#### CONTENTS

	Page No.
Declaration	i
Certificate	ii
Acknowledgement	iii-iv
Introduction	vi
Review of Literature	vi-vii
Materials and Method	vii-ix
Results	ix-xii
Discussion	xii
Summary and conclusion	xii
References	xii
Appendices	xii
Publications	xii
Certificates Of Seminar Presentation	xii
List of Tables	xiii-xiv
List of Figures	xv-xix
Abbreviations	xx-xxiv
Standard units	XXV

# Chapter 1 INTRODUCTION

1.1.	Glucose-6-Phosphate Dehydrogenase enzyme	2-4
1.2.	Glucose-6-Phosphate Dehydrogenase deficiency	4-6
1.3.	Glucose-6-Phosphate Dehydrogenase variants	6-7
1.4.	Haemolysis in Glucose-6-Phosphate Dehydrogenase deficiency	7-10
1.4.1.	Oxidative triggers in Glucose-6-Phosphate Dehydrogenase deficiency	8-10
1.4.2.	Haemolytic sensitivity of Glucose-6-Phosphate Dehydrogenase	10-11
	variants to primaquine	
1.5.	Haemoglobinopathies	11
1.5.1.	Structural disorders of Haemoglobin	11-13
1.5.2.	Thalassaemia syndromes	13-15
1.6.	Prevalence of haemoglobinopathies	15-16
1.7.	Association of haemoglobinopathies with malaria	16
1.8.	Association of Glucose-6-Phosphate Dehydrogenase deficiency with	17
	haemoglobinopathies	
1.9.	Association of Glucose-6-Phosphate Dehydrogenase deficiency with	17
	malaria	
1.10.	Malaria in Northeast India	18
1.11.	Study area	18-19
1.12.	Rationale of the study	19
1.13.	Objectives	20

# Chapter 2 REVIEW OF LITERATURE

2.1.	Glucose-6-Phosphate Dehydrogenase enzyme	22
2.2.	Glucose-6-Phosphate Dehydrogenase deficiency	22
2.2.1.	Global prevalence of G6PD deficiency	22
2.2.2.	Glucose-6-Phosphate Dehydrogenase deficiency in India	22-23
2.3.	Glucose-6-Phosphate Dehydrogenase variants	23
2.3.1.	Glucose-6-Phosphate Dehydrogenase variants identified till date	23-24

2.3.2.	Glucose-6-Phosphate Dehydrogenase variants in Southeast Asia	24-29
2.3.3.	Glucose-6-Phosphate Dehydrogenase variants reported in Indian	29-33
	population	
2.3.4.	Types of mutations involved in various Glucose-6-Phosphate	33-34
	Dehydrogenase mutations	
2.3.5.	Distribution of mutations in Glucose-6-Phosphate Dehydrogenase	34-35
	gene	
2.4.	Haemoglobinopathies	35
2.4.1.	Prevalence of haemoglobinopathies in India	35-36
2.5.	Association of Glucose-6-Phosphate Dehydrogenase deficiency with	36-37
	haemoglobinopathies	
2.6.	Clinical manifestations of Glucose-6-Phosphate Dehydrogenase	37
	deficiency	
2.6.1.	Neonatal jaundice, hyperbilirubinemia and kernicterus	37
2.6.2.	Acute haemolytic anaemia	37-38
2.6.3.	Chronic nonspherocytic haemolytic anaemia	38
2.7.	Approaches taken to treat Glucose-6-Phosphate Dehydrogenase	38-39
	deficiency	
2.7.1	Antioxidant therapy	39-40
2.7.2	Enhancement of Glutathione (GSH) biosynthesis via N-acetyl-	40
	cysteine (NAC)	
2.7.3.	Nicotinamide Adenine Dinucleotide Phosphate (NADPH) generation	40-41
	via complementary pathways	
2.7.4.	Using transcriptional regulators to target upregulation of Glucose-6-	41
	phosphate dehydrogenase	
2.7.5.	Small molecule activators to address Glucose-6-Phosphate	41-42
	Dehydrogenase deficiency	
2.8.	In-silico drug designing for Glucose-6-Phosphate Dehydrogenase	42-43
	variants	

# Chapter 3 MATERIALS AND METHOD

vii

3.1.	Site of the study	46-47
3.2.	Approval from ethical committee	47
3.3.	Informed consent	47
3.4.	Selection of study subjects	47
3.5.	Screening for Glucose-6-Phosphate Dehydrogenase deficiency	49
3.6.	Collection of blood samples	49
3.7.	Clinical evaluation of Glucose-6-Phosphate Dehydrogenase deficient	49-50
	subjects	
3.8.	Screening for abnormal haemoglobin variants by Complete Blood	50
	Count & Haemoglobin-typing	
3.9.	Statistical analysis	50
3.10.	Molecular analysis of Glucose-6-Phosphate Dehydrogenase	50
	deficiency	
3.10.1.	Extraction of genomic DNA	50-51
3.10.2.	Quantification of DNA	51-52
3.10.3.	Polymerase Chain Reaction for amplification of Glucose-6-	52-54
	Phosphate Dehydrogenase gene	
3.10.4.	Restriction Fragment Length Polymorphism	54-55
3.10.5.	Statistical analysis of Glucose-6-Phosphate Dehydrogenase variants	56
3.11.	In silico study of Glucose-6-Phosphate Dehydrogenase variants	56
3.11.1.	Three dimensional structure modeling of Glucose-6-Phosphate	55-56
	Dehydrogenase variants	
3.11.2.	Retrieval of naturally available antioxidant compounds from	56-57
	databases	
3.11.3.	Preparation of ligands and protein structures for docking	57
3.11.4.	Identification of binding pocket and preparation of grid box	57
3.11.5.	Molecular Docking of natural antioxidants with Glucose-6-Phosphate	58
	Dehydrogenase variants	
3.11.6.	Analysis of Molecular docking results	58
3.11.7.	Analysis of drug-likeness and ADME properties	58

3.11.8. Molecular Dynamics simulation

## Chapter 4

### RESULTS

59

4.1.	Prevalence of Glucose-6-Phosphate Dehydrogenase deficiency	61-62
4.2.	Screening for abnormal haemoglobin variants by Complete Blood	62-63
	Count and Haemoglobin-typing	
4.3.	Statistical analysis of results of Haemoglobin-typing	63-64
4.4.	Clinical evaluation of Glucose-6-Phosphate Dehydrogenase deficient	64-66
	subjects	
4.5.	Association between Glucose-6-Phosphate Dehydrogenase	66
	deficiency and gender	
4.6.	Molecular analysis of Glucose-6-Phosphate Dehydrogenase deficient	66
	samples	
4.6.1.	Agarose gel electrophoresis of extracted DNA	66-67
4.6.2.	Quantification of DNA	67
4.6.3.	Polymerase Chain Reaction for amplification of exonic regions of	67-69
	Glucose-6-Phosphate Dehydrogenase gene	
4.6.3.1.	Amplification of exon 3	68
4.6.3.2.	Amplification of exon 4	69
4.6.3.3.	Amplification of exons 4-5	69
4.6.3.4.	Amplification of exons 6-7	69
4.6.3.5.	Amplification of exon 9	71
4.6.3.6.	Amplification of exon 10	71
4.6.3.7.	Amplification of exon 11	71
4.6.3.8.	Amplification of exon 12	71-73
4.6.4.	Restriction Fragment Length Polymorphism	73
4.6.4.1.	Detection of Orissa variant (131C>G) in exon 3 by HaeIII restriction	73
	digestion analysis	
4.6.4.2.	Detection of Namoru variant (208T>C) in exon 4 by NlaIII	73-75
	restriction digestion analysis	

4.6.4.3.	Detection of $A^+$ variant (376 A>G) in exon 5 by FokI restriction	75
	digestion analysis	
4.6.4.4.	Detection of A <sup>-202</sup> variant (202 G>A) in exon 4 by NlaIII restriction	75
	digestion analysis	
4.6.4.5.	Detection of Mahidol variant (487G>A) in exon 6 by HindIII	77
	restriction digestion analysis	
4.6.4.6.	Detection of Mediterranean variant (563C>T) in exon 6 by MboII	77
	restriction digestion analysis	
4.6.4.7.	Detection of Acores (595A>G) in exon 6 by BstUI restriction	77-79
	digestion analysis	
4.6.4.8.	Detection of Kalyan-Kerala/Jamnagar/Rohini variant (949G>A) in	79
	exon 9 by Mnll restriction digestion analysis	
4.6.4.9.	Detection of Chatham variant (1003G>A) in exon 9 by BstXI	79-80
	restriction digestion analysis	
4.6.4.10.	Detection of Guadalajara variant (1159C>T) in exon 10 by HhaI	80
	restriction digestion analysis	
4.6.4.11.	Detection of Union variant (1360C>T) in exon 11 by HhaI restriction	80
	digestion analysis	
4.6.4.12.	Detection of Canton variant (1376G>T) in exon 12 by AfIII	80-82
	restriction digestion analysis	
4.6.4.13.	Detection of Kaiping variant (1388G>A) in exon 12 by NdeI	82
	restriction digestion analysis	
4.6.5.	Statistical analysis of Glucose-6-Phosphate Dehydrogenase variants	82
4.6.5.1.	Variant-wise distribution among the Glucose-6-Phosphate	82-84
	Dehydrogenase deficient subjects	
4.6.5.2.	Gender-wise distribution among the G6PD deficient subjects	84
4.7.	In silico study of Glucose-6-Phosphate Dehydrogenase variants	84
4.7.1.	Three dimensional structure modeling of detected Glucose-6-	84-85
	Phosphate Dehydrogenase variants	
4.7.1.1.	Three dimensional structure modeling and validation of G6PD Orissa	85
	(131C>G)	

х

4.7.1.2.	Three dimensional structure modeling and validation of G6PD	85
	Kalyan-Kerala (949G>A)	
4.7.1.3.	Three dimensional structure modeling and validation of G6PD	85
	Mahidol (487G>A)	
4.7.1.4.	Three dimensional structure modeling and validation of G6PD $\ensuremath{A^+}$	85-88
	(376 A>G)	
4.7.2.	Retrieval of naturally available antioxidant compounds	88
4.7.3.	Molecular Docking of Glucose-6-Phosphate Dehydrogenase variants	88
	with natural antioxidants	
4.7.3.1.	Molecular Docking of G6PD Orissa	92
4.7.3.2.	Molecular Docking of G6PD Kalyan-Kerala	92
4.7.3.3.	Molecular Docking of G6PD Mahidol	92
4.7.3.4.	Molecular Docking of G6PD A <sup>+</sup>	93
4.7.4.	Interaction of the ligands with the Glucose-6-Phosphate	
	Dehydrogenase variants	
4.7.5.	Analysis of drug-likeness and ADME properties of the natural	95-97
	antioxidants	
4.7.6.	Analysis of toxicity of the natural antioxidants	97-101
4.7.7.	Visualization and analysis of best complexes	101
4.7.7.1.	Molecular docking of Orissa with Myricetin	108
4.7.7.2.	Molecular docking of Kalyan-Kerala with Apigenin	108
4.7.7.3.	Molecular docking of Mahidol with Catechin	108
4.7.7.4.	Molecular docking of A <sup>+</sup> with Daidzen	108
4.7.8.	Molecular Dynamics simulation	111
4.7.8.1.	Molecular Dynamics simulation of Glucose-6-Phosphate	111-115
	Dehydrogenase variants	
4.7.8.2.	Molecular Dynamics simulation of Glucose-6-Phosphate	115
	Dehydrogenase variants	
4.7.8.2.1.	Molecular Dynamics simulation of Orissa-Myricetin	115-116
4.7.8.2.2.	Molecular Dynamics simulation of Kalyan-Kerala-Apigenin	116-119
4.7.8.2.3.	Molecular Dynamics simulation of Mahidol-Catechin	119-122

4.7.8.2.4.	Molecular Dynamics simulation of $A^+$ - Diadzen	122-125
Chapter 5	DISCUSSION	
5.1.	Prevalence of Glucose-6-phosphate Dehydrogenase deficiency	129-130
5.2.	Clinical presentation of Glucose-6-phosphate Dehydrogenase	130
	deficient subjects	
5.3.	Co-relation of Glucose-6-Phosphate Dehydrogenase deficiency with	130-132
	haematological indices	
5.4.	Association between Glucose-6-phosphate Dehydrogenase deficiency	132
	and gender	
5.5.	Haemoglobinopathies	132-134
5.6.	Association between Glucose-6-phosphate Dehydrogenase deficieny	134
	and haemoglobinopathies	
5.7.	Glucose-6-phosphate Dehydrogenase variants	134-137
5.8.	In- silico study	137-141
5.9.	Significant findings of the study	141-142
5.10.	Future scope of the study	142-143
Chapter 6	SUMMARY AND CONCLUSION	145-146
REFERENCES		148-184
APPENDICES		186-196

### PUBLICATIONS

#### **CERTIFICATES OF SEMINAR PRESENTATION**