## CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1-9</td>
</tr>
<tr>
<td>2</td>
<td>REVIEW OF LITERATURE</td>
<td>10-39</td>
</tr>
<tr>
<td>3</td>
<td>MATERIALS AND METHODS</td>
<td>40-63</td>
</tr>
<tr>
<td>4</td>
<td>RESULTS</td>
<td>64-84</td>
</tr>
<tr>
<td>5</td>
<td>DISCUSSION</td>
<td>85-99</td>
</tr>
</tbody>
</table>

**SUMMARY, CONCLUSIONS**

100-101

**FUTUROLOGY**

102

**BIBLIOGRAPHY**
Chapter 1. INTRODUCTION
1.1 Inborn Errors of Metabolism
1.2 Lysosomal storage disorders
1.3 Mucopolysaccharidoses
1.4 Biochemistry of glycosaminoglycans
1.5 Urinary excretion of glycosaminoglycans
1.6 Biochemical diagnosis of MPS
1.7 Molecular diagnosis of MPS
1.8 Therapy
1.9 Incidence of Mucopolysaccharidoses
1.9.1 Incidence of MPS in India
1.10 Objectives of the present study

Chapter 2. REVIEW OF LITERATURE
2.1 History of Mucopolysaccharidoses
2.2 Mucopolysaccharidosis (clinical features)
2.2.1 Mucopolysaccharidosis IH (Hurler syndrome)
2.2.2 Mucopolysaccharidosis IS (Scheie syndrome)
2.2.3 Mucopolysaccharidosis I H/S (Hurler – Scheie syndrome)
2.3 Degradation of glycosaminoglycans
2.3.1 Degradation of heparan sulfate
2.3.2 Degradation of dermatan sulfate
2.4 α-L-Iduronidase
2.5 Localization of α-L-Iduronidase gene (IDUA)
2.5.1 The gene structure
2.5.2 Mutations identified
2.5.2.1 Chain termination mutation
2.5.2.2 Splice site mutation
2.5.2.3 Insertion / Deletion mutation
2.5.3 Population studies
2.5.3.1 Druze and Arabs
2.5.3.2 Asian-Indian
2.5.3.3 Japanese
2.5.3.4 Others
2.5.4 Mutation frequencies in different populations
2.5.5 Polymorphisms and nonpathogenic sequence variations in the gene
2.5.6 Genotype – Phenotype correlation
2.6 Prenatal diagnosis
2.7 Carrier detection
2.8 Animal Models
2.8.1 Canine Model
2.8.2 Murine Model
2.9 Therapy
2.9.1 Bone Marrow Transplantation
2.9.2 Enzyme Replacement Therapy
2.9.3 Gene Therapy
2.9.4 Others

Chapter 3. MATERIALS AND METHODS
3.1 Study Group
3.2 Collection of clinical data
3.3 Materials
3.4 Instruments used
3.5 Methods
3.5.1 Urinary GAG analysis
3.5.1.1 Collection of urine samples
3.5.1.2 Isolation of urinary GAGs
3.5.1.3 Quantitation of isolated urinary GAGs
3.5.1.4 Estimation of urinary creatinine
3.5.1.5 Discontinuous cellulose acetate membrane electrophoresis
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5.1.6</td>
<td>Sequential multisolvent thin layer chromatography</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Lysosomal enzyme assays</td>
</tr>
<tr>
<td>3.5.2.1</td>
<td>Collection of blood samples</td>
</tr>
<tr>
<td>3.5.2.2</td>
<td>Isolation of leukocytes</td>
</tr>
<tr>
<td>3.5.2.3</td>
<td>Sonication of leukocytes</td>
</tr>
<tr>
<td>3.5.2.4</td>
<td>Protein Estimation</td>
</tr>
<tr>
<td>3.5.2.5</td>
<td>Assay of alpha L-iduronidase</td>
</tr>
<tr>
<td>3.5.2.6</td>
<td>Assay of GALNS</td>
</tr>
<tr>
<td>3.5.2.7</td>
<td>Assay of beta-galactosidase</td>
</tr>
<tr>
<td>3.5.2.8</td>
<td>Assay of arylsulfatase B</td>
</tr>
<tr>
<td>3.5.2.9</td>
<td>Assay of arylsulfatase A</td>
</tr>
<tr>
<td>3.5.2.10</td>
<td>Assay of beta-D-glucuronidase</td>
</tr>
<tr>
<td>3.5.2.11</td>
<td>Assay of acid phosphatase</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Mutational analysis</td>
</tr>
<tr>
<td>3.5.3.1</td>
<td>DNA isolation and quantitation</td>
</tr>
<tr>
<td>3.5.3.2</td>
<td>PCR amplification of IDUA gene</td>
</tr>
<tr>
<td>3.5.3.3</td>
<td>Agarose gel electrophoresis</td>
</tr>
<tr>
<td>3.5.3.4</td>
<td>Polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>3.5.3.5</td>
<td>Silver staining</td>
</tr>
<tr>
<td>3.5.4</td>
<td>Restriction digestion</td>
</tr>
<tr>
<td>3.5.5</td>
<td>Direct sequencing of PCR products</td>
</tr>
<tr>
<td>3.6</td>
<td>Statistical analysis</td>
</tr>
</tbody>
</table>

**Chapter 4. RESULTS**

4.1 Introduction
4.2 Biochemical studies
4.2.1 Quantitation of urinary GAGs
4.2.1.1 Age dependent GAGs excretion
4.2.1.2 Sex dependent GAGs excretion
4.2.2 Differential diagnosis
4.2.2.1 Discontinuous cellulose acetate membrane electrophoresis
4.2.2.2 Multisolvent sequential thin layer chromatography
4.2.3 Lysosomal enzyme assays
4.2.3.1 Estimation of group I and group II lysosomal enzymes in control children and adults
4.2.3.2 Assay of five lysosomal enzymes in group I patients
4.2.3.3 Assay of three lysosomal enzymes in group II patients
4.2.4 Reanalysis of urinary GAG values after definitive diagnosis
4.3 Carrier detection
4.3.1 IDUA activity in MPS I families
4.3.2 Arylsulfatase B activity in MPS VI families
4.4 MPS and consanguinity
4.5 MPS and community
4.6 Mutational analysis
4.6.1 PCR amplification of IDUA gene
4.6.2 PAGE and SSCP analysis of the PCR amplified exons of IDUA gene
4.6.2.1 Exon II
4.6.2.2 Exons V and VI
4.6.2.3 Exon VII
4.6.2.4 Exon VIII
4.6.2.5 Exon XIII and XIV
4.6.2.6 Mutational analysis of exon IX
4.6.2.6.1 PCR amplification and SSCP of exon IX
4.6.2.6.2 DNA sequence analysis of exon IX
4.6.2.6.3 Confirmation of band shifts in controls
4.6.2.7 Mutational analysis of exon X
4.6.2.7.1 PCR amplification and SSCP of exon X
4.6.2.7.2 DNA sequence analysis of exon X
4.6.2.7.3 Restriction digestion of exon X
Chapter 5. DISCUSSION

5.1 Biochemical studies
5.1.1 Urinary GAG analysis
5.1.2 Collection of urine samples
5.1.3 Quantitative analysis of GAGs
5.1.4 Qualitative analysis of GAGs
5.1.5 Lysosomal enzyme assays
5.1.5.1 Lysosomal enzyme assay in group I patients
5.1.5.2 Lysosomal enzyme assay in group II patients
5.2 Carrier detection
5.2.1 Carrier detection in MPS I families
5.2.2 Carrier detection in MPS VI families
5.3 Consanguinity and MPS
5.4 Mutational analysis
5.4.1 Methodology
5.4.2 Mutational studies
5.4.2.1 Exon IX
5.4.2.2 Exon X

SUMMARY AND CONCLUSION

FUTUROLOGY

BIBLIOGRAPHY