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Study of the prevalence of *Plasmodium* infections by the polymerase chain reaction (PCR) among patients with severe anaemia treated at a rural hospital in southern Ethiopia

Prevalencia de infección por Plasmodium *mediante la reacción en cadena de la polimerasa (PCR) en pacientes con anemia grave atendidos en un hospital rural de sudeste de Etiopía*

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SUMMARY

This study was conducted to determine the current burden of *Plasmodium* infections among patients with severe anaemia attending a rural hospital in southern Ethiopia. A total of 111 patients with severe anaemia (hemoglobin < 7 mg/dL) were included. The *Plasmodium* species causing the infection were identified using a Semi-nested Multiplex PCR. The mean age of the study population was 15 years and 26 (23.4%) individuals tested positive for malaria. Of these, 18 (16.2%) were infected by *P. falciparum*, 4 (3.6%) by *P. vivax*, and 4 by *P. ovale*. No significant associations between the species of *Plasmodium* and the sex of the patient or the haemoglobin values were found. This study showed that *Plasmodium* infections cause severe anaemia in one in four cases.

Key words: Anaemia, *Plasmodium*, Malaria, PCR.

RESUMEN

El estudio se ha llevado a cabo para conocer el impacto de la infección por especies de spp Plasmodium entre los pacientes con anemia grave atendidos en un hospital rural del sudeste de Etiopía. Se incluyeron en el estudio 111 pacientes con anemia grave (hemoglobina < 7 mg/dL). La infección por Plasmodium spp. se llevó a cabo mediante una PCR Semi-nested Multiplex. La media de edad de la población de estudio fue de 15 años; 26 pacientes presentaban infección por Plasmodium spp. (23.4%): 18 (16.2%) fueron para P. falciparum, 4 (3.6%) para P. vivax, and 4 (3.6%) para P. ovale. No encontramos asociación entre el tipo de Plasmodium con el sexo y el los valores de hemoglobina. Este estudio reveló la importancia que la infección por Plasmodium es responsable de la anemia grave en uno de cada cuatro pacientes evaluados.

Palabras clave: *Anemia,* Plasmodium*, Malaria, PCR.*

Anaemia remains a major public health problem affecting about a quarter of the world's population (Haidar, 2010. *J Health Popul Nutr.* **28:** 359-368). Anaemia can result from non-nutritional factors, such as haemorrhage, infection, chronic disease states, or drug toxicity, and from nutritional ones, including deficiencies of iron, certain vitamins, copper, and protein (Zhang *et al.*, 2003. *J Natl Cancer Inst*. **95:** 373-380). Moreover, parasitic diseases, including helminthic infections and *Plasmodium falciparum* and *P. vivax*, have long been recognized as important contributors to anaemia in endemic countries (McDevitt *et al.*, 2014. *Curr Hematol Rep*. **3:** 97-106).

The light microscopy examination of blood smears is still considered the gold standard for laboratory diagnosis of malaria (WHO: *World Malaria Report*, 2008). It is relatively cheap and allows for the quantification of parasitaemia. Polymerase chain reaction (PCR) is a technique that is more sensitive and specific than light microscopy, particularly in situations of low-level parasitaemia (0.7-0.02 parasites/μL) (Hermsen *et al.*, 2011. *Mol Biochem Parasitol.* **118:** 247-251).

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This research was conducted to determine the current burden of *Plasmodium* infections among patients with severe anaemia attending a rural hospital in southern Ethiopia.

A prospective cross-sectional study was performed at Gambo General Rural Hospital from September 2013 to May 2014. Patients with severe anaemia < 7 mg/dL were included. The hospital is a 150-bed rural general hospital located in the West-Arsi zone, 250 km south of Addis Ababa. Most of the population lives in a rural setting and works in agriculture and farming. It is situated at an altitude of 2,200 meters. However, patients can come from endemic areas situated at lower altitudes. The patients with severe anaemia gave informed verbal consent. Pregnant women were excluded. A sample of blood was taken from each patient on filter paper (Whatman© 3MM) for diagnostic confirmation by Semi-nested Multiplex PCR (sPCR) at the National Centre of Tropical Medicine, Institute of Health Carlos III, Spain. Blood samples were stored at 4ºC and transported at room temperature in double zip-lock plastic bags. DNA extraction was performed on the filter paper samples using commercial kits (Speedtools tissue DNA Extraction Kit, Biotools). Haemoglobin levels were determined by laboratory technicians using a portable digital haemoglobin meter (Hemo Control, EKF-diagnostic GmbH, Barleben/Magdeburg, Germany). This study was carried out under anonymous conditions and ethics committee approvals were obtained from the local Research and Publication Committee of the GRH.

A total of 111 patients with anaemia were included. The median age of this investigation population was 15 years (range: 6 months - 65 years; interquartile range: 3-28); 47 (43.3%) were male and 64 (57.7%) were female. The median haemoglobin was 4.7 (range 1.6-6.9; interquartile range: 3.9-5.7). Of 111 cases studied, 26 were positive for malaria (23.4%; 95% confidence interval [CI]: 16.1-32.1%), 18 (16.2%; 95% CI: 10.5-24.1%) were so for *P. falciparum*, 4 (3.6%; 95% CI: 1.4-8.9%) for *P. vivax*, and 4 for *P. ovale*. Patients with *P. falciparum* infection and severe anaemia are younger than those with other *Plasmodium* infections and those with no *Plasmodium* infections (p=0.08, Kruskal-Wallis Test). There was no significant association of malaria and with sex, and the haemoglobin value (Table I).

Plasmodium falciparum and *P. vivax*, have long been recognized as important contributors to anaemia in endemic countries (McDevitt *et al.*, 2014. *Curr Hematol Rep.* **3:** 97–106). So, Molineaux suggested that 'total' falciparum malaria mortality due to anaemia for malaria in Africa is likely to be twice as high as 'direct' malaria mortality (Molineaux, 1997. *Ann Trop Med Parasitol.* **91:** 811-825). Moreover, malaria with anaemia are significant risk factors for poor early childhood neurodevelopment in malaria-endemic areas in rural Africa (Boivin *et al.*, 2016. *Malar J.* **15:** 210). It is a important relevance of association.

This study is relevant because it showed that malaria infections diagnosed by PCR methods cause severe anaemia (< 7 mg/dL) in one of four cases. The PCR technique allows diagnosing more cases of malaria than the conventional light microscopy technique and it could be implemented (Orm *et al.,* 2014. *Bol Mal Salud Amb*. **54:** 95-99). However, other

Table I. Age, gender, and haemoglobin according to *Plasmodium* infection.

	P. falciparum	*P. vivax* and *P. ovale*	No *Plasmodium* infection	Total
Median age (IQR)*	6.5 (0.95-20)	17.5 (1.5-33.75)	20 (3-30)	15 (3-28)
Age group				
<5 years (%)	7 (38.9)	3 (37.5)	22 (25.9)	32 (28.8)
5-18 years (%)	6 (33.3)	1 (12.5)	19 (22.1)	26 (23.4)
>18 years (%)	5 (9.4)	4 (50)	44 (51.8)	53 (47.7)
Sex				
Male (%)	8 (44.4)	4 (50)	35 (41.2)	47 (42.3)
Female (%)	10 (55.6)	4 (50)	50 (58.5)	64 (57.7)
Median haemoglobin (IQR)	4.9 (4-5.8)	3.85 (3.45-4.5)	4.8 (4-5.8)	4.7 (3.9-5.7)

IQR: interquartile range

*Patients with *P. falciparum* are younger than those with other *Plasmodium* infections and without *Plasmodium* infections (p=0.08, Kruskal-Wallis Test).

causes can influence in severe anaemia as nutritional ones including deficiencies of iron, certain vitamins, copper, and protein or parasitic infections. This research has a limitation that it did not evaluate other causes of anaemia, such as helminthic infections, vitamin deficiency, and so on.

In our area one of four cause of severe anaemia is due to *Plasmodium* infection by molecular methods, the introduction of rapid diagnostic tests for initial screening reduce the number of false negative of the conventional light microscopy technique. The strategy of the control of malaria based on better prevention, diagnosis and treatment for this disease implemented by government of Ethiopia set in 2011 (Alemu *et al.,* 2012. *Parasit Vectors*. **5:** 173) will confirm a progressive reduction in incident malaria and after that the episodes of severe anaemia.

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Conflict of interest

The authors report no conflicts of interest with this study.

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