

# Assessment of Some Inflammatory, Cardiac and Coagulation Markers Associated With Moderate Alcohol Consumption in Nnewi, Nigeria

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# **ABSTRACT**

**Background:** Moderate alcohol consumption had been associated with a lower relative threat of chronic heart disease. The precise mechanisms of this lower risk are not always clear.

**Methods:** Thus, this study was designed to determine the inflammatory cytokines, myocardial, and homeostatic responses using the levels of circulating interleukin 1 $\beta$ , IL-10, serum C-reactive protein, absolute lymphocyte counts, creatinine kinase-M, troponin-I and values of activated partial thromboplastin time and prothrombin time. The pre and post experimental design consists of 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/m2) who were randomly selected from residents and staff of Nnamdi Azikiwe University, Nnewi environ. The blood samples collected in EDTA (1.5 mg/ml) were used for absolute white blood cell count and total white blood cell 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 1 $\beta$ , 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<0.05 were considered significant. **Results:** The levels of IL-1 $\beta$ , 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.

**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.

Keywords: Alcohol; Cytokine; Moderate consumption; Nnewi environ

### **INTRODUCTION**

The Moderate alcohol consumption had been associated with a lower relative threat of chronic heart disease. However, the precise mechanisms of this lower risk are not always clear at a glance. However, elevations of High Density Lipoprotein (HDL) cholesterol levels, increases in serum adiponectin levels, reduction in C-Reactive Protein (CRP) serum levels, reduced serum fibrinogen levels and increased insulin sensitivity have all been suggested as possible positive influences of moderate alcohol consumption [1]. It also has been reported that alcohol reduces hyperglycaemia through the inhibition of hepatic gluconeogenesis, with a resulting reduction in plasma glucose

Received:	3- January -2022	Manuscript No:	EJEBAU-22-12756
Editor assigned:	5- January -2022	PreQC No:	EJEBAU-22-12756(PQ)
Reviewed:	19- January -2022	QC No:	EJEBAU-22-12756
Revised:	24- January -2022	Manuscript No:	EJEBAU-22-12756(R)
Published:	31- January -2022	DOI:	10.36648/2248 -9215.10.1.117

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**Citation** Alfred EF, Ihim A, Osakue ON, Onah EC, Ehiaghe IJ, et al. (2022) Assessment of some inflammatory, cardiac and coagulation markers associated with moderate alcohol consumption in Nnewi, Nigeria. Eur Exp Bio. 12:117

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levels. Reduced plasma glucose levels serve to decrease the incidence of hyperglycaemia and hyperinsulinaemia [2]. However, studies had reported that excessive and no consumption of alcohol, led to higher serum levels of CRP compared to moderate alcohol consumption [3-5]. This indicates that excessive alcohol consumption can increase inflammation. Studies have also observed that fibrinogen serum reduce after moderate alcohol consumption. This leads to a reduction in hypercoagulability, which would reduce the risk for CHD events. It is now clear at a glance that moderate alcohol consumption increases HDL-cholesterol, insulin sensitivity and adiponectin levels while decreasing inflammation, all of which have positive effects on the risk for CHD [6]. 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. Findings have been equivocal 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 [7-9]. High triglyceride levels in the blood stream have been linked to atherosclerosis and, by extension, increased risk of CHD and stroke. However, Vu and colleagues [10] reported that low to moderate alcohol consumption reduced triglyceride and LDL-c and increased HDL-c, in particular the HDL2-c subfraction. Interestingly, the researchers found 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 moderate alcohol drinkers [11].

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 [12]. Thus, this study was designed to determine inflammatory cytokines (Th1, Th2), myocardial, and homeostatic responses using the levels of circulating interleukin 1 $\beta$ , 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 low moderate alcohol consumption lower relative threat of chronic heart disease [13]. This will add to the existing level of information in Nigeria on low moderate alcohol consumption, which is beneficial.

### **MATERIALS AND METHODS**

#### **Subjects**

We The pre and post experimental design consists of 50 consented apparently healthy young male with an average age of 28.3  $\pm$  3.0 years and body mass index of 24.7  $\pm$  1.8 (Kg/m2) who were randomly selected from residents and staff of Nnamdi Azikiwe University, Nnewi environ (Table 1).

Table 1: Mean (±SD) values of the baseline characteristics of the participants

All subjects (N=50)	Variables
28.3 ± 3.0	Age (yrs)
1.8 ± 0.07	Height (m)
$69.6 \pm 6.2$	Weight (kg)
24.7 ± 1.8	Body mass index (kg/m2)

#### **Inclusion Criteria**

HPLC this study was delimited to apparently healthy Community participants (HCP) recruited from residents and staff of Nnamdi Azikiwe University, Nnewi environ within 18 and 35 years of age who are willing to participate in the study [14].

#### **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 currently undertaking examination were excluded. Those that engage in strenuous activities such as (professional athletes, welders etc) were excluded [15]. 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 18 years, 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 ten minutes before baseline (pre) blood sample was then collected. The subjects were asked to consume two bottles of any brand of alcohol per day for one month as part of a dietary assessment [16-18]. 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 (1.5 mg/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 [19]. 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 minutes 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 [20-22]. The assay employs an antibody specific for coated on a 96 well plate. Briefly, 100 µl of assay diluents was added to each well. 50 µl of standard or samples was added per well and the mixture was incubated for 2 hours. The solution was discarded and microplates washed four times with 300  $\mu$ l of 1X wash solution. 200  $\mu$ l protein conjugate was added to the standard or samples and covered with a sealing tape and incubated at 250 C for 2 hours [23]. The mixture was discarded and microplates washed four times with 300 µl of 1X wash solution. 200 µl of tetramethyl benzidine substrate was added to each well and incubated in the dark with gentle shaking. 50 µl of stop solution was added to each microplate [27]. The intensity of the color developed was measured at 450 nm wavelength using stat fax® 4700 micro strip reader as previously described by Ehiaghe et al [24].

### **Statistical Analysis**

All numerical results were analyzed with student's t-test using SPSS version 20.0 statistical program. P<0.05 were considered significant [25].

### RESULTS

Quantification Tables 2 and 3 shows the levels (mean  $\pm$  SD) of (interleukin-1 $\beta$ , 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-1 $\beta$ , 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 [26-28].

Table 2: Mean ( $\pm$  SD) values of interluekin-1  $\beta$  (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 pro- tein	Total leucocyte count x 103	Absolute gran- ulocyte count x 103	Absolute Lym- phocyte count x 103
Pre stage	20.03 ± 0.05	15.03 ± 0.05	21.24 ± 0.36	5. 35 ± 1.15	$2.43 \pm 0.88$	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 3: 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-l	platelet count x 103	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

An lon pair reversed phase HPLC method was developed for the simultaneous separation of 15 ribonucleotides with required sensitivity, accuracy, and precision from a single injection.

### **CONCLUSION**

In conclusion this method can be used to closely monitor the intracellular level of nucleotide in in vitro and in vivo studies.

## ACKNOWLEGEMENT

None

### **CONFLICT OF INTEREST**

Authors declare no conflict of interest

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