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Changes in levels of serum beta-carotene, vitamin A and cholesterol in breast cancer of pre and post menopausal Nigerian women

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Abstract

Twenty newly diagnosed breast cancer patients were assessed for serum vitamin A, β - carotene and total cholesterol levels with their matched control (n = 20). The mean age and standard deviation of pre-menopausal breast cancer patients was 34.75± 6.57 while the mean age and standard deviation for controls was 32.00 ±8.79 years. There was no statistical difference in age between the two groups (P< 0.05). There was evidence of decrease in the mean serum vitamin A with increasing stage of advanced breast cancer. However, the mean serum β–carotene level showed significant decrease in trend with increasing stage of advanced breast cancer (P< 0.05). Serum total cholesterol was negatively correlated with serum vitamin A but was found to be positively correlated though non-significantly with serum β–carotene in breast cancer patients and control (r = 0.10, p> 0.95 for cases; r = 0.05, p> 0.05 for control).

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Keywords: β–carotene, vitamin A, breast cancer, menopause

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INTRODUCTION

Plants, fungi and bacteria synthesize carotenoids de novo while animals do not and rather obtain carotenoids from the diet and are enzymatically altered to vitamin A (Miki, 1991). Carotenoids perform a variety of biological functions which include functioning as vitamin A precursors (Matsuno, 1991), scavenger and quencher against free radicals and active singlet oxygen (Miki, 1991), anti-cancer agents (Krinsky, 1989) and immune system enhancers. Actions of carotenoids are either physiological or pharmacological (Olson, 1989) and may be related to intact carotenoid molecule rather than to a specific metabolite.

The inverse relationship between the ingestion of carotenoid – containing fruits and vegetables with the risk of certain forms of cancer has attracted significant attention.

The inverse relationship of blood β–carotene level with breast cancer has hitherto been restricted only to post-menopausal cases (Potischman et al; 1990). Pre-menopausal breast cancer patients has significantly lower blood retinol and β–carotene levels compared to post-menopausal breast cancer patients (Wald et al; 1984).

Reports show that the mean serum vitamin A level decrease significantly with increasing stage of the disease (Potischman et al; 1990; Torun et al; 1995).

Lack of knowledge of the role or relationships of cholesterol and breast cancer might mean that its association with cancer is secondary. However, serum cholesterol has been reported to be positively associated with serum vitamin A in all cancer sites (Kark et al; 1982) while little correlation was found between blood total cholesterol and β–carotene blood levels in breast cancer patients (Potischman et al; 1990; Stahelin et al; 1991).

The objectives of this work are therefore to:

i. predict the trend of vitamin A and β–carotene in advanced stages of breast cancer.
ii. test the hypotheses of low mean total cholesterol blood value in newly diagnosed pre-and post-menopausal breast cancer Nigerian women.
iii. deduce the association of serum total cholesterol to serum vitamin A and β–carotene in newly diagnosed breast cancer patients.

MATERIALS AND METHODS

Test and individuals were selected from weekly checks of patients in the Surgical and Radiotherapic Units of University College Hospital, Ibadan, Nigeria between November 1996 to June 1997. Cases consisted of twenty newly diagnosed histologically proven breast cancer patients (women only) with stages I, II, III and IV of the disease. Control subjects consisted of twenty patients who had negative history of breast cancer and malignant tumour, evidence of hepatic, vascular and metabolic disorders.

5mls 12 hours fasting blood samples were collected from both test and control individuals using sample bottles without anticoagulants. Blood was centrifuged at 3000xg for 10 mins. The sera were separated and stored at – 50^0C and analysed when necessary for various biochemical parameters.

Serum vitamin A (retinol) and β–carotene were assayed spectrophotometrically according to the method of Neeld and Pearson (1963). 1ml serum was pipetted into a centrifuge tube followed by the addition of 1ml of 95% ethanolic KOH and 1.5ml of hexane. The mixture was stoppered and vortexed vigorously for 2mins and centrifuged at 3000xg for 10mins. 2mls of the upper hexane extract was transferred into a dry test tube and the absorbance of the extract read at 450nm against hexane blank with Corning 258 spectrophotometer. The content of the curette was poured into a dry clean test tube. The extract was evaporated to dryness in a water bath (at 40^0C). 0.1ml of chloroform was then added to the residue in the test tube followed by the addition of 0.1ml acetic anhydride. The solution was capped to minimize evaporation and absorbance of the blue colour developed was read at 620nm with Corning 258 spectrophotometer at exactly 30secs after the addition of 1.0ml mixture of trifluoroacetic acid – chloroform reagent against blank containing 0.1ml of choloroform, 0.1ml of acetic anhydride and 1.0ml of trifluoroacetic acid – chloroform

reagent. The vitamin A concentration was then determined from the standard curve.

Serum total cholesterol was determined spectrophotometrically according to the method of Zlatkis et al (1953). The assay involved the addition of a fixed volume of concentrated sulphuric acid, glacial acetic acid and ferric chloride solution to 0.1ml serum. The full colour development is purple and required approximately 1 minute. 0.1ml serum was pipetted into 3ml of glacial acetic acid in a test tube followed by the addition of 2ml of colour reagent. The absorbance of the solution was read at 560nm on Corning 258 spectrophotometer. The serum total cholesterol was then determined from standard curve.

Student's t-test was used for statistical analysis. t-values < 0.05 were considered significant.

RESULTS

It can be observed from the table that pre-menopausal breast cancer patients had significantly lower mean total cholesterol level compared to post-menopausal breast cancer patients ($P< 0.05$). In addition, the result indicated that both pre-and post-menopausal breast cancer patients had lower mean serum total cholesterol compared to their matched control.

Table 1: Mean serum cholesterol level (mg/dl) in different stages of breast cancer

	Pre-menopausal women (< 45yrs)	Post-menopausal women (> 45yrs)
Cases		
Number	12	8
Mean age ± SD (yrs)	34.75 ± 6.57	58.88 ± 7.06
Mean serum cholesterol (mg/dl)	126.31 ± 23.56	180.20 ± 41.34
Number	12	8
Mean age ± SD (yrs)	32.00 ± 8.79	50.75 ± 6.24
Mean serum cholesterol (mg/dl)	174.56 ± 51.77	198.60 ± 39.98

SD = standard deviation

Table 2: Mean serum vitamin A and β-carotene levels in advanced stages of breast neoplasm

Stage of breast cancer	Number	Mean serum retinol level (µg/dl)	Mean serum β-carotene level (µg/dl)
III	6	33.95	149.91
IV	9	31.22	98.02

Table 3: Shows Pearson's correlation between serum total cholesterol with serum vitamin A and β-carotene in breast cancer.

	Cases, n = 20		Controls, n = 20	
	Serum vitamin A (µg/dl)	Serum β-carotene (µg/dl)	Serum vitamin A (µg/dl)	Serum β-carotene (µg/dl)
Serum total cholesterol (µg/dl)	- 0.30	0.10	- 0.14	0.10

The mean serum vitamin A and β-carotene levels in advanced stages of breast malignancy are shown in table 2.

There was significant decrease in the mean serum vitamin A with increasing stage of advanced breast cancer ($P < 0.05$). However, the mean serum β-carotene showed significant decrease with increasing stage of advanced breast cancer.

From the analysed data, serum total cholesterol was negatively correlated with serum vitamin A in both test and control individuals but was found to be positively correlated though non-significantly serum β-carotene in breast cancer ($r = 0.10$ for cases and $r = 0.05$ for controls).

DISCUSSION

From the results obtained in this investigation, serum total cholesterol was negatively correlated with serum vitamin A. This observation is different from the report of Kark et al (1982) where there was positive correlation between serum total cholesterol and serum vitamin A in all cancer sites.

Alternatively, the mean serum β-carotene level showed significant decrease with increasing degree of breast cancer. This observation is similar to the reports of earlier investigators that gave significant decreases of mean blood β-carotene level with increasing stages of the disease (Potischman et al, 1990; Torun et al; 1995). It is suggested that patients with the later stage of breast cancer might have altered their intake to a greater degree than patients with earlier stage of breast cancer.

There is an evidence in this study of significant decrease in trend in the mean serum vitamin A level with increasing stage of advanced breast cancer. This lends support to the earlier report of Ramaswamy and Krishnamorthy (1996).

Result of this investigation also revealed that serum total cholesterol was positively correlated though weakly with serum β-carotene in breast cancer patients. This is in keeping with the evidence of positive correlation between blood total cholesterol and β-carotene in patients with breast cancer (Potischman et al 1990; Stahelin et al, 1991).

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REFERENCES

Kark, J.D.; Smith, A.H. and Hames, C.G. (1982) Serum retinol and the inverse relationship between serum cholesterol and cancer. *Br. Med. J.* **284**:152– 4.

Kark, J.D.; Smith, A.H.; Switzer, B.R. and Hames, C.G. (1981) Retinol, carotene and the cancer cholesterol association. *Lancet* **2**:1371.

Krinsky, N.I. (1989) Antioxidant function of carotenoids. *Free Radic. Biol. Med*. **7**:617 – 35.

Matsuno, T. (1991) Xanthophylls as precursors of retinoids. *Pure Appl. Chem*. **63**: 81 – 88.

Miki, W. (1991) Biological functions and activities of animal carotenoids. *Pure and Appl. Chem.* **63**:141 – 146.

Needl J.B. (Jr.) and Pearson W.N. (1963) Micro- and macro-methods for the determination of serum vitamin A using trifluoroacetic acid. *J. Nutr*. **79**:454 – 462

Olson, J.A. (1989) Provitamin A function of carotenoids. The conversion of beta-carotene into vitamin A. *J. Nutr*. **119**: 105 – 108.

Potischman, N; Mcculloch, C.E.; Byers, T; Nemoto, Y; Stubbe, N; Milch, R; Parker, R; Rasmussen, K.M.; Root, P; Graham, S. and Campbell, T.C. (1990) Breast cancer and dietary and plasma concentrations of carotenoids and vitamin A. *Am J. Clin. Nutr*. **52**: 909 -915.

Ramaswamy, G. and Krishnamorthy, L. (1996) Serum carotene, vitamin A, and vitamin C levels in breast cancer and cancer of the uterine cervix. **Nutr. Cancer 25** : 173 – 177.

Stahelin, H.B.; Eichholzer, M. and Ludin, E. (1991) β-carotene and cancer prevention. The Basel Study. *Am. J. Clin. Nutr*. **53**: 2655 – 95.

Torun, M; Yardin, S. Gonenc, A; Sargin, H; Menesve, A. and Simsek, B. (1995) Serum β-carotene, vitamin E, vitamin C and malondialdehyde levels in several types of cancer. *J. Clin. Pharm. Ther*. **20**: 259 – 63.

Wald, N.J.' Boreham, J.; Hayward; J.L.; Bulbrook, R.D. (1984) Plasma retinol, β-carotene and vitamin E in relation to the future risk of breast cancer. *Br. J cancer* **49**: 321 - 324.

Zlatkis, A; Zak, B; Boyle, A.J. and Mich, D. (1953) A new method for the direct determination of serum cholesterol *J. Lab. Clin. Med.* **41**:486 – 492.