Lipid-soluble antioxidants status and some of its socio-economic determinants among pregnant Ethiopians at the third trimester

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Abstract

Objective: To determine the serum levels of three lipid-soluble antioxidants (retinol, β-carotene and α-tocopherol) in pregnant women attending an antenatal clinic (ANC) in Gondar, Ethiopia.

Design: A cross-sectional study involving laboratory determination of serum levels of retinol, α-tocopherol and β-carotene using high-performance liquid chromatography and questionnaire-based assessment of socio-economic status.

Setting: ANC of a university teaching hospital.

Subjects: Three hundred and twenty-two healthy pregnant women in their third trimester, who attended the ANC.

Results: Mean serum levels of retinol, α-tocopherol and β-carotene were found to be 1.23 ± 0.5, 25.5 ± 0.9 and $0.21 \pm 0.09\,\mu\mathrm{mol\,l}^{-1}$, respectively. The prevalence of vitamin A deficiency ($< 1.05\,\mu\mathrm{mol\,l}^{-1}$) among pregnant women was 38.5%. Women having low retinol (vitamin A) levels were highly likely to live in earth-floored and mud-walled houses, and less likely to own a house and to have a latrine.

Conclusion: The study shows that serum levels of lipid-soluble antioxidants were low among ANC attendees in northern Ethiopia. It also indicates that some socio-economic factors (such as poor housing standard) are associated with vitamin A deficiency.

Pregnancy is characterised by physiological and metabolic changes that alter maternal biochemical and haematological parameters. These changes are the result of altered maternal nutritional requirements, superimposed foetal metabolism and its needs, pregnancy-related physiological adaptations and re-tailored hormonal homeostasis. All these alterations increase the demand of the mother for essential nutrients. Therefore, the supply of adequate nutrients to pregnant women is one of the basic requirements for proper embryonic development, and normal foetal and maternal health. Hence the nutritional status of pregnant women is a concern particularly in regions where nutritional deficiency is a public health problem.

Lipid-soluble antioxidant vitamins have been recognised for many years as among the essential micronutrients involved in normal foetal development, positive pregnancy outcome, and long-term maternal and child well-being. For instance, vitamin A (retinol)-deficient women were found to be more susceptible to frequent infection of mucous surfaces of hollow viscera such as genitourinary, respiratory and intestinal linings because retinol is essential for immune function and integrity of epithelial tissue[1]. However, in countries like Ethiopia in which vitamin A deficiency (VAD) is labelled a public

health problem based on its extensively studied endemicity among children, maternal VAD has received little attention. Most studies and intervention programmes on vitamin A are directed towards childhood deficiency. This is paradoxical, since the nutritional status of the mother, as well as contributing to her own well-being, plays a critical role in the health of her foetus, infant and suckling baby[2].

Furthermore, decreased plasma levels of antioxidant vitamins (A, C and E) are typically associated with pre-eclampsia, eclampsia, premature rupture of the membranes and premature delivery[3–6]. α-Tocopherol is the most powerful fat-soluble natural antioxidant in the blood; it is essential for scavenging free radicals. Retinol and its precursor β-carotene are also antioxidants that similarly fight free radicals. Free-radical-mediated lipid peroxidation and related consumption of the antioxidant vitamins A and E, and also β-carotene, may play a role in the pathophysiological mechanism of pre-eclampsia[3–6].

Many nutritional surveys carried out in the past have shown the existence of a multitude of nutritional deficiencies in African infants and children. However, there are very few reports so far on lipid-soluble antioxidant vitamins (retinol, β-carotene and α-tocopherol) in pregnant mothers in Africa[7,8] in general and in East Africa in particular[9]; a group that is especially

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subjected to nutritional stress. Therefore the principal objective of the present study was to evaluate the serum status of retinol, α-tocopherol and β-carotene of pregnant Ethiopians in the third trimester.

Subjects and methods

Women appearing for the first time late in the second or in the third trimester to attend the antenatal clinic (ANC) of Gondar University teaching hospital (Gondar, Ethiopia) were recruited for the study. All new attendees at the clinic for a period of 2 months were enlisted. First-time attendees were recruited into the study with the intention of eliminating the effect of counselling or any dietary or therapeutic intervention of the ANC follow-up. Women under the age of 16 years, suffering from any debilitating chronic illness or carrying more than one foetus were excluded. A thorough physical assessment was performed to ascertain the health status of the women. All women included in the study gave their informed consent to participate. Information on sociodemographic characteristics and socio-economic status, and dietary, anthropometric and maternity data, were collected by an obstetric nurse using a structured pre-tested format. The study protocol was approved by the institutional ethical committee (nationally accredited) and by the institutional peer review mechanism for the approval of research proposals. The teaching hospital gave its consent for running the study.

Blood samples were taken from the antecubital vein of each woman in two separate containers. One of the serum samples was sent to the clinical chemistry unit of the central laboratory of the hospital for determination of total serum protein and cholesterol. The other sample was centrifuged within an hour and frozen at $-25°C$ until it was transported to the Chemical Pathology Department of Leicester Royal Infirmary, Leicester University (UK), where serum retinol, β-carotene and α-tocopherol levels were analysed by high-performance liquid chromatography (HPLC) as described elsewhere[10].

A value of $1.05\,\mu mol\,l^{-1}$ was used as the cut-off between normal and deficiency retinol level, as recommended by Haskel and Brown[11]. This is based on evidence which suggests that symptoms of deficiency such as xerophthalmia are found in pregnant women at serum retinol levels of $0.7-1.05\,\mu mol\,l^{-1}$, which is a physiologically significant and acceptable level of circulating retinol in children[12]. Similarly, there is no well-established cut-off value for α-tocopherol concentration for pregnant women. In the literature, the adult cut-off for plasma α-tocopherol concentration $(11.6\,\mu mol\,l^{-1})$ is described as inappropriate for pregnant women[13]. Hence, the non-pregnant reference vitamin E level $(24\,\mu mol\,l^{-1})$ that was reported by Lockitch[14] was adapted as a cut-off value. Comparisons of socio-economic status and a number of household characteristics were made between women having normal

and low serum retinol levels. All data are expressed as mean and standard deviation. Statistical analysis and differences between means were tested using the statistical program EPI-Info 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA). All study subjects were provided with multivitamin pills after the study and advised how to enrich their diet with sources of antioxidant vitamins.

Results

A total of 322 women attending the ANC were enrolled in the study. The sociodemographic characteristics of the study population are described in Table 1. The study population was relatively young as illustrated by average age $(24.7 \pm 5$ years), average size of the household (3.5 ± 1.5) and parity (38% nulliparous). Geographically, 62% of the women resided in towns and 38% in rural settings. Some 18.6% of the women were illiterate and 64.6% had less than 6 years of schooling. Almost all of them reported three times daily, regular consumption of

Table 1 Anthropometric, antenatal and demographic characteristics of the study population ($n = 322$)

Characteristic	Number	Percentage	Mean $\pm$ SD
Age (years)			24.7 ± 5
16−19	56	17.4	
20−24	140	43.5	
25−29	82	25.5	
30−34	24	7.5	
≥ 35	20	6.2	
Body weight (kg)			55 ± 7
< 50	52	16.2	
50−59	156	48.5	
60−69	84	26.1	
70−79	25	7.8	
≥ 80	5	1.6	
Body height (cm)			157.3 ± 6
≤ 150	39	12.1	
151−155	125	38.8	
156−160	84	26.1	
161−165	54	16.8	
166−170	16	5.0	
≥ 171	4	1.2	
Gestational age (weeks)			29 ± 7.4
24−28	117	36.3	
29−37	167	51.9	
38−41	38	11.8	
Parity			
Nulliparous	122	37.9	
Para I	100	31.1	
Para II	66	20.5	
Multiparous	32	10.0	
Marital status			
Married	316	97.9	
Single	6	2.1	
Nursed previous baby (months) ($n = 200$)			
≤ 6	8	4.0	
7−12	24	12.0	
13−24	121	60.5	
> 24	47	23.5	

SD − standard deviation.

the typical highland Ethiopian traditional diet. This consisted mainly of flat bread from teff as a basic food along with sauce made of cereal flour (beans, peas, grass-pea and chickpeas), oil from oily seeds, pepper and spices. A 1-month dietary recall indicated that consumption of fish, liver, green leafy vegetables, tomatoes, citrus fruits and papaya was rare and occasional. The women stated that they did not supplement their diet using vitamin pills. However, 45.7% of them occasionally took fruits, vegetables or extra snacks between meals. None of them was receiving multivitamin or iron tablets prior to or during the pregnancy. There were no current pregnancy-related illnesses such as abruptio placentae, hypertension, abnormal fasting blood sugar profile or premature labour prior to the study period. None of them were smokers. A history of contraceptive use prior to the present pregnancy was reported by 44.6% of women. Of the women, 22.5% reported having a history of death of a child.

Serum values of lipid-soluble antioxidant vitamins are illustrated in Table 2. The mean concentration of serum retinol was found to be $1.23 \pm 0.5\,\mu\mathrm{mol\,l}^{-1}$. Serum retinol level $< 1.05\,\mu\mathrm{mol\,l}^{-1}$ was found in 38.5% of the women, with 17.2% of these having serum retinol $< 0.7\,\mu\mathrm{mol\,l}^{-1}$ including 2.7% with a value $< 0.52\,\mu\mathrm{mol\,l}^{-1}$. This means that 124 women out of the 322 (39%) had biochemical VAD. The mean serum β-carotene level was $0.21 \pm 0.09\,\mu\mathrm{mol\,l}^{-1}$. Ten women had undetectable serum β-carotene levels ($< 0.001\,\mu\mathrm{mol\,l}^{-1}$). Mean serum α-tocopherol value was $25.5 \pm 0.9\,\mu\mathrm{mol\,l}^{-1}$. Only 58% (187) of the women had a value $> 24\,\mu\mathrm{mol\,l}^{-1}$ ($10.3\,\mathrm{mg\,l}^{-1}$), which is the reference value of α-tocopherol for non-pregnant women of reproductive age[14]. The mean value of those 187 women with α-tocopherol level above the preconception reference was $33.5 \pm 1.6\,\mu\mathrm{mol\,l}^{-1}$. Serum cholesterol and protein values were found to be $173 \pm 31.3\,\mathrm{mg\,dl}^{-1}$ and $6.16 \pm 0.7\,\mathrm{g\,dl}^{-1}$, respectively.

The socio-economic status of women having marginal and severe VAD ($< 1.05\,\mu\mathrm{mol\,l}^{-1}$) was compared with that of women having normal serum vitamin A status (Table 3). The monthly income of households with VAD women was found to be 420 ± 98 Birr (Ethiopian currency), while that of households without VAD was 600 ± 127 Birr ($P < 0.02$). Women having low vitamin A levels were less likely to own a house ($P < 0.01$). Furthermore, they lived in poor-quality houses, i.e. houses without a private water source (running water) and without a latrine ($P < 0.001$). Living in a house with earth floor and mud walls, and the death of a previous child, were also associated with low serum retinol status, as was sharing the living quarters with cattle, sheep and goats ($P < 0.001$).

Discussion

This is the first cross-sectional study in which serum levels of lipid-soluble antioxidants have been determined in East African women in their third trimester of pregnancy. The findings in this survey provide evidence that a large segment of the studied women had low levels of serum lipid-soluble antioxidants. Furthermore, the results reveal not only that serum retinol was rather low for a large proportion of the women, but also that there were many individuals with marginally normal values. This is reflected in the overall retinol and α-tocopherol levels (which were 1.23 and $25.5\,\mu\mathrm{mol\,l}^{-1}$, respectively) of the study population and in the individual values, which tended to cluster at the lower end of normality. Thus, from the results of this study, it is possible to make a cautious conjuncture that VAD is a problem of public health significance among pregnant women in northern Ethiopia. This inference is further reinforced by the general understanding that those women who took steps to come to the ANC were more conscious about their health status than women who did not. Moreover, the World Health Organization (WHO) states that VAD is a severe problem when the percentage of subjects having biochemical VAD is more than 20%[12]. In this study the proportion of women who had plasma retinol $< 1.05\,\mu\mathrm{mol\,l}^{-1}$ was 38.5%, almost double the figure. stated by WHO to indicate a severe public health problem.

To understand the relative status of the study population, their mean serum antioxidant levels were compared with those of pregnant women elsewhere. Our retinol value of $1.23\,\mu\mathrm{mol\,l}^{-1}$ is lower than that found among Canadian ($2.3\,\mu\mathrm{mol\,l}^{-1}$) and Spanish ($1.77\,\mu\mathrm{mol\,l}^{-1}$) women[15,16]. However, it is comparable

Table 2 Serum values of lipid-soluble antioxidants and other biochemical parameters used as indicators of nutritional status in the study population ($n = 322$)

Parameter	Mean ± SD	Women with an unacceptably low level		Cut-off value
		Number	Percentage	
Retinol ($\mu\mathrm{mol\,l}^{-1}$)	1.23 ± 0.5	124	38.5	1.05
β-Carotene ($\mu\mathrm{mol\,l}^{-1}$)	0.212 ± 0.09	159	49.3	0.22
α-Tocopherol ($\mu\mathrm{mol\,l}^{-1}$)	25.5 ± 0.9	135	41.9	24
Total protein ($\mathrm{g\,dl}^{-1}$)	6.16 ± 0.7	58	18.0	6
Cholesterol ($\mathrm{mg\,dl}^{-1}$)	173 ± 31.3	0	0	260*
Haematocrit	42.6 ± 1.9	9	2.8	37

SD – standard deviation.
* Upper limit of normal.

Table 3 Comparison of socio-economic and sociodemographic characteristics of pregnant women having low and normal vitamin A status

Characteristic	Vitamin A status				Odds ratio	Confidence limit	P-value
	Low (n = 124)		Normal (n = 198)				
	Number	Percentage	Number	Percentage			
House status							
Privately owned	30	24.2	92	46.4	2.79	1.38−5.51	<0.01
Private water source	28	22.5	56	28.2	0.78	0.36−1.68	>0.05
House wall from mud	116	93.5	122	61.6	1.78	1.25−4.96	<0.05
Earth floor	100	80.6	108	54.5	1.44	0.85−2.44	>0.05
Shared with animals	20	16.1	8	4.0	3.89	1.06−15.47	<0.001
Latrine							
Private	30	24.2	124	62.6	2.66	1.33−5.37	<0.01
Communal	50	40.3	42	21.2	1.85	1.06−4.17	<0.05
None	46	37.1	30	15.1	2.39	1.09−5.24	<0.05
Household head works	98	79.0	188	94.9	0.81	0.49−1.33	>0.05
Household head literacy	94	75.8	170	85.8	0.56	0.52−1.42	>0.05
History of child death	54	43.5	34	17.1	2.47	1.18−5.19	<0.05
Use of contraceptive	22	17.7	60	30.6	1.09	0.55−2.17	>0.05

with results reported for Navajo $(1.26\,\mu\mathrm{mol\,l^{-1}})$ and Turkish women $(1.4\,\mu\mathrm{mol\,l^{-1}})$[17,18], whereas it is higher than that reported for Tanzanian women $(0.89\,\mu\mathrm{mol\,l^{-1}})$[9]. On the other hand, the mean β-carotene level found in this study $(0.21 \pm 0.09\,\mu\mathrm{mol\,l^{-1}})$ is not much different from those reported for women in other developing countries. Levels of β-carotene in pregnant women in Malawi[19], postpartum Algerian mothers[7] and pregnant Nepalese women who were not suffering from night blindness[1] were 0.35, 0.19 and $0.24\,\mu\mathrm{mol\,l^{-1}}$, respectively. This low level of vitamin A and its precursor is presumed to be associated with VAD rather than pregnancy-related haemodilution. This assertion is based on the observation that classical functional disturbances of VAD such as poor dark adaptation patterns and abnormal conjunctival cytology were reported[10,20] among women having serum retinol level $<1.05\,\mu\mathrm{mol\,l^{-1}}$.

Information obtained regarding the diet of the women showed that they had a low intake of retinol-containing foods (animal and plant food sources), as has been found previously in Ethiopia and other developing countries[9,21]. In particular, the low level of serum β-carotene in the study population may reflect the absence of carotenoid-containing foods in the regular diet of the women. This association is supported by the fact that serum carotenoids including β-carotene are biomarkers of recent consumption of carotenoid-rich foods such as fruits and vegetables[22,23].

In many studies, socio-economic indices such as household income, mother's literacy and family size have been used as status indicators of socio-economic inequalities to illustrate their association with VAD[1,24−26]. Similarly, in this study, low serum vitamin A was associated with poor household hygiene, and low socio-economic and sociodemographic standing. Criteria used for categorisation were household income, ownership of a house, quality of the house (material from which the house is made), availability of a private toilet and running water, and whether the living quarters are shared with animals. An interesting additional interesting variable used was the association between vitamin A status and nursing the previous baby for a longer duration, but this association was not statistically significant. Other family health status indicators such as maternal age and parity could not be analysed because the study population was relatively young and the parameters were weakened when stratified further. In other studies, for instance in a study done among pregnant women in Sarlahi in rural Nepal and Bangladesh, night blindness was associated with teenage motherhood[1,26] and maternal illiteracy[25]. The risk of having retinol deficiency was associated with the number of family members: the larger the family, the higher the risk of night blindness[25]. The results of the present study are similar to the findings of Gebre-medhin et al.[27]. They showed that the breast milk of Ethiopian women of low socio-economic status contained 281−331 μg of vitamin A and 239−256 μg of β-carotene per litre, whereas in Ethiopian mothers of high socio-economic status, the corresponding values were 362−364 and 262−281 μg l^{-1}, respectively.

In normal adults[13], plasma α-tocopherol concentrations range from 11.6 to $46\,\mu\mathrm{mol\,l^{-1}}$. A cut-off for pregnant women is not well defined[9]. The use of normal adult values for pregnant women is inappropriate. Judgement regarding the serum level of α-tocopherol depends on the fact that there is a steady increase in serum vitamin E level during pregnancy as a compensatory mechanism for the increase in oxygen radical formation as gestation progresses[28,29]. The result obtained in our study, $25.5\,\mu\mathrm{mol\,l^{-1}}$, is almost identical to the non-pregnant reference vitamin E level $(24\,\mu\mathrm{mol\,l^{-1}})$ that was reported by Lockitch[14] and the postpartum concentration obtained by Cikot et al.[28]. After delivery, α-tocopherol concentration returns to the

preconception value. Results of the present study indicate our pregnant mothers at the third trimester have serum α-tocopherol values that would rather be expected preconception and postpartum, i.e. where there is no pregnancy-related oxidative stress. In our study 42% of women had lower serum α-tocopherol than the reference preconception level, indicating a high prevalence of deficiency. Comparisons show that the plasma level of serum α-tocopherol in these third-trimester Ethiopian women is lower than that found in women of developed countries at a similar stage of pregnancy. Dutch and Swiss women have been reported to have values of about 30.9 and 52.2 $\mu mol\,l^{-1}$, respectively[28,30]. Our result is more or less comparable to that in Algerian $(20\,\mu mol\,l^{-1})$[7], Nigerian $(21.1\,\mu mol\,l^{-1})$[8] and Tanzanian $(15.6\,\mu mol\,l^{-1})$[9] women.

Recent studies have indicated that children born to women with the lowest vitamin A levels have a four-fold greater risk of being infected with HIV-1 than those born to women with the highest vitamin A levels[24,30]. Vitamin A is indicated to play a role in enhancing the function of T and B lymphocytes. VAD women are more susceptible to infections of the genitourinary, intestinal and respiratory tracts[1]. There is increasing evidence that free radicals are the most likely promoters of maternal vascular malfunction[3,4]. Their concentration rises when the concentrations of retinol, α-tocopherol, β-carotene and ascorbic acid (lipid- as well as water-soluble antioxidant vitamins) that have the capacity to scavenge them decline. Free radicals can then diffuse to cell membranes, initiating lipid peroxidation by attacking polyunsaturated fatty acids in the cell membranes. Lipid peroxidation of fatty acids causes cell membrane damage. According to recent evidence, damage to the vascular endothelial cell membrane is the cause of pre-eclampsia and eclampsia[4,7,8]. Oxygen radical formation increases as gestation progresses[29]; likewise hypertensive disorders usually manifest themselves at an advanced stage of pregnancy. Furthermore, foetal growth restriction and premature rupture of membranes have been correlated with low maternal concentration of α-tocopherol[8,28,31]. However, longitudinal case–control studies are strongly recommended to determine whether the high rate of deficiency of antioxidant vitamins found among pregnant northern Ethiopian women is accompanied by the described pregnancy-related disorders.

In conclusion, the present study indicates that deficiencies of retinol, β-carotene and α-tocopherol are prevalent among pregnant women in northern Ethiopia, particularly among newcomers to the ANC. Circumstances linked with poverty (living in an impoverished house, poor household hygiene, low income) are indicators of possible retinol deficiency in these women. Therefore, multi-faceted intervention programmes during pregnancy should be designed and implemented. There is a need to strengthen programmes of vitamin A/multivitamin supplementation to pregnant women, along with health education and the promotion of horticultural habitude.

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