β-Carotene is significantly less in children with serum retinol < 20µg/dl

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Abstract:

Vitamin A Deficiency (VAD) is very often considered to be global problem in very small children both in urban and rural areas of many developing countries. Provitamin A carotenoids such as beta-carotene has the potentiality of converting to vitamin A. So, the present study is carried out to estimate beta- carotene concentrations in children with both VAD and in children whose vitamin A concentrations is normal using Liquid Chromatography.

Keywords: Vitamin A Deficiency (VAD), Provitamin A carotenoids, beta-carotene, Liquid Chromatography.

Introduction:

Vitamin A Deficiency (VAD) (serum retinol <20µg/dl) is often observed in very small children both in urban and rural areas of many developing countries. The World Health Organization has classified VAD as a public health problem affecting about one third of children aged 6 to 59 months in 2013, with the highest rates in sub-Saharan Africa (48 per cent) and South Asia (44 per cent).

Moderate VAD in children causes increased risk of diarrhoea and infection such as respiratory or measles whereas severe VAD causes xerophthalmia and blindness (Sommer and West 1996). Dotan et.al (2013) in his study reported pseudotumor cerebri in children to be associated with VAD and also suggested early recognition and appropriate therapy can prevent blindness. It is indicated that vitamin A supplementation can prevent 50% of mortality in children below 5 years old (Kraemer *et al.* 2008). As such, the prevention of VAD is important for child development and growth. Children below 2 years of age may be prone to VAD though VAD can occur in all individuals of any age group since this period requires a high amount of vitamin A to support growth and is a transition period from breastfeeding to other food after 6 months of age.

Beta-carotene is a hydrocarbon carotenoid which plays a vital role as an antioxidant and a precursor of vitamin A (Moore, 1930). Considering its potential, Beta-carotene is under scrutiny and as such several studies are being carried out. Study carried out in children reported that dietary intervention with lycopene or with β-carotene improves and accelerate the recovery from pneumonia among infants and children Mohamed *et al.* (2008).

A study (NNMB Technical Report) in 2006 reported high (62%) sub-clinical prevalence of VAD (serum retinol <20µg/dl) in the eight states of survey i.e. Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Orissa, Tamil Nadu, Maharashtra and West Bengal. In Jharkhand sub-clinical VAD was also reported high (Brahmam *et al.* 2002). A study conducted among rural children of 6-12 years in West Bengal reported 61%

of children have serum retinol <20µg/dl (Arlappa *et al.* 2011). Chauhan *et al.* (2011) carried out a study in slum areas of Ahmedabad and reported 2.9 % prevalence of VAD among children of 5-15 years age.

The aim of the present study was to determine beta carotene concentration both in children with serum retinol <20µg/dl and in children with serum retinol ≥20 µg/dl.

Subjects and Methods:

24 Children less than 2 years old specifically girls (n=13) and boys (n=11) were enrolled in the study after examination by a paediatrician. The children were healthy (absence of cough, runny nose or diarrhoea with ≥ 3 or more watery stools/day in the preceding 10 days including the day of the survey). 8 children less than 6 months of age included in the study are exclusively breastfed. They had no clinical signs of vitamin A deficiency and did not use any supplements containing vitamin A or carotenoids. Written consent was obtained from parents. The study protocol has been approved by the ethical committee of Gauhati University constituted for this purpose.

Care was taken throughout the analysis and extraction to minimize exposure of the samples to air and light to avoid decomposition of the compounds.

Blood samples were collected and centrifuged to separate the serum. The serum samples were analyzed immediately whenever possible otherwise stored at -20^{o}C.

0.5 ml of water and 0.25 ml (0.1-0.3ng/µl serum) of retinyl acetate (internal standard) was added to 0.5 ml serum and vortexed for 5 mins. To the mixture 1 ml ethanol (containing 0.04% BHT) was added and vortexed again. The samples were extracted thrice with hexane. The supernatants were removed, pooled and evaporated in a rotary evaporator and redissolved in 100 µl methanol and 20 µl was injected into the HPLC system for analysis. All the samples are analyzed in duplicate, so the data are presented as mean ± SD.

The chromatography was carried out using the same Shimadzu system composed of two pumps and a photodiode array detector (PDA SPD-M10AVP) as detailed in Chapter 2A and 2C. The separation was achieved on a supelcosil- LC-8 (25cm x 4.6 mm, 5 µm) column. The chromatography was carried out using a step gradient elution mode in which eluent A was MeOH : H_2O (85 : 15) and eluent B was (MeOH : DCM (80 : 20) for both carotenoids and retinol estimation. The channel corresponding with different wavelength values were used to acquire data for the monitoring of retinol and retinyl acetate at 325nm and carotenoids at 450nm. The components from each were estimated from the peak area of the corresponding HPLC chromatogram with the help of standard curves.

The student's t test was used to determine the differences in means. When the calculated value of $t > t_{0.05, \nu}$, then it was considered significant, where ν is the respective degrees of freedom.

Results and Discussions:

Figure 1 shows the HPLC chromatogram of a sample at 325nm and 450nm using mobile phase MeOH:H_2O and MeOH:DCM.

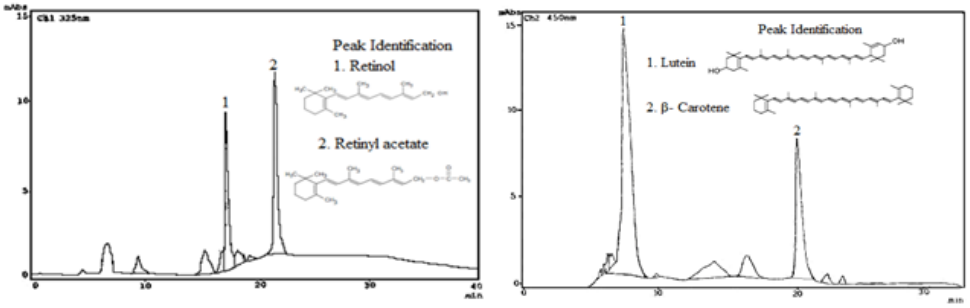

Fig 1: HPLC Chromatogram of a sample at 325nm and 450nm using mobile phase MeOH:H_2O and MeOH:DCM

In the HPLC chromatogram at 325nm the peak at retention time 17.807mins corresponds to retinol and peak at retention time 21.665 mins corresponds to retinyl acetate (internal standard) and at 450nm shows the peak at retention time 7.365mins and 20.118mins corresponding to lutein and β-carotene respectively.

The mean concentrations of lutein and β-carotene in children with serum retinol <20 µg/dl and ≥20 µg/dl is shown in Table 1.

Table 1: Mean ± SD values for Lutein and β-Carotene concentrations in children with serum retinol <20 µg/dl and ≥20 µg/dl

	Serum retinol <20µg/dl	Serum retinol ≥20µg/dl	Calculated t value
Lutein in µg/dl	7.09 ± 4.47	5.37 ± 2.45	1.213
β-Carotene in µg/dl	0.66 ± 0.33	2.10 ± 0.93	4.196
$t_{0.05,22}$=2.074			

In Table 1 mean serum carotenoid concentrations were compared between children with serum retinol <20µg/dl (considered as vitamin A deficiency) and ≥20µg/dl. The differences in the mean lutein concentration of children with serum retinol <20µg/dl and ≥20µg/dl is not statistically significant as calculated t value < $t_{0.05,22}$.Since the tabulated t value for 22 degrees of freedom at 5% probability level is 2.074, so children with VAD (serum retinol <20µg/dl) have significantly lower mean serum concentration of β-carotene which is the precursor of vitamin A.

Provitamin A carotenoids such β-carotene are the natural precursors for retinoids. In order to perform this function, provitamin A carotenoids must be converted by centric oxidative cleavage to all-*trans*-retinal. All-*trans*-retinal can be then reduced to all-*trans*-retinol which on esterification, is stored in large quantities in stellate in the liver and in other tissues such as lung and fat (D'Ambrosio, 2011). The structure of the

carotenoids that determine the provitamin A properties is the β-ionone ring. β-Carotene in this case has advantage over other provitamin A carotenoids since β-Carotene has two such rings in its structure while all other provitamin A carotenoids have only one so it is stoichiometrically equivalent to two molecules of retinol.

Studies carried out Lietz et.al (2001) and Mulokozi et.al (2003) reported that β-carotene is present in the plasma of people with low concentrations of plasma retinol which explains that the cause of the low plasma retinol concentrations is unlikely to be vitamin A deficiency. As Thurnham et.al (2003) reported that plasma retinol concentrations can be depressed both by a severe dietary deficiency of vitamin A but also by inflammation. Thus low retinol concentrations in the plasma in the presence of apparently adequate concentrations of β-carotene are unlikely to be due to a vitamin A deficiency.

But the results of the current study reported that though β-carotene concentration of the entire study group is low but it is observed from the Table 1 that β-carotene concentration is significantly lower in children with VAD (serum retinol<20µg/dl). Most probable explanation of this may be that since these children have very low retinol concentration in their blood which may lead to better bioconversion of β-carotene to retinol, resulting in less β-carotene concentration compared to children with serum retinol ≥20 µg/dl.

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