

## Table of Contents

Chapter	Description	Page
	<b>Preface</b> <b>Abstract</b> <b>Acknowledgement</b> <b>List of Tables</b> <b>List of Figures</b> <b>List of Appendices</b> <b>List of Abbreviation</b>	I –ii iii- vii viii- ix xvii- xviii  xviv xv xvi- xviii
<b>1</b>	<b>Introduction</b> 1.1 Evolutionary process affecting population diversity 1.2 Genetic tools for studying human genetic diversity 1.2.1 Short tandem repeats (STR) 1.2.2 Mitochondrial DNA (mtDNA) and Y chromosome markers 1.3 Population of Indian Sub- continent  <b>OBJECTIVES OF THE STUDY</b>	1 - 7 3 5 5 6 6  7
<b>2</b>	<b>Review of literature</b> 2.1 Toll Like Receptors 2.2 Structure of Toll like receptors 2.3 Signalling pathways of TLR 2.4 MyD88-dependent pathway 2.5 Other molecules 2.5.1 MyD88-independent pathway 2.6 Balanced activation between MyD88- and TRIF-dependent pathways	8- 44 11 12 13 14 14 14 15

2.7 Negative regulation of TLRs	<b>15</b>
2.8 TLR1 and disease association (cluster of differentiation 281-CD281)	<b>17</b>
2.9 TLR2 and disease association (cluster of differentiation 282-CD282)	<b>18</b>
2.10 TLR3 and disease association (cluster of differentiation 283-CD283)	<b>18</b>
2.11 TLR4 and disease association (cluster of differentiation 284-CD284)	<b>18</b>
2.12 TLR5 and disease association (cluster of differentiation 285-CD285)	<b>19</b>
2.13 TLR6 and disease association (cluster of differentiation 286-CD286)	<b>19</b>
2.14 TLR7 polymorphism and disease association (cluster of differentiation 287-CD287)	<b>20</b>
2.15 TLR8 and disease association (cluster of differentiation 288-CD288)	<b>20</b>
2.16 TLR9 and disease association (cluster of differentiation 289-CD289)	<b>20</b>
2.17 TLR10 and disease association (cluster of differentiation 290-CD290)	<b>21</b>
2.18 Correlation among the TLR polymorphic varieties and diseases	<b>21</b>
2.19 TLRs and rheumatoid arthritis	<b>22</b>
2.19.1 Markers of RA	<b>22</b>
2.19.2 Epidemiology of RA	<b>23</b>
2.19.3 Disease etiology	<b>23</b>
2.19.4 Genetic factors	<b>23</b>
2.19.5 Role of Environment in case of Rheumatoid Arthritis	<b>24</b>
2.19.6 Cellular destruction in case of Rheumatoid Arthritis	<b>25</b>
2.19.7 Role of different cells in case of RA	<b>28</b>
2.19.7.1 Monocytes/Macrophages	<b>28</b>

	2.19.7.2 Fibroblast-like synoviocytes	29
	2.19.7.3 T and B cells	29
	2.19.7.4 Neutrophils	30
	2.19.7.5 Dendritic cells	30
	2.19.7.6 NK cells	31
	2.19.8 Diagnosis	32
	2.19.8.1 Laboratory diagnosis	32
	2.19.8.2 RF antibodies	32
	2.19.8.3 Anti-cyclic citrullinated peptide (CCP) antibodies	33
	2.19.9 Different Inflammatory markers in case of RA (ESR and C-reactive protein)	33
	2.19.9.1 Assessment of Disease Activity	34
	2.19.10 Measurement of clinical disease activity	34
	2.19.10.1 Pain, Swollen and tender joint counts	35
	2.19.11 Disease activity scores and indices	35
	2.19.12 Criteria to assess disease activity	36
	2.19.13 Radiological progression	36
	2.19.14 Included Joints	37
	2.19.15 Different Scoring methods for RA	37
	2.19.16 Assessment of function	38
	2.20 Relation of TLRs with RA	39
	2.21 TLRs and typhoid fever	40
	2.21.1 Toll-Like Receptor 4 (TLR4) and Typhoid Fever	41
	2.21.2 Toll-Like Receptor5 (TLR5) and Typhoid fever	43
	2.22 Association of Toll like receptors with HIV	44
<b>3</b>	<b>Materials and Methods</b>	<b>45- 68</b>
	3.1 Sample Collection	47
	3.1.1 Selection criteria of samples	47
	3.1.2 Sample collection strategy	47
	3.1.3 Demographic profile of the samples	48
	3.2 The four populations that were selected for our study include	48

3.2.1 Rajbanshi	48
3.2.2 Rabha	49
3.2.3 Gurkha	49
3.2.4 Muslim	49
3.3 RA Based Study	50
3.3.1 Study population	50
3.3.2 Sample collection strategy	52
3.3.3 RF titre assay	52
3.3.4 Anti-CCP estimation	53
3.4 Collection of the samples from typhoid patients	53
3.4.1 Disease samples of Typhoid patients	53
3.4.2 Collection of the samples	53
3.4.3 Criteria for collection of samples	53
3.5 Collection of the samples from typhoid patients	53
3.5.1 Selection of patients	53
3.5.2 Sample Collection	54
3.6 Disease Sample of HIV patients	54
3.6.1 Selection of patients	55
3.7 Laboratory Analysis	55
3.7.1 Extraction of genomic DNA	55
3.7.1.1 DNA extraction	55
3.7.1.2 Characterization of DNA	57
3.7.1.3 The integrity	57
3.7.1.4 The concentration	57
3.7.1.5 Purity of DNA	57
3.7.1.6 Storage of DNA	58
3.8 Gene specific analysis	61
3.8.1 Primer designing	61
3.8.2 Amplification Check	61
3.9 Statistical analysis	63
3.9.1 Gene frequency	63

	3.9.2 Genotypic frequency	63
	3.9.3 Chi- square and G2 tests	64
	3.9.4 Exact test	64
	3.9.5 Gene diversity Analysis	64
	3.9.6 Genetic distances	64
	3.9.7 Sensitivity and Specificity test for disease	65
	3.9.7.1 Sensitivity	66
	3.9.7.2 Specificity	66
	3.9.7.3 Positive likelihood ratio	66
	3.9.7.4 Negative likelihood ratio	66
	3.9.7.5 Positive predictive value	66
	3.9.7.6 Negative predictive value	67
	3.9.7.7 Test Score calculation	67
	3.9.7.8 Calculating ODD ratio	67
	3.9.7.9 Calculating Relative Risk	67
	3.9.7.10 Relative Risk calculation	68
<b>4</b>	<b>RESULTS</b>	<b>69-93</b>
	4.1 TLR gene frequencies among four different populations	71
	4.2. Study of heterogeneity among the local population(s).	73
	4.2.1 Chi-Square Test	73
	4.2.2 Principal Component Analyses	74
	4.2.3 Genetic distance calculation	75
	Frequency, Distribution and Association study of TLR genes among Rheumatoid arthritis, Typhoid fever and Human Immunodeficiency Virus patients	
	4.3 Association of TLR genes among Rheumatoid arthritis	76
	4.3.1 Diagnostic and Demographic profile	76
	4.3.2 Gene frequency and Chi-square analysis	77
	4.3.3 Relative risk calculation	79
	4.3.4 Odd ratio/ Risk ratio calculation	80

	4.3.5 Sensitivity and Specificity	81
	4.4 Association of TLR genes among typhoid fever patients	82
	4.4.1 Observed frequency and Chi-square analysis	82
	4.4.2 Relative risk calculation	82
	4.4.3 Odd ratio/ Risk ratio calculation	83
	4.4.4 Sensitivity and Specificity	84
	4.5 Association of TLR genes among HIV+ patients	89
	4.5.1 Observed frequency and Chi-square analysis	89
	4.5.2 Relative risk calculation	89
	4.5.3 Odd ratio/ Risk ratio calculation	90
	4.5.4 Sensitivity and Specificity	91
<b>5</b>	<b>Discussion</b>	<b>94 – 106</b>
	5.1 Frequency and distribution study of TLR genes in four different populations in North Bengal region of India	96
	5.1.1 Rajbanshi	96
	5.1.2 Rabha	98
	5.1.3 Gurkha	98
	5.1.4 Muslim	99
	5.2 Frequency, distribution and association study of Rheumatoid arthritis among the patients of Siliguri and adjacent areas	100
	5.3 Frequency, distribution and association study of Typhoid fever among the patients of Siliguri and adjacent areas	104
	5.4 Frequency, distribution and association study of HIV+ patients among the patients of Siliguri and adjacent areas	106
<b>6</b>	<b>Summary and conclusion</b>	<b>107- 112</b>
	6.1 Population- based study	109
	6.2 Rheumatoid arthritis- based study	111
	6.3 Typhoid –based study	112
	6.4 HIV –based study	112
	<b>BIBLIOGRAPHY</b>	<b>113- 147</b>

INDICES	<b>148 -154</b>
APPENDIX A	<b>155 -156</b>
APPENDIX B	<b>157 -158</b>
APPENDIX C	<b>159</b>
APPENDIX D	<b>160</b>
APPENDIX E	<b>161</b>
APPENDIX F	<b>162-164</b>
APPENDIX G	<b>165</b>