

# Vitamin B12 and vitamin B9 (folate) status in Togolese schoolchildren

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## ABSTRACT

**AIM** To determine the status of vitamin B12 and vitamin B9 (folate) and assess the contribution of these vitamins to anaemia in Togolese children.

**METHODS** Red blood cell parameters, vitamin B12 and vitamin B9 were measured in 155 schoolchildren aged 6-15 years old.

**RESULTS** Mean values of vitamin B12 and vitamin B9 were  $623.83 \pm 263.35$  pmol/L and  $10.76 \pm 4.36$  nmol/L respectively. There were no cases of vitamin B12 deficiency. Forty children had a high level of vitamin B12  $>800$  pmol/L. Twenty four children were found to be deficient in vitamin B9 but their haematological profile was dominated by hypochromic microcytic anaemia suggesting iron deficiency anaemia rather than megaloblastic anaemia.

**CONCLUSION** This study suggests that vitamin B12 and vitamin B9 are not important causes of anaemia in Togolese children.

**Keywords:** Togo; children; vitamin B12; vitamin B9; anaemia

## INTRODUCTION

Anaemia is a widespread public health problem with major consequences for human health as well as economic and social development. The causes of anaemia are often complex. <sup>1</sup> World Health Organisation (WHO) estimates the number of anaemic people worldwide to be about two billion people and that approximately 50% have iron deficiency anaemia. <sup>1</sup> Vitamins B12 and B9 (folate) are both required for the synthesis of DNA in the nucleus of the red blood cells and their deficiency causes a macrocytic anaemia. The magnitude of the contribution of vitamin B12 and vitamin B9 to global prevalence of anaemia is largely unknown. <sup>1</sup> In Togo, like in many sub-Saharan African countries, we systematically give folic acid in combination with iron to children. The usefulness of this approach is unknown.

The objective of this study was to determine the status of vitamins B12 and B9 and assess the contribution of these vitamins to anaemia in Togolese children.

## METHODS

This descriptive cross-sectional study was conducted from April 23 to May 4, 2007 at Immaculate Conception of Nyekonakpoe Catholic primary school located in one of the most populous areas of Lomé (capital of Togo). The target population was all students attending that institution. Out of 12 classes in the school, we randomly selected three classes; all children in the selected classes whose parents provided verbal consent for the study were included in the study. All children were healthy as assessed by a paediatrician. Blood samples were drawn by venipuncture into

two 5 ml vacutainer tubes: 5 ml of EDTA tube for determination of haemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) and 5 ml of a plain red-top tube for the determination of vitamin B12 and B9 levels. Serum from the plain red-top tube was immediately separated by centrifugation, aliquoted and frozen at -20°C. Red blood cell parameters were done at the haematology laboratory in the Tokoin Teaching Hospital (CHU Tokoin de Lomé) and vitamin B12 and B9 levels were done at the laboratory INSERM 724/UMRS 954 Faculty of Medicine, University Raymond Poincaré Nancy (France).

The different parameters studied were: socio-demographic characteristics (age, sex, parents' socio-economic status), nutritional status, full blood count parameters, and vitamin B12 & B9 levels. Parents socio-economic status was categorised into three groups on the basis of established scores: low (3-4), medium (5) and high (6) according to the existence or not of electricity, running water, owner or tenant of the dwelling house; each item was coded as 2 if it was present and 1 if it was absent (for example, a dwelling house owner with running water and electricity was classified as having a high socio-economic level score of 6). Nutritional status was assessed from the weight and height of children according to WHO child growth standards 2006.

<sup>2</sup> Full blood count parameters of interest were Hb level, MCV and MCH; these were measured with the automated blood cell counter Sysmex KX21. Anaemia was defined as Hb level less than 11.5 g/dl for children aged 6-11 years, Hb level less than 12g/dl for children aged 12-14 years and girls aged 15 years, and Hb level less than 13g/dl for boys aged 15 years. <sup>3</sup> Vitamin B12 and vitamin B9 levels were measured by radioisotope method with the kit MP BIO ref. 06B264806, lot SNBK 0731 / exp. December 19, 2007 (vitamin B12 range of detection was: 74 – 1480 pmol/L and vitamin B9 range of detection was: 2.3 – 45 nmol/L); the reference values used were validated by the biologists responsible for the study laboratory: normal range for B12 level was 800 <B12>150 pmol/L, and serum folate of <7 nmol/L was indicative of deficiency.

We performed statistical analysis with Epi Info version 3.5.1.2008. All our variables were normally

distributed. The t-test was used to compare means. A p-value of <0.05 was considered statistically significant.

The study was approved by the primary education committee in the commune of Lomé, the school management and the Parents Committee of the Immaculate Conception of Nyekonakpoe Catholic primary school. Informed consent was obtained from the parents of each child participating in the study.

## RESULTS

One hundred and fifty five children, 80 girls and 75 boys participated in the study. The mean age of the study participants was  $7.73 \pm 1.57$  yr (Table 1). The majority (61%) of the parents of the participants had low socio-economic status. Nutritional status was good for 130 children, 25 children had malnutrition (3 over-nutrition, 22 under-nutrition). The mean haemoglobin was  $12.01 \pm 1.21$  g/dl (range 6.30 - 14.20 g/dl). Sixty seven children (43%) were anaemic, all of them had low MCVs and low MCHs (hypochromic microcytic anaemia).

The mean value of vitamin B12 was  $623.83 \pm 263.35$  pmol/L (range 163.00 - 1550 pmol/L). It was  $616.52 \pm 253.68$  pmol/L for girls and  $631 \pm 274.80$  pmol/L for boys. This difference was not statistically significant ( $p=0.87$ ). No cases of vitamin B12 deficiency were noted. Vitamin B12 serum values were above the upper limit of normal (800 pmol/L) for forty children (26%). The mean values of vitamin B9 were  $10.76 \pm 4.36$  nmol/L (range 4.20 - 27.20 nmol/L),  $11.44 \pm 4.15$  nmol/L for girls and  $11.10 \pm 4.58$  nmol/L for boys. This difference was not statistically significant ( $p=0.38$ ). Twenty four children were found to be vitamin B9 deficient, 14 girls and 10 boys. Their mean age was  $7.79 \pm 1.56$  years (Table 2). The mean haemoglobin level among vitamin B9 deficient children was slightly lower (11.42 g/dl) than those of non vitamin B9 deficient children (12.11 g/dl) but this difference was not statistically significant ( $p=0.05$ ).

## DISCUSSION

As there are few studies on vitamin B12 and vitamin B9 in African children, this study was designed to

**Table 1. Characteristics of study participants stratified by gender**

Parameter		Boys (n=75)	Girls (n=80)	Total (n=155)	p value
<b>Age (yr)</b>	Mean	7.70 ± 1.59	7.79 ± 1.55	7.73 ± 1.57	
	Range	(6.00- 15.00)	(6.00 - 15.00)	(6.00 - 15.00)	
<b>Weight (kg)</b>	Mean	21.12 ± 4.50	22.81 ± 6.40	22.00± 5.61	
	Range	(11.00 -35.00)	(14.00 - 46.00)	(11.00 - 46.00)	
<b>Height (cm)</b>	Mean	120.80 ± 8.69	121.86 ± 10.26	121.35 ±9.52	
	Range	(101.00 - 150.00)	(101.00 - 150.00)	(101.00 - 150.00)	
<b>Vitamin B12 (pmol/L)</b>	Mean	631.64 ± 274.80	616.52 ± 253.68	623.83 ± 263.35	NS
	Range	(163.00-1550.00)	(186.00 - 1120.00)	(163.00- 1550.00)	
<b>Vitamin B9 (nmol/L)</b>	Mean	11.10 ± 4.58	10.44 ± 4.15	10.76 ± 4.36	NS
	Range	(4.20 -27.00)	(4.30 - 22.20)	(4.20 - 27.20)	
<b>Haemoglobin (g/dl)</b>	Mean	11.88 ± 1.12	12.12 ± 1.28	12.01 ±1.21	NS
	Range	(6.70 - 13.60)	(6.30 - 14.20)	(6.30-14.20)	
<b>MCH (pg/gr)</b>	Mean	25.70 ± 3.23	26.74 ± 2.62	26.24 ± 2.97	p= 0.029
	Range	(14.70 - 34.60)	(18.00 - 32.20)	(14.70 - 34.60)	

MCH, Mean corpuscular haemoglobin.

**Table 2. Characteristics of vitamin B9 deficient participants**

Parameter		Total (n=24)
<b>Age (yr)</b>	Mean	7.79 ±1.56
	Range	6.00-11.00
<b>Vitamin B12 (pmol/L)</b>	Mean	595.16 ±274.00
	Range	186.00-1082.00
<b>Vitamin B9 (nmol/L)</b>	Mean	5.64 ± 0.83
	Range	4.20 - 6.80
<b>Haemoglobin (g/dl)</b>	Mean	11.43 ± 1.96
	Range	6.30 - 14.20
<b>MCV (fl)</b>	Mean	75.69 ± 8.60
	Range	53.60 - 90.00
<b>MCH (pg/gr)</b>	Mean	25.80 ± 4.22
	Range	14.70 - 34.60

MCV, mean corpuscular volume; MCH, Mean corpuscular haemoglobin

explore for the first time in Togo, the status of these two vitamins in schoolchildren. The predominance of girls in our study is likely related to the general population in Togo where 52% are women (48% men),<sup>4</sup> it could also be the result of the policy of promoting girls' education.<sup>4</sup>

The haemoglobin levels similar to those found in our study were also found 20 years ago in African schoolchildren in the Western Cape.<sup>5</sup> Because the anaemia was predominantly hypochromic microcytic in our study together with the high rate (43% of children) of anaemia, the anaemia in these children is likely to be related to iron deficiency caused by malnutrition and intestinal parasites.<sup>1</sup> We however cannot exclude abnormalities of haemoglobin particularly haemoglobin C.

The levels of vitamins B12 and B9 in our study were similar to those found in young Ugandans.<sup>6</sup> There are also differences between our findings and other studies in vitamin B12 values owing to differences in study populations involving adolescents<sup>7,8</sup> and young people.<sup>6</sup> These values also depend on nutritional status and genetic factors such as polymorphisms and target enzymes which are different in different countries and different geographic areas.<sup>9,10</sup> In Greece, Panpadreou<sup>11</sup> studied the same school age population and concluded that both vitamin B12 and vitamin B9 decreased markedly with increasing age. The

difference between this finding and the findings of our study may be because our study had lower numbers of children over 10 years of age in addition to geographical differences.

We found no cases of vitamin B12 deficiency. However, it is well known that severe deficiency can exist without low levels of vitamin B12. We did not measure homocysteine and/or methyl malonic acid levels in our study. These would be useful in the diagnosis of vitamin B12 deficiency where serum B12 levels were normal.<sup>12</sup> Forty children (26%) were found to have high vitamin B12 levels above the upper limit of normal of 800 pmol/L. The contributing factors to the increased values require further study.

Only twenty-four children were found to have low vitamin B9 levels in our study. It is well known that erythrocyte concentrations of vitamin B9 show more of the state of body stores of folate, nevertheless, the haematological profile of these children like all anaemic children in the study was dominated by hypochromia and microcytosis favouring iron deficiency. This suggests, as also concluded by Lamparelli and Margo<sup>5,13</sup> that the folate deficiency did not contribute to the aetiology of the anaemia.

The reasons why this is so require further study to include peripheral blood smear examination, iron studies, possible role of malaria in reducing folate levels, and the role of genetics in folate deficiency.<sup>10, 14</sup>

## CONCLUSION

This study shows that vitamin B12 deficiency is not common among schoolchildren in Togo and that vitamin B9 deficiency, although common, may not be important in the aetiology of anaemia in this population.

## FOOTNOTES

**Conflicts of interest:** The authors declare no competing conflicts of interest.

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