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Original Research Article

Vitamin A Status of Rural Women from Ahmednagar District, Maharashtra

Niharika Nahata[1], Shobha A Udipi[2], Padmini Ghugre[3], Aparna Thorat[4], Ashwini Salvi[4], Erick Boy[5]

[1]Postgraduate Student, [2]Hon. Director, Integrative Nutrition and Ayurceuticals, Medical Research Centre-Kasturba Health Society and Former Professor and Head, [3]Associate Professor and Incharge Head, [4]Research Assistant,
Department of Food Science and Nutrition, SNDT Women's University
[5]Nutrition Coordinator HARVEST PLUS

Corresponding Author: Aparna Thorat

ABSTRACT

Vitamin A deficiency is a public health problem, especially in low income countries including India. Women of reproductive age, pregnant women and preschool children are vulnerable to vitamin A deficiency. Poor vitamin A status is largely attributable to poor intakes of the vitamin. The present study assessed the dietary intake of vitamin A intake and serum retinol levels of rural women of reproductive age, 15 to 48 years of age). Dietary intakes of 898 women from Ahmednagar district were studied and serum retinol was analyzed for a subsample of 200 non-pregnant women. Mean vitamin A intake was 328.0±394.7µg/day, with intakes being significantly and positively influenced by age, income and possession of cattle. Serum retinol levels of 77 percent of women were <0.7 µmol/L, indicative of deficient vitamin A status. Women whose diets provided less than 30 percent of the recommended dietary intakes had the lowest serum retinol levels. The study indicated that rural women had highly inadequate diets that place them at risk of vitamin A deficiency. This situation needs serious and concerted efforts to improve the vitamin A status of the women

Key words: serum retinol, women, reproductive age, vitamin A intakes, possession of cattle, income.

INTRODUCTION

Vitamin A is an essential fat soluble vitamin, having several important functions in the human body, including cellular differentiation and gene transcription, [1] vision, [2] reproduction, [3,4] immunity [5,6] hematopoiesis. [7] The most common outcome of VAD is xerophthalmia, the leading cause of preventable childhood blindness, anemia and weakened host resistance to infection, which can increase the severity of infectious diseases and mortality. Night blindness is a common feature of pregnant women who are vitamin A deficient. [8] Vitamin A deficiency during pregnancy increases the risk of low birth weight in the offspring who are likely to have poor liver stores of the vitamin. Consequently, various physiological functions in the infant e.g. immunity, vision and growth would be compromised from the very beginning. VAD is linked to increased risk of under five mortality. [9,10]

Vitamin A deficiency (VAD) is one of the major public health concerns of the developing world. [11-13] Reports in the literature indicate that among the South Asian countries, India has the highest prevalence of subclinical vitamin A deficiency. [11,14,15] However, these reports

are at least a decade old and Vitamin A status of women of reproductive age has received less attention, because clinical signs of xerophthalmia are rarely seen in this segment of the population. Studies have indicated that VAD in women of reproductive age may increase morbidity and mortality during pregnancy and the early postpartum period of both mother and infant. [16-18] Thus, women of reproductive age are now being considered as a target group for vitamin A supplementation. [16,19-21]

One of the major causes of VAD is consumption of diets that are markedly low in foods that are good sources of preformed vitamin A or those containing the provitamin β-carotene. The National Nutrition Monitoring Bureau [22] reported that the dietary vitamin A intake of females in rural India is grossly inadequate and that not even 50 percent of the recommended intakes are met in most of the age groups. However, besides this report in 2002, there are few studies reported in the literature from India. Since VAD has serious implications for both mother and child, the present study was undertaken to assess the vitamin A intakes and serum retinol levels in rural women of reproductive age. This investigation was part of a study undertaken to examine the dietary intakes and iron and zinc status of rural women of reproductive age.

MATERIALS AND METHODS

Ethical approval: This investigation was approved by the Departmental Research Committee and the main study on dietary intakes and biochemical measures of iron and zinc status was approved by the Inter Systems Bio Medical Ethics Committee, Mumbai.

Sample Selection: The study sample consisted of 905 women of reproductive age, including pregnant and lactating women, from 30 villages that were selected by systematic random sampling from two blocks – Parner and Sangamner, in Ahmednagar district in Maharashtra state. First, written informed consent was obtained from the *Sarpanch* or *Deputy Sarpanch* / village Head. Then, each potential study participant was provided complete information about the study and written consent was obtained. For individuals who could not read and/or write, the information about the study was read out and explained by the field investigator and the participant's thumb impression was taken on the consent form which was countersigned by a witness.

Inclusion and exclusion criteria: The following criteria were used for the study. Exclusion criteria included women who were suffering from any acute or chronic illness in the past one month or any chronic condition including tuberculosis, HIV/AIDS, any respiratory infections or skin disorders, hepatitis.

Data collection: Information on dietary intakes was collected by trained field investigators using the 24-hour diet recall and weighment method. Each participant was interviewed using the three –pass, 24-hour recall procedure. Data collection on dietary intakes was done in each household at the time of meal preparation, wherever possible. The procedure was briefly as follows:

(i) Each participant was first asked to recall all the food items consumed on the previous day, from the time she woke up in the morning ending with the last food item/beverage at night, including foods prepared and eaten outside the home. Time of consumption of each item was noted.

(ii) Thereafter for each item, the amount consumed was recorded in household measures or numbers and dimensions, whichever was appropriate. Household measures such as katoris, spoons, glasses, cups were equated with standard measures. For items like biscuits, bread, *bhakris*, the number consumed and the dimensions were recorded. Portion sizes

were quantified using models and actual food items which were part of a tool kit provided to each field investigator. The tool kit consisted of samples of commonly used food ingredients to be used as reference including cereals, cereal/millet flours, whole and decorticated pulses, vegetables, bundles of green leafy vegetables. All food items had been measured in terms of size, dimension and weight. The kit also included standardized measuring cups, spoons and utensils commonly used in the village.

(iii) In the third step, for each recipe / preparation, the raw ingredients used for the entire household were measured and / or weighed. For food items prepared/eaten outside the home, recipes were constructed from similar dishes eaten in the home. The yield was then recorded using household measures and thereafter with standard measures. Wherever applicable, numbers, dimensions i.e. thickness and diameter were recorded. If food preparation had been completed before or after the interview was completed, the participant was asked to simulate the preparation of the food items consumed on the previous day.

The weight of the food consumed was then calculated. Food intake data was entered in the CS Dietary software provided by HARVEST PLUS. Intakes of vitamin A and β-carotene were calculated using the software. β-carotene was then converted into retinol equivalents using the values for conversion given by Tang et al. [20] Vitamin A intakes were calculated as retinol equivalents. Adequacy of dietary intakes was calculated as percent of the recommended dietary allowances given by the Indian Council of Medical Research. [23]

The 24-hour diet recall was validated by the weighment method for 40 women who were not part of the study but who resided in the same blocks but in villages other than those included in the study and whose diet patterns were similar to those of the participants.

Blood collection: Among the 905 women, 200 women were willing to give a blood sample. Of these 200 women, 72 were from Parner block and 128 were from Sangamner block.Blood was collected in nonfasting state between 8 am and 10 am by antecubital venepuncture, by a trained phlebotomist under the supervision of a physician. Fifteen ml of blood was drawn using sterile, disposable needles in vacutainers. The vacutainers were packed in ice boxes with pre-frozen gel packs and transferred immediately in an air-conditioned vehicle to the laboratory at Ahmednagar. After bringing the samples to room temperature, two ml of whole blood was kept aside for estimation of haemoglobin. The remaining blood was centrifuged at 2000 rpm for 20 minutes. Aliquots of serum were transferred into individual Eppendorfs, labelled and frozen at -20^{o}C. They were transported in frozen condition to the Department at Mumbai.

Estimation of serum retinol: Serum retinol was estimated by the HPLC method of Cattignani. [24] All steps were carried out in the dark.0.5 ml of serum was first deproteinized using n-hexane, that was evaporated under nitrogen. The sample was thengently mixed and dissolved in 200 µl of HPLC grade methanol for injection.20 µl of the sample was injected in the HPLC column. An isocratic HPLC system (Shimadzu make) comprising of CCPM pump was used, using the following conditions:

Hypersil C_{18} reverse phase column, 250x4.6 mm and particle size 10µm. The mobile phase was 98:2 methanol: water with a flow rate of 2 ml /minute.A UV/VIS detector (LC21 UVD) was used with detection wavelength being 280 nm. The integrating recorder used was SIC-122 model. The detection limit was 0.1 ppm.

Retinyl acetate was used as the internal standard and retinol standard solution (100 ppm concentration was used). The retention times for retinol and retinyl

acetate were 1.5-2 minutes and 3-4 minutes respectively. Peak height ratios were converted to quantity of retinol from standard curve constructed using 2 ppm to 18 ppm of retinol. Concentration of serum retinol was reported as µmol/L.

Statistical Analysis: Among the 905 women who consented to participate in the survey, complete information was available for 898 women. Hence, data was analysed for these 898 women. All data was entered in Microsoft Excel and was analysed using the Statistical Package for the Social Sciences (SPSS) version 16. Influence of age, education level, physiologic status, physical activity, family income, possession of cattle, possession of amenities such as tap and electricity on dietary vitamin A intakes were examined. The tests applied included Karl Pearson's correlation coefficient, analysis of variance (ANOVA), chi-square test and post hoc Bonferroni and Scheffé's test. Analysis of variance and Pearson's correlation coefficient was used for continuous variables and chi-square test was applied for categorical variables.

RESULTS

The mean age of the women was 24.8±3.9 years. Among the 898 participants, 47.8% (n=429) were non-pregnant, non-lactating women, 45.5% (n=409) were lactating and 6.7% (n=60) were pregnant at the time of study.

Dietary Intakes: The mean dietary vitamin A intake was 328.0±394.7 µg/day. The mean dietary adequacy expressed as a percentage of the dietary allowance recommended by the Indian Council of Medical Research (ICMR) was 44.45±54.77%.

***Influence of selected factors*:**

Age: Vitamin A intakes of women in the reproductive age (21 to 39 years) were significantly lower than intakes of older women (Table 1). More than half the women aged 21 to 39 years had intakes that were less than 30% of the RDA. In the entire sample, barely 5% had intakes than met 90 to 100 percent of the RDA.

Table 1: Mean Vitamin A Intake and Percent Adequacy of Dietary Vitamin A in Relation to Women's Age

Age group (years)	n	Intake (RE µg/day) mean ± sd	Intake as % of RDA (mean ± sd)
<20	84	393.6±545.3	45.8±59.9
21-29	708	319.0±364.3	42.8±50.5
30-39	96	310.8±381.6	48.1±62.4
>40	10	674.3±818.3	111.6±136.9
F,p		3.542,0.014	5.462,0.001

Physiological state: Mean dietary intakes did not differ between women who were either pregnant, or lactating or non-pregnant, non-lactating women (F=0.939, p=0.391). Mean intakes in retinol equivalents for the three groups were: pregnant women - 389.9± 422.7µg/day, lactating women -331.1±407.4 µg/day and non-pregnant non-lactating women - 316.8±379.1 µg/day, respectively. However, dietary adequacy was significantly lower for women with longer duration of lactation (F=12.873,p=0,000). Mean adequacy was 36.0±44.0% for women who had been breastfeeding for 6 months or longer compared to women who were in their first six months of lactation (49.6±90.2%).

Family income: Mean dietary intakes and mean adequacy for vitamin A intakes increased significantly with family income (Table 2).

Table 2: Mean Vitamin A Intake and Percent Adequacy of Dietary Vitamin Ain relation to Family Income

Family income (Rs/month)	n	Intake (RE µg/day) mean ± sd	Intake as % of RDA mean ± sd
≤14999	48	209.7±182.0	29.6±30.5
15000-24999	117	259.3±322.9	34.7±42.9
25000-49999	328	321.9±362.8	44.5±53.2
50000-74999	219	318.8±315.8	41.4±37.1
75000-100000	103	369.6±402.8	48.4±51.8
>100000	83	501.7±708.3	48.4±102.1
F,p		5.155,0.000	5.305,0.000

Possession of cattle: Almost 60% of women (n=526) belonged to families who did not own cattle/livestock. These women had significantly lower dietary intakes (F=3.860,p=0.050) and lower adequacy (F=5.951,p=0.015) as compared to women whose families had livestock. Mean intake for women whose families owned livestock

was about 50 µg/day more compared to those women whose families did not own livestock (357.3±385.3 vs 309.2±401.2 RE µg/day). Percent mean adequacy was 41.9±55.8% for those without livestock whereas percent adequacy was 48.0±53.1 for those with livestock.

Other factors: No significant difference was found in mean intakes with women's education level (F= 0.257, p=0.905), possession of amenities like tap (F=0.271,p=0.603), electrification of house (F=0.890, p=0.346), possession of land (F=1.055, p=0.305), type of house (F=1.678,p=0.153), family size (F=1.774,p=0.061).

Serum retinol: The mean serum retinol was 0.59±0.81 µmol/L and varied from as low a level as 0.02 µmol/L to 5.2 µmol/L. The median serum retinol was0.29 µmol/L. Only 20 percent of the women had serum retinol levels in the normal range of 0.7 to 2.8 µmol/L. Three percent of the subjects had serum retinol levels exceeding 2.8 µmol/L.

Relation of serum retinol and dietary intakes: Mean dietary intake of vitamin A was higher among women whose serum retinol levels were greater than 2.8 µmol/L although this was not statistically significant (Table 3). Serum retinol levels were higher for women with better dietary adequacy (Table 4).

Table 3: Mean Dietary Vitamin A Intake According to Serum Retinol Levels

Serum Retinol µmol/L	n	Intake (RE µg/day) mean ± sd	Min-Max (RE µg/day)
<0.7	142	251.9±278.1	18.1-1460.1
21-29	708	218.1±161.5	12.6-639.8
30-39	96	310.8±381.6	41.1-1234.9
F,p		5.413,0.092	

Table 4: Serum Retinol in relation to Mean Percent Adequacy of Dietary Vitamin A Intake

		Serum Retinol (µmol/L)		
Percent Dietary Adequacy	n	Mean	Median	Min-Max
≤30	119	0.49±0.56	0.27	0.02-3.64
30-49.9	26	0.96±1.39	0.36	0.02-5.01
50-69.9	18	0.71±0.76	0.45	0.03-2.61
70-100	7	0.94±1.12	0.96	0.04-3.21
>100	13	0.68±1.08	0.22	0.02-3.20

Mean serum retinol levels of women with dietary adequacy of more than 30% were generally in the normal range, whereas majority of the women who did not meet even 30 percent of the recommended dietary allowance had low serum retinol levels < 0.7µmol/L, a level indicative of vitamin A deficiency. There was a significant difference in the percentage of women with differing levels of dietary adequacy of vitamin A intakes (χ^2=35.385, p=0.000) (Table 5).

Table 5: Distribution of Women According to Serum Retinol and Mean Adequacy of Dietary Vitamin A Intakes

Percent Dietary Adequacy (% RDA)	Percent Women		
	Serum Retinol <0.7 µmol/L	Serum Retinol 0.7-2.8 µmol/L	Serum Retinol >2.8 µmol/L
≤30	68.1	62.9	14.3
30-49.9	12.8	14.3	42.9
50-69.9	7.1	22.9	0.0
70-89.9	3.5	0.0	0.0
90-100	0.7	0.0	14.3
>100	7.8	0.0	28.6

DISCUSSION

Vitamin A deficiency is a major public health problem in poor societies especially in lower income countries, largely attributable to chronically insufficient dietary intakes. Women in the reproductive age constitute a high risk group (WHO,2009). In the present study, majority of women did not meet even 50 percent of the recommended dietary allowance set by the Indian Council of Medical Research. [23] These findings are in line with reports of the National Nutrition Monitoring Bureau. [22] Kennedy et al., (2009) [25] also reported that the median intake of Balinese women did not meet the estimated average requirement (EAR) for vitamin A.

Vitamin A intakes of women in the present study were found to be influenced by age, physiological status and family's

possession of cattle. Women in the age group of 21-29 years had the lowest intakes. Women in this age bracket were not able to meet even 50% of the RDA, making them highly susceptible to vitamin A deficiency compared to women above 30 years of age who were found to have better dietary adequacy. This has serious implications since 21-29 years is a very crucial age for reproduction and lower vitamin A intakes would affect the vitamin A status and vitamin A stores of these women. If these women become pregnant, the mother and the fetus would be at risk and the poor vitamin A status could adversely affect fetal growth and development. Reports in the literature indicate that the risk of night blindness increases with increase in women's age. [26]

Dietary adequacy was found to be better for non-pregnant, non-lactating women than the pregnant and lactating women in the present study. Overall, pregnant and lactating women were not able to meet 50% of the RDA which was also observed by the NNMB for rural population. The NNMB [22] report showed that dietary intakes of vitamin A were much below the recommended levels for 81% of rural pregnant and 90% of lactating women. In the present study, as duration of lactation increased, the dietary adequacy was lower, indicating that after six months, breast fed infants would be at risk of deficiency especially if the complementary foods given to them do not include sources of vitamin A. Also, maternal vitamin A stores would be depleted, putting the mothers also at risk of vitamin A deficiency.

Family income was found to influence dietary intakes and percent adequacy of vitamin A intakes. Overall, the intake of the vitamin increased as family income increased, suggesting that with increasing income, the family's purchasing power improves and the family may be able to purchase vitamin A- rich food sources. Thus women whose family incomes exceeded Rs 100000 per month were better off than their counterparts with lower family incomes. Possession of livestock/cattle was associated with better vitamin A intakes, possibly because these women had a chance to consume some milk which would provide some vitamin A. However, in rural South Indian women, Katz et al [8] observed that women without cattle ownership had lower prevalence of maternal night blindness than those who owned one or more cows.

In the present study, type of house did not significantly influence vitamin A intakes. In contrast, Christian and co-workers reported that majority of the night-blind women in Nepal lived in houses made from mud and thatched walls or roofs. Similarly, Katz et al [8] observed that women living in houses with a concrete roof had lower risk of night blindness during pregnancy compared to women living in houses with tiled/thatched roofs. Semba et al [26] made similar observations on Cambodian women of reproductive age.

Low dietary intakes of these women were clearly reflected in low serum retinol levels. In the present study, only one-fifth of the women had serum retinol levels in the normal range as recommended by the International Vitamin A Consultative Group (IVACG) in 2001. [27,28] More than three-fourths of the women had serum retinol elves indicative of deficiency. Low dietary intakes were clearly reflected in the low serum retinol levels in this group of women. One strength of this study is the relatively large sample size for dietary assessment. However, the number of subjects for serum analysis was relatively small and hence it was not possible to examine the influence of various factors on serum retinol levels. Nevertheless, both sets of data on dietary intakes and serum levels individually indicate the situation of women in rural Maharashtra.

CONCLUSION

The study throws light on the high prevalence of vitamin A deficiency among rural women of reproductive age in Maharashtra and emphasizes the need for immediate attention in order to avoid the

serious health implications for these women and their offspring. Overall, the results of this study emphasize the need to educate the community in general and women in particular about the importance of including vitamin A rich food sources in their diets, frequently and in adequate quantity. Other steps may be required to ensure that pregnant and lactating women have adequate intakes. The study results indicate the need for improving availability and utilization of vitamin A rich foods including milk, eggs, green leafy vegetables and yellow-orange fruits and vegetables. Also fortification of suitable vehicles needs to be considered.

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