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ASSESSMENT OF INFLAMMATORY, CARDIAC AND COAGULATION MARKERS ASSOCIATED WITH ALCOHOL CONSUMPTION IN NNEWI, NIGERIA

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ABSTRACT

Alcohol consumption had been associated with chronic heart disease. The precise mechanisms of this risk are not always clear. This study was designed to determine the inflammatory cytokines, myocardial, and homeostatic responses using the levels of circulating interleukin 1β, (IL-1β), interleukin-10 (IL-10), serum C-reactive protein (CRP), total white blood cell (TLC), absolute granulocyte counts (AGC), absolute lymphocyte counts (ALC), platelet count (PLT), creatinine kinase-M (CK-MB), troponin-I (TI), activated partial thromboplastin time (APTT) and prothrombin time (PT). The pre and post research design consists of fifty (50) consented apparently healthy young male with an average age of 28.3 ± 3.0 years and body mass index of 24.7 ± 1.8 (Kg/m^2) who were randomly selected from residents and staff of Nnamdi Azikiwe University, Nnewi, Nigeria. The subjects were asked to consume 600 ml of any brand of beer per day for one month as part of a dietary assessment. The blood samples collected in EDTA (1.5mg/ml) were used for absolute white blood cell count, platelet count (PLT) and total white blood cell (TLC) count using Sysmex® Automated Hematology Analyzer. Blood anticoagulated with 0.109 M trisodium citrate (9: 1 v/v) were used for the measurement of activated partial thromboplastin time and prothrombin time. Serum interleukin 1β, IL-10, serum C-reactive protein, creatinine kinase-M and troponin-I were evaluated using enzyme-linked immunosorbent assay method. All numerical results were analyzed with student's t- test using SPSS version 20.0 statistical program. P values < 0.05 were considered significant. The levels of IL-1β, IL-10 and CRP were significantly lower at one month post alcohol consumption when compared with pre stage while the levels of TLC, AGC, ALC, CK-MB, TI, PLT, APTT and PT shows no significant difference at one month post alcohol consumption when compared with pre stage. One month alcohol consumption tends to switch the cytokine pattern toward the anti-inflammatory pattern.

KEYWORDS: *Alcohol, cytokine, Alcohol consumption, Nnewi, Nigeria*

INTRODUCTION

Alcohol consumption had been associated with threat of chronic heart disease (Ikehara *et al*., 2008; Arriola *et al.,* 2010; Hvidtfeldt *et al.,* 2010; Ronksley *et al.,* 2011). The precise mechanisms of this risk are not clear. However, elevations of high-density lipoprotein (HDL) cholesterol levels, increases in serum adiponectin levels (Pischon *et al.,* 2005), reduction in C-reactive protein (CRP) serum levels (Pischon *et al*., 2005), reduced serum fibrinogen levels (Brien *et al*., 2011), and increased insulin sensitivity (Sierksma *et al*., 2004) have all been suggested as possible positive influences of alcohol consumption. It also has been reported that alcohol reduces hyperglycaemia through the inhibition of hepatic gluconeogenesis, with a resulting reduction in plasma glucose levels. Reduced plasma glucose levels serve to decrease the incidence of hyperglycaemia and hyperinsulinaemia (Siler *et al*., 1998). However, studies had reported that no consumption of alcohol, led to higher serum levels of CRP compared to alcohol consumption (Imhof *et al.,* 2001; Imhof *et al*., 2004). This indicates that alcohol consumption can increase inflammation. Studies have also observed that plasma fibrinogen reduce after alcohol consumption (brien *et al*., 2011; sierksma *et al.,* 2002; mukamal *et al*., 2005). It is now clear that alcohol consumption increases HDL-cholesterol, insulin sensitivity and adiponectin levels while decreasing inflammation, all of which have positive effects on the risk for CHD. Many researchers have found that alcohol intake increases HDL cholesterol (HDL-c) levels, HDL ("good cholesterol") particle concentration, apolipoprotein A-I, and HDL-c subfractions (Gaziano et al., 1993; Marmot, 1984; Mariann, 2017). Findings have been also reported for other lipids, such as low-density lipoprotein cholesterol (LDL-c) (the estimated amount of cholesterol within LDL particles, or "bad cholesterol") and triglyceride levels (Rimm *et al*., 1999a; Jackson *et al*., 1991; Zhou *et al*., 2020). High triglyceride levels in the blood stream have been linked to atherosclerosis and, by extension, increased risk of CHD and stroke. Mariann, (2017) reported that alcohol consumption reduced triglyceride and LDL-c and increased HDL-c, in particular the HDL2-c subfraction. Interestingly, Mariann (2017) reported a nonlinear effect of alcohol consumption on HDL2-c levels. This supports the findings from other studies that the alcohol-induced changes in HDL-c do not fully account for the lower risk of CHD in alcohol drinkers (Mukamal *et al*., 2005).

Several non-Nigerian epidemiologic and randomized controlled studies have found alcohol consumption decreases coagulation factors such as fibrinogen, which is a CV risk marker at elevated levels (Rimm *et al*., 1999b; Moris *et al*., 2015). Thus, this study was designed to determine inflammatory cytokines (Th1, Th2), myocardial, and homeostatic responses using the levels of circulating interleukin 1β, IL-10, serum C-reactive protein, absolute lymphocyte counts, creatinine kinase-M ,troponin-I and values of activated partial thromboplastin time and prothrombin time as studies have shown

that alcohol consumption lower relative threat of chronic heart disease (Ikehara *et al*., 2008; Arriola *et al*., 2010; Hvidtfeldt *et al*., 2010; Ronksley *et al*., 2011).

MATERIALS AND METHODS

Study Area

This study was delimited to apparently healthy Community participants (HCP) recruited from residents of Nnewi which is a commercial and industrial city in Anambra State, southeastern Nigeria. It is the second largest and second most populated city in Anambra State located in the southern part of the state. Nnewi as a metropolis has one local government area, which is Nnewi North. Nnewi North comprises four quarters: Otolo, Uruagu, Umudim, and Nnewichi.

Subjects

The pre and post experimental design consists of fifty (50) consented apparently healthy young male with an average age of 28.3 ± 3.0 years and body mass index of 24.7 ± 1.8 (Kg/m^2) who were randomly selected from residents and staff of Nnamdi Azikiwe University, Nnewi, Nigeria.

Inclusion Criteria

This study was delimited to apparently healthy Community participants (HCP) recruited from residents and staff of Nnamdi Azikiwe University, Nnewi within 18 - 35 years of age who are willing to participate in the study

Exclusion Criteria

Young male with an underlying history of illness e.g. Hypertension, irregular heart rate, glucose utilization disorders, asthmatics, sickle cell anemia and other forms of anemia were excluded. Those that engage in strenuous activities such as (professional athletes, welders etc) were excluded. Subjects currently on antioxidant supplementation, alcohol and any antimicrobial agents were excluded. Serological evidence of HIV infection, chronic cardiovascular or metabolic diseases, immunosuppressive medication, age less than 18years, and incomplete data records constituted exclusion criteria.

Study Design

Upon arrival at the venue of the research, their height (H) and weight (W) was measured and recorded and they were allowed to rest for at least 10 min before baseline (pre) blood sample was then collected. The subjects were asked to consume 600 ml of any brand of beer per day for one month as part of a dietary assessment. The subjects were encouraged to eat balance diet and avoid any strenuous activity during the course of the research.

Pre and One Month Post Alcohol Blood Samples Collection and Analysis

Blood sample were collected from the ante-cubital vein of the subjects using standard laboratory collection technique at pre and one month post alcohol consumption. The blood samples collected in EDTA (1.5mg/ml) were used for absolute white blood cell count and total white blood cell count using Sysmex® Automated Hematology Analyzer as previously described by Ehiaghe *et al*. (2016). Blood was also anticoagulated with 0.109 M trisodium citrate (9: 1 v/v) for the measurement of activated partial thromboplastin time and prothrombin

time whereas, the blood collected in anticoagulant free vacutainers, subsequently centrifuged at 750 x g for 15 min to obtain serum which were used for the evaluation of interleukin 1, IL-10, serum C-reactive protein, creatinine kinase-M and troponin-I using enzyme-linked immunosorbent assay method. The assay employs an antibody specific for coated on a 96 well plate. Briefly, 100 µl of assay diluents was added to each well. 50µl of standard or sample(s) was added per well and the mixture was incubated for 2 hrs. The solution was discarded and microplates washed four times with 300µl of 1X wash solution. 200µl protein conjugate was added to the standard or sample(s) and covered with a sealing tape and incubated at 25°C for 2hrs. The mixture was discarded and microplates washed four times with 300µl of 1X wash solution. 200µl of tetramethyl benzidine substrate was added to each well and incubated in the dark with gentle shaking. 50µl of stop solution was added to each microplate. The intensity of the colour developed was measured at 450nm wavelength using stat fax® 4700 micro strip reader as previously described by Ehiaghe *et al.* (2013).

Statistical Analysis

All numerical results were analyzed with student's t- test using SPSS version 20.0 statistical program. P values < 0.05 were considered significant.

RESULTS

Table 1 and Table 2 show the levels (mean ± SD) of (interleukin-1β, interleukin-10), C-reactive protein, total white blood cell count, absolute granulocyte, absolute lymphocyte count, creatinine kinase-M, troponin-I, platelet count, activated partial thromboplastin time and prothrombin time at pre and one month post alcohol consumption. The levels of IL-1β, IL-10 and CRP were significantly lower at one month post alcohol consumption when compared with pre stage while the levels of TLC, AGC, ALC, CK-MB, TI, PLT, APTT and PT shows no significant difference at one month post alcohol consumption when compared with pre stage.

Table 1: Mean (±SD) values of interluekin-1 β (ng/L), interluekin-10 (ng/L), C-reactive protein (ng/L), total white blood cell count (Cells/ul), absolute granulocyte (cells/ul) count and absolute lymphocyte count (cells/ul) of the subjects pre and one month post alcohol consumption

Time interval	Interluekin-1 β	Interluekin-10	C-reactive protein	Total leucocyte count x 10^3	Absolute granulocyte count x 10^3	Absolute Lymphocyte count x 10^3
Pre stage	20.03± 0.05	15.03± 0.05	21.24± 0.36	5. 35± 1.15	2.43± 0.88	2.5± 0.79
Post stage	13.40± 0.04	10.04 ± 0.77	13.15± 3.95	3. 84± 0.89	2.02± 0.89	1.44± 0.78
P-value	0.005	0.005	0.001	0.186	0.419	0.817

Table 2: Mean (±SD) values of creatinine kinase-M ((ng/ml), troponin-I((ng/ml), platelet count(cells/ul), activated partial thromboplastin time (seconds), and prothrombin time (seconds) of the subjects pre and one month post alcohol consumption

Time interval	creatinine kinase-M	troponin-I	platelet count x 10^3	activated partial thromboplastin time	prothrombin time
Pre stage	48. 82 ± 3.80	1.54± 0.07	197.40± 41.82	37.60± 1.43	11.20± 1.40
Post stage	50.36 ± 2.17	2.00± 0.51	186.00± 53.60	38.50± 1.40	12.70± 0.95
P-value	0.527	0.221	0.486	0.356	0.305

DISCUSSION

The study revealed a significant lower levels of IL-1 β, IL-10 and CRP one month post alcohol consumption when compared with the pre stage, thus, indicating that one month alcohol consumption tends to switch the cytokine pattern toward the anti-inflammatory pattern. It is also evidence from the study that one post alcohol consumption insignificantly lowers the levels of TLC, AGC, ALC, PLT, APTT and PT in these study. It is possible that moderate alcohol consumption exerts its protective or enhancing effects by modifying inflammatory and hemostatic factors as evidence in this study. It has also been noted that moderate alcohol use reduces fibrinogen levels, clotting factors, and platelet aggregation, which affects hypercoagulability, however, the precise mechanisms governing these reductions are not known, findings from a meta-analysis of 42 studies by Rimm and colleagues (Rimm *et al*., 1999b) suggested that 30 g of alcohol/day (2 standard drinks) was associated with a 7.5 mg/dl decrease in fibrinogen concentration. Similarly, the results from the small randomized crossover trial by Mori *et al*. (2015), found that women consuming alcohol (146 to 218 g/week, ~2 to 3 standard drinks/day) for 4 weeks showed a 14% reduction in fibrinogen levels. Not surprisingly, alcohol consumption has complex and varying effects on platelet function. Studies using different methodologies have shown that low-to moderate alcohol consumption decreases platelet activation and aggregation (Salem and Laposata 2005).

Furthermore, the levels of CK-MB and TI was insignificantly elevated one month post alcohol consumption (Table 2), suggesting an increased mechanical stress during muscle contraction. Thus, it can be extrapolated that alcohol consumption can increase the chance of cardiovascular problem. Studies has also shown that excessive alcohol consumption, more than 30 g per day, has also been associated with hypertension (Beilin and Pudday, 2006), declining ejection fraction (De-Leiris *et al*., 2006), progressive left ventricular hypertrophy (Lucas *et al*., 2005), increased risk of stroke (Klatsky, 2005), dementia (Mukamal *et al*., 2003) and overall mortality (Mukamal *et al*., 2005).

CONCLUSION

One month alcohol consumption tends to switch the cytokine pattern toward the anti-inflammatory pattern. Thus, it is extremely important that alcohol use be constrained to moderate consumption.

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