

Development and validation of a patient-informed educational booklet for G6PD-deficient patients receiving weekly primaquine for vivax malaria in Brazil: an exploratory sequential mixed-methods study

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Abstract

Background

Plasmodium vivax accounts for approximately 80% of malaria cases in the Brazilian Amazon and remains a major challenge to malaria elimination. The introduction of quantitative glucose-6-phosphate dehydrogenase (G6PD) testing and weekly primaquine (0.75 mg/kg for 8 weeks) has improved treatment safety for G6PD-deficient patients. However, little is known about patients' understanding of G6PD deficiency, adherence to weekly treatment, or recognition of the warning signs of hemolysis. This study explored patients' experiences with weekly primaquine therapy and developed and validated a culturally adapted educational booklet.

Methods

An exploratory sequential mixed-methods study was conducted in Manaus (Amazonas) and Porto Velho (Rondônia), Brazil, between May 2023 and December 2024. Twenty-three G6PD-deficient patients receiving weekly primaquine participated in semi-structured interviews analyzed using thematic analysis. The findings guided the development of an educational booklet, which was subsequently validated by 10 experts using the Suitability Assessment of Materials (SAM) and by 10 G6PD-deficient patients. Content validity was assessed using the Item-level Content Validity Index (I-CVI), Scale-level Content Validity Index based on the average method (S-CVI/Ave), and modified kappa.

Results

Two themes emerged: (1) Challenges in communication and understanding of the G6PD deficiency diagnosis, resulting in limited knowledge, confusion, fear, and misunderstanding of the purpose of G6PD testing; and (2) factors influencing adherence to weekly primaquine therapy, including good acceptance of the regimen but frequent misinterpretation of hemolysis signs, particularly dark urine, as an expected treatment effect. The educational booklet demonstrated excellent content validity (S-CVI/Ave = 0.97 among experts and 0.91 among the target audience). Although one cultural adequacy item showed lower agreement, qualitative feedback confirmed the booklet's relevance and acceptability.

Conclusions

Communication gaps at the time of G6PD deficiency diagnosis may hinder patients' understanding of weekly primaquine treatment and delay recognition of haemolysis warning signs, despite generally favourable perceptions of the regimen. The patient-informed educational booklet demonstrated high content validity and acceptability and may support clearer counselling and safer delivery of radical cure. Integrating culturally appropriate patient education into the implementation of quantitative G6PD testing

may strengthen the safe use of 8-aminoquinolines and support *Plasmodium vivax* malaria elimination efforts.

Background

In 2024, Brazil accounted for 24.4% of malaria cases reported in the Americas, remaining one of the main contributors to the regional malaria burden [1]. In the country, malaria is highly concentrated in the Amazon region and is predominantly caused by *Plasmodium vivax*, which represents approximately 80% of reported cases [2]. Despite this persistent burden, Brazil has committed to malaria elimination by 2035 through national strategies that include improved diagnosis, safer radical cure, and implementation of new treatment algorithms[3, 4]. As part of this strategy, the implementation of tafenoquine as a single-dose radical cure for *P. vivax* malaria (300 mg) required the incorporation of quantitative glucose-6-phosphate dehydrogenase (G6PD) testing for all eligible patients using the SD Biosensor [5]. This policy shift enabled systematic G6PD screening among individuals diagnosed with *P. vivax* malaria, substantially increasing the safety of 8-aminoquinoline use, including primaquine, by tailoring treatment regimens to patients' enzymatic status.

After more than 60 years of using primaquine to control *vivax* malaria in Brazil, the incorporation of quantitative G6PD testing into routine malaria care represented a major shift in treatment safety and clinical decision-making [6, 7]. For decades, primaquine had been prescribed without prior identification of patients at risk of hemolysis, which limited the ability of health services to prevent severe adverse events [8]. Consequently, patients with G6PD deficiency in the Brazilian Amazon, where the prevalence of this condition is estimated at 5.6%, could be identified before treatment initiation, reducing the risk of adverse events [9]. Within this context, in 2020, the Brazilian Ministry of Health formally incorporated a modified primaquine regimen into national guidelines, recommending a weekly dose of 0.75 mg/kg for eight weeks for patients with confirmed G6PD deficiency (enzymatic activity ≤ 4.0 U/g Hb), as a safer alternative to daily dosing [10].

Evidence suggests that this weekly regimen reduces the risk of severe hemolysis compared with daily primaquine regimens in G6PD-deficient patients and may be effective in preventing malaria recurrence. In Brazil, a clinical trial reported that weekly primaquine was safe and showed a tendency toward improved efficacy in preventing recurrences over six months of follow-up [11].

In another study conducted in the Brazilian Amazon, patients with *vivax* malaria and G6PD deficiency were treated with weekly primaquine (0.75 mg/kg for 8 weeks). According to healthcare professionals involved in patient management, some individuals experienced challenges with the prolonged treatment duration and expressed concerns about its effectiveness. Reports indicated that some patients discontinued the regimen due to the perception that it was excessively long or less effective compared to previous treatment schemes. However, these observations reflect the perspectives of healthcare professionals, as no study has directly explored the experiences and views of patients with G6PD deficiency [12].

From an implementation science perspective, these findings illustrate that clinical effectiveness alone is insufficient to ensure real-world uptake, as factors such as patients' acceptability play a central role in

shaping the implementation of healthcare interventions [13]. Evidence consistently shows that patients' understanding of treatment rationale, perceived necessity, and alignment with prior experiences are key determinants of adherence, particularly when therapeutic regimens deviate from standard or familiar practices [14]. For G6PD deficiency, safe treatment also depends on patients' ability to understand a genetic enzymatic condition, avoid relevant medication-related risks, and seek timely care when symptoms such as dark urine, jaundice, pallor, or severe weakness occur. These demands are especially challenging in contexts where biomedical concepts may be unfamiliar, and health communication is not always adapted to patients' literacy levels, cultural references, or previous experiences with malaria care [15].

In this context, culturally adapted educational materials are particularly important for conditions such as G6PD deficiency, where safe treatment depends on patients' understanding of genetic risk, medication triggers, and early signs of hemolysis. Evidence indicates important knowledge gaps among key groups, including parents, caregivers, and frontline health professionals, particularly regarding inheritance patterns, drug safety, and clinical management. Therefore, this study aimed to explore the experiences and perceptions of G6PD-deficient patients undergoing weekly primaquine therapy for *P. vivax* malaria in the Brazilian Amazon and to use these findings to develop and validate a culturally adapted educational booklet for this population.

Methods

Study area

This study was conducted in Manaus, Amazonas State, and Porto Velho, Rondônia State, two capitals in the Brazilian Amazon that were prioritized for implementing

point-of-care quantitative G6PD testing as part of the new treatment algorithm for *P. vivax* malaria in Brazil. Both municipalities are located in malaria-endemic areas and report a substantial annual burden of malaria cases, predominantly caused by *P. vivax*. These municipalities include urban, peri-urban, rural, and riverine/peri-riverine populations, with heterogeneous patterns of access to malaria diagnosis, follow-up care, and health information. Such diversity is particularly important for the development of culturally adapted educational materials, as patients may differ in their previous experiences with malaria treatment, familiarity with biomedical terminology, and ability to access health services during an eight-week treatment regimen (Fig. 1).

The research was conducted between May 2023 and December 2024. During this period, more than 20,000 malaria cases were reported in the two municipalities, most of which were attributed to *P. vivax* [16]. Participants were recruited from health services involved in implementing quantitative G6PD testing and prescribing weekly primaquine for individuals classified as G6PD-deficient. **Fig. 1 Study sites: Manaus and Porto Velho, Brazil.** A) Floating houses in the peri-urban area of Manaus; B) Highway in the urban area of Porto Velho; and C) Dirt road in the rural area of Porto Velho. The base used to create the map is from the IBGE (Brazilian Institute of Geography and Statistics), which is freely accessible for creative uses in shapefile format, in accordance with the Brazilian Access to Information Law (12,527/2011) [17].

Study design

This study adopted an exploratory sequential mixed-methods design to develop and validate a culturally adapted educational booklet for individuals with G6PD deficiency undergoing 8-week primaquine treatment (0.75 mg/kg/week) for *vivax* malaria. This design was selected because the development of the educational material required an initial qualitative understanding of patients' knowledge gaps, concerns, language, and lived experiences with G6PD deficiency and the 8-week primaquine regimen.

In this design, qualitative findings were first used to understand patients' experiences and identify knowledge gaps, which subsequently informed the development of educational materials. The resulting material was then evaluated through a structured validation process involving both experts and members of the target population (Fig. 2).

Phase 1: Exploratory qualitative study

Recruitment of individuals with G6PD deficiency

Participants were recruited by purposive sampling, guided by predefined eligibility criteria. This approach was used to identify individuals with direct experience of the phenomenon of interest, as recommended for qualitative studies in implementation research [18]. Within this purposive sample, recruitment sought variation by sex and municipality of residence, aiming to include participants with different experiences of diagnosis, counselling, access to care, and use of weekly primaquine.

Between May 2023 and December 2024, potential participants were identified from malaria surveillance databases in both cities, which included malaria diagnosis, quantitative G6PD results, treatment information, and contact details when available. G6PD deficiency was identified during routine use of quantitative point-of-care testing with the STANDARD G6PD Test (SD Biosensor, Suwon-si, South Korea [19, 20], implemented across health facilities in both cities as part of the treatment algorithm introduced by the Brazilian Ministry of Health [10]. G6PD deficiency was defined as enzyme activity ≤ 4.0 U/g Hb, according to the manufacturer's instructions. Individuals classified as G6PD-deficient were prescribed weekly primaquine at 0.75 mg/kg once weekly for 8 weeks.

Eligible participants were individuals diagnosed with *P. vivax* malaria, classified as G6PD-deficient by health services, and recruited within four months after completing the prescribed 8-week primaquine regimen. This pragmatic cutoff was used to keep the interviews close to the malaria episode and treatment period, while allowing participants to have experienced the full regimen. Among the 192 eligible individuals identified in the surveillance databases, 56 had valid telephone contact information and were invited to participate. Of these, 23 agreed to participate and were interviewed. The 33 individuals who did not participate either declined the invitation or were unavailable for an interview despite repeated attempts to schedule an interview. The remaining 136 individuals could not be contacted because their telephone information was unavailable or inactive.

G6PD status confirmation

To confirm eligibility, all recruited individuals underwent repeat G6PD assessment at the time of the interview. Testing was performed by two trained biochemists from the research team using the same point-of-care device used in the health services. Because the surveillance databases available for this study recorded only whether individuals were classified as G6PD-deficient, without numerical enzyme activity values, repeat testing was conducted to confirm this classification, reduce the risk of operational errors during initial screening, and obtain enzyme activity measurements for the study. Also, at recruitment, participants did not report fever or a new malaria diagnosis after the previous episode. However, the research team did not perform any malaria tests at that time.

Qualitative data collection

We conducted individual, face-to-face in-depth interviews (IDIs) using a semi-structured interview guide developed by the research team (Additional file 1), which included open-ended questions to allow in-depth exploration of relevant topics. The guide was iteratively adjusted during the interviews to incorporate emerging themes and refine questions based on participant responses and observed dynamics.

The interviews were conducted in participants' homes and lasted approximately 30 minutes. Only the participant, the interviewer, and an observer responsible for taking field notes were present. With participants' consent, the interviews were audio-recorded. RND, APCS, LSMB, APSO, HSSG, EARTS, and HAAJ contributed, according to their respective roles, to conducting the interviews, collecting field notes, and transcribing the audio recordings verbatim in Brazilian Portuguese. Quotations selected for publication were translated into English by HSSG and independently reviewed by FLGM to ensure semantic consistency with the original accounts.

The number of interviews was defined during fieldwork, through concurrent data collection and preliminary analysis. After each round of interviews, the research team reviewed field notes and preliminary codes to assess whether new, relevant information was still emerging [24]. Data saturation was considered reached when subsequent interviews no longer generated new codes or themes related to participants' understanding of G6PD deficiency, counselling received, access to health services, use of weekly primaquine, and perceived barriers to treatment completion.

Qualitative data analysis

The data were transcribed without personal identifiers and uploaded into MAXQDA software (2024 version) for thematic analysis, following Braun and Clarke's approach [25]. This approach was chosen for its flexibility in exploring patterns across participants' accounts while preserving the context and meaning of individual experiences with G6PD deficiency and weekly primaquine treatment.

RND and RAF conducted line-by-line inductive coding separately, and the results were reviewed by APCS (Additional file 2). Preliminary categories were developed by RND and RAF and refined through consensus

with APCS and FLGM, culminating in the final themes. Data from IDIs were triangulated with field notebook entries, which were used to contextualize participants' narratives and capture non-verbal cues and situational aspects of the interviews.

Research team and reflexivity for a qualitative approach

The qualitative component of the study was conducted by a multidisciplinary team with experience in qualitative research on malaria, G6PD deficiency, and neglected tropical diseases in the Amazon region [1–3]. The qualitative component was conducted by a multidisciplinary team with experience in malaria, G6PD deficiency, and qualitative research in the Brazilian Amazon. The core qualitative team comprised four researchers with PhD degrees (FLGM, MVGL, MAMB, and APCS) and five with MSc degrees (RND, RAF, LSMB, HSSG, and EARTS), including four women and five men. Team members contributed to study design, interview guide development, data collection, analysis, and interpretation in accordance with their respective roles. All members had formal training or previous experience in qualitative research on malaria or neglected tropical diseases in the Amazon region.

The interviewers had no prior relationship with participants before recruitment. To support reflexivity, field notes were recorded during interviews and discussed in team meetings throughout data collection and analysis. Preliminary codes and emerging themes were reviewed collectively to identify alternative interpretations and reduce the influence of researchers' prior assumptions on data interpretation. The study adhered to the COREQ (Consolidated Criteria for Reporting Qualitative Research) guidelines (Additional file 3) to ensure rigorous and ethical reporting of the research process and findings [26].

Phase 2: Booklet development

Following the thematic analysis conducted in Phase 1, an educational booklet was developed by RND, LSMB, APSO, HSSG, TLG, and ICS (Additional file 4). The development process was based on a theme-to-content matrix, linking the main findings from the interviews to the sections, messages, and visual elements included in the booklet.

The research team held five meetings to review the qualitative themes and identify the information that should be addressed in the material. Interview excerpts and field notes were used to define the main educational messages, with particular attention to participants' doubts about G6PD deficiency, concerns about hemolysis, and difficulties understanding the 8-week weekly primaquine regimen. Decisions about content, wording, and illustrations were recorded in meeting notes and incorporated into successive versions of the booklet.

The booklet structure was organized according to the main educational needs identified in Phase 1. Questions about G6PD deficiency informed the section that explains the enzyme's role in red blood cells. Concerns about hemolysis guided the use of a shield as a visual metaphor for the protective role of G6PD. Difficulties understanding weekly primaquine were informed in the section describing the treatment

schedule and the importance of completing all doses. This process aimed to preserve the connection between participants' accounts and the final educational content.

An interdisciplinary team reviewed the draft material and defined the final messages and visual elements. A professional illustrator with experience in health communication contributed to the visual development of the booklet using CorelDRAW® software. The illustrations were designed to support the explanation of biomedical concepts using simple language and images related to patients' everyday experiences.

One central visual element was the representation of a shield protecting the red blood cell, used as a metaphor for the protective role of the G6PD enzyme. This element was used to explain the relationship between enzyme deficiency, risk of hemolysis, and the need for adapted malaria treatment (Fig. 3).

Phase 3: Booklet validation

The educational booklet was submitted to content validation by a panel of experts using an adapted version of the Suitability Assessment of Materials (SAM) instrument, previously translated and validated in Portuguese. Sixteen experts were invited electronically, of whom ten completed the assessment, corresponding to a response rate of 62.5%. The expert panel was purposively selected to include researchers and health professionals with expertise in malaria diagnosis, treatment, control, G6PD deficiency, health education, or implementation of malaria-related interventions in endemic settings.

Experts completed the assessment through an online form (Additional file 5). The instrument evaluated content accuracy, language, illustrations, layout and presentation, cultural adequacy, motivational appeal, and relevance for patients receiving weekly primaquine treatment. Each item was rated on a five-point scale: not applicable, inadequate, partially adequate, adequate, or fully adequate.

For the quantitative analysis, ratings of "adequate" and "fully adequate" were considered agreement with item suitability, whereas "inadequate" and "partially adequate" indicated the need for revision. Items rated as "not applicable" were analyzed separately and excluded from the denominator when calculating item-level agreement.

Validation with the target audience was also conducted. The target audience comprised patients with G6PD deficiency who had received weekly primaquine treatment for *P. vivax* malaria during 2023–2024. Participants were recruited by purposive sampling from health services involved in the new malaria treatment algorithm. The sample included individuals previously enrolled in Phase 1 (n = 6), to maintain continuity with the exploratory findings, and newly recruited participants who had not taken part in the earlier phase (n = 4), to assess the booklet from the perspective of patients not involved in its initial development.

Participants completed the assessment in person using an adapted SAM-based instrument (Additional file 6). The booklet was evaluated for content, clarity of language, visual presentation, motivational appeal, and cultural adequacy. A three-point scale was used: inadequate, partially adequate, and fully adequate.

Qualitative feedback was also collected through interviews to explore participants' perceptions of the booklet, identify unclear terms or images, and gather suggestions for improvement.

Feedback from experts and target-audience participants was reviewed by the research team and used to refine the final version of the booklet. Revisions focused on improving clarity, reducing technical language, adjusting illustrations and page layout, and ensuring consistency between the educational messages, the qualitative findings from Phase 1, and national recommendations for G6PD deficiency and weekly primaquine treatment.

Validation data analysis

Quantitative data from the expert validation were analyzed using content validity indices. For each item, responses were dichotomized to calculate agreement: ratings of "adequate" and "fully adequate" were considered agreement with item suitability, whereas "inadequate" and "partially adequate" were considered non-agreement. Responses marked as "not applicable" were analyzed separately and excluded from the denominator for the corresponding item.

Content validity was assessed separately for the expert committee and the target audience, in accordance with the recommendations of Polit and Beck [27]. In both phases, the Item-level Content Validity Index (I-CVI) was calculated as the proportion of evaluators who rated each item as relevant. For the expert committee, ratings of 4 ("adequate") or 5 ("fully adequate") on the five-point Likert scale were considered indicative of item relevance. For the target audience, only the highest score (3 = "totally adequate") on the three-point Likert scale was considered indicative of relevance. The Scale-level Content Validity Index based on the average method (S-CVI/Ave) was calculated as the mean of all I-CVI values. To account for chance agreement, the *modified kappa coefficient* (k^*) was calculated for each item following the recommendations of Polit et al. [28]. The probability of chance agreement (P_c) was estimated using the formula: $P_c = [N! / A!(N - A)!] \times 0.5^N$, where N represents the number of valid ratings for each item and A represents the number of evaluators who rated the item as relevant. The modified kappa coefficient was calculated as: $k^* = (I-CVI - P_c) / (1 - P_c)$. According to Polit et al., I-CVI values ≥ 0.78 , S-CVI/Ave ≥ 0.90 , and k^* values > 0.74 were considered indicative of excellent content validity.

Quantitative data from the target audience validation were analyzed descriptively using absolute and relative frequencies for each response category. Qualitative feedback was reviewed by the research team and grouped descriptively into recurrent categories related to clarity, visual presentation, cultural identification, and perceived usefulness. Analyses were conducted in R software v. 4.2 and RStudio, using the tidyverse, readxl and writexl packages.

Results

Phase 1 – G6PD exploratory qualitative study

In phase 1, 23 individuals were interviewed (20 men and 3 women), aged 18 to 83 years. The predominance of male participants reflects the epidemiological profile of G6PD deficiency, an X-linked disorder that is

more commonly expressed in males. All participants had their diagnosis of G6PD deficiency confirmed by the study team and received weekly primaquine therapy (Table 1). None of the participants initially classified as deficient by the healthcare service presented normal G6PD activity upon retesting by the research team.

Table 1
Sociodemographic and clinical characteristics of participants with G6PD deficiency included in Phase 1 of the study (exploratory qualitative interviews).

Characteristic	n (%)
Location	
Manaus	18 (78.3)
Porto Velho	5 (21.7)
Sex	
Male	20 (87.0)
Female	3 (13.0)
Area of residence	
Urban	10 (43.5)
Rural	9 (39.1)
Peri-urban	4 (17.4)
G6PD activity (U/g Hb)	
Mean ± SD	1.82 ± 0.71
Median (IQR)	1.8 (1.3–2.2)
Range	0.9–3.8

Mean ± SD: Mean ± standard deviation; IQR: Interquartile Range.

Thematic analysis of the 23 interviews identified two overarching themes and four interconnected subthemes that describe participants' experiences with G6PD deficiency diagnosis and weekly primaquine treatment. The themes were consistently identified across interviews conducted in both study sites, with no substantially new concepts emerging in the final interviews. These themes are summarized and presented below with illustrative excerpts from the interviews (Fig. 4).

Theme 1: Challenges in communication and understanding of the G6PD deficiency diagnosis

Subtheme 1a: Scarcity of information regarding the G6PD diagnosis

Participants commonly reported that G6PD deficiency was introduced during malaria testing or treatment without sufficient explanation of its meaning or implications. For some, the test was performed as part of routine care, but the reason for testing and the consequences of a deficient result were unclear. Even when explanations were provided, participants described them as technical and difficult to understand, leading to confusion between the enzyme, the test, and the malaria treatment.

"Most of the time they don't say anything to us [...] they don't explain why we cannot take certain medications. I don't know what G6PD is, I don't know. Nobody ever explained it, and I have never studied it either" (Participant 06, Manaus).

"The professionals said that I had low immunity and that I needed to take some vitamins. I took a cocktail of vitamins, but I don't know how things are today" (Participant 08, Manaus).

Conversely, those who did receive some form of explanation still reported significant difficulties in understanding the diagnosis. The main challenge was the lack of clarity in communication, particularly due to the complexity of the subject, which became even more confusing when searching for information online. Many highlighted that, given the technical nature of the topic, their understanding of the diagnosis was compromised. In addition, participants frequently confused concepts such as the enzyme, the diagnostic test, and the treatment, underscoring the need for educational materials to support comprehension of the condition.

"I was confused, because I don't know what G6PD is. So I looked it up on the internet, but I didn't really understand it" (Participant 12, Porto Velho).

"The diagnosis was very confusing for me, because to this day I don't understand much about malaria treatment or G6PD. The health professional said that the treatment was not the usual one, that the medications were stronger, and that I needed to follow it carefully and correctly, otherwise I would feel unwell" (Participant 16, Porto Velho).

The lack of clear information at diagnosis resulted in uncertainty, anxiety, fear, and confusion, leaving many participants uncertain about the implications of living with a lifelong genetic condition. The absence of adequate explanations led to dissatisfaction and raised questions about the lack of specific guidance for managing this genetic condition, including doubts about the possibility of treatments or medications to restore the deficient enzyme.

"I think that if there was a problem (G6PD deficiency), they should have prescribed some medication for me to take [...] they should have told me whether there is a treatment or a medicine to buy. None of this was done. I have had no answers to this day, and I still don't know if treatment exists. That is something I would like to know" (Participant 13, Porto Velho).

"When I received the treatment, I asked: 'Does this (G6PD) have a treatment?' and they said: 'No, it is a diagnosis that some people have'... I was a bit scared at first, but then I calmed down" (Participant 10, Manaus).

Subtheme 1b: G6PD deficiency and its association with treatment

In the absence of adequate counselling at the time of diagnosis, participants constructed their own explanations for the purpose of G6PD testing. Most interpreted the test as an assessment of liver function or the body's ability to tolerate malaria treatment, while others associated it with conditions such as thrombocytopenia, allergy, or low immunity.

"This exam (G6PD test) is for everyone who has malaria. It serves to check how the liver is functioning and whether it will withstand the medication or not, because the medicine is very strong. Depending on the G6PD result, health professionals prescribe the treatment. That is why I am now taking the 8-week primaquine regimen" (Participant 09, Manaus).

"When I was diagnosed with malaria, they told me my platelets were low, which is why I had the weekly treatment. It was three days of medication followed by eight Sundays of medicine" (Participant 19, Manaus).

Theme 2: Key factors influencing adherence to weekly primaquine therapy

Subtheme 2a: Impact of weekly treatment on daily life

Participants evaluated the weekly regimen positively, emphasizing that it caused fewer adverse effects compared to the daily regimen to which they had previously been exposed. They highlighted that the treatment felt lighter and resulted in milder symptoms. Patients with multiple malaria episodes also noted greater attention during the prescription process, considering this aspect positive during the treatment.

"The first time I had malaria, I received fewer medicines, and I remember the treatment lasted about a week and a half. But this time, I found the treatment lighter and I did not feel unwell from the medication" (Participant 16, Porto Velho).

"My treatment was weekly. I could no longer take the daily primaquine because of this thing (G6PD enzyme) [...] so, this time it was different, because in my previous malaria episodes they never did that [...] this last one was more detailed" (Participant 13, Porto Velho).

Although the regimen was longer, participants reported that it did not significantly affect their daily routines, particularly regarding work. This contrasted with their initial expectations that the treatment would be more difficult due to the prolonged disappearance of malaria symptoms and possible persistence of side effects. The main complaints were the inability to consume alcoholic beverages for an extended period and some discomfort during the first days of treatment.

“Regarding work, the treatment did not affect me because I was unemployed. For me, it only affected weekends, since I went a long time without being able to drink alcohol” (Participant 12, Porto Velho).

“I thought the treatment would be harder in daily life, that the symptoms would last longer. But even though it was quite long, I didn’t feel anything” (Participant 16, Porto Velho).

Subtheme 2b: Impressions of hemolysis during treatment

A central safety concern was the normalization of possible hemolysis warning signs. Participants reported receiving limited guidance, often restricted to observing urine colour, without clear information about what changes would require medical attention. As a result, dark or reddish urine was frequently interpreted as an expected effect of primaquine rather than a potential warning sign.

“They didn’t tell me about the reactions the medicine could cause, whether I could feel what I felt (dizziness)” (Participant 22, Manaus).

“At the primary health unit I attended, they only said: ‘Look, you need to watch the color of your urine,’ and that was it. That is why I went to the referral hospital, because I was worried about my red urine” (Participant 06, Manaus).

“For me, it was a shock to have red urine. I even went to the community pastor and told him, ‘Pastor, I am urinating red.’ I was embarrassed to say it, but he told me: ‘No, my son, this is from the medicine, it is like that,’ because he also had malaria many times” (Participant 02, Manaus).

“I think the urine turns red because of the medicine, but I don’t know what the composition of this medicine is” (Participant 20, Manaus).

Phase 2 - Development of the educational booklet

The qualitative findings from Phase 1 were translated into the validation version of the educational booklet. The final material was organized around three main educational needs identified in the interviews: understanding what G6PD deficiency is, recognizing why weekly primaquine was prescribed instead of the usual daily regimen, and identifying warning signs of hemolysis that require prompt medical attention.

The booklet included simple explanations of the role of G6PD in red blood cells, the relationship between G6PD deficiency and malaria treatment safety, the rationale for the 8-week weekly primaquine regimen, and visual guidance on symptoms such as dark urine, jaundice, pallor, severe weakness, and dizziness.

Table 2 summarizes how the main qualitative findings informed the educational content included in the booklet.

Table 2
Qualitative findings informing the design of the educational booklet

Theme	Key finding from qualitative analysis	Incorporation into educational booklet
Challenges in communication and understanding of the G6PD deficiency	Limited understanding due to technical complexity and insufficient explanations from healthcare professionals.	Clear, accessible language with illustrative visual resources.
Key factors influencing adherence to weekly primaquine therapy	Concerns about treatment impact on daily life; poor recognition of hemolysis warning signs.	Graphical representation of warning signs requiring prompt medical attention.

In Phase 3, the expert validation panel comprised ten healthcare professionals and researchers, including seven women and three men. The panel was multidisciplinary, with experts from medicine (n = 2), pharmacy (n = 2), biomedical sciences (n = 2), nursing (n = 1), biology (n = 1), public health (n = 1), and social work (n = 1). Their experience with malaria ranged from 1 to 35 years, supporting the assessment of the booklet from clinical, laboratory, public health, and health education perspectives.

Moreover, 10 participants from the target population were also included in the validation, of whom 6 had previously participated in the exploration interviews, and 4 were newly recruited (Table 3).

Table 3
Characteristics of the target audience included in the validation

Variable	Category	n (%)
Sex	Male	7 (70.0)
	Female	3 (30.0)
Age group	18–39 years	2 (20.0)
	40–59 years	5 (50.0)
	≥ 60 years	3 (30.0)
Education level	Incomplete elementary education	5 (50.0)
	Complete secondary education	2 (20.0)
	Incomplete secondary education	1 (10.0)
	Complete higher education	2 (20.0)
Race/Color	Mixed (Parda)	7 (70.0)
	Black	1 (10.0)
	Indigenous	1 (10.0)
	White	1 (10.0)
Marital status	Married	6 (60.0)
	Single	2 (20.0)
	Divorced	1 (10.0)
	Widowed	1 (10.0)
Occupation	Retired	4 (40.0)
	Unpaid domestic work	2 (20.0)
	Self-employed/informal urban work	2 (20.0)
	Rural work	1 (10.0)
	Skilled technical work	1 (10.0)
Area of residence	Rural	5 (50.0)
	Urban	4 (40.0)
	Peri-urban	1 (10.0)

All items evaluated by the experts reached the predefined I-CVI threshold of 0.78 and were therefore considered valid. Item-level I-CVI values ranged from 0.90 to 1.00. The overall S-CVI/AVE was 0.97, indicating strong content validity of the booklet as a whole. Universal agreement was observed in 14 of the

21 items, resulting in an overall S-CVI/UA of 0.67. Items without universal agreement still achieved satisfactory I-CVI values. Based on expert feedback, only minor refinements were made to wording, layout, and visual presentation. The validation results are presented in Table 4.

Table 4
Results of the validation of the educational booklet with specialists

Domain	Item	I-CVI	Modified Kappa (k*)
1. Content	1.1 The material is easy to understand	1	1
	1.2 The information promotes health education	1	1
	1.3 The material is scientifically consistent	1	1
2. Language	2.1 The reading level is appropriate for the audience	0.9	0.9
	2.2 The messages are presented clearly and objectively	0.9	0.9
	2.3 Common vocabulary words are used	0.9	0.9
	2.4 The content follows a logical sequence	0.9	0.9
	2.5 The information is well structured, with proper grammar and spelling	1	1
	2.6 The texts facilitate learning	1	1
3. Illustrations	3.1 The cover is attractive and consistent with the subject	1	1
	3.2 The illustrations convey essential visual messages that allow readers to understand the main points independently	0.9	0.9
	3.3 The illustrations are adequate in size and number	1	1
	3.4 The illustrations are relevant	1	1
4. Presentation	4.1 The material follows a logical sequence	1	1
	4.2 The font size and style facilitate pleasant reading	1	1
	4.3 The size of titles and headings is appropriate	1	1
5. Cultural adequacy	5.1 The material is culturally appropriate to the audience's logic, language, and experience	0.9	0.9
	5.2 The illustrations are culturally appropriate	0.9	0.9
6. Relevance	6.1 The topics address important aspects that should be reinforced	1	1
	6.2 The material adds knowledge to the target audience regarding self-care	1	1
	6.3 The material is suitable for use by health professionals in educational activities	1	1
S-CVI/Ave		0.97	

I-CVI: Item-level Content Validity Index: S-CVI/Ave: Scale-Level Content Validity Index / Average Calculation Method

The booklet was also evaluated by members of the target audience, who assessed the material using the adapted SAM-based instrument (Table 5). Most items reached satisfactory I-CVI values, and the overall S-CVI/Ave was 0.91, indicating adequate validity of the booklet as a whole. The only item below the predefined threshold was item 5.1, related to cultural adequacy, suggesting that some images, words, or situations could be further refined to better reflect participants' daily experiences.

The motivation domain showed particularly strong results, with universal agreement across all subitems. Participants reported that the booklet encouraged them to read the material to the end and to reflect on self-care related to G6PD deficiency and weekly primaquine treatment. Qualitative feedback also indicated that the material was perceived as clear, useful, and relevant, especially because it addressed the same treatment regimen experienced by participants.

Table 5
Results of the validation of the educational booklet with the target audience

Domain	Item	I-CVI	Modified Kappa (k*)
1. Content	1.1 Is the material easy to understand?	0.9	0.9
	1.2 Is the sequence of content appropriate?	0.9	0.9
	1.3 Is the structure of the booklet adequate?	1	1
2. Writing	2.1 Is the font size appropriate?	0.9	0.9
	2.2 Are the words easy to read?	0.9	0.9
	2.3 Is the text easy to understand?	0.9	0.9
3. Appearance	3.1 Does the material look organized?	1	1
	3.2 Does the cover catch your attention?	0.9	0.9
	3.3 Can you see and understand the images easily?	0.9	0.9
	3.4 Are the images self-explanatory?	0.9	0.9
	3.5 Do the illustrations complement the text?	0.9	0.9
4. Motivation	4.1 Did you feel motivated to read the material to the end?	1	1
	4.2 Did this material motivate you to think about self-care related to G6PD deficiency?	1	1
5. Cultural adequacy	5.1 Do the images, words, and situations presented in this material resemble those in your daily life?	0.7	0.66
S-CVI/Ave =		0.91	

I-CVI: Item-level Content Validity Index; S-CVI/Ave: Scale-Level Content Validity Index / Average Calculation Method

Table 6
Participant's quotes about the booklet

Main Finding	Participant's quotes
Acceptability and Identification	<i>"Here I understood it personally because it was the same medication I took, exactly the same way, since my G6PD was low, I took it only once a week." (Participant 02, validation interview)</i>
Clarity of language	<i>"It is easy to understand [...] when you have some free time, you can pick it up and study it again" (Participant 04, validation interview).</i>
Visual resources	<i>"I thought it was important to have the little pictures, colorful, so we could see it better" (Participant 01, validation interview).</i>
	<i>"I liked this part that shows the disease and the symptoms; it is really good and easy to understand" (Participant 05, validation interview).</i>
Gaps in health communication	<i>"The healthcare professional said, 'yours is different because your enzyme level was low' [...] but then she could not explain it to me." (Participant 03, validation interview).</i>
Valuing health information	<i>"When I have some free time, I can pick it up and study it again, because this is something that is not talked about very much." (Participant 04, validation interview).</i>

Discussion

In this study, the sequential mixed-methods design was central to developing and validating the educational booklet. Rather than developing the material solely from biomedical recommendations, the qualitative phase allowed patients' uncertainties, language, misconceptions, and safety concerns to shape the booklet's core messages. This approach aligns with other studies showing that qualitative data and user involvement strengthen content validity by improving the relevance, comprehensiveness, and comprehensibility of health education materials [33, 34]. In this study, patients' difficulty understanding G6PD deficiency informed the use of simplified explanations and visual metaphors; concerns about weekly primaquine informed the treatment schedule section; and the normalization of dark urine as a "normal" drug effect informed the emphasis on hemolysis warning signs and return-to-care messages. Thus, the validation results should be interpreted not only as an expert assessment of technical adequacy but also as evidence that patient-derived content can improve the acceptability and practical relevance of educational technologies.

This study reveals that communication about G6PD deficiency remains a central challenge in the implementation of safer radical cure strategies for *P. vivax* malaria. Patients' limited understanding of the enzyme, the meaning of G6PD test results, and the rationale for receiving PQ8W was consistently associated with confusion, fear, and uncertainty. These findings are relevant because effective communication is widely recognized as a key determinant of successful implementation of health technologies and services [35, 36]. In addition, communication between health professionals and patients is strongly associated with treatment adherence, with evidence showing that poor communication increases the risk of non-adherence by up to 19% [37, 38].

Communication barriers may be intensified by the patient's altered health status during the malaria episode, unfamiliarity with biomedical concepts, and the low educational level frequently observed in malaria-vulnerable populations. This concern is consistent with evidence from other settings showing poor knowledge among diagnosed G6PD-deficient patients, including limited awareness of hemolysis triggers and of the importance of informing healthcare providers about their condition [39]. When patients do not receive clear and reliable information about the rationale for treatment and potential adverse effects, they may rely on their own interpretations and self-management strategies, including dosage adjustments, treatment interruption, or self-medication without clinical supervision [40]. Therefore, improving counseling about G6PD deficiency, weekly primaquine, and warning signs of hemolysis should be considered a core component of patient safety and adherence support.

Based on these results, it is evident that understanding of G6PD test results and adherence to PQ8W are strongly influenced by how health professionals convey information. Barriers such as communication difficulties, the altered health status of patients at the time of diagnosis, and the low educational level frequently observed in malaria-vulnerable populations may further compromise understanding.

In terms of adherence to the weekly primaquine regimen, the findings reveal that the participants positively evaluated the weekly primaquine regimen, reporting fewer adverse effects and less impact on daily life compared to the daily regimen [41]. Contrary to concerns reported in previous implementation studies [42], in which most patients expressed discomfort with weekly PQ due to its long duration and fear of missed doses or inefficacy, our study showed more favorable perceptions.

However, this acceptance coexisted with a lack of understanding regarding hemolysis signs, which were frequently normalized, minimized or misinterpreted, such as dark urine as a normal drug effect rather than a warning sign. This phenomenon can be explained by the fact that patients tend to rely on their own cognitive frameworks and interpretive resources to identify and make sense of treatment-related symptoms. Consequently, patients may delay seeking medical assistance, thereby raising the likelihood of severe outcomes [43].

Regarding booklet validation, although the material achieved satisfactory overall indices among experts and target-audience participants, the item assessing whether images, words, and situations resembled participants' daily lives did not reach the expected threshold. This finding is important because cultural adaptation should not be understood as a single endpoint, but as an iterative process involving both surface-level elements, such as language, images, characters, settings, and everyday situations, and deeper elements related to beliefs, explanatory models, social experiences, and contextual barriers to health behaviors [44, 45].

In the present study, the qualitative phase supported deeper cultural adaptation by incorporating patients' uncertainty about G6PD deficiency, their interpretations of weekly primaquine dosing, and their concerns about hemolysis into the booklet's content. However, the lower score for cultural adequacy suggests that some surface-level elements may not have fully represented the diversity of Amazonian malaria contexts, particularly urban, peri-urban, rural, and riverine realities. Similar studies with culturally adapted educational materials have shown that end-user validation can reveal limited identification even when content is

technically correct, and that successive revisions using familiar people, settings, and daily activities may improve perceived relevance and acceptance [46, 47]. Nevertheless, this limitation did not undermine the material's perceived importance, usefulness, or overall acceptability. Therefore, the booklet should be interpreted as a patient-informed and culturally sensitive educational technology with satisfactory overall validation, but one that requires further participatory refinement of its visual and contextual elements before broader implementation.

These findings reinforce that communication and health education should not be treated as secondary actions but as central strategies in the implementation of new health technologies [48]. Also, the literature supports that accessible, contextualized, and culturally sensitive educational materials can enhance understanding, promote self-care, and strengthen patients' autonomy [49].

Limitations

This study has limitations. It was conducted in Manaus and Porto Velho and included few women, so the findings may not reflect the experiences of G6PD-deficient patients across the Brazilian Amazon. Recruitment depended on valid telephone contacts: of 192 eligible individuals, only 56 could be reached and 23 were interviewed. Participants who were contacted and agreed to participate may therefore differ from those who were not reached or declined. Interviews were conducted up to four months after treatment, which may have affected recall of counselling, symptoms, and medication use.

Six of the ten target-audience participants had also taken part in Phase 1. This continuity preserved the link between patients' experiences and booklet development, but previous involvement may have favoured positive assessments. Validation was conducted through direct interaction with the research team, which may also have influenced responses. The item on cultural adequacy remained below the predefined threshold, and a second validation round could not be conducted because of logistical constraints.

The study assessed content validity, clarity, and acceptability, but not effectiveness. It did not measure knowledge retention, adherence, recognition of haemolysis warning signs, healthcare-seeking behaviour, or clinical outcomes. Further studies should evaluate the booklet in routine health services and with a broader range of Amazonian populations.

Conclusions

Among the interviewed participants, the eight-week duration of weekly primaquine was not perceived as a major barrier to treatment completion. The main challenges were gaps in communication about G6PD deficiency, the rationale for weekly primaquine, and the recognition of potential haemolysis warning signs. The patient-informed educational booklet showed high content validity and acceptability and may support clearer and culturally appropriate counselling. Integrating patient education into quantitative G6PD testing strategies may contribute to safer radical cure and support *Plasmodium vivax* malaria elimination efforts in Brazil.

Abbreviations

AHA

Acute Hemolytic Anemia

COREQ

Consolidated Criteria for Reporting Qualitative Research

G6PD

Glucose-6-phosphate dehydrogenase

IBGE

Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística*)

I

CVI–Item–level Content Validity Index

IDIs

In–depth individual interviews

K*

Modified Kappa Statistic

PQ

Primaquine

PQ8W

Primaquine 8–week regimen

SAM

Suitability Assessment of Materials

S

CVI/Ave–Scale–level Content Validity Index/Average

SUS

Brazilian Unified Health System (*Sistema Único de Saúde*)

U/g Hb

Units per gram of hemoglobin

Declarations

Ethics statement

The Ethics Review Board at the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado in Manaus (CAAE: 67531223.2.0000.0005) approved the study. Eligible participants provided informed consent before participation, following ethical guidelines in Brazil. For literate participants, written consent was obtained, and each participant received a copy of the consent form. For illiterate participants, verbal informed consent was recorded, accompanied by the signature of a legally authorized witness, as required by Brazilian legislation.

Consent for publication

Written informed consent for publication of anonymized quotations was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

RND, APCS, MVGL, MAMB, and FLGM conceived and designed the study. RND and APCS developed the semi-structured interview guide. RND, APCS, LSMB, APSO, HSSG, EARTS, and HAAJ contributed to participant recruitment, G6PD testing, interviews, field-note collection, and transcription. RND and RAF performed the qualitative coding and analysis, with APCS and FLGM contributing to theme development and interpretation. RND, LSMB, APSO, HSSG, TLG, and ICS developed the educational booklet. FLGM led the quantitative validation analyses. HM, JSMC, YEARS, GCCP, MOM, RSAG, JV, and WM contributed to the interpretation of the findings and critically revised the manuscript. MVGL, MAMB, and FLGM supervised the study. RND, APCS and FLGM drafted the manuscript. All authors read and approved the final manuscript and agreed to be accountable for the work.

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Data Availability

The datasets generated and analysed during the current study are not publicly available because the qualitative data contain potentially identifiable and sensitive information. De-identified qualitative and quantitative data may be available from the corresponding author on reasonable request and subject to ethical and institutional approval. The study instruments and educational booklet are provided as additional files.

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Figures

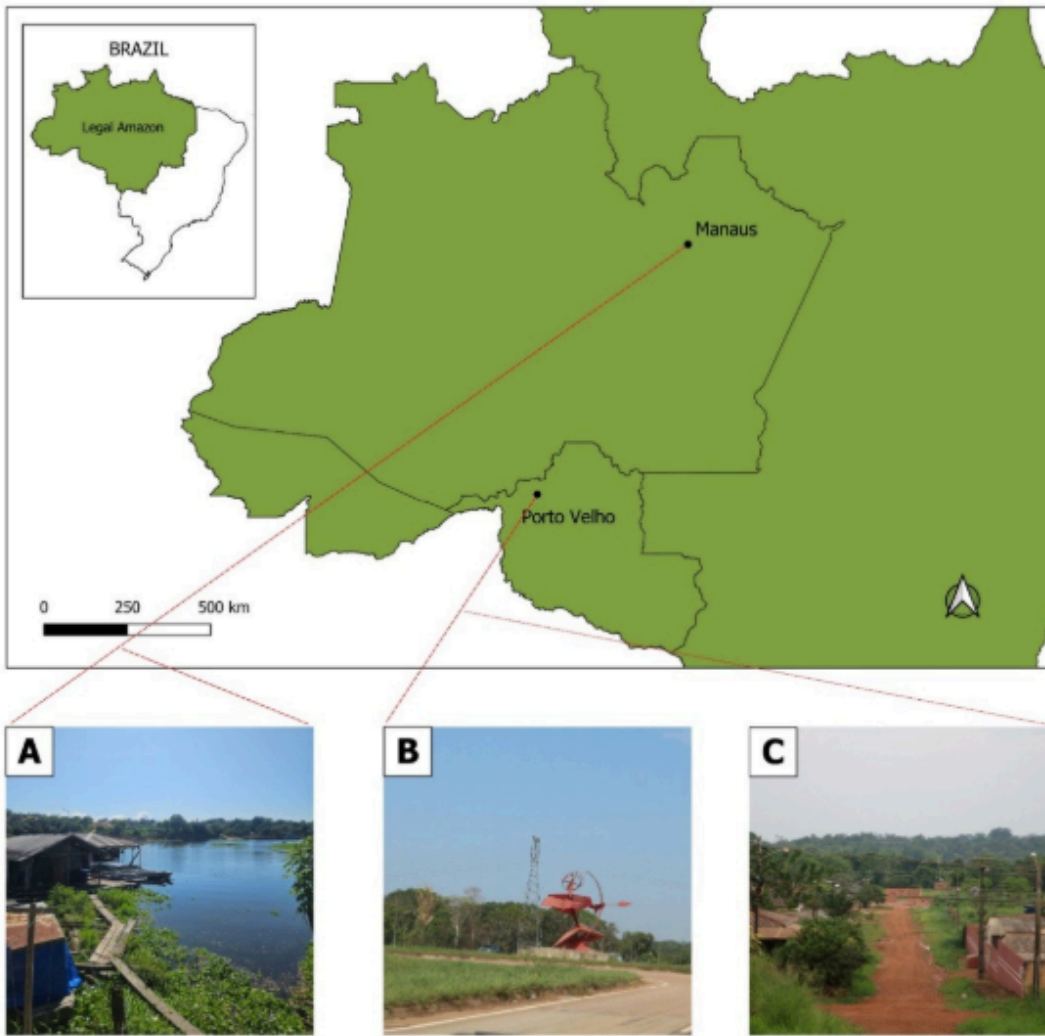


Figure 1

Study sites: Manaus and Porto Velho, Brazil. A) Floating houses in the peri-urban area of Manaus; B) Highway in the urban area of Porto Velho; and C) Dirt road in the rural area of Porto Velho. The base used to create the map is from the IBGE (Brazilian Institute of Geography and Statistics), which is freely accessible for creative uses in shapefile format, in accordance with the Brazilian Access to Information Law (12,527/2011) [17].

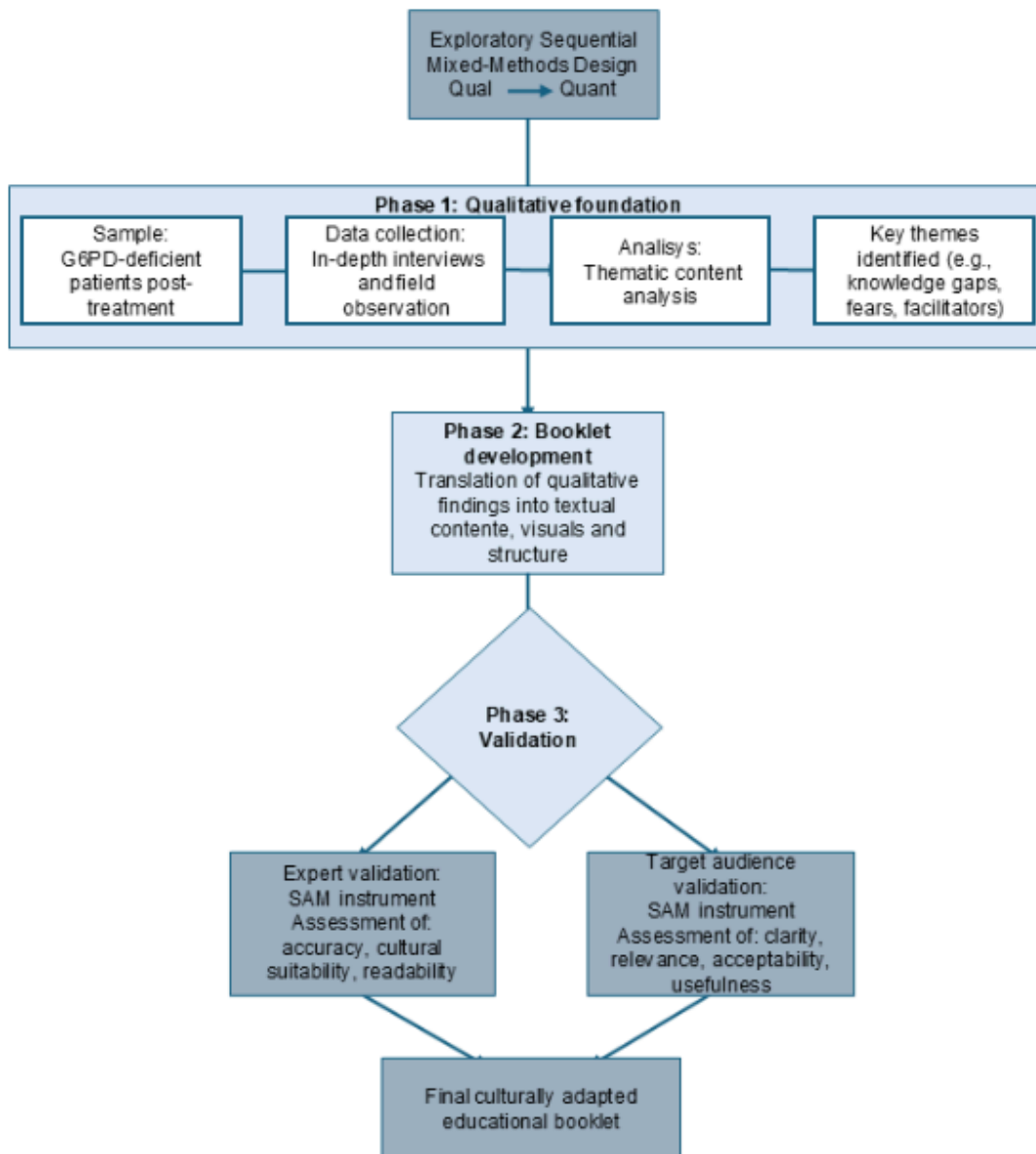


Figure 2

Overview of the exploratory sequential mixed-methods design and the study phases.

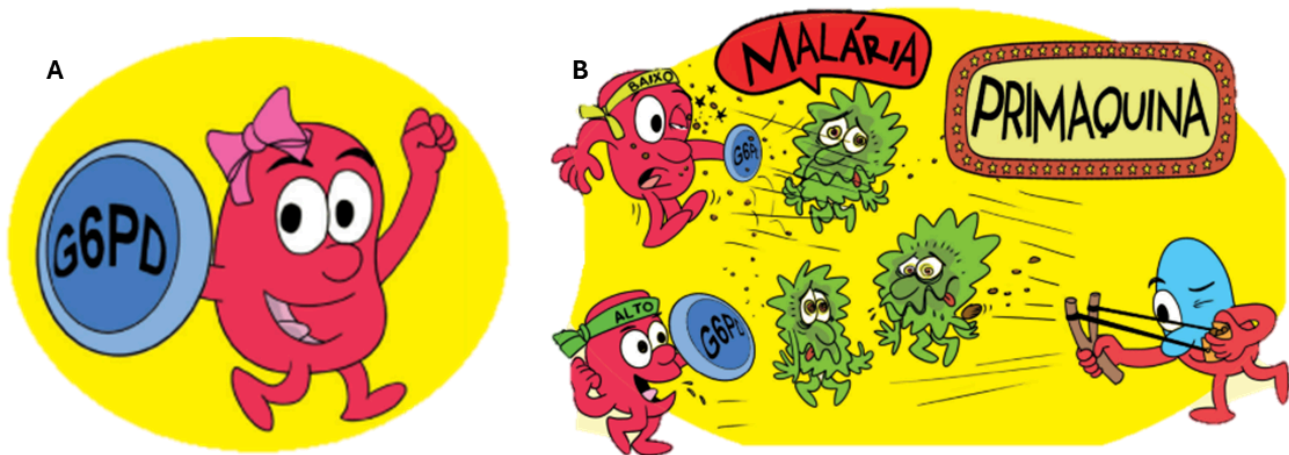


Figure 3

Visual metaphors used in the educational booklet to represent the protective role of the G6PD enzyme. The shield illustrates how enzyme deficiency increases susceptibility to hemolysis during malaria treatment.

Theme 1: Challenges in communication and understanding of the G6PD deficiency diagnosis

Subtheme 1a: Scarcity of information regarding the G6PD diagnosis

***Finding:** The lack of clear information about G6PD deficiency compromises patients' understanding and generates negative feelings about the diagnosis.*

***Evidence:** Reports indicate a lack of explanations about diagnosis and treatment.*

Subtheme 1b: G6PD deficiency and its association with treatment

***Finding:** G6PD is not associated with weekly treatment.*

***Evidence:** The reports highlight misconceptions about the purpose of the test and the justification for weekly treatment with Primaquine.*

Theme 2: Key factors influencing adherence to weekly primaquine therapy

Subtheme 2a: Impact of weekly treatment on daily life

***Finding:** The weekly primaquine regimen was associated with reduced adverse effects and no significant negative impact on daily routines, being perceived as milder and more conducive to treatment adherence.*

***Evidence:** Reports highlight positive experiences, including reduced symptoms, favorable adaptation to the prolonged regimen, and less impact than expected on daily activities, along with greater attention during the prescription process.*

Subtheme 2b: Impressions on hemolysis during treatment

***Finding:** The lack of clear information about signs and symptoms associated with hemolysis has led to uncertainty and concern, especially regarding urine color, which is often interpreted as a normal effect of the medication.*

***Evidence:** The reports highlight limited guidance and the search for additional explanations due to concerns about signs and symptoms.*

Figure 4

Thematic focus and key findings.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile3.CompletedCOREQchecklist..docx](#)
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- [Additionalfile2.Codingframeworkorthemetocontentmatrix..pdf](#)
- [AdditionalFile5Expertvalidationinstrument.pdf](#)

- [Additionalfile6Targetaudiencevalidationinstrument..pdf](#)
- [Additionalfile4.Finaleducationalbooklet..pdf](#)