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Research Article

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The relationship between alcoholism and cardiac biomarkers in non insulin dependent diabetics

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Abstract Non insulin dependent diabetes also known as type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular disease and can have devastating consequences on quality of life. Diagnosis of Acute cardiac event in the early stage of its onset is important in the treatment process, and the development of highly sensitive and specific immunoassays for myocardial proteins such as cardiac Troponin I (cTnI) and Creatine kinase-MB had made it possible. The identification of subjects with high risk of developing cardiac event in the future is more significant as it will provide time to prevent such incidents. Alcohol is also a risk factor for both diabetes and cardiovascular disorder. This paper is aimed at evaluating the relationship between alcohol and cardiac markers like CK-MB, Troponin I and lipid profile in Non insulin dependent diabetes mellitus subjects. A total of 200 adult male (aged 30-70 years) subjects were selected by stratified random sampling technique and by administration of questionnaire. Glucose, lipid profile, and CK-MB levels of these subjects were assayed using 2012 Shenzhen mindray semi-auto Chemistry Analyzer, model BA-88A. Troponin I was assayed by enzyme linked immunosorbent assay (ELISA) method. The blood glucose levels of both men who reported to be diabetic and non diabetic were assayed to reconfirm those who were diabetics and the non diabetics. Statistical package for Social Sciences (SPSS) version 20.0 software was used to analyze the data obtained in this study. Values were expressed as mean ± standard deviation Level of significant was taken at $p \leq 0.05$. The results show that Creatine Kinase-MB is significantly higher in diabetics than in non diabetic male subjects ($p = 0.004$). There are also significantly increased levels of total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, diastolic blood pressure, and troponin I in alcoholic type 2 diabetic men when compared to the corresponding non alcoholic diabetics. Also, the result shows that alcohol positively associated with troponin I, triglyceride, and fasting blood sugar in alcoholic diabetics. From this study, lipid profile levels can still be used in screening populations to identify the subjects with high risk of developing cardiac event which is identified by highly sensitive and specific positive Troponin test. Alcoholic diabetic subjects are at increased risk of cardiovascular disease than non alcoholic diabetic subjects with same anthropometric factors.

Keywords Relationship, alcoholism, cardiac biomarkers, diabetics

Introduction

Cardiovascular disease is more prevalent in type 1 and type 2 diabetes and has remained the leading cause of death among adults with diabetes. Diabetes coexists as a more severe risk factor with other associating risk factors such as heavy alcohol consumption and dyslipidemia [1]. It has been suggested that mild to moderate alcohol consumption reduces the risk of type 2 diabetes mellitus (DM) in men [2], and coronary heart disease (CHD) [3]. In contrast, heavy drinking is associated with an increased risk. This relationship may be attributed to effects on the lipid profile.

However, the association of alcohol consumption, as well as CK-MB and Troponin with the prevalence of overall cardiovascular disease (CVD) remains unclear. The diagnosis of patients with myocardial infarction (MI) is challenging and has social, psychological, and legal implications if the diagnosis turn out to be positive [4]. According to World Health Organization (WHO) definition of MI, there must be the presence of two of the following three features: symptoms of myocardial ischemia, elevation of cardiac marker (protein or enzyme) concentrations in the blood, and a typical electrocardiographic pattern involving the development of Q waves or persistent T wave changes [5].

In line with WHO, the American Heart Association (AHA) case definition for acute myocardial infarction (AMI) requires a proper set of biomarkers and measurements of the same marker at least 6 hours apart [6].

The major cardiac enzyme assessments for the detection of MI includes the triad of Lactate dehydrogenase (LDH), aspartate transaminase and CK-MB which is of heart origin. However the use of CK-MB levels has limited prognostic power [4].

Some proteins with smaller molecular mass such as Myoglobin, Heart fatty acid binding protein (which is more cardio specific) has been developed. They appear more rapidly in the blood following the onset of necrosis and may have a specific role in the early detection of MI. But, neither of these proteins is considered as cardiac markers in clinical practice [5]. A more specific and highly sensitive cardiac Troponins T and/or I which are components of the thin filaments of the sarcomere has been used to identify subjects with small areas of myocardial necrosis. Cardiac Troponins have greater sensitivity and specificity for the diagnosis of MI in acute myocardial ischemia and may be elevated when CK-MB concentrations are not even mildly elevated.

In this study, we evaluated the association between alcohol consumption and cardiac markers such as lipid profile, CK-MB and Troponin I in non insulin dependent diabetes mellitus

Materials and Methods

A total of 200 adult male (aged 30-70 years) subjects were selected by stratified random sampling technique. Alcohol consumption and diabetes was self-reported, and data were collected during direct interview and by administration of questionnaire which included questions asking if the person is a diabetic, on lipid lowering drugs, presently drinks alcoholic beverages. If the latter was answered positively, subjects were asked specifically about the number of drinks per week of each type of their favorite beverage (beer, wine, or spirits). Ethanol consumption was estimated, assuming that concentrations of alcohol were 5% for beer, 12% for wine, and 40% for liquor. Subjects were characterized as diabetics and non diabetics, the diabetics were again grouped as alcoholic and non alcoholic diabetics, the alcoholic diabetics were further categorized as mild drinkers (if consuming 1-15g of ethanol per day), moderate drinkers (if consuming 16-30g of ethanol per day), and heavy drinkers (if consuming more than 30g of ethanol per day) for easy correlation. A total of five milliliter (5ml) of whole blood sample was collected from each participating subjects after an overnight fast of 10-12hours. The samples were dispensed into plane tubes and fluoride oxalate tubes. The samples were spun at 2000 rpm for 10min, separated and stored at -20^{o}c prior to assay as some were not assayed immediately. The glucose, lipid profile, and CK-MB levels of these subjects were assayed using 2012 Shenzhen mindray semi-auto Chemistry Analyzer, model BA-88A. Troponin I was assayed by enzyme linked immunosorbent assay (ELISA) method using a mindray microplate reader, model MR96A. The test analysis was carried out in Dr. Joe Nwiloh Heart Center Diagnostic laboratory, at St. Joseph's Hospital, Adazi-Nnukwu, Anambra State. The blood glucose of both men who reported to be diabetic and non diabetic was assayed to reconfirm those who were diabetics and the actual non diabetics

Result

Table 1 shows the biochemical characteristics of the study population; the diabetics and non diabetics. The results of their mean fasting blood sugar levels were 9.55±4.2 and 5.10±0.89 respectively. The result shows that the mean levels of fasting blood sugar was significantly higher in diabetics ($p = 0.000$) when compared to non diabetics. The mean levels of their triglyceride were 1.3±0.75 and 1.10±0.49 respectively. Triglyceride was also significantly increased in diabetics ($p = 0.019$) when compared to non diabetics.

Their mean levels of CK-MB were 14.79±9.12 and 11.34±6.94 respectively showing that CK-MB was also significantly increased in diabetics (p = 0.004) when compared to non diabetics. The mean levels of total cholesterol (5.85±1.78 and 5.80±2.76 respectively), High density lipoprotein cholesterol (HDL-C) (1.47±0.65 and 1.46±0.83 respectively), low density lipoprotein cholesterol (LDL-C) (3.81±1.54 and 3.81±2.56 respectively) and troponin (0.62±0.39 and 0.64±0.48 respectively), show no statistical significant difference (p>0.05) between the diabetics and non diabetic men.

Table 1: Biochemical characteristics of the study population (N = 200)

Parameters	Diabetics (Test) N= 112	Non Diabetics (Control) N= 88	P-value
FBS (mmol/L)	9.55 ± 4.2	5.10 ± 0.89	0.000***
TC (mmol/L)	5.85 ± 1.78	5.80 ± 2.76	0.877
TG (mmol/L)	1.3` ± 0.75	1.10 ± 0.49	0.019*
HDL-C (mmol/L)	1.47 ± 0.65	1.46 ± 0.83	0.997
LDL-C (mmol/L)	3.81 ± 1.54	3.81 ± 2.56	0.987
Troponin I (ng/ml)	0.62 ± 0.39	0.64 ± 0.48	0.766
CK-MB (units/L)	14.79 ± 9.12	11.34 ± 6.94	0.004**

Mean difference is significant at $p \leq 0.05$; N= No of subjects in the group; FBS= Fasting blood sugar; CK-MB=Creatin Kinase-MB; TC= Total cholesterol; TG= Triglyceride; HDL-C= High density lipoprotein cholesterol; LDL-C= Low density lipoprotein cholesterol.

Result of table 2 showed the biochemical characteristics of alcoholic diabetics and non alcoholic diabetics. The mean diastolic blood pressure levels of the two groups were 85.59 ± 8.11 & 80.56 ± 12.00 respectively, showing that diastolic blood pressure was significantly higher in alcoholic diabetics when compared with non alcoholic diabetics (p = 0.003) The mean TC levels of the two groups were 7.00 ± 2.55 & 5.40 ± 1.60 respectively, showing that TC was significantly higher in alcoholic diabetics when compared with non alcoholic diabetics (p = 0.002) LDL-C was also significantly higher in alcoholic diabetics when compared with non alcoholic diabetics (p = 0.033) with mean levels as 4.30 ± 2.00 & 3.47 ± 1.60 respectively. The mean levels of HDL-C between the groups were 1.74 ± 0.9 & 1.25 ± 0.48 respectively. HDL-C was significantly higher in alcoholic diabetics when compared with non alcoholic diabetics (p = 0.002). The result also showed the mean Troponin levels of the two groups as 0.68 ± 0.53 & 0.56 ± 0.29 respectively, indicating significantly higher troponin I in alcoholic diabetics when compared with the non alcoholic diabetics (p = 0.050).

There are no significant difference (p>0.05) in the mean levels of their body mass index (BMI) (30.83 ± 10.38 & 25.90 ± 4.22 respectively), systolic blood pressures (129.64 ± 17.16 & 132.22 ± 19.35 respectively), glucose, TG, and CK-MB (7.70 ± 3.04 & 7.30 ± 2.7; 1.30 ± 0.89 & 1.14 ± 0.50; and 15.76 ± 8.76 & 18.15 ± 10.54, respectively) between the alcoholic diabetics and the non alcoholic diabetic male subject studied.

Table 2: Biochemical characteristics of alcoholic diabetics and non alcoholic diabetics

Parameters	Alcoholic diabetics N =60	Non-alcoholic diabetics N = 52	P –value
Age (year)	50.59 ± 13.00d	52.98 ± 10.90	0.165
BMI (kg/m^2)	30.83 ± 10.38	25.90 ± 4.22	0.123
SBP (mmHg)	129.64 ± 17.16	132.22 ± 19.35	0.357
DBP (mmHg)	85.59 ± 8.11	80.56 ± 12.00	0.003***
Glucose (mmol/L)	7.70 ± 3.04	7.30 ± 2.7	0.388
TC (mmol/L)	7.00 ± 2.55	5.40 ± 1.60	0.002***
Triglyceride (mmol/L)	1.30 ± 0.89	1.14 ± 0.50	0.330
LDL-C (mmol/L)	4.30 ± 2.00	3.47 ± 1.60	0.033***
HDL-C (mmol/L)	1.74 ± 0.9	1.25 ± 0.48	0.001***
Troponin I (ng/ml)	0.68 ± 0.53	0.56 ± 0.29	0.050**
CK-MB (units/L)	15.76 ± 8.76	18.15 ± 10.54	0.279
AIP	-0.11 ± 0.03	-0.06 ± 0.02	0.296

Mean difference is significant at $p \leq 0.05$; N= No of subjects in the group; BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; CK-MB=Creatin Kinase-MB; TC= Total cholesterol; HDL-C= High density lipoprotein cholesterol; LDL-C= Low density lipoprotein cholesterol.

Table 3: This showed the relationship between alcohol consumption and the cardiac biomarkers. There are no significant statistical relationship observed (p >0.05) between alcohol and total cholesterol, LDL-C, CK-MB, and HDL-C. A significant positive correlation was observed when alcohol was correlated with fasting blood sugar (r = 0.317, p = 0.001), triglyceride (r = 0.237, p = 0.015), and troponin I (r = 0.203, p = 0.037).

Table 3: Relationship between alcohol consumption and the cardiac marker of alcoholic type 2 diabetics

Parameters	R	p-value
Alcohol Vs. Fasting blood suger	0.317	0.001***
Alcohol Vs. Total cholesterol	0.143	0.145
Alcohol Vs. Triglyceride	0.237	0.015**
Alcohol Vs. High density lipoprotein cholesterol	-0.062	0.531
Alcohol Vs. Low density lipoprotein cholesterol	0.144	0.144
Alcohol Vs. Troponin I	0.203	0.037**
Alcohol Vs. CK-MB	0.129	0.190

*** Correlation is significant at 0.001; **Correlation is significant at 0.015 and at 0.037

Table 4a & b: show the biochemical parameters at different levels of alcohol consumption in type two diabetics. In table 4a, the results show the mean levels of total cholesterol of the mild, moderate and heavy alcoholics as 5.23 ± 1.86, 5.75 ± 1.89, and 7.40 ± 2.17 respectively, indicating that total cholesterol was significant across the three levels (p = 0.003). The mean LDL-C levels of the mild moderate and heavy alcoholics were 3.22 ± 1.46, 3.69 ± 1.71, and 5.01 ± 1.9 respectively showing also a significant difference in the mean levels of low density lipoprotein cholesterol across the three levels of alcohol consumption (p = 0.004). The result the also shows the mean levels of Troponin for the three levels of alcoholics as 0.51 ± 0.19, 0.69 ± 0.27, and 1.00 ± 0.93 respectively with a significant difference across the levels (p = 0.041). There was no significant difference in the mean levels of glucose, triglyceride, high density lipoprotein cholesterol, and CK-MB of the levels of alcoholics (p > 0.05).

Table 4b shows that TC was significantly higher in heavy alcoholics when compared with mild alcoholics (p = 0.003) same also in heavy alcoholics when compared with moderate alcoholics (p = 0.030). LDL-C was also significantly increased in heavy alcoholics when compared with mild alcoholics (p = 0.006). Troponin was significantly higher in heavy alcoholics when compared with mild alcoholics.

Table 4a: biochemical characteristics of the mild, moderate and heavy alcoholic diabetics.

Parameters	Mild drinkers (1-15g/day) N=15	Moderate drinkers (16-30g/day) N=20	Heavy drinkers (>30g/day) N=25	p-value
Glucose (mmol/L)	6.72 ± 2.24	6.86 ± 1.96	8.07 ± 2.83	0.160
TC (mmol/L)	5.23 ± 1.86	5.75 ± 1.89	7.40 ± 2.17	0.003**
Triglyceride (mmol/L)	1.04 ± 0.4	1.10 ± 0.63	1.49 ± 0.96	0.112
LDL-C (mmol/L)	3.22 ± 1.46	3.69 ± 1.71	5.01 ± 1.9	0.004**
HDL-C (mmol/L)	1.60 ± 0.84	1.62 ± 0.63	1.76 ± 0.68	0.741
Troponin I (ng/ml)	0.51 ± 0.19	0.69 ± 0.27	1.00 ± 0.93	0.041*
CK-MB (units/L)	11.83 ± 5.14	16.00 ± 6.69	15.52±7.87	0.222

Mean difference is significant at p≤0.05; N= No of subjects in the group; CK-MB=Creatin Kinase-MB; TC= Total cholesterol; HDL-C= High density lipoprotein cholesterol; LDL-C= Low density lipoprotein cholesterol.

Table 4b: multiple comparisons of the biochemical characteristics of the mild, moderate and heavy alcoholic diabetics

Parameters	Groups	p-value
Total cholesterol (mmolL)	Mild Vs. Heavy alcoholics	0.003***
LDL-C (mmo/L)	Moderate Vs. Heavy alcoholics	0.004***
Troponin (ng/ml)	Mild Vs. Heavy alcoholics	0.041*

Discussion

Alcohol consumption is associated with changes in plasma lipid concentrations and the entire body lipid balances in humans [7]. A combination of decreased fatty acid oxidation in mitochondria and increased glycerolipid synthesis is said to be a major pathogenesis of alcoholic hyperlipidemia [8]. It has been established that increased levels of low density lipoproteins (LDL), Triacylglycerides (TG) and total cholesterol (TC) and decreased levels of high density lipoproteins (HDL) are also indicative of increased incidence of cardiac events and are considered as risk factors [9]. In this present study, triglyceride was significantly increased in diabetics when compared to non diabetics (table 1). Hypertriglyceridemia is one of the risk factors in coronary artery disease [CAD] and diabetes mellitus is always associated with raised triglycerides [10]. The increased levels of triglycerides in diabetes may be due to lack of insulin, which normally activates the enzyme lipoprotein lipase [11]. Creatine kinase-MB was found to be significantly increased in diabetics when compared to non diabetics (table 1). Since CK-MB is the most specific isoenzyme for the heart, the elevation in CK-MB levels is significant.

This present study show that alcoholic diabetics have higher blood cholesterol levels when compared with non alcoholic diabetic subjects. This increase may be due to increased synthesis of cholesterol in the tissues and excess cholesterol leaking out into the blood. These elevated levels of cholesterol in the tissues can greatly affect membrane fluidity. We also found in this study an increased level of LDL-C in alcoholic diabetics when compared to non alcoholic diabetics (table 2). Oxidative modification of LDL plays a major role in early atherogenesis [12], with oxidized LDL contributing to the accumulation of cholesterol and oxidized lipids in the arterial wall [13]. Thus it is believed that increased level of LDL than the recommended level is a high risk factor in the development of cardiac event. Studies comparing alcohol consumption and LDL-C are inconclusive as some show a strong positive effect of alcohol on LDL-C [14] while others indicate that genetic factor may be at play. However, this present work agrees with the work done by Lichtennstien *et al.,* (2006) [15], who also got a high plasma level of LDL-C. But disagrees with the result in the work done by Moutawakilou *et al.* (2013) [16] where there was no significant difference in the mean levels of LDL-C of the alcoholics when compared to the non alcoholics. LDL-C is only a measure of the cholesterol mass within the LDL-particle and Lichtennstien *et al.* (2006) [15] considered it to be a risk factor for cardiovascular disease. HDL-C level in this study was significantly higher in alcoholic diabetics when compared with non alcoholic diabetics (table 2). Many studies have reported that mild to moderate alcohol consumption increase HDL-C level in diabetes, conferring a cardioprotective effect on these individual [17]. The increase in the cardio protective HDL fraction obtained in the present study could imply that most of the alcoholics used in this study were mild to moderate alcoholics. HDL-C exerts a protective effect by decreasing the rate of entry of cholesterol into the cell through LDL-C and increasing the rate of cholesterol release from the cell by enhancing reverse cholesterol transport. This helps in removing excess cholesterol from peripheral tissues, and inhibiting the oxidation of LDL-C as well as the atherogenic effects of oxidized LDL-C by virtue of its antioxidant [18] and anti-inflammatory property [19]. Although the there was increase in the mean levels of lipid profile (TC, HDL-C, & LDL-C) of the alcoholic diabetics when compared to non alcoholic diabetics, there was no significant statistical difference observed in the mean levels of the atherogenic index of plasma between the two groups. All the values of lipid parameters of the non alcoholic subjects are within the safe levels for healthy subjects indicating they were having a minimum possibility of developing any cardiac event. Troponin was also significant in alcoholic diabetics when compared to non alcoholic diabetics. According to Laitinen *et al.* (1991), [20] acute alcohol consumption can interfere with calcium metabolism as it can lead to transient deficiency of parathyroid hormone and increase urinary calcium excretion, resulting in loss of calcium in the body. Decreased body calcium, will decrease the amount of calcium entering the cardiac muscle and this in turn will decrease the contractile ability of the heart muscle, leading to low amount of blood reaching the vital tissues and organ and finally giving rise to cell death dut to hypoxia. Once the heart muscle is affected, there is possibility of troponin is leaking into the plasma in a much hihger concentration. So, the increase in the mean level of troponin in the alcoholic diabetics could due to direct effect of alcohol on the heart muscle of due to the effect of alcohol on calcium metabolism.

This present work also showed a significant positive correlation of triglyceride with alcohol (table 3). The increased accumulation of TG in the presence of ethanol may also be due to increased availability of fatty acids and L–

glycerophosphate in the liver, increased disturbed catabolism of VLDL and decreased removal of TG from serum due to diminished lipoprotein lipase activity (LPL) (Grunnet *et al., 1985; and* Djousse *et al.,* 2004). There was also significant positive correlation between troponin and alcohol (table 3).

This study also revealed increased levels of TC, LDL-C, and Troponin in heavy alcoholic type 2 diabetics than in mild and moderate alcoholic type 2 diabetics (table 4a & 4b). Heavy drinking causes alcoholic cardiomyopathy and according to O'Keefe *et al.* (2014) [21], alcoholic cardiomyopathy accounts for 20 to 50 percent of all cases of cardiomyopathy in Western Countries and many alcoholics exhibit some degree of subclinical depression of cardiac function. Thomas *et al.* (1996) [22] also identified several mechanisms by which the negative effects of alcohol on cardiac muscles can be explained. According to him, when an electrical current spreads to the interior of cardiac muscle fibers, it causes the release of large quantities of calcium ions from a network of tubules (i.e., the sarcoplasmic reticulum), which in turn trigger the chemical events that produce muscular contractions. Alcohol alters the permeability of the sarcoplasmic reticulum to calcium ions, and thus reduces the efficiency by which calcium activates muscle contraction. This therefore can cause a reduction in the amount of blood pumped across the body, leading to cell death including the surrounding heart muscle due to hypoxia. If this happens, the protein troponin I will leak out of the dead or damaged heart muscle into the plasma, increasing its concentration in the plasma as seen in this study. Similarly, acetaldehyde (a metabolite of alcohol) and free radicals may contribute to decreased protein (actin and myosin) synthesis as well. Another way that alcohol can induce cardiac muscle damage is by increasing the expression of a certain gene (i.e., c-myc), which can promote programmed cell death, resulting in muscle cell loss and increased the possibility of troponin I leaking out into plasma.

Conclusion

Lipid profile levels can still be used in screening populations to identify the subjects with high risk of developing cardiac event which is identified by highly sensitive and specific positive Troponin test. Alcoholic diabetic subjects are at increased risk of cardiovascular disease than non alcoholic diabetic subjects with same anthropometric factors. The association of hyperlipidemia with alcohol consumption as seen in this study is relevant to the development of atherosclerosis and heart diseases in the drinking population.

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