

1 Serum vitamin A content among malnourished and healthy children in Kisangani City, DRC

2
3 **Summary:** Vitamin A is an essential micronutrient needed by the body for various physiological
4 functions. Its deficiency is associated with several functional disorders. The objective of this study is
5 to determine blood vitamin A levels in malnourished and healthy children.

6 **Methods:** It is a cross-sectional analytical study, consisting of determining the vitamin A content in
7 the blood of children suffering from malnutrition and those in good nutritional status. Our sampling
8 was casual and 59 children aged 6 to 59 months were retained. Among them 30 healthy children
9 chosen from those attending preschool consultation at U HC and 29 malnourished children from those
10 followed at M TNU for the management of malnutrition. The serum vitamin A assay was performed
11 according to the method described by Tietz. Children with serum vitamin A level below $30\mu\text{g} / 100\text{ml}$
12 had vitamin A deficiency and those with a serum level greater than or equal to $30\mu\text{g} / 100\text{ml}$ had good
13 vitamin A status. Percent, average and standard deviation calculations were performed. The Chi square
14 statistical test was used to compare serum vitamin A content in healthy and malnourished children, as
15 well as other maternal parameters for a significance level of 0.05.

16 **Results:** from 59 children examined, 30 or 50.8% were 6-17 months old, the average age was $21.9 \pm$
17 13 months. 45.7% had a serum retinol level between 50-59 $\mu\text{g}/100\text{ml}$; the average value was $46.84 \mu\text{g}$
18 ± 14.27 . The prevalence of VAD was 20.3% and this deficit was more marked in children aged 6-17
19 months that is 50% ($P < 0.14$). Among 12 children with VAD, 10 or 83.3% were the wealthy and two
20 were the malnourished, the difference was statically significant ($P < 0.011$).

21 **Conclusion:** Vitamin A deficiency remains a major health problem in the DRC. This affect all
22 children regardless of their current nutritional status. Supplementation with this vitamin remains one
23 of the palliative solutions.

24 **Key words:** Serum vitamin A, Malnourished and healthy children, Kisangani-DRC.

25 Abbreviations

26 U HC: Umoja Health Center; M TNU: Mwana Mpendwa Therapeutic Nutritional Unit
27 DRC: Democratic Republic of Congo; VAD: Vitamin A Deficiency; UNIKIS: University of
28 Kisangani; ACF: Action contre la faim.

30 Introduction

31 Vitamin A is an essential micronutrient that the body needs to provide the various physiological
32 functions. Its deficiency is associated with several functional disorders [1].

33 Malnutrition remains a major public health problem in sub-Saharan Africa [2]. It occurs in children
34 under five, pregnant women and even in adults and is often accompanied with vitamin A deficiency
35 (VAD) [3]. According to WHO estimates, severe acute malnutrition affects 19 million children under
36 5 years of age and causes approximately 400,000 child deaths each year [4]. About 190 million
37 preschool children, most of whom live in Africa and South-East Asia, are deficient in vitamin A (low
38 plasma retinol levels) and may be considered clinically or sub-clinically deficient in vitamin A. [5] In
39 sub-Saharan Africa, more than 38.5% (> 56 million) of children suffer from chronic malnutrition,

40 nearly 80% have a martial anemia; the risk of blindness due to vitamin A deficiency is 50% and more
41 than 4 million children are born annually with low weight [6].

42 VAD is more common between 6 to 36 months of weaning. [7] Agne and al [8], found a prevalence of
43 15.2% among children aged 6-23 months. This prevalence was 6.2% in pregnant women (serum
44 retinol level $<35 \mu\text{g} / \text{dl}$ or $<0.7 \mu\text{mol} / \text{L}$) [9]

45 In Democratic Republic of Congo (DRC) a national survey among children aged 6 to 36 months on the
46 serum vitamin A content showed that 61.1% of children had a retinolemia rate of less than $0.7 \mu\text{mol} / \text{L}$
47 [10]. This rate indicated a serious situation and had placed the DRC among the countries most affected
48 by vitamin A deficiency in Africa [11].

49 Vitamin A plays several roles in human body: it is involved in growth, in the renewal of epithelial
50 tissues, in the reproductive system, in vision and in the strengthening of immunity; it is an antioxidant
51 [12,13]. The deficiency of this vitamin causes blindness and xerophthalmia, it is an aggravating factor
52 of certain diseases such as respiratory infections, measles, diarrhea, protein-calorie malnutrition and
53 various dermatological lesions [14].

54 Vitamin A supplementation has been shown to reduce malnutrition by 23-30% [14] and infectious
55 diseases in children [15,16]. Since 2000, the DRC Ministry of Health has integrated vitamin A
56 supplementation into routine primary health care activities within the Health Zones by organizing
57 mass activities as well.

58 However, several unknowns persist and provoke interrogations despite the attempts to respond: the
59 proportion of children with an abnormal vitamin A serum level among the so-called "healthy"
60 children, the age most affected by this deficiency, the family structure of these children and the socio-
61 economic conditions of the children parents. The objective of this study is to determine vitamin A
62 blood levels in malnourished and healthy children.

63

64 **Material and methods**

65 Our study took place in the city of Kisangani, Tshopo Province, located in the North-East of the DRC,
66 precisely in Umoja Health Center (U HC) and Mwana Mpendwa Therapeutic Nutritional Unit (M
67 TNU) in Kabondo urbano-rural health zone. The serum vitamin A assay was done at the Biochemistry
68 Laboratory of the Faculty of Science, Kisangani University (UNIKIS).

69 **Material**

70 During our analyzes we used the spectrophotometer, the glass test tubes, the centrifuge (HETTICH
71 brand), the precision balance (KERN EW brand), the syringes, the gloves, and some reagents, namely:
72 the ethanol solution and the petroleum ether solution.

73 Our study population consisted of malnourished children being monitored in the Mwana Mpendwa
74 Therapeutic Nutrition Center and healthy children continuing pre-school counseling sessions at the
75 Umodja Health Center during the study period.

76 **Methods**

77 It was a cross-sectional analytical study, consisting of determining the vitamin A content in the blood
78 of children suffering from malnutrition and those in good nutritional status during the period of our
79 study which took place from 05 July to 25 September 2018.

80 Our sampling was casual. Healthy children were selected from those received at U HC for the
81 preschool consultation and malnourished from those followed at M TNU for the management of
82 malnutrition.

83 Was included in the study any child aged 6 to 59 months whose well informed mother has given the
84 agreement for the child's blood test; excluded were any child aged 6-59 months whose mother had not
85 given her consent. A total of 60 women accepted this deal. The sample consisted of 30 malnourished
86 children and 30 healthy children. In the samples taken from the malnourished, one became insufficient
87 after centrifugation to be analyzed (less than 1 ml of serum) and reduced our samples to 29 for
88 malnourished children giving a total of 59 samples.

89 Data collection was done using a card containing the child's anthropometric parameters: age, sex,
90 weight, height, nutritional status and socio-demographic parameters of the mother: Age, level of
91 education, main occupation.

92 **Serum dosage of vitamin A**

93 After blood collection, approximately 3 ml were collected in a syringe and brought to the laboratory
94 for direct analysis.

95 The serum vitamin A assay was done according to the method described by Tietz [17].

96 Put 1ml of serum in a centrifuge tube, add 2ml of 95% ethanol and 2ml of petroleum ether. Stir
97 vigorously for 10 minutes then take 1 ml of the ether phase, that is to say supernatant liquid and put it
98 in the spectrophotometer vat, then read the absorbance at 440 nm against the petroleum ether.

99 The carotenoid content in the serum was determined as follows:

$$100 \mu\text{g of carotenoids} / 100 \text{ ml} = \frac{\mu\text{g carotenoid} / \text{vat} \times \text{dilution} \times 100}{\text{ml of serum}}$$

101
102 After the determination of carotenoids in the serum, evaporate the ether phase in the water bath
103 between 40 to 45 ° C, dissolve the residue by adding 1 ml of a 20% solution of antimony trichloride.

104 Read at 620 nm using antimony trichloride as blank.

$$105 \text{The content of vitamin A in 100 ml of serum} = \frac{\mu\text{g vitamin A} / \text{vat} \times \text{dilution} \times 100}{\text{ml of serum}}$$

$$106 \mu\text{g vitamin A} / 100 \text{ ml Serum} = \mu\text{g vitamin A} / \text{vat} \times 200 - (0.075 \times \mu\text{g carotenoid} / 100 \text{ ml.})$$

107
108 The nutritional status assessment was based on nutritional software, ENA of SMART 2011 shared by
109 ACF. The classification of nutritional status was made according to WHO 2006 standards which
110 expresses the weight/ height ratio of children in standard deviation or z-score. If this report is:

- 111 ○ <-3 z-score or edema: the child suffers from severe acute malnutrition
- 112 ○ <-2 z-score and $\geq - 3$: the child suffers from moderate acute malnutrition and
- 113 ○ $\geq - 2$ z-score: the child is in good nutritional condition

114 All children with serum vitamin A levels below 30 $\mu\text{g} / 100\text{ml}$ had vitamin A deficiency and those with
115 a serum level greater than or equal to 30 $\mu\text{g} / 100\text{ml}$ had good vitamin A status Tietz [17].

116 **Statistical analysis:**

117 The collected data were encoded into an Excel version7 workbook and imported into the SPSS 20
 118 software. Percent, average and standard deviation were calculated. The Chi square statistical test was
 119 used to compare serum vitamin A content in healthy and malnourished children, as well as other
 120 maternal parameters such as main activity, level of education, the number of children under five in the
 121 household for a significance level of 0.05. The data were presented in the form of frequency tables.

122 **Ethical consideration**

123 The blood sample was taken from the child whose mother has given her agreement by signing or
 124 fingerprinting the consent form after being well informed.

125

126 **Results**

127 Age, sex and serum vitamin A content of children

128 Table I presents the children by age group, sex and serum vitamin A content according to the sampling
 129 center.

130 Table I. Distribution of cases by age, sex and serum vitamin A content

Sampling center	M TNU N=29		U HC N=30		Total		P-val
	n	%	n	%	n	%	
Age (months)							
6-17	15	50	15	50,	30	50,8	0.014*
18-29	7	58,3	5	41,7	12	20,3	
30-41	4	44,4	5	55,6	9	15,3	
42-53	1	33,3	2	66,7	3	5,1	
54-59	2	40	3	60,0	5	8,5	
sex							
Male	17	58,6	15	50	32	54,3	0,50*
Female	12	41,4	15	50	27	45,7	
Ratio	1,4		1,0		1.2		
Vitamin A content (µg / 100ml)							
20-29.9	2	6,9	10	33,3	12	20,3	0,001*
30-39.9	5	17,2	5	16,6	10	17	
40-49.9	0	0	2	6,6	2	3,3	
50-59.9	18	62,1	9	30	27	45,7	
60-69.9	4	13,7	4	13,3	8	13,6	

131 * = statistical test of Chi square

132 This table shows that 30 children or 50.8% were 6-17 months old, the average age was 21.9 ± 13
 133 months; the sex ratio was 1.2; 27 children or 45.7% had a serum retinol level between 50-59 µg /
 134 100ml, the average value was 46.84 µg ± 14.27.

135

136

137

138 3.2 Vitamin A status by age and sampling center

139 Table II shows the vitamin A status of children by age and sampling center

140 Table II. Vitamin A status by age and sampling center

Status vitamin A	<30µg/100ml N= 12		≥30 µg/100ml N=47		Total		P-val
Age (months)	n	%	n	%	n	%	
6-17	6	50	24	51,1	30	50,8	0,561*
18-29	3	25	9	19,1	12	20,3	
30-41	3	25	6	12,8	9	15,3	
42-53	0	0	3	6,4	3	5,1	
54-59	0	0	5	10,6	5	8,5	
Consultation Center							
M TNU	2	6,9	27	93,1	29	49,2	0,011*
U HC	10	33,3	20	66,7	30	50,8	

141 * = Statistical test of Chi square

142 It emerges from this table that, 12/59 children (20.3%) had vitamin A deficiency and this deficit was
 143 more marked in children aged 6-17 months that is 50%;

144 Among 29 malnourished children, two (6.9%) were vitamin A deficient and among thirty healthy
 145 children 10 that is 33.3% were vitamin A deficient. Ten of the 12 children with vitamin A deficiency
 146 that is 83.3% were consulted at Umoja Health Center.

147

148 3.3 Mother's profile and vitamin A status

149 Table III presents the socio-demographic profile of the mother as well as the vitamin A status of the
 150 children according to the two sampling centers.

151

152 Table III. Socio-demographic profile of mother and vitamin A status of the child

Age (year)	M TNU		U HC		Total		Statut Vit A (µg/100ml)		P-val
	n	%	n	%	n	%	<30	≥30	
15-19	4	13,8	9	30	13	22	4	9	0,348*
20-29	13	44,8	14	46,7	27	45,8	4	23	
30-39	9	31,1	6	20	15	25,4	4	11	
40-49	3	10,3	1	3,3	4	6,8	0	4	
Level of study									
Illiterate	2	6,8	0	0	2	3,4	0	2	0,005*
primary	13	44,9	1	3,3	14	23,7	1	13	
secondary	14	48,3	27	90	41	69,5	11	30	
Sup / university principal activities	0	0	2	6,7	2	3,4	0	2	

Household	14	44,9	17	56,7	31	52,5	8	23	0,685*
Small business	7	24,1	3	10	10	17	2	8	
Farmer	8	27,5	3	10	11	18,6	0	11	
Teacher	0	0	4	13,3	4	6,8	1	3	
Other ©	0	0	3	10	3	5,1	1	0	
Number of children <5 years									
1-2	15	55,5	20	66,7	35	35	8	27	0,245*
3-5	14	45,5	10	33,3	24	24	4	20	

153 * = Statistical test of Chi square, © = cutter, maid.

154 This table shows that, 45.8% of mothers were 20-29 years old, the average age was 26.22 ± 7.4 years;
 155 41 mothers or 69.5% had a secondary level of education, this distribution was statistically significant
 156 with the vitamin A status of the child ($P = 0.005$); more than half of the mothers were housewives.

157 Discussion

158 Vitamin A status by age and consultation center

159 It is evident from our results that the majority of the children were 6 to 17 months old. This result may
 160 be due to the fact that the mothers are active to bring their children to the consultation when they are
 161 even small while this interest decreases as the child grows.

162 It emerges from this table that, 12 children or 20.3% had vitamin A deficiency (VAD) and that this
 163 deficit was more marked in children aged 6-17 months or 50%. Retinolemia increases from birth to
 164 adulthood, Agne and al [8] in their study conducted in Senegal found a prevalence of VAD of 15.2%
 165 among children aged 6-23 months. Rongwang and al [18] found a prevalence of 3.08% among
 166 pregnant women. Ajuluchukwa and al [22] found a prevalence of VAD of 65%. In view of these
 167 different results we can say that vitamin A deficiency is widely prevalent among people at risk such as
 168 children and pregnant women. Indeed, in a well-nourished breastfeeding woman, breast milk contains
 169 the necessary amount of Vitamin A to cover the infant's needs, at six months, this quantity no longer
 170 covers all needs and a complementary diet is the rule. In the case where this diet is not well conducted
 171 it leads to micronutrient deficiency among which vitamin A deficiency.

172 The national survey conducted in 1998 in DRC among children aged 6 to 36 months established that
 173 61.1% of the children examined had a retinolemia rate of less than $0.7 \mu\text{mol} / \text{l}$ [11]. In our study our
 174 results are paradoxical; on 29 malnourished children; 2 that is 6.9% had VAD and on 30 healthy
 175 children; 10 that is 33.3% had VAD out of the total of 12 children with VAD. Ten children(83.3 %)
 176 on a total of twelve with VAD were consulted at Umoja Health Center so were in good health and 2
 177 children that is 16.7% (2/12) at Mwana Mpendwa Therapeutic Nutritional Unit therefore were
 178 malnourished; the observed difference was statistically significant ($p = 0.011$). Our result would be
 179 justified by vitamin A supplementation in all malnourished children who come to the nutritional
 180 center. In addition, in ready-to-use therapeutic foods administered to malnourished the amount of
 181 vitamin A is well represented.

182 The 33.3% of children with vitamin A deficiency observed in healthy children is consistent with the
183 WHO estimate of more than 33.3% of preschool children who suffer from clinic deficiency in this
184 vitamin [14].

185 Since 2000, the DRC Ministry of Health has integrated vitamin A supplementation into routine
186 primary health care activities within health zones by also organizing mass activities in response to the
187 outcome of the health survey. It is therefore important to insist on infant feeding by integrating foods
188 rich in provitamin A such as: green leaves, orange fleshed sweet potato, carrots, meat, fish, eggs, and
189 especially to improve culinary practice with palm oils that are naturally rich in this vitamin and whose
190 impact on health is not visible. A study conducted by Lusamaki and al [23] showed that the quality of
191 palm oil sold at the central market of Kisangani at an affordable price to all levels of the population
192 had already lost more than 53% of provitamin A before its use in the household compared to the first
193 quality oil which is rich in provitamin A but which is expensive and to which certain population has
194 not access.

195 Sociodemographic profile of mother and vitamin A status

196 According to the result of Table 3, it appears that, 45.8% of mothers were between the ages of 20-29,
197 the average age was 26.22 ± 7.4 years; this average is lower than that found by Seck and al [24], who
198 was 28 years old in a study of women's knowledge of malaria; on the other hand, it corroborates that
199 of Lukuka and al, [25] who found the mean age of 26 in parturient. Ajuluchukwa [22] in his study of
200 vitamin A deficiency in pregnant women found an average age of 23.67 ± 6.11 years. Our result would
201 be explained by the fertility of women during this period.

202 Forty-one mothers or 69.5% had a secondary level of education, this distribution was statistically
203 significant with the status of the child ($P = 0.005$); Chun Yang and al [26] found a significant
204 difference between serum vitamin A levels in pregnant women with higher education level and those
205 with low level of education. Breast milk is an ideal source of vitamin A, promotes rapid growth, plays
206 the role of antioxidant and immune barrier, however, many factors modulate its composition such as
207 diet, socioeconomic level, and nutritional status of mother [27]. It is well known that the education
208 level of the mother allows her to comprehend and practice the counsels given at prenatal consultation
209 and obtain information on the role of vitamin A in her body. Women who have studied often have a
210 high socioeconomic level that allows them to provide good nutrition in their household.

211 Conclusion: At the end of our study, it appears that vitamin A deficiency remains a major health
212 problem in the DRC and that supplementation with this vitamin remains one of the palliative solutions.
213 This would affect all children regardless of their current nutritional status.

214 Recommendation: Given this result, we recommend mothers to feed their children well with foods rich
215 in provitamin A, such as green leaves, oils, some tubers and colored cereals, as well as foods of animal
216 origin.

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UNDER PEER REVIEW