Anti-Oxidant Status of Male Adults with and without Prostate Cancer in Ibadan, Nigeria*

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Abstract

Background: Recent studies show increasing prostate cancer incidence in Nigeria. Significant correlations identified between diet and prostate cancer occurrence, indicate that low antioxidant status could contribute to the aetiology of prostate cancer. Methods: This cross-sectional study determined selected antioxidants (lycopene, beta-carotene and retinol) status of 10 (Experimental group) and 17 (Control) male adults with and without PC, recruited from the Urological Section of the Surgical Out-patients’ Department, University College Hospital and the General Out-patient Clinic of the Ring Road State Hospital, Ibadan, Oyo State, Nigeria. Demographic characteristics were assessed using a semi-structured, interviewer-administered questionnaire. Daily antioxidant intakes were assessed and analyzed using 24-hour dietary recall, and an adapted version of the food database “Total Diet Assessment”. Serum lycopene, beta-carotene and retinol were determined using High Performance Liquid Chromatography. Results: The mean age of the PC and the control (72.8 ± 6.2 years and 59.8 ± 4.8 years) was significantly different (p = 0.001). Mean daily lycopene intake (1408.4 ± 233.2 µg) of the PC was significantly lower (P = 0.030) than the controls (3862.3±316.2µg). The mean serum lycopene (19.8  ± 13.2 ng/ml), beta-carotene (43.6 ± 26.0 ng/ml) and retinol (362.2 ± 304.3ng/ml) of the PC were significantly lower (p = 0.008, 0.040 and 0.033 respectively) than the values (70.8  ± 49.8 ng/ml, 57.6 ± 47.7 ng/ml and 395.4 ± 275.6 ng/ml respectively) of the controls. Significant inverse correlations were observed between the dietary lycopene intake (r = −0.396, p = 0.041) and serum lycopene (r = −0.502, p = 0.008) with PC; while a significant positive association was observed between dietary intake of retinol with PC (r = 0.394, p = 0.042). Conclusion: The study has revealed low anti-oxidant status, and an inverse association between lycopene status and prostate cancer in the elderly men.

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1. Introduction

Prostate cancer (PC) has emerged as a major public health problem in nations with an affluent culture and an aging population [1] [2]. Globally, it is the third most common type of cancer in men, and the sixth most common cancer overall [3]. Contrary to the global ranking, recent hospital and cancer registry data show increasing prostate cancer incidence in Nigeria, which was previously regarded as a low incidence region [4] [5]. Prostate cancer, the number one cancer in Nigerian men, constitutes 11% of all male cancers [6].

As previously low risk countries have become more westernized, the incidence of prostate cancer has risen. The expense of screening programs, diagnostic tests, initial therapies, management of therapeutic complications, and the treatment of metastatic disease add significantly to national health care budgets for aging men [1]. The 2006 estimate of national expenditure for prostate cancer care in the US was USD 9.862 billion [7]. Significant correlations have been identified between dietary habits and prostate cancer occurrence [8]. Systemic oxidative stress plays an important role in the development and progression of cancer [9]. Antioxidants function by suppressing metabolic pathways, inhibiting cellular damage and lowering PSA in prostate cancer cell lines [10].

There is some limited evidence of benefits from lycopene in the treatment of established prostatic cancer, apparent in the form of modulation of biomarkers of disease, such as reductions in serum PSA levels, and oxidative metabolites of DNA damage (8-hydroxy-2’-deoxyguanosine) in the diseased prostatic tissues obtained from patient groups taking dietary lycopene supplements [11]. Debulking of tumour volume, resulting in a reduction in surgical margin involvement at radical prostatectomy, has also been described in patients supplemented with lycopene before surgery [12].

Retinoids have been shown to inhibit prostate cancer cell growth in vitro and to suppress prostate carcinogenesis through a signalling pathway that involves both nuclear hormone receptors and cytoplasmic carriers [13]. Both the esterification of all-trans retinol to retinyl esters and the levels of lecithin: retinolacyltransferase, were shown to be greatly decreased in human prostate cancer cell lines and in patient tumor samples [14].

In a re-analysis of Physician’s Health Study data, a 32 percent reduction in prostate cancer incidence was indicated for men receiving beta-carotene supplementation who were in the lowest quartile of plasma beta-carotene [15]. In Nigeria and West Africa as a whole, there is little documentation of the antioxidant status of male adults; and the relationship between antioxidant status and prostate cancer is not well explored.

This study therefore aimed at determining the dietary intake and serum levels of selected antioxidants (retinol, beta-carotene and lycopene) of male adults with and without prostate cancer; and exploring the relationship between their antioxidant status and prostate cancer.

2. Methodology

2.1. Subjects

The study was a cross-sectional, descriptive study. We recruited 10 subjects with localized prostate cancer (PC) from the Urology division of the Outpatient Department of Surgery of the University College Hospital, Ibadan, Oyo state, Nigeria. The controls were 17 male adults (>50 years) living in the same city, who had low serum PSA and low Prostate Symptom Scores, recruited from the General Outpatients Department of Ring Road State Hospital, Ibadan, Oyo state, Nigeria. The inclusion criteria were histologically confirmed non-metastatic prostate cancer, high serum PSA, age above 50 years, absence of acute illness and informed consent of willingness to participate in the study. The exclusion criteria were the presence of other histologically proven malignancies, chronic diseases of the liver and kidneys and inflammatory diseases of the urogenital tract. Subjects were selected by simple random sampling (ballot).

2.2. Data Collection

A semi-structured interviewer-administered questionnaire was used to quest for demographic characteristics.
Antioxidant intakes/day were assessed using 24-hour dietary recall. Serum lycopene, beta-carotene & retinol were determined using reversed phase High Performance Liquid Chromatography.

2.3. Antioxidant Intake Assessment
Retinol, beta-carotene & lycopene intakes/day were assessed using a pre-validated, structured 24-hour recall questionnaire. During pre-validation, a structured food frequency questionnaire was tested but discarded due to the short attention span of the subjects. Two 24-hour dietary recalls spaced 8 days apart were carried out to estimate usual food intake.

Due to the absence of a suitable dietary intake assessment computer software containing Nigerian foods, we analysed the subjects’ antioxidant intakes using “Total Diet Assessment Version 3.0 for Windows” after adapting it with the retinol, lycopene and beta-carotene contents of Nigerian foods. Estimation of lycopene intake was done using values from United States Department of Agriculture food database [16] due to the absence of published data on the lycopene content of Nigerian foods. We analysed beta-carotene intakes based on the beta-carotene content of Nigerian foods [17] and retinol based on the retinol values in Nigerian Food Composition Tables [18].

2.4. Serum Analysis
A single 5 ml blood sample was drawn from each subject by venipuncture by a phlebotomist. Samples were transported to the laboratory immediately, centrifuged at 2500 rpm for 10 minutes to obtain the serum which was stored in a freezer at −20°C for 2 weeks [19] until use. Samples were protected from photo degradation by transport, storage, extraction and chromatography under dimmed natural lighting, excluding direct sun and fluorescent light at all times and by using foil-wrapped test tubes.

2.5. Chromatography
High Performance Liquid Chromatography was carried out at the Central Science Laboratory of the Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

2.6. Extraction of Antioxidants in Human Serum
A method similar to that described by Tzeng et al. [20] was used for extraction. Serum samples were thawed and the antioxidants were extracted and analyzed. Analar grade solvents including ethanol & hexane (obtained from the laboratory of the Department of Human Nutrition, University of Ibadan, Oyo State) were used for the extraction of the antioxidants from the serum. 200 μl of serum was placed in a centrifuge tube. 1 ml of distilled water and 70 μl of ethanol solution containing 0.01% ascorbic acid were added for the precipitation of protein and protection of carotenoids. 2 ml hexane was added for the extraction of carotenoids, and the mixture was centrifuged at 2500 rpm for 20 minutes, after which the hexane layer was collected and evaporated to dryness under nitrogen gas. The residue was dissolved in 100 μl of mobile phase and 20 μl was drawn and injected into the HPLC system.

2.7. HPLC Analysis of Antioxidants in Human Serum
The HPLC system comprised of a C18 column (Agilent Eclipse XDB, 4.6 × 150 mm, 5 μm particle), a Rheodyne model 7161 injector (Rheodyne Co., CA, USA), an Agilent model 1100 pump (Agilent Co., CA, USA), and Agilent model 1100 UV-VIS detector. A Chem-station software was used to process data. HPLC grade methanol was used for the detection of the carotenoids and retinol. Standard beta-carotene (Catalogue No. C9750), standard retinoic acid (minimum 98% HPLC, Catalogue No. R2625) and Standard lycopene (Part No. ASB-00012550-005, Lot No. 00012550-771) all in pure crystalline form were obtained from Sigma Chemical Company.

After several tests using methanol, dichloromethane and acetonitrile in various volume combinations, we established the most suitable mobile phase to be a binary solvent system of methanol/deionised water (95:5, v/v) degassed by ultrasonication for 10 minutes. The UV detector was set at a wavelength of 272 nm for detecting retinol and the carotenoids respectively. The flow rate was 1 ml/min and column temperature was 25°C. Retinol,
beta-carotene and lycopene in serum samples were identified by comparing the retention times and absorption spectra of unknown peaks with reference standards.

Stock standards were prepared as follows: 1.6 mg of lycopene was dissolved in 10 mls of methanol; 25 mg of beta-carotene was dissolved in 25 mls of methanol and 12.5 mg of retinol was dissolved in 25 mls of methanol. Each stock standard was made up to 100 mls respectively with mobile phase to get the working standards. Flow rate was 1 ml/min and column temperature was 25˚C.

Retinol, beta-carotene and lycopene in serum samples were identified by comparing the retention times and absorption spectra of unknown peaks with reference standards. Because of the absence of a suitable internal standard, retinol and the carotenoids were quantified using absolute calibration curves. Five concentrations 0.1 μg/ml, 0.2 μg/ml, 0.5 μg/ml, 0.8 μg/ml and 1 μg/ml were prepared for each antioxidant. After injection into HPLC, the calibration curve of each carotenoid was made by plotting peak area (y) against concentration (x). The amounts of carotenoids and retinol were calculated from the following regression equations:

β-carotene: \[ y = 0.342x + 0.1863 \]
Lycopene: \[ y = 0.0278x + (-0.2541) \] and
Retinol: \[ y = 0.0113x + 0.1081 \]

2.8. Recovery Study

Human serum was spiked with 20 μl of combined standards with a concentration of 20 ng/ml each. The spiked serum was then extracted as described above. After HPLC analysis, the recovery of each antioxidant was obtained by dividing the calculated concentration by the added concentration. Duplicate analyses were carried out and the data were expressed as means ± standard deviations.

2.9. Data Analyses

Statistical analyses were done using SPSS for Windows version 16.0 [21] at the 95% confidence level. Means with their standard deviations and differences in means between groups (Student’s t-test) were computed for food items consumed, dietary and serum retinol, lycopene and beta-carotene. Spearman’s rank correlation was used to assess the relationship between the serum and dietary antioxidant status and prostate cancer. Student’s t-test was used to evaluate mean differences between the groups.

2.10. Ethical Considerations

Ethical Clearance was obtained from the University of Ibadan/University College Hospital Ethics Committee (UI/UCH Institutional Review Board). We obtained informed consent of willingness to participate in the study from the subjects that were recruited for the study. Full disclosure on the procedures, risks, discomfort and benefits was made to the patients and we adhered strictly to all confidentiality steps.

3. Results

The mean age of the PC and the control (72.8 ± 6.2 years and 59.8 ± 4.8 years) were significantly different (p = 0.001). Socio-demographic characteristics such as marital status, education, occupation and income did not differ significantly (p > 0.05). The mean serum PSA (1.72 ± 0.47 ng/ml) of the controls were significantly lower (p = 0.032) than that of the PC (134 ± 50.7 ng/ml).

Table 1 shows the top 20 food items frequently consumed by the respondents (mean intake in grams and the percentage of respondents consuming each item). The mean intakes of staple foods such as “eba”, “laafun” cooked rice and bread (575.0 g, 493.7 g, 273.8 g, and 229.0 g respectively) were higher in the PC group than the control group. Similarly, a higher proportion of the PC group consumed fish, beef and milk (70.0%, 60.0% and 50.0% respectively) than the control (52.9%, 29.4% and 0.0% respectively) group. The mean intake of tomato stew (67.3 g) was higher in the control than the PC (45.0 g) group.

Table 2 shows the mean daily antioxidant intake of the respondents. Mean lycopene intake (1408.4 ± 233.2 μg) of the PC was significantly lower than the value (3862.3 ± 316.2 μg) of the control (p = 0.030). No significant difference was observed in the retinol and beta-carotene intake (558.2 ± 147.5 μg RAE and 13.4 ± 8.2 mg) of the PC versus (545.6 ± 870.5 μg RAE and 10.1 ± 8.3 mg) of the control respectively (p = 0.964 and 0.321 respectively).
Table 1. Top 20 food items frequently consumed by the respondents.

<table>
<thead>
<tr>
<th>Food Items</th>
<th>Control g (%)</th>
<th>PC g (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize pap</td>
<td>186.7 (5.8)</td>
<td>250.0 (40.0)</td>
<td>0.893</td>
</tr>
<tr>
<td>Cold, stiffened maize pap (eko)</td>
<td>316.7 (17.6)</td>
<td>380.0 (30.0)</td>
<td>0.071</td>
</tr>
<tr>
<td>Cooked rice</td>
<td>203.0 (47.0)</td>
<td>273.8 (60.0)</td>
<td>0.038*</td>
</tr>
<tr>
<td>Bread</td>
<td>161.0 (29.4)</td>
<td>229.0 (40.0)</td>
<td>0.028*</td>
</tr>
<tr>
<td>Garri (eba)</td>
<td>475.0 (23.5)</td>
<td>575.0 (10.0)</td>
<td>0.049*</td>
</tr>
<tr>
<td>Yam flour meal (amala)</td>
<td>492.2 (52.9)</td>
<td>480.0 (50.0)</td>
<td>0.343</td>
</tr>
<tr>
<td>Cassava meal (laafun)</td>
<td>412.5 (11.8)</td>
<td>493.7 (20.0)</td>
<td>0.025*</td>
</tr>
<tr>
<td>Bean pudding (moimoi)</td>
<td>208.3 (17.6)</td>
<td>205.0 (20.0)</td>
<td>0.982</td>
</tr>
<tr>
<td>Bean cake (akara)</td>
<td>187.5 (23.5)</td>
<td>180.0 (10.0)</td>
<td>0.540</td>
</tr>
<tr>
<td>Boiled beans</td>
<td>238.9 (52.9)</td>
<td>297.5 (40.0)</td>
<td>0.540</td>
</tr>
<tr>
<td>Cooked fish</td>
<td>65.6 (52.9)</td>
<td>75.0 (70.0)</td>
<td>0.035*</td>
</tr>
<tr>
<td>Cooked beef</td>
<td>80.0 (29.4)</td>
<td>110.0 (60.0)</td>
<td>0.046*</td>
</tr>
<tr>
<td>Milk, filled, powdered</td>
<td>-</td>
<td>48.0 (50.0)</td>
<td>*</td>
</tr>
<tr>
<td>Egg</td>
<td>65.0 (5.8)</td>
<td>130.0 (30.0)</td>
<td>0.575</td>
</tr>
<tr>
<td>Tomato Stew</td>
<td>67.3 (76.4)</td>
<td>45.0 (40.0)</td>
<td>0.013*</td>
</tr>
<tr>
<td>African Spinach stew (eforiro)</td>
<td>79.2 (35.2)</td>
<td>85.0 (50.0)</td>
<td>0.693</td>
</tr>
<tr>
<td>Corchorus leaf soup (ewedu)</td>
<td>63.3 (58.8)</td>
<td>70.0 (60.0)</td>
<td>0.294</td>
</tr>
<tr>
<td>Fluted pumpkin soup (egusi)</td>
<td>-</td>
<td>100.0 (40.0)</td>
<td>*</td>
</tr>
<tr>
<td>Banana</td>
<td>50.0 (40.0)</td>
<td>150.0 (30.7)</td>
<td>0.512</td>
</tr>
<tr>
<td>Pineapple</td>
<td>90 (53.5)</td>
<td>155.0 (10.0)</td>
<td>0.681</td>
</tr>
<tr>
<td>Oranges</td>
<td>190.0 (5.8)</td>
<td>245.0 (20.0)</td>
<td>0.642</td>
</tr>
</tbody>
</table>

*aT-test not conducted because one group had no intake of these food items; *Significant difference detected.

Table 2. The mean daily antioxidant intake of the respondents.

<table>
<thead>
<tr>
<th></th>
<th>Control intakes (SD)</th>
<th>PC intakes (SD)</th>
<th>DRI intakes (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene (µg)</td>
<td>3862.3 ± 316.2</td>
<td>1408.4 ± 233.2</td>
<td>*</td>
<td>0.030*</td>
</tr>
<tr>
<td>Beta-carotene (mg)</td>
<td>10.1 ± 8.3</td>
<td>13.4 ± 8.2</td>
<td>*</td>
<td>0.321</td>
</tr>
<tr>
<td>Retinol (µg RAE)</td>
<td>545.6 ± 870.5</td>
<td>558.2 ± 147.5</td>
<td>900</td>
<td>0.964</td>
</tr>
</tbody>
</table>

*aNo DRIs have been established for lycopene and beta-carotene; *Significant difference detected.

In Table 3, the serum antioxidant status of the respondents is presented. The mean serum lycopene (19.8 ± 13.2 ng/ml), beta-carotene (43.6 ± 26.0 ng/ml) and retinol (362.2 ± 304.3 ng/ml) of the PC were significantly lower than the serum lycopene (70.8 ± 49.8 ng/ml), beta-carotene (57.6 ± 47.7 ng/ml) and retinol (395.4 ± 275.6 ng/ml) of the controls (P = 0.008, 0.040 and 0.033 respectively).

Correlations between antioxidant status and prostate cancer are presented in Table 4. A significant inverse association (p = 0.041) was observed between dietary lycopene intake and PC. Similarly, serum lycopene was significantly inversely correlated with PC (p = 0.008). There was a significant positive association between their retinol intakes and PC (p = 0.042), while non-significant correlations were observed between their dietary intakes of retinol, serum beta-carotene; serum retinol and PC respectively.

4. Discussion

The relationship between age and prostate cancer has been established, with age being a compelling, non-mod-
Table 3. Mean serum lycopene, beta-carotene and retinol status of the respondents.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene (ng/ml)</td>
<td>70.8 ± 49.8</td>
<td>19.8 ± 13.2</td>
<td>0.008*</td>
</tr>
<tr>
<td>Beta-carotene (ng/ml)</td>
<td>57.6 ± 47.7</td>
<td>43.6 ± 26.0</td>
<td>0.040*</td>
</tr>
<tr>
<td>Retinol (ng/ml)</td>
<td>395.4 ± 275.6</td>
<td>362.2 ± 304.3</td>
<td>0.033*</td>
</tr>
</tbody>
</table>

*Significant difference detected.

Table 4. Correlations between antioxidant status and PC.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>PC r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary intake of lycopene</td>
<td>−0.396</td>
<td>0.041*</td>
</tr>
<tr>
<td>Dietary intake of beta-carotene</td>
<td>−0.217</td>
<td>0.278</td>
</tr>
<tr>
<td>Dietary intake of retinol</td>
<td>0.394</td>
<td>0.042*</td>
</tr>
<tr>
<td>Serum lycopene</td>
<td>−0.502</td>
<td>0.008*</td>
</tr>
<tr>
<td>Serum beta-carotene</td>
<td>−0.064</td>
<td>0.751</td>
</tr>
<tr>
<td>Serum retinol</td>
<td>0.084</td>
<td>0.678</td>
</tr>
</tbody>
</table>

*Significant correlation.

A number of studies have examined retinol, beta-carotene and lycopene intakes and circulating levels in relation to the risk of prostate cancer and have suggested a protective effect of these antioxidants against prostate cancer. In the current study, the indication that dietary retinol intake was positively associated with prostate cancer is in agreement with older studies (in which a weak positive association between retinol intake and advanced prostate cancer was shown), [24] but is in contrast with more recent findings that dietary retinol is not associated with prostate cancer [25]-[27]. The mean dietary beta-carotene intake of the respondents in the PC group was significantly higher than the suggestion of an intake of 3 to 6 mg/day of β-carotene from food sources to maintain plasma β-carotene concentrations in the range associated with a lower risk of various chronic disease outcomes [27]. This high beta-carotene intake could be attributable to a high mean consumption by the subjects, of a vegetable soup cooked with palm oil (“efo riro”) which has been shown to be rich in beta-carotene [17]. No significant association was observed between beta-carotene intake and PC. This observation agrees with the findings that dietary beta-carotene intake is not associated with the risk of prostate enlargement [26] [28] [29].

Lycopene is a promising component for the chemoprevention of prostate cancer [30]. Regular intake of lycopene has been repeatedly associated with a reduced risk of developing the disease [31]. The significant inverse association detected between lycopene intake and prostate cancer in this study is consistent with the findings that evidence exists for inverse associations of dietary intake of lycopene with PC [13] [32] [33]. The serum lycopene levels observed in the present study were very low in comparison with the findings of the European Prospective Investigation into Cancer and Nutrition (EPIC) study [28]. The differences in mean serum lycopene observed in the present study are significant and are consistent with the findings of significantly lower serum lycopene levels in patients with prostate cancer compared with controls [9]. These low levels of serum lycopene could be attributable to the non-consumption of many lycopene-rich foods such as water melon, red-fleshed guava and others by the PC but may also be because patients with prostate cancer lack the ability to isomerize dietary lycopene and therefore do not absorb it efficiently [34]. The most common lycopene source in the diets of the subjects were bell pepper and tomatoes as contained in tomato stew which serves as an accompaniment to starchy staple foods consumed in the study area. This tomato stew was consumed in larger quantities by the control group.

In the present study, a statistically significant inverse association was found between serum lycopene levels and PC. This is in contrast with the indication [13] that there is no association between plasma lycopene and
prostate cancer, but are however consistent with the indication of an inverse association between serum lycopene and prostate cancer [35]-[37].

This study is one of the first to document the antioxidant status of elderly men in Nigeria and their association with Prostate cancer. Among its limitations are the small sample size, un-matched age of the subjects and cross-sectional design, therefore the explanatory power of this study is limited. The sample size used in this study was small due to the low patient turn-out in the study area at the time of the study. Furthermore, subjects were randomly sampled according to the inclusion criteria, (one of which was age above 50 years). However, the cross-sectional methodology may be quite relevant for current symptoms of Lower Urinary Tract Symptoms, compared to evaluating nutrients consumed a few years prior [38]. Furthermore the use of 24 hr dietary recall data in estimating nutrient intake does not provide a reliable estimate of an individual’s intake due to day-to-day variation [39]. Nevertheless the method is fast and easy to administer, particularly taking into consideration the short attention span of the elderly [40].

5. Conclusion

The study indicated evidence of low antioxidant status among elderly men with prostate cancer in Ibadan, Oyo State, Nigeria. Significant inverse associations were indicated between dietary and serum lycopene status and prostate cancer, while dietary retinol intake was significantly positively associated with prostate cancer.

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**List of Abbreviations**

1) PC: Prostate Cancer  
2) WCRF: World Cancer Research Fund  
3) AICR: American Institute of Cancer Research  
4) PSA: Prostate Specific Antigen  
5) DNA: Deoxyribonucleic Acid  
6) US: United States of America  
7) USD: US Dollar  
8) HPLC: High Performance Liquid Chromatography  
9) USDA: United States Department of Agriculture  
10) Rpm: Revolutions Per Minute  
11) UV-VIS: Ultraviolet-visible