Original Research Article

Assessment of vitamin D status in patients suffering from uncomplicated malaria in a health center in the District of Abidjan (Côte d'Ivoire).

Abstract:

Aims

The pathophysiology of *Plasmodium falciparum* infection is most often associated with anemia and immune deficiency. Given the important role of vitamin D in the synthesis of hemoglobin and in the stimulation of the immune system, it would be essential to assess the vitamin D status of patients with malaria in order to improve the quality of treatment management.

Methodology

A thick drop and a blood smear were used to determine parasite density and parasite species respectively. The complete blood count was performed using an automated analyzer labelled Sysmex XN 1000i. Biochemical parameters such as calcium and phosphorus were determined using the Cobas C311 Hitachi. The Vidaswas used to determine the concentrations of 25 (OH) -vitamin D.

Comment [LB1]: Please add space between words!
Use instead "A hormonology analyzer, VIDAS (Bio-Mérieux, France) was used to…."

Results

The results showed a decrease in 25 (OH) -vitamin D concentrations in relation to the parasite density and anemia observed in patients with uncomplicated malaria.

Conclusion

Vitamin D status in patients with uncomplicated malaria could therefore represent an essential biomarker in the monitoring of antimalarial treatment.

Keywords:*Plasmodium falciparum*, Vitamin D, Parasitology, Côte d'Ivoire

1.Introduction

Malaria is a real public health problem in Côte d'Ivoire, it is the leading cause of morbidity with 43% of the reasons for consultation in the country's medical care facilities with an incidence of 155 ‰ in the general population and 291 ‰ in children of less than 5 years [1]. The fight against malaria is one of the

Comment [LB2]: Replace with "under 5 years of age"

eight Millennium Development Goals (MDGs) of the Millennium Declaration signed in 2000 by more than 180 countries and institutions whose goal was to control malaria infection in 2015 [2].

The pathophysiology of malaria infection is most often associated with symptoms such as fever, anemia, which can lead to organ failure, which is often severe and fatal in the case of *Plasmodium falciparum*. Immunocompromised peopleand children are the most exposed to malaria morbidity and mortality [3,4]. The recent discovery of the physiological role of vitamin D in cell immunity, differentiation and proliferation justifies a growing interest in this hormone. Indeed, numerous studies have shown that vitamin D plays an important role in the regulation of the immune system and can therefore potentially protect against infections [5, 6]. The presence of receptors for this vitamin in all types of cells and tissues, especially in immune cells such as macrophages, lymphocytes and epithelial cells, allows this vitamin to exert these immunostimulatory actions.

However, secondary hyperparathyroidism-induced inhibition of erythropoiesis caused by increased production of 1,25-dihydroxivitamin D may be the cause of the anemia observed in patients with uncomplicated malaria.

Therefore, the objective of this study is to provide a good understanding of the mechanisms underlying malaria infection in order to help improve the medical management of this pathology.

2.Material and methods

2.1 Study site, type and population

This is a prospective study for experimental purposes carried out within the department of medical and fundamental biochemistry of the Pasteur Institute of Côte d'Ivoire (IPCI) from September 25 to October 12, 2020. It focused on one hundred (100) cases,comprised of fifty (50) patients suffering from uncomplicated malaria and fifty (50) controls without malaria in outpatient consultation in a Community-based Urban Health Center (CSU-COM) in the District of Abidjan. The ages of the patients ranged from twelve (12) to sixty-five (65) years.

2.2 Inclusion and non-inclusion criteria

Anyone who gave informed consent to participate in this study was included in the study. Diabetics, hypertension and pregnant women were not excluded in the study population to avoid biasing the results.

2.3 Materials

The biological material consists of serum and EDTA whole blood from malaria patients and non-malaria controls subject.

The technical equipment consists of an automated hematology analyzer, the Sysmex XN-1000i for the complete blood count (CBC), a biochemical automated, the Cobas C311 (Roche Diagnostic, France) for the determination of calcium and phosphorus, a hormonology analyzer, VIDAS (Bio-Mérieux, France) for the determination of 25 (OH) D and a microscope for reading thick drops.

Vitamin D, calcium, phosphorus and CBCassay kits and GIEMSA for slide staining were used to perform this study.

2.4 Methods

Blood sample of 5 ML were taken from selected patients at the elbow, drop into the EDTA (EthylenDiamine Tetra-Acetic Acid) tubes with purple caps for the determination of the hematological parameters and tubes without anticoagulant with red cap for the assay of vitamin D. The tubes with red

Comment [LB3]: Sentence reformulation (grammatical errors)!

Comment [LB4]: Please add space between words!

Comment [LB5]: Please delete “one hundred”, “fifty” and use only numbers!

Comment [LB6]: Use only numbers!

Comment [LB7]: “people with hypertension”

Comment [LB8]: “ml”

caps were centrifuged at 3000 rpm for 5 minutes, then aliquots were constituted with the sera and stored at -20 ° C until the actual assay of vitamin D and phosphocalcic balance.

For the Thick Drop (GE), using a micropipette, a 15 µL drop of blood was taken into EDTA tubes and placed on clean glass slides. With the tip of the slide, the drop was spread onto another slide in circular motions from the center outwards to make a drop 2 cm in diameter. The blood on the slide was dried at room temperature and stained with Giemsa stain diluted 1/10 for 15 minutes [7]. The coloring was gently rinsed with tap water to prevent detachment of the GE and then dried at laboratory temperature. Using immersion oil, the slides were viewed under a microscope with a × 100 objective to determine the parasite density which corresponds to the number of asexual forms of the parasite on 200 white blood cells with the rate of 8,000 leukocytes / mm^3 as standard rate.

To perform the blood smear (FS), a 5 µL drop of blood was applied to the end of a slide using a micropipette. Then, a second slide was placed in contact with the drop of blood at an angle of inclination of 45 degrees and then the blood is spread in the dihedral thus formed. The spreader is pushed quickly and evenly, while keeping the same inclination, and gradually raised at the end of the spread. The smear is stopped about 1 to 2 cm from the other end of the slide. The FS was dried at room temperature then fixed with methanol. Then, the entire surface of the slide was covered with Giemsa diluted 1 / 10th for 15 min. The slide was gently rinsed with tap water and then dried at laboratory temperature. Using immersion oil, the slides were viewed under a microscope at × 100 objective to determine the species of plasmodia.

Hematological markers such as hemoglobin level (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin content (TCMH) were determined using Sysmex XN -1000i, Japan.

The vitamin D assay is a quantitative test for measuring total 25 (OH) D in human serum and plasma. This assay was carried out using an automatic device, the VIDAS® (BioMérieux), the principle of which is based on the immuno-enzymatic method by competition with a final detection in ELFA fluorescence (Enzyme Linked Flueorescent Assay).

Biochemical parameters such as calcium and phosphorus were determined using the Cobas C311.

2.5 Statistical analysis

The data obtained were processed and the graphs produced using Excel software version 2010 Graph Pad prism 5. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The difference is significant when p-value <0.05.

3. Results

The study included 100 participants, 50 of whom had uncomplicated malaria and 50 negative controls. The sex ratio was 32 for females and 18 for males in both patients and controls. The study population was divided into five (5) age groups namely [12-15 years], 17 patients or 34%; [16-24 years old]. 12 patients or 24%; [25-34 years], 09 patients or 18%; [35-49 years], 07 patients 14% and [50-65 years], 05 patients or 10% both in patients with uncomplicated malaria and in non-malarial controls.

The results of vitamin D status of the study population were presented according to age group, type of anemia and parasite density for malaria patients and phosphocalcic balance.

Regarding the age groups (**Table I**), the youngest, that is to say [12-15], presents the highest rates of vitamin D deficiency, i.e. 67% in patients and vitamin deficiency. D (33%) in controls.

Table I: Distribution of vitamin D status according to age

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Comment [LB10]: µl

Comment [LB11]: Explain what is GE!

Comment [LB12]: 8.000

Comment [LB13]: "was"

Comment [LB14]: "was"

Comment [LB15]: You can delete this part!

Comment [LB16]: Reformulate the sentence!

Comment [LB17]: Is not mandatory this explanation!

Comment [LB18]: Use instead "50 with uncomplicated malaria and 50 individuals included in the control group."

Comment [LB19]: "control group"

Comment [LB20]: Delete this!

Comment [LB21]: Reformulate like this "[12-15 years] - 17 patients (34%); [16-24 years old] - 12 patients (24%); [25-34 years] - 9 patients (18%); [35-49 years] - 7 patients (14%) and [50-65 years] - 5 patients (10%)."

Comment [LB22]: Table 1

Comment [LB23]: category [12-15] years

Comment [LB24]: Reformulate this sentence (there are grammatical mistakes)!

Comment [LB25]: Table 1

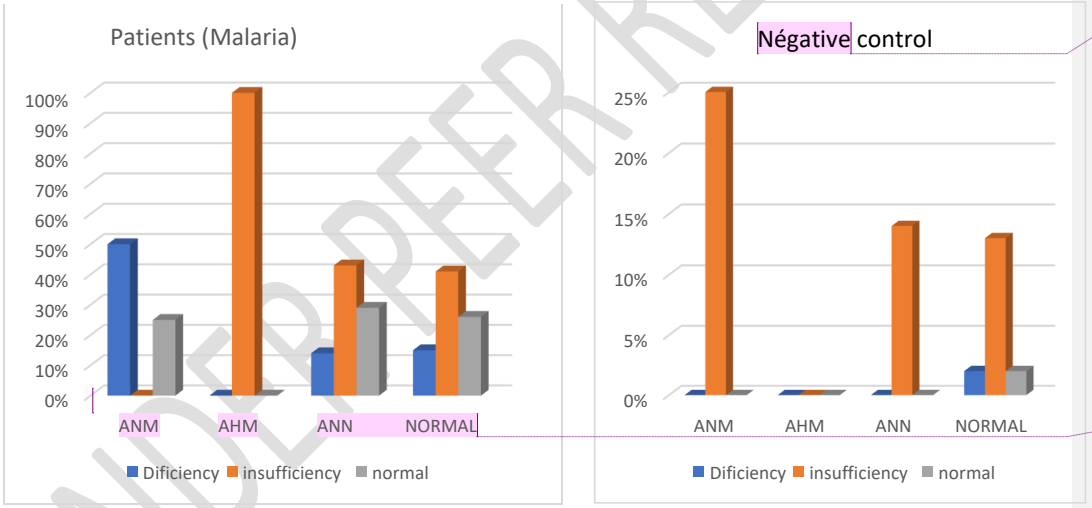
	PATIENTS				Control				
Age (yrs)	D	I	N	T	C	I	N	T	TG
[12-15]	67%	0%	0%	67%	0%	33%	0%	33%	100%
[16-24]	11%	53%	26%	89%	0%	5%	5%	11%	100%
[25-34]	20%	40%	30%	90%	0%	10%	0%	10%	100%
[35-49]	15%	38%	15%	69%	8%	23%	0%	31%	100%
[50-65]	0%	40%	40%	80%	0%	20%	0%	20%	100%
TG	**17%**	**42%**	**25%**	**83%**	**2%**	**13%**	**2%**	**17%**	**100%**

D = deficiency; I = insufficiency ; N = normale ;T = total; TG = total général

Comment [LB26]: Use * D = deficiency; I = insufficiency ; N = normale ;T = total; TG = total general

Based on hemoglobin level, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) values, anemias are classified as microcytic normochromic anemia (MNA), microcytic hypochromic anemia (MHA) and normochromic normocytic anemia (NNA). The results showed a predominance of vitamin D insufficiency with microcytic hypochromic anemia in patients and microcytic normochromic anemia in the controls. However, most of the patients are just under 50% presenting with normochromic microcytic anemia and vitamin D deficiency (**Figure 1**).

Comment [LB27]: "control group"



Comment [LB28]: "Negative"

Comment [LB29]: "MNA", "MHA", "NNA"

Figure 1 : Distribution of vitamin D status according to the type of anemia

Comment [LB30]: "Fig. 1."

The parasite density was divided into two classes: one of 1-80 trophozoites / µL of blood and the other of 80 trph / µL and above. According to the results obtained, the class of 80 trophozoites / µL of blood and above is the most represented in all the statuses: Deficiency, insufficiency and normal. The results showed that even with a high parasitemia, some patients had normal vitamin D status (**Figure 2**).

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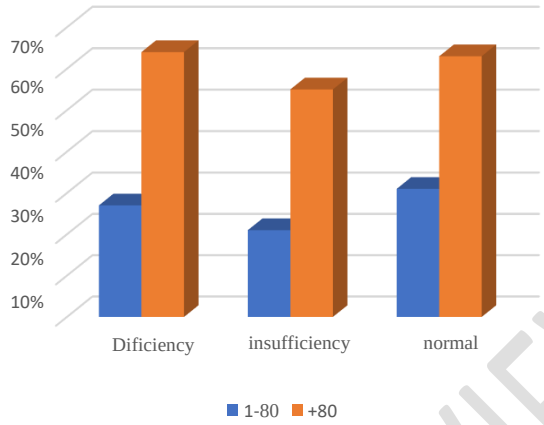

Figure2 : Distribution of vitamin D status according to parasite density

Comment [LB34]: "Fig. 2."

Regarding the phosphocalcic balance, the calcium and phosphorus concentrations did not vary according to the pathological condition and regardless of the age group (**Table II**).

Comment [LB35]: "Table 2"

Table II: Distribution of calcium and phosphorus concentrations.

Comment [LB36]: "Table 2"

	PATIENTS		CONTROL		
Age (yrs)	**Calcium concentrations (mg/L)**	**Phosphorus concentrations (mg/L)**	**Calcium concentrations (mg/L)**	**Phosphorus concentrations (mg/L)**	**p-value**
[12-15]	92 ± 1,42	40 ± 2,01	93 ± 1,03	41 ± 1,29	P > 0,05
[16-24]	91 ± 2,23	42 ± 1,15	92 ± 1,53	42 ± 0,31	
[25-34]	94 ± 0,87	41 ± 1,32	94 ± 0,46	43 ± 0,78	
[35-49]	95 ± 0,54	42 ± 0,79	93 ± 1,42	42 ± 0,89	
[50-65]	93 ± 1,94	43 ± 0,98	95 ± 1,36	42 ± 1,53	

4. Discussion

Comment [LB37]: The authors could look for other similar studies to compare the results!

The very young age of the study population shows that adolescents and young people are the most exposed to malaria infection [3, 4]. During this study, the results showed a high prevalence of hypovitaminosis D in the studied population and would affect 73% of this population of which 75% would present a deficiency and 25% with a 25 (OH) D deficiency. Hypovitaminosis D is not strongly related to age because all age groups are concerned. However, 25 (OH) D insufficiency and deficiency are found mainly in the relatively young population with a prevalence of 23% for the 25-35 age group, 22% for 16-24 years and 18% for 35-49 years. Indeed, children and young people constitute a group at risk of hypovitaminosis D. In growing children, vitamin D deficiencies are frequent, puberty also constitutes a period at risk of vitamin D deficiency. A study has shown that nearly 25% of adolescents aged 10 to 15 years have a 25 (OH) D deficiency (level less than 10 ng / ml) related to the increased demand for skeletal

calcium [8]. Therefore, vitamin D supplements would represent the method of choice for achieving optimal vitamin D status in infants and children. In subjects older than 50 years of age, no vitamin D deficiency has been observed. In this same age group, only 5% presented a vitamin D deficiency. These results are in contradiction with the data published by many authors affirming that people over the age of 70 are more exposed to hypovitaminosis D [9, 10]. These data could be explained by a diet richer in vitamin D and increased sun exposure of this category of people [11,12].

The study of the relationship between vitamin D status and different types of anemia showed that anemic malaria patients mostly had vitamin D deficiency and vitamin D insuffiency 5 times higher than in controls. This would indicate that an insufficiency or a deficiency in vitamin D would expose to anemia during the malaria infection. Indeed, in view of its important role in maintaining bone homeostasis, in recent years there has been particular interest in the regulatory capacities of vitamin D on several major physiological systems including the immune system [13, 14]. The majority of cells in the immune system have been shown to express the specific vitamin D receptor mainly after activation. It has also been shown a link between vitamin D and hemoglobin, one of the hypotheses focused on the effect of vitamin D during the production of red blood cells by the hematopoietic bone marrow or the ability of this vitamin to regulate the immune inflammation, which is a known catalyst for anemia [15]. This observation could explain the normochromic or microcytic hypochromic anemia observed in malaria patients. Likewise, another study found a link between vitamin D and anemia. Indeed, children with a high level of vitamin D are less likely to suffer from anemia than those with an insufficient level. To determine this relationship between vitamin D and anemia, the authors followed 9,400 children aged 2 to 18 and measured their hemoglobin level. The results showed that the lower the vitamin D level, the lower the hemoglobin level and the higher the risk of anemia. In children with vitamin D levels below 20 ng / mL, the risk of anemia is increased by 50% compared to children with vitamin D above 20 ng / mL. In addition, this study also showed that any increase of 1 ng / mL of vitamin D would reduce the risk of anemia by 3% [16]. In subjects with uncomplicated malaria, vitamin D deficiency is 65% compared to almost zero deficiency in negative controls. This result shows that the higher the parasite density, the more vitamin D deficiency is increased, thus showing a link between the increase in the number of trophozoites in the blood and the decrease in serum vitamin D concentrations. These results agree with those obtained by **Luong and Nguyen** [17]. Indeed, in the case of severe *falciparummalaria*, mild hypocalcemia is common and simultaneously associated with an excessively low serum parathyroid hormone (PTH) level [18]. Many studies have suggested a role for vitamin D in the pathophysiology of malaria. Thus, the mortality rate in mice infected with *Plasmodiumberghei* was improved by the addition of cod liver oil or vitamin D and dicalcium phosphate to quinine [19]. In addition, high levels of vitamin D reduced the ability of *P. berghei* to penetrate the erythrocyte membrane [20]. Also, vitamin D and its derivatives inhibited the intraerythrocytic growth of *P. faciparium in vitro*[21]. In addition, **Ray et al.** [22] showed more increased expression of the vitamin D receptor (VDR) in patients with *P. vivax* malaria. They suggested some association between VDR polymorphism and disease severity. These results suggest that there is a relationship between vitamin D and malaria. Apart from these cases of pathology causing serum vitamin D deficiency, there is also a functional deficiency, which is frequently hereditary. It is currently accepted that a whole panel of genes influence the metabolism of vitamin D, since they lead to changes in vitamin D receptors, transport molecules, but also enzymes necessary for the conversion of vitamin D [23]. Some chronic diseases have been shown to be frequently associated with characteristic genetics, including polymorphic vitamin D receptors, which interfere with the exploitation of calcium. People in this situation must have significantly higher levels of vitamin D in their blood in order for it to be able to perform its functions properly in the body [24]. At the same time, these circumstances may explain the link between vitamin D and many pathologies. Also, the liver being the seat of the first hydroxylation in position 25 of vitamin D, all severe liver pathologies, reducing liver function by more than 90%, will prevent sufficient synthesis of 25 (OH) D, [25]. The phosphocalcic balance did not undergo any significant variation, which

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would suggest that hypovitaminosis D would not have affected the phosphocalcic metabolism of patients suffering from uncomplicated malaria.

5. Conclusion

Knowledge about Vitamin D has progressed in recent years as evidenced by the very large number of recent publications on the subject. Any deficiency or insufficiency in vitamin D has many consequences in several pathologies, mainly bone. It is therefore important to know the prevalence of hypovitaminosis D. The results of our study reveal that a large part of the population is deficient and therefore requires vitamin supplementation to reach normal levels. However, a cautious approach is advised before recommending the use of vitamin D in the treatment of malaria. It would therefore be interesting to have more in-depth studies on the entire metabolic pathway of this vitamin from its synthesis to its biological action to better understand the level of interaction between this vitamin and uncomplicated malaria infection.

Ethical approval

The patient'sselections were carried out in accordance with the provisions of the 1964 Declaration of Helsinki and the relevant regulatory provisions. Informed consent was required for each participant or parents or legal guardians of the adolescents. A consent form was signed by them before any inclusion in the study.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Comment [LB40]: Correct the grammar mistakes! Reformulate the whole discussion part!

Comment [LB41]: This sentence is not a conclusion!

Comment [LB42]: Missing: **Acknowledgments**
A brief acknowledgment section may be given after the conclusion section just before the references. The acknowledgments of people who provided assistance in manuscript preparation, funding for research, etc. should be listed in this section. All sources of funding should be declared as an acknowledgment. Authors should declare the role of the funding agency, if any, in the study design, collection, analysis and interpretation of data; in the writing of the manuscript. If the study sponsors had no such involvement, the authors should so state.

Comment [LB43]: Before "References"

AUTHORS' CONTRIBUTIONS
Authors may use the following wording for this section: " 'Author A' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author B' and 'Author C' managed the analyses of the study. 'Author C' managed the literature searches…… All authors read and approved the final manuscript."

CONSENT (WHERE EVER APPLICABLE)
No manuscripts will be peer-reviewed if a statement of patient consent is not presented during submission (wherever applicable). This section is compulsory for medical journals. Other journals may require this section if found suitable. It should provide a statement to confirm that the patient has given their informed consent for the case report to be published. Journal editorial office may ask the copies of the consent documentation at any time.
Authors may use a form from their own institution or Patient Consent Form 1.0. It is preferable that authors should send this form along with the submission. But if already not sent during submission, we may request to see a copy at any stages of pre and post publication.
If the person described in the case report has died, then consent for publication must be collected from their next of kin. If the individual described in the case report is a minor, or unable to provide consent, then consent must be sought from their parents or legal guardians.
Authors may use the following wording for this section: "All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by ...

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Comment [LB44]: Respect the requirements of the journal and correct all references!