

Short Communication

Serum Levels of Selected Vitamins and Trace Elements in Nigerian Consumers of Alcoholic Beverage: A Suggestion for DNA Hypomethylation

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Summary: Folic acid, vitamins and Zinc play essential role in DNA methylation but alcohol consumption is known to affect the levels of these micronutrients leading to risk of developing various illnesses and certain cancers. This study determined the levels of DNA methylation dependent-micronutrients (folate, vitamin B₁₂, vitamin B₆, zinc and selenium) and homocysteine as a suggestion for DNA methylation status in Nigerian alcohol consumers compared with non-consumers of alcohol. Venous blood (5ml) was obtained from thirty-four males that consume alcoholic beverages for at least 10 years and thirty-two male controls that did not consume alcoholic beverages at least 10 years. Serum concentrations of folate, vitamin B₁₂, vitamin B₆, homocysteine (Hcy), selenium (Se) and zinc (Zn) were determined using High Performance Liquid Chromatography (HPLC) and Atomic Absorption Spectrophotometry (AAS) as appropriate. Independent Student t-test was used to compare the mean values between alcohol consumers and control. Mean differences were considered significant at $p < 0.05$. The mean serum levels of Zn and Se were significantly raised in alcohol consumers when compared with non-alcohol consumers while the mean levels of Vitamin B₆ and Hcy were significantly reduced in alcohol consumers when compared with non-alcohol consumers. There were no statistically significant differences in the mean serum levels of Vitamin B₁₂ and folate in alcohol consumers when compared with non-alcohol consumers. Since vitamin B₆ and Hcy are required for DNA methylation, reduced vitamin B₆ and Hcy levels in consumers of alcoholic beverages might suggest DNA hypomethylation in alcohol consumers.

Keywords: Illnesses, Cancers, DNA methylation, alcohol, micronutrients

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Manuscript Accepted: June, 2016

INTRODUCTION

There are evidences pointing to a causal link between alcohol consumption and infectious diseases such as tuberculosis, pneumonia (Samokhvalov et al., 2010; Rehm et al., 2009) as well as cancers (Nelson et al., 2013; Rehm and Shield, 2013; IARC, 2012; Seitz et al., 2012). In addition to direct effects of alcohol on immune responses, nutritional changes associated with alcohol use may be accountable for increased cancer risk among chronic alcohol-users (Boffeta et al., 2006; Calder and Jackson, 2000). Alcohol use has been reported to modulate dendritic cell function (Szabo et al., 2010) and interfere with major histocompatibility complex 1-restricted antigen presentation (Osna, 2009). Also among Nigerian consumers of alcoholic beverages, reduced transferrin and zinc as well as reduced level of NO, total white blood cell and neutrophil count (Olaniyi et al., 2010) have been reported. Although, understanding the mechanism by which alterations in immune responses account for increased cancer risk in alcohol users is evolving.

However, since epigenetic alteration which is a hallmark of cancer development (Esteller, 2008) has been associated with increased cancer risk (Portela and Esteller, 2010), this study posits that nutritional factors associated with DNA methylation (an important epigenetic mechanism of transcription control) may contribute to carcinogenesis in alcohol consumers.

A number of bioactive food components have been demonstrated to reduce cancer susceptibility by modifying epigenetic events such as DNA methylation (Kim, 2005; Davis and Uthus, 2004). In one-carbon metabolism, folate plays an important role in the synthesis of methionine which is a precursor of methylation donor S-adenosyl methionine (SAM). Folate transfers methyl group to homocysteine when in the form 5-methyl-tetrahydrofolate and also acts as a cofactor in enzyme methionine synthase which requires vitamin B₁₂ as cofactor (Ho et al., 2011). Vitamin B₆ also functions as a cofactor for several enzymes involved in homocysteine regeneration, methionine synthesis and metabolism of folate (Davis

and Uthus, 2004). Selenium has been proposed to react with homocysteine to form selenohomocysteine limiting homocysteine availability in the methionine cycle (Ho et al., 2011). Zinc acts as key cofactor in several enzymes involved in the folate and methionine/transsulfuration pathway, a key pathway for generating methyl donation equivalents such as SAM and betaine. Betaine-homocysteine methyltransferase, methionine synthase and serine hydroxymethyltransferase are zinc-dependent (Ho et al., 2011). Chronic reduction in any of these nutrients is hypothesized to affect DNA methylation patterns and susceptibility to carcinogenesis.

This study determined the level of the DNA methylation associated micronutrients (vitamin B₁₂, vitamin B₆, folate, zinc and selenium) as well as homocysteine in Nigerian regular consumers of alcohol compared with non-alcoholic consumers as predictor of DNA methylation status. Knowledge gained will provide additional information on the mechanism of alcohol induced susceptibility to cancers and other illnesses.

MATERIALS AND METHODS

Participants:

Thirty-four (34) male participants (38.18±10.68 years) that consumed 500ml alcoholic beverages daily for 10years were recruited for this study. Thirty-two (32) male controls (36.24±9.66 years) were selected based on their responses to questionnaire that they did not drink alcoholic beverages for at least 10 years and gamma glutamyl transferase (GGT) values above 30 U/L. Cigarette smokers or those with high parasite densities, abnormal liver functions, pathogenic infections (including HIV and pulmonary tuberculosis), and abnormal renal functions as presented by blood, urine, sputum and stool tests were excluded. Those on any form of compulsory medication/special diet (nutrient supplement) and those that declined their consents were also excluded.

Sample collection

Venous blood (5ml) was aseptically obtained from the antecubital fosa vein, using pyrogen free needle and syringe, into plain serum bottle and allowed to clot. After clotting, the blood sample was spun at 4,000 x g for 5 minutes; serum was obtained and stored at -20°C until analysis within 1 week.

Biochemical Analysis

Serum concentrations of micronutrients (Zn and Se) were determined using Atomic Absorption Spectrophotometry (Buck Scientific, 210, Atomic Absorption Spectrophotometer, Connecticut, USA). The levels of micronutrient vitamins (Folate, B₆, B₁₂) and homocysteine were determined by High Performance Liquid Chromatography method using WATERS 616/626 (USA) machine.

Statistical Analysis

The data obtained were analyzed using statistical package for social sciences (SPSS) version 17.0. Independent Student's t-test was used to compare the mean values between subjects and controls. Values were considered significant at p<0.05.

RESULTS

The mean serum levels of Zn and Se were significantly raised in alcohol consumers when compared with non-alcohol consumers. The mean levels of Vit B₆ and Hcy were significantly reduced in alcohol consumers when compared with non-alcohol consumers. There were no statistically significant differences in the mean serum levels of Vit B₁₂ and Folate in alcohol consumers when compared with non-alcohol consumers.

DISCUSSION

Alcoholism has been implicated as a strong risk factor for cancer, particularly of the upper aero-digestive tract - head and neck cancers (Marsitet et al., 2009), liver (Hernandez-Vargas et al., 2010; Calvisiet al., 2007) colorectum (Schernhammer et al., 2010) and breast (Seitz et al., 2012; Pelucchiet al., 2011). Alteration in DNA methylation pattern was demonstrated in these cancers (Seitz et al., 2012; Schernhammer et al., 2010; Hernandez-Vargas et al., 2010; Marsit et al., 2009). Methyl group supply by the one carbon metabolism pathway underscores the role of nutrition in modulating DNA methylation.

Vitamins (folate, vitamin B₆ and vitamin B₁₂) play central roles in the one carbon metabolic pathway such that any factor that alters circulating levels or metabolism of these vitamins may also affect availability of methyl group for DNA methylation. This present study observed reduced level of vitamin

Table 1: Comparison of micronutrient concentrations between alcohol consumers and control

Variables	Subjects (n=34)	Controls (n=32)	T	p
Zn (µg/dl)	130.56±13.72	87.45±24.44	8.905	0.000*
Se (µmol/l)	1.36±0.52	1.06±0.44	2.505	0.015*
Hcy (µmol/l)	6.15±1.28	6.76±0.82	-2.273	0.026*
Vit B ₆ (nmol/l)	25.00±7.8	79.00±6.5	-4.827	0.000*
Vit B ₁₂ (pg/ml)	350.22±42.49	324.32±13.72	1.646	0.105
Folate (ng/ml)	17.93±6.35	16.08±3.12	1.483	0.143

*Significant at p<0.05

B₆ in alcohol consumers when compared with non-alcohol consumers. This is similar to previous reports (Medici et al., 2010; Mennen et al., 2003; Ganji and Kafai, 2003; Gloria et al., 1997); which demonstrated reduced vitamin B₆ in alcohol consumers. Vitamin B₆ functions as a cofactor for several enzymes involved in homocysteine regeneration, methionine synthesis and metabolism of folate in the one carbon metabolism pathway. In women carrying *MTHFR* 677 T allele, vitamin B₆ deficiency was associated with a 1.8fold increased risk of DNA hypomethylation (La Merrill et al., 2012). Global DNA hypomethylation in the colon has also been demonstrated in vitamin B₆ deficiency (Figueiredo et al., 2009). Thus alcohol induced reduced vitamin B₆ might affect DNA methylation status in alcohol consumers.

Homocysteine (Hcy) a metabolic intermediate of the one carbon pathway and a known risk factor for atherosclerotic cardiovascular diseases has been shown to be increased in patients with colorectal (Kato et al., 1999) as well as head and neck squamous cell carcinomas (Sun et al., 2002). Conditions that are also associated with alcohol consumption. This present study observed reduced homocysteine level in alcohol consumers when compared with non-alcohol consumers. This contradicts previous reports of alcohol induced hyperhomocysteinemia (Cravo et al., 1996; Hultberg et al., 1993). Decreased level of homocysteine in alcohol consumers may be explained by increased (though insignificant) levels of folate and vitamin B₁₂ which are components of the remethylation pathway for homocysteine disposal.

Trace elements can indirectly affect one-carbon metabolism, methyl donor availability and ultimately DNA methylation and cancer susceptibility. Our study reported higher serum selenium level in alcohol consumers when compared to control. This is in concert with the results obtained in NHANES III (Dixon et al., 2002) as well as Galan et al. (2005), though the reverse was the case in some previous reports (Kafai and Ganji, 2003; Manariet al., 2003). Selenium as selenoproteins is an antioxidant, hence raised level of selenium in alcoholics compared to control may be considered beneficial. However, selenium has been proposed to react with homocysteine to form selenohomocysteine (Ho et al., 2011), a reaction which may limit homocysteine availability in the methionine cycle. Raised selenium levels in alcohol consumers may also explain reduced level of homocysteine observed in this group.

The present study observed significantly increased serum zinc in alcohol consumers when compared to non-alcohol consumers. Our finding is however similar to the report of Riitta Hartoma et al. (1977) who observed elevated serum zinc in alcoholics with normal liver. The type, volume and duration of alcohol consumption might explain differences in the results.

Adequate intake of vitamin B₆ is important in the maintenance of adequate serum level of Hcy. Vitamin B₆ takes part in the conversion of tetrahydrofolate to 5, 10-methylene tetrahydrofolate as a cofactor of serine hydroxymethyltransferase (Anderson et al, 2012) needed for DNA methylation. In this study, reduced vitamin B₆ and Hcy levels in consumers of alcoholic beverages might suggest DNA hypomethylation in them. Therefore, use of vitamin B₆ supplement is advised for consumers of alcoholic beverages.

REFERENCES

- Calder PC, Jackson AA (2000). Undernutrition, infection and immune function. *Nutr. Res. Rev.* 13(1):3-29.
- Cravo M, Gloria L, Selhub J, Nadeau MR, Camilo ME, Resende MP, et al. (1996). Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B₁₂ and B₆ status. *Am. J. Clin. Nutr.* 63:220-4.
- Boffeta P, Hashibe M, La Vecchia C, Zatonski W, Rehm J (2006). The burden of cancer attributable to alcohol drinking. *International J. Cancer* 119(4):884-887.
- Calvisi DF, Ladu S, Gorden A, Farina M, Lee J, Conner EA, et al. (2007). Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. *J. Clin. Investigation* 117(9):2713-2722.
- Davis CD, Uthus EO (2004). DNA methylation, cancer susceptibility, and nutrient interactions. *Exp. Biol. Med.* 229:988-995.
- Dixon JB, Dixon ME, O'Brien PE (2002). Reduced plasma homocysteine in obese red wine consumers: a potential contributor to reduced cardiovascular risk status. *European J. Clin. Nutr.* 56(7):608- 614.
- Esteller M (2008). Epigenetics in cancer. *New England J. Med.* 358(11):1148-1159.
- Figueiredo JC, Grau MV, Haile RW, Sandler RS, Summers RW, Bresalier RS, et al. (2009). Folic acid and risk of prostate cancer: Results from a randomized clinical trial. *J. Natl Cancer Inst.* 101: pp. 432-5.
- Galan P, Viteri FE, Bertrais S, Czernichow S, Faure H, Amaud J, et al. (2005). Serum concentrations of β-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur. J. Clin. Nutr.* 59:1181-1190.
- Ganji V, Kafai MR (2003). Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentration in the third National Health and Nutrition Examination Survey, 1988-1994. *Am. J. Clin. Nutr.* 77:826-33.
- Gloria L, Cravo M, Camilo ME, Resende M, Cardoso

- JN, Oliveira AG, et al. (1997). Nutritional deficiencies in chronic alcoholics: relation to dietary intake and alcohol consumption. *Am. J. Gastroenterol.* 92:485-9.
- Hernandez-Vargas H, Lambert MP, Le Calvez-Kelm F, Gouysse G, McKay-Chopin S, Tavtigian SV, et al. (2010). Hepatocellular carcinoma displays distinct DNA methylation signatures with potential as clinical predictors. *PLoS One* 5(3):e9749.
- Ho E, Beaver LM, Williams DE, Dashwood RH (2011). Dietary factors and epigenetic regulation for prostate cancer prevention. *Adv. Nutr.* 2:497-510.
- International Agency for Research on Cancer (IARC). A Review of Human Carcinogens: Personal Habits and Indoor Combustions, vol. 100E. Lyon: IARC 2012.
- Kafai MR, Ganji V (2003). Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third National Health and Nutrition Examination Survey, 1988–1994. *J. Trace Elements Med. Biol.* 17: 13–18.
- Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, et al. (1999). Serum folate, homocysteine and colorectal cancer risk in women: nested case-control study. *Br. J. Cancer* 79: 1917-1922.
- Manari AP, Preedy VR, Peters TJ (2003). Nutritional intake of hazardous drinkers and dependent alcoholics in the UK. *Addict. Biol.* 8:201–210.
- Mennen LI, de Courcy GP, Guillard JC, Ducros V, Zarebska M, Bertrais S, et al. (2003). Relation between homocysteine concentrations and the consumption of different types of alcoholic beverages: the French supplementation with antioxidant vitamins and minerals study. *Am. J. Clin. Nutr.* 78:334–8.
- Hultberg B, Berglund M, Andersson A, Frank A (1993). Elevated plasma homocysteine in alcoholics. *Alcohol. Clin. Exp. Res.* 17:687.
- Kim YI (2005). Nutritional epigenetics: Impact of folate deficiency on DNA methylation and colon cancer susceptibility. *J. Nutr.* 135 (11):2703-2709.
- La Merrill M, Torres-Sanchez L, Ruiz-Ramos R, Lopez-Carrillo L, Cebrian ME, Chen J (2012). The association between first trimester micronutrient intake, *MTHFR* genotypes, and global DNA methylation in pregnant women. *J. Matern Fetal Neonatal Med.* 25(2):133-137.
- Marsit CJ, Christensen BC, Houseman EA, Karagas MR, Wrensch MR, Yeh R, et al. (2009). Epigenetic profiling reveals etiologically distinct patterns of DNA methylation in head and neck squamous cell carcinoma. *Carcinogenesis* 30(3):416-422.
- Medici V, Peerson JM, Stabler SP, French SW, Gregory JF III, Virata MC, et al. (2010). Impaired homocysteine transsulfuration is an indicator of alcoholic liver disease. *J. Hepatol.* 53:551-7.
- Nelson DE, Jarman DW, Rehm J, Greenfield TK, Rey G, Kerr WC (2013). Alcohol-attributable cancer deaths and years of potential life lost in the United States. *Am. J. Public Health* 641–8.
- Olaniyi J, Obikoya M, Akinosun O, Arinola G (2010). Leucocyte counts, humoral immunity and nitric oxide level in Nigerian consumers of alcoholic beverages. *Report and opinion* 2(6):67-70.
- Osna NA (2009). Hepatitis C virus and ethanol alter antigen presentation in liver cells. *World J. Gastroenterol.* 15:1201–1208.
- Rehm J, Samokhvalov AV, Neuman MG, Room R, Parry CD, Lönnroth K et al. (2009). The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review. *BMC Public Health* 9:450.
- Rehm J, Shield KD (2013). Alcohol and mortality: global alcohol-attributable deaths from cancer, liver cirrhosis and injury in 2010. *Alcohol Research: Current Reviews* 35(2), 174–183.
- Rittahartoma T, Sotaniemi EA, Pelkonen O, Ahlqvist J (1977). Serum zinc and serum copper and indices of drug metabolism in alcoholics. *European J. Clin. Pharmacol.* 12(2):147-151.
- Samokhvalov AV, Irving HM, Rehm J (2010). Alcohol consumption as a risk factor for pneumonia: systematic review and meta-analysis. *Epidemiology and Infection* 138(12):1789–95.
- Sun CF, Haven TR, Wu TL, Tsao KC, Wu JT (2002). Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: a potential new tumor marker. *Clin. Chem. Acta.* 321: 55-62.
- Szabo G, Wands JR, Eken A, Osna NA, Weinman SA, Machida K, et al. (2010). Alcohol and hepatitis C virus—interactions in immune dysfunctions and liver damage. *Alcohol. Clin. Exp. Res.* 34:1675–1686.
- Pelucchi C, Tramacere I, Boffetta P, Negri E, La Vecchia C (2011). Alcohol consumption and cancer risk. *Nutr. Cancer* 63(7):983-990.
- Schernhammer ES, Giovannucci E, Kawasaki T, Rosner B, Fuchs CS, Ogino S (2010). Dietary folate, alcohol and B vitamins in relation to LINE-1 hypomethylation in colon cancer. *Gut* 59(6):794-799.
- Seitz HK, Pelucchi C, Bagnardi V, La Vecchia C (2012). Epidemiology and pathophysiology of alcohol and breast cancer: Update 2012. *Alcohol and Alcoholism* 47(3):204-212.