International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: 10.7439/ijbr
CODEN: IJBRFA

Original Research Article

Dietary implication of high malondialdehyde, reduced vitamin D and total antioxidant status of prostate cancer subjects in Ibadan

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Abstract

A number of studies have investigated the roles of lipid peroxidation/ anti-oxidative balance as risk factors of prostate cancer. However, no study has yet to relate these factors with dietary habits of individuals in this environment. The objective of this study was to assess the role of lipid peroxidation and its relationship with vitamin D, calcium and dietary habits in individuals with prostate cancer. A total of sixty four (64) male participants (32 patients and 32 age-matched apparently healthy individuals as control) were recruited. Blood samples were analyzed for Malondialdehyde (MDA), Total antioxidant status (TAS), Calcium and Albumin spectrophotometrically; Prostate specific antigen (PSA) using immune radiometric assay; and Vitamin D by high performance liquid chromatography. Participants with prostate cancer had a significantly higher ($p \leq 0.05$) mean plasma level of MDA and PSA but lower TAS and vitamin D when compared with the control group. The plasma levels of MDA had a significant positive correlation with plasma PSA while negative with TAS in both groups. A significant proportion of the study participants that consumed dairy and smoked food regularly had a higher mean plasma level of MDA when compared with those that did not regularly as well as the control. This study has provided support for the hypothesis that lipid peroxidation and oxidative stress is associated with prostate cancer. A peculiar relationship exists between dairy and smoked food in prostate cancer which may be linked with increased lipid peroxidation.

Keywords: Lipid peroxidation, Antioxidant status, Diet Factors.

1. Introduction

Prostate cancer is the most common male cancer in the developed world, second only to lung cancer worldwide, and is the sixth most common cause of cancer death among men [1]. It has become the number one cancer in men with increasing incidence and morbidity in men of black African ancestry [2]. Its incidence and prevalence in black men is in multiples of those from other races in several studies, however, the reason for this is not yet clear [3]. In Nigeria, one of the earliest epidemiological studies done was by Nkposong and Lawani (1973) [4] from the University College Hospital, Ibadan, who reported a low but increasing incidence of prostate cancer. In recent times however, reports have shown that prostate cancer has become the top male cancer and fourth commonest cancer in Nigeria [5].

The risk factors for prostate cancer that can be considered established are age, race/ethnicity, and family history [6]. Many other factors are still being investigated as regarding the etiology of prostate cancer. A common example of such is the oxidant/antioxidant balance in the prostate gland. One of the common features associated with cancer cells is increased reactive oxygen species generation and most cancer cells exhibit elevated oxidative stress with increased metabolic activity and production of ROS [7].

Lipid peroxidation is one of the most investigated consequences of reactive oxygen species (ROS) actions on membrane lipid structure and functions. The oxidative damage of lipids, DNA and proteins has been shown to ultimately lead to outcomes such as disorganization, dysfunction, and destruction of membranes, enzymes and proteins [8].

Free radical damages can accumulate over time and may thereby contribute to cell injury and development of human diseases including atherosclerosis, diabetes, cancer, chronic inflammatory diseases and neurodegenerative diseases as well as in the process of aging [9,10, 11].

However, the idea of lipid peroxidation as a solely destructive process has changed during the past decade. It has been shown that lipid hydroperoxide and oxygenated products of lipid peroxidation initiators (that is ROS) can participate in the signal transduction cascade [12]. It has also been shown that lipid peroxidation and ROS are triggers and essential mediators of apoptosis, which eliminates precancerous and cancerous, virus-infected and otherwise damaged cells that threaten health [13, 14].

It has been hypothesized that lipid peroxidation represents a protective mechanism in breast cancer and this anticarcinogenic effect have also been associated with the potential anticarcinogenic effects of dietary factors, including soy [15], marine n-3 fatty acids [16], isothiocyanates (ITCs) [17], green tea [18], vitamin D [19], and calcium [20,21]. Moreover, numerous observational studies have found supplemental calcium and vitamin D to be associated with reduced risk of common cancers [22,23]. Diet on the other hand has been proposed to play a role in carcinogenesis. Few epidemiologic studies have linked greater intake of dairy products, animal protein and fats to higher prostate cancer risk [24].

Gago-Dominguez *et al.*, (2005) [25] suggested that many dietary factors have both antioxidant and pro-oxidant properties; and in answering the question of which property is responsible for their anti-cancer effect raised the possibility that a deficiency in lipid peroxidation, not an increased antioxidant potential may be detrimental for cancer.

Although a number of studies have investigated the role of lipid peroxidation and its balance with the level antioxidants in prostate cancer, such studies did not however relate these with the dietary habits of the individuals examined. This study therefore examined the relationship between the extent of lipid peroxidation, antioxidant status and the dietary habits of individuals with prostate cancer.

2. Subjects and methods

The study was approved by the University of Ibadan / University College Hospital (U.I/U.C.H) Joint Ethics Review Committee and informed consent was obtained from each of the participants prior to specimen collection.

2.1 Study design

A total of sixty four (64) male participants of above fifty years of age were recruited for this study. Thirty two (32) of the participants with prostate carcinoma were recruited from the Surgery department (Surgical out-patient), University College Hospital, Ibadan, Oyo State. Thirty (32) apparently healthy, age-matched control subjects were also recruited. Exclusion criteria included participants less than 50 years of age, participants on multivitamins, food supplements or therapy at recruitment. Diagnosis of prostate cancer was by clinical examination, histological investigation, and biochemical result of prostate specific antigen (PSA). A data collection form which contains items on the demographic characteristics, nutritional history (which included participants' dietary recall and the frequency of consumption of major food items) and diagnosis was used for participant recruitment. Data collection was by personal interview and informed consent was obtained from all the participants after educating them on the benefit and relevance of the study.

2.2 Sample collection and preparation

Ten (10) mls of blood sample was collected from each subject by venepuncture after overnight fasting, into lithium heparinised bottle. The blood samples were centrifuged at 5000g for 15 minutes and the plasma were separated into labeled plain bottles and stored at -20 ^{0}C (aliquots for vitamin D at -80 ^{0}C) until analysis.

2.3 Method of analysis

Plasma Total antioxidant status (TAS), Malondialdehyde (MDA), calcium and albumin were determined using standard colorimetric methods. TAS was determined by the method described by Koracevic *et al.*, 2001 [26]. Malondialdehyde was estimated by spectrophotometry as described by Buege and Aust, 1978 [27]. Albumin was determined spectrophotometrically using bromocresol green (BCG) as described by Doumas *et al*., 1971 [28]. Calcium was determined by spectrophotometric method, which is based on modified Ortho-cresolphtalein complex methodology (Biggs and Moorehead, 1974) [29]. 25(OH) Vitamin D was determined using high performance liquid chromatography. Estimation of total PSA was by Immuno Radiometric Assay kit prepared by Institute of Isotopes Ltd, 1535 Budapest. (Product code: RK-10CT).

2.4 Statistical analysis

Statistical analysis was performed using SPSS software version 19.0. All data were expressed as mean±standard deviation (SD) for study participants and controls. Independent T-test was used to compare the difference between the means of

the two groups studied, while 2×2 Chi-square test was used to compare the difference between the percentage frequencies of consumption of diets of the study participants and controls. Pearson's correlation was used to investigate the relationship between prostate specific antigen and other biochemical parameters studied. *P*-value < 0.05 was considered significant.

3. Result

A total of sixty four (64) subjects participated in the study, which included thirty two (32) participants with histopathological diagnosis of prostate cancer, and thirty two (32) apparently healthy individuals as control.

3.1 Malondialdehyde (MDA)

The extent of lipid peroxidation which is measured by the level of plasma MDA is shown in Table 1. The mean plasma MDA was significantly higher (P < 0.05) in patients with prostate cancer than in controls, (3.797 ±1.215 versus 2.775±0.826).

3.2 Total Antioxidant status

Patients with prostate cancer had a mean plasma TAS of 0.913±0.793 which was significantly lower (P < 0.05) than the mean plasma level of the control group (1.076±0.113).

3.3 Vitamin D

Mean plasma 25(OH)vit D was lower in patients with prostate cancer (30.362±8.639) compared to the control group (67.183±7.043).

3.4 Calcium and Albumin

There was no significant difference in the plasma level of total calcium and albumin between the case and control groups (P < 0.05).

Table 1: Comparison of biochemical parameters in study participants and controls

Parameter	Control n = 32	Cases n = 32	T	*P*-value
Malondialdehyde (×10^{-5} U/mg prot.)	2.775±0.826	3.797 ± 1.215	3.934	0.000*
Total Antioxidant Status (mmol/l)	1.076 ± 0.113	0.913 ± 0.793	6.705	0.000*
Albumin (mg/dl)	4.225 ± 0.375	4.309 ± 0.370	-0.906	0.906
Vitamin D (ng/dl)	67.183 ± 7.043	30.362 ± 8.639	18.452	0.000*
Prostate Specific Antigen (ng/mL)	1.491 ± 1.047	118.86 ± 128.3	-5.174	0.000*
Calcium (mg/dl)	8.83 ± 0.831	8.81 ± 0.708	0.081	0.936

Table 2 represents the frequency distribution of the rate of consumption of different food substances taken by both the study and control groups. Participants with prostate cancer accounted for a higher percentage of regular consumption of milk/dairy products (65.6%) and smoked food (46.9%) which were significantly different (P= 0.045 and 0.035 respectively) from their control counterpart with lesser percentages of 40.6 and 21.9 respectively. This pattern of distribution is further illustrated by Fig.1; a bar chart showing the frequency distribution of intake of milk/dairy and smoked food in both case and control subjects.

To further determine the relationship that exist between the two implicated diets (milk and smoked food) and the studied biochemical variables, each group (case and control) was split into regular and non regular consumers. The mean values of the biochemical parameters of the subgroups were compared using independent T-test. This is presented in Tables 3 and 4. Here, no significant difference existed in the mean values of the biochemical parameters tested while categorizing both control and study participants on regular and non regular intake of milk. However, the level of MDA was significantly higher in PCa patients that took smoked food regularly than those who did not (P= 0.010).

Table 2: Frequency distribution on the consumption of certain diets among control and study participants

Diet	Regular (n,%)	Non regular (n, %)	P-value
Dairy (Milk)			
Control (32,100)	13, 40.6	19, 59.4	0.045*
Cases (32,100)	21, 65.6	11, 34.4	
Fish			
Control (32,100)	23,71.9	9, 28.1	0.777
Cases (32,100)	23,71.9	9, 28.1	
Chicken			
Control (32,100)	9, 28.1	23, 71.9	0.784
Cases (32,100)	10, 31.3	22, 68.8	
Nuts			
Control (32,100)	7, 21.9	25, 78.1	0.171
Case (32, 100)	12, 37.5	20, 62.5	
Beef			
Control (32,100)	13, 40.6	19, 59.4	0.451
Cases (32,100)	16, 50.0	16, 50.0	
Pork			
Control (32,100)	1, 3.1	31, 96.9	1.000
Cases (32,100)	1, 3.1	31, 96.9	
Egg			
Control (32,100)	9, 28.0	23, 71.9	0.777
Cases (32,100)	8, 25.0	24, 75.0	
Smoked food			
Control (32,100)	7, 21.9	25, 78.1	0.035*
Cases (32,100)	15, 46.9	17, 53.1	
Fruits/Vegetables			
Control (32,100)	25, 78.1	7, 21.9	0.396
Cases (32, 100)	22, 68.8	10, 31.2	

Figure 1: Frequency distribution on the intake of milk and Smoked food among patients with prostate cancer and control.

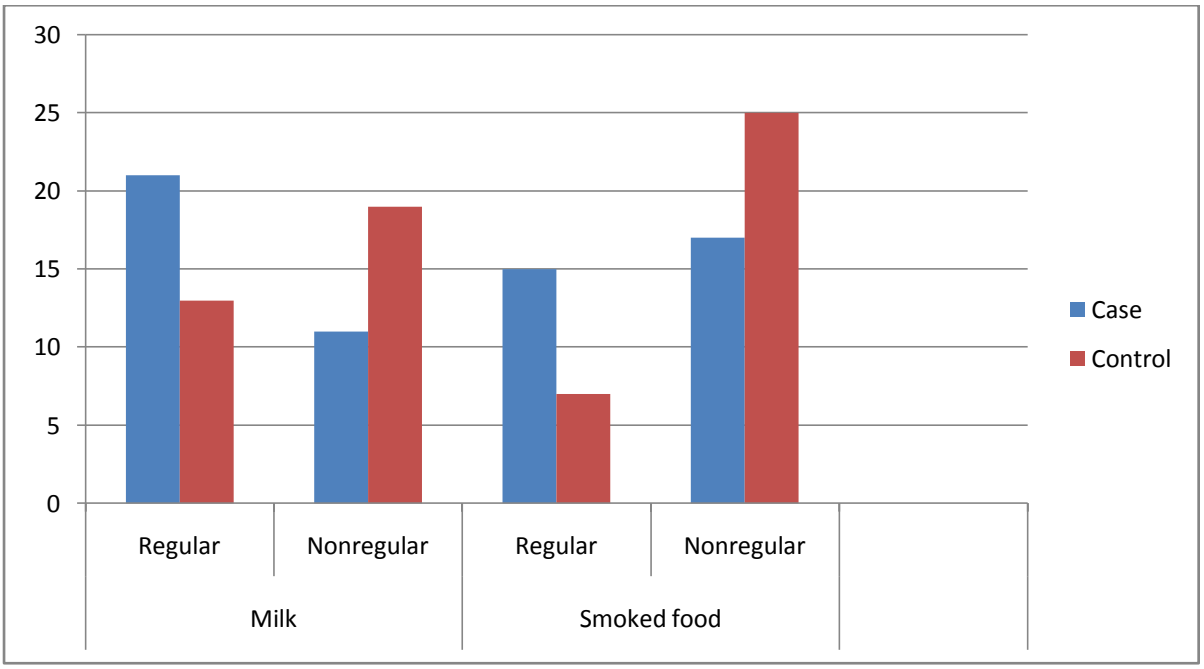

Table 3: Comparison of biochemical parameters by regular and non regular intake of dairy (milk) in study participants and controls (mean±SD)

Parameter	Regular	Non regular	T	*P*-value
Malondialdehyde (×10^{-5} U/mg prot.)				
Control	2.76±0.75	2.67±0.88	0.293	0.772
Case	4.01±1.06	3.24±1.29	1.40	0.171
Total Antioxidant Status (mmol/l)				
Control	1.078 ± 0.125	1.072 ± 0.118	0.147	0.884
Case	0.900 ± 0.086	0.933 ± 0.072	-1.172	0.250
Vitamin D (ng/dl)				
Control	65.62 ± 4.82	67.35 ± 7.24	-0.769	0.448
Case	30.25 ±9.04	30.25 ± 8.40	0.347	0.731

Table 4: Comparison of biochemical parameters by regular and non regular intake of smoked food in study participants and controls (mean ±SD)

Parameter	Regular	Non regular	T	*P*-value
Malondialdehyde (×10^{-5} U/mg prot.)				
Control	2.27 ± 0.85	2.84 ± 0.85	-1.63	0.105
Case	4.30 ± 1.32	3.20±1.14	2.750	0.010*
Total Antioxidant Status (mmol/l)				
Control	1.13 ± 0.05	1.06 ± 0.13	1.390	0.175
Case	0.900 ±0.08	0.933 ± 0.03	-1.029	0.312
Vitamin D (ng/dl)				
Control	65.70 ± 5.88	66.87 ± 6.50	-0.455	0.670
Case	31.60 ±8.19	30.13 ± 7.40	0.475	0.638

Using Pearson correlation, the levels of plasma PSA was compared with the other biochemical parameters in the study which is presented in Table 5. There was a strong positive correlation between the levels of PSA and MDA which was significant in both case and control population. Similarly, there was a significant correlation between the levels of PSA and TAS which appeared to decrease with increase in PSA.

Table 5: Pearson's Correlation between Prostate specific antigen and biochemical parameters in study participants and controls

	Control	Cases
Albumin (mg/dl)	r = -0.033	r = -0.241
	P = 0.858	*P* = 0.183
MDA (×10^{5} U/mg prot.)	r = 0.434*	r = 0.887*
	P = 0.013	*P* = 0.000
TAS (mmol/L)	r = -0.323	r = 0.812*
	P = 0.071	*P* = 0.000
Vit. D (ng/dl)	r = -0.110	r = -0.361*
	P = 0.548	*P* = 0.043
Calcium (mmol/L)	r = 0.099	r = 0.244
	P = 0.590	*P* = 0.178

4. Discussion

Lipid peroxidation which is effectively indicated biochemically by the level of Malondialdehyde is one of the most investigated consequences of reactive oxygen species (ROS) actions on membrane lipid structure and functions. The association of lipid peroxidation with prostate cancer is still in clear debate. It has been argued that products of lipid peroxidation are triggers and essential mediators of apoptosis, which eliminates precancerous and cancerous cells that threaten health [13]. Furthermore, this anticarcinogenic effect can be associated with the potential anticarcinogenic effects of dietary factors such as vitamin D [19] and calcium [21].

In this study, the level of plasma MDA was significantly higher in participants with prostate cancer than the control. It also had a positive significant correlation with the level of prostatic specific antigen. This is in agreement with the study conducted by Oparinde *et al*., (2013) [30]. However, it is not clear whether this is a cause-effect relationship with regards to increased free radical levels leading to the development of cancer or vice versa; although Freeman and Solomon (2004) [31] had earlier demonstrated that fats accumulate in solid tumors and that cholesterol homeostasis breaks down in the prostate with aging and with transition to the malignant state. This could probably account for the susceptibility to lipid peroxidation and increase in the system.

Another major finding of our study was that the total antioxidant status was significantly reduced in subjects with high PSA values and this inverse relationship shows that oxidative stress could be positively associated with prostate cancer. In 2009, Oluyemi *et al.,* [32] reported a similar finding with individual antioxidants such as superoxide dismutase, catalase and vitamin E which was also supported by the observations made by Sosanya *et al.,* (2014) [33]. Meanwhile, from an earlier study we suggested that the reason for which there was no significant difference in the level of total antioxidant status was probably because subjects took enough antioxidants that protected them from oxidative stress as at the period of study [34]. The report of this research however differ from the hypothesis advocated by Gago-Dominguez *et al.,* (2005) [26] supporting a deficiency in lipid peroxidation and increased antioxidants as a procarcinogenic factor.

According to Khandrika *et al.,* (2009) [8], processes associated with proliferation, apoptosis, and senescence may be a result of the activation of signaling pathways in response to intracellular changes in ROS levels. Thus, the excessive production of ROS or inadequacy in normal cell's antioxidant defense system (or both) can cause the cell to experience oxidative stress and the increased ROS may play a broader role in cellular processes associated with initiation and development of many cancers including prostate cancer. Though recent studies have indicated that oxidative stress is higher in the epithelium of prostate cancer patients than men without the disease the association of ROS-mediated oxidative stress and prostate cancer risk remains to be elucidated. Theories abound regarding their role in initiation of prostate cancer, and include but not limited to, failure of antioxidant defense mechanism (due to persistent oxidative stress) leading to inherited and acquired defects in the defense system. Other areas of defect are mitochondrial DNA mutations, chronic inflammation, defective DNA repair mechanism and apoptosis etc. All these finally lead to the development of prostate cancer. Thus, many of the factors that are associated with prostate cancer like aging, imbalance of androgens, antioxidant system, dietary fat, and pre malignant conditions like high grade prostate intraepithelial neoplasia etc. may be linked to oxidative stress [7].

Furthermore, the results of this study showed a strong significant difference in the level of vitamin D between the case and control group which also consistently had an inverse relationship with the level of PSA. This is consistent with findings from a number of previous studies [22, 35]. The biochemical evidence to support a role for vitamin D in prostate cancer includes the demonstration of vitamin D receptor (VDR) and the anti-proliferative, apoptotic and prodifferentiation activities of $1\alpha,25(OH)_2D$ and its analogs in prostate cells in vitro and in vivo [37]. Attempts to account for reasons for deficiency of vitamin D in prostate cancer has been however inconclusive. As early as 1990, Schwartz and Hulka [37] while stating their hypothesis that vitamin D deficiency may be related to risk of prostate cancer, made an observation that black race, northern latitudes, and older age all appeared to be positively correlated with greater risk of prostate cancer as well as vitamin D deficiency.

Meanwhile, Philippe *et al.,* 2014 [38] in a review has suggested an alternative reason for which vitamin D could be deficient in cancer including prostate cancer. They were curious to know whether low 25(OH)D might be the result, rather than the cause, of physiological disturbances involved in diseases including prostate cancer. In their conclusion, they opined that low level of vitamin D could be the result of inflammatory processes involved in the occurrence and progression of diseases especially cancer. Inflammation is believed to be the common factor between most non-skeletal health disorders and low 25(OH) concentrations.

In this study, regular consumption of milk/dairy and smoked food was associated with increased risk of prostate cancer. Several studies have consistently linked dairy intake with prostate cancer [24]. While some have attributed its prostate cancer causing potential to high calcium - low vitamin D pathway, others have associated it with its ability to increase androgen which has been suggested to increase cell division, activation of proto-oncogenes, and inactivation of tumor suppressor genes [39]. The prostate is an androgen-regulated organ, thus, they are strong candidates as major contributors to prostate carcinogenesis. The association of smoked food with prostate cancer is consistent with our earlier reported study [40] where we particularly

noted that when muscle meat, including beef, pork, fish and poultry, is cooked using high temperature methods, such as grilling directly over an open flame, they form chemicals like Heterocyclic Amines (HCA's) and Polycyclic Aromatic Hydrocarbons (PAHs) which generally are risk factors for cancer. In addition, a clear positive association between regular consumption of smoked food and lipid peroxidation seem to have been revealed. Smoke on the other hand is a well known environmental oxidant with high potential for oxidative stress hence carcinogenesis. This pattern has been reported by other studies [41].

5. Conclusion

This study has provided support for the hypothesis that lipid peroxidation and oxidative stress is associated with prostate cancer. It has also shown a relationship for vitamin D in prostate cancer which is not necessarily linked to the extent of lipid peroxidation but may be looked into as a therapeutic factor in preventing the disease state. A role for diet, especially dairy and smoked food in prostate cancer which may be linked with increased lipid peroxidation has also been established.

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