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A novel method for identification of hemoglobin variants by modified sample preparation followed by LC coupled with MALDI MS/MS

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Around 7% of the global population carries an abnormal hemoglobin gene. Over 330,000 infants are born annually with hemoglobinopathies and it is the major cause of morbidity and mortality in early childhood. The treatments rely heavily on the diagnosis of hemoglobin variants. The routine/conventional techniques used for the identification of mutation in hemoglobin variants have their own limitations like co-migration of variants in electrophoresis and co-elution in HPLC. The WHO (2002) report on Genomics and Health has emphasized on the development of precise molecular techniques for screening of hemoglobin disorders. A sensitive, robust and reproducible method was thus developed to identify single substitution mutations in the hemoglobin disorders from sequence of the entire globin chains. The method was MS compatible and dealt with certain limitations like difficulty in getting complete sequence coverage. Different methods like using organic solvent, digestion with a different protease and combining results, treating the digestion mixture with 10% acetonitrile prior to incubation and combining the separation power of LC coupled with MALDI MS/MS were tried for standardization and optimization of protocol. Finally, we optimized a method using an organic solvent and heat denaturation step prior to digestion resulting in 100% sequence coverage in the β chains and 95% sequence coverage in the α chains. A hemoglobin variant database was created to specify the search and reduce the search time. All the mutations were thus identified using a non-targeted approach and this method could be used in future for regular screening of any single mutation in hemoglobin variants.

Biography

Pushpanjali Dasauni is pursuing her PhD from University of Delhi South Campus, New Delhi, India and will submit the same by this year-end. She is expert in most of the proteomics tools like MALDI TOF-TOF and 2D-DIGE and works with various biophysical tools like FTIR etc. She is developing novel diagnostics tools for fast, cost-effective and accurate detection of hemoglobin disorders. She has published papers in reputed journals and she is a Life Time Member of Protein Society, India.

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