TABLE OF CONTENTS

ABST	RACT	11	
ABST	TRAK	v	
ACK	NOWLEDGEMENT	viii	
TABLE OF CONTENTS			
LIST	LIST OF ABBREVIATIONS		
LIST	LIST OF TABLES		
LIST OF FIGURES			
LIST	OF APPENDIX	xxii	
СНА	PTER 1 : INTRODUCTION		
1.1	General Introduction	1	
1.2	Human Herpes Virus 6 (HHV6)	2	
	1.2a Morphology and Ultrastructure	3	
	1.2b Genetic Characteristics	3	
1.3	Human Herpes Virus 6 Variants A and B	5	
1.4	Cell Tropism and Growth Properties of HHV6A and HHV6B	8	
1.5	Animal Models of HHV6 Infection	10	
1.6	6 Human Herpes Virus 6 Infection		
	1.6a Primary Infection	11	
	1.6b Latent Infection	12	
1.7	Major Antigens of Human Herpes Virus 6	14	
1.8	Mode of HHV6 Transmission	17	
	1.8a Transmission through Sera	20	

	1.8b	Transmission through Saliva	21
	1.8c	Transmission through Breast Milk	22
1.9	Detect	ion of Human Herpes Virus 6 Infections	23
1.10	Epider	miology of Human Herpes Virus 6	27
1.11	Human Herpes Virus 6 Associated Diseases		
1.12	Protein Analysis and Structure 3		
1.13	Immu	nogenic Epitope Analysis and Mapping	34
1.14	Multipin Peptide Synthesis		
1.15	Prediction of Antigenicity by Hydropathy, Accessibility and Secondary Structure		
1.16	Objectives of Present Study		44
СНА	PTER T	TWO: MATERIALS AND METHODS	
2.1	Materials		
2.2	Samp	les	45
	2.2a	Sera Samples	45
	2.2b	Cord Blood Samples	46
	2.2c	Saliva Samples	46
	2.2d	Breast Milk Samples	48
2.3	HHV	6B Stock	48
2.4	Materials for Separation, Preparation and Harvesting of Human Cord Blood Mononuclear Cells (HCBMC)		

	2.4a.	Phosphate Buffered Saline (PBS)	48
	2.4a.	Human Interleukin-2 (IL-2)	48
	2.4b.	Phytohemaglutinin (PHA) Reagent	49
	2.4c	RPMI 1640 Cell Culture Medium	49
	2.4d	The RPMI 1640 Growth Medium	49
	2.4e	RPMI 1640 Maintenance Medium	50
Methods for Separation, Preparation and Harvesting of Human Cord Blood Mononuclear Cells (HCBMC)			
	2.5a	Lymphocyte Separation	50
	2.5b	Infection with HHV6	50
	2.5c	Cell Harvesting and Preparation of Slides	51
	2.5d	HHV6 Negative Control Slides	51
Indirect Immunofluorescence Assay (IFA) for Detection of IgG and IgA Antibodies against HHV6 Antigens			52
Electron Microscope Observation of HHV6 Virus in HCBMC			52
Peptide Synthesis of the p101 Nucleocapsid Protein of HHV6 Using the Multipin Peptide Synthesis Method			53
	2.8a	Chemistry of Peptide Synthesis	53
	2.8b	Selection of Synthesis Regions and Generation of the Synthesis Schedule	55
	2.8c	Pre-synthesis Preparation	55
	2.8d	Weighing Amino Acids and Activating Chemicals	56
	2.8e	N, N - Dimethylformamide (DMF) Filtration	50
	2.8f	Deprotection of Pins	5
	2.8g	Amino Acid Couplings	5

2.5

2.6

2.72.8

	2.8h	Washing of Pins	39	
	2.8i	Acetylation of Terminal Amino Groups	59	
	2.8j	Side - chain Deprotection	60	
2.9		e – Linked Immunosorbent Assay (ELISA) on Samples against 01 Synthesized Peptides	62	
СНАБ	CHAPTER THREE: RESULTS			
3.1	HHV6	Cell Culture	68	
	3.1a	Light Microscopy	68	
	3.1b	Electron Microscopy	68	
3.2		ion of HHV6 IgA Antibody Prevalence in Age Groups en 0 – 20 Years by IFA.	72	
	3.2a	Detection of Specific Serum IgG	72	
	3.2b	Detection of Specific Serum IgA	72	
3.3	Immu Antige	nofluorescence Assay for Detection of Antibodies against HHV6	76	
	3.3a	Serum IgA	76	
	3.3b	Serum IgG	76	
3.4	Linear	Epitope Mapping of p101 Nucleocapsid Protein of HHV6	79	
	3.4a	Epitope Mapping of the HHV6 p101 Carboxyl Terminal Protein	79	
	3.4b	Conjugate Scanning	80	
	3.4c	IgG Epitopes of HHV6 p101 Carboxyl Terminal Protein in Serum	82	
	3.4c	(i) HHV6 IFA-positive Sera	82	
	3.4c	(ii) HHV6 IFA-negative Sera	85	
	3.4c	(iii) Comparison of IgG Epitopes in HHV6 IFA-positive and HHV6- negative Sera	88	

	3.4d	(i) HHV6 IFA-positive Breast Milk	92
	3.4d	(ii) HHV6 IFA- negative Breast Milk	95
	3.4d	(iii) Comparison of IgA Epitopes in HHV6 IFA-positive and HHV6- negative Breast Milk	98
	3.4e	Salivary IgA Epitopes of HHV6 p101 Carboxyl Terminal Protein	101
	3.4f	Cord Blood IgG Epitopes of HHV6 p101 Carboxyl Terminal Protein	105
3.5	Compari Epitope	son Studies Between IgG-Serum, IgA-Saliva and IgA-Breast Milk based on p101 Carboxyl Terminal Peptide Profiles	105
	3.5a	Serum and Breast Milk	105
3.6	Hydro	pathy Plots, Surface Accessibility and Secondary Structure	107
	3.6a	Hydropathy Plot Studies	107
	3.6b.	Surface Accessibility Analysis	108
	3.6c	Secondary Structure Studies	108
CHAPTER FOUR: DISCUSSION			
4.1	Overv	view	125
4.2	Immu Cord	nofluorescence Assay (IFA) on HHV6 Infected Human Blood Mononuclear Cells (HCBMC)	128
	4.2a	Detection of Specific Antibodies to HHV6 in HCBMC	128
	4.2a	(i) Serum IgG	128
	4.2a	(ii) Salivary IgA	129
	4.2a	(iii) Breast Milk IgA	129
	4.2b	Age Prevalence Study of IgA and IgG Antibody to HHV6	130
4.3	Pepti HHV	de Synthesis and Selection of Antigenic Peptides of the 6 p101 Carboxyl Terminal Protein	132

	4.3a	Antigenic Regions of HHV6 Carboxyl Terminal-recognized by Serum IgG Antibodies	133
	4.3b	Antigenic Regions of HHV6 Carboxyl Terminal p101 Protein-recognized by Breast Milk IgA Antibodies	136
	4.3c	Saliva IgA Antibody Reactivity to HHV6 Carboxyl Terminal Peptides	137
.4	Comparison of HHV6 Epitopes Defined by Serum, Breast Milk and Saliva Antibodies		
	4.4a	Potential Use of HHV6 Peptide ELISA for Investigation on HHV6 Infection	145
	4.4b	IgA HHV6 in Breast Milk	146
1.5	Assoc	niation of Hydropathy, Surface Accessibility, Secondary tures and Pepscan Analysis with reference to HHV6 p101 Peptides	147
1.6	Conc	lusions	153
REFI	REFERENCES		