RESEARCH COMMUNICATION

Serum Antioxidant Vitamin Levels of People in Khon Kaen, Northeastern Thailand

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Abstract

Three antioxidant vitamins, the α- and β-carotenes as well as vitamin E, were measured in sera of a normal population in Northeastern Thailand using HPLC. The mean serum β-carotene level of females was significantly higher than the value for males, i.e, 37.55 (95%CI=34.59-40.51) versus 32.97 (95%CI=30.01-35.93) μg/dl. The β-carotene level tended to decrease as age increased, particularly in the male population. The mean serum α-carotene level was also higher in females than in males, i.e., 7.08 (95%CI=6.57-7.59) and 6.26 (95%CI=5.77-6.75) μg/dl, respectively. The average serum α-tocopherol (Vitamin E) level of the whole population was 1.08 (95%CI=1.04-1.12) μg/dl and did not show age or sex differences. In general, the serum antioxidant vitamins of smokers were lower than those of the non-smokers but a significant difference was observed only for α-tocopherol. Alcohol drinking resulted in slightly lower serum β-carotene values, whereas coffee or tea drinking and betel nut chewing did not cause any differences with these three antioxidant vitamins. However, we report higher in serum α-carotene levels of people in Ban Fang district than in Chonnabot district. The results from our study give the base line data of serum antioxidant vitamins in a Thai population and also suggest future intensive study on the relationship of dietary intake and cancer prevention.

Key Words : Carotenes - α-tocopherol - antioxidants - serum - Thailand - HPLC

Asian Pacific J Cancer Prev, **4**, 147-152

Introduction

Several epidemiologic and blood chemistry investigations have provided information about relationship between diet and disease. Certain serum parameters are associated with chronic diseases, including cancer. It has been generally assumed for many years that the intake of dietary carotenes and plasma carotene concentrations have an inverse association with cancer incidence (Mc Laron et al., 1967; Stahelin et al., 1984; Normura et al., 1985; Menkes et al., 1986). Besides β-carotene and α-carotene, other vitamins like vitamin C and E have similarly been found to decrease risk of several cancers (Hinds et al., 1984; Wald et al., 1984; Peng et al., 1998; Rumi et al. 1999). A high-performance liquid chromatography method for combined analysis of these antioxidant vitamins has been developed (Milne and Botnen, 1986; Thurnham et al., 1988), which is relatively quick and practical with small amounts of samples.

Cross-sectional studies of dietary intake, vitamin supplements and cigarette smoking behavior have revealed relations to serum antioxidant vitamin levels (Davis et al., 1983; Willett et al. 1983). Because it is reported that the high incidence of liver cancer in the northeast population of Thailand might have a relation to behavior of dietary intake as well as other risk factors (Vatanasapt et al., 1990a, 1990b; Sriamporn et al., 1993), we have focused on measuring the

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serum levels of the antioxidant vitamins α- and β-carotenes and α-tocopherol, to provide baseline data for people in the northeast of Thailand.

Materials and Methods

1. Sample Collection

The blood samples used in this study were obtained from the people who had no disease history in Chonnabot and Ban Fang Districts of Khon Kaen Province who participated in a mobile cancer screening programme during 1990. Those found with any abnormality from a physical investigation, such as oral cavity mass, breast mass, thyroid gland enlargement or from ultrasonography and Pap smear were excluded from this study.

Information of tobacco smoking, Betel nut chewing and alcohol drinking was obtained from questionnaires.

Overnight fasting venous blood was drawn, transferred to a microtube and kept in an ice-box. When the samples arrived at the laboratory in the University, the serum was immediately separated, and stored at –80°C until used for analysis, which was within 2 months of the sample collection.

2. Chemicals

Standard α- and β-carotenes, α-tocopherol,α-tocopherol acetate, sodium dodecyl sulfate (SDS) and butylated hydroxy toluene (BHT) were purchased from the local distributor of Sigma Co. The solvents including acetonitrile (HPLC grade), chloroform, methanol, n-hexane, n-heptane were products of Merck. PTFE filters were obtained from Whatman Ltd.

3. Analysis of Serum Antioxidant Vitamins

The HPLC technique as described by Thurnham et al (1988) was slightly modified to determine the serum levels of α-, β- carotenes and α- tocopherol. α-Tocopherol acetate was used as an internal standard. Rapidly, 0.25 ml of serum was mixed with 0.25 ml of 10 mM SDS reagent (Burton et al., 1985) in a light protected test tube. Then 0.5 ml of 40 μM tocopherol acetate in ethanol was added and vigorously mixed for 1 minute. 1 ml of 0.05% BHT in n-heptane was then added to the mixture which was continuously shaken for 2.5 minutes. After centrifugation at 2,500 g for 10 minutes, 0.7 ml of supernatant was separated and dried under nitrogen at 40°C. All steps were carried out under dimmed natural light. The dried sample was dissolved in 0.25 ml of mobile phase (acetonitrile: methanol: chloroform = 47:47:6). After filtering through a 2 μm PTFE filter, the filtrate was injected into an HPLC system (Perkin-Elmer 410). A Nova pak C18 column (3.9 X 150 mm, 4μm) connected with a guard column was used at a flow rate of 2 ml/minute. α- and β-carotene peaks were detected at 450 nm whereas α-tocopherol and α-tocopherol acetate peaks were observed at 292 nm. The % recovery of tocopherol acetate and the area under the peaks were used to calculate the vitamin amount. α-and β-carotenes were expressed as μg/dl, and tocopherol as μg/dl. The acceptable inter-batch and intra-batch coefficient of variations (CV_s) were within 10% and 5%, respectively. The limit of recovery was generally more than 60%.

4. Statistical Analysis

The t-test was used to test for statistical difference in comparison of two means and ANOVA (Analysis of Variance) was used to test statistical difference in comparison of more than two means. A 95% Confidence Interval (CI) was estimated for each individual mean.

Results

The total number of samples collected was 560, with age range 30-93. However, some obtained sera were not sufficient for analysis of all vitamins, thus the number of measured vitamins were not the same.

Figure 1 shows the chromatogram of standard vitamins. The retention times of the α-, and β-carotenes peaks were 6.74 and 7.27 minutes at 450 nm, whereas the values for tocopherol and tocopherol acetate were 2.35 and 2.69 minutes at 292 nm, respectively.

Tables 1-4 and Figures 2-4 demonstrate the mean levels of serum β- and α-carotene in the male, female and total population, showing sex and age differences. The serum β-carotene of the total population was 35.47 μg/dl (95% CI = 33.35-37.59). The female value was significantly higher than that for males, with mean levels of 37.55 μg/dl (95% CI = 34.59-40.51) and 32.97 μg/dl (95% CI = 30.01-35.93), respectively ($p<0.025$). Although there was no statistical difference, serum β-carotene levels of the male population decreased as age increased. Conversely, the female serum β-carotene levels significantly increased in proportion to the age ($p<0.025$). Generally, the female serum α-carotene level

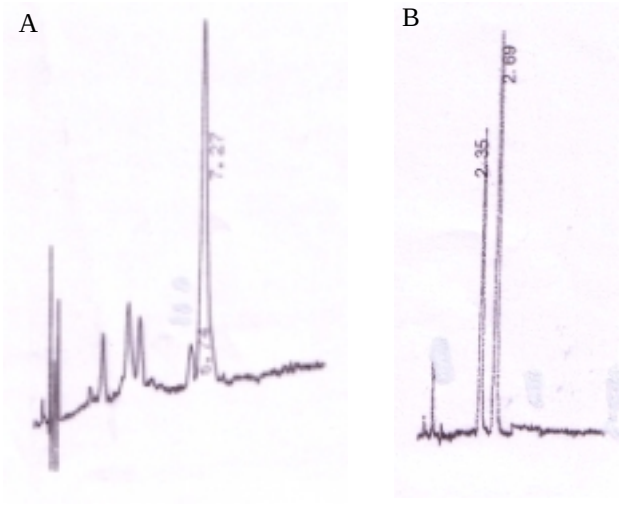

Figure 1. HPLC Chromatogram of Standard Vitamins. Retention times were 6.74 and 7.27 minutes detected at 450 nm for α- and β-carotenes (A); and 2.35 and 2.69 minutes detected at 292 nm for α-tocopherol and tocopherol acetate (B), respectively.

Table 1. Serum Antioxidant Vitamins of People in Khon Kaen, Northeast Thailand

	β-carotene Mean (μg/dl) ± SEM	95% CI	α-carotene Mean (μg/dl) ± SEM	95% CI	α-tocopherol Mean (mg/dl) ± SEM	95% CI
Male	32.97±1.51* (n=254)	30.01-35.93	6.26±0.25* (n=243)	5.77-6.75	1.07±0.03 (n=255)	1.01-1.13
Female	37.55±1.51 (n=296)	34.59-40.51	7.08±0.26 (n=292)	6.57-7.59	1.08±0.02 (n=304)	1.04-1.12
Total	35.47±1.08 (n=540)	33.35-37.59	6.70±0.18 (n=535)	6.35-7.05	1.08±0.02 (n=559)	1.04-1.12

*significant difference from female (p<0.025)

Table 2. Serum β-carotenes of People in Khon Kaen, Northeast Thailand within Various Age Ranges

Age	Male		Female		Total	
	Mean (μg/dl) ± SEM	95% CI	Mean (μg/dl) ± SEM	95% CI	Mean (μg/dl) ± SEM	95% CI
≤35	39.24±5.93 (n=16)	27.62-50.86	36.92±4.55 (n=25)	28.00-45.84	37.82±3.57 (n=41)	30.82-44.82
36-45	32.48±2.16 (n=102)	28.25-36.71	35.16± 2.53** (n=117)	30.20-40.12	33.91±1.6 (n=219)	30.77-37.05
46-55	31.57±3.08 (n=65)	25.53-37.61	39.60±2.77 (n=81)	34.17-45.03	36.02±2.0 (n=146)	32.10-39.94
56+	27.23±3.42* (n=32)	20.53-33.93	38.37± 3.93 (n=40)	30.67-46.07	33.42±2.72 (n=72)	28.09-38.75
Total	32.97±1.51* (n=254)	30.01-35.93	37.55± 1.51 (n=296)	34.59-40.51	35.47±1.08 (n=540)	33.35-37.59

*significant difference from females (p<0.025)
**significant difference when compared to 46-55 and 56+ years in females (p<0.025)

Table 3. Serum α-carotenes of People in Khon Kaen, Northeast Thailand within Various Age Ranges

Age	Male		Female		Total	
	Mean (μg/dl) ± SEM	95% CI	Mean (μg/dl) ± SEM	95% CI	Mean (μg/dl) ± SEM	95% CI
≤35	6.91±0.90 (n=16)	5.15-8.67	8.15±0.88 (n=25)	6.43-9.87	7.66±0.66 (n=41)	6.37-8.95
36-45	6.46±0.39 (n=102)	5.70-7.22	6.85±0.4 (n=116)	6.07-7.63	6.67±0.29 (n=218)	6.10-7.24
46-55	6.52±0.50 (n=64)	5.54-7.50	7.29±0.49 (n=80)	6.33-8.25	6.95±0.35 (n=144)	6.26-7.64
56+	5.23±0.60 (n=31)	4.05-6.41	6.53±0.69 (n=39)	5.18-7.88	5.95±0.48 (n=70)**	5.01-6.89
Total	6.26±0.25* (n=243)	5.77-6.75	7.08±0.26 (n=292)	6.57-7.59	6.70±0.18 (n=535)	6.35-7.05

* significant difference from females (p<0.025)
** significant difference when compared to ≤35 years (p<0.025)

was higher than that of males at every age range. The average level in females was significantly higher than in males, with mean values of 7.08 μg/dl (95% CI = 6.57-7.59) and 6.26 μg/dl (95% CI = 5.77-6.75), respectively. However, there were no differences between male and female serum α-tocopherol levels.

Tables 5-7 show the serum vitamin levels of people with respect to risk factors. Although the constant cigarette smoking group tended to have lower serum α- and β-carotene levels than those who were not smoking, there was

Table 4. Serum α-tocopherol of People in Khon Kaen, Northeast Thailand within Various Age Ranges.

Age	Male		Female		Total	
	Mean (mg/dl) ± SEM	95% CI	Mean (mg/dl) ± SEM	95% CI	Mean (mg/dl) ± SEM	95% CI
≤35	1.10±0.08 (n=17)	0.94-1.26	1.03±0.06 (n=24)	0.91-1.15	1.06±0.0 (n=41)	1.06-1.06
36-45	1.13±0.05 (n=103)	1.03-1.23	1.04±0.03 (n=120)	0.98-1.10	1.08±0.03 (n=223)	1.02-1.14
46-55	1.03±0.05 (n=69)	0.93-1.13	1.13±0.06 (n=81)	1.01-1.25	1.08±0.04 (n=150)	1.00-1.16
56 +	0.99±0.07 (n=36)	0.85-1.13	1.12±0.05 (n=46)	1.02-1.22	1.06±0.04 (n=82)	0.98-1.14
Total	1.07±0.03 (n=255)	1.01-1.13	1.08±0.02 (n=304)	1.04-1.12	1.08±0.02 (n=559)	1.04-1.12

no statistical significance. However statistical significance was observed for the serum tocopherol level ($p<0.01$). The serum β-carotene levels of alcohol consumers, who were mostly male, were not different from the non-alcohol drinking group. There were no differences in serum vitamin levels between people who did or did not drink coffee/tea.

For the betel nut-chewing group, the levels of all three serum vitamins were not different from the non-chewing group. It is interesting to note that α-carotene levels of people in Ban Fang district were significantly higher than those of people in Chonnabot district ($p<0.01$).

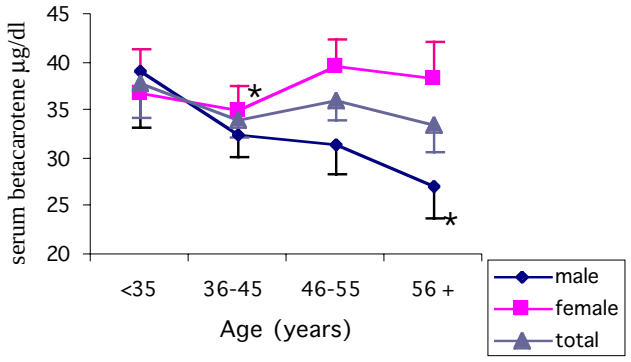

Figure 2. Serum β-carotene of People within Various Age Ranges (mean±SEM)
*significant difference from female at $p<0.025$
**significant difference from 46-55 and 56+ years in female

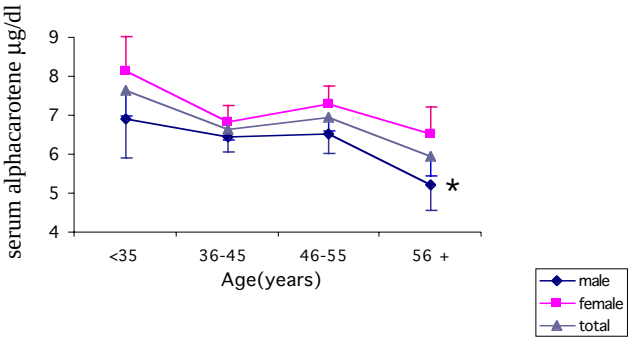

Figure 3. Serum α-carotene of People within Various Age Ranges (mean±SEM)
*significant difference from ≤35 years ($p<0.025$)

Discussion

The base line data obtained in this study indicate sex differences in serum antioxidant vitamins in people of Northeastern Thailand. Serum β-, and α-carotenes of females were higher than those of males, whereas serum α-tocopherol levels were not different.

The age difference of serum β-, and α-carotenes observed in males could reflect the possibility of lower carotenoid dietary intake in males or more absorption in females as reported earlier (Willett et al., 1983; Russell-Briefel et al., 1985 and Roidt et al., 1988). The findings that serum β-, and α-carotenes of cigarette smokers were lower than in non-smokers also tended to confirm the results of a previous study (Stryker et al., 1988). However, whereas the authors also reported the inverse relationship of serum α-, and β-carotenes in both cigarette smokers and alcohol drinking group, we only found a tendency for lower values of these three antioxidant vitamins in the alcohol drinking population with no statistical significance. This may result from the small numbers in the studied samples.

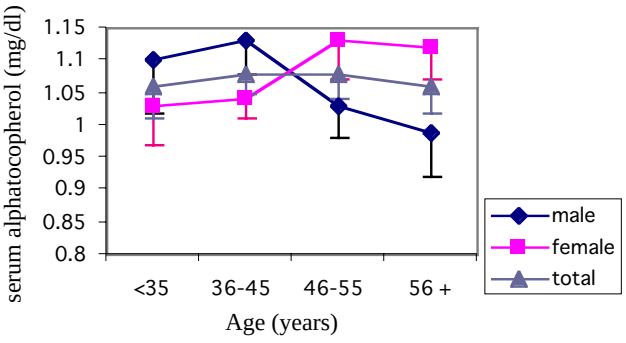

Figure 4. Serum α-tocopherol of People within Various Age Ranges (mean±SEM)

Table 5. Serum β-carotenes of People in Khon Kaen, Northeast Thailand with Various Behaviors.

	β-carotene Mean (μg/dl) ± SEM	95%CI
Cigarette Smoking		
Regular	33.02±1.74 (n=197)	29.61-36.43
Sometime	38.77±6.00 (n=15)	27.01-50.53
Non-smoking	37.29±1.45 (n=319)	34.45-40.13
Alcohol drinking		
Regular	31.82±5.61 (n=25)	20.82-42.82
Sometime	32.81±2.31 (n=109)	28.28-37.34
Non-drinking	38.53±1.67 (n=256)	35.26-41.80
Coffee/Tea drinking		
Regular	31.71±4.83 (n=27)	22.24-41.18
Sometime	35.23±9.3 (n=8)	17.00-53.46
Non-drinking	35.85±1.20 (n=446)	33.50-38.20
Betel nut chewing		
Regular	35.67±2.24 (n=120)	31.28-40.06
Sometime	38.23±4.31 (n=34)	29.78-46.68
Non-chewing	35.46±1.30 (n=385)	32.91-38.01
District		
Ban-Fang	37.34±1.66 (n=251)	34.09-40.59
Chonnabot	34.19±1.43 (n=290)	31.39-36.99

Table 6. Serum α-carotenes of People in Khon Kaen, Northeast Thailand with Various Behaviors.

	α-carotene Mean (μg/dl) ± SEM	95%CI
Cigarette Smoking		
Regular	6.18±0.28 (n=195)	5.63-6.73
Sometime	5.96±0.85 (n=15)	4.29-7.27
Non-smoking	7.41±0.41 (n=315)	6.61-8.21
Alcohol drinking		
Regular	7.47±1.08 (n=25)	5.35-9.59
Sometime	5.94±0.37 (n=107)	5.21-6.67
Non-drinking	7.51±0.47 (n=254)	6.59-8.43
Coffee/Tea drinking		
Regular	6.48±0.87 (n=26)	4.77-8.19
Sometime	6.78±2.08 (n=8)	2.70-10.86
Non-drinking	7.01±0.30 (n=442)	6.42-7.60
Betel nut chewing		
Regular	6.56±0.39 (n=117)	5.80-7.32
Sometime	5.72±0.62 (n=33)	4.50-6.94
Non-chewing	7.41±0.34 (n=383)	6.74-8.08
District		
Ban-Fang	7.29±0.28 (n=248)*	6.74-7.84
Chonnabot	6.66±0.41 (n=287)	5.86-7.46

* significant difference from people in Chonnabot ($p<0.01$)

Table 7. Serum α-tocopherol of People in Khon Kaen, Northeast Thailand with Various Behaviors

	α-tocopherol Mean (mg/dl) ± SEM	95%CI
Cigarette Smoking		
Regular	1.02±0.03 (n=206)	0.96-1.08
Sometime	1.16±0.07 (n=16)	1.02-1.30
Non-smoking	1.10±0.02 (n=324)*	1.06-1.14
Alcohol drinking		
Regular	1.13±0.09 (n=27)	0.95-1.31
Sometime	1.03±0.04 (n=117)	0.95-1.11
Non-drinking	1.08±0.03 (n=259)	1.02-1.14
Coffee/Tea drinking		
Regular	1.04±0.05 (n=29)	0.94-1.14
Sometime	1.03±0.14 (n=8)	0.76-1.30
Non-drinking	1.07±0.02 (n=457)	1.03-1.11
Betel nut chewing		
Regular	1.10±0.03 (n=125)	1.04-1.16
Sometime	1.01±0.05 (n=35)	0.91-1.11
Non-chewing	1.07±0.02 (n=394)	1.03-1.11
District		
Ban-Fang	1.05±0.03 (n=256)	0.99-1.11
Chonnabot	1.09±0.02 (n=300)	1.05-1.13

* significant difference from regular smoking ($p<0.01$)

It was surprising to find higher serum β-, α-carotenes of people in Ban-Fang district than in Chonnabot district. The report of cancer incidence from the Khon Kaen population-based cancer registry showed that the incidences of cancer during 1992-1995 of Channabot district were higher than that in Ban Fang district (273 per 100,000 in males, 171.1 per 100 000 in females in Chonnabot district and 146.4 per 100,000 in males, 105.7 per 100 000 in females in Ban Fang district). The incidence of cancer in males is higher than in females in both districts (Vatanasapt et al. 1998). Recently, Wallström et al. (2003) reported the results from the Malmö Diet and Cancer study in Sweden that non-smokers had higher serum β-carotene concentrations than smokers. They also demonstrated a positive association of serum β-carotene and α-tocopherol with fruit and vegetable and vitamin supplementation in non-smokers. Therefore, the explanation for our findings of higher serum β-, and α-carotenes of people in Ban-Fang may result from the higher antioxidant consumption than the people in Chonnabot district. However, studies on intensive dietary intake program and the cancer incidence of these people should be performed according to the previous report of the lower antioxidant vitamins in tissues and blood of cancer patients (Peng et al., 1998; Mireskandari et al., 1999; Rumi et al., 1999). Moreover, indigenous edible vegetables, reported to have high antioxidant vitamins (Sripanidkulchai et al., 2002), should be recommended to be consumed for possible protection against tissue damage by free radicals, and the prevention of cancer.

Acknowledgement

This research was a part of the main project on "a prospective study of cancer and other outcomes in a rural

population in Thailand" , which was approved on February 11, 1997 by the research ethic committee, Faculty of Medicine, KKU. We were deeply grateful to Professor Vanchai Vatanasapt for his support in this study.

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