# Table of Contents

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chapter 1.0</strong> INTRODUCTION</td>
<td>1-3</td>
</tr>
<tr>
<td><strong>Chapter 2.0</strong> REVIEW OF LITERATURE</td>
<td>4-51</td>
</tr>
<tr>
<td>2.1 DIABETES MELLITUS – AN OVERVIEW</td>
<td>4</td>
</tr>
<tr>
<td>2.2 CLASSIFICATION OF DIABETES MELLITUS</td>
<td>5</td>
</tr>
<tr>
<td>2.2.1 Type 1 Diabetes Mellitus (T1DM)</td>
<td></td>
</tr>
<tr>
<td>2.2.2 Type 2 Diabetes Mellitus (T2DM)</td>
<td></td>
</tr>
<tr>
<td>2.2.3 Gestational Diabetes Mellitus (GDM)</td>
<td></td>
</tr>
<tr>
<td>2.2.4 Other Specific Types of Diabetes</td>
<td></td>
</tr>
<tr>
<td>2.3 DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS</td>
<td>8</td>
</tr>
<tr>
<td>2.4 GLOBAL PREVALENCE OF TYPE 2 DIABETES</td>
<td>8</td>
</tr>
<tr>
<td>2.5 EPIDEMIOLOGY OF TYPE 2 DIABETES – AN INDIAN SCENARIO</td>
<td>9</td>
</tr>
<tr>
<td>2.6 ECONOMIC IMPACT OF DIABETES IN INDIA</td>
<td>14</td>
</tr>
<tr>
<td>2.7 DIABETES RELATED COMPLICATIONS</td>
<td>15</td>
</tr>
<tr>
<td>2.7.1 Macrovascular complications</td>
<td></td>
</tr>
<tr>
<td>2.7.2 Microvascular complications</td>
<td></td>
</tr>
<tr>
<td>2.8 GENETIC BASIS OF T2DM: CLASSICAL EVIDENCES</td>
<td>18</td>
</tr>
<tr>
<td>2.8.1 Prevalence of T2DM in different ethnic groups</td>
<td></td>
</tr>
<tr>
<td>2.8.2 Familial aggregation</td>
<td></td>
</tr>
<tr>
<td>2.8.3 Twin studies</td>
<td></td>
</tr>
<tr>
<td>2.8.4 Heritability of intermediate phenotypes</td>
<td></td>
</tr>
<tr>
<td>2.9 ENVIRONMENTAL RISK FACTORS ASSOCIATED WITH T2DM</td>
<td>19</td>
</tr>
<tr>
<td>2.9.1 Age at onset of T2DM</td>
<td></td>
</tr>
<tr>
<td>2.9.2 Obesity and abdominal adiposity</td>
<td></td>
</tr>
<tr>
<td>2.9.3 Dietary habits</td>
<td></td>
</tr>
<tr>
<td>2.9.4 Physical inactivity</td>
<td></td>
</tr>
<tr>
<td>2.9.5 Migration and stress</td>
<td></td>
</tr>
<tr>
<td>2.9.6 Smoking</td>
<td></td>
</tr>
<tr>
<td>2.9.7 Intrauterine growth retardation ('thrifty phenotype' hypothesis)</td>
<td></td>
</tr>
</tbody>
</table>
2.9.8 Genetic susceptibility ('thrifty genes' hypothesis)
2.9.9 Dyslipidemia
2.9.10 Hypertension

2.10 PATHOPHYSIOLOGY OF TYPE 2 DIABETES

2.11 CELLULAR MECHANISMS OF INSULIN RESISTANCE IN T2DM
2.11.1 Insulin signalling
2.11.2 Insulin-stimulated phosphorylation cascades

2.12 REGULATION OF GLUCOSE METABOLISM
2.12.1 Regulation of glycogen synthesis
2.12.2 Regulation of gluconeogenesis

2.13 REGULATION OF LIPID METABOLISM
2.13.1 Free fatty acids

2.14 STRATEGIES OF THE SEARCH FOR T2DM SUSCEPTIBILITY GENES
2.14.1 Genome-wide scan approach
2.14.2 Candidate gene approach (Association studies)
   2.14.2.1 Calpain-10 (CAPN10) gene
   2.14.2.2 Peroxisome proliferator-activated receptor γ gene
   2.14.2.3 Ecto-nucleotide pyrophosphatase/phosphodiesterase-1
   2.14.2.4 Angiotensin converting enzyme gene (ACE)
   2.14.2.5 Paroxonase 1 gene (PON1)

Chapter 3.0 AIMS AND OBJECTIVES

Chapter 4.0 RESEARCH DESIGN AND METHODS
4.1 HUMAN SUBJECTS
4.1.1 Why study Khatri Sikhs?
4.1.2 Recruitment of study subjects
4.1.3 Informed consent
4.1.4 Diabetes questionnaire

4.2 PHENOTYPIC EVALUATION OF STUDY SUBJECTS

4.3 ANTHROPOMETRIC MEASUREMENTS

4.4 BIOCHEMICAL MEASUREMENTS
5.3 PREVALENCE OF DIABETES RELATED COMPLICATIONS IN NISDS SUBJECTS

5.4 METABOLIC ESTIMATIONS OF NISDS SUBJECTS

5.5 CALPAIN 10 (CAPN 10) GENE POLYMORPHISM AND T2DM

5.5.1 Genotyping of SNP-43 of Calpain 10 gene
  5.5.1.1 Genotype and allelic frequencies of SNP-43
  5.5.1.2 Association between clinical and metabolic traits and genotypes

5.5.2 Genotyping of SNP-19 of Calpain 10 Gene
  5.5.2.1 Genotype distribution and allele frequencies
  5.5.2.2 Association between clinical and metabolic traits and genotypes

5.5.3 Genotyping of SNP-63 of Calpain 10 gene
  5.5.3.1 Genotype distribution and allele frequencies
  5.5.3.2 Association between clinical and metabolic traits and genotypes

5.6 Pro12Ala POLYMORPHISM IN EXON B OF PPARγ GENE

5.6.1 Genotype distribution and allele frequencies
  5.6.2 Association between clinical and metabolic traits and genotypes

5.7 K121Q POLYMORPHISM OF ENPP1/PC-1 GENE AND T2DM

5.7.1 Genotype distribution and allele frequencies
  5.7.2 Association of clinical and metabolic traits with the genotypes of K121Q polymorphism of ENPP1/PC1 gene

5.8 ASSOCIATION OF ACE (I/D) GENE POLYMORPHISM WITH T2DM

5.8.1 Genotype distribution and allele frequencies
  5.8.2 Association between clinical and metabolic traits and genotypes

5.9 ASSOCIATION OF Gln192Ala POLYMORPHISM OF PON 1 GENE WITH T2DM

5.9.1 Genotype distribution and allele frequencies
  5.9.2 Association between clinical and metabolic traits and genotypes of Gln192Ala polymorphism of PON 1 gene
4.5 DERIVED MEASURES
4.5.1 Homeostasis model assessment index
4.5.2 Body fat content

4.6 REAGENTS USED

4.7 PREPARATION OF SOLUTIONS
4.7.1 0.5M EDTA solution
4.7.2 Tris-Cl (pH 8.0) buffer
4.7.3 10x TE (Tris - EDTA) solution
4.7.4 Proteinase K solution
4.7.5 5X TBE buffer
4.7.6 50x TAE buffer
4.7.7 Gel-loading dye (6x)
4.7.8 Ethidium bromide solution

4.8 EXTRACTION OF GENOMIC DNA FROM HUMAN BLOOD SAMPLES
4.8.1 Procedure
4.8.2 Determination of concentration, yield, and purity of DNA

4.9 GENETIC POLYMORPHISM OF CANDIDATE GENES IN T2DM
4.9.1 Genotyping of CAPN10 gene polymorphism
  4.9.1.1 SNP-43 (CAPN10-g.4852G/A)
  4.9.1.2 SNP-19 (CAPN10-g.7920indel32bp)
  4.9.1.3 SNP-63 (CAPN10-g.16378C/T)
4.9.2 Genotyping of Pro12Ala Polymorphism in Exon B of PPARG Gene
4.9.3 Genotyping of K121Q polymorphism in ENPP1 gene
4.9.4 Genotyping of insertion/deletion polymorphism in ACE gene
4.9.5 Genotyping of Gln192Ala polymorphism of PON1 gene

4.10 STATISTICAL ANALYSIS

Chapter 5.0 RESULTS
5.1 BASELINE CHARACTERISTICS OF NSDS SUBJECTS
5.2 CLINICAL AND ANTHROPOMETRIC CHARACTERISTICS OF NSDS SUBJECTS
Chapter 6.0 DISCUSSION

6.1 ENVIRONMENTAL FACTORS ASSOCIATED WITH T2DM IN KHATRI SIKH POPULATION

6.1.1 Demographic and socioeconomic factors

6.1.2 Clinical characteristics associated with T2DM in Khatri Sikh population
   6.1.2.1 Age at onset of T2DM
   6.1.2.2 Obesity and abdominal adiposity
   6.1.2.3 Body fat distribution
   6.1.2.4 Dyslipidemia

6.2 GENETIC RISK FACTORS ASSOCIATED WITH T2DM IN KHATRI SIKH POPULATION

6.2.1 Association of three SNPs of calpain-10 gene with T2DM
   6.2.1.1 Relationship between SNP-43 and type 2 diabetes
   6.2.1.2 Relationship between SNP-19 and type 2 diabetes
   6.2.1.3 Relationship between SNP-63 and type 2 diabetes

6.2.2 Association of Pro12Ala polymorphism of PPARγ2 gene with T2DM

6.2.3 Association of ENPP1/PC-1 K121Q Polymorphism with T2DM

6.2.4 Association of ACE (I/D) Gene Polymorphism with T2DM

6.2.5 Association of Gln192Ala Polymorphism of PON1 Gene with T2DM

Chapter 7.0 SUMMARY AND CONCLUSIONS

Chapter 8.0 BIBLIOGRAPHY

Chapter 9.0 APPENDIX