Clinica Chimica Acta 442 (2015) 130–135

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Determination of serum lipoprotein lipase using a latex particle-enhanced turbidimetric immunoassay with an automated analyzer

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ARTICLE INFO

Article history:
Received 19 December 2014
Received in revised form 18 January 2015
Accepted 20 January 2015
Available online 26 January 2015

Keywords:
Lipoprotein lipase (LPL)
TG-rich lipoproteins
Latex particle-enhanced turbidimetric immunoassay
Automated analyzer
ELISA

ABSTRACT

Background: Lipoprotein lipase (LPL) plays a central role in triglyceride-rich lipoprotein metabolism by catalyzing the hydrolysis of triglycerides. Quantification of serum LPL is useful for diagnosing lipid disorders, but there is no rapid method of measuring LPL for clinical use.
Methods: We developed a rapid and sensitive latex particle-enhanced turbidimetric immunoassay (LTIA) serum LPL using latex bead-immobilized anti-LPL monoclonal antibodies. The assay was performed on a Hitachi 7700 P analyzer and evaluated for its validity as a method of quantitating the serum LPL concentration in parallel with ELISA.
Results: Dilution tests using LTIA produced a calibration curve from 0.5 to 800 ng/ml. Within-run CV was obtained in the range of 2.2–5.5%. No interference was observed in the testing of specimens containing potentially interfering substances such as bilirubin-F and C, hemoglobin, triglycerides and rheumatoid factor. A strong correlation between LTIA and ELISA was confirmed ($n = 40$, $r = 0.967$, $y=0.99x-1.86$). The normal range of LPL in pre-heparin serum was 50–77 ng/ml and in post-heparin plasma 354–410 ng/ml, respectively.
Conclusion: The LTIA assay is applicable in quantitating the concentration of LPL in both pre-heparin serum and post-heparin plasma. This assay is more convenient and faster than ELISA and highly suitable for clinical routine analysis.

1. Introduction

Lipoprotein lipase (LPL) plays a major role in the metabolism and transport of lipids and lipoproteins [1,2]. It is the enzyme responsible for the hydrolysis of core triglycerides (TG) in chylomicrons (CM) and very low density lipoproteins (VLDL), producing CM remnants and VLDL remnants, respectively. Determination of LPL in plasma has typically been routinely carried out by ELISA after the intravenous injection of heparin (with its activity and concentration). However, it is also known that a comparatively high LPL concentration (ranging approximately 30–100 ng/ml in normal controls) is found in the pre-heparin serum, with an undetectable level of LPL activity, indicating that the majority of circulating LPL is catalytically inactive, but still a ligand for the receptors [3–6].

The LPL concentration and activity in the post-heparin plasma have been clinically used for the detection of LPL deficiency [2], but in general not for the diagnosis of lipid disorders or the risk of cardiovascular disease. This is because heparin injection dissociates LPL from the blood vessel endothelium, so the result does not necessarily reflect the physiological or pathophysiological concentration of circulating LPL.

An LPL-ELISA assay using specific monoclonal antibodies was reportedly developed previously by Brunzell et al. [7] and Ikeda et al. [8] for the detection of LPL in human plasma, which involved the administration of a heparin injection to the patients before the measurement of the plasma LPL concentration. Considering the assay time and the technical steps required for the quantitative measurement by ELISA, this method is not suitable for large-scale epidemiological studies or routine clinical laboratory assay.

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http://dx.doi.org/10.1016/j.cca.2015.01.016
0009-8981/

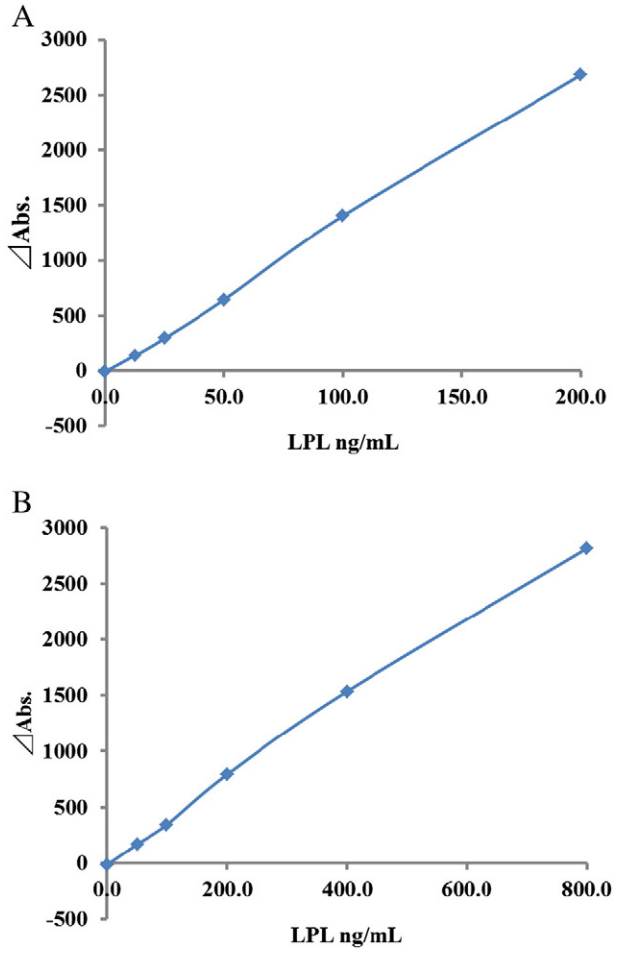


Fig. 1. Linearity of LTIA. Linearity tests of LPL were performed using LTIA on a H7700 P analyzer. Two calibrators for the LPL concentrations are shown for the pre-heparin serum (Fig. 1A) and post-heparin plasma (Fig. 1B).

Therefore, there remains a need for a reliable, rapid and automated assay for the LPL concentration that has both good sensitivity and good calibrator stability, in particular if the measurement of the pre-heparin serum LPL concentration is going to be clinically meaningful and useful. LPL concentrations in the pre-heparin serum have been intensively investigated by Shirai and his colleagues the last decades using LPL-ELISA, revealing the clinical significance of the pre-heparin LPL concentration in cardiovascular and diabetic diseases [9–16].

We recently showed the possibility that the LPL concentration in the pre-heparin serum is replaceable with the LPL activity in the post-heparin plasma based on a comparison between them [17]. Therefore, the measurement of the LPL concentration in the pre-heparin serum will be able to provide more practical clinical applications in TG-rich patients without the need of a heparin injection using an automated LPL assay. As the serum pre-heparin LPL concentration is sufficiently high so as to measure it with a latex assay system, we developed a rapid and sensitive latex particle-enhanced turbidimetric immunoassay (LTIA) using latex bead-immobilized LPL-specific antibodies. The performance of the LTIA was evaluated on a Hitachi H7700 P automated analyzer. We compared its analytical properties with a commercially available ELISA assay [18] in normal volunteers, with and without heparin injection.

Table 1
Within-assay precision.

	Low 32.0	Mid 100.0	High 280.0
1	28.6	93.5	279.4
2	29.7	97.5	280.4
3	30.4	97.6	291.2
4	31.7	97.7	286.1
5	30.7	93.4	296.2
6	28.8	91.5	293.6
7	29.7	95.6	293.2
8	27.3	98.3	293.2
9	26.8	96.3	284.6
10	27.5	97.2	295.8
Mean (ng/ml)	29.1	95.9	289.4
S.D. (ng/ml)	1.60	2.30	6.25
CV (%)	5.5	2.4	2.2

2. Materials and methods

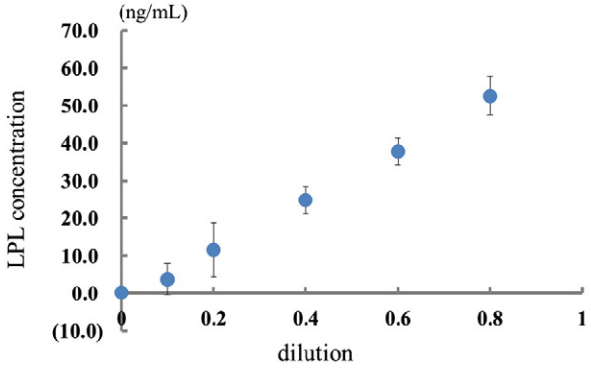
2.1. Reagents

Polystyrene latex particles were obtained from Fujikura Corporation and bovine serum albumin (BSA) from Sigma, respectively. Interfering reagents, containing bilirubin F and C, hemoglobin, triglycerides and rheumatoid factor, were from Sysmex. All of the chemicals and reagents were of the highest available grade.

2.2. Preparation of blood samples

The study was conducted in relatively healthy young volunteers (some were overweight or obese) in a male (n = 19) and female (n = 21) population (Caucasian 25, Asian 5, Hispanic 4, African American 3, others 3) with a median age of 24 years and BMI of 24 at the University of California, Davis, USA. All of the volunteers were injected with heparin (50 unit/kg BW) for the LPL activity assays [19]. The University of California at Davis Institutional Review Board approved the experimental protocol and the subjects provided written informed consent to participate in the study. Two hundred forty healthy volunteers (male = 170, female = 70, median age of 26 years and median BMI of 21.6) were recruited at Tenshi College (Sapporo, Japan), with obtained written informed consent and University Ethical Committee approval [20].

The prepared serum was used for the experiments on precision, sensitivity, cross-reactivity, dilution and recovery as well as the normal range of healthy controls.



Dilution	0	0.1	0.2	0.4	0.6	0.8
Theoritical value (ng/mL)	0.0	5.4	10.7	21.4	32.2	53.6
S.D. (ng/mL)	0.35	1.64	2.77	1.42	1.39	2.01
2.6SD (ng/mL)	0.92	4.25	7.20	3.69	3.62	5.23
average (ng/ml)	0.12	3.69	11.55	24.81	37.79	52.66

Fig. 2. Analytical detection limit estimated as the concentration equal to the mean absorbance of 10 replicates of the zero calibrator plus 2.6.