The Effect of Sugar Fortification with Vitamin A on Serum Retinol and Retinol Binding Protein of Khalwa Students at Risk of Vitamin A Deficiency
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Abstract
Background: Vitamin A deficiency (VAD) is a public health problem in Sudan
Objectives: The aim of this study is to test the effect of sugar fortified with vitamin A on VA status of Khalwa students (informal education).
Methods: Sixty Khalwa students age 6-11 years were included in the study. Each student consumed about 28g of the fortified sugar for thirty days. Blood samples were collected before breakfast, to measure serum retinol (SR) and retinol-binding protein (RBP) of children before and after consuming the fortified sugar. Data including socioeconomic status and dietary intake of VA rich food were obtained. Anthropometric measurements and clinical examinations to detect signs of VAD were performed.
Results: Mean SR was 32.42 ±15.43ig/dl and mean RBP was 20.96 ±14.32mg/l, which increased significantly to 49.08 ±12.2ig/dl and 30.42±15.95mg/l respectively after consumption of the fortified sugar (P=0.000 for SR and p<0.005 for RBP). Serum retinol 30 days response (SR30DR) and retinol binding protein 30 days response (RBP30DR) both showed levels indicating severe VAD as a public health problem at baseline among 60%. Most cases were from low socio-economic status and 43.3% were mildly malnourished.
Conclusion: sugar fortification improved SR and RBP of the studied subjects who were considered at risk of VAD and would be expected to reduce the prevalence of VAD in Sudan.

Key words: Africa, malnutrition, measles.

Vitamin A deficiency is one of the leading causes of child mortality in sub-Saharan Africa. The percentage of preschool children who were at risk of vitamin A deficiency was 42%. By controlling vitamin A deficiency, deaths of 650,000 children can be prevented every year.1 Sudan is classified as one of the least developed countries with manifestation of food deprivation2. It is one of the 26 countries where vitamin A deficiency, xerophthalmia, and night blindness are considered as public health problems3. In 1999, the prevalence of night-blindness was estimated to be between 0.01% - 1 %.4 In 2004, vitamin A deficiency problem in Sudan was identified to be in the category of clinical according to WHO Eastern Mediterranean Region categorization of VAD as public health problem5. The most recent survey in Sudan in 2005 reported that there were 99 informal schools, which are known as “Khalwas”. The number of students studying in these Khalwas was 10,752 students. These students were at risk of VAD and the incidence of night blindness among them was 0.9%6. The use of vitamin-A-fortified foods contributes significantly to dietary intake and improves vitamin A status of at-risk populations. An evaluation of the mandatory national sugar fortification program in Honduras showed that it was an effective intervention7,8. Countries such as Zambia have implemented sugar fortification, but the programs are too new to be evaluated for its effectiveness. Kenya and South Africa were also considering the development of sugar fortification program9. This study was conducted on a group at risk
of VAD to assess the effect of introducing sugar fortified with vitamin A in the form of retinol palmitate on their vitamin A nutritional status. Assessment was based on measuring vitamin A status prior to and after consumption of the fortified sugar for 30 days.

This study will enable us to determine whether vitamin A deficiency is a problem among Khalwa students and to introduce a practical method to eradicate this problem.

**Materials and Methods:**

The study included 60 students aged 6-11 years from rural and urban Khalwas. The first rural group was 24 students, from a Khalwa in Jabal Awlia Province, Khartoum State and the second was an urban group of 36 students, from a Khalwa in Western Omdurman Province, Khartoum State. Of these 30 resident students were included. Those who have been supplemented with capsules within the last six months were excluded.

Fortification of sugar was performed with vitamin A as retinol palmitate. The dose was designed according to the daily recommended dietary allowances (RDA) issued by WHO\(^{10}\). The RDA for the subjects within the age group 6-11 is 300-575μg/day as retinol\(^{11}\).

**The Subjects:**

All students were interviewed by a questionnaire, which included dietary and socioeconomic data. Anthropometric measurements and clinical examinations were done.

Weight for height Z-score was used to classify the nutritional status into: normal (> -1), mild (-1 — -2), moderate (-2 — -3), and severe (< -3) with reference to the criteria of WHO\(^{12}\). Clinical examination to detect signs of VAD was done.

**Biochemical analysis:**

This was done for thirty subjects who agreed to give blood samples. Five milliliters of fasting blood samples were collected. Blood was allowed to clot and serum was pipetted into 2 epindorf tubes closed tightly and stored at -20°C till time of analysis of SR and RBP. SR was determined using HPLC (Bieri et al, 1979)\(^{13}\). Radial immunodiffusion method was used for determination of RBP\(^{14}\).

A second blood sample was collected after consumption of the fortified sugar for one month. SR and RBP were reevaluated to determine the percentage increase and their 30 Days Response (30DR) which was reported to be a more sensitive indicator of inadequate vitamin A status than only estimating serum level of vitamin A when blood levels were above 20μg/dl\(^{15, 16}\). A cut-off point of 20% increase within 30 days was used to identify a deficient level at baseline and the effectiveness of the fortified sugar\(^{17}\).

Data were analyzed using SPSS Analysis of variances for quantitative data at 95% confidence interval (P≤0.05).

**Results**

Most of the subjects were malnourished with low weight for height Z-score. Only 15% were found to be normal while 43.3% had mild malnutrition. Moderate malnutritional status was found in 30% of the Khalwa students, and severe malnutrition was observed in 11.7%.

The frequency of vitamin A rich food intake was assessed. Results have shown that the percentages of students who “always” consume green/yellow vegetables and fruits, red meat/liver, and egg were 16.7%, 18.3%, and 5% respectively (Figure 1).

Low socioeconomic status was seen in 49.3% where all children showed signs of VAD whereas 16.7 % had high status. Out of 11 students who had infection, eight were found to have VAD. A percentage of 18.3% of the total subjects suffered from infectious diseases mainly malaria and upper respiratory tract infections. Signs of VAD were detected in 72.2% of those who were infected.
In our study group clinical signs of VAD were detected in 51.7% of the students. These signs were within grades 1-5 according to the WHO clinical classification of vitamin A deficiency. Most of the students had an adequate level of SR (76.7%). Marginal level was recorded among 20.0%, whereas only one student was deficient. Mean serum retinol before supplementation was $32.42 \pm 15.43 \mu g/dl$, which increased to a mean of $49.1 \pm 12.2 \mu g/dl$ after consumption of fortified sugar. The mean of RBP before and after sugar consumption were $20.96 \pm 15.43$ mg/l and $28.8 \pm 15.95$ 43mg/l respectively. No student had a high level of RBP, however, 6.6% changed to high after consumption. Similar percentage of students had low and medium levels (46.7%); whereas, two students proved to be deficient (Table 1).

Table 1: RBP Levels Before and After Sugar Consumption.

| Classification | RBPI | | | RBPII |
|----------------|------| | |------|
|                | No.  | %  | No. | %     |
| Deficient      | 2    | 6.6| -   | -     |
| Low            | 14   | 46.7| 8   | 26.7  |
| Medium         | 14   | 46.7| 20  | 66.7  |
| High           | 0    | 0  | 2   | 6.6   |
| Total          | 30   | 100| 30  | 100   |

Table 2 shows the students 30 days response to the introduction of sugar fortified with VA regarding SR and RBP levels. Both SR30DR and RBP30DR have shown levels indicating severe deficiency among 60% of the subjects. The SR30DR showed that equal percentage of students (20%) had moderate and mild VAD. RBP30DR indicated moderate VAD level in 13.3% of the students, and mild VAD in 26.7%.

**Discussion**

Malnutrition with low weight for height Z-score was found in most of the subjects. Only 15% were found to be normal. This high prevalence of malnutrition was attributed mainly to the type and mode of nutrition in the Khalwa. Khalwa diet is composed of porridge made of millet called (Dibliba) taken with soup made of dried okra (Bamia or Weka), some oil, and onions. These students spent most of their time in the Khalwa depending on this diet which is very poor in its VA contents.

**Table 2: Serum Retinol and Retinol Binding Protein 30 days Response (S30DR).**

<table>
<thead>
<tr>
<th>Level of importance as a public health problem</th>
<th>SR30DR No.</th>
<th>%</th>
<th>RBP30DR No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>6</td>
<td>20.0</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td>Moderate</td>
<td>6</td>
<td>20.0</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>Severe</td>
<td>18</td>
<td>60.0</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

The mean time they spent in the Khalwa was $9.6 \pm 6.7$ months. Our finding showed that the intake of vitamin A rich food is not very frequent and this might explain Khalwa students’ vulnerability to the risk of VAD. This result was supported by Coutsoudis.

Malnutrition is an important factor that affects vitamin A status as was reported earlier. Mahalanabis had observed that in addition to the severe PEM and poor socioeconomic status, recent measles, prolonged diarrhea are also important risk factors or prognostic indicators for exophthalmia. All the students from low socioeconomic class have signs of VAD. This goes with the WHO/UNICEF report in 2004.

Our finding of signs of VAD in 72.2% of those who were infected supports the suggestions that there is a close association between vitamin A deficiency and infections.

VAD is a major nutritional problem among Khalwa student since 51.7% had signs of VAD (Figure 3). Significant increase was recorded in the mean serum retinol after supplementation (from $32.42 \pm 15.43 \mu g/dl$ to $49.1 \pm 12.2 \mu g/dl$). An
adequate level was reported by most of the students (76.7%), while 20.0% had marginal status, and only one (3.3%) was deficient. Similar results were obtained by others. There was a significant increase in the mean RBP after sugar fortification.

A similar result was obtained by Bloem et al. This increase in RBP might be explained by the fact that increased pool of native apo-protein accumulates in the liver during VAD which is released to the plasma quickly after retinol uptake resulting in increased RBP level.

**Conclusion:**
Sugar fortification with vitamin A resulted in significant improvement of Vitamin A status in our patients. This method can be applied nationally as a strategic interventional program to reduce the high risk of Vitamin A Deficiency.

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