

LIST OF FIGURES

Figure 1. 1 Global distribution of malaria (WHO report, 2015).....	4
Figure 1. 2 Distribution of malaria in INDIA (WHO report, 2015).....	5
Figure 1. 3 Approximate malaria cases in each region due to <i>P. vivax</i> , 2015 (WHO report, 2015).....	8
Figure 1. 4 Distribution of malaria vectors in relation to physio-geographic regions of India (Dev et al., 2013).	10
Figure 1. 5 Female <i>Anopheles</i> mosquito using proboscis for her blood meal. (http://www.neomosquito.com/understanding-mosquitoes/).....	11
Figure 1. 6 Life cycle of <i>Plasmodium</i> species (Image from www. http://vivaxmalaria.com/template_disease.htm).....	13
Figure 1. 7 Thin blood smear of <i>Plasmodium vivax</i> with different blood stages (http://www.cdc.gov/).....	16
Figure 1. 8 Development and function of monocyte (Ginhoux et al., 2014).....	23
Figure 1. 9 Diagrammatic representation of the chemokine receptor (Murdoch et al., 2000).....	27
Figure 1. 10 Model of chemokine receptor activation and signal transduction for IL-8 and neutrophils (Murdoch et al., 2000).	28
Figure 1. 11 Flow chart explaining the different settings for diagnosis and drug selection for the treatment of malaria (http://www.nvbdc.gov.in/).....	32
Figure 2. 1 Isolation and analysis of genomic DNA from <i>P. vivax</i> infected individuals. Ethidium bromide stained 0.8% agarose gel electrophoresis of (A) Genomic DNA isolated from infected individuals and (B) PCR amplification of human β_2 microglobulin gene from the <i>P. vivax</i> genomic DNA (negative control). 62	

Figure 2. 2 Analysis of PCR products from <i>P. vivax</i> genomic DNA.	63
Figure 2. 3 0.8% agarose gel stained with EtBr showing confirmation of AMA1 (A), MSP7 (B) and WARP (C) inserts in pJET1.2 vector by restriction digestion.....	64
Figure 2. 4 0.8% agarose gel stained with EtBr showing confirmation of AMA1 (A), MSP7 (B) and WARP (C) inserts in pET30a (+) vector by restriction digestion.....	65
Figure 2. 5 Coomassie stained 10% SDS-PAGE with whole cell extracts of <i>E. coli</i> BL21 (DE3) transformed with either pET30a(+) or pET30a(+)-AMA-1 (A), pET30a(+)-MSP7 (B) and pET30a(+)-WARP (C)	67
Figure 2. 6 Silver stained 10% SDS-PAGE of purified AMA-1, MSP7 and WARP by nickel affinity chromatography	69
Figure 2. 7 AMA1, MSP7 and WARP gene position in <i>P. vivax</i> genome. All the genes are highlighted with yellow background. (Maps were generated in www.plasmodb.org)	70
Figure 2. 8 Schematic representation of the cloning strategy.	71
Figure 3. 1 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with serum of patient 1	82
Figure 3. 2 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 2	82
Figure 3. 3 Western blot analysis of recombinant antigen AMA-1 and WARP with serum of patient 3	83
Figure 3. 4 Western blot analysis of recombinant antigen MSP7 and WARP with serum of patient 4	83
Figure 3. 5 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 5	84

Figure 3. 6 Western blot analysis of recombinant antigen AMA-1 and WARP with serum of patient 6	84
Figure 3. 7 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 7	85
Figure 3. 8 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 8	85
Figure 3. 9 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 9	86
Figure 3. 10 Western blot analysis of recombinant antigen AMA-1 and WARP with serum of patient 10	86
Figure 3. 11 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 11	87
Figure 3. 12 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 12	87
Figure 3. 13 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 13	88
Figure 3. 14 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 14	88
Figure 3. 15 Western blot analysis of recombinant antigen MSP7 with serum of patient 2-3 & 5-7.....	89
Figure 3. 16 Western blot analysis of recombinant antigen MSP7 with serum of patient 8-11	89
Figure 3. 17 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with serum of control 1	90

Figure 3. 18 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with serum of control 2.....	90
Figure 3. 19 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with no serum.....	91
Figure 3. 20 Phagocytosis assay of monocytes of healthy individual 1. Fluorescence microscopy showing phagocytosis of latex beads. Phase contrast (i), fluorescent (ii) and superimposed images (iii) are shown for monocytes treated and untreated with the recombinant antigens.....	93
Figure 3. 21 Phagocytosis assay of monocytes of healthy individual 2. Fluorescence microscopy showing phagocytosis of latex beads. Phase contrast (i), fluorescent (ii) and superimposed images (iii) are shown for monocytes treated and untreated with the recombinant antigens.....	95
Figure 3. 22 Phagocytosis assay of monocytes of healthy individual 3 Fluorescence microscopy showing phagocytosis of latex beads. Phase contrast (i), fluorescent (ii) and superimposed images (iii) are shown for monocytes treated and untreated with the recombinant antigens.....	97
Figure 3. 23 Phagocytosis index: Percentage of macrophages that phagocytosed single latex beads, in the presence and absence of recombinant antigen, were plotted.	99
Figure 3. 24 Phagocytosis index: Percentage of macrophages that phagocytosed multiple latex beads, in the presence and absence of recombinant antigen, were plotted	99
Figure 3. 25 Nitroblue tetrazolium (NBT) reduction by treated and untreated healthy monocytes (i, ii, iii) viewed under 40x magnification.....	100

Figure 4. 1 Analysis of RNA and cDNA preparation of patient 1. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared from an individual infected with <i>P. vivax</i>	112
Figure 4. 2 Analysis of RNA and cDNA preparation of patient 2. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared of an individual infected with <i>P. vivax</i>	113
Figure 4. 3 Analysis of RNA and cDNA preparation of patient 3. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared from an individual infected with <i>P. vivax</i>	113
Figure 4. 4 Analysis of RNA and cDNA preparation of patient 4. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared from an individual infected with <i>P. vivax</i>	114
Figure 4. 5 Analysis of RNA and cDNA preparation of patient 5, 6 and 7. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared from an individual infected with <i>P. vivax</i>	114
Figure 4. 6 Analysis of RNA and cDNA preparation of patient 8. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared from an individual infected with <i>P. vivax</i>	115
Figure 4. 7 Analysis of RNA and cDNA preparation of patient 9. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared from an individual infected with <i>P. vivax</i>	116
Figure 4. 8 Analysis of RNA and cDNA preparation of patient 10. Ethidium bromide stained 0.8% agarose gel electrophoresis of total RNA and β -actin amplicon from cDNA prepared from an individual infected with <i>P. vivax</i>	116

Figure 4. 9 Analysis of cDNA prepared from healthy individual-1 (β actin PCR) after stimulation of (A) monocytes with recombinant antigens and (B) monocytes derived from PBMCs treated with antigens	117
Figure 4. 10 Analysis of cDNA prepared from healthy individual-2 (β actin PCR) after stimulation of (A) monocytes with recombinant antigens and (B) monocytes derived from PBMCs treated with antigens	118
Figure 4. 11 Analysis of cDNA prepared from healthy individual-3 (β actin PCR) after stimulation of (A) monocytes with recombinant antigens and (B) monocytes derived from PBMCs treated with antigens of healthy individual 3	119
Figure 4. 12 Analysis of chemokine receptor expression in patients (n=10). Results were normalized to the expression of a housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).....	120
Figure 4. 13 Analysis of chemokine receptor expression: (A) Chemokine receptor levels in monocytes of healthy individuals (n=3) treated with recombinant antigens (B) Chemokine receptor levels in monocytes of healthy individuals (n=3) following treatment of peripheral blood mononuclear cells (PBMC) with recombinant antigens. Results were normalized to the expression of a housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). *P < 0.05, **P< 0.01 and ***P< 0.001.....	121