker for estimating the prevalence of malaria. The stability of IgG antibodies against PvMSP1-19 for nine months after infection indicates the importance of this marker for surveillance[23], especially in areas of low transmission settings. The study by Liu *et al.* further illustrates that despite a decline in quantitative IgG levels over time, PvMSP1-19 IgG antibodies persist above baseline at 9 months post-infection, indicating sustained seropositivity following a clinical Pv infection[63]. The PvMSP1-19 exhibited 79.2% sensitivity and 94.8% specificity[64]. In a different study in a region characterized by low malaria transmission and the presence of both Pv and Pf, individuals quickly developed antibodies against PfMSP1-19 and PvMSP1-19. This emphasizes the intricate nature of antibody responses in co-endemic settings and suggests that MSP1-19 can serve as a valuable marker for detecting recent exposures to distinct *Plasmodium* species[65].

5.5. Merozoite surface protein 1 paralog-19 (MSP1P-19)

A novel vaccine candidate designed for the blood stage of *vivax* malaria infections is *P. vivax* merozoite surface protein 1 paralog-19 (PvMSP1P-19). It is situated upstream of the *MSP1* gene and displays resemblances in size, molecular mass, and cystine residue composition[66]. One study has shown that antibodies against PvMSP1P-19 persist for up to nine months after infection, highlighting their importance in malaria and their potential as markers for monitoring the disease[66]. Moreover, a separate investigation found that PfMSP1P-19 specific IgG antibodies exhibit a sensitivity of 83% and specificity of 77% when tested with archived plasma samples[67]. These findings underscore the potential utility of MSP1P-19 in combating malaria and assessing immune responses over time.

5.6. Merozoite surface protein-3 (MSP-3)

MSP-3, a soluble 48 kDa protein found on the merozoite surface, plays a crucial role in the invasion of red blood cells, making it a promising target for malaria treatments and vaccines. Within the family of merozoite surface proteins, namely PvMSP3α (PVX_097720) and PvMSP3β (PVX_097680), significant sequence variations are observed across laboratory and field isolates. Both PvMSP3α and PvMSP3β display immunogenicity during natural infections[68]. Notably, investigations have highlighted a substantial genetic diversity among clinical Pvmsp-3α isolates, particularly within specific geographic areas of the Indian subcontinent. This remarkable allelic variability underscores PvMSP-3α's potential as a potential serological marker for differentiating field isolates[69]. Additionally, MSP3.10 emerged as a promising marker for Pv, as among a panel of eight serological markers, PvMSP3.10 was identified as capable of predicting recent exposure to Pv within individuals occurring within the previous nine months[44].

Each serological marker contributes differently to malaria surveillance in various epidemiological contexts, each with advantages and limitations. They are valuable tools for comprehending the dynamics of malaria and directing intervention strategies because of their unique characteristics.

All the relevant descriptions of the most promising serological markers for both Pf and Pv have been characterized and depicted in Table 1.

6. Seroprevalence studies in India based on different *Plasmodium* antigens

In the diverse landscape of India, where malaria remains a significant public health concern, understanding the seroprevalence of the disease across different regions and populations is imperative for effective control and prevention strategies.

Several studies conducted in India, as described below, have focused on both Pf and Pv antigens.

Several studies on serological markers in India for both Pv and Pf are described in this section. In a study involving malaria-infected individuals in India, researchers detected antibodies against the PfMSP-3 protein. The *N*-terminal domain, with variable amino acids, showed a stronger antibody response than the conserved *C*-terminal domain. All serum samples, including those from Pf infections, exhibited seropositivity against MSP-3, suggesting it is a potential serological marker for Pf infections. Importantly, no cross-reactivity was observed with Pv and Pm infections, indicating the specificity of the antibodies against MSP-3 protein for distinguishing Pf infections from other malaria species[70].

In a parallel study led by Deshmukh *et al*[71], evaluating PfMSP3N antigenicity in samples from malaria-endemic regions in Africa and India, results revealed notable seropositivity of 75% in Liberian samples and 67.9% in Indian samples for MSP3N, highlighting the significance of MSP-3 antigen in triggering immune responses across diverse populations affected by malaria. Additionally, we conducted sequence alignment of the *MSP3* gene from various parasite species using the EMBOSS needle alignment tool[72] to determine sequence identity between Pf *vs.* Pv and Pf *vs.* Pm. The gene sequence were retrieved from PlasmoDB database[73] with corresponding ID Pf (Pf3D7 1035400), Pv (Pvx 110940), and Pm (PmUGo1-o6022700). From the analysis, we found that the identity match between Pf *vs.* Pv was 12% (Supplementary Figure 1) and Pf *vs.* Pm was found to be 15.2% (Supplementary Figure 2).

Moreover, a distinct research study focused on assessing antibody responses toward PvAMA-1 and PvMSP1-19 antigens among individuals residing in three regions with endemic malaria in India:

Table 1. Overview of antigen target common for both Pf and Pv for potential in serosurveillance.

No.	Serological marker	Life cycle stage	Homology between Pf and Pv sequences	Half life	Sensitivity	Specificity
1	AMA-1	Pre-erythrocytic and merozoite (micronemes)[52]		PvAMA-1 ~1.1 to ~1.6 year[39]		
2	CSP	Sporozoite (pre-erythrocytic stage)[57]	25%[21]	~8.5 months- ~1 year[39]	PvCSP (VK210) 61.42%[57]	PvCSP (VK210) 97.14%[57]
3	ETRAMP	Erythrocytic stage[60]			PvETRAMP 4: 62% PvETRAMP 5: 48% PvETRAMP 8: 46% PvETRAMP 9: 43.8% PvETRAMP 10.3: 40% PvETRAMP 11.2: 76.8%[50]	PvETRAMP 4: 97.5% PvETRAMP 5: 95% PvETRAMP 8: 95% PvETRAMP 9: 100% PvETRAMP 10.3: 97.5% PvETRAMP 11.2: 97.5%[50]
4	MSP1-19	Merozoite[63]	45%[21]	PfMSP1-19: ~6-~7.5 months PvMSP1-19: ~1.1-~1.6 year[39]	PvMSP1-9 79.2%[64]	PvMSP1-19 94.8%[64]
5	MSP1P-19	Merozoite[67]			PfMSP1P-19 83%[67]	PfMSP1P-19 77%[67]
6	MSP-3	Merozoite[69]	20%[21]			

Note: MSP: merozoite surface protein; CSP: circumsporozoite protein; AMA-1: apical membrane antigen; ETRAMP: exported protein trafficked to the apical membrane; GLURP: glutamate-rich protein. Pf: *Plasmodium falciparum*; Pv: *Plasmodium vivax*.

Chennai, Nadiad, and Rourkela. The study revealed a general seroprevalence of 40.6% for PvAMA-1, while PvMSP1-19 exhibited a significantly higher seroprevalence at 62.4%. Notably, the findings were striking in Chennai, showing a substantial seroprevalence for both antigens-47% for PvAMA-1 and 80.3% for PvMSP1-19. This points towards the prevalent existence of Pv in the region. These findings suggest PvMSP1-19's heightened immunogenicity in the Indian population, emphasizing its potential as a serological marker warranting further investigation[74].

Furthermore, a cross-sectional survey was conducted from June to October 2007 in the Ghaziabad district of Uttar Pradesh. The study analyzed immune responses to synthetic peptides derived from crucial Pv antigens CSP, MSP1, AMA1, and gametocyte surface antigen-1 (GAM1) across different age groups. It revealed that adults exhibited a higher frequency of antibody responses to these antigens than younger children. Among 304 cases, 66% were seropositive for all peptides, while 13% showed no response. A significant correlation was observed between antibody responders and their capability to recognize stage-specific epitopes. This study emphasizes age-related variations in immune responses to specific Pv markers in the region[75]. Strengthening seroprevalence studies in India is essential to reinforce efforts in malaria surveillance, requiring a broadening of focus to encompass additional antigens, such as ETRAMP and GLURP, to gain a more thorough understanding of malaria transmission dynamics.

7. Role of antibody–based serosurveillance in infectious diseases beyond malaria

Global public health organizations have acknowledged serosurveillance as a fundamental approach for monitoring various infectious diseases, including trachoma, onchocerciasis, dengue, and COVID-19. Modern tools like multiplex assays have revolutionized serological testing by allowing the simultaneous detection of multiple protein targets. This advancement not only enhances cost-effectiveness but also minimizes sample volume and testing time. Technologies such as cytometric bead arrays and Luminex xMAP enable the assessment of antibody responses to numerous Pv proteins, significantly advancing our understanding of immunity and malaria exposure[46]. Furthermore, the emergence of lateral flow immunochromatographic devices is promising for future disease surveillance, as they have shown promise in detecting a range of infectious diseases like HIV and malaria[21].

In Haiti, a nationwide serosurvey conducted from December 2014 to February 2015 utilized serological multiplex bead assays to simultaneously assess exposure to 11 pathogens using 15 antigens. This method efficiently provided comprehensive insights into population exposure to multiple infectious diseases. The multiplex assay's ability to analyze various antigens in a single panel greatly enhanced surveillance efforts, offering a detailed understanding of disease prevalence and dynamics within the country[76].

8. Summary

This review emphasizes the pivotal role of serosurveillance in tackling challenges associated with malaria diagnosis, burden, and elimination. The above-mentioned markers have demonstrated a significant antibody response in malaria transmission areas for both Pf and Pv. As these markers are also being considered as potential vaccine candidates for malaria, further extensive studies are necessary to validate whether these markers can indeed be utilized for serosurveillance purposes in the future. The possible use of non-invasive samples such as saliva and urine is at various stages of modification and if found feasible and accurate in further research, it may be useful in conducting field based studies for serosurveillance.

9. Limitations and considerations of serosurveillance

The implementation of malaria serosurveillance poses several challenges that require careful consideration. Choosing the right sample size, timing, and frequency of sampling is essential[77], especially in light of the various populations and environments that are involved. Large sample sizes from multiple age groups are necessary to estimate transmission intensity accurately in pre-elimination settings[78]. However, it is essential to acknowledge the ethical concerns raised by population screenings and the requirement for appropriate infrastructure. The current limitations of serosurveillance, particularly concerning Pv, include a restricted focus on a small number of antigens[21]. This study is limited to a literature search conducted exclusively on the PubMed database and focuses solely on potential markers shared between Pf and Pv. Furthermore, the primary challenge in serosurveillance is the extensive genetic polymorphism of recombinant antigens. To overcome the constraints associated with antigenic diversity, exploring new serological markers while considering their genetic variability is crucial[79]. Concurrently, concerns persist regarding antigen diversity and cross-reactivity among various *Plasmodium* species[80]. Developing serosurveillance tests may require considering multiple antigens or variants to enhance sensitivity based on different transmission conditions[81]. A solid understanding of factors influencing antibody acquisition and maintenance is necessary for valid transmission intensity estimates in diverse populations and settings[82]. A major limitation is the inability to diagnose actively infected individuals due to the time lag for antibody development and their persistence even after parasite elimination[36].

10. Conclusions

The increased emphasis on serosurveillance underscores its crucial role as a promising tool in confirming malaria elimination. Various serological markers such as MSP-1, AMA-1, and CSP have been widely utilized for both Pf and Pv. Nevertheless, there remains an urgent need for further exploration and validation, especially for developing reliable diagnostic tests. Although significant progress has been made, the journey toward comprehensive and reliable serosurveillance demands additional attention, especially for exploring and validating other potential serological markers such as ETRAMP, and MSP-3. The continued pursuit of more extensive seroepidemiological studies involving these markers is crucial for enhancing and strengthening serosurveillance efforts. In this pursuit, the collaborative efforts of researchers, clinicians, and public health authorities are essential to ensure the robustness and effectiveness of serosurveillance strategies, ultimately contributing to the global endeavor to eliminate malaria. (1) Future aspects of serosurveillance in malaria elimination: The future of serosurveillance in malaria elimination involves a multidimensional approach, combining scientific research, technological advancements, and collaboration with public health programs to deploy these tools effectively in diverse settings. (2) To determine the endemicity and the transmission dynamics: Comprehensive seroprevalence studies are required to validate serological exposure markers in different endemic areas. This will help to determine the robustness and applicability of these markers across diverse malaria-endemic regions. (3) Integration into malaria elimination programs: Incorporating serosurveillance tools into malaria elimination initiatives can enhance efficacy by providing additional monitoring methods and targeting interventions.

As we look ahead, in future, the primary method for serosurveillance will likely be point-of-care tests for convenient antibody detection. However, it is crucial to further develop, validate, and standardize these assays, including creating positive controls, to enhance accuracy, reliability, and widespread use in monitoring malaria transmission dynamics[46]. The practicality and accessibility of point-of-care tests, like lateral flow assays, make them accessible, especially in remote areas. This shift will empower community health workers to conduct real-time malaria surveillance, enhancing efforts towards elimination in hard-to-reach regions[83].

Conflict of interest statement

The authors declare no conflicts of interests.

Acknowledgments

The authors would like to thank the University Grants Commission (UGC) for VM fellowship, and ICMR-NIMR for Institutional support.

Funding

The authors received no extramural funding for the study.

Authors' contributions

NN, PKB, and VM conceptualized the study; VM did the literature review; VM and KV analysed and drafted the manuscript; NN, PKB, and KV critically reviewed the manuscript. All authors read and approved the final manuscript.

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Edited by Zhang Q, Lei Y, Pan Y

```
Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 785
# Identity:      94/785 (12.0%)
# Similarity:   158/785 (20.1%)
# Gaps:         469/785 (59.7%)
# Score: 173.5
#
#
#=======================================

EMBOSS_001         1 MKSFINITLSLFLLHLYIYINN---VASKEIVKKYNLNLRNAILNNNSQI
47
                        ..|::...|...||:..::   :...|..:..:.||||....|:|.
EMBOSS_001         1 ---MRNLSCVTFYFALYVLSSHGGGILCSEAGENKSTNLRNGFTVNSSL-
46

EMBOSS_001        48 ENEENVNTTITGNDFSGGEFLWPGYTEELKAKKASEDAEKAANDAENASK
97
                       ||.....:..|.|.|.::           ....||.::...:.:....
EMBOSS_001        47 --EEGDQAELNDNSFDGSDY-----------SNQGEDLQEVIKNEQRVGG
83

EMBOSS_001        98 EAEEAAKEAVNLKESDKSYTKAKEACTAASKAKKAVETALKAKDDAEKSS
147
                     |           :|.|:.......|..|..|.......::...||:|...
EMBOSS_001        84 E-----------EEDDEEILDEASASLAKEKYGNIRRGSVLFGDDSEVHG
122

EMBOSS_001       148 KAD----------------SISTKTKEYAEKA--------------KNAY
167
                     ::|                :|||...|:|::.              :...
EMBOSS_001       123 ESDWLENDMNMMWGTGRSANISTDHGEHADQGWQSGWSAPWDAVGQEEGQ
172

EMBOSS_001       168 EKAKNAYQKANQ---------------AVLKAKEASSYDYILGW----EF
198
                     |:.:...|:..|               .|....|.:.:|....|    |.
EMBOSS_001       173 EEGQEEGQEEGQEEGQEEGQQEEQPVEQVANPDEEAPHDAPAVWEQNDEE
222

EMBOSS_001       199 GG----------GVPEHKK------------------EENMLSHLYV--S
218
                     ||          |||..|.                  ||::|..:.|  |
EMBOSS_001       223 GGDKIRWEYEEEGVPTPKNVHESLKVDPLLFDQVDVAEEDVLPSVDVLPS
272

EMBOSS_001       219 SKDKENISKEND----DVLDE--------------KEEEAEETEE-----
245
                     ::|.|..|..|:    ||..|              .||:..|.||
EMBOSS_001       273 AEDAEGESPSNESAGKDVAQEGVDNPQEGGENAQDDEEKVHENEENSPND
322

EMBOSS_001       246 EELEEKNEEET-------------------ESEISEDEEEEEEEEEKEEE
276
                     ||...:|||..                   :.|:.||.:|:.||:.||.|
```

```
EMBOSS_001        323 EEKVHENEENAPNDEEKVHENEENVKPESQQQEVPEDAQEQVEEQAKEVE
372

EMBOSS_001        277 NDKKK--------------------EQEKEQSNENNDQKKDMEAQNLISK
306
                      ..:::                    |:||.|..|..|::.:...|. ...
EMBOSS_001        373 EPEEQQQMPPLEEEPAEIVEVEEVAEEEKPQEQEEQDEQGEQGEQG-EQG
421

EMBOSS_001        307 NQNNNEKNVKEAAESIMKTLAGLIKGNNQIDSTLKDLVEELSKYFKNH--
354
                      .|....:.|:||.:.         :|....:..::::|||.:.|...
EMBOSS_001        422 EQGEQGEQVQEAIDE---------QGPEPEEEHMQEVIEELQEAFDEQGP
462

EMBOSS_001        355 --------------------------------------------------
354

EMBOSS_001        463 QPEEEHMQEVIEELQEAFDEQGPQPEEENMQEVIKELQEAFDEQVPQPEG
512

EMBOSS_001        355 --------------------------------------------------
354

EMBOSS_001        513 ENVQEVIEELQEVAEGEQMPEPEEELVQEVIEELQEVEREEKMPEPEIEY
562

EMBOSS_001        355 --------------------------------------------------
354

EMBOSS_001        563 VQEVIDELKEATEEAQMPEPEEELVQEVIDELKEATEEEQMPEPEIEYVQ
612

EMBOSS_001        355 --------------------------------------------------
354

EMBOSS_001        613 ELIEELQEVTEEKQPAEKPEEQPEEQPAEQPAEQPAQQPAEQPEEQPAED
662

EMBOSS_001        355 --------------------------------------------------
354

EMBOSS_001        663 EAEDEAEEPQEEDGDEECAESSGQSFFRRIHIREYIDIRSIKNSAGNMVK
712

EMBOSS_001        355 -----------------------------------     354

EMBOSS_001        713 NVFSIFGGDNGLVGMFKDFAGNFAKNGENINDKME     747
```

Supplementary Figure 1. The *MSP-3* gene was aligned using the EMBOSS needle tool to compare sequences between Pf and Pv.

```
Aligned_sequences: 2
# 1: EMBOSS_001
# 2: PmUG01_06022700
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 729
# Identity:     111/729 (15.2%)
# Similarity:   188/729 (25.8%)
# Gaps:         390/729 (53.5%)
# Score: 244.5
#
#
#=======================================

EMBOSS_001         1 MKSFINITLSLFLLHLYIYINN------VASKEIVKKYNLNLRNAIL---
41
                     |.:.:|:|..:|.|::|:.|||      |.|.:.:||.:.|||...|
PmUG01_060227      1 MGNLLNLTFYIFFLNMYMNINNATIIDGVQSDDHMKKRDYNLRKGSLTKS
50

EMBOSS_001        42 --------------------------------NNNSQIENEENVNTTITG
59
                                                     |....:||..|.|.....
PmUG01_060227     51 KFPRTPEDYLKTDLGGFQKMGEGSYYEINLRDNRTDLLENVNNSNNPSVE
100

EMBOSS_001        60 ND--FSGGEFLWPGYTEELKAK-----------------KASEDAEK--A
88
                     :|  .....||.....||||..                 :.|.::||  .
PmUG01_060227    101 DDGNIKYRSFLRSDVREELKKNIEMQQEDKNSQSKSQLPENSLESEKKNI
150

EMBOSS_001        89 ANDAE--NASKEAEEAAKEAVNLKESDKSY---TKAKEACTAASKAKK--
131
                     |||:|  |..|:.|..|||..||:|..:..   .:.|:|.|...:.|.
PmUG01_060227    151 ANDSESRNDEKKEEVKAKEDENLEEKREEVDLKAETKDALTKQEEEKSRQ
200

EMBOSS_001       132 --------------------------------------------AVETAL
137
                                                                 .:|.:.
PmUG01_060227    201 TEFESPRGPKDNLDEQEKSANLPKDMAPRNLESDDNKQTQLPLPELEESN
250

EMBOSS_001       138 KAKDDAEKSSKADSISTKTKEYAEKAKNAYEKAKNAYQKAN-QAVLKAKE
186
                     |::.|.||:...::....|.:..|..|...:..|....:.| ::|::.||
PmUG01_060227    251 KSQLDEEKNEDKENDKDNTDKNVEDGKKGEKDGKKDRTEGNLESVVQMKE
300

EMBOSS_001       187 ASSYDYILGWEFGGG-------VPEHKKEENMLSHLYVSSKDKEN---IS
226
                               |.|.|       .|..||.:|:.|:  ..|:|.:|   .|
PmUG01_060227    301 ----------EAGSGGKPPQETFPGGKKLKNIDSN--EGSEDGKNKLPTS
338

EMBOSS_001       227 KENDDVLD------------------EKEEEAEETE--EEELEEKNEEET
256
                     :::||:.:                  ||:...|..:  ||::|.|::|:.
```

```
PmUG01_060227    339 QQSDDLQNALASSVTPEETVNAKDKLEKQPPQENPKLLEEDVEHKHQEDE
388

EMBOSS_001       257 ESEI------SEDEEEEEEEEEKEEENDKKKEQEKEQSNENNDQKKDMEA
300
                     :.|:      .:.||||||::::|:|.:.|:||:||.......|.:::..
PmUG01_060227    389 QKELQKLKHQKQQEEEEEEKKKREKEREMKEEQKKEVKEPAKTQTENVFE
438

EMBOSS_001       301 QNLISKNQNNN----EKNVKEAAESIMKTLAGLIKGNNQIDSTLKDLVEE
346
                     |..|....|::    ||:|.:..|...:   |..:...:.:...::..||
PmUG01_060227    439 QPNIPPRDNDDIYHKEKDVNKRKEEEKR---GEEEEKREEEEVKREEEEE
485

EMBOSS_001       347 LSKYFKNH------------------------------------------
354
                     ..|..|..
PmUG01_060227    486 KEKRRKKRRGSKKRRRGRKRGEEEEKRGEEEEKRGEEAVKEKKRKKREER
535

EMBOSS_001       355 --------------------------------------------------
354

PmUG01_060227    536 KREEEEEKRGEEEEKRGEEEKEEKRQEEEEEKRGEEEEKRGEEAVKREEE
585

EMBOSS_001       355 --------------------------------------------------
354

PmUG01_060227    586 EEKREEAVKREEEEEKREEETEKREEKEEKKQQVEKEEEEVKKEEEAVKRE
635

EMBOSS_001       355 --------------------------------------------------
354

PmUG01_060227    636 EEVEKKEEEKEKMEVHQEGAETYNEEDRKKDYGDVINFKNSAEKSIHSTID
685

EMBOSS_001       355 -----------------------------    354

PmUG01_060227    686 SIDTEKTVSDTLHGLSKDMTNYFVKDMNI    714
```

Supplementary Figure 2. The *MSP-3* gene was aligned using the EMBOSS needle tool to compare sequences between Pf and Pm.