

Enzymatic Determination of Electrophoretically separated LDL-Cholesterol from Sera of Cardiac Patients

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Summary: The lipoproteins have been isolated electrophoretically from the blood samples of patients having suffered from type-III, hyperlipoproteinemia but survived of Myocardial infarction. The LDL fraction from the strip is cut and the cholesterol extracted. The cholesterol is determined using immobilized cholesterol esterase/oxidase column in a flow system.

Introduction

Estimation of cholesterol is highly significant in atherogenesis, the pathological deposition of lipids, particularly cholesterol and its esters in the vascular bed. Major portion of cholesterol (70%) is present in low density lipoproteins and hence is of great clinical significance [1].

Various methods are used for isolating lipoproteins in practicable quantities, these include,

chromatography [2], ultracentrifugation [3], precipitation [4] and some other semiautomated colorimetry [5]. Electrophoresis has been performed on various supporting media's [8,7]. The use of cellulose strips for the purpose offers the advantage of better resolution, shorter running time, the smaller samples size, and less trouble from adsorption of the lipoproteins on to the medium. In this paper we report the separation of lipoproteins on cellulose acetate strips. The LDL

band is cut from the strip and dissolved in aqueous solution with saline and surfactant. The samples are injected into a flow system containing the cholesterol esterase followed by cholesterol oxidase column for the estimation of cholesterol of the LDL fraction.

Results and Discussion

A control group of thirty men, matched for age and socio-economic status, were selected from the attendants of the patients. The blood samples were analysed for cholesterol (total LDL). The enzymatic method already established was used and the result compiled are given in Table-1, there is marked increase in the total and LDL cholesterol of patients. The data shows that the middle aged category is more susceptible to cardiac diseases.

In Table-2, the results of the patients with type III, hyperlipoproteinemia are enlisted. There is also marked increase in their total as well as LDL cholesterol showing their susceptibility to cardiac diseases.

Experimental

Reagents and apparatus

Cellulose acetate membrane, stain (Fat Red 7B) and electrophoresis complete unit were purchased from Gelman Sciences, USA. All reagents used were obtained from Sigma, unless otherwise stated. Double distilled/deionized water was used throughout the experiment.

Collection of the specimens (blood samples)

Fresh blood samples from over night fasted normal and patients of Myocardial infarction and type III, hyperlipoproteinemia were collected in the morning and centrifuged at $1500 \times g$ for 30 min. Plasma was separated in 1 mg/ml of EDTA containing tubes, stored at 4°C for no more than 24 hrs, and analysis performed. No history of the lipids in the diet of these patients was recorded.

Preparation of cellulose acetate membrane (CAM) strips and horizontal electrophoresis

Strips of cellulose acetate intended for the analysis of a single sample was 15×2.5 cm. The

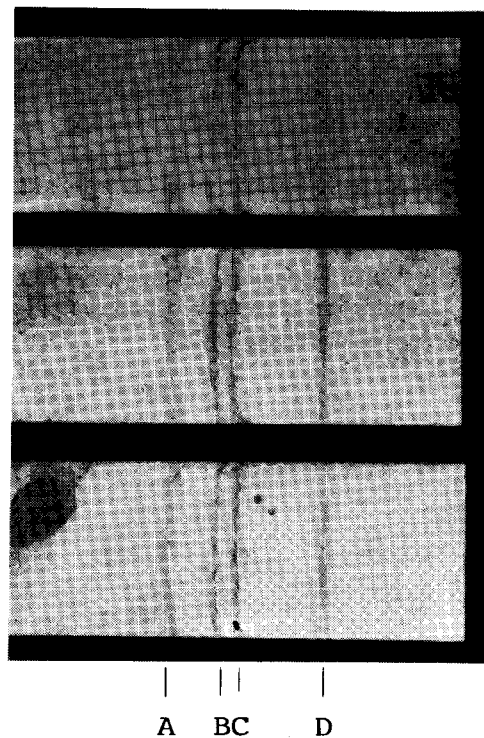
Table-1: Distribution of various lipoproteins in cases of cardiac patients (Myocardial infarction) and respective control groups according to age, (mg/dl of blood mean ± 7 SD)

Age group	Total cholesterol		LDL cholesterol	
	Cases	Controls	Cases	Controls
25 - 40	289 \pm 5.2	200 \pm 31.0	205 \pm 32.3	118 \pm 22.9
41 - 60	321 \pm 39.2	213 \pm 27.1	246 \pm 40.2	129 \pm 21.0
60 - 80	280 \pm 38.1	196 \pm 27.9	194 \pm 30.0	122 \pm 18.9

Table-2: LDL cholesterol values in sera of nine patients with type III, hyperlipoproteinemia, (mg/dl of whole blood)

Age years	Total cholesterol*		LDL-cholesterol*	
	Cases	Control	Cases	Control
43	331.96	207.28	219.63	150.00
38	320.02	202.31	214.00	148.07
32	319.22	202.30	214.30	147.77
63	326.06	209.28	217.00	153.00
58	324.06	204.30	216.00	152.07
37	330.06	211.27	219.24	149.00
39	320.00	200.30	215.50	152.00
70	318.90	200.31	213.00	146.99
68	328.00	200.39	217.53	146.09

*Mean of three readings.



A = HDL
C = LDL.
B = VLDL.
D = Chylomicron.

Photograph of lipoproteins separated on cellulose Acetate Membrane.

cathode side was marked for the application of the sample. The strips were marked at least 2 cm to the edge of the membrane. The membrane floated on fresh electrolytes and submerged completely and soaked for at least 5 min. The membrane removed with forceps and excess buffer blotted off with clean filter paper.

Plasma sample of 2 μ l/cm was uniformly applied to the mark made on damp strips, using the special applicator. After applying the sample the moistend strips with filter paper strips on both sides were positioned in the horizontal deluxe chamber which contained 250 ml of barbital buffer (0.05 M, pH 8.6) in each reservoir. Electrophoresis was started and a constant current of 0.5 mA/cm was applied. The analysis time of 40 min was sufficient enough to resolve lipoproteins. The strips were removed and floated on the surface of the fixation solution (5% trichloroacetic acid in 2.5% formaldehyde) for several seconds, then submerged completely and soaked for 20 min. Staining was done for 15 min by immersing the strips into the tray of working stain (Fat Red 7B), clear sharp bands appear on the CAM strips. The LDL band cut down and their aqueous solution with saline and surfactant were prepared by the method as applied to the cholesterol standard preparation [8]. Immobilization of enzymes and flow system used for the determination of cholesterol and cholesterol ester is described elsewhere [9].

Conclusion

The present study indicates that there is highly significant correlation of increased total cholesterol with an increase in LDL cholesterol concentration coupled with the risk of Coronary heart disease.

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References

1. A.H. Slyper, *J. Amer. Med. Assoc.*, **272**, 305 (1994).
2. L.J. Seman, J. L. Jenner, J.R. Mcnamara and E.J. Scheafer, *Clin. Chem.*, **40**, 400 (1994).
3. T.R. Red Grave and L.A. Carlson, *J. Lipid Res.*, **20**, 217 (1979).
4. H. Wieland and D.Siedel, *J. Lipid Res.*, **24**, 904 (1983).
5. H. Heckers, U. Gobel and U. Kleppel, *J. Internal Medicin*, **235**, 192 (1994).
6. R.P. Noble, *J. Lipid Res.*, **9**, 693 (1968).
7. H. Weiland and D. Siedel, *Hand Book of Electrophoresis*, Vol. 3, CRC Press, p. 83 (1983).
8. M. Masoom and A. Townshend, *Anal. Chim. Acta*, **174**, 293 (1985).
9. M. Masoom, in "Biochemistry for development", M.Atta-ur-Rahman ed., p. 55 (1988).