Age-related factors associated with placental malaria and adverse pregnancy outcomes in rural Burkina Faso

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- Study grant from the Académie de Recherche et d'Enseignement Supérieur (ARES) to attend the 10th European Congress on Tropical Medicine and International Health, Antwerp 2017.

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THESIS OUTLINES

Chapter one provides an overview of the burden of malaria in the world, with a rapid drop from 2010 to 2014, and a stagnation thereafter, mostly in sub-Saharan Africa. It showed also the current strategy against malaria consisting of more targeted and tailored interventions. This chapter highlights the burden of malaria in pregnancy worldwide. The prevalence of peripheral and placental malaria was provided alongside the parasite species involved. The factors associated with placental malaria as far as the deleterious effect of the infection on the fetus and the newborn were presented. The role of ultrasound evaluation of fetal biometry in understanding and quantifying the prejudicial effect of malaria throughout the pregnancy was acknowledged.
In **Chapter two** we contextualized the situation of malaria in pregnancy and its adverse birth outcomes to Burkina Faso, a sub-Sahara African endemic country where Plasmodium falciparum is the predominant parasite species. The low availability of ultrasound in rural areas and the lack of harmonization in the use of fetal biometric references within the country were also pointed out.
Chapter three presented the rationale and objectives of the study. It showed that the study was in line with the current approach recommending targeted and tailored interventions against malaria to hasten the progress toward its control and elimination. Therefore, the general objective was to contribute to the improvement of the control of malaria in pregnancy through adequate identification of those most at risk who could benefit from specific interventions. The process of such identification was addressed consistently through the three objectives of the study.
Chapter four summarized the methodological approach used including the exploration of the effect of age on the association of maternal factors with placental malaria and adverse birth outcomes and the comparison of fetal biometric reference charts.
Chapter five described the results of our study in detail, showing that the risk of placental malaria was increased in women younger than 30 years, and adolescent women were more at risk of delivering low birth weight or preterm neonates as compared to adult mothers. We drew attention to the lack of agreement between reference charts in assessing fetal biometric measurements in malarious pregnant women from rural Burkina Faso.
In chapter six we discussed concerns of the age-related burden of malaria in pregnancy and the possible inclusion of data on placental malaria when projecting to use pregnant women to monitor malaria. We underline the interplay between malaria and undernutrition that could be more prejudicial

to young pregnant women than their older counterparts, as far as symptomatic malaria. We underlined also that the use of many fetal biometry references as currently practiced did not allow reliable estimation and comparison of adverse fetal outcomes related to malaria in pregnancy within and across countries.

In **chapter seven** we highlighted the need of considering maternal age in designing and implementing interventions against malaria in pregnancy. We also, pointed out that these interventions should incorporate nutritional, and socio-cultural components. Last but not least, we called for a harmonization of the practice of fetal biometry assessment within the country.

SUMMARY

Malaria control and elimination remained challenging in sub-Saharan Africa where pregnant women and their fetuses are mostly affected. The current strategy proposed by the WHO and its partners for accelerating progress toward malaria elimination is to use quality data for targeting and tailoring interventions toward those needing them most.

The overall objective of our study was to contribute to improving the control of malaria in pregnancy through adequate identification of high-risk groups that could be potentially targeted by programs for specific actions or strategies. Specifically, we aimed at 1) determining the prevalence of placental malaria and assessing whether its associated factors are varying with age; 2) determining the low birth weight or prematurity prevalence in newborns from mothers infected with malaria and its burden in teenagers; 3) accurately estimating fetal biometry in pregnant women with malaria in rural settings for gestational age and abnormal fetal size determination.

We analyzed data from pregnant women treated for malaria in the framework of a clinical trial and explored interactions between age and maternal factors. We found that young pregnant women with low gravidity, anemia, and presenting with symptoms like fever were more at risk of placental malaria and delivered more low birth weight or preterm neonates than their older counterparts. Also, the estimation of the fetal size in these women varied with the reference biometric chart used.

Our findings are in line with the approach in the fight against malaria. They point out the need for comprehensive interventions targeting young pregnant women including malaria prevention and treatment but also nutritional support. Ultrasound practice should be standardized, particularly the choice of ultrasound reference biometric chart for accurate gestational age and fetal size estimation. This might benefits greatly the fetuses of the young mothers affected by malaria in pregnancy

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LIST OF ABBREVIATIONS

AC	Abdominal Circumference
AL	Arthemether-Lumefantrine
ANC	Antenatal Care
ASAQ	Artesunate-Amodiaquine
BC	Before Christ
BMI	Body Mass Index
BPD	Biparietal Diameter
CRUN	Clinical Research Unit Of Nanoro
DSA	Demographic Surveillance Area
ECDC	European
EIR	Entomological Inoculation Rate
FCFA	Franc De La Communauté Financière Africaine
FGR	Fetal Growth Restriction
FL	Femur Length
GTS	Global Technical Strategy
HBHI	High Burden High Impact
HC	Head Circumference
HDN	Health District Of Nanoro
HIV	Human Immunodeficiency Virus
IPTp	Intermittent Preventive Treatment During Pregnancy
IRS	Indoor Residual Spray
ITN	Insecticide-Treated Bed Net
LLIN	Long-Lasting Insecticide-Treated Bed Net
LMIC	Low And Middle Income Country
MiP	Malaria In Pregnancy
MQAS	Mefloquine-Artesunate
NAAT	Nucleic Acid Amplification Technic
NINA-LAMP	Non-Instrumented Nucleic Acid -Loop-Mediated Isothermal Amplification
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PM	Placental Malaria

PREGACT	Safe And Efficacious Artemisinin-Based Combination Treatments For African Pregnant Women With Malaria
RDT	Rapid Diagnostic Test
SMC	Seasonal Chemoprophylaxis
SP	Sulfadoxine-Pyrimethamine
UK	United Kingdom
US	United States
WBC	White Blood Cell
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1. Overview of malaria

1.1.1. Definition

Malaria is an infectious vector-borne parasitic disease. The pathogen is a protozoan of the genus *Plasmodium* parasitizing the red blood cells. The parasite is transmitted to a human when the anopheles female mosquito bites him for feeding his blood.
Malaria is an ancient disease. Records of the cyclical fever characterizing the infection date from at least 5500 BC. In the 5th century BC, Hippocrates described in detail fevers occurring each third day (tertian) and each fourth day (quartan). Up to the 19th century, the disease was thought to be caused by the miasm emanating from marshes. At the end of the century, several scientific discoveries allowed a better comprehension of the disease. In 1880, Alphonse Laveran discovered the causative agent of the disease. In 1897 Ross assumed for the first time that the mosquito was the vector transmitting the disease to humans. This assumption was confirmed one year later by Grassi [1].

Parasites

The disease was caused by five *Plasmodium* species: *P.falciparum*, *P. malariae*, *P.vivax*, *P.ovale*, and *P. knowlesi*. *Plasmodium ovale* has two subspecies, *P.o. wallikeri* and *P. o. curtisi* [2]. *Plasmodium falciparum* and *P.vivax* are the more prevalent species and have specific geographical distribution. *Plasmodium falciparum* predominated in the World Health Organization (WHO) African Region where it is responsible for 99.7 % of the estimated cases in 2018. This parasite was also found in 71 %, 65 %, and 50 % of the cases, in the regions of Eastern Mediterranea, Western Pacific, and South East Asia, respectively [3]. *Plasmodium vivax* is the main parasite in South East Asia, the Americas, and temperate areas [3-5]. Africa and Asia are the areas where *Plasmodium malariae* and *P.ovale* were encountered the most. However, a marginal number of cases can be found elsewhere [4, 6]. *Plasmodium knowlesi* is naturally hosted by primates and is significantly prevalent in Malaysia [7, 8].

Vectors

The Anopheles infected female mosquito transmits malaria to humans during its blood meal. There are more than 40 species competent for malaria

transmission [2, 4]. Their distribution varies geographically. A map of the predominance of the vectors by geographical areas was realized in 2012. Three species are prevalent in Africa: *An. gambiae*, *An. Arabiensis*, *An. Funestus*. *An arabiensis* is more capable to survive in areas with less humidity than the two other species. Europe and the Middle East are home to species like *An. atroparvus*, *An.messeae*, *An. Superpictus* and *An. sergentii*. From the north to the south of the Americas, the following species can be encountered: *An. freeborni*, *An. quadrimaculatus s.l.*, *An. pseudopunctipennis*, *An. darlingi*, and *An. albitarsis s.l. Anopheles. stephensi* and *An.fluviatilis s.l.* are prevalent in India and the west of Asia while *An. dirus* and *An. minimus* predominate in Southeast Asia. In the Pacific, *An.farauti*, *An. koliensis* and *An. punctulatus* are among the species or complex retrieved in these settings [9].

1.1.2 Global burden of malaria

Cases

In 2018, the number of malaria cases was estimated at 228 million worldwide. The African region on the WHO shared the greatest part of this burden with 213 million (93 %) of estimated cases. The following regions were South-East Asia and East Mediterranea accounting for 3.4 % and 2.1 % of the estimated cases, respectively [3].

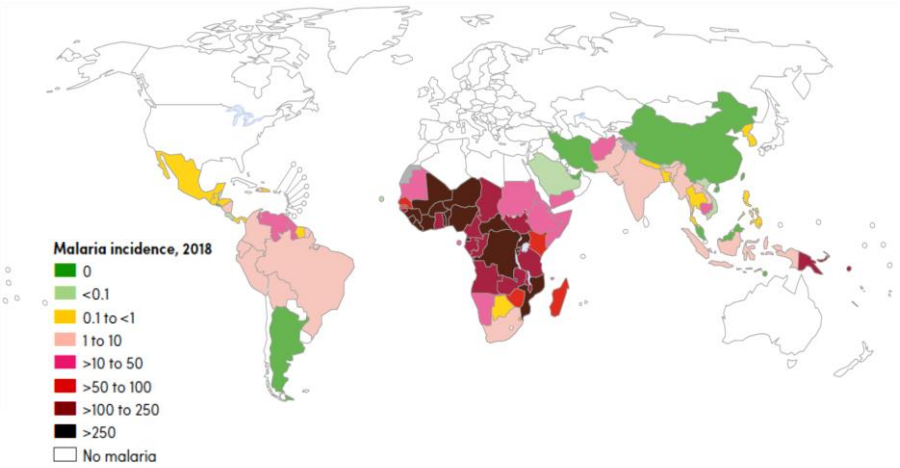

Figure 1 : Map showing malaria cases (per 1000 persons) in 2018 *Source: WHO world malaria report 2019. https://www.who.int/publications-detail/world-malaria-report-2019#*

Deaths

Malaria kills more people than armed conflicts [4]. In 2015, the deaths due to this disease (438,000 – 631,000) were estimated to be three folds those due to armed conflicts (167.000) [10-13]. The 2019 report attributed 405 000 estimated deaths to malaria, with 94 % of them occurring in the WHO African Region. Twenty countries in this region and India shared 85 % of the global estimated deaths [3].

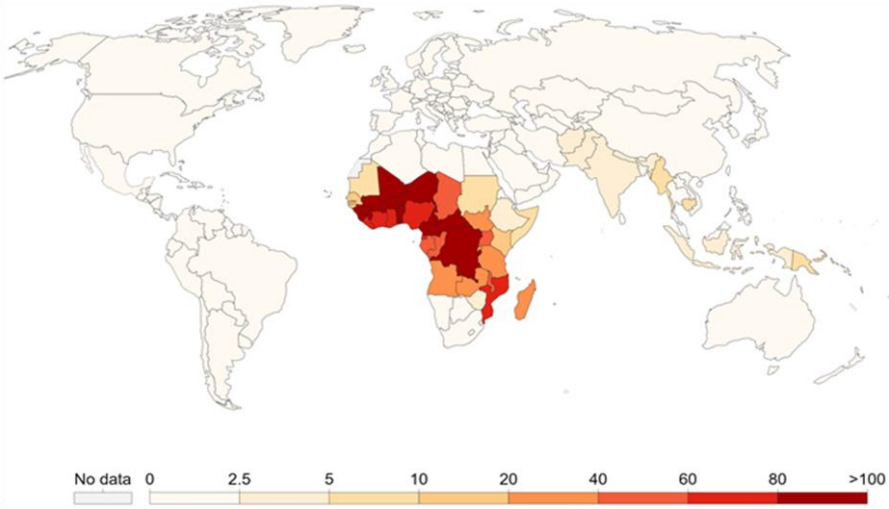

Figure 2 : Map showing malaria deaths (per 100,000 persons) in 2017. *Source Institute for Health Metrics and Evaluation. Download from https://ourworldindata.org/malaria*

Trends

Globally, the malaria incidence rate per 1000 people at risk decreased from 71 cases in 2010 to 57 cases in 2018. However, this global trend masks period-and geographical heterogeneity. Indeed, the incidence rate remained roughly at the same level from 2014 to 2018. Also, the incidence rate dropped markedly between 2010 and 2018 in the WHO African region (22 % decrease) and more in the South East Asia region (70 % decrease). The decline was marginal in other regions and an increase was even reported in the Americas. At the start of 2016, 91 territories and countries were regarded as endemic [14]. Thirty-one countries where malaria transmission persisted between 2015 and 2018 were about to meet the objective of at least a 40 % reduction of incidence by 2020. This means that unless rapid progress, the

Global Technical Strategy for malaria 2016 – 2030 goals for 2025 and 2030 will not be reached [3].
Modeling studies conducted separately for *P.falciparum* and *P.vivax* in 2019 showed that cases of- and deaths from- malaria decreased substantially, thanks to improved control interventions.
Incidence and mortality due to *P.falciparum* decreased by 27.9 % and 42.5 %, respectively. In endemic regions, cases dropped from 232.3 million in 2005 to 193.9 million in 2017. Over the same period, deaths decreased from 925,000 to 618 700. In sub-Saharan Africa, incidence fell from 213 million to 182.7 million, and mortality from 821,500 to 545,200. [15].
A downward trend was also reported in studies modeling the burden of *P.vivax* malaria. The global decrease was estimated at 41.6 % between 2000 and 2017. The regions of America, South-East Asia, and the Western pacific halved at least the number of cases [16].
This global optimism should be mitigated as some regions remained highly affected. In Sub-Saharan Africa, the risk remains important with 90.1 % of the population living in endemic areas in 2017 [17]. As in the report from the WHO, these studies emphasized that there was a stagnation in several countries in the five preceding years which was hidden by the global downward trend. A rise in cases was even reported particularly in politically or economically unstable areas [3, 16].

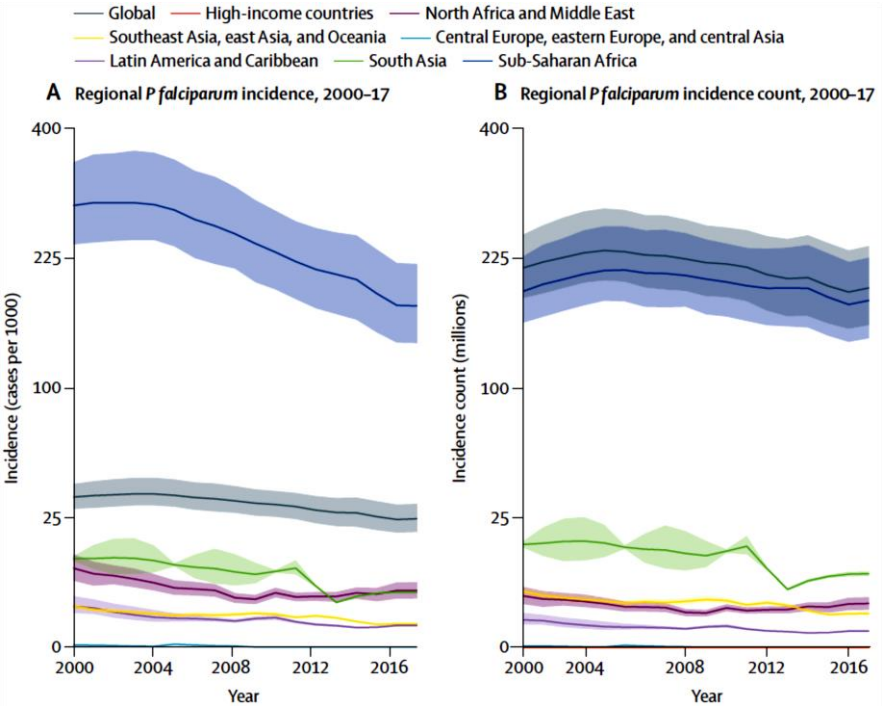

Figure 3 : *Plasmodium falciparum* incidence and count evolution from 2000 to 2017. *Source Weiss et al 2019.*

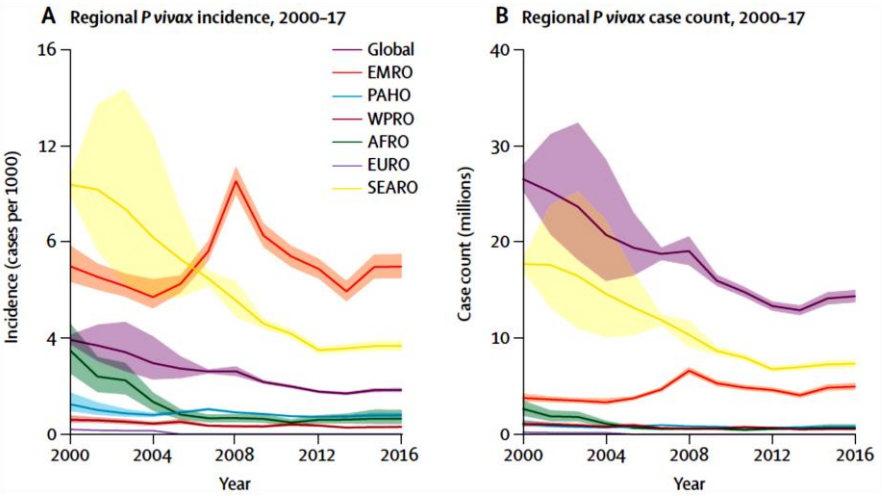

Figure 4 *Plasmodium vivax* incidence and count evolution from 200 to 2017. *AFRO: Regional Office for Africa, EURO: Regional Office for Europe, EMRO: Eastern Mediterranean Regional Office, PAHO: Pan American Health Organization, SEARO: Regional Office for South-East Asia, WPRO: Regional Office for the Western Pacific. Source Battle 2019.*

Malaria elimination and eradication

There is a growing consensus that malaria eradication is the most convenient option in terms of health, economics, and social improvement, mostly in high burden countries [18, 19]. Modeling analyses projected the achievement of this ambitious goal [18-20] at the earliest by 2040 [18] or at the latest by 2050 [19]. This demands innovative- approaches, tools, and financial modalities [18, 19].

According to the WHO, the number of countries moving toward elimination is increasing based on the number of indigenous cases reported. Countries reporting less than 10,000 indigenous cases were 49 in 2018, compared to 40 in 2010, while those with less than 100 indigenous cases grew from 17 to 25 over the same period [3].

In 2017, 20 countries that were endemic in 2000 were malaria-free for a minimum of one year [21]. In 2016, 21 countries were ascertained by the WHO as being able to eliminate malaria by 2020 and were named "the eliminating countries for 2020 (E-2020)". Seven of these countries already reached this objective (Timor-Leste, Algeria, Paraguay, China, Malaysia, El Salvador, and Iran). Ten of the remaining 14 countries with persisting transmission are on the right way but the number of indigenous cases raised between 2017 and 2018 in two others, Costa Rica and Comoros [3].

Despite the reduced incidence and mortality observed in high transmission settings like sub-Saharan Africa, there is little progress toward malaria elimination in 2017 [22]. In the past, these countries were regarded as controlling malaria, meaning that they aimed at reducing the incidence and deaths from malaria through uninterrupted interventions. Nowadays, as the fight against malaria is directed toward its eradication [18, 19], this theoretical delineation between control and elimination is no longer considered. All endemic countries are regarded as being in a continuous process, moving toward the final objective of eliminating malaria nationwide [20]. To stimulate the progress toward this goal in these countries, the WHO and Roll Back Malaria Partnership to End Malaria set up a new, more offensive, and more focused strategy called "High Burden to High Impact (HBHI)" in 2018. This approach owned and led specifically by each high burdened-country is based on 4 key points:

- transposition of political engagement into concrete actions,
- a departure from the "one-size-fits-all" approach to a more appropriate analysis and use of quality data to maximize impact,

- tailoring of the global recommendations from the WHO to the local specific context, and
- improved coordination of the response to malaria in collaboration with the other sectors [23].

This will ensure that suitable interventions are delivered to the right place and those needing them most.

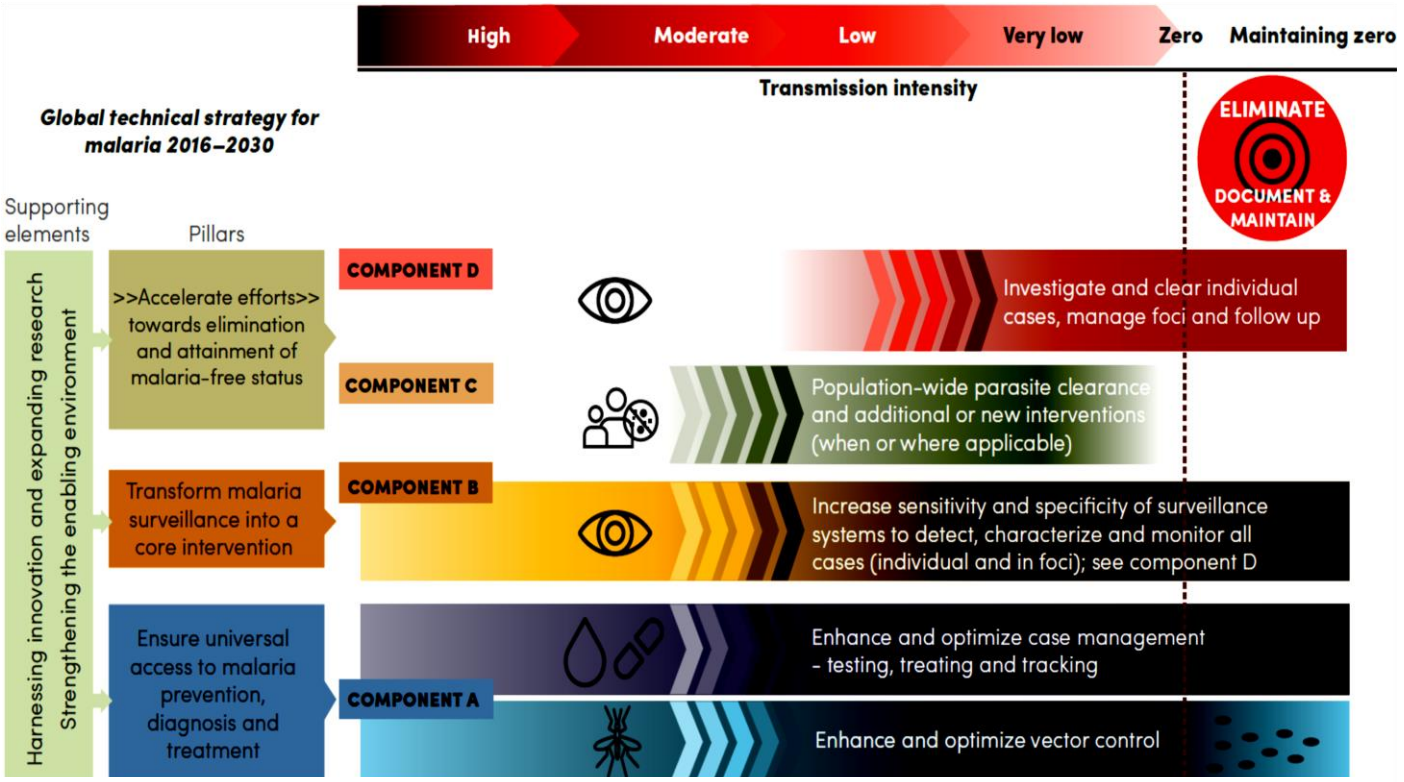

Figure 5 : Representation of the strategies and actions for eliminating malaria.
Source WHO https://apps.who.int/iris/bitstream/handle/10665/254761/9789241511988-eng.pdf?sequence=1

Malaria in Europe

Malaria cases and epidemics were common in Europe until the early 20th century. During the 1930s an approximate annual attack rate of 15 – 30 % and mortality of up to 73.7 deaths per 100,000 people were recorded in some places. Malaria was the second disease in childhood and the 9th cause of death irrespective of age in Greece [24]. However, the combination of environmental engineering, drug therapy, and insecticide spraying resulted in the successful eradication of the disease in the 1970s. Nowadays, malaria is imported with immigrants and international travelers representing the largest part of the cases encountered in France, Italy, Germany, and the United Kingdom [25]. In 2018, the European Centre for Disease Prevention and Control (ECDC) reported 8347 confirmed cases in the European Economic Area [26].
In Belgium malaria imported cases followed a three-phase trend between 1998 and 2017 (Figure 6). The cases decreased from 300-350 to 200 – 250 between 1998 and 2006 and then, remained constant till 2009. Since 2009, an upward trend was observed until 2017 [27]. Most of the cases originate from Africa with *Plasmodium falciparum* retrieved in the majority of the cases [27-29].

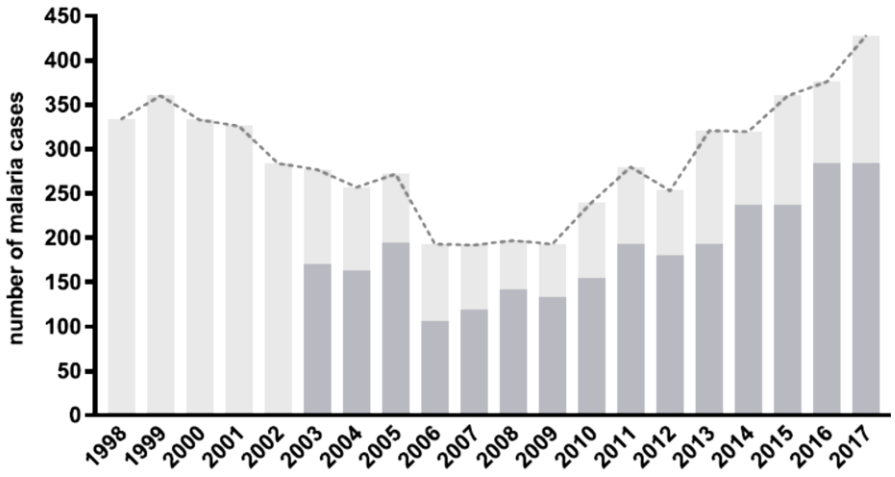

Figure 6 : Malaria cases imported to Belgium from 1998 to 2017.
The number of malaria cases are in light grey bars and dashed line. The referred cases are in dark grey bars. Sources Sciensano and Institute of Tropical Medicine. Figure taken from Rovira-Vallbona et al 2019.

Funding

About US$ 8.9 billion were invested in the fight against malaria between 2006 and 2010 [30]. Investments remained stable in the following years. In 2018, the US$ 2.7 billion disbursed were insufficient compared to the US$ 5.0 need to reach the first two intermediate objectives of the Global Technical Strategy (GTS). Approximately, $ 102 billion are necessary for the total expenditure for malaria programs during the period 2015- 2030. Africa alone needs 63 % of this amount while scaling up the coverage in Long-Lasting Insecticide-Treated Nets (LLINs) and Indoor Residual Spray (IRS) to 90 % by 2025 will represent 55 % of the spending [19]. About 30 % of the malaria funding comes from the local governments of endemic countries, while international providers sourced 70 %. The WHO African region benefited from about 75 % of the funding for 2018. Roughly, domestic investment serves half for malaria control and case management and a half for research and development together with private and philanthropical sources [3]. Africa received a large part of the international funding directed to research [3, 31]. Between 1997 and 2013, 333 grants representing US$ 814.4 were awarded to the continent. A rise in the investment for basic and drug research was observed between 2017 and 2018 [3].
Rapid advancement in controlling malaria was shown to be led by increased financing [32]. Rapid progress toward malaria eradication needs to double the current funding by 2025. This requires the sustainability of international investment but also scaling up domestic funding from the countries concerned [18]. Investment in malaria is cost-effective [18, 19, 33]. Reduction of malaria incidence by 50 % and delaying the development of artemisinin resistance above 1 % between 2015 and 2025 in sub-Saharan Africa is projected to cost $ 570 million annually while generating $ 6279 – 47 307 million benefits. This means a return of $36 for every $1 disbursed [33]. Also, eradicating malaria would result in 11 million deaths prevented and an economic gain exceeding US$ 2 trillion [18].

Challenges

The fight against malaria is facing several challenges.
Vector control is essential to malaria elimination. Indeed, Insecticide-Treated Nets (ITN) and Indoor Residual Spray (IRS) contributed to preventing respectively 68 %, and 10 % of the 663 million malaria cases averted between 2000 and 2015. According to WHO, the decrease of more than 50 % of the malaria incidence rate in 2016 was the result of the increased use of ITNs [34,

35]. However, these gains could be lost due to the decreasing susceptibility and expanding resistance of malaria vectors to the insecticides used to impregnate bed nets [36]. Nevertheless, even if ITNs lose the biological effect of insecticides, they protect physically humans from mosquito bites [37]. Also, *P. falciparum* became resistant to artemisinin-based combination therapies (ACTs) [38]. Another challenge is the socio-cultural beliefs about malaria. Different populations have various thoughts about the disease depending on their education, religion, and culture. These factors may influence their adoption of preventive and curative methods against malaria. Therefore, the involvement of communities in the process of malaria elimination should require dealing with such factors and often correcting erroneous knowledge [36, 39, 40]. Managing malaria elimination interventions was foreseen to be challenging for national programs [19]. Some authors argued that this may happen as these programs were built primarily for controlling the disease [36]. Other analyses showed that management issues already existed and were the cause of the present slow progress. The reason is that the management topic is not addressed in malaria meetings or by funders. Therefore the personnel of malaria programs including managers is not trained and skilled accordingly [19].

1.2. Malaria in pregnancy

1.2.1. Prevalence of malaria in pregnancy

Peripheral infection

Global data on Malaria in Pregnancy (MiP) are estimates of the risk rather than observed cases. In 2007, 125.2 million pregnant women were estimated to be exposed to malaria worldwide. The distribution of this exposure to malaria risk by WHO regions was as follows: 61.8 % in South East Asia and the Western Pacific, 24.2 % in Africa, 10.5 % in Eastern Mediterranea and Europe, and 3.4 % in the Americas. Pregnancies exposed to the risk of *P.falciparum* infection alone were 32.3 million, those exposed to *P.vivax* infection alone were 39.9 million, and those exposed to co-infection with *P.falciparum* and *P. vivax* were 53.0 million. Geographical estimates by continent showed that pregnant women from Asia-Pacific and those from Africa were the most exposed to *P.falciparum* and *P.vivax*. Indeed, India, Indonesia, and Pakistan were among the five Asian countries where pregnant women were the most exposed to both malaria parasites. In Africa, Nigeria and the Democratic Republic of Congo were the African countries classified

among the five countries with the greatest exposure to *P.falciparum* [41]. Another modeling study conducted in Africa in 2014, showed that without protective measures against malaria, 12.4 million out of 27.6 million pregnancies would have been at risk of *P.falciparum* infection in 2010. The probability for a woman to be exposed to malaria exceeded 0.6 in the first and second times pregnant women in this analysis. When all gravidities were taken into account, the probability for the majority of the countries on the continent was higher than 0.4. This probability seemed to increase from East to West and from South to North [42]. A similar trend was also found in the 2019 world malaria report: West and Central Africa subregions presented the greatest likelihood of exposure (35 %) while a lower risk was found in East and Southern Africa [3].

Prevalence observed in malaria in pregnancy studies varies greatly even within the same transmission setting or country. In 2019, in high transmission settings, prevalence exceeding 50 % was reported in Nigeria (57.7 %) [43], in Burkina Faso (53 – 55 %) [44], and Uganda (51 %) [45]. Lower prevalence was also found in other studies: 37. 8 % in Sudan in 2017 [46], 30.84 % in a multicentric study involving Burkina Faso, Mali, the Gambia, and Ghana in 2015 [47], 20.4 % in Cameroon in 2013 [48], 15.8 % in Guinea in 2019 [49]. *Plasmodium falciparum* was the species more encountered in malaria cases from high transmission settings [43-49].

In low transmission settings prevalence is very weak and *P. falciparum* and *P.vivax* are found in variable proportions. A study conducted in Papua New Guinea, Guatemala, Brazil, Colombia, and India in 2017 reported an overall prevalence of *P.vivax* malaria ranging from 0.4 % using microscopy to 7.0 % using polymerase chain reaction while *P. falciparum* prevalence was 0.5 % by microscopy [50]. In another study in Brazil in 2018, a higher prevalence of 8.0 % for *P. vivax* and 8.9 % for mixt infection was found [51].

Placental infection.

Estimates done in 2014 showed that 11.4 million pregnancies (41.2 %) out of 27.6 million would have experienced placental malaria (PM) in absence of specific prophylaxis in Africa. Prevalence of at least 50 % was found in some high transmission-countries like Nigeria (69.6 %) in 2012 [52], Ghana (62.3 %) in 2014 [53], Sudan (59.3 %) in 2017 [46] and Burkina Faso (50 %) in 2019[44]. However, prevalence lower than 50 % was reported in other studies conducted in countries with high transmission: 32 - 33 % in the Gambia and Burkina Faso in 2019 [54], 44.6 % in Uganda in 2019 [45], 18.5 % in Sudan in 2017 [55], 14.8 % in Guinea in 2019 [49], and 8 % in Tanzania in 2015

[56]. *Plasmodium falciparum* was found in most of the cases in the above-mentioned studies.
Outside Africa, the prevalence of malaria did not reach so high levels. Also, these infections involve *P. vivax* alone or with *P. falciparum*. In Bolivia, a prevalence of 2.7 to 7.8 % was found for *P. vivax* and 0.4 % for *P.falciparum* in 2013[57]. Placental malaria was found in 12.4 % of the participants in a study conducted in Papua New Guinea, Guatemala, Brazil, Colombia, and India, irrespective of the parasite species in 2017 [50].

1.2.2. Factors associated with malaria in pregnancy

Environmental factors

Several environmental factors are associated with malaria in pregnancy. In 2019, the prevalence of peripheral infection was greater in women delivering in the rainy season than those delivering in the dry season in a multicentric study conducted in Burkina Faso, the Gambia, and Benin. The inverse was observed with placental malaria with women delivering in the dry season being more at risk. [54]. Also, rural pregnant women were more at risk of both peripheral and placental malaria than urban dwellers and this did not seem to depend on the magnitude of the transmission in studies conducted from 2013 to 2020 in Uganda, Papua New Guinea, Brazil, and Ethiopia [51, 55, 58, 59]. A study conducted in Uganda in 2019, showed that the material used for house construction influenced the prevalence of peripheral malaria in pregnancy. Women living in a house built using traditional material experienced more malaria than those living in modernistic habitation. The same study reported that the household socio-economic level influenced the risk of malaria infection with the lowest wealth status being subject to more infection compared to the lower level. In Brazil, a low wealth index was also associated with an increased risk of malaria infection during pregnancy in 2018 [45, 51]. Women who earned less than 28 000 F CFA per month were subject to more placental infection than those earning more in Cameroon in 2013[48].

Maternal factors

Age
Young age was reported as a risk factor for peripheral and placental malaria in pregnancy in several studies from highly endemic countries. In Uganda and Ethiopia, the risk of peripheral malaria decreased as maternal age increase in

2013, and 2020, respectively [59, 60]. In Indonesia, adolescent mothers aged 16 years or less were more at risk of peripheral malaria during their pregnancy than their older counterparts in 2008 [61]. In a low transmission setting of Brazil, women aged 30 years and above experienced less peripheral malaria during pregnancy as compared to those aged 13 – 20 years in 2018 [51].
Placental malaria was common in teenage mothers (aged less than 20 years) in Cameroon and Nigeria in 2013 and 2019, respectively [43, 48]. In another study in Nigeria in 2018, the age of up to 25 years was reported with an increased risk of placental malaria [62].

Parity

Parity conditions the risk of peripheral malaria in pregnant women. First and second-time mothers were more prone to peripheral malaria in Uganda in 2019 [45], Ethiopia in 2013 and 2020 [58, 60], Burkina Faso in 2014 [63]. Studies from Indonesia reported also such results sooner in 2008 [61].
A study conducted in Burkina Faso, Benin, and the Gambia showed a reduced risk of placental malaria in women having at least three previous pregnancies [54]. The prevalence of placental malaria was greater in pregnant women of low gravidity in Uganda in 2017 and 2020 [64, 65], Sudan in 2017 [46], and Tanzania in 2015 [56].

Education

The prevalence of peripheral malaria in pregnancy was greater in women who did not attend a formal school or those with poor educational levels in Uganda in 2013 [59] and Burkina Faso in 2014 [63]. The risk of placental malaria was increased in pregnant women with such schooling level in Nigeria in 2019 [43].

Anemia

Anemia was found associated with peripheral malaria in pregnancy in Burkina Faso in 2014 [63] and Indonesia where transmission is high, but also in studies involving low transmission countries like Brazil, Guatemala, Colombia in 2017 [50]. In Malawi and Papua New Guinea, anemia and placental malaria were associated in 2013 and 2017 [55, 66]. Surprisingly, placental malaria risk was lower in anemic women in Sudan in 2012 [67].

Human immunodeficiency virus infection

Immunodeficiency virus (HIV) was associated with a greater risk of peripheral malaria infection in pregnancy in 2013 in Uganda [59] may be due to the decreased immunity.

Fever

Fever is a common symptom of malaria rather than a risk factor. Peripheral parasitemia was more prevalent in febrile women and those reporting fever

24 hours before hospital attendance in Indonesia in 2008 [61]. Fever was also found in presence of placental malaria in Tanzania in 2015 [56].

Use of preventive and curative tools

Pregnant women using Sulfadoxine-Pyrimethamine (SP) for intermittent preventive treatment of malaria in pregnancy (IPTp) were less prone to peripheral, and placental infection in Nigeria in 2012, Burkina Faso, the Gambia, and Benin in 2019. The magnitude of the protective effect increase with the number of doses taken [52, 54].

Peripheral malaria in pregnancy was less frequent in women who slept under a bed net before being enrolled in a study in Papua New Guinea in 2015, and Uganda in 2016 [68, 69]. In 2020, in Ethiopia, this protective effect was also seen in women who utilized their insecticide-treated bed net but not in those possessing but not using it [60]. Another study conducted in Guinea in 2019 showed that the benefit of a long-lasting insecticide-treated bed net was guaranteed only for women using it regularly [49]. In the same year in Nigeria, women of childbearing aged less than 30 years underutilized bed nets [70]. Even if not pregnant this presents a risk as it was thought that malaria in pregnancy could be due to infection occurring before- and persisting throughout- the pregnancy [71].

The prevalence of placental malaria was higher in non-users of bed net in Sudan in 2017 [46] or long-lasting insecticide-treated bed nets (LLINs) in Nigeria in 2012 [52].

The lack of indoor residual spray in the precedent year exposed pregnant women to an increased risk of asymptomatic parasitemia in pregnancy in Ethiopia in 2020 [58].

Nutritional status

A study conducted in 2016 reported that undernutrition was found alongside malaria in endemic countries. It lowers the immune capacity of the pregnant woman against malaria and aggravates the detrimental effect of malaria on fetal growth. On the reverse, malaria can decrease the assimilation of nutrients in the pregnant woman [72].

Other maternal factors associated with placental malaria

Women in which peripheral malaria was diagnosed were more susceptible to placental malaria than those with undetectable parasitemia in Sudan in 2017 and Malawi in 2013 [46, 66]. The likelihood of detection of malaria in the placenta increased with repeated peripheral infections in Uganda in 2020 and 2017 [64, 65]. An increased prevalence of placental malaria was reported in women presenting with symptoms in Papua New Guinea in 2017 [55]. Blood group O seemed to reduce the occurrence of placental malaria in Sudan in

2017 [73] and at least in first-time mothers in Ghana in 2014 and a metanalysis in 2019 [53, 74]. The pathways of this protective effect are not fully understood. Some authors thought that blood groups influence the pathogenesis of malaria in infected individuals. Indeed, a study conducted in 2007 in Mali and Kenya, a comparison of children with blood group O to those with other blood groups found that severe malaria was less prevalent in the first. The authors linked, this reduced prevalence of severe malaria to the lesser rosetting phenomenon observed with blood group O compared to other blood groups [75].

Malaria knowledge

Women who were not educated on how to prevent malaria during pregnancy and who did not visit antenatal clinics presented a higher risk of being infected in Ethiopia in 2020[60].
In 2016, a study conducted in the Gambia showed that Gambian women used terms that incompletely define malaria. Also, they confounded symptoms of malaria to those of pregnancy. For malaria treatment, they relied firstly on traditional herbal medicine because modern pharmaceutical medications are regarded as risky and less effective for them. For these reasons, they adhered poorly to treatment against malaria [39]. Approximately, one pregnant woman out of 3 knew adequately malaria, and 1 out of 4 cited intermittent preventive treatment in pregnancy as a preventive intervention in a study conducted in Malawi in 2020. Also, less than 20 % of them knew prematurity or smallness as consequences of malaria in pregnancy [76]. Furthermore, the use of bednet increased among pregnant women alongside knowledge of malaria after an educational intervention in Pakistan in 2020 [77].

Health care system and program factors

A review of guidelines regarding malaria prevention and treatment in four sub-Sahara African countries in 2014 revealed some concerns. In some countries, guidelines for intermittent preventive treatment during pregnancy did not follow those issued by the WHO. As an example, this prevention was not allowed before 20 weeks of gestation and was stopped after 36 weeks of gestation in two countries. A document recommended three doses of IPTp for pregnant women with HIV without clearing if sulfadoxine-pyrimethamine or cotrimoxazole should be used. Also, some documents showed truncated or incoherent guidelines regarding the specific treatment of malaria for each trimester of pregnancy. A recommendation for treating pregnant women

confirmed without malaria upon microscopic examination of blood smear was even found, contradicting the WHO policy of "test, treat and track" [78, 79].
In 2012, the number of doses of sulfadoxine-pyrimethamine was increased from two to at least three for intermittent preventive treatment of malaria in pregnancy. This update took up to 2015 to be integrated into the malaria policies of the concerned countries. Only 7 among the 17 countries concerned entirely operationalized this update by 2018 [80]. An analysis including data of 32 sub-Saharan Africa countries in 2015 showed that antenatal care visits attendance raised after the augmentation of the number of doses of sulfadoxine-pyrimethamine for preventing malaria in pregnancy in 2012. About 76.6 % of women attended at least 3 visits. However, during 72. 9 % of all antenatal care visits, health care providers missed the occasion to give IPTp [81].
In 2018, it was acknowledged that the successful countrywide campaign of distribution of 6 million long-lasting insecticide-treated bed nets in Zambia benefited from concertation between all the stakeholders including partners outside the domain of health. The use of mobile phones allowed the transmission of data without delay [82].
In 2016 it was estimated that only 30 % of women in urban settings benefited from obstetrical ultrasound scanning during their pregnancy in low-income countries where malaria is endemic like sub-Saharan Africa. This proportion dropped to 6 % in rural settings [83]. The first reason is that fetal imaging is not included in the package of standard routine care in peripheral health facilities [84]. In places where this possibility is offered, people cannot afford it because of its high cost. The personnel qualified for performing ultrasound and managing the subsequent required care or interventions is also insufficient [83]. National guidelines and recommendations framing the usage of ultrasonographic imaging in pregnancy are unavailable, leaving the way for inappropriate and detrimental practices [84, 85].

1.2.3. Pathogenesis of and immunity to malaria in pregnancy

Parasite live cycle

The malaria parasite development has a phase in the mosquito and a phase in the human (Figure 7). When the mosquito bites the human to feed on the blood it releases the Plasmodium as sporozoites that reach the liver cells within an hour. After multiplying and segregating as merozoites they rupture the liver cells' walls, flow into the blood. However, merozoites of *P.vivax* and

P.ovale are capable to stay in dormancy in the liver for a long time and are called hypnozoites. In the blood, the merozoites penetrate the erythrocytes and initiate the asexual phase of their reproduction. This corresponds also to the clinical phase of malaria. The merozoite becomes a trophozoite and then a schizont that disrupts the red blood cell, releasing new merozoites that can infect other erythrocytes. This cycle takes 36 to 72 hours to be completed. Without treatment, the cycles synchronize triggering fever that is repeated with the same periodicity. Some merozoites transform into female and male gametocytes resting in the capillaries of the skin, where they are ingested by the mosquito during its blood meal. The male gametocyte multiplies into eight flagellar microgametes in the gut of the mosquito while the female gametocyte becomes a macrogamete after a maturation process. The fusion of the microgamete and the macrogamete forms a zygote which becomes an ookinete by elongation. It escapes the lumen of the stomach of the mosquito and remains fixed on the external wall as an oocyst. After a series of multiplication, it forms sporozoites that reach the salivary gland of the mosquito. The development of the parasite in the vector from the sexual stage to the infectives forms lengths 7 – 10 days [4].

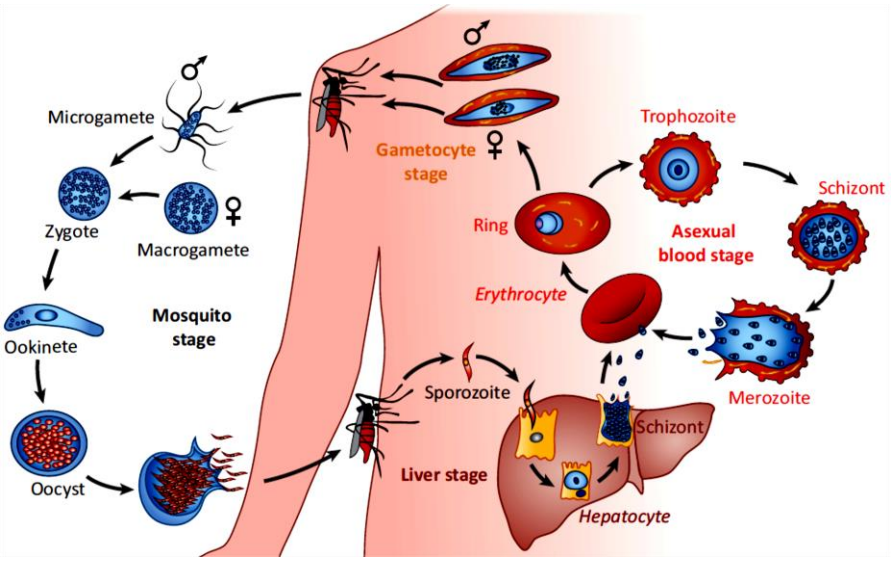

Figure 7 Plasmodium life cycle *Source: Maier et al 2019*

Pathogenesis

The time from the infection to the onset of clinical manifestations of malaria is specific to each parasite and is known as the incubation period. This time is 10 to 14 days for *P.falciparum* or *P. knowlesi*, 18 days or more for *P. malaria*, 14 to 21 days for *P.vivax* and *P.ovale* At the end of this period, the parasitemia is generally above a theoretical threshold of 100 parasites /µl. Symptoms like fever occur with the same periodicity as the parasite cycle [2]. Red blood cells infected by *P.falciparum* can escape the splenic destruction and sequestrate into the blood vessels of diverse organs obstructing these vessels and damaging their walls leading to severe malaria. This phenomenon of cytoadherence is possible because of the expression of a protein named *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) by the parasite at the surface of the parasitized red blood cell. This protein belongs to a group of proteins encrypted by the var gene family. There are different subgroups of this protein with different receptors on the endothelium of the cells. The clinical manifestation reflects the subgroup of proteins and the organ implicated. Neurologic manifestation like coma involves the binding on intercellular adhesion molecule-1 and endothelial protein C in the brain [86, 87]. Sequestration of the parasites in the lungs could lead to respiratory distress[88, 89]. The microcirculation obstruction and damages to the cells can be worsened by the rosetting phenomenon, corresponding to the binding of infected red blood cells to uninfected erythrocytes [90].
Plasmodium falciparum sequestrate and accumulate frequently in the intervillous spaces of the placenta. The receptor in the placenta is Chondroitin Sulfate A (CSA) and the subgroup of PfEMP1 binding on this receptor is the variant VAR2CSA [88, 91, 92]. *Plasmodium vivax* has been suspected to be responsible for some placental alterations in some studies [93-96]. As a response to the infection of the placenta, monocytes invade this organ leading to inflammation[97]. The adverse maternal and fetal effects of placental malaria occur as a response to a cascade of reactions. Placental malaria prevents the trophoblast from migrating and invading the uterus [98]. The complement is activated disturbing the angiogenesis of the placenta and affecting fetal circulation [99]. The result of the disturbed placental angiogenesis is a reduced size of this organ as the villi grow and develop insufficiently [100]. The inflammation leads to a discharge of cytokines [97] disorganizing the transportation and delivery of nutrients [101, 102], and dysregulating the hormonal axis [103]. All the reactions contribute at least partially to maternal anemia [97]. Other factors contributing to anemia are the destruction of red blood cells in the spleen [90], the vessels, and the bone

marrow, as far as the dysfunction of the erythropoiesis [93]. Maternal death by severe malaria is probably the result of sequestration of parasites extending to multiple organs including the placenta, and the brain [104]. Negative consequences in the fetus consist of fetal growth restriction (FGR), preterm birth (PTB), low birth weight (LBW), and stillbirth [88, 105]. The mechanisms involved in placental malaria and leading to fetal growth restriction are shown in figure 8.

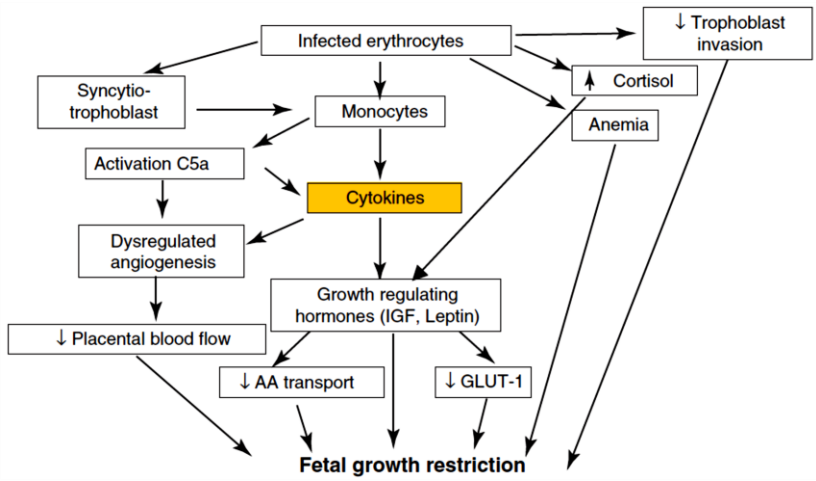

Figure 8 Mechanisms involved in fetal growth restriction during malaria in pregnancy. *Source: Umbers et al 2011*

In high and stable transmission settings, vulnerability to malaria in pregnancy depends on age and parity. Reports dating from the earlier 2000s showed that young women are more prone to malaria than their adult counterparts and that pregnancy accentuates this vulnerability in endemic settings. Within women of the same gravidity, malaria affected more those who are younger in some studies [106-108]. In recent studies, age was also found associated with both peripheral and placental infection [48, 51, 59-61]. Therefore, the mechanisms that could explain the relationship between age and malaria in pregnancy should be explored [97] or updated.

Low parity was a risk factor for both Peripheral and placental malaria [45, 54, 56, 61]. Biological data support this epidemiological finding. It has been noticed that the accumulation of monocytes in the placental was more frequent in the placentas from primipara [97]. In vitro studies showed that antibodies that prevent the infected red blood cells to bind to the CSA were more abundant

in multigravidae than in primigravidae [88, 109].
In settings where transmission is low increasing parity does not confer protection against malaria in pregnancy [89, 98]. In Mozambique, malaria prevalence in pregnant women decreased between 2005 and 2010. Concomitantly, a decrease in the level of antibodies against malaria and a rise in the deleterious consequences of *P.falciparum* parasitemia was observed in pregnant women [110].

1.2.4. Malaria in pregnancy-related adverse birth outcomes

In sub-Saharan Africa, 12-20 % of stillbirths were attributable to malaria in pregnancy in a study conducted in 2017. The likelihood of stillbirth raised twofold in presence of *P.falciparum* in peripheral or placental blood at delivery [89]. A systematic review conducted in 2006 found that insecticide-treated bed net protected against placental malaria and reduces the occurrence of stillbirths [111].
Malaria in pregnancy was associated with low birth weight in Sudan in 2017 and Uganda in 2020 [46, 64]. In 2014, a modeling study estimated that 11.4 million women with placental malaria would have delivered 900 000 low birth weighted neonates in Africa [42]. Low birth weight, in turn, raised the likelihood of neonatal death. Intrauterine growth restriction and prematurity are two ways by which low birth weight occurs. In endemic settings, malaria is responsible for up to 70 % of the intrauterine growth restriction probably through the altered provision of oxygen and nutrients to the fetus[98]. Infection occurring in the second trimester was more likely to result in low birth weight than that occurring in the third trimester in a systematic review conducted in 2013 [112]. The fetus's maximal development period corresponds to the second trimester and when compromised could result in fetuses of suboptimal size [113]. Prematurity was also found associated with malaria in pregnancy [114]. In Papua New Guinea both low birth weight and preterm birth were associated with chronic placental malaria in 2015 [68]. In Uganda, placental parasitemia was a risk factor for preterm delivery in 2017 [65].
Congenital malaria is another adverse event directly linked to malaria in pregnancy. It corresponds to the appearance of asexual parasites in the blood of a neonate in the first week of his life, in association with at least one symptom. A systematic review and meta-analysis issued in 2020 reported a prevalence of clinical congenital malaria of 39. 5 % in Africa. In other regions, the prevalence increased to 56.3 % [115].

1.2.5. Diagnosis of malaria in pregnancy

Clinical symptoms

In high transmission settings, pregnant women who acquired some degree of immunity carry parasites without manifesting any symptom [88]. However, they remain capable to infect mosquitoes and perpetuate the transmission cycle [116].

Uncomplicated malaria

Pregnant women lacking immunity in high transmission settings and those living in low transmission areas can complain of fever, cold, headache, and pains at different parts of their body, anorexia, nausea, and vomiting. [117]

Severe malaria

Plasmodium falciparum is incriminated in nearly all cases of severe malaria. This form of malaria involves major organ dysfunction like neurological impairment, (coma, prostration), respiratory disorders, severe anemia, and other biological abnormalities [2].

Histology

This diagnostic tool is intended for placental malaria diagnostic only and is more useful in research settings where it is the reference method. It can reveal parasites sequestrating in the placental when they are undetectable in the peripheral blood [118]. Placental biopsies can be processed either by freezing the fresh samples or by fixing them using buffered formalin and embedding them in paraffin. Histological findings are classified as acute, chronic, or past according to Bulmer [118]. The patterns of placental malaria were reported in figure 9.

Practically, histology is difficult to perform routinely at health facilities. It is costly and needs some type of equipment and qualification for processing and interpretation. Furthermore, the interpretation varies from one study to another. Polymerase chain reaction performs more than histology in diagnosing placental malaria [119]. However, in studies conducted in Uganda in 2020, histology detected malaria in 43.9 % of the placental compared to 10.1 % for loop-mediated isothermal amplification, and 3.5 % for microscopy [64].

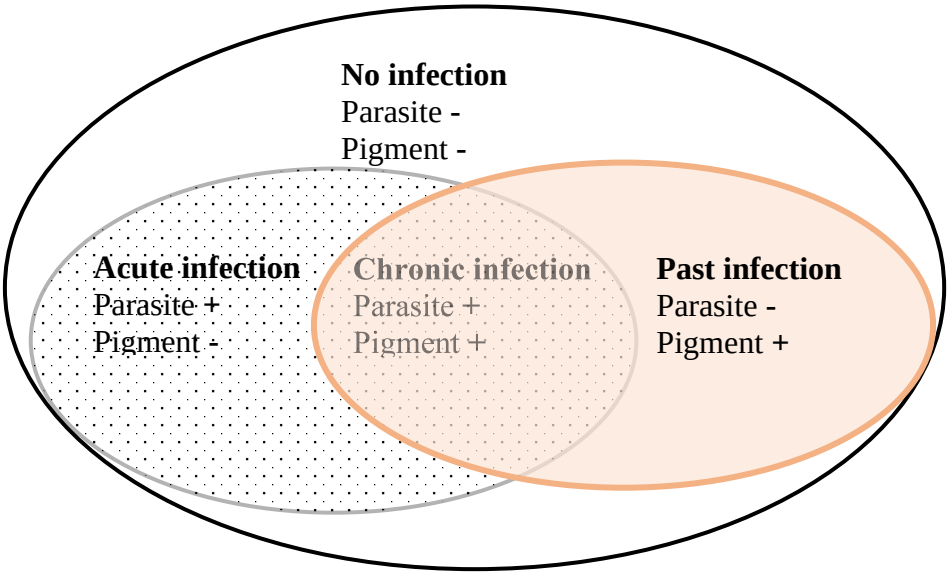

Figure 9 Histopathological diagnosis of placental malaria
Note: +: present; -: absent.

Microscopy

Microscopy is the standard method for peripheral malaria diagnosis through the reading of stained blood smears but could be used for placental malaria diagnosis also [120]. It required equipment, energy supply, and experience. Also, it is unable to identify malaria when density is under 50 parasites/µl [121]. For this reason, it performs less in diagnosing malaria in pregnancy because parasites sequestrate in the placenta and cannot be found at the periphery. In Sudan, microscopy detected malaria in the peripheral blood of 37.8 % of pregnant women while 58.9 % were identified with placental malaria in 2017 [46]. However, placental malaria is not systematically diagnosed in all pregnant women with peripheral infection. In Uganda malaria was found in 43.9 % of the placentas of women with peripheral infection during their pregnancy in 2020 [64]. Nevertheless, microscopy detection capacity was lower (8.8 %) than histology (61.7 %) for placental malaria diagnosis in another study in Uganda in 2019 [122].

Rapid Diagnostic Tests

These tests use the lateral flow immunoassay technic to diagnose malaria [123]. They reveal the presence of the parasite antigens in the blood. Most of

the devices can recognize the histidine-rich protein 2 (HRP2) for *P. falciparum* or the lactate dehydrogenase (LDH) identifying all plasmodium species [124]. However, they are less able to detect *P. knowlesi*. Rapid diagnostic tests are used worldwide [124] because they are simple, fast, and do not need high qualification. In 2018, 412 million tests were commercialized, and 259 were delivered through the national malaria programs [3]. Rapid diagnostic tests operate optimally at densities above 200 parasites/µl [121]. This threshold is projected to be lower for the new "ultrasensitive" tests [125]. Surprisingly, hyperparasitemia limits also, the capacity of rapid diagnostic tests, and this is known as the "prozone effect" [126]. Since 2008, *P. falciparum* lacking the HRP2 gene was found in Peru. As a result, rapid diagnostic tests remained negative in presence of such parasites [127].
The sensitivity of rapid diagnostic tests assessed during antenatal visits is low compared to PCR the reference method, ranging from 75.7 % to 42. 6 % in Burkina Faso in 2016 [128].

Molecular diagnostic tests

Malaria diagnosis benefited from the contribution of the advances in biomolecular technics like polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP).

Polymerase Chain Reaction
Polymerase Chain Reaction also called PCR is a technology that allows the multiplication of a fragment of nucleic acid. Polymerase Chain Reaction technics are useful for detecting low-density parasitemia or identifying species in mixed infection and assessing therapeutic efficacy [129, 130]. It remains sensitive for parasitemia lower than 1 parasite/µl. Therefore, it has better performance than microscopy and rapid diagnostic tests [68].
In low resource settings, Polymerase Chain Reaction use is restricted to reference laboratories because of the costs and the level of qualification needed[129].
Polymerase Chain Reaction detected malaria in peripheral blood of 66 % of pregnant women in Papua New Guinea in 2015, while microscopy diagnosed only 34.4 %[68].

Loop-mediated isothermal amplification
Loop-mediated isothermal amplification is a nucleic acid amplification technic (NAAT), with performance comparable to PCR. This method has

advantages over PCR: its cost is lower and its implementation does not need important equipment. It is faster and less complicated to operate than PCR [131]. In Colombian apyretic women, the sensitivity of LAMP was higher (100 %) than that of microscopy (79.5 %) and rapid diagnostic tests (76.9 %), nested-PCR being the reference in 2018. LAMP agrees perfectly with nested PCR and performs similarly in detecting placental infection in peripheral blood. Its sensitivity (88.9 %) is four times that of microscopy and rapid diagnostic tests (22.2 %) in placental blood [132].

1.2.6. Treatment of malaria in pregnancy

Treatment of uncomplicated malaria

According to 2015 WHO guidelines and a 2019 publication, quinine and clindamycin are taken for seven days for treating *P. falciparum* malaria in the first trimester, Alternatively, artemisinin-based combination treatments or oral artesunate with clindamycin could be used. Chloroquine or quine (in regions with resistance to chloroquine) is suitable for malaria involving other species than *P.falciparum* [38, 133].
In the second and third trimesters, pregnant women can benefit from artemisinin-based combination treatments (ACTs) like artemether-lumefantrine, artesunate-amodiaquine, or dihydroartemisinin-piperaquine [134]. These therapeutics are semi-synthetized from qinghaosu or Artemisia annua [4, 135, 136] and are currently acknowledged for treating malaria in most of the endemic countries [135, 136]. They integrate the rapid but short action of the artemisinin derivative to the slow but persisting effect of another drug [137]. Their efficacy exceeds 98 % over 28 days follow-up even on areas where parasites are resistant to old drugs according to the results of a large multicentric clinical trial published in 2016 [130].
Unfortunately, parasites resisting ACTs arise in the subregion of the Great Mekong and is spreading [135].

Treatment of severe malaria

This form of the disease is caused most of the time by *Plasmodium falciparum*. However, *P. vivax* is now recognized as being able to cause such a form of the disease. The treatment for pregnant women is identical for both species. Artemisinin derivatives are used from the second trimesters because of embryotoxicity and teratogenicity concerns in early pregnancy in studies on animals. A study conducted in 2020 in Burkina Faso, Mozambique, and

Kenya showed that the occurrence of low birth weight or smallness for gestational age was not greater in newborns from mothers exposed to artemisinins in the first trimester than those from mothers not exposed [138]. Nevertheless, they could be used in that trimester if maternal life is threatened. Pregnant women infected by *P. vivax* are recommended to take primaquine after delivery to clear completely the parasites sequestrated in the liver and avoid recurrence [10, 139].

1.2.7. Prevention of malaria in pregnancy

Indoor residual spray

The number of people protected by the indoor residual spray of insecticides decreased from 180 million in 2010 to 93 million in 2018. One reason explaining this drop is the rise in the costs induced by the replacement of the pyrethroids which are no more effective because of the resistance [3]. However, indoor residual spray demonstrated a protective effect against malaria in pregnancy including placental malaria in Uganda in 2016 [140].

Intermittent preventive treatment during pregnancy

Sulfadoxine-Pyrimethamine (SP) is used for preventing malaria during pregnancy in women without human immunodeficiency virus. The doses should be taken monthly, starting in their second trimester [10]. The drug is distributed during the antenatal visits. According to the 2019 world malaria report, the number of pregnant women covered with at least three doses is increasing but remains under 50 % globally in 2018. Also, 18 % of women visiting antenatal clinics at least once were not provided with the treatment [3]. In 2014, a systematic review reported that the use of this prophylaxis is beneficial for both the mother and her fetus. Indeed, pregnant women receiving at least two doses lower the risk of being parasitized by 61 % while placental parasitemia and low birth weight risk are reduced by 55 % and 27 %, respectively. The prophylactic effect of sulfadoxine-pyrimethamine can be compromised by the resistance already reported in some settings [141]. The possibility of replacing it with Dihydroartemisinin-piperaquine was evaluated in addition to new strategies like intermittent screening and treatment in Kenya in 2015, and Malawi in 2016 [142, 143].

Prevention using bed nets

The aim of bed nets usage is to prevent contact between the mosquito and humans. Different types of bed nets are used, but those impregnated with insecticides are recommended.
An insecticide-treated bed net is a bed net immersed in pyrethroid insecticidal solution. Long-lasting insecticide-treated nets are industrial nets. They have insecticides directly embodied during the manufacturing process. According to WHO, the drop of 50 % in malaria burden in 2016 was the result of increased utilization of bed nets [34].
The bed net had a protective effect against peripheral infection in pregnancy in Papua New Guinea in 2015 and Uganda in 2016 [68, 69]. The possession of a net was not associated with protection against malaria in pregnancy in Ethiopia in 2020 [60]. In Ghana, pregnant women owning bednets did not use them because they were not sufficiently informed and encouraged by providers in 2019. Other pregnant women did not sleep under bednets because of the irritation caused by the insecticide used to impregnated them [144]. In Guinea, women who used them daily were more protected than those using them intermittently in 2019 [49]. Young women aged less than 30 years underused bed nets in Nigeria in 2019 [70], and could be exposed to a higher risk of malaria if they become pregnant [71]. Placental malaria risk was higher in Nigerian and Sudanese women who did not use bed nets in 2012 and 2017, respectively [46, 52].

Vaccine

The most known vaccine is the RTS, S/AS01, utilizing the circumsporozoite protein of *P. falciparum* [145]. It was only evaluated in infants and children aged 5-17 months in phase 3 clinical trials. Its efficacy was about 36 % in this latter group, after four years of follow-up. Pilot studies are conducted in Ghana, Kenya, and Malawi to assess the public health potential of this vaccine [146].
The PRIMVAC vaccine based on the VAR2CSA and intended specifically for placental malaria undergoes its first time evaluation in humans in Burkina Faso and France in 2020 with encouraging results [147].

1.3. Contribution of ultrasound in studies on malaria in pregnancy

Ultrasound scanning is increasingly used in studies on malaria in pregnancy. It allowed more reliable estimates of gestational age. The negative impact of

malaria on fetal growth was better studied using ultrasound. In Africa and Asia, prospective studies conducted from 2012 to 2015 revealed also the contribution of malaria infection in the first three months of the pregnancy to maternal anemia, miscarriages, impaired fetal growth, and low birth weight. [148-150]. Doppler ultrasound allows the measurement of the resistance of the umbilical artery, which reflects the circulation of the blood from the fetus to the placenta. In the Democratic Republic of Congo, low umbilical artery resistance was detected in the third trimester in first-time pregnancies beginning with malaria infection, in 2012. The fetuses of these women experienced more fetal growth restriction than those of multigravidae without early malaria infection [151].

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CHAPTER 2: MALARIA IN PREGNANCY IN BURKINA FASO

2.1. Country general context

Burkina Faso is a West Africa country covering an area of 272 960 km^2. It is surrounded to the North-East and North-West by Niger and Mali, and from South to South-East by Côte d'Ivoire, Ghana, Togo, and, Benin (Figure 10) [1]. The population was 14,017,262 in 2006 and was estimated to 20, 244,080 inhabitants in 2018 with 51.7 % women [2]. The life expectancy at birth is 61 years [3]. The population is mostly young. One-third are teenagers and 64.8 % are under the age of 24. A large part (76 %) of women of 15 – 24 years old reside in rural areas [4]. In 2018, 55 % of women aged 18 – 24 years are married and 31 % gave birth the first time by age 18 in rural areas. In urban areas, these proportions are 18 % and 11 %, respectively. The total fertility rate was 5.2 births /woman. Adolescent (women aged 15 -19years) fertility rate was 102 births/1,000 women [4]. The maternal mortality rate was 28/100,000 live births in 2010. The literacy rate in individuals of at least 15 years old is lower in women (26.1 %) than in men (44.3 %) [5]. The country has 13 administrative regions, subdivided into 45 provinces encompassing 351 departments or communes, and 8895 villages [1]. The size of the road network was 15 304 km in 2014, including 3642 km of asphalt road [6].

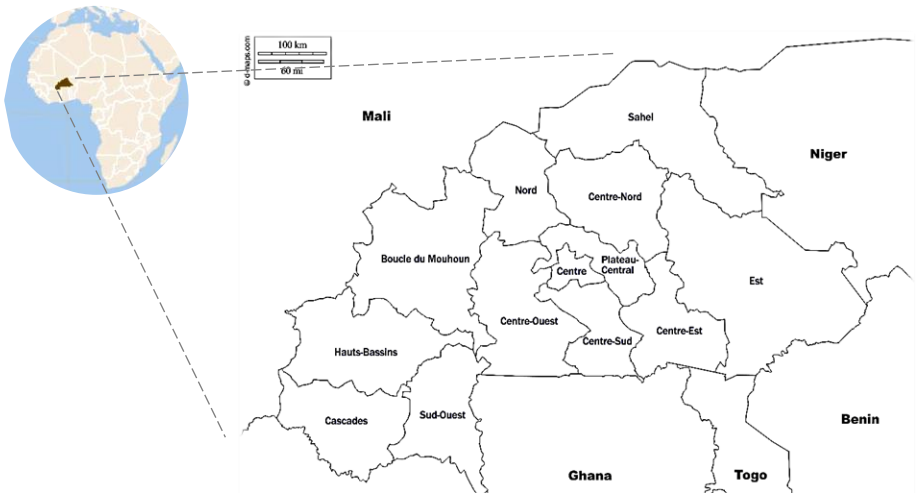

Figure 10: Map of Burkina Faso with the 13 administrative regions.
Source Enquête sur les indicateurs du paludisme (EIPBF) 2017 – 2018 and http://d-maps.com/m/africa/burkina/burkina18.gif

The climate is sudano-Sahelian in Burkina Faso. The rainy season takes place from June to October, followed by the dry season from November to May. The country can be divided into three climatic regions according to the duration of the rainy season and the average rainfall. The Sahelian region corresponds to the northern part of the country and has a rainy season of 3 to 4 months with average rainfalls ranging between 400 mm and 600 mm. The Sudanian region corresponds to the Southern part of the country. In this region, the rainy season extends beyond 5 months with average rainfalls above 1,000 mm. The sudano-Sahelian region lies between the above-mentioned regions with a rainy season of 3 to 4 months and average rainfalls between 600 mm and 1,000 mm.
The three main rivers are Nakambe, Nazinon, and Mouhoun [1].
Burkina Faso is a low-income country, with 55.3 % of its population living with less than $ 1.90 a day in 2011. In 2018, the country was ranked in the 182nd position out of 189 countries, according to the human development index [7]. Three inhabitants out of four are farmers [8].
In 2018, the public health system accounted for 1955 health and social promotion centers, 167 medical centers with or without surgical capacity, 172 single dispensaries, 9 single maternities 70 health districts, 8 regional hospitals, and 6 national teaching hospitals [5]. The health and social promotion center is the most peripheral health facility. It comprises at least a dispensary, maternity, an immunization service, and a pharmacy. The system has a pyramidal structure with three levels in which technical and administrative aspects overlap (Figure 11). From an administrative view, the top of the pyramid corresponds to the central level that includes the office of the minister of health, and the secretariat general. The health policy is defined and its implementation is coordinated at this level. It ensures also global data processing. The intermediate level corresponds to the 13 regional health directorates. This level implements the national health policy at the regional level and supervises the activities of the health districts. These later correspond to the bottom and the first level of the pyramid.
Technically, the district is the operational component of the health system. It consists of peripheral health centers called “centres de santé et de promotion sociale (health and social promotion centers)”, medical centers, and the district hospital with surgery capacity(center medical avec Antenne chirurgicale). The peripheral health facilities and medical centers are the gateways to the system. They carry out inclusive health services [1].
Patients’ reference system includes successively the district hospitals, the regional hospitals, and the national teaching hospitals.

The health personnel is insufficient with 0.09 physician/1 000 inhabitants, and 0.9 nurses or midwife/ 1 000 inhabitants, in 2017 [9]. In the same year, these indicators were 3.1 physician/1 000 inhabitants and about 19 nurses or midwives/1 000 inhabitants in Belgium [10]. The health system share of the total budget of Burkina Faso was 11 % in 2018 [11].

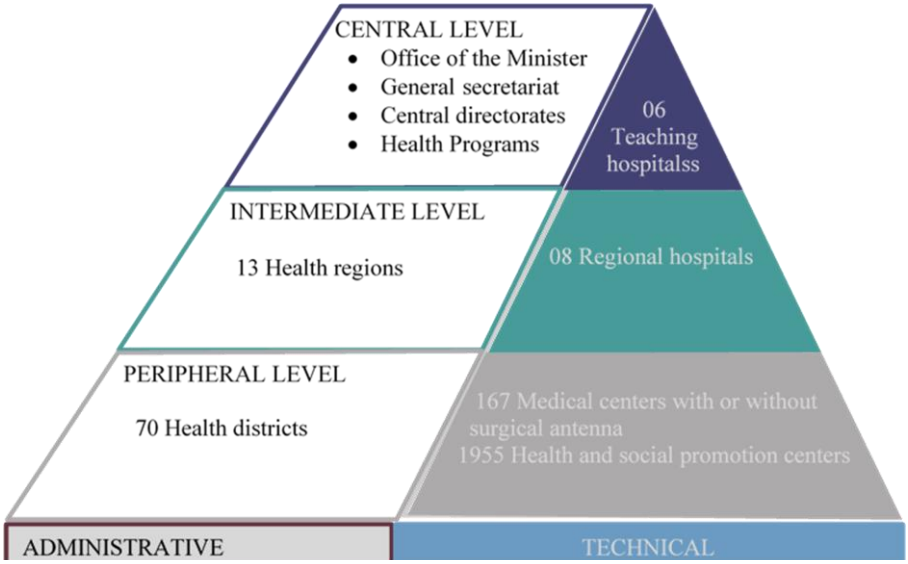

Figure 11 Administrative and technical organisation of the health system of Burkina Faso. *Adapted from Plan strategique national de lutte contre le paludisme 2016 – 2020, Ministry of Health Burkina Faso)*

The private sector, the communities, and traditional healers contribute also to the health care provision.
Most of the 533 private health facilities are located in the two main urban cities of the country, Ouagadougou, and Bobo-Dioulasso.
In 2018, the community-based sector accounted for 239 community-based associations, 6,084 community health workers, and Non-Governmental Organizations. They provide basic health care to communities, including the management of cases of uncomplicated malaria. They are also involved in health promotion and in social and resource mobilization to ensure continuity and accessibility of health care.
Traditional medicine is recognized as a health care provider in Burkina Faso. A dedicated technical direction of the ministry of health supervises the activities of traditional healers. They are also trained to identified symptoms of severe malaria and to refer cases to the health centers [1, 5].

The nosological profile of the country was dominated by malaria that accounted for 41 % of external consultations at the peripheral health centers, followed by acute respiratory tract infections (27 %) and infected wounds (6 %) in 2018. Severe malaria (21%), anemia (6 %), and pneumonia(4 %) were the three main reasons leading to hospitalization in medical centers and hospitals. In the health districts, the leading causes of death were severe malaria (16 %), neonatal infection(7 %), and prematurity(6 %) [5]. In the same year, 80 % of births were attended by skilled health staff [3]. This was maybe due to the increase in the health personnel, and the number of private and public health facilities [11]. Also, the coverage of first and second antenatal visits were around 80 % and 70 %, respectively. Since 2016, pregnant women and children under five benefit from free of charge health care.

2.2. The burden of malaria in Burkina Faso

In 2018, malaria continued to be the leading motive for consulting, hospital stay, and death in the public health centers of Burkina Faso[5]. The country shared 7 % of the total estimated malaria cases worldwide, and 3 % of estimated deaths attributable to malaria [12]. A total of 11,463,808 cases were reported. About 7,474 deaths were notified among the 721,174 people presenting with severe malaria to the health facilities [11].

Three malaria parasites are responsible for the infections occurring in Burkina Faso. *Plasmodium falciparum* is retrieved in 90 % of infections. Few cases are attributable to *Plasmodium malariae* and *Plasmodium ovale* [1].

Malaria vectors are *Anopheles gambiae ss*, *Anopheles coluzzii*, *Anopheles arabiensis*, and *Anopheles funestus* [13, 14].

Malaria is endemic with and the number of cases raises during the rainy season. Transmission patterns can be superimposed on the climatic regions of the country (Figure 12). In the South-West where the climate is Sudanian-type, malaria is transmitted throughout the year. The transmission duration is four to six months in the sudano-Sahelian region corresponding to the central part of the country. In the North, the transmission period is shorter (two to three months). An estimated average annual entomological inoculation rate of 100.6 was reported for *P.falciparum* over 25 years [15]. The number of infectious bites/person /day reaches 2.35 during the rainy season but drop to 0.03 – 0.22 during the dry season. [13]. About 60 % of the infections occur between July and November [1].

There is a substantial drop in the malaria mortality rate between 2015 and 2018. This put the country on the road to attain the objective of the WHO

global technical strategy of a 40 % reduction of malaria mortality rate by 2020. In contrast, malaria incidence stagnates over the same period, and the 40 % reduction objective is unlikely to be reached by 2020 [12].
Malaria affects pregnant women and children aged less than 5 years most [1].

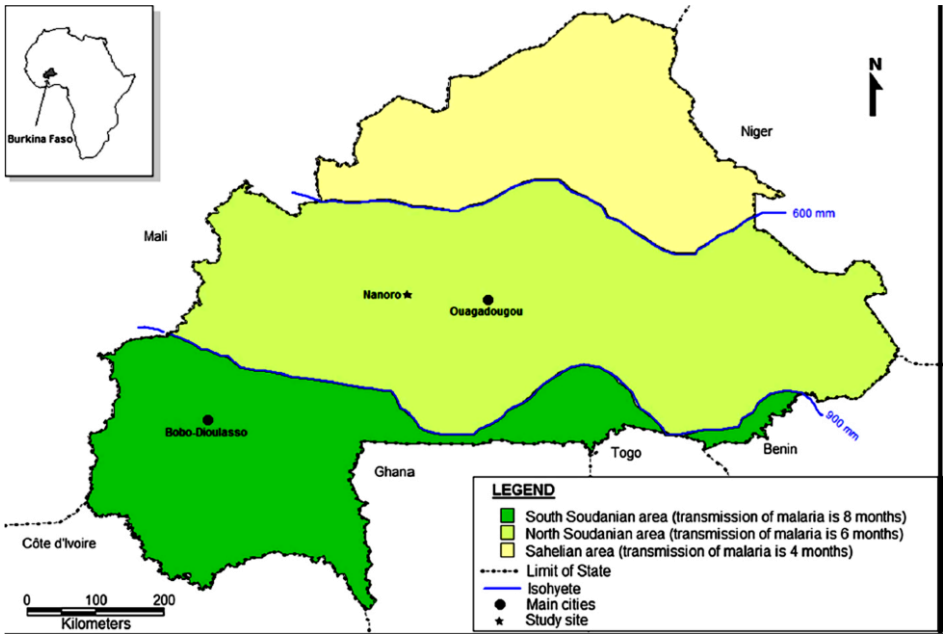

Figure 12 : Strata of malaria transmission in Burkina Faso *Source Centre Muraz, 2010*

Malaria control pillars

The National Malaria Control Program (NMCP) created in 1991 coordinates the fight against malaria according to WHO recommendations. Its activities are focused on early detection and management of cases in health care centers and the community, preventing malaria in pregnancy by intermittent treatment, provision and promotion of the usage of treated bed nets, and indoor residual spray.

- Detection and management of cases in health care centers and community

The WHO recommends testing every presumed case of malaria before treating it [16]. Malaria rapid diagnostic tests (RDTs) were made available either at the health facilities or in the community through the community health workers [11, 12]. In 2018, 13,026,870 RDTs were distributed.[12] The

percentage of cases confirmed by microscopy or RDTs was 88.9 % with 77.7 % positivity [11]. The pharmaceutical sector was not part of the RDTs distribution network. This is a missed opportunity to increase the case confirmation rate as some patients seek care there. Indeed, between December 2014 and June 2016, 6738 suspected cases of malaria were reported by 45 pharmacies out of 191 in the two main cities of Burkina Faso. However, less than 6 % of these suspected cases received confirmation either at the pharmacy or elsewhere [17].

Malaria treatment was changed in 2005. Artemisinin-based combination therapies replaced Chloroquine because of the confirmed resistance of parasites to this drug. Nowadays, the malaria treatment policy is based on Artesunate-Amodiaquine and Artemether-Lumefantrine for treating uncomplicated cases. Severe malaria was treated with quinine, artesunate, or artemether, all through injection. Treatment containing an artemisinin derivative was not allowed in the first trimester of pregnancy. More than 10 million treatment courses including ACTs, injectable -artesunate, and artemether were distributed in the health facilities and the communities in 2018 [11].

- Intermittent preventive treatment

Sulfadoxine-pyrimethamine (SP) is the drug chosen by the NMCP for the prevention of malaria during pregnancy. In Burkina Faso, SP remains efficacious with a small prevalence (11 %) of the triple mutation of the gene of the parasite associated with resistance in 2015 [18]. In 2014, the number of doses of SP was scaled-up from 2 to a minimum of three according to WHO recommendation. A minimum of one month shall be observed between two consecutive doses [1]. The proportion of women who took three doses or more of SP in Burkina Faso in 2017 and 2018 passed 50 % [8, 12].

As SP is delivered as part of the antenatal care package, its coverage and efficacy are highly dependent on the number and timing of antenatal visits. In Burkina Faso, most of the women start their antenatal visits late [19], and sometimes, 44 % of them did the four recommended visits [20]. Therefore, they are exposed to malaria infection during the first part of their pregnancy [21].

- Bed net use and indoor residual spray

Bed net use and indoor residual spray are two strategies for vector control. Insecticidal treated nets (ITNs) are made available free of charge to the population during mass campaigns distribution (84 %) and at antenatal care visits (7 %) [8]. The number of long-lasting insecticidal treated bed nets(LLINs) distributed between 2016 and 2018 reached 2 million. In the

general population, three households out of four had at least one ITN but only 44 % used it [12]. Among pregnant women, 74 % lived in households with at least one ITN, and 57.7 % slept under an ITN the night preceding the survey [8].

Since 2009 residual spraying started in the South-West district of Diébougou [8]. However, this strategy is not widely implemented [12].

- Seasonal Malaria Chemoprevention

Chemoprophylaxis against malaria in under-fives has been carried out between 1972 – 1974, using chloroquine. This strategy has been abandoned because of the resistance of malaria parasites to the precited drug. In 2014, Burkina Faso adopted the seasonal malaria chemoprevention (SMC) as recommended by the WHO. This new tool combines two antimalarials sulfadoxine-pyrimethamine plus amodiaquine and was first set up in seven health districts. One year later, it was extended to ten others [1]. Nowadays, SMC is fully operational across the country with coverage reaching 100 % of the targeted population at each of the four passages [11] conducted during the high transmission season (June to October).

- The financial and technical partners of the malaria program

The National Malaria Control Program receives both financial and technical support from African and international partners. Collaborators from Africa include the West African Economic and Monetary Union, the Alliance for International Medical Action, and non-governmental organizations (NGOs) Help, and Terre des Hommes. International partners outside Africa are the Malaria Consortium, United States Agency for International Development, the Global Fund, the World Bank, and the United Nations International Children Emergency Fund.

Some partners support only technical activities for malaria control. At the national level, the NMCP collaborates locally with the health and family direction for the control and management of malaria in pregnancy. Also, four local research centers carrying out operational research on malaria provide evidence and guidance on which NMCP can base its decisions. Technical partners in Africa are the West African Health Organization (WAHO), the NGOs Plan Burkina, and Croix-Rouge. The international organizations involved are the WHO, the Roll Back Malaria partnership, Rotary, and Italian cooperation [1, 12].

2.3. Prevalence of and factors associated with malaria in pregnancy in Burkina Faso.

Peripheral infection

Malaria remained highly prevalent in pregnant women in Burkina Faso. In 2018, 534,905 cases were reported, including 31,996 with severe malaria and 7 deaths [11]. Incidence of uncomplicated cases varied over time. In 2012 a monthly incidence rate of 39.2 per 1,000 women was reported [22]. From 2015 to 2017, the estimated crude annual cumulative incidence per 1,000 pregnant women increased from 310 to 567 [23]. Contrary, the incidence of severe malaria was constant from 2013 to 2018. The fatality rate of severe malaria decreases substantially during the same period [24].

Factors associated with peripheral infection in pregnant women

Several factors influence the distribution of malaria in pregnancy (MiP). The number of uncomplicated and severe cases culminates during the rainy season [23-25]. There is also a spatial variation in the relative risk of MiP. Communities situated in the regions of Center-West, Center-East, Cascades (South), East, and South-West were the most at risk of uncomplicated malaria. Those in the regions of Mouhoun (in the western part of the country), Hauts-Bassins neighboring the South-West, and the Sahel (in the far North) presented the lowest risk of uncomplicated cases but the highest risk of death from severe MiP. Pregnant women in health districts which were insufficiently prepared to prevent and manage malaria during pregnancy were exposed to an increased risk of death from severe malaria [23, 24]. Rural residence was also associated with an increased risk of MiP [26].
At the individual level, the use of IPTp-SP reduced the risk of MiP. The protective effect was stronger as the number of doses increases [22, 25, 26]. Adequate coverage with three doses was reported to be associated with a decreased fatality rate of severe MiP [24]. Malaria in pregnancy is more prevalent in adolescent pregnant women than in adults [19]. Women pregnant or giving birth for the first or second time were also more prone to MiP compared to those with higher gravidity or parity [22, 26-28]. Low educational level is also a risk factor of MiP [27].
Maternal infection may result in anemia [19, 27]. Low birth weight can occur as the result of infection occurring in the last two trimesters of the pregnancy [22].

Placental malaria

There is no nationwide data for placental malaria (PM) as for the peripheral infection. Most of the data on placental malaria were collected in the framework of clinical trials or epidemiological studies. Therefore, the prevalence varied from one study to another, probably influenced by study-design, settings, and the diagnostic tool used.
A study conducted in 2004 at the health district of Koupela using microscopic examination of placental blood smears reported a prevalence of parasitemia of 15.9 % [26]. Between 2004 and 2006, the prevalence of parasitemia using the same diagnostic method was (15.6%) at the health district of Boussé and 22.7 % at the health district of Boromo [28, 29]. Lower prevalence (7.1 %) was found at Saint Camille medical center of Ouagadougou between 2013 – 2014 [30]. Studies using a histological examination of placental biopsies were carried out at the health district of Nanoro from 2009 to 2015. They found a prevalence of placental malaria of any type of at least 50 % [25, 31]. Past or chronic placental malaria (presence of malaria pigment with or without parasites) was the most encountered pattern [25].
Placental infection was associated with peripheral infection. Indeed, 81.4 % of pregnant women with placental infection presented with a peripheral infection while this proportion was only 14.4 % among those without placental infection [32].

Factors associated with placental malaria

The prevalence of placental malaria is influenced by the season and residence. A higher incidence is reported in the rainy season and rural areas than in the dry season and urban cities, respectively. The infection is also more prevalent in primigravidae and secundigravidae women, and in women who did not attend school [25, 26]. The use of IPTp-SP is protective, with a dose-dependent effect [25].
Placental malaria is linked to low birth weight and preterm birth [31, 32].

Low birth weight and prematurity in malaria in pregnancy studies

Infant death in the first year of life is largely due to low birth weight and preterm birth complications [33, 34]. A study analyzing nation representative data reported a prevalence of low birth weight of 13.4 % in Burkina Faso, regardless of whether the mothers were infected by malaria during their pregnancy. There are spatial disparities with mothers from rural areas being

more exposed than those of urban areas. Also, the risk of giving birth to a low birth weight neonate was higher in young women, women of low parity, and underweighted women [35].
Most of the studies on malaria in pregnancy assessed low birth weight and prematurity as poor fetal outcomes. However, the estimation of their prevalence included both newborns from mothers with and without malaria. Low birth weight was found in 11 % to 36.5 % of newborns, while 2.06 % to 50 % born prematurely [25, 31, 36]. Pregnant women infected in their first or second trimester were more prone to deliver low birth weight babies [22]. Other maternal factors potentially leading to the delivery of a low birth weight infant regardless of the malaria infection status included adolescence, first pregnancy, underweight, no use of bed net [25, 31, 36]. This risk decreased in mothers using IPTp-SP, with a dose-dependent effect [25]. Newborns of the female gender were found to be more prone to low birth weight [36]. Preterm birth was associated with congenital malaria in a study conducted in a tertiary hospital over 10 years [37]. Nulliparous women supplemented weekly with iron presented an increased risk of delivering prematurely [31]. Both low birth weight and preterm birth were influenced by season and picked during the rainy season [31, 36].

2.4. Ultrasound assessment of fetal biometry in malaria in pregnancy studies.

In Burkina Faso, few obstetrical ultrasound scans are performed routinely in public health facilities [11]. Fetal biometry was assessed in some malaria in pregnancy studies. The aim of these examinations was mostly to provide more reliable and accurate gestational age [31, 38-40]. The crown-rump length was measured to exclude women in their first trimester of pregnancy [31]. Biparietal diameter, head circumference, abdominal circumference, and femur length were used for pregnancy dating in the second or third trimesters [31, 38, 39]. Besides gestational age estimation, obstetrical ultrasound scanning aimed also at numbering the fetuses, locating the pregnancy, and confirming fetal viability [38].
Most of the studies did not report on the fetal biometry charts on which they based their estimations [31, 38-40]. Probably, they referred to the chart built-in the ultrasound device instead of a local chart [41]. As the equipment was not described in some studies, the identification of the chart used was not possible in all cases [22, 38].

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CHAPTER 3: STUDY RATIONALE AND OBJECTIVES

3.1. The rationale of the study

After a rapid decrease in malaria morbidity and mortality from 2010 to 2014, a stagnating phase followed since 2014. The concern remains in sub-Saharan Africa that bears the heaviest part of the global burden of malaria [1, 2]. Progress toward malaria elimination is minimal in this subregion [3-5]. Pregnant women are particularly affected by malaria in these settings [6, 7]. Moreover, placental infection occurs when parasites sequestrate in the placenta damaging this organ [8-10] and causing harm to the mother and her fetus [8].

There is a need for a rapid improvement of malaria control in sub-Saharan African countries before the spreading resistance of vectors and parasites shrinks the potential of current insecticides and antimalarials [1, 2]. As a response to this concern, the WHO and its partners set up an initiative called "from high burden to high impact". This plan aims at reducing the morbidity and mortality of malaria in the ten countries of this subregion with the highest burden of malaria, and India. One of the key elements of this approach is to depart from the "one-size-fits-all" practice and use strategically quality data for targeting and implementing malaria control interventions [11]. This of particular interest in pregnancy as the risk of MIP and its adverse birth outcomes are age-dependent and parity-specific [7, 12, 13]. Therefore, the identification of those at the highest risk will allow tailored control interventions for optimal impact [2, 14]. This information can be refined by exploring the impact of the interplay of age with gravidity and other maternal factors in their association with placental malaria and with LBW or PTB.

Another challenge to meet for accelerating malaria control and elimination in sub-Saharan Africa is to identify the hidden reservoir of parasites that sustain the transmission [14]. Pregnant women are regarded as "silent sustainers" of malaria in endemic regions. Indeed, infections are asymptomatic most of the time. Parasites sequestrated in the placenta causing parasitemia to be undetectable in the peripheral blood with routine diagnostic tools. Also, pregnant women carry gametocytes the sexual stage of the parasite that infects the mosquito [15-17]. Therefore, some authors suggest using pregnant women as a complement for malaria transmission monitoring. Their opinion is reinforced by the strong correlation between the prevalence of malaria found in children in household surveys and the prevalence in pregnant women. However, the prevalence in primigravidae was closer to that of the children than the prevalence in multigravidae [6, 18, 19]. This demonstrates that parity-specific immunity influences this relationship and should be taken into account. Age-related immunity contributes also to regulating malaria

infection during pregnancy [7]. Therefore it should be considered alongside parity-specific immunity for a better assessment of the correlation between the prevalence in pregnant women and that in children.

Adverse fetal outcomes related to MiP are objectivated with the use of ultrasound that allows fetal biometry measurement and comparison to reference curves [8, 20-22]. However, the accuracy of this evaluation depends on the choice of the reference chart [23, 24]. In low and middle-income countries, several charts from Caucasian populations or preinstalled in the ultrasound device software are used within the same country without official recommendations or institutional guidelines [25]. This may yield conflicting results in studies assessing the adverse birth outcomes related to MiP and their associated risk factors. Confusion could also occur when translating these results into public health actions [23, 24, 26].

Burkina Faso is among the 11 HBHI countries that together shared 70 % of the global burden of malaria in 2018 [1, 11].

Both peripheral and placental malaria is highly prevalent within the country [20, 27, 28]. Recent national representative studies revealed an increase in the cumulative incidence of MiP between 2015 and 2017 [27]. This means an increase in the likelihood of placental infection. Low birth weight and preterm birth were also reported to be linked to both peripheral and placental malaria [28, 29]. To our knowledge, there is no official nor an institutional recommendation for the choice of reference chart for fetal biometry assessment using ultrasound.

The National Malaria Control Program needs information evidencing those with the highest risk of placental malaria and its adverse birth outcomes among pregnant women to tailor and improve its actions toward this population. Also, it should be aware that the amplitude of adverse fetal outcomes attributable to MiP and their associated factors might be influenced by the reference chart used for ultrasound assessment of the fetal biometry measurements.

The conceptual framework of our study is presented in figure 13.

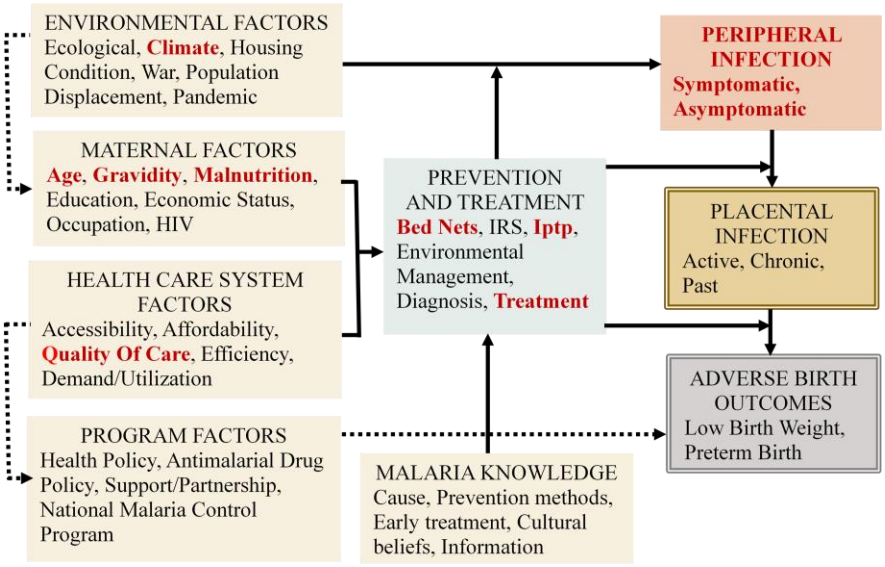

Figure 13: Conceptual framework of the factors associated with placental malaria and adverse birth outcomes. *Factors included in this study are in red bold font. Solid arrows represent direct relationship and dashed arrows represent indirect relationship. (Adapted from https://www.measuremalaria.org/wp-content/uploads/2020/02/ms-20-184-1.pdf*

3.2. Objectives of the study.

3.2.1. General objective

Contribute to improving the control of malaria in pregnancy through adequate identification of high-risk groups, potential targets of programs specific actions, or strategies

3.2.2. Specific objectives

To determine the prevalence of placental malaria in Burkina Faso and to assess whether its associated factors are varying with age.

To determine the low birth weight or prematurity prevalence in newborns from mothers infected with malaria in rural Burkina Faso and its burden on adolescents

To accurately estimate fetal biometric measurements in pregnant women with malaria in rural Burkina Faso for gestational age and abnormal fetal size determination

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CHAPTER 4: METHODS

4.1. Study settings

The PREGACT study was conducted at the clinical research unit of Nanoro (CRUN) in the health district of Nanoro (HDN). The study participants were recruited at the peripheral health centers of Nanoro and Nazoanga (Figure 14).

Nanoro is a rural health district belonging to the Central West region. It is distant from the capital town Ouagadougou by about 90 km.

Approximately, 158 127 inhabitants lived within the district area in 2013 [Data from the National Institute of Statistics and Demography].

Health care is provided in 18 primary health centers. Patients needing further investigations or specialized interventions are referred to the district hospital called "Centre Medical avec Antenne Chirurgicale". The latter is co-operated by the Italian religious order of Saint Camille and the government. Each peripheral health center has maternity, a dispensary, and an immunization program. The district hospital comprises eight departments: surgery, internal medicine, pediatrics, pharmacy, maternity, dentistry, nutrition, outpatient service, and laboratory services.

The CRUN is installed within the district hospital. Its activities are carried out within its demographic surveillance area accounting for about 60,000 inhabitants in 24 villages. Seven peripheral health facilities and the referral hospital were located within this demographic surveillance area (DSA). All research participants are identified, selected, and followed-up through this system [1]

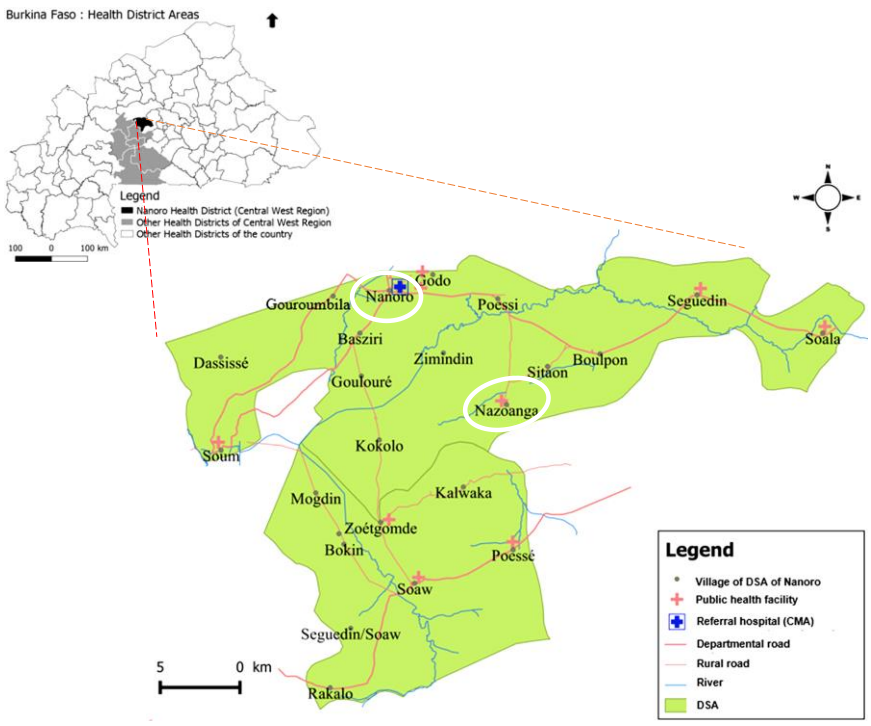

Figure 14: Demographic surveillance area of Nanoro. *Source: Burkina Faso, Base Nationale de Découpage du Territoire (BNDT, 2006) ; shapefile downloaded from www.maplibrary.org. Created by Eli Rouamba, 2018*

Three ethnic groups populated the DSA: Mossi (90 %), Gurunsi (7.9%), and Fulani(1.7 %). In 2010, the male/female ratio was 77/100. This ratio is explained by a higher out-migration of men aged 20 – 40 years than women of the same age, mainly to Côte d'Ivoire. The population mostly young is representative of rural communities in low and middle-income countries. The birth rate is high with a total fertility rate of 5.6.

Overall, 3 out of 4 inhabitants had a low education level. The main activities were subsistence agriculture and cattle keeping. The climate is sudano-Sahelian-type with rains starting in June and ending in October. Average, rainfalls are between 450 mm/year and 700 mm/ year. From November to May the weather becomes more and more dry and hot. Temperature ranges from 17°C in December to 43°C in April [1].

Malaria incidence is high in the DSA. During the period from 2010 to 2014, the annual incidence of malaria ranged from 559/1000 inhabitants to 646/1000 inhabitants in the health district of Nanoro [2]. Pregnant women are particularly vulnerable. A study conducted at the district hospital in 2012

reported a prevalence of malaria infection during pregnancy of 42.6 % [3]. Malaria transmission is seasonal. The number of cases culminated during the rainy season, from June to November, followed by an intermediary incidence phase from November to March. This intermediary season is characterized by the absence of rainfalls. However, persisting bodies of water, favorable temperature, humidity, and post-season farming activities, enable mosquito and larval survival and thus, malaria transmission. The low transmission period lengths from April to June [2].

4.2. Study design

The study was a secondary analysis of the data collected at Nanoro and Nazoanga, the two sites of Burkina Faso participating in the PREGACT multicentric open-label randomized non-inferiority clinical trial (trial registration number NCT00852423). Seven sites in Africa carried out the activities of the PREGACT trial including Nanoro and Nazoanga in Burkina Faso. This trial assessed the safety and efficacy of four drugs against malaria in pregnancy: Artemether-Lumefantrine(AL), Artesunate-amodiaquine(ASAQ), Dihydroartemisinin-Piperaquin (DHAPQ), and Mefloquine-Artesunate(MQAS). Each site evaluated three out of the four treatments. The treatments tested at Burkina Faso sites were ASAQ, AL, and MQAS [4].
The present study followed prospectively a cohort of women with singleton pregnancies from their second or third trimester till delivery. It assessed also the newborns.

4.3. Study participants

Study participants were pregnant women aged 15 – 49 years included in the PREGACT trial and their newborns. A complete list of the inclusion/exclusion criteria in the main trial was provided in table 1 below

Table 1 Inclusion and exclusion criteria of the main trial

Inclusion criteria	
	Pregnancy between 16 and 37 weeks gestation; Infection by *P.falciparum* regardless of the density or presence of symptoms; Hb ≥ 7g/dL; Fifteen years old minimum; Living within the health facility area; Desiring to deliver at the health center; Desiring to comply with the requirements of the study; Aptitude to give informed consent.
Exclusion criteria	
	Previous allergy to the study drugs; Known previous complicated pregnancies or poor obstetrical history such as recurrent eclampsia or stillbirths; Previous or current major sickness with potential to affect the outcome of pregnancy including heart disease diabetes mellitus, severe renal illness, or active tuberculosis; Ongoing treatment with antiretrovirals or prophylaxis using cotrimoxazole; Severe malaria or major illness needing hospitalization at the time of screening; Willing to move outside the study zone before giving birth or delivery at a parent's residence outside the study area. Previous participation in the study or concomitant enrollment in another study. Inability to take oral medication Known recent (1 week) antimalarial treatment or antimicrobials with antimalarial activity (clindamycin, azithromycin, etc).

Source :Protocol ITMP0308-NCT00852423 Amendement 3, version1.0, 17 January 2011

4.4. Follow-up of the participants and data collection

The participants received study treatments at random. After the three days of treatment, they were followed -up actively for 63 days with regular visits every week. Afterward, participants visited the antenatal clinic once a month till delivery. However, they could attend the study clinic each time they feel sick. At each visit, the study medical personnel recorded the medical history, checked the presence of signs and symptoms, and took the body temperature. It carried out thick and thin blood smears and measured Hb level weekly from days 7 to 28 and on day 63. Bilirubinemia, creatinine, and alanine aminotransferase (ALAT) were performed on days 7 and 14. At delivery, the pregnancy outcome was noted. The newborn was weighted and checked for congenital abnormalities. Maternal Hb was measured and placenta samples were taken.
The mother and the infant were seen between 4 and 6 weeks and one year after delivery for assessing whether adverse events occurred. [4].
Data were collected in individual source documents and transferred to an electronic case report form (e-CRF) developed using MACRO (Infermed, UK).

4.5. Malaria diagnosis

Light microscopy was used to diagnose peripheral malaria. Thick and thin blood smears were prepared using blood drawn by finger puncture. A 3 % Giemsa solution was used to stain the blood smear for 30mn. Parasitemia was determined based on the number of trophozoites counted per 200 leucocytes or 500 leucocytes in the case where the number of trophozoites was <10/200 leucocytes, assuming a count of leucocytes of 8000/µl.
When 100 fields were examined and asexual parasites were not found the result was considered negative [4]. Two independent microscopists read each slide. When the first readings mismatched on the presence of parasites, the species of the parasites, or when the difference between the densities of the parasites overpassed 50 % a third distinct reading was done. The final parasitemia was the average of the two most close densities between the three readings [4, 5].
After delivery, biopsies were collected from the maternal face of the placenta for subsequent diagnosis of malaria diagnosis using the histopathological examination. These samples were conserved in 10 % neutral buffered formalin and incorporated in paraffin wax. They were maintained at 4°C awaiting histological assessment. Sections of the paraffin were 4 mm thick.

Staining was performed using hematoxylin-eosin [4]. Histopathological classification performed by Ismail et al was used to define placental malaria patterns. Acute infection was defined in the presence of parasites and the absence of malaria pigment. Chronic infection corresponded to the presence of both parasite and malaria pigment. In cases with past infection, malaria pigment is present but parasites are absent. The absence of both parasite and malaria pigment was considered as negative [6].

4.6. Ultrasound procedures

Each pregnant woman benefited from an obstetrical ultrasound assessment at inclusion in the main study. The ultrasound device used was the portable FFsonic UF-4100 from Fukuda Denshi©. Standard scanning was carried out using a 3.5 MHz. For thinner women, a 5.0 MHz probe was used. The four fetal biometric parameters measured were abdominal circumference(AC), biparietal diameter (BPD), femur length(FL), and head circumference (HC).
The measurements of HC and BPD were done in a transversal view of the fetal head. The cavum septi pellucidi discontinued the midline echo constituted by the fax cerebri at the anterior third of its length. The thalami were symmetrical at each side of the midline. The biparietal diameter was measured from the external table of one parietal to the internal table of the other parietal. The ellipse was placed around the external border of the skull to measure the HC. The abdominal circumference was measured in a transverse section showing the stomach bubble together with the anterior third of the umbilical vein. The bladder and the kidneys were not visualized. The ellipse tool was placed on the external border of the abdomen. Femur length was calculated when the screen entirely displayed the diaphysis. The calipers were then positioned at the external borders of the femoral bone extremities [7, 8].
The Hadlock formula integrated into the ultrasound device software was used to calculate the gestational age in complete weeks from the four-parameter [9]. Women in their first trimester of pregnancy were excluded based on gestational age calculated from crown-rump length.
Two physicians were committed to the obstetrical ultrasound examination during all the study period according to the quality assurance and quality control system. They were centrally trained before the study beginning and periodically retrained on-site by skilled obstetricians. Ultrasound measurements were performed following standard operating procedures. Besides, every first week of the month, the second committed clinician carried out internal quality control by repeating the measurements. Also,

every third week of the month, the measurements were reiterate by the second clinician on one participant [7]. The procedures are summarized in table 2.

Table 2: Summary of the procedures in our study

Day / **Procedure**	0	1	2	3	7- 63	Unscheduled	Delivery
Medical history	x						
Informed consent	x						
Clinical assessment	x						x
Viability of the fetus	x			x	x	x	
Ultrasound scan	x						
Blood smears	x	x	x	x	x	x	x
Hémoglobin	x						
Treatment	x	x	x				
Placental sampling							x

Source :Protocol ITMP0308-NCT00852423 Amendement 3, version1.0, 17 January 2011

4.7. Sample size

A simulation was used to determine the sample size for the PREGACT clinical trial. A mean PCR-adjusted cure rate of 95 % at day 63 was chosen as recommended by the WHO. Boundaries of the 95 % confidence interval for the difference in cure rates were assumed to be within 5 %. The power was set to 95 % for each of the six in pairs comparisons, and 80 % for the overall assumption that therapeutic efficacy would be identical for all of the four treatments. The sample size was increased to a total of 3480 participants taking into account a 20 % loss to follow-up. This corresponds to 870 participants for each treatment and 290 participants per treatment arm in each country [4, 5]. In Burkina Faso three treatment arms were evaluated, therefore, 870 participants were recruited.

4.8. Ethical considerations

The ethical committee of the University of Antwerp in Belgium, the national or local ethics committees, and the relevant national regulatory authorities approved the PREGACT study. Written or thumb-printed informed consent was provided by all participants before inclusion in the study [4, 5].

4.9. Statistical analysis

For all papers, normally distributed quantitative variables were summarized using the mean followed by the standard deviation. The median and interquartile range or the range (minimum and maximum) described not normally distributed quantitative data. The proportion was used to present qualitative variables. The comparison of two independent means was based on the t-test of Student's while a Wilcoxon signed-rank test was used to compare paired means when their differences were not gaussian. The first paper and the second paper analyzed the effect of age on the association between maternal factors and malaria in pregnancy-related outcomes namely placental malaria and low birth weight or prematurity. In both analyses, the multivariable logistic regression models included interactions of age and other maternal factors. For the first paper, variables that were not significantly associated with placental malaria in bivariate or multivariable analysis were not included in the final models. The odds ratio of the association of a factor with malaria in pregnancy and the corresponding 95 % confidence interval was computed by age category when the interaction of this factor with age was statistically significant.

The patterns of the evaluation of fetal biometric measurements using the equations of Salomon were compared to those yielded by the equations of the Intergrowth 21st study in the third paper. Individual z scores were computed for abdominal circumference, biparietal diameter, femur length, and head circumference from the two sets of equations separately and used for subsequent comparisons. The linear relationship between the two sets of equations was assessed by linear regression. Agreement between the two charts was analyzed using the method of Bland and Altman, by graphing individuals scores averages on the horizontal axis, while their differences were reported on the vertical axis. The formula used for the calculation of the limits of agreement was: mean ± 1.96 standard deviations. The pre-specified perfect agreement corresponded to limits of agreement extending from -0.5 to 0.5 as in France [10]. The intraclass correlation coefficient calculated from a random-effect one-way analysis of variance was used to estimate the reliability of the two sets of equations.

The statistical software STATA® version 14 to 16 from Statacorp LLC is used to carry out all the analyses.

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CHAPTER 5: RESULTS

5.1. Result 1: Age-related factors associated with placental malaria

Abstract

Background: Malaria in pregnancy can result in placental infection with fetal implications. This study aimed at assessing placental malaria (PM) prevalence and to assess if factors associated with PM are depending on age.
Method: The data were collected in the framework of a clinical trial on treatments for malaria in pregnant women (the PREGACT study). Placental malaria was diagnosed by histopathological detection of parasites and/or pigment on placenta biopsies taken at delivery. Factors associated with PM were assessed using logistic regression.
Results: Out of 745 biopsies examined, PM was diagnosed in 86.8 % of women. Acute, chronic and past PM were retrieved in 11 (1.5 %), 170 (22.8 %), and 466 (62.6 %) women, respectively. A modifying effect of age was observed in the association of gravidity or anemia at study start with pooled PM. In women under 30, gravidity ≤ 2 was associated with an increased prevalence of pooled PM but in women aged 30 years or more, gravidity was no more associated with pooled PM (OR 6.81, 95 % CI 3.18 – 14.60; and OR 0.52, 95 % CI 0.10 – 2.76, respectively). Anemia was associated with pooled PM in women under 30 (OR 1.96, 95 % CI 1.03 – 3.72) but not in women aged 30 years or more (OR 0.68, 95 % CI 0.31 – 1.49). Similarly, the association of gravidity with past-chronic PM depended also on age. A higher prevalence of active PM was observed in women under 30 presenting with symptomatic malaria (OR 3.79, 95 % CI 1.55 – 9.27), while there was no significant increase in the prevalence of active PM in women with symptomatic malaria when aged 30 years or more (OR 0.42, 95 % CI 0.10 – 1.75). In women with chronic PM, the prevalence of low birth weight or prematurity was the highest (31.2 %) as compared with past PM or no PM.
Conclusion: Placental malaria prevalence was high in women with P. falciparum peripheral infection in Nanoro, especially in younger women with low gravidity, with anemia, or with symptomatic malaria. This underlines the importance of preventive measures and timely diagnostic and treatment of malaria in young pregnant women.

Keywords: pregnancy, placenta, malaria, risk factors, Burkina Faso

5.1. 1. Background

In 2018, 11 million pregnant women were infected by malaria worldwide. According to the World Health Organization, more than one third (35 %) of these infections occurred in the West-African region [1]. Plasmodium falciparum is the parasite responsible for a large majority of cases of malaria [2]. Despite the endemicity of malaria in sub-Saharan African countries, up to 60 % of pregnant women are already infected when seen at their first antenatal care visit. Placental malaria (PM) has been reported up to 40 % at delivery [3]. The mechanism of PM is the binding of infected red blood cells reaching the placenta to chondroitin sulfate A and their accumulation in the intervillous spaces [4]. Placental malaria can result in maternal anemia[5] and in low birth weight (LBW) or preterm delivery [5-7] which can induce neurocognitive and other health disorders later in life [8, 9].
Pregnant women acquire protective immunity against PM as their age increase. That is why younger pregnant women are more prone to PM than their older counterparts [10, 11]. We hypothesized that this immunity may influence the relationship between maternal characteristics and PM. While factors associated with placental malaria have been studied widely, there is little information on the potential interaction between maternal age and other maternal risk factors [7, 12-18]. Few studies assessed factors associated with PM in Burkina Faso where malaria is endemic with seasonal transmission [17, 19]. In a study conducted in Nanoro in 2013, approximately 43 % of pregnant women attending antenatal care visits presented with peripheral parasitemia and therefore were susceptible to PM [20].
The current study aims to assess the prevalence of PM in Burkina Faso and to assess if factors associated with PM are depending on age.

5.1.2 Methods

Study settings and population

The present study used data collected in the framework of a multicenter, randomized, open-label trial of treatments for malaria in pregnant women entitled: “safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria (PREGACT)” which is registered under the number NCT00852423. The latter evaluated the efficacy and safety of four artemisinin-based combination treatments (ACT) in pregnant women

with uncomplicated P. falciparum malaria. Detailed methodology and study results were published elsewhere [21, 22].

Recruitment and follow-up

Briefly, second or third trimester pregnant women with microscopically confirmed P. falciparum peripheral infection, who were willing to give informed consent, were enrolled and treated with one of the four ACTs at random and followed up until delivery. All participants were provided a long-lasting insecticidal bed net upon inclusion.

Data collection

At inclusion, obstetrical, medical, demographic, and anthropometrics, data were recorded. The use of malaria prevention methods before the inclusion in the study was documented. Peripheral malaria infection was confirmed and parasites density and species determined by microscopic examination of thick and thin blood smear stained with Giemsa [21]. Hemoglobin level was determined using a portable HemoCue (Hb301 Hemocue®, Angelholm, Sweden). Gestational age and fetal viability were confirmed ultrasonographically using a portable Fukuda Denshi® machine (FFsonic, UF – 4100).

Peripheral malaria parasitemia occurring after the cure of the initial episode until delivery was recorded.

Delivery date and mode, gestational age, and newborn characteristics were collected. Also, a biopsy of the placenta was collected.

Placental malaria diagnosis

Biopsies pieces were collected from the maternal side of the placenta, the earliest after delivery, and kept in a neutral buffered 10 % formalin. Subsequent procedures comprised embedment in paraffin wax, sliced in 4 mm thick, and staining with hematoxylin-eosin. Upon examination, diagnosis of PM was defined as follows. Acute PM infection was considered in presence of parasites and the absence of malaria pigment. Chronic PM infection was considered in presence of both malaria parasites and pigment. A past PM was considered in absence of malaria parasites but the presence of malaria pigment. No PM infection was considered when there was no malaria parasites nor pigment [23]. Active PM comprised acute or chronic placental infections and represented all placentas with parasites, while past-chronic PM referred to past or chronic infections, corresponding to all placentas with

pigment. Gravidity did not include the present pregnancy. Symptomatic malaria referred to cases where: (1) parasite density was above 2000/mm3 irrespective of symptoms, or (2) a parasitemia regardless of the density but with axillary temperature ≥ 37.5°C at inclusion, or (3) there was a parasitemia regardless of the density but with a minimum of three of the following symptoms: fever in the past 24 hours, muscular- and articular pain, head pain, tiredness or weakness, seizures [22]. Recurrent malaria was defined as the occurrence of a malaria infection after initial treatment and before delivery regardless of the number of episodes. Anemia was defined as a hemoglobin level lower than 11g/dl. Underweight was defined as a pregnancy body mass index < 18.50 kg/m2. Preterm birth was defined as a birth occurring before 37 weeks of gestation and low birth weight was defined as birth weight below 2500 g. The season at delivery was either rainy (July to December) or dry (January to June) [17].

Statistical analysis

The gestational age at delivery was calculated from the estimation done by ultrasonography at inclusion. The association of placental malaria with maternal characteristics was assessed using logistic regression. Only factors associated with PM with p-value < 0.05 in the univariate and multivariate analysis were kept for the final models. Modifying effect of age was assessed by introducing interactions in logistic regression. Interactions of age with the following maternal characteristics were considered: gravidity, anemia, symptomatic malaria at study start, recurrent malaria during follow-up, and season at delivery. When an interaction was statistically significant, the odds ratios of the association of these variables with placental malaria and their 95 % confidence intervals were estimated by age category. Outcome variables were either pooled PM, active PM, or past-chronic PM. Distribution of low birth weight (LBW) and prematurity by placental malaria status was also outlined.

The statistical significance level was set at 0.05. All analyses were performed using

STATA® statistical software version 16 (Statacorp LLC, Texas, USA).

Ethics

The PREGACT study was approved by the ethics committee of the University of Antwerp in Belgium and the National Ethics Committee of Burkina Faso. Written or witnessed thumb-printed informed consent was obtained from all participants before inclusion and any study procedure was undertaken.

5.1.3. Results

Characteristics of study participants.

From June 2010 to April 2013, 870 pregnant women in their second or third trimester were recruited into the PREGACT trial at Nanoro and Nazoanga sites. Among the 847 women who delivered, 779 had a placental histology assessment. After excluding 13 twins and 21 stillbirths or malformations, the remaining cohort included 745 participants (Figure 1).

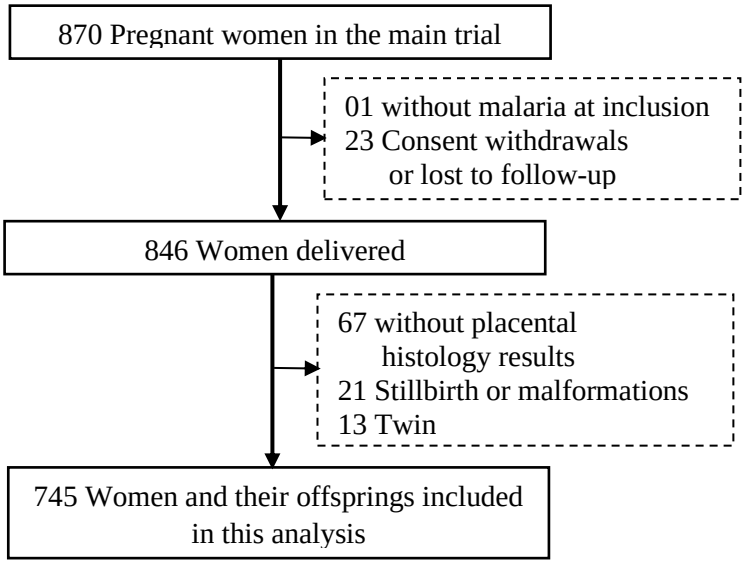

Figure 1: Flow-chart of the pregnant women and their newborns included

At inclusion, 588 (78.9 %) women were under 30 years, 355 (47.7 %) had less than 3 previous pregnancies, 308 (41.4 %) had symptomatic malaria, and 447 (74.2 %) had symptomatic malaria (Table 1).

Table 1 Mother baseline, follow – up and newborn characteristics

Mother baseline	
Age (years)	
median (IQR)	23 (20 – 29)
< 30 years	588 (78.9)
Gravidity	
median(IQR)	3 (1 – 4)
≤ 2*	355 (47.7)
No use of bednet the night before recruitment	370 (49.7)
Did not received IPTp before recruitment	648 (87.0)
Anemia at recruitment (Hb < 11 g/ dl)	553 (74.2)
Underweight at recruitment (BMI< 18.5 kg/m^2)	46 (6.2)
Symptomatic malaria at recruitment*	308 (41.4)
Treatment at random*	
AL	242 (32.5)
ASAQ	255 (34.3)
MQAS	247 (33.2)
Mother follow - up	
Recurrent malaria during follow-up*	289 (38.9)
Delivery in rainy season	447 (60.1)
Newborn	
Girl *	358 (48.1)
Birth weight* (g)	
mean ± sd	2838 ± 439
< 2500g	133 (17.9)
Preterm birth (gestation < 37 weeks)	94 (12.6)
Low birth weight or preterm	175 (23.5)

Number are n (%) unless stated.
IQR: interquartile range; IPTp: intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine; Hb: hemoglobin; BMI: body mass index; AL: artemether-lumefantrine; ASAQ: artesunate-amodiaquine; MQAS: mefloquine – artesunate; sd: standard deviation;
* *Missing data for one participant for gravidity, symptomatic malaria, treatment, newborn gender, birth weight and for two women for recurrent malaria.*

Patterns and distribution of placental malaria

Overall, PM was diagnosed in 647 (86.8 %) women. Parasites were seen in 181 (24.3 %) of the placentas and pigment in 636 (85.4 %). Acute, chronic, and past placental malaria was diagnosed in 11 (1.5 %), 170 (22.8 %) and 466 (62.6 %) women, respectively. (Figure 2).

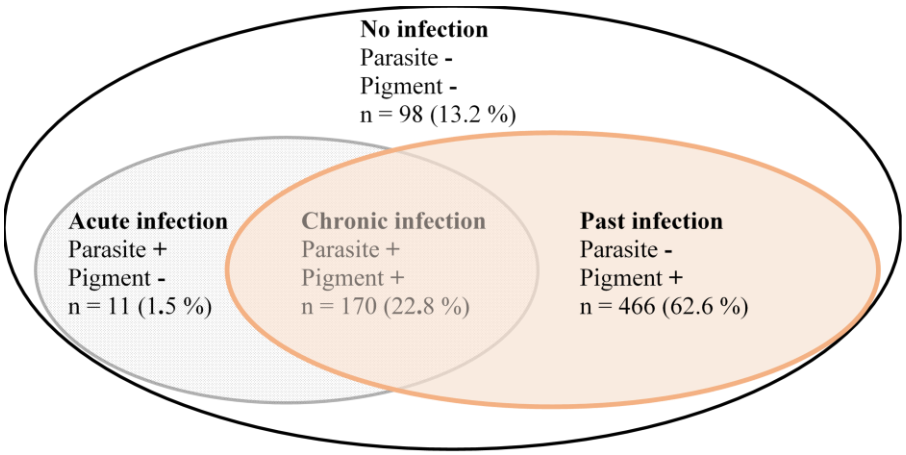

Figure 2 Histopathological diagnosis and distribution of placental malaria. *Note: +: present; -: absent.*

Past infection prevalence was 69.4 % in women delivering in the dry season. Women under 30 years of age experienced more past PM than women of 30 years or over (64.8 % and 54.1 %, respectively). The past PM was more frequent when gravidity ≤ 2 than when gravidity > 2 (65.1% and 60.3%, respectively). Chronic PM distribution followed a trend similar to past PM for maternal age groups (25.9 % in women < 30 years and 11.5 % in women ≥ 30 years) and gravidity groups (31.0 % in women with gravidity ≤ 2 and 15.4 % in women with gravidity > 2). Acute infections were scarce and observed mostly in women of 30 years or more (3.8 %) (Table 2).

Factors associated with placental malaria

In univariate analysis, age < 30 years, gravidity ≤ 2, anemia, symptomatic malaria at inclusion, recurrent malaria during follow-up, and rainy season at delivery were associated with pooled PM (Table 3), with past-chronic PM (Figure 3), and with active PM (Figure 4). Women who did not sleep under a

bed net the night before their enrollment into the main trial had a higher prevalence of active PM than women who slept under a bed net.

Table 2 Placental malaria distribution by maternal factors

Maternal Factors	N	Placental malaria			
		No	Past	Chronic	Acute
Baseline					
Age (years)					
< 30	588	50 (8.50)	381 (64.8)	152 (25.9)	5 (0.9)
≥ 30	157	48 (30.6)	85 (54.1)	18 (11.5)	6 (3.8)
Gravidity					
≤ 2	355	12 (3.4)	231 (65.1)	110 (31.0)	2 (0.6)
> 2	390	86 (22.1)	235 (60.3)	60 (15.4)	9 (2.3)
Bednet use previous night					
No	370	46 (12.4)	215 (58.1)	105 (28.4)	4 (1.1)
Yes	375	52 (13.9)	251 (66.9)	65 (17.3)	7 (1.9)
Received IPTp before study start					
Yes	97	8 (8.2)	68 (70.1)	17 (17.5)	4 (4.1)
No	648	90 (13.9)	398 (61.4)	153 (23.6)	7 (1.1)
Anemia at study start(Hb <11g/dl)					
Yes	553	61 (11.0)	349 (63.1)	137 (24.8)	6 (1.1)
No	192	37 (19.3)	117 (60.9)	33 (17.2)	5 (2.6)

Table 2 Placental malaria distribution by maternal factors (continued)

Maternal Factors	N	Placental malaria			
		No	Past	Chronic	Acute
Baseline (continued)					
Underweight at study start(BMI <18.5kg/m^2)					
Yes	46	3 (6.5)	30 (65.2)	13 (28.3)	0 (0.0)
No	699	95 (13.6)	436 (62.4)	157 (22.5)	11 (1.6)
Symptomatic malaria at study start*					
Yes	308	24 (7.8)	191 (62.0)	91 (29.5)	2 (0.6)
No	436	74 (17.0)	275 (63.1)	78 (17.9)	9 (2.1)
Treatment at random*					
AL	242	29 (12.0)	159 (65.7)	50 (20.7)	4 (1.7)
ASAQ	255	31 (12.2)	161 (63.1)	61 (23.9)	2 (0.8)
MQAS	247	38 (15.4)	146 (59.1)	58 (23.5)	5 (2.0)

Numbers are frequencies (percentages)
IPTp: intermittent preventive treatment in pregnancy; AL: arthemether-lumefantrine; ASAQ: artesunate-amodiaquine; MQAS: mefloquine-artesunate; Hb: hemoglobin; BMI: body mass index.
**One woman with chronic placental malaria missed data for symptomatic malaria, treatment at random and recurrent malaria. Another with past placental malaria missed data for recurrent malaria and delivery season*

Table 2 Placental malaria distribution by maternal factors (continued)

Maternal Factors	**N**	**Placental malaria**			
		No	**Past**	**Chronic**	**Acute**
Follow-up					
Recurrent malaria during follow-up*					
Yes	289	19 (6.6)	172 (59.5)	94 (32.5)	4 (1.4)
No	454	79 (17.4)	293 (64.5)	75 (16.5)	7 (1.5)
Season at delivery*					
Rainy	447	35 (7.8)	259 (57.9)	144 (32.2)	9 (2.0)
Dry	297	63 (21.2)	206 (69.4)	26 (8.8)	2 (0.7)

Numbers are frequencies (percentages)
IPTp: intermittent preventive treatment in pregnancy; AL: arthemether-lumefantrine; ASAQ: artesunate-amodiaquine; MQAS: mefloquine-artesunate; Hb: hemoglobin; BMI: body mass index.
**One woman with chronic placental malaria missed data for symptomatic malaria, treatment at random and recurrent malaria. Another with past placental malaria missed data for recurrent malaria and delivery season*

Table 3 Maternal factors associated with pooled placental malaria in univariate analysis

Factors	**Pooled placental malaria [n / N (%)]**	**OR (95 % CI)**
Age (years)		
< 30	538 / 588 (91.5)	4.74 (3.03 - 7.40)
≥ 30	109 / 157 (69.4)	1
Gravidity*		
≤ 2	343 / 355 (96.6)	8.09 (4.34 - 15.08)
> 2	304 / 390 (77.9)	1
Bednet use previous night		
Not used	324 / 370 (87.6)	1.13 (0.74 – 1.74)
Used	323 / 375 (86.1)	1
Received IPTp before study start		
Yes	89 / 97 (91.8)	1.79 (0.84 - 3.82)
No	558 / 648 (86.1)	1
Anemia at study start (Hb < 11g/dl)		
Yes	492 / 553 (89.0)	1.93 (1.23 - 3.01)
No	155 / 192 (80.7)	1
Underweight at study start (BMI <18.5kg/m^2)		
Yes	43 / 46 (93.5)	2.25 (0.69 - 7.41)
No	604 / 699 (86.4)	1
Symptomatic malaria at study start*		
Yes	284 / 308 (92.2)	2.42 (1.49 - 3.93)
No	362 / 436 (83.0)	1
Treatment at random*		
AL	213 / 242 (88.0)	1.34 (0.79 - 2.25)
ASAQ	224 / 255 (87.8)	1.31 (0.79 - 2.19)
MQAS	209 / 247 (84.6)	1

Table 3 Maternal factors associated with pooled placental malaria in univariate analysis (continued)

Factors	**Pooled placental malaria [n / N (%)]**	**OR (95 % CI)**
Recurrent malaria during follow-up*		
Yes	270 / 289 (93.4)	2.99 (1.77 - 5.06)
No	375 / 454 (82.6)	1
Season at delivery		
Rainy	412 / 447 (92.2)	3.17 (2.03 – 4.94)
Dry	234 / 297 (78.8)	1

OR: odds ratio; CI: confidence interval; IPTp: intermittent preventive treatment in pregnancy; AL: arthemether – lumefantrine; ASAQ: artesunate – amodiaquine; MQAS: mefloquine – artesunate; Hb: hemoglobin; BMI: body mass index.

** missing data for one participant for symptomatic malaria at study start, treatment and for two women for recurrent malaria.*

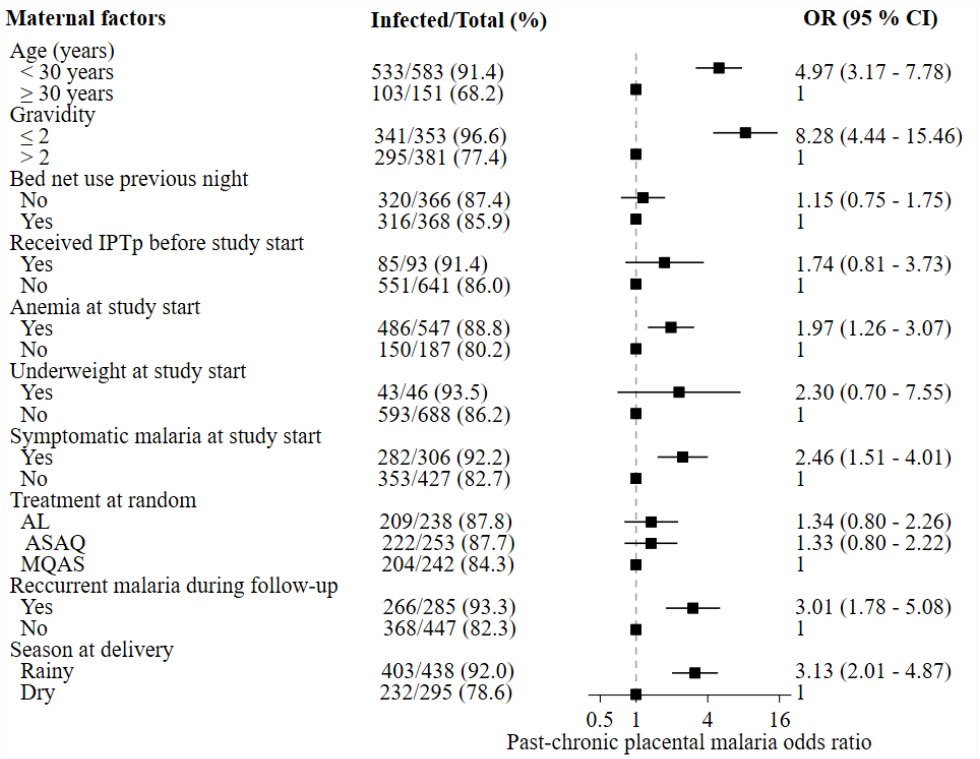

Figure 3 Maternal factors associated with past-chronic placental malaria in univariate analysis

OR: odds ratio; CI: confidence interval; IPTp: intermittent preventive treatment during pregnancy with sulfadoxine - pyrimethamine AL: arthemether - lumefantrine; ASAQ: artesunate - amodiaquineMQAS: mefloquine - artesunate; Anemia: hemoglobin < 11g/dl; Underweight: body mass index < 18.5 kg/m^2 Rainy season: July-December; Dry season: January-June

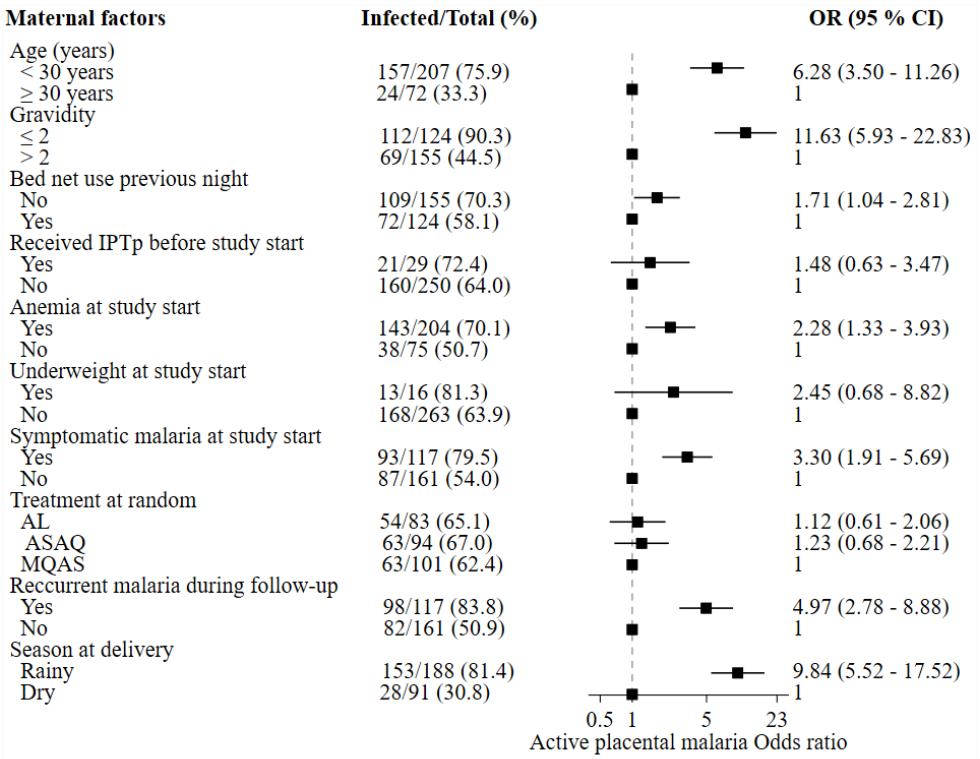

Figure 4 Maternal factors associated with active placental malaria in univariate analysis

OR: odds ratio; CI: confidence interval; IPTp: intermittent preventive treatment during pregnancy with sulfadoxine - pyrimethamine AL: arthemether - lumefantrine; ASAQ: artesunate - amodiaquineMQAS: mefloquine - artesunate; Anemia: hemoglobin < 11g/dl; Underweight: body mass index < 18.5 kg/m^2 Rainy season: July-December; Dry season: January-June

In multivariable analysis, there was a modifying effect in the association of maternal characteristics with PM. In women under 30 years of age, gravidity ≤ 2 was associated with a higher prevalence of pooled PM but in women of 30 years or more, gravidity was no more associated with pooled PM (OR 6.81, 95 % CI 3.18 – 14.60; and OR 0.52, 95 % CI 0.10 – 2.76, respectively). Anemia at enrollment was also associated with pooled PM in women under 30 years of age (OR 1.96, 95 % CI 1.03 – 3.72) but not in women of 30 years or more (OR 0.68, 95 % CI 0.31 – 1.49) (Table 4).

A modifying effect was also observed in the association of gravidity with past-chronic PM (Table 5).

Table 4 Maternal factors associated with pooled placental malaria in multivariable analysis

Factors	**OR (95 % CI)**
Recurrent malaria during follow-up	
Yes	2.02 (1.15 – 3.55)
No	1
Season at delivery	
Rainy	3.24 (1.99 – 5.29)
Dry	1
Age < 30 years	
Gravidity ≤ 2	6.81 (3.18 – 14.60)
Gravidity > 2	1
Age ≥ 30 years	
Gravidity ≤ 2	0.52 (0.10 – 2.76)
Gravidity > 2	1
Age < 30 years	
Anemia at study start	1.96 (1.03 – 3.72)
No anemia at study start	1
Age ≥ 30 years	
Anemia at study start	0.68 (0.31 – 1.49)
No anemia at study start	1

OR : odds ratios ; CI : confidence interval.

Table 5 Maternal factors associated with past-chronic placental malaria in multivariable analysis

Factors	**OR (95 % CI)**
Recurrent malaria during follow-up	
Yes	2.01 (1.15 – 3.54)
No	1
Season at delivery	
Rainy	3.16 (1.94 – 5.13)
Dry	1
Age < 30 years	
Gravidity ≤ 2	7.74 (3.65 – 16.44)
Gravidity > 2	1
Age ≥ 30 years	
Gravidity ≤ 2	0.46 (0.08 – 2.72)
Gravidity > 2	1

OR : odds ratios ; CI : confidence interval; Hb: hemoglobin.

Symptomatic malaria was associated with active PM in women under 30 years of age (OR 3.79, 95 % CI 1.55 – 9.27). The association was not significant in women of 30 years or more (OR 0.42, 95 % CI 0.10 – 1.75) (Table 6).

Table 6 Maternal factors associated with active placental malaria in multivariable analysis

Factors	**OR (95 % CI)**
Recurrent malaria	
Yes	2.77 (1.35 – 5.70)
No	1
Delivery in rainy season	
Yes	6.92 (3.38 – 14.14)
No	1
Gravidity	
≤ 2	6.01 (2.62 – 13.80)
> 2	1
Age < 30 years	
Symptomatic malaria	3.79 (1.55 – 9.27)
Asymptomatic malaria	1
Age ≥ 30 years	
Symptomatic malaria	0.42 (0.10 – 1.75)
Asymptomatic malaria	1

OR: odds ratio; CI: confidence interval;

Distribution of low birth weight or prematurity by placental malaria patterns

LBW or prematurity was observed in 31.2 % and 23.4 % of newborns from mothers with chronic PM and past PM, respectively. Among newborns from women without PM, 13.3 % were LBW or preterm. Surprisingly, none of the newborns from mothers with acute PM was LBW or preterm. (Figure 5).

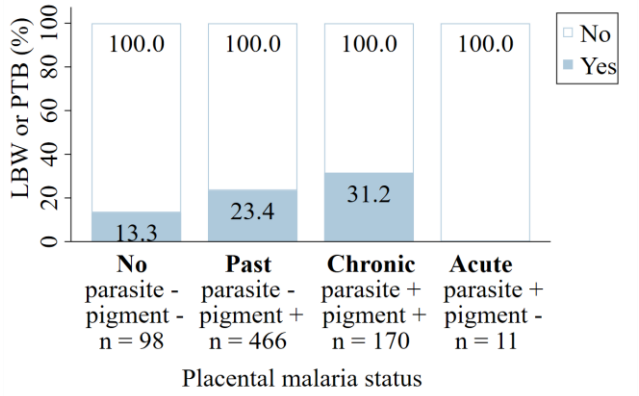

Figure 5 Low birth or prematurity by placental malaria status

Note: +: present; -: absent; LBW: low birth weight; PTB: preterm birth

5.1.4. Discussion

Our study showed that PM prevalence was high in this cohort of pregnant women with P. falciparum peripheral infection at inclusion. Past infections were the predominant PM pattern, followed by chronic infections, acute infections being scarce. A modifying effect according to age was noticed in the association of gravidity, anemia, and symptomatic malaria with PM. Our data also evidenced a higher prevalence of LBW or prematurity in newborns from mothers with chronic PM compared to newborns from mothers with past PM or without PM infection.

We reported 86.8 % of women with PM. Placental malaria is expected to be more prevalent in mothers with peripheral infection than in mothers without detectable peripheral infection, as reported in Malawi, in India, and a metanalysis [15, 24, 25]. The lower PM prevalence in Malawi (78.3%) compared to our study could be explained by the peri-urban characteristic of the Malawi study location. In addition, malaria in pregnancy burden is usually higher in rural areas like in our study setting than in urban areas [26]. Peripheral infections in our study were all due to Plasmodium falciparum, the malaria parasite with the greatest potential to accumulate in the placenta [27]. We hypothesized that this contributed to the higher prevalence of PM in our study compared to an Indian study (64.1 %) where mixed Plasmodium falciparum and Plasmodium vivax peripheral infections occurred as well [15].

A lower proportion (56 % to 58 %) of infected placentas compared to our study were reported in the same area in another multicentric study. This difference could be explained by the inclusion of both women with and without peripheral infection in the latter [17].
In our study, 13.2 % of the placentas were uninfected. Such discordance between peripheral and placental malaria was already reported in Nigeria. Authors attributed this to peripheral parasitemia of low density occurring at the beginning of the pregnancy [14].
In the present study, past infections were the most frequent, followed by chronic infections. The number of acute infections was low. A similar trend of PM distribution with a reduced magnitude was observed in Sudan and another study conducted in Nanoro [16, 17]. In the latter, more than two-thirds of women received at least 2 doses of sulfadoxine-pyrimethamine (SP) for malaria prevention during their pregnancies compared to only 13 % who took two doses of SP in our study before enrollment in the trial. Thus, we hypothesized that the higher number of SP doses contributed to clear the peripheral asymptomatic parasitemia and then to lower the load of parasites reaching the placenta. This is consistent with the dose-dependent protective effect of SP against PM [17]. In a study conducted in Papua New Guinea, acute infections were the most frequent (38 %) PM pattern. The perennial transmission of malaria in this study area could explain the difference with our study conducted in an area where malaria transmission is seasonal [28].
The association of gravidity, anemia at study start, and symptomatic malaria at study start with PM was modified by maternal age.
Placental malaria affects primigravidae or secundigravidae more than multigravidae [7, 13, 17, 29]. In our study, pooled PM and past-chronic PM were more frequent in younger women with lower gravidity. These findings suggest that for a certain pattern of PM, the protective effect of the parity specific immunity depends on the maternal age [10].
Association between anemia and placental malaria was already reported [7, 24]. In our study, a higher prevalence of pooled PM was observed in anemic women aged less than 30 years. Infected red blood cells bind to the vascular walls in the bone marrow. This results in a lower production of red blood cells contributing to anemia [30, 31]. In malaria-endemic regions, older pregnant women acquire antibodies that prevent infected red blood cells from binding to the vascular walls [2, 32]. We infer that younger pregnant women are producing less protective antibodies and this is why they are more prone to both anemia and PM [2, 26]. Pregnant women could benefit from iron and folic acid supplements without being exposed to an increased risk of malaria compared to those receiving folic acid only [33]. However, the benefit of this

supplementation for their offspring was questionable as a study conducted at the Nanoro site reported an increased risk of prematurity in women supplemented with iron and folic acid compared to women supplemented with folic acid alone [34].

In Papua New Guinea, symptomatic malaria was associated with PM [7]. In our study, symptomatic malaria was associated with active PM in women aged less than 30 years of age. Younger women lack acquired immunity against malaria, as women residing in regions of low transmission, and experiencing more clinical episodes [11, 35]. Some authors proposed to use symptomatic malaria as a proxy for PM diagnosis antenatally [36]. In sub-Saharan malaria-endemic regions, this would underestimate the burden of PM as peripheral infections are often asymptomatic [20].

Reduced birth weight and preterm birth were more prevalent with chronic infections [7, 37]. In our study, the composite outcome of LBW and prematurity was also more frequent in newborns from mothers with chronic PM. The fetal growth restriction caused by the inflammatory response to chronic infection was cited as a possible contributing factor of LBW [6].

The present analysis had some limitations. The study was not designed primarily to assess factors associated with PM. The study was conducted in a rural area where malaria is highly prevalent compared to urban areas [26]. Therefore, the inference should be limited to such settings.

However, our study has several strengths. The participants were recruited at peripheral health centers, the first level of contact with the health system and this reduces the chances of a selection bias. The prospective design was also an advantage. Analysis exploring interactions of age with other maternal factors are more relevant to understand how factors are associated with PM.

5.1.5. Conclusion

Placental malaria is frequent in women with peripheral P. falciparum infection in Nanoro, especially in younger women with low gravidity, with anemia, or with symptomatic malaria. This underlines the importance of preventive measures and timely diagnostic and treatment of malaria in young pregnant women.

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5.2. Result 2: Low birth weight and prematurity in teenage mothers in rural areas of Burkina Faso

Abstract

Background: Adolescence is associated with adverse fetal outcome, particularly in -resources limited settings. We assessed the association between mother's age and low birth weight or prematurity in Nanoro, a rural health district of Burkina Faso.

Methods: We collected data on mothers and their newborns in the framework of the "Safe and Efficacious Artemisinin-based Combination Treatments for African Pregnant Women with Malaria" clinical trial. Low birth weight or prematurity was defined as adverse fetal outcome. Logistic regression was used to compare its occurrence in teenagers and in women aged ≥ 20 years.

Results: From June 2010 to November 2013, 870 pregnant women enrolled in the PREGACT study were treated for a Plasmodium falciparum infection and followed up until delivery.

Of the 823 women with singleton live-borns, 205 (24.9 %) were teenagers of whom 44 (5.3%) were minors (15 -17 years). Up to 91.7 % of adolescents presented with anemia at entry.

The incidence of adverse fetal outcome in teenagers was 39.8 %, increasing to 50.0% in minors. Anemic adolescents were significantly at higher risk of delivering low birth weight or preterm babies compared to their older counterparts.

In multivariate analysis, teenagers with both anemia and fever presented the highest and significant odds ratios of adverse fetal outcome, whatever was their BMI: teenagers with anemia, fever and high BMI at entry, AOR = 3.46, 95 % CI: [1.40; 8.58], teenagers with anemia, fever and low BMI at entry, AOR = 2.86, 95 % CI: [1.14; 7.13].

Conclusion: Teenager's pregnancy is associated with adverse fetal outcome in the rural health district of Nanoro, mainly when teenagers experiment anemia and fever. In low resources setting, multidisciplinary approach including education and setting up favorable socio-economic environment are needed to prevent early motherhood.

Key words: Teenagers; low birth weight; preterm birth; rural; Burkina Faso.

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Low Birth Weight and Prematurity in Teenage Mothers in Rural Areas of Burkina Faso

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Abstract

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The incidence of adverse fetal outcome in teenagers was 39.8%, increasing to 50.0% in minors. Anemic adolescents were significantly at higher risk of delivering low birth weight or preterm babies compared to their older counterparts.

In multivariate analysis, teenagers with both anemia and fever presented the highest and significant odds ratios of adverse fetal outcome, whatever was their BMI: Teenagers with anemia, fever and high BMI at entry, AOR=3.46, 95% CI: (1.40, 8.58), teenagers with anemia, fever and low BMI at entry, AOR=2.86, 95% CI: (1.14, 7.13).

Conclusion: Teenager's pregnancy is associated with adverse fetal outcome in the rural health district of Nanoro, mainly when teenagers experiment anemia and fever. In low resources setting, multidisciplinary approach including education and setting up favorable socio-economic environment are needed to prevent early motherhood.

Keywords: Teenagers; Low birth weight; Preterm birth; Rural; Burkina Faso

Introduction

In 2010, 36.4 million women aged 20-24 years had given birth to a live child before their 18th birthday. This was much more common in West and Central Africa (28%) than in Eastern Europe and Central Asia (4%). Similar proportions were found for giving birth before the 15th birthday, i.e. 6% and 0.2%, respectively. In Burkina Faso, the prevalence of a first birth before 18 was estimated at 28% [1].

Teenage pregnancy is a concern worldwide as it is associated with low birth weight (LBW) and preterm birth (PTB) [2-4]. Pregnancy in teenagers can result in adverse maternal outcome such as severe anemia or death and compromise their socio-economic opportunities [5]. Children from teenage mothers are more likely to be stunted at their second birthday, to develop glucose disorder and problems at school [3]. They are more often hospitalized and will contribute with more than half to the following generation of adolescent mothers [6]. Moreover, PTB complications are one of the main causes of death among children aged less than 5 years [7]. LBW has also been associated to an increased neonatal mortality and hospitalization [8,9]. Premature infant's experience also more temperature instability, hypoglycemia, respiratory distress and jaundice [10]. Later in life they are more likely to present impaired neurodevelopment, impaired learning capacity, and impaired behavior. Previous studies that reported an association of LBW or PTB with maternal age remains controversial about mechanisms involved [8,11-13].

Studies focusing on adverse fetal outcome in adolescents are scarce in Burkina Faso, despite a high proportion of them that could further increase in the upcoming decades [1]. This study aimed at assessing the prevalence of adolescents' pregnancy in Burkina Faso and its association with LBW or PTB in the rural setting of Nanoro and Nazoanga.

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Materials and Methods

Study settings

This study was nested in the PREGACT multicenter clinical trial (trial registration NCT00852423). Details of the methodology and results have been reported elsewhere [14]. The study was designed to assess the efficacy and the safety of four artemisinin based combinations treatments against malaria in pregnancy. Seven sites in four countries were involved of whom two in Burkina Faso. The present analysis uses data collected from all pregnant women enrolled in Burkina Faso.

Recruitment

Pregnant women attending the antenatal care clinic in Nanoro or Nazoanga were screened for malaria using a rapid diagnostic test. If positive, study protocol and procedures were explained and a written informed consent obtained. Women included in the trial were those residing within the health center catchment zone, aged of at least 15 years, with a pregnancy between 16 and 37 weeks, with *Plasmodium falciparum* infection, a hemoglobin level of at least 7 g/dL, willing to deliver at the health facility, and to adhere to the study required procedures. They should be able to provide written informed consent also. Exclusion criteria were history of illness which may affect pregnancy outcome, history of allergy to the study drugs, history of known obstetric complication or bad pregnancy history, current use of cotrimoxazole or antiretroviral treatment, evidence of intake of antimalarial drug or antimicrobials with antimalarial activity, inability to take oral treatment, or requirement of hospitalization because of serious illness at screening. Women intending to leave the study catchment area prior to delivery and those currently participating to another study were also excluded. In Burkina Faso, women were randomized to one of the three following treatments: Artesunate-Amodiaquine (ASAQ), Arthemether-Lumefantrine (AL) and Mefloquine-Artesunate (MQAS). Randomization was done using balanced incomplete block design. Participants were treated and observed the first three days by study nurses.

At recruitment study staff recorded demographics, anthropometrics, medical and pregnancy history. A portable ultrasonography device allowed assessment of the fetal viability.

Follow-up

Visits were scheduled over 9 weeks after treatment, at days 3, 7 and then once a week. Subsequently, women were encouraged to attend the antenatal clinic monthly until delivery and at any time they felt unwell, regardless to the schedule.

At each scheduled and unscheduled visit, physical examination was performed. Weight, height and temperature were measured. Signs and symptoms since last visit were collected. A blood sample was taken for malaria smears while hematology was done on days 7, 14, 28 and 63 and biochemistry at days 7 and 14 only.

Recurrent malaria infection was treated as per the malaria national program guidelines. After delivery, the newborn was weighted within 72 h. The gestational age was determined using the Ballard score [15].

Laboratory methods

Thin and thick blood smears were obtained from each participant to determine parasite density. A portable HemoCue device (Hb301 Hemocue®, Angelholm, Sweden) was used to determined maternal hemoglobin level. Detail laboratory methods have been published elsewhere [14,16].

Ethics

The PREGACT study was approved by the ethics committee of the University of Antwerp, Belgium, the Institutional Ethics committee of Centre Muraz and the Ethics committee of the Ministry of Health, Burkina Faso [16]. All data were coded, and no participant's identifiers appeared either in the study case report form, database and previous publications. Informed consent was obtained from the study participants and, for those minor of age, from their representatives

Definitions and statistical analysis

Maternal age was determined at entry from the date of birth in the health booklet or self-reported by the participant. Teenager was defined as an individual between the age of 15 and 19 years according to the WHO definition [17]. Teenagers were divided in two subgroups: 15-17 and 18-19 years old.

The dependent variable was adverse fetal outcome defined as the occurrence of LBW (<2500 g) and/or PTB (gestational age<37 weeks). The weight was measured within 72 h after birth using digital scales and Ballard score was assessed at birth by the study physician [15].

Baseline pregnancy BMI (weight in kg divided by square of height in m), fever (body temperature ≥ 37.5°C) and anemia (hemoglobin level<11 g/dl) were covariates analyzed along with maternal age.

As part of the PREGACT quality assurance system, all data were entered into an electronic case report form built under MACRO software (InferMed, Kings Clinical Trial Unit, and King's College London, UK). They were checked for consistency and all queries were resolved before locking the database for statistical analysis.

Univariate logistic regression was used to assess whether teenagers had a higher adverse pregnancy outcome than adults at each level of the covariates. Adjustments were then made using a multivariate logistic regression. All covariates and their interaction terms with maternal age were included in the model independently of their statistical significance. The estimated coefficients including those of the interactions, their standard errors and their correlation coefficients were used to derive adjusted odds ratio and 95% confidence interval for each combination of the covariates.

A p-value ≤ 0.05 was considered as a statistical significant result. All analyses were performed with STATA 14.1 IC® software (StataCorp LP, Texas USA).

Results

From June 2010 to November 2013, 870 pregnant women in their second or third trimester were enrolled in the trial and randomized to one of the three treatment groups. A total of 846 women delivered while 23 withdrew or were lost to follow-up. One woman was excluded by the investigator because of protocol violation (no malaria infection at inclusion). Among the 859 offspring, 847 were live births including 24 twins. Relationship with adverse fetal outcome was assessed on the 823 singleton live births and their mothers. A total of 14 newborns had missing data: 8 lack information on birth weight and 7 were not assessed for gestational age at birth. The large majority of women (73.0%, 601/823) were in their second trimester of pregnancy, there were 205 (24.9 %) teenagers, including 44 (5.3%) minors (15-17 years). More than a quarter 233 (28.3%) were primigravidae and up to 74.6% had anemia (Figure 1 and Table 1).

Half (50.2 %) of the teenagers had a low BMI (≤ 21kg/m^2), 18.5% had fever at entry. There was a significantly higher proportion of anemia in

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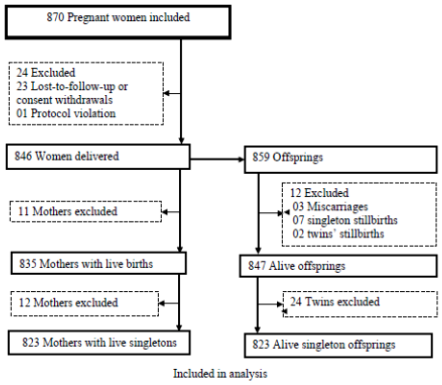

*Solid outlined rectangles represent the number of participants at inclusion, delivery and analysis. Participants excluded at each step were in dashed rectangles. At the left side are the mothers and at the right side their newborns

Figure 1: Flow chart of the participants.

		Maternal age (years)			
	Overall N=823	Teenagers			Adults
		15-17 n=44	18-19 n=161	All teenagers n=205	20-45 n=618
Recruitment site					
Nanoro	552	33 (6.0)	114 (20.7)	147 (26.6)	405 (73.4)
Nazoanga	271	11 (4.1)	47 (17.3)	58 (21.4)	213 (78.6)
Treatment arm					
ASAQ	278	14 (5.0)	65 (23.4)	79 (28.4)	199 (71.6)
AL	277	13 (4.7)	48 (17.3)	61 (22.0)	216 (78.0)
MQAS	268	17 (6.3)	48 (17.9)	65 (24.3)	203 (75.7)
Pregnancy trimester					
Second	601	36 (6.0)	125 (20.8)	161 (26.8)	440 (73.2)
Third	222	8 (3.6)	36 (16.2)	44 (19.8)	178 (80.2)

*ASAQ: Artesunate-Amodiaquine; AL: Arthemether-Lumefantrine; MQAS: Mefloquine-Artesunate

Table 1: Recruitment and randomization characteristics by maternal age group.

teenagers than in adults (91.7% versus 68.9 %, p-value<0.001). Among the 823 newborns, 113 (13.9%) were premature and 148 (18.1%) had a LBW (Table 2).

The overall incidence of adverse fetal outcome was 27.1%, increasing up to 39.8% among teenagers and up to 50.0% in minors (15-17 years). In teenagers, most LBW infants were born from pregnancies at term, the highest incidence, 31.8%, being in minors. Teenagers were significantly at higher risk of delivering LBW or PTB babies compared to older women, regardless of their BMI (BMI of 16-21, OR=2.07, 95% CI: (1.30, 3.29), BMI of 21-30, OR=2.56, 95% CI: (1.54, 4.24)). Teenager's babies had also an increased risk of LBW or PTB whatever was the temperature at entry. Newborns from anemic teenage mothers were at higher risk of adverse fetal outcome than those from anemic adults. (OR=2.24, 95% CI: (1.54, 3.25)) (Figures 2 and 3).

In multivariate analysis, teenagers with both anemia and fever

		Maternal age (years)			
	Overall N=823	Teenagers			Adults
		15-17 n=44	18-19 n=161	All teenagers n=205	20-45 n=618	p-value*
Pregnancy BMI						0.70
16-21	423	24 (54.5)	79 (49.1)	103 (50.2)	320 (51.8)	
21-30	400	20 (45.5)	82 (50.9)	102 (49.8)	298 (48.2)	
Fever at inclusion						0.12
Yes	125	9 (20.5)	29 (18.0)	38 (18.5)	87 (14.1)	
No	698	35 (79.5)	132 (82.0)	167 (81.5)	531 (85.9)	
Anemia at inclusion						<0.001
Yes	614	41 (93.2)	147 (91.3)	188 (91.7)	426 (68.9)	
No	209	3 (6.8)	14 (8.7)	17 (8.3)	192 (31.1)	

*p-value is for all teenagers versus adults; BMI: Body Mass Index (kg/m²)

Table 2: Obstetrical and clinical characteristics of teenagers and adults pregnant women.

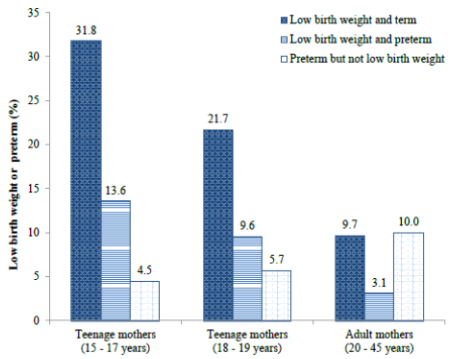

Figure 2: Low birth weight and prematurity in teenagers and in adult women's newborn.

Adjustment covariates			AOR (95% CI)
BMI	Fever	Anemia	
<21	Yes	Yes	2.86 (1.14-7.13)
<21	Yes	No	2.46 (0.62-9.76)
<21	No	Yes	1.97 (1.19-3.26)
<21	No	No	1.70 (0.54-5.29)
≥ 21	Yes	Yes	3.46 (1.40-8.58)
≥ 21	Yes	No	2.98 (0.79-11.24)
≥ 21	No	Yes	2.39 (1.35-4.22)
≥ 21	No	No	2.06 (0.68-6.26)

*AOR: Adjusted Odds Ratio; CI: Confidence Interval; BMI: Body Mass Index (kg/m²)

Table 3: Multivariate odds ratio of low birth weight or prematurity of a newborn from a teenage mother compared to an adult mother.

presented the highest and significant odds ratios of delivering LBW or PTB babies, regardless of the BMI: Teenagers with anemia, fever and low BMI at entry, AOR=2.86, 95% CI: (1.14, 7.13), teenagers with anemia, fever and high BMI at entry, AOR=3.46, 95% CI: (1.40, 8.58) (Table 3).

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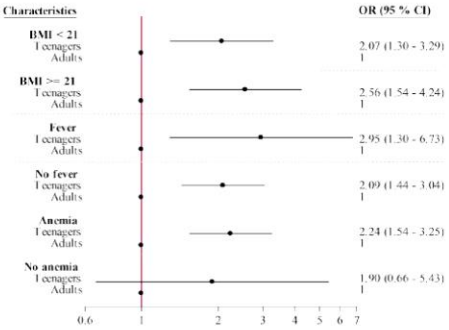

*OR: Odds Ratio; CI: Confidence Interval; BMI: Body Mass Index (kg/m²)

Figure 3: Univariate odds ratio of LBW or prematurity of a newborn from a teenager mother compared to an adult mother. The red solid vertical line is the reference line. Black dots are estimated odds ratios. Horizontal black solid lines are 95% confidence intervals. At the right side of the red line the risk of LBW or PTB increase while it decrease at the left side. An estimate is not statistically significant when its confidence interval cross the red line.

Discussion

The analyses nested in the main PREGACT trial offered the opportunity to investigate consequences of teenagers' pregnancies on their offspring. In rural Burkina Faso, in Nanoro and Nazoanga, about a quarter of pregnant women in the study were teenagers. Adverse fetal outcomes, particularly LBW and PTB, were frequent among teenagers, regardless of the BMI. In addition, anemia and fever increased such a risk. Such level of childbearing by adolescents has been reported by studies done in other sub-Saharan countries: 21.1% in Zimbabwe, 24.4% in Cameroon and 24% in a multi-country study in sub-Saharan Africa [12,18,19]. This is also consistent with the distribution of teenage pregnancies in the world, the highest rates being recorded in the developing countries [1].

Some literature shows that teenager's mothers share more unfavorable socio-economic characteristics than adults [11,20]. Most of the time they were unemployed or earned less income, live in rural areas, have low educational level and do not use effective contraceptive methods [20-23]. We did not assess the socio-economic background of our participants. Nevertheless, Burkina Faso like others West African countries is a low resources country where 67 % of the teenage girls aged 15-19 years live in rural areas. A large proportion of rural adolescents (83.3%) are out of school and only 3% use a modern contraceptive method [24]. A study conducted in inpatient neonates in Burkina Faso reported a higher rate (33%) of adolescent mothers [25]. This is in line with publications stating that newborns from adolescent mothers have a higher risk of hospitalization compared to those form non-adolescent mothers [6].

In our study teenagers LBW and PTB deliveries were 34.0% and 16.3%, respectively. In other sub Saharan countries the rates were lower, but not homogenous [2,12,19,21,26]. This maybe reflects behavioral and cultural diversity among pregnant adolescents across countries [27,28]. Higher proportions (LBW 38.9%, PTB 27.7%) were found in India [29]. We link this to the greater proportion (11%) of young teenagers (15-17 years) in this study, given that inverse relationship between maternal age and adverse fetal outcome [4,30,31].

In this study we did not aim at exploring factors associated with LBW or PTB. Our focus was on the potential risk of these adverse fetal outcome associated with the status of being a teenager.

In our univariate analysis adolescence was associated with LBW or PTB, as already demonstrated elsewhere [3,4,18,19,21,30,31]. Conversely studies from Nigeria and Brazil showed no association between maternal age and LBW or preterm birth [32,33]. The urban setting and the hospital-based recruitment in these studies may explain their finding.

In multivariate analysis anemic teenagers delivered more LBW or PTB newborns than their adults counterparts. Indeed anemia is more frequent in adolescents than in adults and it is linked to LBW and PTB [4,8,12,23,27,34]. The high frequency of anemia in teenagers may be explained by the inadequacy of their diet to fill the iron demand induced by their rapid growth and the menstruation's onset [35,36]. Also, adolescents' intake of iron folic acid for anemia prevention is lower compared to adults [29].

The risk of adverse fetal outcome in teenagers was the highest when they presented both anemia and fever. This is not surprising, considering the interplay of fever with anemia, and the role of malaria in the occurrence of fever anemia and LBW in endemic areas [37].

Although low BMI was frequent in teenagers and could indicate biological immaturity, this did not lead to a significant increased risk of adverse fetal outcome in our study as hypothesized elsewhere [2,31,38].

Our analysis excluded twins and this could underestimate the outcome, even if their number is limited. It was also not exhaustive concerning commonly analyzed covariates.

Nevertheless, the prospective design represents strength of our study. In addition, data were collected in an accurate and standardized way.

Importantly, the interplay of maternal age with the covariates was pointed out in our analysis. Inadequate number of antenatal visits has often been cited as deleterious factor of fetal outcome [12,39]. However, a free provision of quality health care including antenatal package was provided throughout the duration of the PREGACT trial.

Conclusion

Being a teenager is associated with adverse fetal outcome in the rural health district of Nanoro, mainly when teenagers experiment anemia and episodes of fever. In low resources setting, multidisciplinary approach including education and setting up favorable socio-economic environment are needed to prevent early motherhood.

Acknowledgement

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Ethics approval and consent to participate

This research has been conducted according to the declaration of Helsinki and to the ICH Good Clinical Practices guidelines. The PREGACT study was

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approved from the Ethics committee of the University Hospital, Antwerp, Belgium, the Institutional Ethics committee of Centre Muraz and the Ethics committee of the Ministry of Health, Burkina Faso. All the participants or their representatives gave informed consent before entering the study.

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5.3. Result 3: Fetal biometry assessment with Intergrowth 21st's and Salomon's equations in rural Burkina Faso

Abstract

Background: Ultrasound scanning during the 2nd or the 3rd trimester of pregnancy for fetal size disturbances screening is heavily dependent of the choice of the reference chart. This study aimed to assess the agreement of Salomon and the Intergrowth 21st equations in evaluating fetal biometric measurements in a rural area of Burkina Faso, and to measure the effect of changing a reference chart.

Methods: Data collected in Nazoanga, Burkina Faso, between October 2010 and October 2012, during a clinical trial evaluating the safety and efficacy of several antimalarial treatments in pregnant women were analyzed. We included singleton pregnancies at 16-36 weeks gestation as determined by ultrasound measurements of fetal bi-parietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL). Expected mean and standard deviation at a given gestational age was computed using equations from Salomon references and using Intergrowth 21st standard. Then, z-scores were calculated and used subsequently to compare Salomon references with Intergrowth 21st standards.

Results: The analysis included 276 singleton pregnancies.

Agreement was poor except for HC: mean difference -0.01, limits of agreement -0.60 and 0.59. When AC was used as a surrogate of fetal size, switching from the reference of Salomon to the standards of Intergrowth 21st increased ten times the proportion of fetuses above the 90th percentile: 2.9 % and 31.2 %, respectively.

Mean differences were larger in the third trimester than in the second trimester. However, agreement remained good for HC in both trimesters.

Difference in the proportion of AC measurements above the 90th percentile using Salomon and Intergrowth 21st equations was greater in the second trimester (2.6 % and 36.3 %, respectively) than in the third trimester (3.5 % and 19.8 %, respectively). The greatest difference between the two charts was observed in the number of FL measurements classified as large in the second trimester (6.8 % and 54.2 %, using Salomon and Intergrowth 21st equations , respectively).

Conclusion: The agreement between Intergrowth 21st and Salomon equations is poor apart from HC. This would imply different clinical decision regarding the management of the pregnancy.

Key words: biometry, references, standards, Burkina Faso

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RESEARCH ARTICLE Open Access

Fetal biometry assessment with Intergrowth 21st's and Salomon's equations in rural Burkina Faso

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Abstract

Background: Ultrasound scanning during the 2nd or the 3rd trimester of pregnancy for fetal size disturbances screening is heavily dependent of the choice of the reference chart. This study aimed to assess the agreement of Salomon and the Intergrowth 21st equations in evaluating fetal biometric measurements in a rural area of Burkina Faso, and to measure the effect of changing a reference chart.

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Conclusion: The agreement between Intergrowth 21st and Salomon equations is poor apart from HC. This would imply different clinical decision regarding the management of the pregnancy.

Keywords: Biometry, References, Standards, Burkina Faso

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Background

Ultrasound scanning during the 2nd or 3rd trimester of pregnancy allows fetal anthropometrics measurement [1] and screening for fetal size disturbances by comparison to reference values [2, 3].

Biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) are the most commonly measured parameters [4, 5].

Abnormal fetal biometric measurements could reflect underlying health issues like microcephalia, aneuploidy, genetic syndrome of skeletal dysplasia [6].

Early detection of abnormal fetal size helps obstetrician initiating further monitoring, planning and managing delivery in terms of labor induction or caesarian section [6, 7].

However, abnormal fetal size identification depends heavily on the choice of reference values. There is more than eighty references charts in the world [4]. A switching from one reference chart to another could raise tenfold the likeliness of identifying abnormal biometric measurements [8]. This could lead to expensive and stressful monitoring with additional investigations [8, 9]. Also, fetal size depends of ethnicity and use of inadequate reference may result in harmful medical decisions [10, 11]. For example, there is a risk of fetal loss in cases in which karyotype is demanded because of the invasive sampling method used [8]. Screening for fetal size disturbances using inappropriate reference chart may affect research conclusions and health policies as well [8, 10]. Thus some authors recommended to use local reference for screening in a specific population [11–14]. In high-income countries, [4, 5] local biometric reference charts were adopted. Clear guidelines and recommendations for screening of abnormal fetal size and subsequent management were also put in place [5, 15–21]. In low- and middle-income countries (LMICs), and particularly in sub-Saharan Africa, local charts are either lacking or not implemented where available [22–25]. The latter mostly refer to European charts [26], or charts preprogramed by default in the ultrasound device software [11–14, 27].

In 2014, the international fetal and newborn growth consortium for the twenty-first century (Intergrowth 21st) published fetal biometric standard equations based on selected healthy pregnancies from eight countries [10, 27]. The aim was to provide charts that could be used anywhere in the world and to solve the issue of inadequate references [27]. Settings where local reference charts are lacking or not implemented are likely to replace the charts they are currently using by this new chart. However, knowing the variation between nomograms in assessing fetal size and the clinical implication, it would be cautious to check whether adaptation is needed before adopting or replacing a chart in a specific population [14].

In Burkina Faso, no locally-adapted fetal biometry charts have been adopted or recommended in obstetric ultrasound practice. Rather, French references or other European references set by default in the ultrasound machines are used. Indeed in France, Salomon equations were recommended until 2018 [28].

Both the Salomon and Intergrowth 21st equations allow the calculation of a mean and a standard deviation by gestational age and the computation of a standardized z-score for the individual fetus. The objective of the present analysis was to assess the difference between z-scores derived from Salomon and from Intergrowth 21st equations using fetal biometric measurements in pregnant women from rural areas of Burkina Faso, and to measure the effect of changing a reference chart.

Methods

Study settings and population

The current dataset is from the trial "Safe and efficacious artemisinin-based combination treatment for African pregnant women with malaria" (PREGACT) conducted from June 2010 to August 2013 [NCT00852423 (ClinicalTrials.gov)]. The primary study evaluated the efficacy and safety of four artemisinin-based combinations treatment in women with malaria in the 2nd and 3rd trimester of pregnancy. The trial was implemented in four countries, namely Burkina Faso, Ghana, Malawi, and Zambia. Methods and results have been already published [29, 30]. This analysis used data collected in Nazoanga, Burkina Faso, where fetal biometric measurements at inclusion by ultrasound were carried out, to exclude women in the 1st trimester.

Ultrasound

This study was cross-sectional. Ultrasonographic examination of the pregnant women was performed once at inclusion. However, three participants had their scan repeated a second time at screening to confirm gestational age as per the quality assurance system put in place [31,

Table 1 General characteristics of the mothers

Age (years)	23 (20; 29)
Gravidity	3 (1; 4)
Parity	2 (0; 3)
Weight (kg)	54 ± 6
Height (cm)	162 ± 7
Body mass index (kg/m^2)	20.5 ± 1.7
Symphysis-fundal height (cm)	24 (21; 28)
Gestational age (weeks)	25 (21; 29)
Hemoglobin (g/dL)	10.1 ± 1.2

Numbers are mean ± standard deviation or median (IQR)
IQR interquartile range

32]. A Fukuda Denshi© portable ultrasound scanner FFsonic UF-4100 with a 3.5 MHz or 5.0 MHz probes was used for transabdominal examination according to woman's thinness.

Four biometric parameters were measured. BPD and HC were both obtained in a transverse view of the fetal head with the following landmarks: midline echo corresponding to the fax cerebri, its anterior third interrupted by the cavum septi pellucidi, symmetry of thalami at each side. BPD was measured from the inner to the outer wall of the skull. HC measurement was realized by placing an ellipse around the outer border of the skull. AC was measured by applying the ellipse on the external border of the abdomen in a cross-sectional plane showing the stomach bulbe and the anterior third of the umbilical vein. FL measurement was obtained in a plane where the femoral diaphysis was fully visible, with calipers placed on the both ends. All measurements were done according to specific standard operating procedures [31, 32]. Gestational age in complete weeks was automatically derived from the four anthropometric measurements according to Hadlock formula [32, 33]

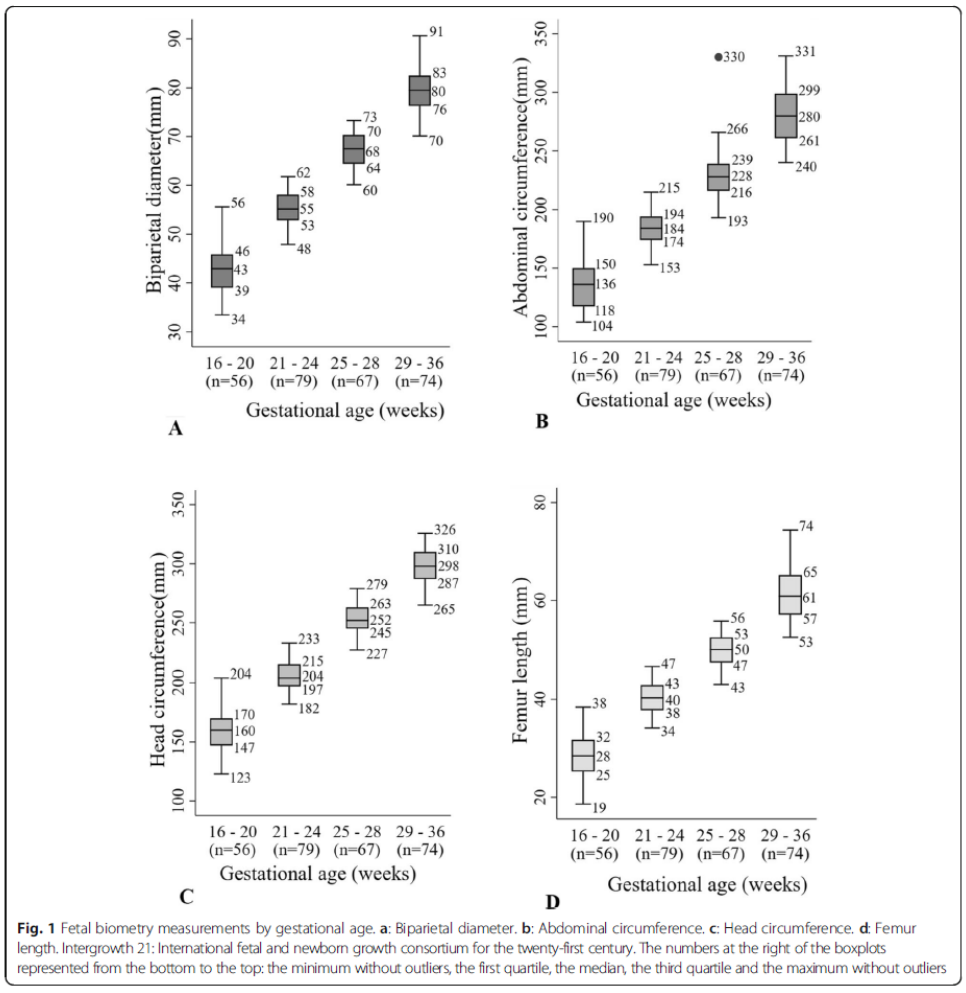

Fig. 1 Fetal biometry measurements by gestational age. **a**: Biparietal diameter. **b**: Abdominal circumference. **c**: Head circumference. **d**: Femur length. Intergrowth 21: International fetal and newborn growth consortium for the twenty-first century. The numbers at the right of the boxplots represented from the bottom to the top: the minimum without outliers, the first quartile, the median, the third quartile and the maximum without outliers

Statistical analysis

Three participants have their scan repeated once at screening to confirm gestational age as per the quality assurance system [29, 30]. We took this into account by averaging the two measurements for each biometric parameter.

The expected mean and expected standard deviation for BPD, HC, AC and FL for the gestational age were computed for each fetus using equations from Salomon [16] and Intergrowth 21st [27]. The z-scores [(Observed value − Expected mean)/Expected SD] of the two equations were compared. Square-diagonal scatter plots were

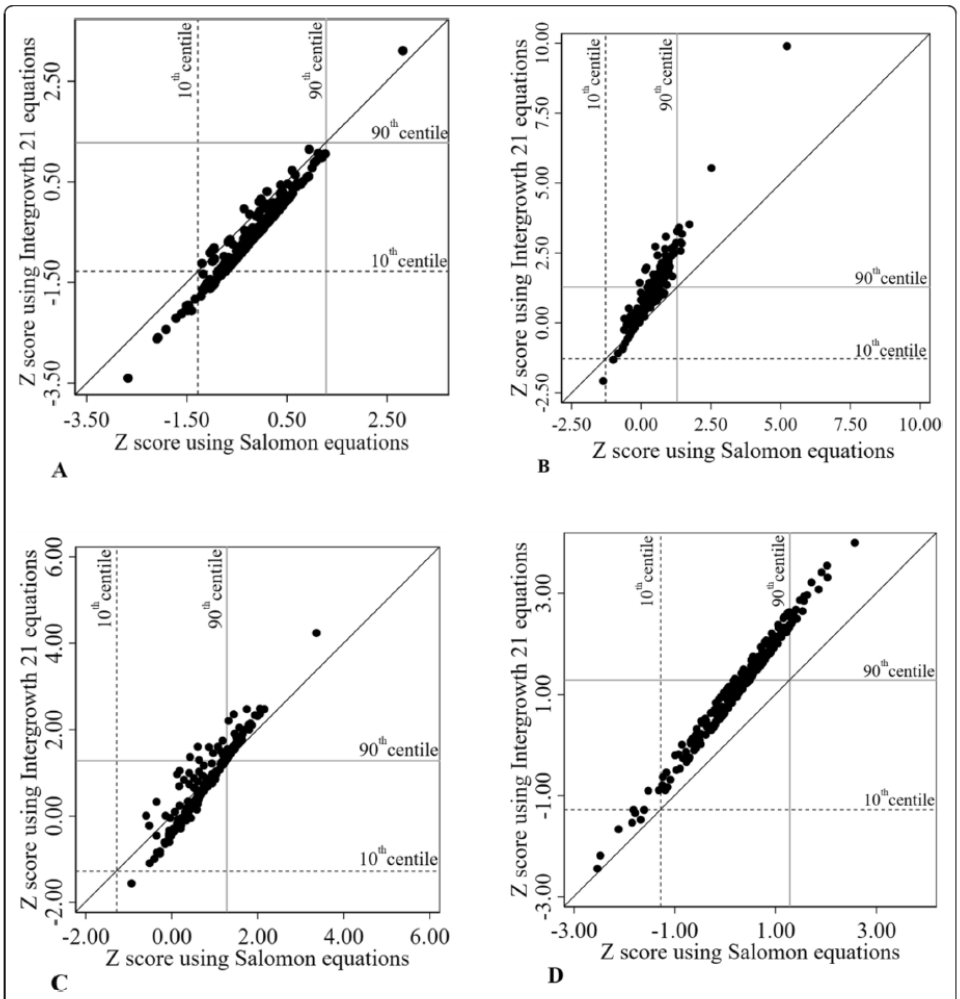

Fig. 2 Number of standard deviations from the mean using Salomon or Intergrowth 21 eqs. **a**: Biparietal diameter. **b**: Abdominal circumference. **c**: Head circumference. **d**: Femur length. Intergrowth 21: International fetal and newborn growth consortium for the twenty-first century. Dashed horizontal and vertical grey lines referred to a z score of − 1.282 corresponding to the 10th centile. Solid horizontal and vertical grey lines referred to a z score of 1.282 corresponding to the 90th centile. The black oblique line is the perfect concordance line where the z-scores from Intergrowth 21st and Salomon equations are equal

drawn to allow visual evaluation of the relationship between the two sets of z-scores. All differences (Intergrowth 21st z-score - Salomon z-score) were compared using paired t test and Wilcoxon signed ranks test. A *p*-value < 0.05 was considered as statistically significant. Linear relationship was checked by performing linear regression of Intergrowth 21st z-scores by Salomon z-scores.

Level of agreement between the charts was checked using Bland-Altman analysis. Individual scores averages were plotted horizontally against their differences vertically. Limits of agreement (LOA) were obtained by applying the following formula: mean of differences ±1.96 standard deviation.

A mean difference of zero and limits of agreement within − 0.50 and 0.50 were considered as a good agreement [34].

Reliability between the two charts was expressed by the intraclass correlation coefficient (ICC), calculated from a random effect one-way analysis of variance. Reliability was considered as weak, good or excellent if ICC values were < 0.40, between 0.40 and 0.75, or > 0.75, respectively.

Abnormal measurement referred to either smallness (z score < − 2.00 corresponding to the 2.5th centile, z score < − 1.282 corresponding to the 10th centile) or largeness (z-score > 1.282 corresponding to the 90th centile, or z-score > 2.00 corresponding to the 97.5th centile).

The effect of replacing one chart with another was measured using AC as a surrogate of fetal weight [2, 9].

STATA® statistical software version 15.1 StataCorp LLC, Texas, USA, was used for all analyses.

Ethics

Ethical approvals were obtained for the PREGACT study from the ethics committee of the University of Antwerp, Belgium, the institutional ethics committee of the Centre Muraz and the ethics committee of the Ministry of Health, Burkina Faso. Study participants or their legally authorized representative (for minors/not emancipated) signed (or thumb printed if illiterate) an informed consent form, before entering the study [29, 30]. The data were anonymized.

Results

Out of the 285 pregnant women recruited in Nazoanga, 9 were excluded: 6 because of consent withdrawal and 3 because of twin pregnancy. Therefore, 276 participants were included in the current analysis. Recruited women were young (median age: 23 years), had several

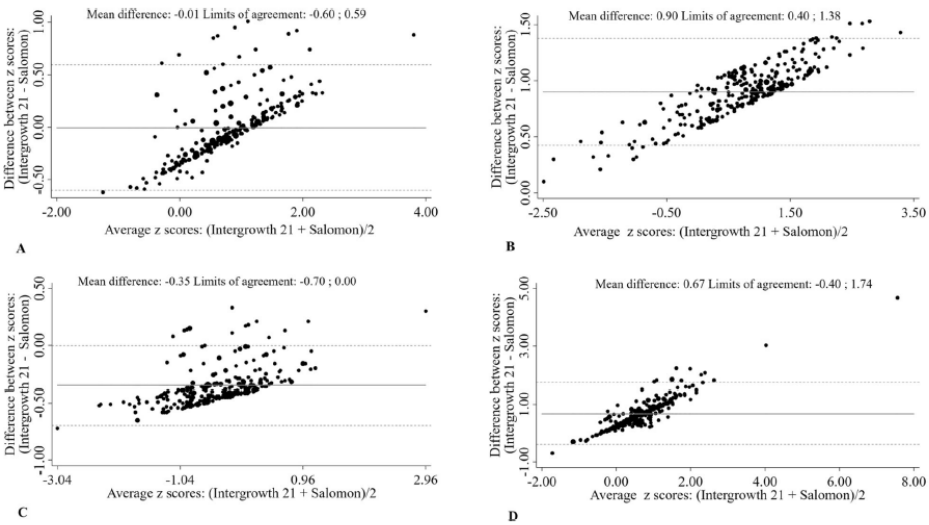

Fig. 3 Agreement between z scores using Salomon or Intergrowth 21 equations. **a**: Head circumference. **b**: Femur length. **c**: Biparietal diameter. **d**: Abdominal circumference. Intergrowth 21: International fetal and newborn growth consortium for the twenty-first century. Dashed horizontal grey lower line represents the lower limit of agreement between the z-scores from Intergrowth 21st and Salomon equations. Solid horizontal grey line represents the mean difference between the z-scores from Intergrowth 21st and Salomon equations. Dashed horizontal grey upper line represents the upper limit of agreement between the z-scores from Intergrowth 21st and Salomon equations

pregnancies (median gravidity: 3), and at inclusion had a median gestational age of 25 weeks (Table 1).

Median and interquartile range (IQR) of the measured parameters increased with increasing gestational age (Fig. 1).

Visual comparison by scatter plots of Intergrowth 21st 's to Salomon's z scores showed that they were underestimated in the low values of BPD (Fig. 2a), AC (Fig. 2b), and HC (Fig. 2c), and overestimated in the high values of AC (Fig. 2b), and HC (Fig. 2c); all z scores of FL were overestimated (Fig. 2d).

The two sets of z scores agreed poorly except for HC. The mean difference (− 0.01) was closed to zero and the limits of agreement (− 0.60 and 0.59) were closed to the prespecified values of − 0.5 and 0.5. (Fig. 3a). Reliability ranged from good to excellent (see Additional file 1, Supplemental Table 1).

There was a strong linear correlation between the z scores by Intergrowth 21st equations and the z scores by Salomon equations. The slopes of linear regression of z scores using Intergrowth 21st equations over the z scores using Salomon equations ranged from 1.11 for BPD to 1.78 for AC (Fig. 4).

The percentages of fetal anthropometrics classified either as small or large are reported in Table 2. Globally, the number of measurements considered as large was greater than that of measurements considered as small, except for BPD. Also, percentages of fetuses with abnormal z scores by Intergrowth 21st equations were higher than those by Salomon equations. The effect of replacing Salomon reference by Intergrowth 21st standards was shown using AC as surrogate of fetal size. Large fetuses (above the 90th percentile) proportion using Salomon eqs. (2.9%) was decupled when Intergrowth 21st equations were used (31.2%).

In the second trimester, the agreement between HC z scores using Intergrowth 21st equations and z scores using Salomon equations remained good: mean difference 0.03; limits of agreement − 0.62 and 0.68 (Table 3). The proportions of large fetuses based on AC measurements above the 90th percentile were 36.3% by

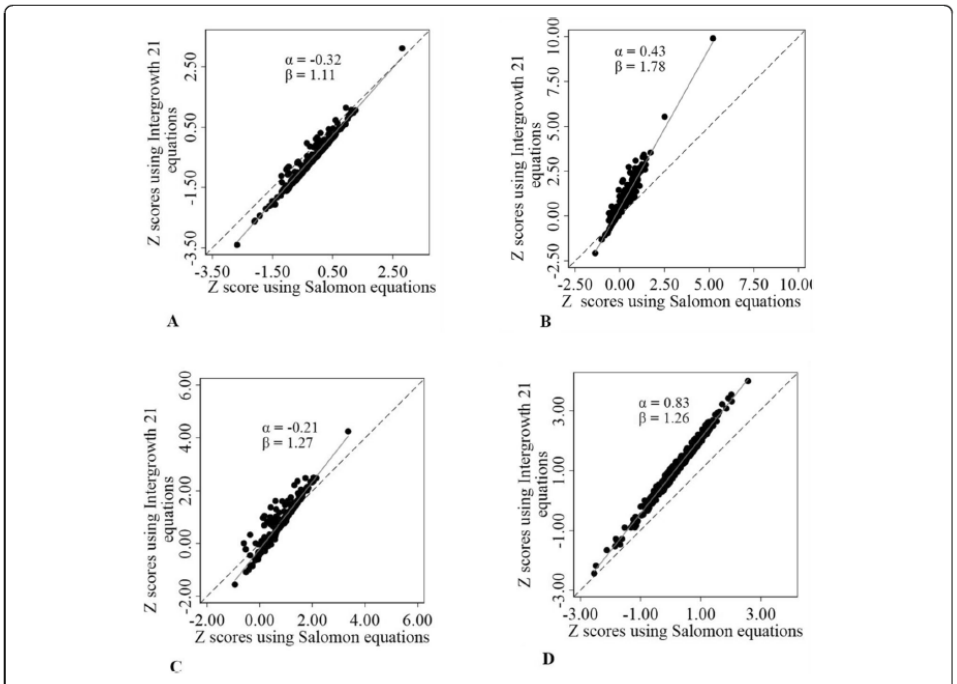

Fig. 4 Regression of Intergrowth 21 z-scores with Salomon z-scores. **a**: Biparietal diameter. **b**: Abdominal circumference. **c** Head circumference. **d**: Femur length. Intergrowth 21: International fetal and newborn growth consortium for the twenty-first century. Dashed oblique black line represents the perfect agreement between the z-scores from Intergrowth 21st and Salomon equations. Solid oblique grey line represents the linear regression fitted line

Table 2 Fetuses with abnormal z scores using Intergrowth 21st or Salomon equations

Anthropometrics	Abnormal z score n (%)			
	< 2.5th centile	< 10th centile	> 90th centile	> 97.5th centile
Biparietal diameter				
Intergrowth 21	10 (3.6)	39 (14.1)	1 (0.4)	1 (0.4)
Salomon	3 (1.1)	14 (5.1)	1 (0.4)	1 (0.4)
Mc Nemar test	n.a	< 0.001	n.a	n.a
Abdominal circumference				
Intergrowth 21	1 (0.4)	3 (1.1)	86 (31.2)	33 (12.0)
Salomon	0 (0.0)	1 (0.4)	8 (2.9)	2 (0.7)
Mc Nemar test	n.a	n.a	< 0.001	< 0.001
Head circumference				
Intergrowth 21	0 (0.0)	1 (0.4)	68 (24.6)	19 (6.9)
Salomon	0 (0.0)	0 (0.0)	52 (18.8)	4 (1.4)
Mc Nemar test	n.a	n.a	< 0.001	< 0.001
Femur length				
Intergrowth 21	2 (0.7)	6 (2.2)	137 (49.6)	49 (17.8)
Salomon	3 (1.1)	10 (3.6)	18 (6.5)	3 (1.1)
Mc Nemar test	n.a	n.a	< 0.001	< 0.001

n.a not applicable; Intergrowth 21: international fetal and newborn growth consortium for the twenty-first century

Intergrowth 21st equations and 2.6% by Salomon equations. However, the greatest difference in large biometric measurements between the four parameters was observed in FL (6.8% and 54. 2%, using Salomon and Intergrowth 21st equations respectively) (Table 4).

In the third trimester mean difference between HC z scores was − 0.09 and limits of agreement were − 0.52 and 0.35 (Table 5). Large fetuses detected by AC z scores above the 90th percentiles were 19.8 and 3.5% using Intergrowth 21st and Salomon equations, respectively (Table 6).

Discussion

The aim of our study was to determine the differences between fetuses' size patterns estimated by Salomon references or Intergrowth 21st standards in a sub-Saharan African rural population, rather than estimating the actual status of smallness or largeness.

The differences between the means of z-scores of the four biometric parameters estimated by the two methods were all statistically significant. Intergrowth 21st equations gave the greatest scores, particularly for FL. Therefore, the charts agreed poorly, except for HC.

These findings revealed differences between our population and the populations used for the charts [1, 10]. Indeed, Salomon's chart was developed on the basis of a cohort of pregnant women followed up in France which probably is ethnically different from our cohort of Burkinabe pregnant women, [16] possibly explaining the observed discrepancies [12–14]. Nevertheless, the development of Intergrowth 21st equations included African pregnant women [27] but had greater means of z-scores, meaning that other factors than ethnicity could explain the differences observed. Intergrowth 21st equations were derived on the basis of healthy, well-nourished women [10] and thus describe growth under optimal conditions [1, 10]. References of Salomon imposed few constraints regarding adequacy of the nutritional or health status [16]. Our study population included malaria-infected pregnant women living in rural Burkina Faso [29]; on average observed measures were more distant from standards and closer to references, suggesting that ideal fetal growth conditions were not fulfilled. Nevertheless, the negative means of BPD z-scores were probably due to systematic variations in head measurement methods as already shown in another publication [34]. BPD was obtained by placing calipers in the center of the width of the skull bone, from outer-to-outer and from outer-to-inner margins, in the study of Salomon's, Intergrowth 21st study and in our study [16, 27, 32].

Despite these disparities, both charts agreed roughly on HC measurements. This finding reinforces the choice of HC as a single "non-fat marker" for comparison of fetal size across populations [4, 10].

It was recently shown that FL z scores between Intergrowth 21st and Salomon's equations were largely divergent in France, [34] a difference also observed in our study that may be due to the evolution in ultrasound technology [35]. Indeed, recent ultrasound equipment's such as those used in the Intergrowth 21st study have thinner beam and yield

Table 3 Agreement and reliability of fetal biometrics z scores using Intergrowth 21st and Salomon equations in the second trimester

Anthropometrics	Mean ± SD	p-value*	Median (IQR)	p-value†	Range
Biparietal diameter					
Intergrowth 21	− 0.48 ± 0.72		− 0.47 (− 0.98; − 0.04)		−3.40; 3.11
Salomon	− 0.15 ± 0.63		− 0.12 (− 0.60; 0.24)		−2.68; 2.81
Difference	− 0.33 ± 0.21	< 0.001	− 0.40 (− 0.48; − 0.22)	< 0.001	− 0.72; 0.33
LOA	− 0.74; 0.07				
ICC	0.85				
Abdominal circumference					
Intergrowth 21	1.05 ± 1.18		0.95 (0.35; 1.59)		−2.07; 9.90
Salomon	0.29 ± 0.63		0.23 (−0.03; 0.58)		−1.37; 5.22
Difference	0.77 ± 0.60	< 0.001	0.69 (0.40; 1.01)	< 0.001	− 0.70; 4.68
LOA	− 0.40; 1.94				
ICC‡	0.54				
Head circumference					
Intergrowth 21	0.75 ± 0.81		0.72 (0.14; 1.29)		−1.56; 4.24
Salomon	0.72 ± 0.61		0.67 (0.31; 1.14)		−0.94; 3.36
Difference	0.03 ± 0.33	0.18	−0.02 (− 0.22; 0.23)	0.85	−0.62; 1.01
LOA	−0.62; 0.68				
ICC	0.89				
Femur length					
Intergrowth 21	1.21 ± 0.92		1.34 (0.63; 1.81)		−1.53; 4.00
Salomon	0.36 ± 0.71		0.47 (−0.07; 0.77)		−1.85; 2.57
Difference	0.85 ± 0.21	< 0.001	0.88 (0.73; 0.97)	< 0.001	0.21; 1.43
LOA	0.43; 1.27				
ICC	0.63				

* Paired t test *p* value
† Wilcoxon signed ranks test *p* value
‡ Intraclass correlation calculation for abdominal circumference z scores excluded one participant with extreme values (9.9 using Intergrowth equations and 5.22 using Salomon equations)
LOA limits of agreement, *ICC* intraclass correlation coefficient

Table 4 Abnormal z scores using Intergrowth 21st or Salomon equations in the second trimester

Anthropometrics	Abnormal z scores n (%)			
	< 2.5th centile	< 10th centile	> 90th centile	> 97.5th centile
Biparietal diameter				
Intergrowth 21	4 (2.1)	16 (8.4)	1 (0.5)	1 (0.5)
Salomon	1 (0.5)	4 (2.1)	1 (0.5)	1 (0.5)
Abdominal circumference				
Intergrowth 21	1 (0.5)	3 (1.6)	69 (36.3)	28 (14.7)
Salomon	0 (0)	1 (0.5)	5 (2.6)	2 (1.1)
Head circumference				
Intergrowth 21	0 (0)	1 (0.5)	49 (25.8)	15 (7.9)
Salomon	0 (0)	0 (0)	34 (17.9)	3 (1.6)
Femur length				
Intergrowth 21	0 (0)	2 (1.1)	103 (54.2)	32 (16.8)
Salomon	0 (0)	4 (2.1)	13 (6.8)	2 (1.1)

Table 5 Agreement and reliability of fetal biometrics z scores using Intergrowth 21st and Salomon equations in the third trimester

Anthropometrics	Mean ± SD	p-value*	Median (IQR)	p-value†	Range
Biparietal diameter					
Intergrowth 21	−0.91 ± 0.70		−0.89 (−1.31; −0.44)		−2.62; 0.78
Salomon	− 0.53 ± 0.64		−0.49 (− 0.90; − 0.11)		−2.10; 1.00
Difference	−0.39 ± 0.07	< 0.001	−0.38 (− 0.44; − 0.34)	< 0.001	−0.53; − 0.22
LOA	−0.52; − 0.25				
ICC	0.85				
Abdominal circumference					
Intergrowth 21	0.81 ± 0.71		0.75 (0.29; 1.21)		−0.95; 2.89
Salomon	0.35 ± 0.43		0.36 (0.02; 0.63)		−0.68; 1.45
Difference	0.45 ± 0.31	< 0.001	0.37 (0.25; 0.64)	< 0.001	−0.27; 1.48
LOA	−0.16; 1.07				
ICC	0.66				
Head circumference					
Intergrowth 21	0.76 ± 0.70		0.70 (0.39; 1.21)		−0.87; 2.49
Salomon	0.85 ± 0.48		0.79 (0.58; 1.17)		−0.28; 2.05
Difference	−0.09 ± 0.22	< 0.001	− 0.09 (− 0.18; 0.07)	0.001	−0.59; 0.44
LOA	−0.52; 0.35				
ICC	0.92				
Femur length					
Intergrowth 21	1.09 ± 1.15		1.15 (0.53; 1.75)		−2.44; 3.55
Salomon	0.07 ± 0.90		0.13 (−0.38; 0.55)		− 2.54; 2.02
Difference	1.02 ± 0.26	< 0.001	1.04 (0.91; 1.16)	< 0.001	0.10; 1.53
LOA	0.50; 1.53				
ICC	0.65				

* Paired t test *p* value
† Wilcoxon signed ranks test *p* value
LOA limits of agreement, *ICC* intraclass correlation coefficient

Table 6 Abnormal z scores using Intergrowth 21st or Salomon equations in the third trimester

Anthropometrics	Abnormal z scores n (%)			
	< 2.5th centile	< 10th centile	> 90th centile	> 97.5th centile
Biparietal diameter				
Intergrowth 21	6 (7.0)	23 (26.7)	0(0)	0(0)
Salomon	2 (2.3)	10 (11.6)	0(0)	0(0)
Abdominal circumference				
Intergrowth 21	0(0)	0 (0)	17 (19.8)	5 (5.8)
Salomon	0(0)	0 (0)	3 (3.5)	0(0)
Head circumference				
Intergrowth 21	0(0)	0 (0)	19 (22.1)	4 (4.7)
Salomon	0(0)	0 (0)	18 (20.9)	1 (1.2)
Femur length				
Intergrowth 21	2 (2.3)	4 (4.7)	34 (39.5)	17 (19.8)
Salomon	3 (3.5)	6 (7.0)	5 (5.8)	1 (1.2)

smaller FL [35, 36] than older machines as in our study [31] and in Salomon's [16].

When AC was used as a proxy of fetal size estimation [2], the proportions of small fetuses were low for both charts which may indicate the difficulty ultrasonography has in identifying small fetuses [15, 37]. However, the proportions of both small and large fetuses were higher with Intergrowth 21st equations than with Salomon equations and a similar trend was remarked with HC, suggesting the tendency of Intergrowth 21st equations to underestimate the size in small measurements and to overestimate it in high measurements [3, 34]. Thus, the choice of one or another of these references would implies very different medical interventions, follow-up, and resources allocation as well as stress put on patients [4, 27].

It is well documented that pregnancies affected by malaria, as in our study, are subject to fetal growth restriction [31, 38]. Thus, the number of small fetuses was expected to be high even if differences would be found between the charts. Surprisingly, this number was very low and the number of large fetuses was high. We suspect gestational age determination to be a possible cause of such situation. Indeed, pregnancies were dated late owing to the study design, [29] using a combination of fetal biometry measurements [33]. Late dating is less accurate than early dating. However, this is common practice in sub-Saharan Africa where almost three out of four pregnant women attend their first antenatal clinic during the second or third trimester, or not at all [39]. Although the combination of fetal anthropometric measurement is the recommended method at this stage of pregnancy, [40] it could produce redundant relationship when used for determining both gestational age and fetal size [4]. This is of particular concern in areas where malaria in pregnancy is common such as in Burkina Faso [41]. Gestational age could be underestimated in case of symmetric fetal growth restriction [33], hiding the adverse effect of malaria [42]. However, the difficulty for estimating the gestational age applies to both sets of equations when calculating z scores. Therefore, the differences between the charts are probably not due only to pregnancy dating problems, as shown by the positive and significant slopes in linear regressions. In addition, pregnant women included in this clinical trial, besides malaria, did not have any other chronic or major disease with adverse effect on fetal growth [29].

This is a post-hoc analysis and thus, has some limitations. Physicians performing the ultrasound scans are not professional sonographers even if trained ad hoc. In addition, the study design was not conceived to evaluate two different methods for the assessment of fetal biometry. Moreover, the use of European references equations may not be appropriate for African populations. Gestational age determined in late pregnancy is also another limitation because of less accuracy. Our study population prone to malaria was quite selected and this maybe introduce a bias. However, a recent study showed that the difference between the two charts remained while using fetal biometric measurements from pregnant women as healthy as those in the Intergrowth 21st study [43].

Conclusion

The agreement between Intergrowth 21st and Salomon equations, besides HC, was poor. This would imply different clinical decisions regarding the management of the pregnancy and the delivery. Encouraging women to attend antenatal clinics earlier and to use preventive measures against malaria such as long-lasting insecticidal bed nets, would probably be much more beneficial than just dating gestation or determining fetal size.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12884-020-03183-5.

Additional file 1: Supplemental Table 1. Agreement and reliability of fetal biometrics z scores using Intergrowth 21st and Salomon equations.

Abbreviations

AC: Abdominal circumference; BPD: Biparietal diameter; FL: Femur length; HC: Head circumference; ICC: Intraclass correlation coefficient; INTERGROWTH – 21st: International fetal and newborn growth consortium for the twenty-first century; LLIN: Long lasting insecticidal net; LOA: Limits of agreement; PREGACT: Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria

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Authors' contributions

UDA and JPV conceived the PREGACT trial. Data were collected by ZSH, TMC, VI and HT; RR supervised the conduct, monitoring and data management of the PREGACT trial; BB and RA suggested the idea of the paper. BB analyzed the data, and drafted the manuscript; All authors reviewed the manuscript and approved the version to be submitted.

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The funding institutions were not involved in designing the trial, and in collecting, analyzing, interpreting the data and in writing the manuscript.

Availability of data and materials

Data supporting the findings are not publicly available. Access could be granted upon motivated request to Tinto Halidou.

Ethics approval and consent to participate

The PREGACT study received approvals from- the Institutional Review Board of the Institute of Tropical Medicine, Antwerp, Belgium; the Ethics committee of the University Hospital, Antwerp, Belgium; the Institutional Ethics

committee of Centre Muraz and the Ethics committee of the Ministry of Health, Burkina Faso.
All the participants provided a written informed consent form before being enrolled in the study. Datasets were anonymized.

Consent for publication
Not applicable.

Competing interests
No conflicts of interest.

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Supplemental Table 1 Agreement and reliability of fetal biometrics z scores using Intergrowth 21st and Salomon equations

	Mean ± SD	p-value*	Median (IQR)	p-value†	Range
Biparietal diameter					
Intergrowth 21	-0.62 ± 0.74		-0.63 (-1.08 ; -0.15)		-3.40 ; 3.11
Salomon	-0.27 ± 0.65		-0.30 (-0.67 ; 0.16)		-2.68 ; 2.81
Difference	-0.35 ± 0.18	< 0.001	-0.39 (-0.45 ; -0.31)	< 0.001	-0.72 ; 0.33
LOA	-0.70 ; 0.00				
ICC	0.86				
Abdominal circumference					
Intergrowth 21	0.98 ± 1.06		0.88 (0.31 ; 1.46)		-2.07 ; 9.90
Salomon	0.31 ± 0.57		0.28 (-0.02 ; 0.59)		-1.37 ; 5.22
Difference	0.67 ± 0.54	< 0.001	0.57 (0.33 ; 0.90)	< 0.001	-0.70 ; 4.68
LOA	-0.40 ; 1.74				
ICC	0.56‡				

Supplemental Table 1 (continued): Agreement and reliability of fetal biometrics z scores using Intergrowth 21st and Salomon equations

	Mean ± SD	p-value*	Median (IQR)	p-value†	Range
Head circumference					
Intergrowth 21	0.75 ± 0.78		0.72 (0.24 ; 1.27)		-1.56 ; 4.24
Salomon	0.76 ± 0.57		0.71 (0.40 ; 1.14)		-0.94 ; 3.36
Difference	-0.01 ± 0.31	0.79	-0.06 (-0.21 ; 0.16)	0.11	-0.62 ; 1.01
LOA	-0.60 ; 0.59				
ICC	0.90				
Femur length					
Intergrowth 21	1.17 ± 1.00		1.27 (0.63 ; 1.77)		-2.44 ; 4.00
Salomon	0.27 ± 0.78		0.34 (-0.13 ; 0.75)		-2.54 ; 2.57
Difference	0.90 ± 0.24	< 0.001	0.92 (0.77 ; 1.05)	< 0.001	0.10 ; 1.53
LOA	0.43 ; 1.38				
ICC	0.64				

Supplemental Table 1 (continued): Agreement and reliability of fetal biometrics z scores using Intergrowth 21st and Salomon equations

* *Paired t test p value.*
† *Wilcoxon signed ranks test p value.*
‡ *Intraclass correlation calculation for abdominal circumference z scores excluded one participant with extreme values (9.9 using Intergrowth equations and 5.22 using Salomon equations).*
LOA: limits of agreement; ICC: intraclass correlation coefficient.

CHAPTER 6: DISCUSSION

6.1. Main results

Our study aimed at identifying pregnant women with the highest risk of malaria in pregnancy and adverse birth outcomes.
Placental malaria was highly prevalent and varied with maternal age. The prevalence of pooled placental malaria was higher in women under 30 with one or two previous pregnancies than in older women with the same gravidity. The prevalence of pooled placental malaria was also higher in women under 30 with anemia than in older anemic women. The active placental malaria was more frequently diagnosed in women with symptoms at inclusion when they were under 30 than in their older counterparts. In our cohort of pregnant women infected by *P.falciparum*, low birth weight, or preterm birth depended also on age, with a higher prevalence in newborns from adolescent mothers than in those from adults mothers. Anemic adolescents were more at risk of delivering low birth weight or preterm birth newborns than anemic adults. The association between adolescence and low birth weight or preterm birth was stronger when anemia and fever were present simultaneously. Accurate gestational age determination and subsequent fetal growth restriction and preterm birth identification are conditioned by the choice of the reference chart of fetal biometry to which measurements are compared. In our study, when abdominal circumference was used as a surrogate of fetal size, replacing Salomon's chart with that of the Intergrowth 21st study increased tenfolds the proportion of large fetuses.

6.2. Discussion

Pregnant women as a sentinel population for malaria transmission monitoring

Despite a substantial increase in the coverage of prevention and treatment commodities, malaria transmission remains intense in sub-Saharan areas in 2018 [1]. Placental malaria prevalence was 86.8 % in our study. This could be explained by the rural location of the study [2-4], and the inclusion of pregnant women with *P. falciparum* confirmed peripheral infection [2, 5, 6]. Prevalence exceeding 40 % was reported in Uganda in 2019 and 2020 and up to 69 % in Nigeria in 2012 regardless of the status of peripheral infection and location of the studies. These countries are concerned by the HBHI approach, [4, 7, 8]. Prevalence between 10.3 % and 22.4 % was reported in India in 2013 and 2014 [9, 10].

As malaria in pregnancy is mostly asymptomatic in these areas [11, 12] and prevalence correlates with that of the children [13, 14], some authors proposed to use this unrevealed parasite reservoir to monitor malaria transmission [2, 14-16].

Pregnant maintain the cycle of infection by carrying gametocytes and are probably the source of 10 % infection of mosquitoes [17, 18]. Placental malaria is present even if parasites are undetectable in the peripheral blood by routine tools [11, 19]. Therefore, a more complete estimation of the burden of malaria in pregnancy may include placental infection [16, 20]. However, a diagnostic method suitable for routine practice has to be found. Histological examination of placental biopsies the reference method cannot be performed at antenatal clinics, because it is costly, time-consuming, and need a high level of qualification [2]. Also, microscopy and rapid diagnostic tests using placental blood perform less than the histopathological method [21]. An alternative method could be non-instrumented nucleic acid -loop-mediated isothermal amplification (NINA-LAMP). As a nucleic acid amplification technic (NAAT), it performs better than rapid diagnostic tests and light microscopy, without needing many instruments, much time and energy as the other NAATs [22, 23].

Another aspect to take into account regarding placental infection diagnosis when using pregnant women for malaria transmission monitoring is the cultural and social representation of the placental itself. Most African societies consider that placenta is sacred. In a study conducted in Benin in 2018, the participants were afraid of losing their babies because they thought that the research staff members were selling the placentas for financial and/or

mystic practices. This situation escalated to choler and threats against the research team members after the death of a baby whose placenta was collected without the parents directly witnessing the procedure. Thus, without adequate communication and a trusty environment between health workers and the community, fears and rumors could prevent the collection of placental blood or biopsies for malaria diagnosis as it happened in Benin during this study [24, 25]. A way to avoid such a situation in sub-Saharan African countries was to effectively involve the key members of the community like the chief, the mayor, and the leaders of opinion as authentic partners throughout the steps of the implementation of new interventions. They could help their community to better understand and take ownership of the activities [26].
Temporal analysis of MiP transmission yielded a time lag of up to three months between the rainfall event and the culmination of infections in Burkina Faso in 2020 [27]. This means that infections may occur during the dry season. This assumption is supported by the peripheral parasitemia diagnosed in pregnant women in West African countries in 2018 and a higher prevalence of placental malaria in this season than in the rainy season in a multicentric study conducted in Benin, the Gambia, and Burkina Faso in 2019 [24, 28]. Therefore, monitoring malaria in pregnancy should be performed in both rainy and dry seasons.

The effect of the interplay of parity-specific and age-related immunity on the risk of placental malaria and its adverse birth outcome

Malaria risk is heterogeneous in pregnant women. First or second-time mothers are more susceptible to placental malaria as per the well-documented parity-specific immunity in high transmission settings [29-33]. Besides, maternal age has an important role in malaria risk in pregnancy [29]. Some studies conducted in Sudan in 2017 and Nigeria in 2018 to 2019 reported an increased risk of placental malaria in younger women up to the age of 24 than in older women [31-33]. The parity-specific immunity against placental malaria was overpassed when the entomological inoculation rate (EIR) exceeded 100 as demonstrated in a mathematical model in 2013 [34]. As EIR generally passed this threshold in most malaria-endemic countries in sub-Sahara Africa [35], pregnant women should face the same risk of placental malaria regardless of their parity. However, the interaction between age and gravidity found in our study suggests that this is plausible in older women which remain protected by immunity related to age. In younger women, parity-dependent immunity persists, with lower gravidities being more exposed. Thus, maternal age is an important factor to consider together with

parity either for monitoring or for implementing actions against malaria in pregnancy in endemic settings of sub-Sahara Africa [28, 30, 36]. National estimates of malaria come generally from surveys conducted among children under five [16]. An analysis done in 2015 found that in sub-Saharan African endemic settings, malaria prevalence in children was found to be closer to the prevalence in primigravidae than to the prevalence in multigravidae [20]. As placental malaria risk varied with age, we suppose that targeting young pregnant women with low gravidity may provide estimates of prevalence more in line with those obtained from children.

Targeting young mothers for malaria interventions is an important question in sub-Sahara.

There is a dangerous triad constituted by placental malaria, low birth weight or preterm birth and adolescence. Indeed, apart from the younger age, placental malaria was associated with low birth weight in Papua New Guinea in 2015, Sudan in 2019, and Nigeria in 2013 [11, 37, 38]. It was also associated with preterm birth in another study in Papua New Guinea and Uganda in 2017 [3, 11, 39]. Among young women, adolescent mothers gave birth to a higher number of babies with low birth weight or preterm birth [40, 41] than older mothers in East Africa and rural Uganda in 2020, in line with our findings. It was estimated in 2010 that newborns from teenage mothers represent more than half of the upcoming generation of adolescent mothers [42]. This supposes that the future generation of teenage mothers could be low birth weight or preterm birth infants, who grew with impaired health status and reduced social and economic potential [43, 44].

In Burkina Faso, 3 out 4 women of 18 – 24 years old live in rural areas where MiP is highly prevalent in studies conducted in 2006 and 2020 [45, 46]. Half of them get married and one-third gives birth by age of 18 according to the fifth round report of the performance monitoring and accountability project (PMA 2020) in 2017 [45]. This picture of the status of young girls could be inferred in sub-Sahara Africa [47, 48]. In 2019, the region accounted for about 218 million people aged 15 – 24 years with approximately half of them being women. This number is projected to increase to approximately 283 million individuals in 2030 [49]. This means that the number of pregnancies at higher risk of placental malaria and its negative consequences may increase if an effective malaria control strategy targeting this group of women was not implemented.

Anemia in young pregnant women: is there an interplay of MIP and undernutrition?

A systematic review and meta-analysis conducted in 2016 found a 42.7 % prevalence of anemia in pregnancy in low and middle-income countries [50]. An analysis of data from 1995 to 2011 showed that West Africa and South Asia were the most affected [51].

Our study reported on the interplay of maternal age and anemia in assessing their link with placental malaria and its adverse birth outcomes. Women aged less than 30 years with anemia were more at risk of placental malaria than older anemic women. This may be due to a convergence of multiple factors toward the occurrence of anemia including malaria and maternal nutritional status.

Apart from maternal age, placental malaria was also associated with anemia in stable malaria transmission settings of Papua New Guinea in 2017, Malawi in 2013, and Tanzania in 2015 [3, 52, 53].

MIP itself causes anemia through immunity reactions. During a MiP episode production of red blood cells decreases [54], while they are increasingly cleared in the spleen [55]. The complement is also excessively activated and this is involved in the biological mechanisms of both placental malaria and anemia [56]. The above-mentioned phenomena might be exacerbated in young women leading to an increased susceptibility to both placental malaria and anemia.

Apart from plasmodium, intestinal parasites were found associated with anemia in pregnant women in Ethiopia in 2019. *Ascaris lumbricoides* (24.9 %) and hookworms (11.2 %) were the main intestinal parasites retrieved in this study [47].

Malnutrition is an important contributor to anemia in pregnant women [55]. Statistics on malaria and undernutrition showed that they share the same geographical areas [57]. Failure to meet the increased maternal and fetal nutritional needs induced by pregnancy lead to both macronutrients and micronutrients insufficiency [58]. Micronutrient insufficiency is frequent in women of reproductive age. Anemia is one of the most predominant consequences due to iron deficiency in low and middle-income countries [59]. Younger pregnant women were reported to experienced more anemia than their older counterparts in studies conducted in Ethiopia in 2019, Ghana in 2019, and the Democratic Republic of Congo in 2015[60-62] regardless of the malaria infection status. Some authors hypothesized that nutrients deficiency is increased in young pregnant women because of higher nutritional demand in link with immature biological status [61, 63]. Pregnant

women lack macronutrients because they consume fewer fats, proteins, and carbohydrates [64].
Malaria and undernutrition probably interact [64]. Indeed, populations in low and middle-income countries including pregnant women suffer from both undernutrition and malaria [59, 64]. Nutrition interferes with immunity. It could modulate the capacity to regulate and eliminate infections like malaria in an individual [65]. In the reverse, an infection like malaria could drain the nutritional store as the protein destruction and energy consumption raise as a response to the inflammation [64, 66].
Therefore, the higher prevalence of placental malaria in young anemic women in our study might be an illustration of such an interplay of malaria and nutritional status in pregnancy in endemic settings.
Also, we assume that the higher prevalence of low birth weight and preterm birth in newborns from anemic adolescents mothers than newborns from adult counterparts in our study might follow the same reasoning. Our assumption is reinforced by the results of systematic reviews and meta-analyses conducted in resources constrained settings in 2013 and 2016 reporting that newborns from anemic pregnant women experience more low birth weight and preterm birth than those of nonanemic women [50, 67]. Also, common pathogenesis mechanisms like complement activation or a reduced flow of nutrients are seen in placental malaria, anemia, and low birth weight or preterm birth [68, 69]. Interestingly, a study conducted in Burkina Faso in 2015 found that the number of preterm birth and SGA peak in the rainy season coinciding with a rise in malaria cases and a descending trend in maternal body mass index (BMI) and mid-upper arm circumference towards their lowest annual values. The reverse was observed during the dry season [70].
Contrary, the association between underweight and placental malaria was not significant and pregnancy BMI did not affect the association between low birth weight or preterm birth in presence of anemia in our study. The relationship might be masked by the steady rise in BMI as gestation advances [71]. Thus, using this marker for assessing the association between placental malaria and nutritional status may be misleading. However, pre-pregnancy BMI is difficult to obtain in practice as most pregnant women initiate antenatal visits late in sub-Saharan Africa [64].
Interventions targeting both malaria and nutritional insufficiencies [64] would contribute to reducing the prevalence of anemia and its adverse birth outcomes in young pregnant women. Intermittent preventive treatment with sulfadoxine-pyrimethamine was shown to reduce effectively the prevalence of both placental malaria and anemia in a meta-analysis in 2014 [72]. Women who sleep under bed net are less prone to anemia in Rwanda in 2019 [73].

Iron supplementation benefits the mother by reducing the risk of anemia without exposure to an increased risk of peripheral infection [74] or placental malaria even if administrated to nonanemic women [75]. However, an increase in preterm birth was reported with supplementation with iron plus folic acid compared to folic acid alone in Burkina Faso in 2019 [76]. Meta-analyses conducted in 2017 and 2019 found that multiple micronutrient supplements (MMS) reduced the prevalence of low birth weight and possibly preterm birth [77, 78]. A study conducted in Bangladesh and Burkina Faso in 2019 showed that up to 20,000 deaths and 35,000 additional preterm birth could be prevented by substituting iron plus folic acid with multiple micronutrient supplements [79]. Macronutrient supplementation using lipid-based nutrients supplements prolonged gestation duration in anemic women with reduced BMI during the rainy season in Burkina Faso in 2015 [70].

Symptomatic malaria in young pregnant women increases the risk of placental infection and worsen the effect of anemia on the fetus

Our results showed that women aged less than 30 years and presenting with symptomatic malaria at inclusion were more at risk of active placental malaria. Other studies reported on the association between symptomatic malaria and placental malaria without assessing the effect of maternal age [3, 7].

Clinical symptoms occur in infected individuals with weak immunity because they are not able to regulate the proliferation of the parasites [80]. Fever means that the density of parasitemia is high exceeding the pyrogenic threshold [81] and that the tolerance fitness of the individual is overwhelmed [82, 83]. In our study, the prevalence of symptomatic malaria at inclusion was 45 % in women under 30 and 27 % in older women ($p<0.001$). This correlated with an increased prevalence of a peripheral density of at least 2000 parasites/µl in women under 30 than in older women (35 % versus 12 %, respectively, $p < 0.001$), consistent with previous studies [84]. Also, 72 % of symptomatic women had parasitemia of this density while none between asymptomatic women was diagnosed with such parasite load [Data not shown]. These observations suggest that women aged less than 30 years with weaker immunity were also more likely to have parasites reaching and sequestrating in their placenta than their older counterparts.

The risk of prolonged parasitemia was higher in patients presenting with severe anemia than in those without anemia. Malaria symptoms like fever correlate with parasitemia [81], suggesting that anemic patients are likely to experience fever. The transposition of this in MiP might be that pregnant

women with anemia are at risk of fever which in turn was associated with low birth weight [11]. Thus, our finding that the prevalence of low birth weight and preterm birth in newborns from anemic adolescents was exacerbated by fever is plausible.
Symptomatic malaria episodes in low immunity young pregnant women should benefit from rapid diagnostic confirmation and treatment to prevent evolution toward complication [85]. Young pregnant women could be of specific interest as their knowledge of malaria symptoms and treatment is poor. They delay antenatal visit initiation because of cultural and social beliefs. They are sometimes socially and financially dependent [86, 87].

Ultrasound in malaria in pregnancy studies: beyond the accurate assessment of fetal biometry.

Obstetrical ultrasound is increasingly used in studies on malaria in pregnancy in endemic countries. Fetal biometry measurements performed during the sonographic scan is used to accurately estimate gestational age and subsequently assess preterm delivery and fetal size or growth [12, 76, 88-93]. Most of these studies conducted in sub-Saharan Africa used nomograms from developed countries or set by default in the software of the ultrasound machine [94, 95]. As malaria and undernutrition can decrease the weight of the fetus, the use of such reference may result in wrong or biased estimates misleading the clinical decision [92]. Therefore some authors recommended customizing the references to the specific context [96-99].
Also, there is a lack of local recommendations or guidelines for the choice and use of a fetal biometric chart [100, 101]. This allows the use of multiple charts within the same country or health setting and may result in contradictory findings. Indeed in our study, the rate of large fetuses according to the AC z scores above the 90^{th} centile was multiplied by ten when the Salomon chart was replaced by the Intergrowth 21^{st} chart. In line with our results, a study comparing different charts in Congo in 2009 found that developed countries' charts overestimated the 10^{th}, 50^{th}, and 90^{th} centiles of the fetal weight compared to the local chart [102]. Another study conducted in Tanzania in 2012 showed that percentiles of the local chart were smaller than those of Congolese and "White population" charts. The latter overestimated the rate of small for gestational age newborns at the threshold of the 10^{th} percentile [103]. Ultrasound detection of macrosomic fetuses may help in planning the caesarian section to prevent the occurrence of obstructed labor and its negative consequences during delivery [94]. However, the results found in our study, in Congo and Tanzania suggest that the

appropriateness of the decision, the number of interventions, the workload for the medical personnel would depend on the reference chart used. Convergently, the amount of anxiety, the cost for the parents, and the share of the health budget of a government would depend on the choice of a reference chart.
Generally, ultrasound examination in a clinical trial or epidemiological study is performed within a quality assurance system by trained people with proper supervision [88, 104-106]. In routine practice, the lack of guidelines and recommendations in the context of poverty may allow the purchase and use of obsolescent equipment that would generate images of poor quality [94]. Also, unskilled or less qualified people could profit from this unregulated environment to performed sonographic examinations, possibly leading to overuse and misdiagnosis both harmful to pregnant women and their fetuses. Symphysis fundal height measurement, last menstrual period, and Ballard score were compared to obstetrical ultrasound for estimating gestational age and preterm birth at delivery. The correlation between each of these methods and ultrasound (the reference) ranged from low to modest in pregnant women with malaria attending antenatal visits in their second or third trimester in Ghana, Zambia, Malawi, and Burkina Faso. The authors concluded that among the four methods, symphysis fundal height measurement performed the best in detecting prematurity [104].

6.3. Limitations and strengths

Our study was quite was conducted in a rural environment and with data of pregnant women who are all infected by *P.falciparum*, limiting its generalisability. However, the results could be used to tailor actions and target specific groups at a district level [107]. As a secondary analysis, we are limited by the inclusion and exclusion criteria used in the primary clinical trial from which we took the data. The study did not exhaustively analyze key variables and this may influence the results.
Nevertheless, our study has some strengths. It addresses the interplay between the parity-specific and age-related immunity against malaria in pregnancy. It is relevant to the current approach recommended for malaria control in high transmission settings. Our study is with that of Cisse et al published in 2000 the rare studies addressing the concern of fetal biometric reference in Burkina Faso [108].
Our study aimed at exploring the extent of the age-related risk of malaria in pregnancy. Therefore, we set the cut-off of maternal age group at 30 years, overpassing that of 24 years commonly used for the definition of the status of

youth [109, 110]. However, we did not expect that the number of women aged at least 30 years with gravidity ≤ 2 would be too low (7) in our study as the total fertility rate in women aged more than 30 years was between 2.1 and 2.49 live births in 2017 [48]. Therefore, our results need to be confirmed by other studies using the same cut-off for maternal age but with a greater number of subjects in this specific group in other malaria transmission strata of Burkina Faso.

CHAPTER 7: CONCLUSION AND PUBLIC HEALTH KEY MESSAGES

7.1. Conclusion

The WHO and individual authors emphasize the need and use of quality data to guide the decision process and to develop malaria control and elimination strategies adapted to a specific area or populations more at risk [15, 107, 111]. Our study showed that even pregnant women are regarded as a group with a higher risk of malaria compared to the non-pregnant population, the level of risk is not the same for all of them. We showed that young pregnant women are more exposed to placental malaria. Consistently, we found also that among pregnant women who experienced malaria, young mothers, specifically, adolescents are more at risk of delivering low birth weight or preterm newborns. Accurate gestational age determination is the prerequisite for reliable estimates of preterm birth and fetal growth restriction, the main causes of low birth weight occurring in pregnancies affected by malaria. Our study pointed out the lack of agreement in evaluating fetal size using different biometric reference charts. In line with the approach currently proposed by WHO and its partners, young pregnant women could be a priority target for the control of malaria in pregnancy in sub-Saharan Africa with intense transmission settings like Burkina Faso. They could benefit from comprehensive healthcare including malaria and nutritional interventions. This would contribute to rapid progress toward malaria control and elimination [28, 36, 112]. A consensus regarding fetal biometry assessment is also needed to accurately address the relationship between adverse pregnancy outcomes and malaria in pregnancy in both clinical and research perspectives.

7.2. Public health key messages

The fight against malaria has attained a crucial step in sub-Saharan Africa. While an increasing number of countries in other regions are moving toward malaria elimination, in the former the indicators are either stagnating or regressing. Nowadays, opinions converge on a shift from a "one-size-fits-all" approach to strategies suited to the transmission pattern of a specific geographical area or targeting groups with the highest risk [15, 107, 113]. The initiative "from high burden to high impact " by the WHO aims at accelerating progress towards the reduction of malaria morbidity and mortality in the 11 countries sharing the greatest part of the global burden of malaria [113].

Pregnant women may provide useful data on malaria trends mostly in rural areas that are difficult to reach [20]. Their data could be used to stratify and plan the assignment of resources, to explore clusters of transmission, and to assess advancement toward elimination [16, 20]. However, we assume that estimates in young peaucigravidae might be more reliable than those of the whole group. If placental malaria should be taken into account in these estimates, appropriate diagnostic tools and socio-cultural factors should be addressed.

Excess risk of placental malaria and adverse birth outcomes in young mothers may be due to lesser use of preventive methods against malaria [114]. It is reported that the use of IPTp intake was associated with a decrease in low birth weight and preterm birth prevalence [53, 115, 116]. Therefore, a fast reduction of the transmission and burden of MIP [117] could be achieved by scaling up the uptake of key malaria interventions in adolescents [28] and peaucigravidae [118] pregnant women.

Screening for anemia as a surrogate of placental malaria will allow identification of those most at risk [52] like young pregnant women. This could be done concomitantly with malaria diagnosis using the same finger puncture at the antenatal visit [52]. Young pregnant women, mostly adolescents are more vulnerable and could benefit from specific attention [57, 63]. Strategies ensuring the provision of both macronutrients and micronutrients might be beneficial in areas where undernutrition is common [119] and in those most affected like young pregnant women. The research could continue exploring novel supplements like L-arginine [120].

The communities should benefit from health education including messages on the harmful effect of malaria in young pregnant women. The adolescent should be informed about the prevention and treatment of MiP [86, 87]. Prevention of early pregnancies could help to reduce the burden of malaria. This could be achieved by helping young girls accessing and remaining at

school. Education in turn would facilitate delaying first sex and first marriage. Also, the use of modern contraceptives could reduce the chances of the occurrence of early and undesired pregnancies [121].

Access to ultrasound services in obstetrics in sub-Saharan Africa is low, estimated to approximately 30 % in urban cities, and 6 % in rural settings [94, 122]. However, the advent of portable and cheap ultrasound devices might grant access to an increasing number of pregnant women mostly in rural African settings [94, 122, 123]. If the ultrasound assessment of fetal biometry is not framed within a national policy the concerns regarding mismatched estimates of gestational age and fetal size at different locations within the same country might increase as the number of women undergoing ultrasound scans rises. Conversely, the set up of guidelines and recommendations as part of comprehensive health care during pregnancy would harmonize ultrasound practice and the results yielded would be more reliable. This might contribute to optimizing the benefit toward those at the highest risk of malaria and its adverse fetal outcome.

The pandemic of COVID 19 may threaten the fragile gains of the fight against malaria in sub-Saharan Africa. An increase of 20 % in the number of cases and a twofold rise in the number of deaths would occur as a result of a concomitant decrease of 75 % in ITNs and antimalarials coverage [124, 125]. Pregnant women and children would be excessively affected by the indirect excedent mortality attributable to the disturbance in malaria control programs related to the pandemic of COVID-19 [124]. This suggests that appropriate action should be undertaken to protect young pregnant who may be more exposed to placental malaria while their fetuses would experience more adverse birth outcomes

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