RESEARCH BRIEF

Heparin Co-factor II Thrombin Complex as a Biomarker for Mucopolysaccharidosis: Indian Experience

SANJEEV KUMAR PANDEY, ANKUR SINGH, AP DUBEY, *TK MISHRA AND SEEMA KAPOOR

*From Departments of Pediatrics and *Biochemistry, MAMC and Associated Lok Nayak hospital, New Delhi, India.*

Correspondence to:
Dr Seema Kapoor,
M-439, Ground Floor,
Guruharkishan Nagar,
Paschim Vihar, New Delhi, India.
drseemakapoor@gmail.com
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Background: Serum heparin cofactor II-thrombin complex (HCII-T) is an emerging biomarker for mucopolysaccharidosis disease (MPS I and MPS II). **Methods:** Seventeen cases (6 MPS I and 11 MPS II) and sixty healthy controls were enrolled in study, conducted from September 2008 to December 2012. The mean ± SD age of MPS1 (n=6, 5 males) and MPS II was 7.02 ± 3.25 and 5.2 ± 2.15 years, respectively. Disease status was confirmed by clinical features and enzyme assay. Urinary glycosaminoglycans were measured in spot urine samples and expressed in relation to creatinine content. HCIIT measurement was done using sandwich ELISA at enrolment and after 12 and 24 months of recruitment. **Results:** Urinary glycosaminoglycans and HCIIT were elevated in all patients compared to their healthy controls. Both markers could not discriminate between the type of mucopolysaccharidosis. **Conclusion:** Heparin Cofactor II Thrombin Complex is a good biomarker for mucopolysaccharidosis I and II.

Keywords: *Diagnosis, Glucosamino-glycans, Mucopolysaccharidosis, Screening*

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The mucopolysaccharidosis (MPS) are progressive, multisystemic disorders that are caused by genetic defects in the catabolism of glycosaminoglycans (GAG) [1,2]. Considering the complexity of the disease and the advent of enzyme replacement therapy for both these disorders [3-5], there exists a need for a robust biomarker. Serum Heparin Cofactor II Thrombin has emerged as a biomarker for certain subtypes of MPS. Considering the scarcity of literature and lack of Indian studies, we studied its role in MPS l and MPS II [6].

METHODS

After approval from Institute Ethical Committee, the study was conducted from September 2008 to December 2012. Seventeen cases (6 MPS I and 11 MPS II) and 60 healthy controls were enrolled in the study after getting informed consent. Disease status was determined based on clinical presentation and enzyme assay on dried blood spot [7,8]. Blood samples were collected with sterile technique from both cases and controls and serum was separated and stored at –80 degree Celsius till analysis. Urinary glycosaminoglycans were estimated in early morning samples, as 24-hour collection is difficult in pediatric age group and more so in neurologically impaired children. Prior to this, normative age stratified data had been established in the laboratory for our population. The estimation was done by using Dimethylmethylene blue as the binding reporter dye [9]. HCII T was measured using ELISA method from commercially available kits (Affinity Biologicals, Hamilton, ONUS). Both urine and blood samples were collected thrice; at start of enrolment, one year after enrolment, and two years after enrolment.

RESULTS

The mean (SD) age of MPS I (*n*=6, 5 males) and MPS II (*n*=11, all males) patients was 7.02 (3.25) and 5.2 (2.15) years, respectively. Spot urinary GAG were measured and were stratified age-wise. This was the first investigation used in a case suspected with MPS. Urinary GAG were markedly elevated in all patients with no significant change in between MPS I and MPS II. The median (interquartile range) HCII T in MPS I and MPS II was 455 (350-500) ng/mL and 340 (265- 530) ng/mL, respectively, as compared to 39 (12-60) ng/mL in normal children. No significant difference was observed in levels of HCII-T over the next 2 years (***Fig*.1**). One patient who had access to therapy showed decline in values with follow up: at start of therapy (330 ng/mL), 18 months (215 ng/mL), and at 28 months (135 ng/mL) after therapy.

DISCUSSION

(HCII T) and urinary glycosaminoglycans were elevated

in both MPS I and MPS ll patients as compared to controls at the beginning of enrolment. Follow up levels of these two biomarkers at end of one year and two year showed consistently high levels from previous ones, highlighting the increase in disease burden status. Both these biomarkers were unable to predict the type of MPS.

Glycosaminoglycans are widely used primary biomarkers in MPS but have certain limitations. Estimation of GAG depends widely on age, hydration and kidney status of affected cases. There is also a wide range of normalcy in infancy. Another drawback is the need for 24-hour urinary collection, which is difficult in uncooperative children. Advantages of HCII-T include: one time collection of blood, estimation independent of renal function, and less time consuming. Likely disadvantage with HCII-T include: invasive procedure, expensive and inability to differentiate among MPS I, MPS II and MPS VI as it is elevated in all conditions with accumulation of dermatan sulphate.

We found a significant difference in the levels of HCII-T found in healthy controls with compared to the disease group, as reported previously [6, 11-13].

Regarding ability to adequately predict the course of disease, we found increase in level of both biomarkers in both group of patients without any significance. The plausible reason for this could have been the short duration of follow up or inclusion of attenuated phenotype, which may demonstrate slow progression and wide variability in the course of disease. We found a fall in both urinary biomarker (glycosaminoglycans) and blood biomarker (HCII-T) values in one patient at end of 18 and 28 months of initation of therapy, it is difficult to derive any conclusion regarding comparative efficacy of the biomarkers from this single case.

To conclude, HCII-T is a reliable screening biomarker for MPS. However, considering the limitation to differentiate between subtypes and to predict the course in attenuated phenotypes, we need to move from organ- or pathway-specific biomarkers to a panel of biomarkers to correlate with prediction, typification, disease progression and response to therapy.

Contributors: SKP: was involved in planning the study and drafting the manuscript; AS: was involved in case enrolment and follow up of cases; APD: guidance at various satges of study; TKM: was involved in helping and guiding the laboratory work; and SK: critically reviewed the manuscript, made the diagnoses and will act as guarantor for the manuscript.
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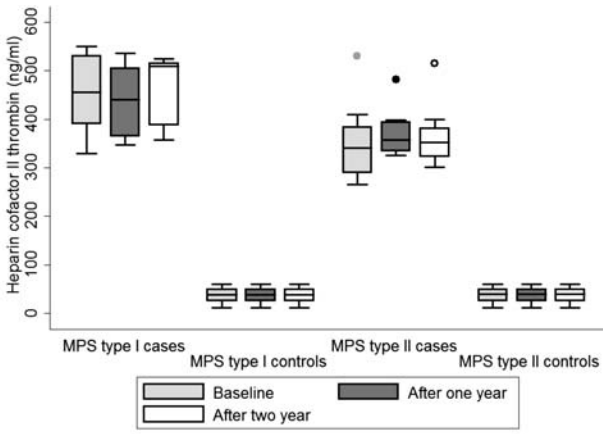

FIG.1 *Box plot showing Heparin Cofactor II Thrombin levels in controls and MPS Type I and MPS Type II patients at 1st visit (Baseline) and at different time intervals.*

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WHAT THIS STUDY ADDS?

- Heparin Cofactor II Thrombin Complex is a good biomarker for Mucopolysaccharidosis I and II.

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