Biochemical and biophysical changes in plasma and erythrocyte membranes of alcohol consuming type 2 diabetics: A clinical study

Ellutla Naresh

Sri Krishnadevaraya University

Ananda Vardhan Hebbani

Vaddi Damodara Reddy

Varadacharyulu NCh (✉ vdp_1975@yahoo.co.in)

Research Article

Keywords: Alcohol, Type 2 Diabetes, Erythrocyte membrane, HB1Ac, Lipid peroxidation, Nitric oxide

Posted Date: December 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1202784/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background and aims:

Effects of alcohol consumption on blood glucose levels is unpredictable and more so with a known type 2 diabetic. Since type 2 diabetes is a chronic condition with impairment of glucose metabolism, influence of excess alcohol consumption in such a derailed metabolism is ought to be investigated. Our aim was to understand the interpolating relationship between the metabolisms of glucose and alcohol, by investigating the biochemical and biophysical changes in plasma and erythrocytes respectively.

Methods

We performed a clinical study with 20 human subjects wherein non-alcoholics, non-diabetics were considered as controls and the test subjects were categorized as alcoholics, diabetics and alcoholic diabetics. Findings were analysed against the control group.

Results

Increased plasma AST, ALT, ALP, and LDH enzyme activity; higher levels of nitric oxide, thiobarbituric acid reactive species (TBARS) both in plasma and erythrocyte lysate; higher fasting and postprandial glucose, glycated haemoglobin levels (Hb1Ac) levels; elevated levels of erythrocyte membrane total cholesterol / phospholipids (C/P) ratio and altered erythrocyte membrane fluidity in the alcoholic diabetics was noted.

Conclusion

Alcohol induced oxidative and nitrosative stress during its metabolism and its worsening effects in type II diabetics leading to a failure in the overall metabolic homeostasis is evident from the study.

1. Introduction

Complications associated with diabetics consuming alcohol had been a point of concern globally from a very long time [1]. While people rely on alcohol consumption generally for social reasons, being an addictive drug, they generally end up becoming chronic alcoholics. The global diabetes prevalence in 2021 is estimated to be a large number i.e. nearly 800 million and alcohol drinking has been known to associated with incidental diabetes and glycemic status [2]. According to previous meta-analysis studies, light-to-moderate alcohol consumption was shown to be inversely associated with the incidence of type 2 diabetes [3, 4, 5]. Global health reports reveal that diabetes stands in second position and alcohol in the third position on the global scale of total deaths [6, 7].

It is well known that alcohol affects various physiological and bio-chemical events of the human body leading to a broad spectrum of metabolic disorders [8]. Ingested alcohol readily enters the circulation exposing all the cells and tissues to a continued shock of alcohol and its metabolites, for very long durations. Thus it is predictable that red blood cells and the other biochemical constituents in the circulation significantly get influenced because of the alcohol. Studies on the assessment of oxidative damage on RBC membranes of alcoholic diabetics are proved to

have close relationship with an associated biochemical and biophysical changes [9]. Biochemical changes in plasma and RBC membrane of diabetics and alcoholics were studied separately in human subjects [8, 10]. Nitric oxide is reported to play an important role in various physiological processes. It is also widely known that alcohol causes a significant physiological damage enhancing the burden of non-communicable diseases, and also known that its abuse among those diagnosed with diabetes has been on a consistent rise globally [11]. It is generally opined that the impact of alcohol consumption on blood glucose levels of a known diabetic is not always the same. The reasons for such an unpredictable outcome of alcohol consumption by a diabetic would probably be because of the varying carbohydrate contents of the drinks, usage of anti-diabetic drugs, their varying patterns of appetite and physical exercises they may be performing [12]. However, limited studies are available on the biochemical effects of alcohol on diabetic subjects. In the present study we have focused on the alcohol induced biochemical changes in some important blood constituent's viz., glucose, lipids with a special emphasis on the physico-chemical changes on the RBC membrane, which are the best model membranes used in several toxicological and membrane investigations. Hence, the present study is undertaken with a view to investigate the same.

2. Materials And Methods

Subjects for the study:

Four groups of human male volunteers, each consisting of twenty members aged between 35-60, residing in Anantapur town, Andhra Pradesh, India, were the subjects for the present study. The subjects were selected for the study based on information through a specially designed questionnaire. These volunteers were categorized into four groups viz., controls (who were non-alcoholics and non-diabetic), alcoholics (who consumed 70-120 g alcoholic beverage/day for the past 7-10 years), Diabetics (NIDDM patients who are on metformin, glycomate medication as prescribed by the physician), Alcoholic diabetics (NIDDM patients who consume 70-120 g alcoholic beverage/day for the past 7-10 years and also are on metformin, glycomate medication as prescribed by the physician). The beverages consumed by the chosen alcoholics include 80 proof hard liquors such as whisky, rum, Gin and brandy of various brands containing up to 40% alcohol. All the volunteers were well explained about the experimentation, a written consent was obtained and were asked to continue with their normal regular local diet throughout the period of study. Enough care was taken to prevent the effects of diet, water or sampling time, and daily activities of the subjects. The chosen subjects were not on medication for any other known chronic diseases or illnesses and were free from use of any other drugs and anaesthetics. The study was approved by our institutional ethical committee. Base line characteristics of the selected subjects are presented in Table-1.

Blood collection and Experimentation:

Venous blood samples were collected from volunteers into heparin test tubes after overnight fasting and were used for analysis immediately. Biochemical studies using plasma, erythrocyte lysate and erythrocyte membrane were carried out.

Plasma glucose and HbA1C:

Plasma glucose was estimated by GOD-POD enzymatic method using Monozyme diagnostic kit [13]. Plasma HbA₁C was estimated by using ERBA diagnostic kit.

Determination of plasma and erythrocyte total nitrate and nitrite levels

Nitrite and nitrate levels have been determined in plasma and erythrocyte as mentioned above [14]. Plasma and red cell lysate samples were treated with 30% zinc sulphate to deproteinize samples followed by centrifugation at 4000 g for 5 minutes. Nitrite is measured using Griess reagent from 1.0 ml plasma aliquots and erythrocyte lysate (1 percent sulfanilamide, 2.5 percent phosphoric acid, and 0.1 percent 1-naphthylethylene diamine). One millilitre of supernatant aliquots was spun with cadmium granules separately for 90 minutes for nitrite conversion, and Griess was then added to the nitrate. The amounts of nitrite have been calculated using a typical sodium nitrite curve.

Estimation of plasma Enzymes and Lipid Profile

Activities of plasma aspartate transaminase (AST; EC 2.6.1.1), alanine transaminase (ALT; EC 2.6.1.2), alkaline phosphatase (ALP; EC 3.1.3.1) and gamma glutamyl transferase (gGT; EC 2.3.2.2) were measured. Total cholesterol (TC), phospholipids, HDL-C and triglycerides (TG) were determined using commercially available kits (Erba Mannheim, Germany) as described earlier [15]. LDL-C and VLDL-C were calculated using the formula described previously [16].

Erythrocyte membrane preparation

Erythrocyte membranes were prepared as described previously [17]. The red blood cells were lysed with 5mM phosphate buffer (P^H 8.0) and spun at 15000 x g for 30 minutes after being rinsed with phosphate buffered saline (P^H 7.2). For analysis, we selected membrane ghosts that were devoid of haemoglobin after another wash with 5 mM phosphate buffer.

Determination of erythrocyte membrane TBARS

The produced malondialdehyde was used to determine the amount of lipid peroxidation (LPO) by treating the samples with 2ml of thiobarbituric acid reagent, as reported before [17, 18].

Erythrocyte membrane total cholesterol, phospholipids and C/P ratio

Erythrocyte membrane lipids were extracted as described previously [19]. Methanol (5 ml) was added to the lysed membrane preparations, followed by the addition of chloroform (10 ml). The filtrate was removed from the mixture after 30 minutes, and the residue was utilised for another extraction. The pooled filtrates were used for the estimation of cholesterol content [20] and phospholipids [21].

Statistical Analysis

Data were subjected to statistical analyses, values are means S.D. of 20 subjects in each group. Two-sided paired Student's *t*-test was performed for finding significant difference between the groups. A *p* < 0.05 was considered statistically significant.

3. Results

General characteristics and haematological profile of the controls, alcoholics, Diabetics and alcoholic diabetics volunteers participated in the study was presented in Table 1. Blood constituents, plasma, platelets, RBC and WBC are exposed to alcohol for long time. Analysis of blood cells and their constituents provide valuable information related to the effects of alcohol and metabolic status of the subject in alcoholic diabetes. Data presented in Table-

2 revealed the information related to the changes in haematological Parameters such as concentration of Hb counts of RBC, WBC, platelets, haematocrit, MCV and MCH. Results of the study revealed that alcoholic diabetics showed significant difference in various parameters compared to diabetics and alcoholics.

The concentrations of plasma glycated haemoglobin levels as well as glucose in both fasting and post prandial levels in different groups viz, control, alcohol, diabetics were compared to alcoholic diabetics. While alcoholics and diabetics showed significant ($p < 0.05$) increase in HbA1c levels and the data presented in Figure 1a, the hike in alcoholic diabetics is more pronounced compared other groups. Similarly, glucose levels also found to be elevated in diabetics and alcoholic diabetics in both fasting and postprandial conditions and the data presented in Figure 1b. Not significant increase was seen in alcoholics compared to controls.

The amounts of nitrite and nitrate in plasma and erythrocyte lysate from alcoholics, diabetics and alcoholic diabetics were measured to determine NO production and the data presented in Figure 1c. When alcoholic diabetics were compared to their respective control individuals, the levels of nitrite and nitrate in plasma and erythrocyte lysate were significantly ($p < 0.05$) higher. Moreover, alcoholics and diabetics were showed elevated NO levels compared to controls. There was a substantially larger degree of change in erythrocyte NO concentration in alcoholic diabetics.

Levels of total cholesterol, triglycerides and lipoprotein patterns in plasma of controls, alcoholics, diabetics and alcoholic diabetics were measured and the data presented in Table 2. A significant ($p < 0.05$) increase in plasma cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol followed by a significant ($p < 0.05$) decrease in HDL-cholesterol is evident from the data in alcoholics, diabetics. However, these alterations are more pronounced in alcoholic diabetics.

Alcohol intake has been linked to a variety of metabolic alterations in erythrocyte membrane. The oxidative stress state is determined by measuring TBARS levels. In the present study we measured TBARS levels in control and alcoholic, diabetics and alcoholic diabetics and the data was presented in Figure 1d. Alcoholics and diabetics individual's erythrocyte membranes had substantially higher TBARS levels than controls. More prominent increase was observed in erythrocyte membrane TBARS levels of alcoholic diabetics subjects than the all other groups.

Alcohol induced cellular damage was determined by measuring plasma enzyme levels in controls and other experimental groups, the data was presented in Table 3. Alcoholics and diabetics showed elevated AST, ALT, ALP and LDH levels compared to controls. However, the hike is more pronounced in alcoholic diabetics compared to all other experimental groups.

Alcohol affects membrane fluidity, assaying membrane total cholesterol and total phospholipids gives an idea about membrane fluidity. We analyzed erythrocyte membrane cholesterol and total phospholipids in control and alcoholic, diabetics and alcoholic diabetics and the data was presented in Table 4. In comparison to control groups, we found a substantial rise in total cholesterol and the resulting C/P ratio in alcoholic diabetics compared to alcoholics. Moreover, alcoholic diabetics had more total cholesterol, phospholipids, and, as a result, a higher C/P ratio than alcoholics.

4. Discussion

Maintenance of stable levels of blood glucose is a finely regulated mechanism in which hormones, tissues and various other factors play a role. It is well known that hyper glycemia is a characteristic feature in diabetics due to disturbed metabolism and hormones [12]. Observed further increase in glucose levels in diabetics consuming alcohol in present study clearly suggested the alcohol provoked hyperglycaemia in alcoholic diabetics. Results of the present study clearly indicated that alcohol consumption caused a continuous and prolonged increase in blood glucose level along with hike in protein glycation in diabetics. This is evident from the observed increase in HbA1C concentrations in alcoholic diabetics. In general increase in blood glucose (hyper glycemia) occurs when there is increase in hepatic glycogenolysis and/or enhanced hepatic and renal gluconeogenesis and/or decrease in utilization of glucose by tissues. Hence the contribution by liver and kidney through the former two reasons is expected in causation of hyperglycaemia diabetics consuming alcohol. But in present study further increase in blood glucose in alcoholic diabetics confirmed the enhancement of glucose by alcohol consumption in diabetes also.

Elevated levels of glucose in the medium or blood are known to cause membrane damage or cell death of red cells, cultured pericytes, kidney cells and retinal cells [1]. However, Biochemical mechanisms that result in membrane damage and cell death were not known. Prolonged Hyperglycaemia leads to the glycosylation of a number of proteins. Haemoglobin is also glycosylated and cause changes in their activity, solubility and susceptibility to degradation. The glycosylation of haemoglobin occurs by a non-enzymatic reaction between glucose and amino terminal valine of β -chain and this on rearrangement resulting protein called HbA1C is a good index of uncontrolled diabetes mellitus [18]. Collagen fibres, antithrombin III are also glycosylated. These changes may possibly favour the accelerated blood vessel damage that occurs in alcoholics with diabetes.

Higher activities of AST, ALT, ALP and LDH in alcoholic diabetics are recorded in this study. Derangement in carbohydrate metabolism is enhanced in alcoholic diabetics suggesting hepatic and renal dysfunctioning leading increased ROS. Increased ROS may damage membrane and cause leaking cellular contents in to blood stream. Increased plasma enzyme levels might be due to increased cellular membrane damage in alcoholics and Diabetic alcoholics. Increased Total cholesterol and plasma lipoproteins, LDL, VLDL and TG with a decrease in HDL in alcoholic diabetics suggested cardiac risk and lipid abnormalities. Circulating levels of VLDL, LDL and HDL are considered to be powerful indicators of for cardiovascular diseases(CVD) [22]. Observed increase in total cholesterol, LDL-C, VLDL-C, triglycerides with significantly decreased HDL-C in alcoholic diabetics when compared with other groups of the present study suggested cardiovascular risk in these groups. The cardio vascular risk is as follows alcohol diabetics>diabetics>alcohol>controls. Furthermore results of this study showed increased plasma cholesterol, triglycerides with a decrease on phospholipids. In general hyperlipidemia is a complication of alcohol toxicity leading to cardio vascular problems and other abnormalities. Accumulation of fat in the liver is chronic alcohol intake acts as stimulus for the secretion of lipoprotein in to the blood stream and also the development of hyper lipidemia. In general HDL is considered to be a beneficial protein that helps in scavenging cholesterol from extra hepatic tissues and Presence of lecithin cholesterol, acyltransferase (LCAT) brings it to liver. Increased Plasma cholesterol, triglycerides, VLDL, LDL and atherogenic index with a decrease in HDL concentration observed in alcoholics in comparison with teetotallers suggested cardiac risks as well as hepatic dysfunction in alcoholics. Also in the present study, alcoholics showed Increased NO Production (elevated levels of nitrite and nitrate) when compared to controls. Nitric oxide (NO) mediated regulation in hepatic production or secretion of apolipoprotein particles, increasing triglycerides and lipases and decreasing the removal of circulating HDL might have played a role in the observed effects [23, 24]. Per oxidation of lipids received much attention in recent years. In present study, the observed increase in lipid per oxidation indicates the damage of

tissues and liver *in vivo*, where it may be a cause of atherosclerosis and other complication associated with alcoholism. Furthermore increased lipid per oxidation is an indicative of enhanced oxidative stress.

The levels of plasma nitrite and nitrate, the end products of NO metabolism the reliable indicators of NO production. Increased concentrations of NO₂ and NO₃ In plasma of alcoholics and alcoholic diabetics. In present study suggested over production of NO in alcoholics. Probably this might be responsible for various abnormalities in lipid profile and the activities of enzymes in alcoholics. Earlier studies revealed that alcohol induced hepatic cytosolic NO production in rats [25]. NO Plays an important role in alcohol induced events. Nitration of lipids and proteins is a common process under nitrosative stress. Multiple physiological action of NO is interesting and its interacts with molecules and free radicals in alcoholics experimentally in plasma lipids, membrane phospholipids/proteins and in intracellular metabolism playing a major role in the causation of observed alteration in lipid profile, protein profile and also in other events in cells and membranes of alcoholics, diabetics and alcoholic diabetics. Increased in plasma nitrite and nitrate levels in alcoholic diabetics suggested an increased production of nitric oxide in the body affecting several physiological activities leading to pathological complications in alcoholic diabetics. Higher lipid per oxidation in alcoholic diabetics is recorded in this study. Also in the present study, alcoholics showed increased NO production (elevated levels of nitrite and nitrate) when compared to controls. NO mediated regulation in hepatic production or secretion of apolipoprotein particles, increasing triglycerides lipases and decreasing the removal of circulating HDL might have played a role in the observed effect [23, 24].

Increased erythrocyte membrane cholesterol and phospholipids contents in alcoholics-diabetics observed in the present study indicated the transfer of cholesterol and phospholipids from plasma to erythrocyte membrane [26]. A Subsequent hike in C:P ratio suggested a decrease in erythrocyte fluidity of alcoholics in the present study. In general alcohol perturbs the bilayer and thereby increases the fluidity of the membranes probably the observed decrease in membrane fluidity in membrane of chronic alcoholic diabetics in present study may be an adaptive change leading to an increased tolerance chronic alcoholic.

Increased lipid per oxidation with an increase in plasma Nitrites and Nitrates in alcoholics in this study strongly suggested enhanced oxidative stress with an increase in NO production leading to generation of several free radicals. A decrease in membrane fluidity observed in alcoholics in the present study may be an adaptive biochemical change in alcoholics to counteract the fluidizing effect of alcohol. This study also strengthens and confirms the earlier reports [27] where in the role of NO in alcohol induced changes in lipid profile of alcoholics was being demonstrated.

In conclusion, the present study revealed that oxidative stress is increased leading to pathological consequences and damage to biomembranes in alcoholic diabetics. Fluctuations in cholesterol and phospholipids lipid concentrations in RBC membrane with an increased in C:P ratio in this study suggested either rigidification/fluidization of membrane due to cholesterol which acts as a stabilizing agent in biomembranes.

Declarations

Acknowledgments: Authors thank DST-SERB for providing financial assistance to Dr.V.Damodara Reddy under Ramanujan Fellowship (SB/S2/RJN-043/2014 dated 17.12.2015)

Conflict of Interest statement

Authors declare that there are no competing interests.

References

1. Wiss D. A.. The Relationship Between Alcohol and Glycohemoglobin: A Biopsychosocial Perspective. *BioResearch open access* 2019; 8(1), 146–154.
2. Munukutla S, Pan G, Deshpande M, Thandavarayan RA, Krishnamurthy P, Palaniyandi SS. Alcohol Toxicity in Diabetes and Its Complications: A Double Trouble? *Alcohol Clin Exp Res*. 2016;40(4):686–97.
3. Howard AA, Arnsten JH, Gourevitch MN. Effect of alcohol consumption on diabetes mellitus: A systematic review. *Ann Intern Med* 2004; 140: 211–219.
4. Koppes LU, Dekker JM, Hendriks HFJ. Moderate alcohol consumption lower the risk of type 2 diabetes; a meta-analysis of prospective observational studies, *Diabetes care* 2005; 28: 719–725.
5. Li XH, Yu FF, Zhou YH, et al. Association between alcohol consumption and the risk of incident type 2 diabetes; as systematic review and dose- response meta –analysis. *Am J Clin Nutr*. 2016; 103: 818–829.
6. Zarate A. Diabetes mellitus in Mexico. *Diabetes Care* 1961; 14:672–675.
7. WHO-Global status report on alcohol and health: 2014
8. Kavitha G, Reddy VD, Padmavathi P, Varadacharyulu NC. Association between plasma glucose and glycoproteins in alcoholic smokers compared to alcoholics and teetotalers. *Diabetes & Metabolic syndrome: Clinical Research and Reviews* 2010; 4: 155-159.
9. Parmahamsa M, Rameswara Reddy K, Varadacharyulu N, Changes in composition and properties of erythrocyte membrane in chronic alcoholics, *Alcohol and Alcoholism*, 2003; 39(2), 110–112.
10. Bulle S, Reddy VD, Padmavathi P, Maturu P, Puvvada PK. *J Clin Bio Nutr*. 2016;1:63–69.
11. Babor T, Rehm J, Jernigan D, Vaeth P, Monteiro M, and Lehman H. Alcohol, diabetes, and public health in the Americans. *Rev Panam Salud Publica* 2012;32:151–155.
12. Alessi SM, Foster NC, Rash CJ, Van Name MA, Tamborlane WV, Cengiz E, Polsky S, Wagner J. Alcohol Use and Clinical Outcomes in Adults in the Type 1 Diabetes Exchange. *Can J Diabetes*. 2020 Aug;44(6):501–506.
13. Trinder P. Determination of glucose by glucose oxidase method. *Ann Clin Biochem*. 1969; 6:24–26.
14. Padmavathi P, Reddy VD, Kavitha G, Maturu P, Varadacharyulu NC. Chronic cigarette smoking alters erythrocyte membrane lipid composition and properties in male human volunteers. *Nitric oxide* 2010; 23:181–186
15. Padmavathi P, Reddy VD, Narendra M, Varadacharyulu NC. Bidis – hand-rolled, Indian cigarettes: induced biochemical changes in plasma and red cell membranes of human male volunteers. *Clinical Biochemistry* 2009; 42: 1041–1045.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*. 1972;18(6):499–502.
17. Hebbani AV, Reddy VD, Asha BM, Nallanchakravarthula V. Protective effect of *Terminalia arjuna* against alcohol induced oxidative damage in rat erythrocyte membranes. *J Ayur Int Med*. 2021, 12, 330–339.
18. Begum MS, Saradamma B, Reddy VD, Padmavathi P, Maturu P, babu Ellutla N, Thippannagari L, Varadacharyulu NC. Influence of green tea consumption on cigarette smoking-induced biochemical changes in plasma and blood. *Clinical Nutrition Experimental*. 2017 Dec 1;16:1–2.

19. Peeterways, Hanahan, Characterization and qualification of red cell lipids in normal man. *Journal of lipid research*.1964; 5:318–328.
20. Zlatkis A, Zak B, Boyle A.A new method for the direct determination of serum cholesterol. *J Lab Clin Med*.1953;41:486–492.
21. Connerty, H.V., Brings R.A.and E.H. Easton. *Clin Chem*.1961; 317: 589.
22. Baer D.J, Judd J.T, Clevidence B.A.,Muesing,R.A., Campbell,W.S., Brown,E.D And Taylor,P.R.Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. *Clinical Nutrition*.2002; 75:593–599.
23. Dreon D.M, Krauss, R.M. Alcohol, Lipids and lipoproteins, In; Zahkari, S., Wassef, M (Eds), *Alcohol and the cardiovascular system*. 1996; 369-391.
24. Leighton F, Miranda- Rottmann, S, Urmequiaga, I.A central role of eNos in the protective effect of wine against metabolic syndrome. *Cell Biochemistry and Function*.2005; 24: 291–298.
25. Reddy KR, Reddy VD, Padmavathi P, Kavitha G, Saradamma B, Varadacharyulu NC. Gender differences in alcohol-induced oxidative stress and altered membrane properties in erythrocytes of rats. *Indian J Biochem Biophys*. 2013; 50: 32–39.
26. Paramahamsa M, Aparna S, Varadacharyulu N. Alcohol-induced alterations in blood and erythrocyte membrane in diabetics, *Alcohol and Alcoholism*, 2002, 37(1), 49–51.
27. Kavitha,G.,DamodaraReddy,V.,Paramahamsa,M.,Akhtar,p.Md&Varadacharyulu,N.C.Role of nitric oxide in alcohol- induced changes in lipid profile of moderate alcohol and heavy alcoholics.*Alcohol*.2008;42:47–53.

Tables

Table 1. General characteristics and Haematological profile of chronic alcoholics, diabetics, alcoholic diabetics and controls.

Parameter	Controls	Alcoholics	Diabetics	Alcoholic diabetics
Age	35 - 60	35 - 60	35 - 60	35 - 60
Sex	Male	Male	Male	Male
Alcohol consumption	Do not drink	70-120 g/day	Do not drink	70-120 g/day
Chronic diseases	Not found	Not found	Not found	Not found
Diabetic history	No	No	10 years	10 years
Alcohol history	No	5 Days/week	No	5 Days/week
Drugs	No	No	As per Physicians prescription	
Socio-economic status	Middle and Lower income	Middle and Lower income	Middle and Lower income	Middle and Lower income
Hb (g/dl)	11.42 ± 0.47	11.72 ± 0.42	10.32 ± 0.59*	12.52 ± 0.62*
RBC (10 ⁶ mm ⁻³)	4.28 ± 0.07	4.13 ± 0.08	4.09 ± 0.05	3.53 ± 0.03**
WBC (10 ³ mm ⁻³)	7340 ± 167.47	8960 ± 165.7*	5440 ± 37.22**	4340 ± 127.38**#
Platelets (Lacks/cumm)	181600 ± 6915	2146600 ± 4723*	171330 ± 5415**	236600 ± 3713**#
Hematocrit (%)	49.04 ± 0.67	46.56 ± 1.08	39.46 ± 1.27**	52.36 ± 1.35**#
MCV (fl)	115.12 ± 2.31	112.21 ± 2.80	107.07 ± 4.93*	121.48 ± 2.72 **#
MCH (pg)	26.81 ± 1.18	27.52 ± 1.32	27.69 ± 2.21	29.52 ± 3.45
MCHB (g%)	23.36 ± 1.98	25.12 ± 2.84	26.09 ± 1.79	29.01 ± 3.67

Values are mean ± SD of 20 human volunteers in each group. A $p < 0.05$ is statistically significant between groups. *indicates significantly difference from controls, **indicates significantly difference from alcoholics and diabetics. # indicates significantly different from alcoholics.

Table 2. Changes of plasma lipoproteins in chronic alcoholics, diabetics and alcoholic diabetics in comparison with controls.

Parameter	Controls	Alcoholics	Diabetics	Alcoholic diabetics
Total cholesterol	162.7 ± 13.42	209.3 ± 15.22*	193 ± 18.05*	232.2 ± 9.03**
Triglycerides	92.7 ± 3.12	119.3 ± 5.26*	153 ± 8.05*	202.2 ± 9.03**#
HDL-Cholesterol	38.6 ± 3.48	25.4 ± 2.27*	26.4 ± 3.71*	34.1 ± 3.12**
LDL-Cholesterol	21.1 ± 1.97	19.3 ± 0.54	41.2 ± 3.43*	45.1 ± 2.67*
VLDL-Cholesterol	15.1 ± 1.22	13.7 ± 1.09	19.4 ± 1.62*	25.2 ± 1.42**

*Values are mean ± SD of 20 samples of each group. *indicates significantly difference from controls, **indicates significantly difference from alcoholics and diabetics. # indicates significantly different from alcoholics. Values are expressed as mg/dl protein.

Table 3. Changes of plasma enzymes in chronic alcoholics, diabetics and alcoholic diabetics in comparison with controls.

Parameter	Controls	Alcoholics	Diabetics	Alcoholic diabetics
AST	12.71 ± 1.01	20.32 ± 1.43*	19.46 ± 1.34	29.60 ± 1.63*
ALT	10.39 ± 0.83	21.56 ± 1.29*	19.78 ± 1.2*	29.80 ± 2.08**
ALP	65.5 ± 8.1	92.4 ± 11*	97.2 ± 5.1*	99.6 ± 9.5*
LDH	13.2 ± 0.5	17.9 ± 1.2*	18.3 ± 1.5*	21.3 ± 1.7*

*Values are mean ± SD of 20 in each group. All the values are expressed as IU/l. *indicates significantly difference from controls, **indicates significantly difference from alcoholics and diabetics.

Table 4: Erythrocyte membrane total cholesterol, total phospholipids and C/P ratio in control, diabetics and alcoholic diabetics.

Parameter	Controls	Alcoholics	Diabetics	Alcoholic diabetics
Total cholesterol	100.8 ± 2.56	123.3 ± 3.57*	122.60 ± 3.42*	124.47 ± 3.73 *
Total phospholipids	113.4 ± 4.76	129.3 ± 5.34*	143.7 ± 8.17	98.52 ± 3.71
C/P ratio	0.88	0.88	0.85*	1.26**

Values are mean ± SD of 20 samples of each group. Values are expressed as mg/mg protein. *indicates significantly difference from controls, **indicates significantly difference from alcoholics and diabetics.

Figures

Figure 1: Effect of alcohol consumption on a) Hb1Ac, b) glucose levels, c) nitrite and nitrate levels, and d) TBARS in Diabetics.

Figure 1

Effect of alcohol consumption on a) Hb1Ac, b) glucose levels, c) nitrite and nitrate levels, and d) TBARS in Diabetics. Values are mean $\pm$ SD of 20 human volunteers in each group. *indicates significantly difference from controls, **indicates significantly difference from alcoholics and diabetics. # indicates significantly different from alcoholics. Hb1Ac and glucose levels expressed as mg/dl. Nitrite and nitrate levels expressed as μ mol/mg protein, TBARS levels expressed as μ mol/l.