Reliable Determination of Anaemia Prevalence, among 2175 Children Admitted to Campus University Hospital of Togo

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Summary

INTRODUCTION

Anaemia prevalence among children under 5 years old, in the world in 2011 according to WHO was at 62.8%. WHO-AFRO in 2015 stated that in most Africa countries, laboratory infrastructures and testing quality remain in budding stages. The purpose of this study was to ensure the accuracy of the haemoglobin test, for a reliable estimation of the anaemia prevalence among children with comparison of this prevalence to some previous national data.

MATERIALS AND METHODS

This retrospective descriptive study was performed from May 2017 to February 2018 on quality control and patient's outcome registers, at the Campus University Hospital Laboratory. The accuracy of The Sysmex XN-1000® was evaluated by using the Clinical Laboratory Improvement Amendments criterion. The anaemia prevalence was assessed with WHO anaemia criteria.

RESULTS

The accuracy conformity rate of haemoglobin test was 100%. The prevalence of anaemia, ranging from 50.2% to 68.6%, was higher among children aged of 12 to 14. The highest prevalence of severe anaemia (22.8%) as well as moderate anaemia (29.1%) was observed among 5 to 11 years children.

CONCLUSION

The Sysmex XN-1000® provides accurate haemoglobin test results. The anaemia prevalence in 2018 among children admitted to Campus University hospital and the previous ones are still high.

Key Words: Sysmex XN-1000®, Haemoglobin, children, Lomé.

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Introduction

Defined as a decrease in haemoglobin concentration below the reference values, anaemia is a global and common public health issue in Africa. According to the World Health Organization (WHO), in 2011, the prevalence of anaemia in Africa was 62.8% among children under 5 years of age [1]. The main cause of this anaemia was nutritional deficiencies frequent among children in Africa, and often associated with helminthiasis and/or malaria [2]. So, the alert to the increase in anaemia prevalence among children should motivate not only medication, but also anaemia nutritional management.

In Togo, this alertness is published by the teaching hospitals including the Campus University Hospital. With 24-beds paediatric service with 10 cradles, the Campus University Hospital also offers laboratory services which include a clinical haematology consultation unit where 30% of children admitted monthly were monitored for haemoglobin level, for the study purpose. WHO-AFRO in 2015, stipulated that in most African countries the laboratory infrastructure and test quality remain in nascent stages [3]. It was therefore necessary to ensure that the automated analyser Sysmex XN-1000® gives accurate haemoglobin test results to avoid risk of inadequate care or unnecessary transfusion [2,4]. The purpose of this study was to ensure the accuracy of the haemoglobin test results provided by the Sysmex XN-1000®, for a reliable estimation of the anaemia prevalence among children and a comparison of this prevalence to some previous national data.

Materials and Methods

This retrospective descriptive study was performed from May 08th, 2017 to February 23rd, 2018 over quality control and patient's outcome register, at the Campus University Hospital Laboratory Departments. The study involved 2,175 children aged up to 14 years old, tested for haemoglobin level and admitted from June to December 2017. Also 3 schemes of proficiency testing results performed from April to October 2017. The available national data on anaemia prevalence from 1990 to 2018 were found from google search engine by targeting PubMed and WHO publication.

Sampling and Ethical Aspects

Medical laboratory scientists at the laboratory, trained in good phlebotomy practice, performed the collection of venous whole blood samples in EDTA anticoagulant (Ethylenediamine Tetra-Acetic Acid) tubes. The study was carried out in accordance with the bioethics standards of the Declaration of Helsinki and in accordance with ISO 15189:2012 requirements [5,6]. It was not necessary to obtain the agreement of the national ethics committee for this study.

Measurement with the Sysmex XN-1000® Automated Analyser

The XN-1000® automated device manufactured by Sysmex corporation (Japan) was used to measure haemoglobin concentrations with Sysmex reagents produced in Germany. This analyser performs a quantitative measurement of haemoglobin by using Sodium Lauryl Sulfate (SLS), a cyanide-free reagent that lyses the erythrocytes. Hydrophilic groups of SLS bind to haem and form a stable coloured complex (SLS-Haem) which is tested using a spectrophotometric method. The optical density is measured at 633 nanometres (nm) by a photosensitive sensor and is inversely proportional to haemoglobin concentration of the sample. A dilution 1:747 of the sample with CELLPACK (diluent used with Sysmex automated haematology analysers) that is performed with SLS-Hb method minimizes interferences caused by a probable turbidity in the sample.

This method has been tested in accordance with the Haemiglobincyanide reference method (Cyanmethaemoglobin, HiCN), an international standard method to determine haemoglobin concentrations in blood sample [7,8]. This analyser was used in the study as reference equipment for the assessment of the prevalence of anaemia.

Criteria for Evaluating the Accuracy of the Measurements

The exploitation of the results of 3 External Quality Assessment (EQA) schemes conducted in 2017 has helped to determine the compliance for accuracy using the performance criteria (average of participants in the peer group ± 7.0%) established by the scientific organisation, CLIA (Clinical Laboratory Improvement Amendments) [9,10]. This EQA service was offered by the Canadian organisation "Oneworld Accuracy®" which sent five (5) samples of different levels of concentrations for testing. The values obtained with the Sysmex XN-1000® automated analyser were compliant if they were within the range of acceptable values of the CLIA. The requirements for determining the target value were in accordance with the requirements of ISO 17043 [11] and ISO 13528 [12]. To define the acceptable level of accuracy, the criterion "compliant rate of results from 5 samples ≥ 80%" defined by WHO-AFRO was used [3].

Criteria for the Diagnosis of Anaemia

The prevalence of anaemia has been established according to the WHO criteria (See Table 1). Haemoglobin concentrations are expressed in g/dl with their equivalent in mmol/l.

Statistical Analysis

Microsoft's Excel® 2016 spreadsheet was used to record the data. These data were analysed by SPSS 21 software (IBM 3.4 version 2018) for the determination of means ± standard deviation (for data with normal distribution determined by the Kolmogorov-Smirnov test) as well as the calculation of CVs. This same software was used to determine prevalence expressed in prevalence (%) with the confidence intervals (CI) at 95%. The main non-parametric statistical tests used were: The Chi-square test and the Fischer's exact test if necessary and the Student Mann-Whitney U test for the plot of differences. The comparison of the prevalence of the other studies with those obtained in our study was computed with the software R version 3.5 (Foundation for Statistical Computing-Vienna, 2018). The 2-prop Z-test was used. The null hypothesis was that the p found in our study does not differ from that of the comparison authors. The significance level used was 5%.

RESULTS

Accuracy Confirmation of the Reference Analyser, XN-1000®

The Campus University Hospital Haematology Laboratory Department participated in 3 EQA schemes. The results are presented in Table 2. The table also presents the level of accuracy of the haemoglobin test results.

Table 2 shows a compliance rate of 100% accuracy level for the 15 samples submitted, compared to CLIA performance specifications.

Determination of the Prevalence of Anaemia on XN-1000®

The study population recruited was 0 (6 months) to 14 years of age. The prevalence of anaemia across age groups was 50.2%; 58.7% and 68.6% respectively among children of 0 to 4 years old; 5 to 11 years old and 12 to 14 years old. Among children aged of 12 to 14 years, the prevalence of anaemia was higher in girls (72.3%) than in boys (64.8%).

Table 3 presents the prevalence of the various grades of anaemia: mild, moderate and severe. It also presents demographic information for each age group and averages of haemoglobin levels.

Three publications about anaemia prevalence in Togo were registered. Table 4 presents their anaemia prevalence and type of study. It shows the p-value of prevalence comparison between our study and each of them.

Discussion

The verification of the analytical performance of the XN-1000® analyser was necessary to meet the requirements stipulated in paragraph 5.5.1.2 of ISO 15189 [6]. Determining the prevalence of anaemia with reliable equipment enables appropriate public health decision-making. Our results showed a 100% accuracy level of the XN-1000® for the haemoglobin test compared to the CLIA specifications for the quarterly EQAs of March, July and October 2017. The highest prevalence of severe anaemia (22.8%) as well as moderate anaemia (29.1%) was observed among children aged 5 to 11 years. Twenty-five (25.5%) of children aged 6 to 59 months (1/2 to 4 years) had moderate anaemia. Among children aged 12 to 14 years, the prevalence of anaemia was higher in girls (72.3%) than in boys (64.8%). The haemoglobin tests realised to study the prevalence of anaemia were performed from May to December 2017, a period during which the performance of the XN-1000® was confirmed to be 100% compliant. Also, the requirement of a compliance rate of at least 80% over two successive WHO-AFRO schemes has been met.

The comparison of each prevalence by age group, obtained in the 3 studies [14,15,16] with those obtained in our study shows that the previous studies were focused on children under 5 years of age. The 5-11 and 12-14-years' age groups whose prevalence of anaemia in our study was 58.7% and 68.6%, respectively, were not taken into account. These school age groups were also at risk of nutritional deficits in iron, vitamins B9, B12 and vitamin A; very often involved in these anaemias [2]. Tariku et *al.* in 2017 in Ethiopia found among 391 children aged 6 to 14, recruited from schools, a prevalence of 37.3% [17]. A lower prevalence than that found in our study (58.7 to 68.6% respectively in children 5-11 and 12-14 years old). In fact, the children in our study were recruited from the hospital and therefore targeted already sick children. Muthayya *et al* in 2007 in India, among boys and girls aged 5 to 15 years and schooled in the Bangalore district, with twice-yearly administration of Albendazole 400mg and vitamin A (200,000 IU) found a lower prevalence. Among girls, the prevalence of anaemia was 13.6% and it was 12.0% among boys [18]. Concerning children under the age of 5, the prevalence in the study by Dop et *al*., in 1992 in Togo was low because it only involved 25 new-born children ($p < 0.001$) [14]. The prevalence found by Aboubakari *et al*. in 2014, from a representative demographic study was higher ($p < 0.001$) [16]. All of these studies used the WHO criteria. The study of Aboubacar et *al.* showed that our study should be extended to children from other towns in Togo.

Anaemia was observed among more than half of the 2,175 children aged of 0 to 14 years, admitted to the Campus University Hospital (50.2 to 68.6%), but at various grades and ages. The low rate of 50.2% was observed in children under 4 years of age. This low rate could be due to the sharing of long-acting insecticide-treated nets (LLINs) by the National Malaria Control Program (PNLP: Programme National de Lutte contre le Paludisme). The prevalence of severe anaemia was thus lower in these children aged 0 to 4 years (7.90%) compared to 22.80% in children aged 5 to 11 years and 22.30% in those aged 12 to 14 years old. In 2008 in Sudan, Sanou et *al.* found a similar prevalence of 5.3% severe anaemia among children of 0-5 years of age [19]. On the other hand, a study carried out in Tanzania in 2013 by Rehemah et *al.* among 448 children under the age of 5 years admitted to the hospital stated a prevalence of severe anaemia of 27.7% with 33.0% of moderate anaemia. The factors associated with these prevalence levels of

anaemia were the presence of malaria and sickle cell disease [20]. In Cape Verde, West Africa, another similar study among 993 children aged 0 to 4 years, detected an overall prevalence of 51.8% of anaemia compared to 50.2% in our study. This study showed that children living in poor households were having more risk of developing anaemia with vitamin deficiencies: vitamins A or retinol (found in fish oil, liver, butter, egg yolk), Vitamin B9 or folic acid (found in vegetables, bowels, meat, egg yolk) and Vitamin B12 or Cobalamin (found in meat, liver, milk, animal products) [21,22]. In Togo, according to the World Bank, the poverty rate was 55.1% in 2015 [23]. This high rate could explain such a high prevalence of anaemia among children.

A study carried out in India in 2016 by Ritu *et al*. in hospital among children aged 2 to 12 years old, detected a prevalence ranging from 44.0% to 66.0% according to WHO criteria. This study highlighted that these rates were associated with food habits and various parasitic infestations [24]. A next study will assess the nutritional habits of anaemic children with the aim of nutritional education. Also, the types of anaemia with socio-demographic, nutritional (micronutrient deficient) and associated infections factors should be assessed. The measurement of iron, ferritin, folates and serum albumin concentrations should be combined with an assessment of the family's eating habits.

Conclusion

This study demonstrated that Sysmex XN-1000® analyser provides accurate haemoglobin test results. It also demonstrated that the prevalence of anaemia in pre-schoolers and schoolchildren admitted to the university hospital is high, and that this situation requires further investigation for effective management. The comparison of this study with some preliminary studies to assess the prevalence of anaemia carried out in Togo; shows that it is essential to also give attention to children aged 5 to 14 in order to promote the management of anaemia in schoolchildren. This will help to reach an improvement in their academic results.

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Conflict of Interest

The authors declare that they have no conflict of interest with respect to the publication of this article.

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Appendix

Table 1: Haemoglobin Concentrations in Anaemia [13]

	Mild anaemia		**Moderate anaemia**		**Severe anaemia**	
Population	**g / dl**	**mmol / l**	**g / dl**	**mmol / l**	**g / dl**	**mmol / l**
Children 0-4 years	10.0-10.9	550.0-599.5	7.0-9.9	385.0-544.5	<7.0	<385.0
Children 5-11 years	11.0-11.4	605.0-627.0	8.0-10.9	440. 0-599.5	<8.0	<440.0
Children 12-14 years	11.0-11.9	605.0-654.0	8.0-10.9	440.0-599.5	<8.0	<440.0

Table 2: Accuracy Level of the Haemoglobin Measurement Test with the XN-1000®

N° Samples	Values XN-1000® (mmol/l)	Participating Laboratories (n)	Assigned Value (mmol/l)	Acceptable Limits (mmol/l)	Conclusion
EQA March 2017 A	1001.0	12	962.5	891.0-1034.0	Compliant
EQA March 2017 B	330.0	12	321.2	291.5-346.5	Compliant
EQA March 2017 C	764.5	11	73.3	682.0-786.5	Compliant
EQA March 2017 D	1006.5	11	963.6	891.0-1034.0	Compliant
EQA March 2017 E	770.0	11	737.0	682.0-792.0	Compliant
EQA July 2017 A	335.5	17	331.1	308.0-357.5	Compliant
EQA July 2017 B	753.5	17	735.9	682.0-792.0	Compliant
EQA July 2017 C	3410	17	334.9	308.0-357.5	Compliant
EQA July 2017 D	979.0	17	959.2	891.0-1028.5	Compliant
EQA July 2017 E	990.0	17	960.9	891.0-1028.5	Compliant
EQA October 2017 A	737.0	18	728.2	676.5-781.0	Compliant
EQA October 2017 B	995.5	15	961.9	891.0-1034.0	Compliant
EQA October 2017 C	1006.5	17	968.0	896.5-1039.5	Compliant
EQA October 2017 D	753.5	17	728.2	676.5-781.0	Compliant
EQA October 2017 E	335.5	17	322.9	297.0-346.5	Compliant

* Conversion rate: mmol/l = g/dl x 55

Table 3: Prevalence of Anaemia in Children

Age groups	N-Gender (M/F)	Age (M±SD) Year	Mild anaemia			Moderate anaemia			Severe anaemia		
			%	CI (95%)	M±SD g / dl	%	CI (95%)	M±SD g / dl	%	CI (95%)	M±SD g / dl
0-4 years (n= 994)	535/459	2.1±1.2	16.8	14.7-19.1	10.5±0.3	25.5	22.8-28.1	8.6±0.8	7.9	6.3-9.7	5.8±1.2
5-11 years (n = 898)	479/418	7.6±1.9	6.8	5.2-8.6	11.2±0.1	29.1	26.1-32.0	9.5±0.9	22.8	20.1-25.6	6.6±1.1
12-14 years (n = 283)	142/141	12.9±0.8	17.7	13.2-22.5	11.51±0.3	28.6	23.8-34.4	9.4±0.9	22.3	17.2-27.2	6.6±1.2

Conversion rate: mmol/l = g/dl x 55, M ± SD: Mean ± Standard deviation, CI: Confidence interval, N-Gender: Number of males / females

Table 4: Comparison of Different National Prevalence of Anaemia

Age groups	Our study			Dop *et al*, 1992 [14]				WHO, 2005 [15]				Aboubakari *et al*, 2014 [16]			
	n	%	Study	N	%	Study	P	n	%	Study	p	N	%	Study	p
0-4 years	994	50.2	Retrospective	125	30.0	Cross-sectional	<0.001	1030	52.4	Regression-based	0.3444	2890	70.9	Retrospective	<0.001
5-11 years	898	58.7	Retrospective	No available data				No available data				No available data			
12-14 years	283	68.6	Retrospective	No available data				No available data				No available data			