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Vitamin B and Antioxidants in Relation to Treatment Duration in Cervical Cancer Patients in Ibadan

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Abstract

In this study we evaluated the variations in vitamin B namely cyanocobalamin, folate and pyridoxine, choline, protein and glucose-6-phosphate dehydrogenase (G6PD) activity in cervical cancer patients at pretherapy, when patients showed up at the clinic and at various times after the commencement of chemotherapy and radiotherapy (using one month and two months respectively). The subjects comprised 32 patients who had visited the Department of Radiotherapy and had been diagnosed with cancer of the cervix (cervical cancer) and 32 age matched control. There was a significant ($\rho<0.05$) decrease in the B vitamins and choline in the different groups relative to control. There was a marked variation in plasma protein at two months compared to control and G6PD significantly decreased ($\rho<0.05$) at one month and two months of treatment relative to control. The study showed the relationship between low levels of vitamin B group, choline, G6PD and plasma protein with cancer progression and changes accompanying treatment and a probable need for supplementation with immune boosters during therapy.

Introduction

Cervical cancer is the second most common cancer in women all over the word, next to breast cancer; around half a million new cases are diagnosed and over two hundred thousand deaths are attributed to the disease annually [1]. The incidence of cervical cancer is most common in developing countries, where routine cervical screening programs are largely absent due to lack of information and poverty [2]. Infection with the human papillomavirus (HPV) is the established major risk factor for cervical cancer [3]. Although HPV infection is common, only a small proportion of HPV infections persist and go on to promote the development of invasive cervical cancer. Other factors must influence the susceptibility to infection with HPV and the development of HPV-induced neoplastic changes to invasive cervical cancer. In addition to promoter-site hypermethylation, a decrease in global DNA methylation is also a common feature of carcinogenesis [4].

Most importantly, dietary factors continue to play a complex and multifaceted role in the aetiology of cancer. Apart from cigarette smoking and chronic inflammation and infection, nutrition accounts for up to one third of the total cause of cancer [5]. So, man cannot shy away from taking nutrient, most especially micronutrient (which includes vitamins and some small nutrients such as choline). Of more grave importance are even the B complex vitamins because they form the bulk of co-factors and prosthetic groups of many enzymes in the body. Therefore serve as a determinant for proper execution of many processes in the system such as gene expression which requires absolute accuracy while being done. Disruption in gene expression or mutation in DNA or gene product has been confirmed to be the most common cause or resulting-cause of the cancer types and Ames [6] reported that a deficiency of any of the micronutrients: folic acid, Vitamin B_{12}, Vitamin B_6, niacin, Vitamin C, Vitamin E, iron, or zinc, mimics radiation in damaging DNA by causing single and double strand breaks, oxidative lesions, or both. Folate deficiency causes extensive incorporation of uracil into human DNA (4 million/cell), leading to chromosomal breaks.

Adequate folate status is thought to be an important determinant of normal DNA methylation status, through provision of methyl groups by 5-methyltetrahydrofolate [7,8]. There is epidemiologic evidence which suggests that folate deficiency contributes to cancer risk at several sites in the body [9], but the findings in studies with respect to folate and cervical cancer risk have been rather inconsistent. Case-control studies that estimated dietary folate intake have generally not been supportive of a protective role for folate [10-13], whereas case-control studies that used biochemical measures of folate status [14-18] were more convincing. Out of four randomized controlled trials using folic acid only one showed a significantly protective effect [19-22]. Folate and vitamin B_{12} may have modulating effect in the risk cervical cancer development. Folate has been recognized as a contributing factor to ensure reproductive health and there is a growing body of evidence that reduced dietary folate may increase the risk of cervical cancer.

Water-soluble vitamins have varying half-life in the body; vitamin C could be up to forty days, some 6-8 days riboflavin for both absorption and elimination from the body hence rapid assay methods have been used previously and plasma samples are stabilized by addition of ascorbic acid [23-26]. The above informed our decision to evaluate the levels of folate, choline, pyridoxine and cyanocobalamin, using high performance liquid chromatography (HPLC) a very rapid assay method, protein and glucose-6-phosphate dehydrogenase (G6PD) using biochemical assay of the parameters in plasma of both patients and healthy controls at both pretreatment of disease and at varying times after the commencement of therapy.

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Materials and Methods

Sample collection

All clinical samples were collected from women attending the Radio medicine Clinic, Radiotherapy department of the University College Hospital, Ibadan, Nigeria following local or regional referral. Informed signed consent was taken in the clinic at the time of the appointment. The use of human samples was approved by University of Ibadan research ethics committee. Women were only excluded from the study if their clinic records indicated other types of cancer. Thirty two cervical cancer patients were enrolled for the study and age matched controls were chosen from Ibadan North metropolis, for the study.

Plasma collection

Blood was collected in heparinized tubes to avoid clotting and stored in refrigerator. Plasma was then separated by centrifuging the heparinized blood in a cold centrifuge at 3000G for 15 minutes; the supernatant was collected into a plain sample tube. These samples were stored at −20°C until needed for various assays.

Vitamin B_{12} assay

Cyanocobalamin level determined using Waters 616/626 HPLC USA. This machine runs the sample via a suitable mobile phase and employs the interaction between the analyte and it solid phase to separate the content of the sample. Equipment was calibrated by pre-run with varying standard Vitamin B_{12} and values of the vitamin in each sample is generated automatically from the calibration curve. Briefly, sample was prepared by adding 0.5 ml of the plasma into clean beakers, 25 ml of 0.2 M HCl was added and the mixture warmed in a water bath for 30 min, cooled, pH was adjusted 4.5 and it was centrifuged at 2000 rpm for 20 min. Supernatant was used for HPLC analysis. Mobile and stationary phase for the assay were mixture of acetic acid, $KMnO_4$ and H_2O_2 for mobile phase and Zorbax Sil 222 respectively (C18 reversed phase stationary phase), flow rate of 1.5 ml/min, column temperature to 35°C. And detection was at 450-500 nm.

Folate assay

Assay for plasma folate level was done on Waters 616/626 HPLC USA. Plasma sample was prepared by adding 0.5 ml to tubes in which 7 ml of ascorbic acid, 3.5 ml 2 M NaOH, 5.0 ml of 1 N HCl and mixture was shaken for 30 min before centrifugation at 15000 rpm for 30 min. Supernatant was used for folate content determination, while folate working standard of diglutamate trihydrochloride solution was 0.0, 2.0, 4.0, 6.0, 8.0 ppb. Methanol was used as mobile phase, Zorbax Sil 222 stationary phase (C18 reversed phase column), flow rate of 1.0 ml/min, column temperature 40°C and detector wave length was 358 nm.

Choline assay

Choline plasma levels of both cervical cancer subjects and normal control was determined using Waters 616/626 HPLC USA. 10 ml of methylalcohol was added to centrifuge tubes containing 0.25 ml of blood plasma, it was covered and shaken mechanically for 1½ hours prior to centrifugation at 5000 rpm for 45 minutes. The supernatants obtained were used for choline HPLC determination.

Vitamin B_6 (pyridoxine) assay

Waters 616/626 HPLC USA was used for pyridoxine level determinations. 0.25 ml of the plasma was added to 12.5 ml of 0.2 N HCl, mixture was warmed in a water bath for 15 min, cooled and the pH was adjusted to 2.5. Mixture was shaken mechanically for 30 min, centrifuged at 1000 rpm for 10 min and the supernatant was used for HPLC. Mobile phase was a mixture of acetic acid, $KMnO_4$ and H_2O_2, Zorbax Sil 222 stationary phase, flow rate of 1.5 ml/min, column temperature of 35°C and detector wave length was 450-500 nm.

Protein concentration determination

The protein concentration of stored samples was determined by Biuret method as described by Gornall et al. [27] with some modifications, potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions as cuprous oxide. The protein and biuret reagent complex was read at 540 nm using BSA as standard.

G6PD activity determination

Plasma G6PD activity were determined by using G6PD Assay Kit manual, Cayman Chemical company, Ann Arbor, MI, U.S.A. Principle is by measuring reduced nicotinamide adenine dinucleotide phosphate (NADPH) produced, because oxidized nicotinamide adenine dinucleotide phosphate is an hydrogen acceptor for G6PD, and NADPH has a characteristic absorption at 340 nm.

Statistical Analysis

The SPSS software package was used for statistical analysis of data and values obtained from the study were expressed as mean ± standard deviation when compared using the student t-test and results were regarded as significant at $\rho<0.05$.

Results and Discussion

This work was done to estimate the effect of radiotherapy and chemotherapy, at different periods of treatment, on vitamins B_{12}, B_6, B_9, choline, G6PD activity and plasma protein content of cervical cancer patients and their age/weight matched controls at varying times after the commencement of treatment. Anthropometric measurements were taken for the patients at pretherapy (treatment onset), one month and two months respectively while that of the controls was once, the height in meters and weight in kg was used to calculate the body mass index (BMI) and the results are shown on Table 1. Generally, cancer patients experience loss in body weight due to burden from the disease and there was further slight decrease in body weight after commencement of medication at one month as well. This might be due to adverse effect of both chemotherapy and radiotherapy which are known to cause nausea, vomiting and weight loss [28]. This may have produced an initial weight loss in the cervical cancer patients and it was only after two months that there was some improvement in body weight.

This research was done to estimate the effect of radiotherapy and chemotherapy, at different periods of treatment, on vitamins B_{12}, B_6, B_9, B_1, G6PD activity and plasma protein content of patients and controls at varying times. Obtained results for these assays are shown on Tables 2 and 3. The four parameters differed significantly ($\rho<0.05$) in all three periods (pretherapy when patient enrolled at the clinic, one and two

Radiotherapy and chemotherapy Period (month)	Weight kg	Height M	BMI (kg/M^2)
Control	60.88 ± 3.25	1.55 ± 0.05	25.2 ± 1.26
Pretherapy	63.28 ± 10.04	1.60 ± 0.098	24.7 ± 4.59
28 days/one month of therapy	60.93 ± 3.81	1.59 ± 0.05	24.2 ± 1.54
56 days/two months of therapy	61.33 ± 5.15	1.61 ± 0.025	23.7 ± 1.87

Data is shown as mean ± standard deviation

Table 1: Variation in body mass index in kg/M^2 in control subjects and cervical cancer patients undergoing radiotherapy/chemotherapy at different periods.

Radiotherapy/Chemotherapy Period (month)	Vitamin B_{12} (µg/L)	Vitamin B_9 (µg/L)	Vitamin B_6 (µg/L)
Control	195.18 ± 5.72^{bcd}	10.64 ± 0.51^{bcd}	21.93 ± 2.68^{bcd}
Pretherapy	113.07 ± 14.43^{a}	5.33 ± 0.92^{a}	4.61 ± 0.75^{a}
28 days/one month of therapy	99.25 ± 6.57^{a}	4.84 ± 0.58^{a}	4.59 ± 0.38^{a}
56 days/two months of therapy	104.68 ± 10.59^{a}	6.21 ± 0.67^{a}	4.33 ± 0.88^{a}

Data are shown as mean ± standard deviation, differences are significant at (ρ<0.05) when compared with: ^{a} control group, ^{b}Zero (Onset), ^{c} One, ^{d} Two

Table 2: Variation in cobalamin (Vitamin B_{12}), folate (vitamin B_9) and pyridoxine (vitamin B_6) in plasma of control subjects and cervical cancer patients undergoing radiotherapy/chemotherapy.

Radiotherapy and chemotherapy Period (month)	Protein (mg/mL)	Choline (µg/L)	G6PD (µM/Min/mg protein)
Control	2.55 ± 0.54^{bcd}	68.25 ± 3.84^{bcd}	0.35 ± 0.22^{bcd}
Pretherapy	2.30 ± 0.22^{a}	39.01 ± 3.14^{a}	0.35 ± 0.05^{a}
28 days/one of therapy	2.36 ± 0.40^{a}	35.00 ± 2.99^{a}	0.31 ± 0.06^{a}
56 day/two of therapy	1.92 ± 0.71^{a}	38.51 ± 1.73^{a}	0.22 ± 0.07^{a}

Data are shown as mean ± standard deviation, differences are significant at (ρ<0.05) when compared with: ^{a}control group, ^{b}Zero (Onset), ^{c}One, ^{d}Two

Table 3: Variation in protein, choline and G6PD in blood plasma of control subjects and cervical cancer patients undergoing radiotherapy/chemotherapy.

months later on treatment regimen) relative to control. This signifies that there is a marked relationship between the level of the vitamins at onset (diagnosis), commencement of treatment and regression of cancerous growth in cervical cancer patients with increase in treatment (chemotherapy and radiotherapy) period, thus confirming the anti-pathologic effect of the vitamins. This is not surprising as serum vitamin B_{12} levels in women with normal cytological smears were significantly higher than those with both high and low grade cervical dysplasia (ρ<0.001) [29]. Low vitamin B_{12} serum levels were significantly statistically associated with increased low-grade and increased high-grade cervical dysplasia risk.

Choline level was significantly different (ρ<0.05) with reference to the control as in Table 3. It has been shown that defect in one-carbon metabolism also plays an important role in cervical cancer prognosis. Substantial amount of the body content protein is involved in carcinogenesis and in the healing process; therefore it is not a surprise to find the effect on protein concentration as shown on Table 3 of patients undergoing chemotherapy and radiotherapy and control subjects. In this study, there was a significant difference (ρ<0.05) in protein content of the case samples at onset and after commencement of both radiotherapy/chemotherapy relative to control. G6PD activity was found to differ significantly (ρ<0.05) with respect to the control at the pretherapy and one month after the commencement of chemotherapy and radiotherapy as shown on Table 3. At two month of treatment (chemotherapy and radiotherapy) G6PD activity was markedly different (ρ>0.05) with respect to the control. There was no noticeable difference in G6PD at pretherapy and one month after the commencement of treatment, however, activity decreased further during the first two month of undergoing treatment after which a G6PD. Inferably it can be proposed that any substance that can mimic the G6PD induction might also aid the regression and eradication of cancerous growth.

G6PD is a cytosolic Mg^{2+} dependent enzyme that catalyzes the first reaction in pentose phosphate pathway [30]. This pathway also generates NADPH which biological scavenges reactive oxygen species (ROS), thus recycling the enzyme, GSH reductase attains full functional status to maintain the level of GSH in the system, thus protecting the cell from oxidative damage [31]. NADPH is also involved in fatty acid oxidation, lipid biosynthesis and is the substrate for NADPH oxidase in the activation of macrophages and polymorphonuclear leukocytes to produce oxygen radicals to destroy pathogens. Since red blood cell does not contain mitochondria, pentose phosphate shunt remains it only source of NADPH; therefore, defense against oxidative damage depend on G6PD. Hence G6PD deficiency becomes especially lethal in red blood cells, where oxidative stress would result in hemolytic anemia [32].

We observed a significant difference (ρ<0.05) in protein content of the case samples in comparism with the control. G6PD activity was found to differ significantly (p<0.05) with respect to the control at the pretherapy and one month after the commencement of chemotherapy and radiotherapy as shown on Table 3 as well. At 2nd of treatment G6PD activity was markedly different (ρ>0.05) with respect to control. There was no noticeable difference in G6PD at pretherapy and one month after the commencement of treatment, however, G6PDactivity decreased further during the first one month of undergoing treatment. Inferably it can be proposed that any substance that can mimic the G6PD induction might also aid the regression and eradication of cancerous growth.

Women in many developing countries, due to low income, take diets that are very low in folate; this, in combination with the lack of screening facilities for cervical neoplasia, may exacerbate the risk of cervical cancer [2,33]. Data obtained for folate (vitamin B_9) in both control and the patients are shown in Table 2. Poor folate status may contribute to cancer risk through effects on one-carbon metabolism and DNA methylation, folate is directly involved in DNA methylation via one-carbon metabolism and it was observed that lower folate and vitamin B_{12} status were associated with HPV infection [34]. Thus suggesting a possible role of folate and vitamin B_{12} in modulating the risk of cervical cancer and HPV infection In a previous study Piyathilake et al. [35], observed that improving the folate status in human papilloma virus infected women may reduce the risk of cervical cancer. We observed a decrease in folate status in this study, with an almost 50% reduction at the pretherapy, prior to treatment commencement compared to control, however patients after two months on treatment regimen had better folate status than at onset.

Conclusion

In conclusion, we observed a significant difference (ρ<0.05) in vitamins B_{12}, B_9, and B_6, choline and protein content in the plasma of cervical cancer patients undergoing chemotherapy and radiotherapy for different periods compared to control. Routine monitoring of these vitamins and supplementation might be helpful in improving lives of cervical cancer patient on chemotherapy/radiotherapy.

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