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Immunochemical detection of glycated lens crystallins and their circulating auto-antibodies in human serum during aging

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The aim of this investigation was to use lens specific glycated crystallins as an immunogen to detect (i) human glycated crystallins and (ii) circulating auto-antibodies to glycated crystallins in human serum during aging. Polyclonal antibodies were produced against human total lens proteins (40-80 years) in rabbits. The specificity of the antibodies produced were determined by antibody capture assay, using the purified human lens (HMW+α, HMW+α-glycated, β-, β-glycated γ- and γ-glycated) crystallins as antigens. The cross-reactivity of these lens specific antibodies against rat β-, β-glycated, γ- and γ-glycated lens crystallins was also analyzed. A non-competitive ELISA methodology was developed for the detection of circulating lens crystallins in human sera, using HMW+α, HMW+α-glycated, β- and β-glycated crystallins from human and rat γ-, and γ-glycated crystallins as immobilized antigens. Further, these polyclonal antibodies were able to detect both natural and *in vitro* glycated crystallins, their IC_{50} values were: Human total lens protein (55 ng), HMW+ α (16.45 ng), HMW+ α-glycated (273 ng), β- (37.82 ng), β-glycated (260 ng), γ- (105.34 ng) and γ-glycated (313 ng). The immunochemical analysis of human serum indicated a significant change ($p<0.001$) in the levels of circulating β-glycated and γ-glycated crystallins in the age group of 40-80 years with respect to their control groups. However, there was no consistent significant change in the levels of HMW+ α-glycated crystallins in the age group of 40-80 years with their respective controls. Notably, the levels of serum β-glycated crystallins was found to be 3 folds higher than that of HMW+ α-glycated and β-glycated crystallins in the age group of 70-80 years. Circulating auto-antibodies to HMW+ α-, β- and β-glycated crystallins were detected in the serum of both apparently normal and cataract patients in the age group of 40-80 years by antibody capture assay. The levels of these auto-antibodies were significantly higher ($p<0.05$ & <0.001) at every time point with their respective controls. Auto-antibodies to γ-gly crystallin was found to be 2 and 3.2 fold higher as compared to the levels of auto-antibodies to γ-gly and HMW+γ-gly crystallins, respectively. During the course of aging, leakage of lens crystallins (HMW+α, HMW+ α-glycated, β-, β-glycated γ- and γ-glycated) elicit an immune response resulting in the formation of auto-antibodies in cataract patients (40-80 years) as compared to age matched controls. For the very first time these specifically designed polyclonal antibodies to lens specific glycated crystallins were able to detect the early leakage of glycated crystallins. This immunochemical method reported may find an application for the early detection of cataract.

Biography

Mala Ranjan has completed her PhD at the age of 46 years from Osmania University and M.Phil in the School of Biological Sciences, University of Wales, Swansea, U.K. Her thrust area of research is application of advanced glycation end products (AGEs) as clinical Biomarkers. She is focus towards the Diabetic complications associated problems. Presently she is working on major project (Biological monitoring of advanced glycated end products of protein in Diabetic Neuropathy: Prospective role of antiglycating dietary agents) funded by University Grants Commission, New Delhi, India. She has published 12 papers in reputed journals. She is the currently working as Professor of Biochemistry in the prestigious organization (St.Francis College for Women) of Hyderabad, India.

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