人類血清中過度糖化最終產物快速檢驗方法的研發

Development of a Rapid Assay for Advanced Glycosylation End Products in Human Serum

中文摘要

蛋白質的氨基和還原糖類反應，並經過一系列的修飾作用，可產生帶有螢光，無法復原(irreversible)的黃褐色物質，稱之為過度糖化最終產物(Advanced Glycosylation End Products, AGEs)。AGEs 大量累積於體內組織中，是引發糖尿病併發症主要因素之一。血清中低分子量的 AGEs 和糖尿病併發症關係密切，但臨床上尚無方便而快速的方法可用來檢驗血清中的 AGEs。本研究以 RNase 和高濃度 Glucose 產生 RNase- AGEs，再用以生產特異性的抗 AGEs 抗體，並用此一抗體製備抗 AGEs 抗體披覆之乳膠而研發一種免疫混濁分析法來定量血清中 AGEs 濃度的檢驗法。本分析使用單一試劑，可直接在生化自動分析儀(如 Olympus, AU-600) 測定血清中過度糖化最終產物的濃度，本分析法之校正曲線成線性(R^2 =0.9953)，精密度良好(within run C.V=1.8%~2.9%; between run C.V=4.5~6.1%)，回收率(recovery rate)達 98.4%。我們分析了健康成年人(4.6 ± 1.5, n=39)，健康老年人(4.9 ± 1.4, n=40)，糖尿病病人(7.8 ± 4.8, n=89)，糖尿病併發末期腎臟病病人(16.3 ± 6.0, n=12)，末期腎臟病病人(17.2 ± 7.7, n=28)的血清。溶血、黃疸、膽固醇(<320 mg/dl)、三酸甘油脂(<500mg/dl)不會干擾測試。此種方法可以運用於臨床常規檢驗。

英文摘要

Non-enzymatic reaction of protein and carbohydrate may produce a series of brown fluorescent advanced glycosylation end products(AGEs), AGEs accumulation in tissue have been linked to diabetic complications.

However, convenient and rapid assay for serum LMW-AGE is currently unavailable. In the present study, we produced RNase-AGEs by incubating RNase with high concentration of glucose, The RNase-derived AGEs were then used to immunize rabbit and produce AGEs-specific polyclonal antibodies. We used the anti-AGEs antibodies to coated latex and used it to develop an immunoturbidimetry, which can be use in autoanalyzer(eg. Olympus, AU-600). By the calibration curve(R^2 =0.9953), recovery rate(98.4 %), The precision was better than those in competitive ELISA,

with a within day of C.V=2.9 %; between day of C.V=6.1 %. The interference was verified by hemolysis, icterus, lipemic serum(cholesterol <320 mg/dl; triglyceride<500mg/dl) not interfered respectively. We are currently

using this assay to measure serum LMW-AGEs in normal individuals(young 4.6±1.5, n=39; elderly 4.9±1.4, n=40), diabetic patients(7.8±4.8, n=89) and patients with end stage renal disease without(17.2±7.7, n=28) or with DM(16.3±6.0, n=12). The assay will be proven useful in predict the diabetic complications in diabetes.