BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF HEMOGLOBIN - ADVANCED GLYCATION END PRODUCT AND ITS RELEVANCE TO DIABETES MELLITUS

by

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CERTIFICATE

This is to certify that the thesis entitled “Biochemical and Biophysical Characterization of Hemoglobin - Advanced Glycation End Product and its Relevance to Diabetes mellitus” being submitted by Mr. Vigneshwaran, N., to the Indian Institute of Technology Delhi, India for the award of the “Doctor of Philosophy in Biomedical Engineering”, is a bonafide research work carried out by him. He has worked under our guidance and supervision.

To the best of our knowledge the thesis has reached the requisite standard. The results presented in this thesis have not been submitted in part or full to any other university or institution for award of degree or diploma.

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ABSTRACT

Diabetes mellitus is a metabolic disorder that is characterized by an elevation of fasting plasma glucose level caused by a relative or absolute deficiency in the hormone, insulin. Measurement of glycated hemoglobin is widely used for routine monitoring of long-term glycemic status in patients with diabetes mellitus. The approved reference method for the measurement of HbA1c in the human blood was given by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Inspite of the high accuracy and less inter-laboratory variation in these methods, routine analysis becomes laborious for large number of samples apart from use of sophisticated instruments. The recent suggestion is that Hb-AGE measurement may provide a better index of long-term tissue modification by advanced glycation end products.

The Hb-AGE accounts for 0.42% of the circulating hemoglobin in normal human beings while in hyperglycemic patients, this value is raised to 0.75%. But, the routine immunological analysis of Hb-AGE is a tedious and time-consuming process. A simple and easy approach is that of, the intrinsic molecular fluorescence nature of Hb-AGE was established by this research work. Hb-AGE was purified from the in vitro glycated hemoglobin in immunoaffinity column using monoclonal antibody which had been immobilized on cyanogen bromide activated Sepharose as a matrix. Competitive ELISA method was standardized using the monoclonal antibody for further quantification of Hb-AGE. The autofluorescence nature of Hb-AGE was observed with the excitation/emission wavelengths of 308/345 nm, which was completely absent in both HbA1c and Hb. The relative quantum yield of Hb-AGE was calculated to be 0.19 using
quinine sulfate as the standard. The fluorescence polarization study supported the specificity of monoclonal antibody towards Hb-AGE.

The isoelectric point (pI) of Hb-AGE was found to be lower than other two fractions (Hb and HbA1c) demonstrating the extended glycation over a period of four months. Hb-AGE analysis by MALDI-TOF-MS showed an increased mass of 618 Da in glycated β chain while the HbA1c fraction showed only an increase of 326 Da when compared to their respective β chains. The thiobarbituric acid assay and thermodynamic analysis were carried out to differentiate HbA1c and Hb-AGE.

A mathematical model was developed to simulate the formation of Hb-AGE under in vitro condition using fluorescence analysis and validated with the results obtained from the in vivo study. Both in vivo and clinical studies supported the formations of autofluorescent adduct, Hb-AGE during the sustained hyperglycemic conditions.

A growing body of epidemiological evidence indicates "epidemic" of type 2 diabetes globally. A reliable long-term monitoring of glycemia using the Hb-AGE as a marker will reduce management errors and hospital visits of the patients. It opens up the scope for research in clinical subjects and development of biosensors based on autofluorescence or difference in molecular mass to detect Hb-AGE as a long term monitoring index for diabetes mellitus.
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