Comparative analysis of dietary pattern, anthropometry and serum ascorbate status of persons living with or without non-Hodgkin's lymphoma

Abiodun Adeoso,[1] Tola Atinmo[2]

[1]Nutrition & Dietetics Department, Yaba College of Technology, Lagos; [2]Human Nutrition Department, University of Ibadan, Ibadan Nigeria

Abstract

Recent upsurge of cancer cases across the globe is of concern to all and many studies shows the relationship between nutrient and the immune system and consequently cancer. This work aims to compare the dietary pattern, anthropometry and serum ascorbate status of the persons living with and those living without non-Hodgkin's lymphoma (NHL). A case-control study was conducted using blood samples of eight patients diagnosed for NHL at the University College Hospital (UCH) Ibadan, while eight (8) volunteers were the control group. Socio-economic characteristics, medical history, food preferences, anthropometric indices were retrieved from questionnaires. Ascorbic Serum assay done with ultraviolet absorption spectrophotometry method using Klett-summerson photoelectric. Students' *t*-test and Chi-square were used to test the educational levels fruit consumption. 25% of the respondents suffering from NHL skipped lunch and dinner, but none skipped breakfast. 66.67% of the cases and 100% of the control have their weight normally distributed. Cases had 11.11% slightly underweight and 11.11% obese. 25% of the population of the respondents had normal range of 0.4 mg/100 of serum ascorbate, while six had low serum ascorbate levels.

Introduction

Cancer is a group of diseases in which cells are aggressive (grow and divide without respect to normal limits), invasive (invade and destroy adjacent tissues), and/or metastatic (spread to other locations in the body). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited in their growth and do not invade or metastasize although some benign tumor types are capable of becoming malignant (www.sciencedaily.com/terms/cancer).[1] Cancer may affect people of all ages, even fetuses, but risk for the more common varieties tends to increase with age. Lymphoma and multiple myeloma however, are cancers that begin in the cells of the immune system. (Cancer Research UK, 2007).[2]

Increased risks of Non-Hodgkin's lymphoma have been related to animal protein.[3,4] and total fat intake[5,6] but fat from fish showed a protective effect.[7]

Vitamin C is a highly effective antioxidant that protects the body's cells against reactive oxygen species that are generated by immune cells to kill pathogens. Primarily through this role, the vitamin affects several components of innate and adaptive immunity; for example, vitamin C has been shown to stimulate both the production[8] and function[9,10] of leukocytes (white blood cells), especially neutrophils, lymphocytes, and phagocytes. Nearly all cancers are caused by abnormalities in the genetic material of the transformed cells. In experimental studies, vitamin C has improved the effectiveness of chemotherapy in inducing lymphoma cell death[11] and vitamin C has been found increase serum levels of antibodies.[12] Lymphoma is the name applied to a group of blood cell tumors[13] that develop from lymphatic cells. They grow at different rates and affect different kinds of lymphocytes. The two main categories of lymphomas are Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL). Symptoms may include enlarged lymph nodes, fever, and drenching sweat.[14]

There are many different types of NHL. Over the years, experts have used a variety of terms to classify these different types. Lymphoma starts when a white blood cell called a lymphocyte is damaged and begins to reproduce rapidly.[15] Aggressive lymphomas, also known as intermediate- and high-grade lymphomas, tend to grow and spread quickly and cause severe symptoms. Non-aggressive lymphomas, also called indolent or low-grade lymphomas, tend to grow quite slowly and cause fewer symptoms early in the disease course.

Due to the influence of nutrient intake on immune system function[16] exploring its potential effects is of particular interest in etiologic studies of NHL. It was recently reported that high consumption of dairy products or fried red meat was associated with increased risk of NHL and several subtypes in men and women, whereas fruit and vegetable intake was inversely associated with risk of NHL in women.[17]

Building on these results, researchers aimed to examine associations with a broad range of macro- and micronutrients to gain a better biologic understanding of how diet may affect NHL development. Few findings have been replicated among previous dietary studies of NHL. Three studies detected a positive association between consumption of animal protein and/or fat, or saturated fat, and NHL risk.[18-20]

Correspondence: Abiodun Adeoso, Nutrition & Dietetics Department, Yaba College of Technology, Lagos, Nigeria.
Tel.: +2348023347774.
E-mail: abioduntaylor@yahoo.com

Key words: Cancer; dietary pattern; non-Hodgkin's lymphoma; serum ascorbate.

Acknowledgements: the support of the following Persons and Organizations were remarkable during the course of data collection and analysis: I acknowledge the support of Professor T. Atinmo and Dr. (Mrs.) Fadupin for their supervisory role during the course of carrying out the work as well as the Laboratory Technologist, all of Human Nutrition Department, University of Ibadan for their Technical support.
The Management of the University College Hospital (UCH) is appreciated for the ethical clearance as well as the support given to me by the medical staff of the Hospital.

Contributions: the first author was a master's student of the second author at the University of Ibadan, Nigeria. The second author supervised the work.

Received for publication: 19 August 2017.
Accepted for publication: 5 July 2018.

Journal of Public Health in Africa 2018; 9:768
doi:10.4081/jphia.2018.768

Materials and Methods

Study area

The study was carried out at the University College Hospital (UCH) Ibadan. The study was a case-control study (analytical study design).

Ethical issue

Ethical clearance was sought and approval was given in November 2007 by the Ethics committee of the University College Hospital (UCH).

Subjects

All eight NHL Patients of the University College Hospital (UCH).

Participant selection

All patients diagnosed to be suffering from NHL at the clinic volunteered to participate in the research after counselling and they were assured that their names and identity would not be published.

Sample size

Blood samples of all the eight Subjects living with NHL who reported at the University College Hospital (UCH) Ibadan at the time of the study were used as the Cases while four adult volunteers were used as the control group.

Data source and Sampling procedure

A total number of 16 respondents took part in the study, which comprise eight (8) subjects living with NHL and eight (8) control i.e. those without NHL. Both the cases and the control group filled the structured questionnaires with 63 questions and food frequency table targeted at measuring both socio-demographic status, food and nutrient intake. Food Frequency Questionnaires (FFQ) with a check-list of food items arranged under their food classes and number of times that particular food was consumed in a week were used in securing information about their food and nutrient intake. Blood samples were collected from both case and control groups through the use of one (1) new syringe and needle for a subject through the assistance of a nurse within the ward.

The blood samples were drawn into normal serum bottles, kept in an ice bag from the Hospital to the Departmental Laboratory (a travel distance of about 30 min). The bottles were immediately centrifuged for 15 min to separate the hemoglobin from the serum on reaching the laboratory. The sera which form the supernatant were decanted into new serum bottles and frozen at 0 until the time the assays were done two weeks later.

Reagents/equipment for serum assay

In order to realize the objective of the study, serum assay using ultraviolet absorption spectrophotometry method was carried out using materials which include: Klett-summerson photoelectric colorimeter with green filter (number 50) and Klett tubes, 5% trichloroacetic acid (TCA) stored in cold 60^{C} water bath, 2, 6- Dichlorophenol-indophenol; 100mg salt dissolved in 50ml of hot distilled water. Refrigerated thiourea, 9N sulfuric acid (3 parts water plus 1 part concentrated acid). 2 g of 2, 6- Dichlorophenol-Indo- Phenol; and 4 g thiourea were dissolved in 100 mL 9N sulfuric Acid; this is "DT Mix". Filter when necessary. Add 85% sulfuric acid (85ml of concentrated acid plus 13 ml of distilled water).

Ascorbic acid – *working standard* 1 ml of the ascorbic acid standard to 100ml water, with 5% TCA. Make fresh with every run.

The procedure used to prepare the treatments is as below:

Sample & sample blank: Pipette 2.0ml of serum into a centrifuge tube. Add 6.0 ml of 5% TCA and mix thoroughly with a vortex mixer. Centrifuge off protein residue. Pipette 2.0 ml of supernatant into Klett tubes for all the samples and sample blank.

Ascorbate standard and standard blank: Ascorbate Standard: Pipette 2.0ml of working ascorbate standard into Klett tube. Ascorbate Blank: Pipette 2.0 ml of 5% TCA into Klett tube. Add 1 drop indophenol reagent to all tubes and mix well. Add 0.5 ml of DT mix to Ascorbate standard and to all samples; do not treat any blanks at this point. Incubate all tubes for one hour in a 60^{o}C water bath. Cool in the ice water. Add 0.5 ml of DT mix to sample blank and ascorbate blank only. While samples remain in ice bath slowly pipette 2.5ml of 85% sulfuric acid to all tubes and mix well. Adjust Klett to zero using ascorbate blank. Read optical density of ascorbate standard and record. The sample Optical Density was read and recorded using the 6305 Jenway Ultraviolet/Visible Spectrophotometer. This process was repeated for each corresponding sample.

The *total ascorbate per 1 litre of blood*:

$$\frac{\text{Optical Density of sample} \times 2}{\text{Optical Density of standard}} = \text{mg of total ascorbate per 100 mL}$$

Normal values for serum: 0.4 – 1.5 mg per 100 mL.

Data analysis

SPSS version 11.0 (Statistical Package

Table 1. Socio-demographic status of respondents (sex, age, marital status, religion, educational qualification).

Variables	Frequency		Percentage (%)
Sex			
Male	NHL	8	50.00
	WNHL	4	25.00
Female	NHL	Nil	Nil
	WNHL	4	25.00
Age			
<20	NHL	1	6.25
	WNHL	Nil	Nil
20-39	NHL	2	12.50
	WNHL	5	31.25
40-59	NHL	4	25.00
	WNHL	3	18.75
60-89	NHL	1	6.25
	WNHL	Nil	Nil
Marital status			
Single	NHL	3	18.75
Single	WNHL	2	12.50
Married	NHL	5	31.25
Married	WNHL	6	37.50
Religion			
Christianity	NHL	3	18.75
Christianity	WNHL	5	31.25
Islam	NHL	5	31.25
Islam	WNHL	3	18.75
Educational qualification			
Primary	NHL	1	6.25
Secondary	NHL	4	25.00
Secondary	WNHL	4	25.00
Tertiary	NHL	3	18.75
	WNHL	4	25.00
Occupation			Frequency
Student	NHL	2	12.50
Student	WNHL	2	12.50
Farmer	NHL	1	6.25
Petty trader	NHL	1	6.25
Artisan	NHL	3	18.75
Artisan	WNHL	2	12.50
Civil servant	NHL	1	6.25
	WNHL	4	25.00

NHL, person living with non-Hodgkin lymphoma; WNHL, without NHL.

for Social science) was used for calculation of mean, standard deviation, frequencies and percentages of the data. The Student's *t*-test was used to analyze since the Subjects were given equal treatment. Chi-square was used to test the educational levels and advantage of fruits and vegetable consumption.

Results

Results are summarized in Tables 1-5.

Discussion

Socio-demographic status indicates all respondents living with NHL are male. The youngest age of respondent living with NHL is less than 20 and the oldest is over 60 years. The food frequency table shows that the cases consume relatively normal portions of starchy solid foods. Occupation of respondents shows all control group are public servant, 50% of respondents living with NHL are artisan, with one farmer, student and public servant each.

It is widely known that consumption of nutrients that suppress inflammation, prevent oxidation, or mediate normal DNA methylation may decrease the risk of developing several types of NHL.[17]

Five respondents received treatment immediately after NHL was diagnosed, while others had 1-2 months, 5-6 months and more than 6 months intervals. None had family history of NHL or any other cancer.

Feeding habits of the respondents could not be really ascertained as they were not fed in my presence. The respondents fed well according to the data given. Seven (7) take three (3) meals daily i.e. breakfast, lunch and dinner. Only an insignificant percentage lost appetite as the disease progresses. 75% of the respondents ate three square meals although in small portions. This shows that respondents and care givers understand the importance of adequate nutrition in the management of cancer.

Table 5 indicates that the mean serum ascorbate value for the cases was 0.27 mg/l or 100 ml, while that of the control group was 0.32 mg/l. A significant difference of $P<0.05$ was observed in the ascorbate level of both the cases and control groups. After the assay, the result above indicates the low ascorbate level of both the control group and the NHL patients.

The table 4B above shows that six (75%) of the cases and all controls eight (100%) have their weight normally distributed, while one of the respondents with NHL was slightly underweight (12.50%)

Table 2. Food frequency analysis of cases.

Variables	Frequency	Percentage (%)
How do you describe your attitude towards Your meals?		
I enjoy taking meals	5	62.5
I have no special emotions towards my meals	1	12.5
I don't really enjoy my meals	2	25
Total	8	100
Do you enjoy fried food than boiled food		
Yes	6	75
No	2	25
Total	8	100
Fruit intake		
Daily	1	12.5
Once weekly	3	37.5
Twice weekly	1	12.5
Thrice weekly	1	12.5
More than thrice weekly	2	25
Total	8	100
Description of starchy food portion (solids)		
Large (3 or more evaporated milk size)	5	62.5
Normal (2 evaporated milk size)	3	37.5
Normal (1 evaporated milk size)	-	-
Total	8	100
Vegetable consumption		
Daily	1	12.5
Once weekly	3	37.5
Twice weekly	1	12.5
Thrice weekly	1	12.5
More than thrice weekly	2	25
Total	8	100

Table 3. Medical history of respondents.

Interval of treament after diagnosis of NHL	Frequency	Percentage (%)
Immediate	5	42.00
1-2 months	1	8.00
5-6 months	1	8.00
>6 months	1	8.00
Not applicable (control)	8	33.00
Family history of NHL	Frequency	Percentage
Yes	0	0
No	16	100
Other diseases	Frequency	Percentage
Yes	3	25
No	9	75

Table 4. Feeding habits of cases.

	Frequency	Percentage (%)
Meals taken		
Lunch + dinner	1	8.00
B/fast+lunch+dinner	6	50.00
B/fast+ lunch	2	17.00
B/fast + dinner	2	17.00
Snack after b/fast +lunch+ dinner	1	8.00
Meals skipped		
Breakfast	1	8.00
Lunch	2	17.00
Dinner	2	17.00
None	7	58.00
Reason for skipping meals		
Money	2	17.00
Time	-	-
Lack of appetite	2	17.00
Dieting	1	8.00
Tiredness	1	8.00
Not applicable	6	50.00

Table 5. Serum ascorbate level of respondents and body mass index distribution of cases.

Serum ascorbate	Normal	Sub Normal	Mean	Sd	Percentage (%) n s	
Cases	2	6	0.270	0.217	12.50	36.50
Control	6	2	0.537	0.215	37.50	12.50
Total	8	8			50.00	50.00

Body mass index	Frequency Cases control	Percentage (%) Case control
Slightly underweight	1 -	11.11 -
Normal	6 8	66.67 100
Obese	1 -	11.11 -
Total	8 8	100 100

and another one with NHL was obesed.

The respondents' BMI were normally distributed about their weight and height measurements. The reason for the obesity of just one of the respondents was due to previous overeating habit and low physical activity. The underweight may be as a result of loss of appetite and the destruction of the normal cells experienced as the proliferation of the cancer cells progresses.

It is becoming increasingly evident that risk for development degenerative disease of which cancer is one increases with more DNA damage, which in turn is dependent majorly on nutritional status and other complex factors such as gene alteration.

Conclusions and Recommendation

The study shows that NHL can affect anyone irrespective of age or sex and that the disease causes a depletion of serum Vitamin C. It is therefore recommended that aggressive Nutrition education should be embarked upon by the Ministry of Health and other Agencies to inform Nigerian of the need to consume healthy diets to reduce cancer risk. Nigerians should be advised to modify their diets by consuming a wide variety of food, which include less fatty food of polyunsaturated source, plant-based diet high in fibre, citrus fruits high in vitamin C, beans and vegetables. Women should be taught how to make quality meals with small amount of money, be encouraged to prepare meals using indigenous food items and avoid processed or packaged convenient foods like the Westerners.

Government should also develop a strategy against the four major cancers in the community- liver and prostate cancers (in men), Breast and cervical Cancers (in women). Control of these major cancers would drastically reduce mortality rate from cancers in Nigeria. National Health Insurance Scheme (NHIS) should include treatment of cancers in the scheme for those using the services to enable masses access treatment facilities accessible at affordable prices.

References

1. ScienceDaily. Cancer. Available from: www.sciencedaily.com/terms/cancer
2. Cancer Research UK (Jan 2007). UK cancer incidence statistics by age. Retrieved on 2007-06-25.
3. Zheng T, Holford TR, Leaderer B, et al. Diet and nutrient intakes and risk of Non-Hodgkin's Lymphoma in Connecticut women. Am J Epidemiol 2004;159:454-66.
4. Chiu BC, Cerhan JR, Folsom AR, et al. Diet and risk of Non-Hodgkin's Lymphoma in older women. JAMA, 1996;275:1315-21.
5. Purdu MP, Bassani DG. Dietary factors and risk of Non- Hodgkin's Lymphoma by histologic sub-type: a case control analysis. Cancer Epidemiol Biomarker Prev 2004;13:1665-76.
6. Fritschi L, Ambrosini GL, Kliewer EV, et al. Dietary fat and protein in relation to risk of non-Hodgkin's lymphoma among women. J Nat. cancer Inst 1990;91:1751-8.
7. Ward MH, Zahm SH, Welsenburger DD, et al. Dietary factors and non-Hodgkin's lymphoma in Nebraska (United States). Cancer Cause Control 1994;5:422-32.
8. Prinz W, Bortz R, Bregin B, Hersch M. () The effect of ascorbic acid supplementation on some parameters of the human immunological defense system. Int. J Vietnam Nut Res 1977;47:248-57.
9. Jariwalla RJ, Harakeh S. Antiviral and immunomodulatory activities of ascorbic acid. () Harris JR, ed. Subcellular Biochemistry. Ascorbic Acid Biochem Biomed Cell Biol. New York: Plenum Press: 1996, pp 215-231.
10. Anderson R, Oosthuizen R, Maritz R, et al. The effects of increasing weekly doses of ascorbate on certain cellular and humoral immune functions in normal volunteers. Am J Clin Nutr 1980;33:71-6.
11. Chen Q, Espey MG, Sun AY, et al. Pharmacologic doses of ascorbate act as a` prooxidant and decrease growth of aggressive tumor xenografts in mice. Proc Natl Acad Sci U S A 2008;105:11105-9.10.1073/pnas.0804226105.
12. Feigen GA, Smith BH, Dix CE, et al. Enhancement of antibody production and protection against systemic anaphylaxis by large doses of vitamin C. Res Comm Chem Pathol Pharmacol 1982;38:313-33.
13. Taylor EJ. Dorland's illustrated medical dictionary (29th edition). Philadelphia: Saunders; 2000.
14. Leukemia and Lymphoma Society; 2013 - retrieved 20th June 2014. Available from: www.lls.org
15. Cancer.org. Available from: www.cancer.org/acs/groups/documents
16. Calder PC, Kew S. The immune system: a target for functional foods? Br J Nutr 2002;88:S165-77.
17. Chang ET, Ekström Smedby K, Zhang SM. Dietary factors and risk of non-Hodgkin lymphoma in men and women. Cancer Epidemiol Biomarkers Prev 2005;14:512-20.

Cunningham AS. Lymphomas and animal-protein consumption. Lancet 1976;2:1184-6.

18. Chiu BC, Cerhan JR, Folsom AR. Diet and risk of non-Hodgkin lymphoma in older women. J Am Med Assoc 1996;275:1315-21.
19. Chen K, Suh J, Carr AC, et al. Vitamin C suppresses lipid damage in vivo even in the presence of iron over-load. Am J Physiol Endocrinol Metabol 2000;279:E1406-212.