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LEUKOCYTE LIPID PROFILE IN CHRONIC ALCOHOLICS

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Abstract : Polymorphonuclear leukocytic (PNML) lipid content was studied in chronic alcoholic and normal control subjects. Chronic alcoholic subjects showed abnormal liver function and abnormal serum lipid profile. PMNL obtained from chronic alcoholic subjects exhibited decreased free cholesterol and phospholipid contents, with a significant increase in cholesterol to phospholipid ratio. Although, there was no change in the free fatty acids, the levels of triglycerides were significantly elevated.

Key words : polymorphonuclear leukocyte chronic alcoholics lipid profile

INTRODUCTION

Circulating Polymorphonuclear Leukocyte (PMNL) are metabolically the most active and are in some way a unique biological system for evaluating biochemical phenomena. They are often the ideal choice as a tissue in the human system for studying biochemical alteration in an abnormal metabolic state.

Leukopenia is frequently associated with alcoholism (1) particularly in cirrhotic patients with hypersplenism (2). Alcohol also impairs granulocyte function like diminished leukocyte mobilisation (3), decreased granulocyte adherence (4) and defective chemotaxis (5, 6). In addition suppression of bactericidal activity of human serum has been noted in presence of ethanol (7, 8). These aberrations in the functional ability of membrane could be due to the well known membrane perturbing action and memberane fluidizing effect of ethanol (9). It is also recognised that lipid composition, which determines cellular membrane structure, is aberrated in chronic alcoholism (10, 11).

Although, significant alteration in the lipid composition of leukemic leukocyte have been reported (12, 13), no attempts have been made to estimate and evaluate the composition of lipids in PMNL obtained from chronic alcoholic subjects.

It was, therefore, thought worthwhile to investigate in details the alteration, if any, in lipid morphology of PMNL obtained from alcohol subjects as compared to healthy normal controls.

METHODS

Subjects : Twentyeight alcohol dependent male subjects (35-55 yrs) admitted to the Govt: Medical College and Hospital, Nagpur were chosen for the study. All the subjects had a history of 10-15 years of alcohol consumption with a daily consumption of alcohol of about 150-200 g and a period of 5-10 days abstinence from alcohol per month.

Materials :Dextran (Mol. Wt. 1,50,000 to 200,000) was obtained from BDH Chemicals, England and Ficoll-paque from Decruz Corporation, Bombay, India. All other chemicals and reagents used were of analytical grade.

During the period of hospitalization the subjects received diet composed of 40% carbohydrate, 35% fat and 22% proteins. Care was taken to choose 30 controls of similar age group and body weight receiving more or less similar diet. None of the above groups, were obese, had, any renal or other complication, hypertension, diabetes, jaundice, anaemia or infection at the time of this study.

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Analytical method : Sera of all the groups were collected after overnight fast and within 15-36 hr. after the last drink and analysed for lipid profile. PMNL were isolated by the dextran sedimentation method (14). Lipids were extracted by the method of Bagdade and Ways (16), cholesterol by the method of Courchaine et al (17) and triglycerides and free fatty acids by the methods of Kaplan & Lee (18) and Duncombe (19) respectively. Proteins were estimated by the method of Lowry et al (20) using bovine serum albumin as standard.

RESULTS

Table I represents the assessment of liver function of normal and chronic alcoholic subjects. Chronic alcoholic subjects exhibited abnormal hepatic function as indicated by altered levels of SGOT, SGPT and Alkaline phosphatase as compared to normal controls. The serum total protein and bilirubin levels remained unaltered.

TABLE I : Assessment of liver function of control and chronic alcoholic subjects.

Serum chemistry	*Units*	*Control (30)*	*Chronic alcoholic (28)*
Asparate Transaminase	U/L	15.44±8.02	40.9 ±9.2*
Alanine Transaminase	U/L	8.50±4.8	25.8 ±6.4*
Total Protein	g/dl	5.4 ±1.8	6.01±2.1
Alkaline Phosphatase	KAU/L	6.21±3.4	11.2 ±2.9*
Total Bilirubin	Mg/dl	0.14±0.02	0.18±0.1

*P < 0.01 (Student's 't' test)
Number of subjects are shown in parenthesis.

Table II depicts the serum lipid status of normal control ad chronic alcoholics. Although there was no significant change in the serum cholesterol, free fatty acid and total serum phospholipid levels, a significant increase in serum triglyceride of chronic alcoholics as compared to normal controls, was observed.

TABLE II : Serum lipid status of control and chronic alcoholic subjects.

Serum lipid profile (mg/dl)	*Control (30)*	*Chronic alcoholic (28)*
Cholesterol	189 ±18.5	203 ±20
Free Fatty Acid	8.4± 2.1	9.2± 2.7
Triglyceride	131.2±14.1	170.8±16.4*
Phospholipid	194.3±18.7	198.7±21.6

*P< 0.01 (Student's 't' test)
Number of subjects are shown in parenthesis.

Table III depicts the lipid composition of PMNL obtained from normal and chronic alcoholics. Although there was no significant change in the content of total cholestarol, free cholesterol decreased significantly. A significant decrease in the contents of total phospholipids was observed. There was no change in the contents of free fatty acids, however, the levels of triglyceride were slightly elevated. A significant increase in the cholesterol to phospholipid ratio was also observed in the PMNL obtained from chronic alcoholics.

TABLE III : Lipid composition of polymorphonuclear leukocytes in control and chronic alcoholic subjects.

Lipid fraction (mg/g protein)	*Control (30)*	*Chronic alcoholic (28)*
Total Cholesterol	32.4 ±6.6	34.8 ±7.2
Free Cholesterol	25.2 ±5.9	15.3 ±4.6*
Total Phospholipids	29.6 ±8.1	18.4 ±6.3*
Free Fatty Acids	12.2 ±3.3	15.6 ±3.7
Triglycerides	24.8 ±5.7	35.8 ±6.2**
Cholesterol Phospholipid	1.19±0.19	1.79±0.21*

*P < 0.01, **P <0.05,
Number of subjects are shown in parenthesis.

DISCUSSION

The effects of alcohol upon the plasma lipid levels are variable and are related to the dose and duration of ingestion. Experienced clinicians have long recognised the association between alcohol ingestion and hyperlipidemia, specifically hypertriglyceridemia (21). The chronic alcoholic patients

chosen in this study exhibit an abnormal hepatic function with changes in serum lipid pattern specially hypertriglyceridemia. However, none of the subjects developed cirrhosis.

The small but significant increase in the PMNL triglyceride could be due to its increased synthesis in alcoholic condition. Earlier studies have indicated higher hepatic accumulated of triglycerides following administration of ethanol (22). Alternatively, increased triglyceride influx from the plasma in this condition cannot be ruled out since, there is a rapid exchange of triglycerides as compared to other lipids between the cellular components of human body cell and plasma (23, 24).

Earlier animal experiments have shown elevated tissue cholesterol levels due to alcohol (10, 25). The decrease in free cholesterol and an insignificant increase in total cholesterol observed in the prevent study denotes higher esterification in PMNL obtained from chronic alcoholics. It may be pointed out that cholesterol is closely linked to phospholipids and is primarily concerned with the organisation of cellular memberaneous structure in controlling the microviscosity and permiability of cell surface membrane (26). Hence, the observered alteration in the cholesterol to phospolipid ratio in PMNL from chronic alcoholic denotes gross alteration in membrane characteristics.

Since there is no *de novo* synthesis of fatty acids in leukocytes (27), it is possible that any influx of fatty acids into the chronic alcoholic PMNL would immediately be utilized for the esterification of cholesterol and for synthesis of triglycerides, thus rendering no chage in its own contents.

Exposure to alcohol is known to bring about a change in the plasma membrane lipid composition (9-11, 25). It is not known at present whether the changes in the lipid composition of PMNL obtained from chronic alcoholics is due primarily to alcohol or is secondary to liver dysfunction. However, the overall alteration in the lipid composition of PMNL obtained from chronic alcoholic subjects would cause variation in the selective entrance of certain mircronutrients and changes in the morphological characteristics of the cell membrane. These alterations could be corelated with the defective PMNL membrane functional ability observed in chronic alcoholics (1-8).

REFERENCES

1. Eichner ER, Hillman RS. The evolution of anaemia in alcoholic patient. *Am. J Med Sci* 1971; 50: 218-224.
2. McFarland W, Libre EP. Abnormal leukocyte response in alcoholism. *Ann Int Med* 1963; 59: 865-873.
3. Brayton RG, Stokes PE, Scwartz M. Effect of alcohol and various diseases on leukocyte mobilization, phagocytosis and intercellular bacterial killing. *N Engl J Med* 1970; 282: 123-128.
4. Gluckman SJ, MacGregor RR. Effect of acute alcohol intoxication on granulocyte mobilisation and kinetics. *Blood* 1978; 52: 551-564.
5. Klepser RG, Nungester WJ. The effect of alcohol upon the chemotatic response of leukocytes. *J Infec Dis* 1937, 65: 196-203.
6. De Meo AN, Anderson BR. Defective chemotaxis associated with a serum inhibitor in cirrhotic patents. *N Engl J Med* 1972; 286: 735-740.
7. Kaplan NM, Braude AI. Hemophilus influenzae infection in adults. Observations on immunised disturbance. *Arch Int Med* 1958; 101: 515-523.
8. Fierer J, Finley F. Deficient bactericidal activity against E. Coli in patients with Cirrhosis of the liver. *J Clin Invest* 1979; 63: 912-921.
9. Seeman P. The membrane actions of anaesthetics and tranquilizers. *Pharmacol Rev* 1972; 24: 583-595.
10. Goldstein DB. In membrane mechanisms of drug abuse C. W. Sharpen and I. G. Abood eds. *Allen R. Liss, N. Y.* 1979; P 151.
11. Wing DR, et al. Change in membrane lipid contents after chronic ethanol administration with respect to fatty acy composition and phospholipid type. *Biochem Pharmacol* 1984; 33: 1625-1633.
12. Gottfried EL. Lipid patterns of Leukocytes in health and diseases. *Semin Heamatol* 1972; 9: 241-250.
13. Kidson C. Lipid synthesis in human leukocytes in acute leukemia. *Australasia Ann Med* 1961; 10: 282-286.

14. Nath N, Chari SN. Superoxidedismutase in diabetic polymorphonuclear leukocytes. *Diabetes* 1984; 27: 889-894.

15. Ways P, Hanahan BJ, Characterization and quantification of red cell lipids in normal man. *J Lipid Res* 1964; 5: 318-329.

16. Bagdade JD, Ways PO. Erythrocyte membrane composition in exogenous and endogenous hypertriglyceridemia. *J Lab Clin Med* 1970; 75: 53-59.

17. Courchaine AJ, Miller WH, Stein DB Jr. Rapid semimicro procedure for estimating free and total cholesterol. *Clin Chem* 1959; 5: 609-613.

18. Kaplan A, Lee VF. A micromethod for the determination of serum triglycerides. *Proc Soc Expt Biol Med* 1965; 118: 296-301.

19. Duncombe WG. Colorometric microdetermination of long chain fatty acids. *Biochem J* 1963; 88: 7-14.

20. Lowry et al. Protein measurement with folin-phenol reagent. *J Biol Chem* 1951; 193: 265-275.

21. Jones DP, Losowsky MS, Davidson CS. Effects of ethanol on plasma lipids in man. *J Lab Clin Med* 1963; 62: 675-682.

22. Lieber CS. Metabolism and metabolic effects of alcohol. In Medical Clinics at North America. *McGookas Eds* 1984; 68: 3-31.

23. James AT. Lovelock JE, Joan Webb. The lipids of whole blood. I-Lipid biosynthesis in human blood *in vitro Biochem J*. 1959; 73; 106-115.

24. Marks EA, Gellhorn A, Kidson C. Lipid Synthesis in human leukocytes, platelets and erythrocytes. *J Bio Chem* 1960; 235: 2579-2584.

25. Chin JHG, Parsons IM, Goldstein DB. Increased cholesterol contents of erythrocytes and brain membranes in ethanol tolerant mice. *Biochem Biophys Acta* 1978; 513: 358-363.

26. Inbar M, Shinitizky M. Cholesterol as a bioregulator in the development and inhibition of leukemia. *Proc. Nat Acad Sci (USA)* 1974; 71: 4229-4231.

27. Majerus PW, Lastra RR. Fatty acid biosynthesis in human leukocytes. *J Clin Invest* 1967; 46: 1596-1602.