

Development of a method for the detection of glycated low density lipoprotein in serum

B.P.Galhena, M.I.Thabrew and O.P.K.S. Keerthisena Department
of Biochemistry and Clinical Chemistry, Faculty of Medicine,
University of Kelaniya

A.Chandresekera

Department of Oral Medicine, Faculty of Dental Sciences. University of Peradeniya

U.IIlangasekera

Department of Medicine, Faculty of Medicine, University of Peradeniya.

ABSTRACT

Complications of atherosclerosis are the leading cause of death in diabetic patients. Recent work on the pathogenesis of atherosclerosis implicates "oxidized" low density lipoprotein (LDL) as a key factor in the initiation of atherosclerotic lesions. Oxidized LDL is the product of two post-secretory modifications either direct in-vivo oxidation or LDL oxidation subsequent to glycation termed as glycooxidation.

It has been suggested that non-enzymatic glycation of apoprotein B (Apo B) could promote generation of "oxidized" LDL.

Previous estimation of glycated LDL was done by either (a) an ELISA technique based on a reaction between monoclonal antibody against carboxymethyllysine (CML) which is an end product formed during LDL glycooxidation or (b) agarose gel electrophoresis of LDL separated from serum by ion exchange chromatography, and staining with a lipoprotein specific stain. (Oil Red-O)

The objective of the present study was to determine if glycated LDL in whole serum could be identified by subjecting serum to agarose gel electrophoresis followed by Oil Red-O staining, thus avoiding the use of monoclonal antibody, which is very expensive to purchase or the separation of LDL by ultra centrifugation.

Serum was pooled under conditions from healthy individuals, 14 hours once informed consent was obtained. Pooled serum was divided into three equal aliquots and stored at - 20°C. Protein content was measured in serum (as previously described by Lowry et. al.), prior to in-vitro glycation. Subsequently, two serum aliquots were dialyzed separately in separate dialysis tubes with 50,000 Dalton pore size. During dialysis, one sample was subjected to in-vitro glycation while the second was kept as the control. In vitro glycation was performed by incubating the dialysis tube filled with serum, in phosphate buffered saline (PBS at pH7.4) containing 200mmol/l glucose for seven days at 37°C in the dark. The phosphate buffered saline that contained

200 mol/l HDTA to prevent auto-oxidation of lipoprotein was changed every 48 hours to minimize possible microbial growth. The control serum was incubated as above, but without glucose in the incubating medium. Subsequently serum electrophoresis was done in 5% agarose gel and stained with Oil Red-O according to the method proposed by Noble et. al. Three adjacent lanes on the gel were loaded with samples of. (1) Serum dialyzed against PBS + Glucose (2) Serum dialyzed against PBS only and (3) Undialyzed serum. Gel was run overnight, fixed for two hours, stained with Oil Red-O and the separated bands regions visualized by densitometry. LDL usually migrates in the β region.

The α and β bands in the dialyzed samples were identified in comparison with the respective bands in the undialyzed samples. An increased band thickness (both α and β) was observed in both glycated and controlled samples compared to the original sample, probably due to protein concentration during dialysis. With regard to β band of the glycated serum, there was a significant ($p < 0.05$) increase in migration ($R_f = 0.415$), compared to that of the control serum ($R_f = 0.304$). It can therefore be concluded that agarose gel electrophoresis followed by Oil Red-O staining can be used for detection of glycated LDL in whole serum.