UNCONQUERABLE STANCE OF *PLASMODIUM FALCIPARUM* DEPENDS ON ARRESTED DEVELOPMENT

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ABSTRACT

Plasmodium falciparum has conquistador prowess that depends on its knack for arrested development. This phenomenon is poorly defined/understood; therefore stakeholders neglect its relevance in malaria control strategies. This study provides requisite knowledge and motivation to interested parties to now begin to factor in arrested development of P. falciparum in order to prepare Sub Sahara Africa to strategize for malaria elimination. Reviews of published works and anecdotes were used to generate the reports embodied in this study. P. falciparum arrested development was redefined and contextualized in the light of current laboratory findings that prove that absolute arrest is incompatible with life. During arrest, metabolism is only down-regulated while essential processes continue. Arrested P. falciparum is found in all stages of its complex forms which permits it to cope with inhibitory substances thereby enabling it to exist asymptomatically while modulating some notable genes to resist chemotherapy or mobilizing the resources of the host to shield itself to frustrate host reactions. Arrested development is a defensive strategy par excellence that P. falciparum depends on for relevance in ways that are strikingly complicated. Progress in malaria control would depend on pressuring P. falciparum and its vector to change their habit through strict use of house screening. Man can also apply the principle of arrest to deal with sociopolitical problems especially in Africa.

Keywords: *Plasmodium falciparum*, Arrested development, Malaria control, Antimalarial, Mosquito vector control

INTRODUCTION

Malaria occurs in all six WHO regions where an estimated 3.3 billion people are at risk of infection and development of the disease; while 1.2 billion people are classified as high risk (WHO, 2014). Recent report estimated that 219 million cases of malaria occurred globally resulting in 435,000 deaths with the burden concentrated in the WHO African Region which accounted for 92 % of all malaria cases and children aged less than 5 years accounting for 61 % of all deaths (WHO, 2018). Specifically the bad news for Nigeria is that most global cases are suffered by Nigerians and about 19 % of global deaths are also Nigerians (WHO, 2018). The foregoing data may not be sacrosanct because there is heterogeneity in malaria prevalence (Tadesse *et al*., 2015; Idris *et al*., 2016). The situation may worsen in years with adequate rainfall (Sharma, 2012). Malaria could waver in intensity at the local, regional and national level, becoming more resilient because both vectors and parasites acquire resistance that facilitate them to stabilize thereby guaranteeing their diversification into various ecotypes (Sharma, 2012).

Malaria is caused by some *Plasmodia* which are transmitted to humans by the bite of infected *Anopheles* mosquitoes. There are five *Plasmodium* species known to infect man: *Plasmodium falciparum*, *P. vivax, P. ovale, P. malariae and P. knowelsi*, all of which have a distinct global distribution and clinical outcome. *P. falciparum* engender one of the most dreaded global health problems. The genome is made up of 14 chromosomes, 23 million base pairs and about 5279 genes (Luthra and Luthra, 2003). It produces up to 30,000 merozoites/hepatocyte that have 2 – 3 times more merozoites than other species; in red cells, it has 10 times higher parasite density, infects erythrocytes of all ages but has preference for young cells; it also produces more merozoites from red cells than others hence associated with anaemia (Francine *et al*., 2016). Its fever is more acute because of the faster rate of parasitaemia with shortest duration of erythrocyte development cycle (Zimmerman *et al*., 2004). It is the cause of the most virulent form of malaria associated with severe disease and mortality, accounting for 99.7 % cases in WHO African Region (WHO, 2018). The liver is the destination organ of infective sporozoites from mosquitoes. Its infection is remarkable and estimated to be up to 0.066 % higher than *P. vivax* (Sattabongkot *et al*., 2006). Besides morbidity and mortality, the social and economic burden of malaria, specifically in underdeveloped countries, remains enormous (Murphy and Breman, 2001; Sachs *et al*., 2001; WHO, 2018).

Since the discovery of malaria life cycle in 1897 by Ross, man had been waging relentless wars to eradicate malarial parasites without complete triumph over them. There is persistent clarion call to put in place surveillance strategies to deal with the spread of drug resistance and eliminate parasites (Dokunmu *et al*., 2019). Meeting this obligation requires that greater familiarity with certain biological factors inherent in the parasite (which are not completely divulged) that mediate this unconquerable quality is unveiled. This conundrum is particularly significant in *P. falciparum* malaria whose conquistadorial attributes could be likened to one who is an all-round achiever. Therefore, the pathogenic qualifications of *P. falciparum* present it as a parasite paradigm par excellence of an all-round victor.

The implication of the foregoing is that the parasite continues to persist and ravage peoples across the globe and particularly Sub-Saharan Africans despite the efforts of researchers who had been responding by devising one strategy after another without achieving commensurate success in parasite containment. This study is directed at the general public and interested parties, particularly health care providers to acquaint them with why this failure subsists. Implicitly, the language of the subject would be kept simple enough for all and sundry and provide some basic relevant characteristics and capabilities of the parasite when it is manifesting this biologic property. In other words, this study is ultimately geared to appraise public health stake-holders to begin to narrow their strategies of checkmating this parasite to its veritable form which is the backbone of this parasite's invincibility. This paper omits the parasite's life cycle peregrinations but focuses on the biological attribute that contributes to this unconquarability and versatileness – the developmental arrest – graphically presented by Arden (2010) who described it as a phenomenon where an animal appears to be doing nothing during severe existential threat when in fact it is bracing up to fight or flee. Arrested development is the single factor which mediates *P. falciparum* hardiness that all concerned must be familiar with to engender renewed anti-malaria campaigns to seriously challenge the scourge of malaria globally and particularly prepare Sub Sahara Africa to strategize towards malaria elimination.

MATERIALS AND METHODS

The literature review was done by the research team who used *P. falciparum*, malaria, arrested development (and its synonyms like dormancy, hypobiosis, diapauses etc.), malaria-endemic regions, as search items to isolate relevant peer reviewed articles from Google Scholar database. Anecdotes, authors' experiences and informal

discussions with chronic malaria sufferers, healthcare givers and co-workers were other sources of information. Lastly the authors deliberated the foregoing data to have the common ground that are articulated in this work.

RESULTS AND DISCUSSION

Broad View of Arrested Development: Malaria is likened to the *Ficus lutea* tree which when cut down soon germinates from other points below the ground. Quite a number of people wonder if mosquitoes are not being wrongly accused as malaria vectors. This is because, to them, they had burnt leaves, applied repellents, installed mosquito nets, used insecticide sprays and powders to keep mosquitoes at bay yet the malaria fever is still recurrent. This informal group concludes that even without renewed encounter with mosquitoes and their bites, certain activities precipitate the episode of malaria. As would be expected, subjective list of items which precipitate episodes of malaria abound. One claims it is palm wine, another says it is beer, raw groundnut, dry groundnut, a particular vegetable, long treks, etc. These observations may in fact be true as Shanks and White (2013) noted the following could reactivate malaria: anything which weakens resistance – fatigue, heat, chill, dissipation, other illness, gunshot injuries, parturition, immunosuppression secondary to severe trauma, sufficient physical stress, etc. Certain factors in diet determine if *P. falciparum* would grow or arrest (Asahi *et al*., 2014). Arden (2010) noted behavioural arrest do occur in larger terrestrial animals: man's activities could induce a deer to arrest. Another way of putting arrested development in perspective is noting how most human activities were halted during the early part of Covid-19 lock-downs (between March and April, 2020) in most countries where transportation, commerce, sports, schools, etc. were put on hold.

The above scenario points to the fact that, whether one perceives malaria and its vector as a biologist or a lay person, the disease could resume attack of its hapless victims with or without obvious mosquito bites. The scenario of arrested development could alternatively be understood if *P. falciparum* is perceived as lying low (whether in cocoons like coconuts or not, just as humans can hide in underground bunkers when under heavy artillery) but that raw groundnuts, alcohol, gunshots, typhoid, etc. could awaken them to resume its pathogenesis. However, it may be pertinent to note that there is a distinction between true relapse which is attributed to awakening due to arrested development following the awakening of hypnozoites of *P. vivax* (which does not occur in *P. falciparum* infection) and recrudescence which is resurgence of malaria after parasite attain a critical number to initiate fever which occurs in *P. falciparum* infection. The biological basis involves the formation of arrested parasites that are capable of re-activating weeks to years after a primary blood-stage infection causing recurring episodes of malaria (Shanks and White, 2013; Joyner *et al*., 2015). The common factor in both is that malaria eventually follows from forms present earlier without mosquito intermeddling.

What is found from anecdotal accounts and experimentally are that: i) *P. falciparum* may strike patients clinically even where any of the forms of the parasites are not found in blood stream (submicroscopic, see below), ii) the pathogen could manifest clinically when critical number in patients is reached (in primary infections in patients and in recrudescence in those who had had the primary attack previously) and iii) patients may have variable numbers of the haemoparasites without displaying any illness (asymptomatic) even among pregnant women where more than 51.5 % were observed in one study (Francine *et al*., 2016). All the foregoing could arise from dormant forms which are reactivated through unknown mechanism that could renew malaria clinically (Voorberg-van der Wel *et al*., 2013).

In literature, the phenomenon is diverse, so that authors attempt explanation using different terminologies. *Plasmodium* arrested development occurs *in-vivo* in animals (LaCrue *et al*., 2011). It is also referred to as hypobiosis, diapause, dormancy, or quiescence (O'Brien *et al*., 2011; Dembele *et al*., 2014). A

small fraction of parasites under chemotherapeutic pressure becomes cytostatic or dormant (Hoshen *et al*., 2000). Dormancy state was also noted in bacterium, *Staphylococcus aureus,* where a small representative population called persister cells become metabolically inactive but do not undergo any genetic change such that they could become tolerant to antibiotics for example (Fauvart *et al*., 2011; Wood *et al*., 2013). However, the experiences of the authors' search for parasites in peripheral blood from clinical pyrexia cases suggests that many slide 'malaria parasite negative' results did have active clinical malaria because such patient promptly recovered when placed on antimalarials. In fact, it is estimated that 98.5 % *P. vivax* infection may be undetected by microscopy (Howes *et al*., 2018). With the growing developments in molecular techniques, this category of submicroscopic and asymptomatic malaria is looming large. Substantial reservoirs of asymptomatic and submicroscopic *Plasmodium* infection now commonly occur in places with low infection rate (Waltmann *et al*., 2015). About 13.5 % of subjects had asymptomatic malaria parasitaemia by microscopy or polymerase chain reaction (PCR) in children adopted from endemic areas (Adebo *et al*., 2015). How else should *Plasmodium* parasites in asymptomatic persons be perceived? In asymptomatic, the parasite is certainly the arrested forms awaiting revitalization. All these suggest that the terms – asymptomatic malaria parasitaemia and submicroscopic infection are practically synonymous; and in *P. falciparum* parasitaemia, arrested forms may be encountered at different phases of parasite growth and when induced by inhibitors as illustrated in Figure 1 a and b.

Malaria infection is regarded as asymptomatic when malaria infection is ongoing without registering fever or its related signs like rigors within the past 48 hours; and defined as submicroscopic when detected by PCR from persons with malaria slides negative by microscopy (Walldorf *et al*., 2015).

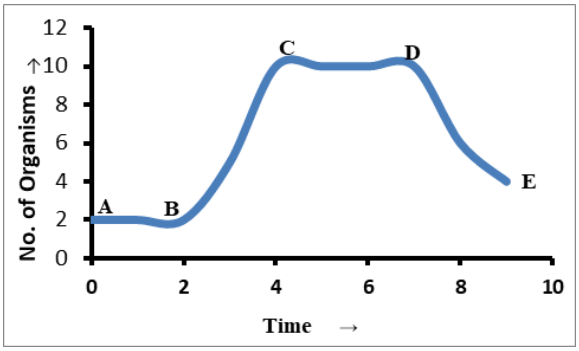

Figure 1a: Growth curve of microorganisms

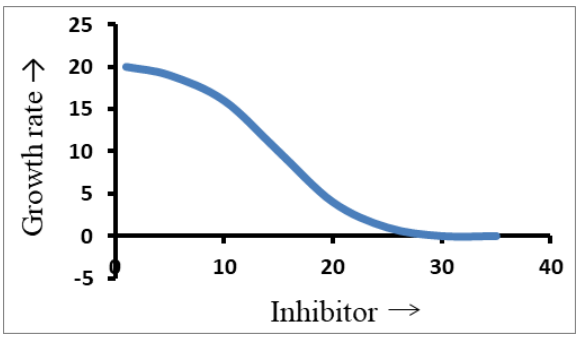

Figure 1b: Growth rate in the presence of an inhibitor

Figure 1: Location of possible growth arrest in growth curves. *A: typical growth curve seen in textbooks of microbiology: A – B is the lag phase when the organism is just introduced to the medium and mobilizing metabolic enzymes; B – C is the log phase where there is astronomical growth; C – D is the stationary phase when the organism is not growing but retain their numbers and viability; D – E is the declining phase where the organism begin to reduce in number, viability and arrested forms. Malaria parasites arrest at all stages of the growth curve (see discussion on Forms of P. falciparum participating in arrested development). This potential could be mediated by the kind of nutrition they obtain from animal hosts (Asahi et al., 2014). B: shows the result of growth rate in the presence of an inhibitor. Growth arrest occurs after a critical level of inhibitor concentration is reached. A typical example is where increasing levels of neocuproine that induce arrest of P. falciparum ring forms (Asahi et al., 2014)*

There may be no standard definition of asymptomatic malaria; however, popular definitions involve the detection of asexual or sexual parasites and an absence of any acute clinical symptoms of malaria (usually fever) during a specified time frame (Lindblade *et al*., 2013). Males have higher odds of asymptomatic infection than females, while season and age of those at risk may influence the extent of both submicroscopic and asymptomatic infections (Walldorf *et al*., 2015). The detection of a large number of subpatent malaria in normal

population without evidence by usual diagnostics raises the issue of choosing convenient route to attain malaria eradication (Tadesse *et al*., 2015).

Re-Definition of Arrested Development and Concept of an Elastic Gradient: Arrested development is a transitory pause of development. In a of nematode, it can occur at a particular point in its premature parasitic development, and where such an interruption is capable of living or existing under varying external environments, it could occur only in certain host, certain situation or at certain times of the year, and often affecting only a percentage of the worm (Ugwu and Onyali, 2005). Researchers had traditionally defined arrested development as a cessation of metabolic activity in an organism. This definition depended on the level of technology available to researcher at the time. However, emerging data contend with such absolutism as metabolism in a living cell cannot really stop. Inducing arrested development activates growth retardation yet there is an accumulation of ubiquitinated proteins involved in DNA repair (Dogovski *et al*., 2015). There is also evidence that mitochondrial activity is ongoing because it is critical for the survival of dormant parasites (Peatey *et al*., 2015). It has been demonstrated that most metabolic pathways were only down-regulated in antimalarial induced dormancy whereas fatty acid and pyruvate metabolic pathways remained active (Chen *et al*., 2014). Synthesis of RNA is thought to cease in terminal arrested gametocytes but at the same time signaling enzymes like protein kinases are expressed in this quiescent stage of the life cycle (Reininger *et al*., 2005; Baker, 2010).

For a motion-based detection technique, freezing will certainly prevent detection (Arden, 2010). Detection of biological activities in arrested intracellular pathogen will depend on the level of sophistication and availability of applicable molecular techniques which at present is not really accessible to most researchers in Sub Sahara Africa. Indeed, it had been observed that there is no specific molecular marker available for detecting *Plasmodium* hypnozoites (Sattabongkot *et al*., 2006). Arrested development is better conceived as a continuum with one end showing no detectable arrest with the organism in its optimal metabolic phase and the other end showing maximal rate of arrest allowable for that form of the organism before death. Mid way between the two extremes, interplay of inducing factors, context and biological endowments of the organism determine the level of arrested development. Therefore, it is safe to theorize that as dormancy induction comes to play, the organism's arrested development increases inversely with its metabolic activities and directly with the inducer up to a point until it reaches a plateau such that absolute arrest does not take place. Absolute arrest would imply the death of the organism.

As an accomplished parasite, *P. falciparum* is intracellularly located enabling very close relationship with its host cell and so well situated to manipulate its host and therefore could pose as player friend or foe that can literally change the goal post when it suits him. As an example, some years back, artemisinin-mefloquine was a preferred therapy for malaria which *P. falciparum* resisted prompting the introduction of artemisinin-piperaquine as replacement was also subsequently resisted; but recently, it is curiously reverting to mefloquine sensitivity (Lim *et al*., 2015). Moreover, the parasites are able to show different sensitivities to artemisinin depending on whether it is the vegetative form or the arrested forms (Reyser *et al*., 2020). Despite the robust immune challenges mounted against it by hosts, it is able to surmount them all to not only proliferate but also switch modes to make it accessible to other hosts. Sometimes it appears some host cells eagerly await this conquistador as they literally dole out the red carpet by way of adorning themselves with receptor cites that facilitate their invasion (Paul *et al*., 2015). In mosquitoes that express fibrinogen-related protein 1(FREP1), midgut invasion by a number of *Plasmodium* species facilitate the anchoring ookinetes and its subsequent penetration into the peritrophic matrix and the epithelium (Zhang *et al*., 2015). *P. falciparum* is able to literally announce its location in the vascular system so that its vector can easily pick it up

(Kelly *et al*., 2015; Cator, 2017) the way pseudoscientific drug hawkers search for clientele; it can also switch modes to make certain tissues vulnerable (Avril *et al*., 2012; Joice *et al*., 2014), switch mode to replicate either asexually or sexually (Rono *et al*., 2018) or switch modes to lie low to suit itself (Luthra and Luthra, 2003). These fluid properties of arrested parasites are captured in Figure 2 to putatively express these sentiments which shows the parasite as unruffled when challenged (in human context).

Figure 2: Putative reactions of *P. falciparum* in a red cell when challenged with chemotherapy. *Three malaria parasites show what could be compared to human facial expressions. These emoticons depict how arrested Plasmodium parasites could react whenever they are confronted with ineffectual challenges such as chemotherapy. The emoticons were arrived at when colleagues were asked to make a face to an opponent to show that his/her subversive plans/actions were of no use. More people showed a scornful face (lower left), lesser number had a grinning face (top), while the least number were a happy laughing face (lower right)*

Forms of *Plasmodium falciparum* Participating in Arrested Development: The infective form of *Plasmodium*, slender and motile sporozoites, is known to arrest in the liver (Gomes-Santos *et al*., 2011). The parasite has non-photosynthetic plastid known as apicoplast that do contain DNA that code for apicoplast associated proteins and its deficiency could cause them to be arrested as schizonts just before merozoite release (Haussig *et al*., 2014). Glutathione reductase-null *Plasmodium* parasites do have normal haematological development in man but become arrested in the definitive host (Pastrana-Mena *et al*., 2010). This may be due to the glucose deprived internal environment of the mosquito (Boysen and Matuschewski, 2011). Calcium-dependent protein kinase-5 (PfCDPK5) is expressed in normal invasive merozoite; but in parasites deficient in PfCDPK5, egresses from schizont were not possible; rather, parasite arrested as mature schizonts (Dvorin *et al*., 2010). When PfCDPK5 is knockdown, parasites remain ensnared in host cell; however, the enzyme exists with micronemes which are parasite organelles that could be discharged with the help of the enzyme to enable the parasite egress to avoid its being arrested (Absalon *et al*., 2018).

The ring stage seems to be the form most amenable to artemisinin induced arrest (Grobler *et al*., 2014). The dormancy in this stage protected early 'ring-stage' intra-erythrocytic parasites from short-term artemisinin treatment (O' Brien *et al*., 2011). Physically, dormant rings are small rounded forms with a dark staining nucleus when subjected to Giemsa stain (LaCrue *et al*., 2011). *Plasmodium* can also arrest at the trophozoite stage during erythrocytic cycle when induced with berenil (Das *et al*., 1997). Even in a particular stage, arrest could be induced in early or late stage depending on the inducing substance. Cyclohexylamine, a spermidine synthase inhibitor induce *P. falciparum* to achieve complete arrest at the early trophozoite stage (Becker *et al*., 2010), while mid trophozoite stage arrest was obtained with a different agent (Naik *et al*., 2003).

The gametocytes which may be found in 1 % of infected individuals (Mawili-Mboumba *et al*., 2017) are most commonly detected in children (Ouédraogo *et al*., 2010; Bousema and Drakeley, 2011). They are by default the sexual stages of the malaria parasite and are produced in the human host but do not cause clinical disease but remain in a state of arrest until ingested by a feeding mosquito (Sinden *et al*., 2010; Venugopal *et al*., 2020). Gametocytes appear to be developmentally arrested at the G_0 (a normal stage during a cell development cycle

when it cannot replicate) phase in the vertebrate host (Baker, 2010). Transmission of malaria parasite from vertebrate host is mediated exclusively by mature arrested gametocytes (Luthra and Luthra, 2003). Disruption of *P. falciparum* male development gene-1 (pfmdv-1) results in a dramatic reduction in mature gametocytes (Furuya *et al*., 2005). A group of insertional gametocyte deficient mutants does not form stage I gametocytes, ostensibly because they likely arrest during commitment pre-stage I development (Ikadai *et al*., 2013). Knockdown of calcium-dependent protein kinases in sexual stages resulted in developmentally arrested parasites and prevented mosquito transmission (Sebastian *et al*., 2012). In mosquitoes, ookinetes generate oocysts that are arrested early in their development (Laurentino *et al*., 2011).

Conclusion: Arrested development is a defensive strategy par excellence that biological entities have used to survive hard times. *P. falciparum* had been effectively using the strategy for survival in ways that are strikingly complicated and involving all stages of the parasite unlike *Ancylostoma* species that arrest only at a particular stage (Ugwu and Onyali, 2005). Its successes are dependent on this biological propensity. After arrest, comes reactivation. Unfortunately, the *modus operandi* of reactivation from this arrested state is yet to be unveiled (Vooberg-Van Der Wel *et al*., 2013).

Man can also apply the principle of arrest to deal with sociopolitical problems especially in Africa where cruel leaders impose hardship, poverty and diseases to their own people (Boyi, 2019). One discussant opined that: "whatever material need one is facing can be ignored, one week after without that need, the situation would 'normalize' and you can go on with your life happily, soon after you would forget that the need existed in the first place." *P. falciparum* 'normalized' forms fortifies it sufficiently to ignore or withstand effects of whatever artilleries are hauled at it in form of antimalarials. Moreover, the parasite intracellular locations makes it privy to every scheming of man because they co-mingle with our genes – parasites had been located in nucleus (Silvie *et al*., 2006) and seem to be a step ahead of whatever adaptation that man muster.

Malaria parasite still have two windows through which their vulnerability could be taken advantage of: one is through prevention of arrested development or reactivation of all arrested forms and applying chemotherapy when they are most vulnerable; the other is adoption of an indirect fight by tackling the parasite's mosquito vectors through pressure techniques to alter their behaviour. Since defeating mosquitoes militarily with insecticides is also not possible presently owing to its quick acquisition of resistance and evasive behaviour, it behooves on man to pressure mosquitoes to change their habit which is easier to accomplish. Denial of blood meals consistently will force mosquitoes to abandon haematophagy and look for alternatives like other non-blood meal dependent mosquitoes. Already developed methods of house screening will deny mosquitos' access to man and his domestic animals (Ugwu and Onu, 2012). If indoor and outdoor screenings are maintained for some time, mosquitoes may ultimately change their habit of haematophagy and head *Plasmodium* permanently into attrition.

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