CHAPTER 3

TO STUDY THE IMMUNE REACTIVITY OF PATIENTS TO THE RECOMBINANT ANTIGENS AND STUDY THE IN VITRO EFFECT OF THE RECOMBINANT ANTIGENS ON MONOCYTE FUNCTION

"What we do in life echoes in eternity"

-GLADIATOR

Chapter 3

To study the immune reactivity of patients to the recombinant antigens and study the in vitro effect of the recombinant antigens on monocyte function

INTRODUCTION

The number of malaria cases per year has decreased in recent years but still is quite high. In endemic areas with limited laboratory support, clinical diagnosis is the basis of therapeutic care. Accurate diagnosis is the only way of effecting rational therapy as to avoid non-specific effects, resistance, and to save cost on alternative drugs (Wongsrichanalai et al., 2007). Due to the emergence and spread of drug resistance and hence the requirement of more expensive drugs, confirmation of the disease before treatment initiation have gained attention (Liddell et al., 2004).

Malaria rapid diagnostic tests (RDTs) helps to diagnose malaria by detecting parasitic antigens in human blood. RDTs are quite easy to perform and interpret, requires less time and limited training. Malaria antigens currently targeted by RDT are HRP-2, pLDH, and *Plasmodium* aldolase (Mouatcho et al., 2013 and Moody et al., 2002).

HRP: *P. falciparum*-infected RBC synthesize three histidine-rich proteins, HRP-1, HRP-2, and HRP-3 (Rock et al., 1987). HRP-1 is present in all *P. falciparum* parasites but very less in Gambian isolates. HRP-2 is secreted as a water soluble protein and is found in all *P. falciparum* parasites. Out of the three HRP-1 and HRP-2 are more frequently found in falciparum. HRP-2 was the first antigen used to develop as RDT and is produced during asexual stages and young gametocytes of *P. falciparum*.

pLDH and aldolase: A glycolyric pathway enzyme**,** pLDH, is also used as an antigen for RDT. It is produced all throughout the sexual and asexual stages of the parasite (Makler et al., 1998). In *Plasmodium*, different isomers of pLDH are present and their detection constitutes a second approach to RDT development. pLDH from *Plasmodium* spp. in the presence of human host LDH can be measured by using the substrate 3-acetylpyridine adenine dinucleotide (APAD), an analogue of NAD, in an immunocapture assay. The Michaelis-Menten constants are similar for human and parasite LDH, the turnover number of the pLDH in the presence of APAD is much greater than that of the human enzyme with the same cofactor.

PROBLEMS WITH RDTs

Cross-reactions with autoantibodies: One of the problems with RDTs is that autoantibodies like rheumatoid factor can cross react to give a false positive tests for malaria (Lee et al., 2014). Patients with positive rheumatoid factor have shown that the false positive reactions are higher with the PfHRP2 tests using IgG capture antibody (16.5% to 83%) compared to the PfHRP2 tests using IgM antibodies (6.6%) and the pLDH test (3.3%).

Cross reactions between *Plasmodium spp.*: Cross reaction of antigens may also lead to false results. PfHRP2 with non-falciparum malaria could cross react and provide a false result for *P. falciparum*.

Sensitivity: Sensitivity of RDTs for the diagnosis of *P. falciparum* malaria is >90% at densities above 100 parasites per µL blood but the sensitivity decreases markedly below that level of parasite density. >95% sensitivity is achieved at parasitemia of ~500 parasites/µL, but this high parasitemia is seen in only a minority of patients. LDH test is more sensitive to *P. vivax* malaria than to *P. falciparum* malaria.

False Positivity: False positive results can occur due to lower level of parasitemia, persistence of antigens due to sequestration and incomplete treatment, delayed clearance of circulating antigen (free or in antigen-antibody complexes) and cross reaction with non-falciparum malaria or rheumatoid factor. Proportion of persistent positivity has been linked to the sensitivity of the test, type of test, degree of parasitemia and possibly the type of capture antibody.

False negativity: On the other hand, false negative tests have been observed even in severe malaria with parasitemia >40000 parasites/µl. This is due to genetic heterogeneity of PfHRP2 expression, deletion of HRP-2 gene, presence of blocking antibodies for PfHRP2 antigen or immune-complex formation, prozone phenomenon at high antigenemia or to unknown causes.

Interpretation: RDTs have been reported to be useful and easy tools for field surveys in remote areas like forests and villages, it has been found that the experience and the level of training of the field staff can influence the sensitivity and specificity of these tests.

10% of the total leukocyte population in human blood represents monocytes. One of the important properties of the monocytes is to remove apoptotic cells and helps in maintaining homeostasis. They also represent a connecting link between inflammation and the innate defence against microorganisms to adaptive immune responses. Monocytes have a large number of receptors that can detect not only microorganisms, but also some lipid moiety. Once stimulated monocytes can release effector molecules involved in the defence against pathogen, and can differentiate into dendritic cell or tissue macrophages (Auffray et al., 2009).

In malaria infected individuals the parasites is associated with various cells like the infected RBCs, kuffer cells, splenic macrophages and bone marrow. Merozoites causes major changes in the infected RBCs by expression of parasitic proteins on its surface which in turn is detected by the circulating monocytes. In response monocytes can limit parasitemia by phagocytosis, antibody dependent cell inhibition and cytokine production (Antonelli et al., 2014).

PHAGOCYTOSIS

One of the effective way to reduce parasitemia is via phagocytosis of infected erythrocytes by monocytes/macs. But, the efficiency of this process may differ among individuals, resulting in varying degrees of protection and susceptibility to malaria (Chua et al., 2013). Availability of opsonin regulates the efficiency of monocyte/mac phagocytosis. Opsonins bind to infected erythrocytes to enhance their interaction with monocyte/mac phagocytic receptors. Complement-opsonised ring-stage infected erythrocytes are phagocytosed by monocytes/macs via complement receptor 1 (CR1 or CD35) (Silver et al., 2010). In malaria-immune individuals, phagocytosis of antibody-opsonised infected erythrocytes and merozoites is mediated via FcγRs and its efficiency depends on the expression of FcγRs by various monocyte subpopulations (Langhorne et al., 2008). Monocytes can express three main types of FcγRs: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI and FcγRIIa mediates the uptake of antibody-opsonised infected erythrocytes, whereas FcγRIIb is an inhibitory receptor that reduces the immune responses (Tebo et al., 2002 and Hunter et al., 1998).

Another type of immune mechanism contributing to protection mediated by monocytes/macs is non-opsonic phagocytosis. Scavenger receptor CD36 directly binds the whole infected erythrocyte to monocyte/mac phagocytic receptors and initiates

phagocytosis. The efficiency of CD36-mediated phagocytosis is dependent upon various factors, including parasite adhesion phenotype. For example, infected erythrocytes from placenta binds to chondroitin sulphate A rather than CD36, thus allowing parasites to avoid early clearance by monocytes/macs. CD36-deficiency has been reported in malaria-endemic regions (Aitman et al., 2000 and Lee et al., 1999).

In this chapter the purified recombinant antigens, AMA1, MSP7 and WARP were used as a diagnostic tool with 14 serum samples of *P. vivax* infected individuals. The role of these antigens on the monocyte phagocytosis was also studied with the help of latex beads and nitro blue tetrazolium assay.

MATERIALS AND METHODS

CHLOROFORM METHANOL PRECIPITATION

400 µl methanol was added vortexed and centrifuged at 13000 rpm for 5minutes to 100 µl of the purified samples. 200 µl chloroform was added, vortexed and centrifuged at 13000 rpm for 5minutes. 300 µl distilled water was added, vortexed and centrifuged at 13000 rpm for 10minutes. The aqueous layer was discarded. 300 µl methanol was added, vortexed and centrifuged at 13000rpm for 10minutes. Methanol was removed to the maximum possible extent and the pellet was allowed to dry at 37ºC (Wessel et al., 1984). The pellet was resuspended in 15 µl of RPMI 1670 media.

BRADFORD ASSAY

Coomassie Brilliant Blue G (100 mg) was dissolved in 50 mL of methanol, 100 mL of 85% H_3PO_4, and made up the volume to 200 mL with distilled water. The solution was kept in dark brown bottle 4°C.

Table 3. 1 Components and their composition used for the preparation of Bradford reagent.

S.No.	Reagent	Concentration
1.	Coomassie Blue G 250	0.5mg/mL
2.	Methanol	25%
3.	H3PO4	42.5%

WESTERN BLOT ANALYSIS

Induced proteins were run on a 10% SDS PAGE followed by electro blotting on to a nitrocellulose membrane with a pore size of 0.20 µm. After electrophoresis the gel was equilibrated in transfer buffer for 15 mins along with the nitrocellulose membrane and whatman filter papers, cut to the size of gel. The electro blot transfer assembly was set up by keeping the gel towards the cathode (-) and membrane towards anode (+) sandwiched between whatman filter papers, in the transfer cassette. This was then placed in the transfer chamber and transfer was carried out at 25mV for 17 hrs. After transfer, the nitrocellulose membrane was was stained with 1% Ponceau S for 45 minutes and de-stained with distilled water. The position of molecular weight marker was marked using a pencil and also transfer was confirmed, so a negative result in western blot analysis means no reaction with sera and not because of no antigen tranfer. The membrane was treated with 2% gelatin as a blocking solution for 1 hour. It was then incubated in 1:1000 diluted serum sample of *P. vivax* infected individual for 2 hours, followed by 3 washes with 0.1% PBS-Tween 20 for 1 minute each. 1:1000 diluted goat antihuman IgG HRP antibody (Genei), which is the secondary antibody, was incubated with the blot for 1 hour and was washed again with 0.2% PBS-Tween

20. It was subsequently developed by treating the blot with 1:10 diluted TMB/H_2O_2 solution (Genei). Reaction was stopped by washing the blot with distilled water.

Reagents:

1. **Transfer buffer**

Table 3. 2 Composition of tank buffer used for western blot

S. No.	Name	Final Concentration
1.	Tris base	25mM
2.	Glycine	192 mM
3.	Methanol	20%

2. **Ponceau S Stain:** 1% Ponceau S in 5% acetic acid.
3. **Phosphate Buffer Saline:** 25 mM Na_2HPO_4 (pH 7) in 0.85% NaCl.
4. **Blocking Solution:** 2% Skim Milk (w/v) in PBS.
5. **PBS Tween:** 0.2% Tween 20 (v/v) in PBS.

ISOLATION AND STIMULATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS) AND MONOCYTES

PBMCs were isolated from healthy individuals (n=3) by density gradient centrifugation. 10ml of blood was layered on 10 ml of Histopaque 1077 (Sigma) and centrifuged at 800xg for 20 min at 20°C. After the centrifugation, the buffy coat obtained was collected and washed twice with Dulbecco's phosphate-buffered saline (DPBS). Finally, 1 x 10^6 PBMCs were resuspended in RPMI-1640 (Sigma) medium containing 10% fetal calf serum (Gibco) and were seeded on an 8-well chamber slide for 2 hours in a 5% CO_2 incubator at 37°C. After 2 hrs, the non-adherent cells were washed vigorously twice with DPBS. The adherent cells were used as monocytes for further experiments. For stimulation, the recombinant antigens were cloned and

expressed in *E. coli* and further purified using Ni-affinity chromatography before being used (explained in chapter 2).

PHAGOCYTOSIS AND RESPIRATORY BURST ASSAYS

The monocytes of healthy individual (n=3) were stimulated with the recombinant antigen in 8 well chamber slide (Nunc, Thermo Scientific, USA) as mentioned above. The media was removed and fluorescent latex beads (Sigma L3030) were added at an MOI of 20:1, followed by incubation at 37ºC for 60 minutes. To remove any loose beads the chambers were washed twice. For phagocytosis assay, the cells were treated with 0.5 ml of 0.2% trypan blue in PBS for quenching non-specific fluorescence for 5 minutes and allowed to air dry following which the slides were sealed in 50% glycerol/ PBS and further examined under a fluorescence microscope (CX-41, Olympus, JAPAN). For respiratory burst assay, cells were treated with 2mg/ml of Nitroblue tetrazolium (NBT) (Sigma) in DPBS for 30 minutes at 37°C and 5% CO_2. The cells were then washed with DPBS and fixed with methanol for 1 min and examined under a light microscope for viewing NBT reduction. The following equation was used to determine the rate of phagocytosis: (cells that have phagocytosed)/ (total number of cells per field of view) and (cells that have phagocytosed more than one bead)/ (total number of cells per field of view).

STATISTICS

Student's t-test and ANOVA was performed followed by Fisher's LSD test for phagocytosis rate compared to that of the control.

RESULTS

WESTERN BLOT DIAGNOSIS OF VIVAX MALARIA WITH RECOMBINANT ANTIGEN

Genomic DNA from the whole blood of a malaria patient was used as templates to amplify the coding regions of apical merozoite antigen (AMA1), merozoite surface protein (MSP) 7 and von Willebrand factor A domain-related protein (WARP) of *Plasmodium vivax*. Each amplified DNA fragment was cloned into a pET30a (+) plasmid to induce the expression of histidine tagged protein in *E. coli* by IPTG. The protein of interest was purified using nickel affinity chromatography. The purified samples were separated on 10% SDS-PAGE followed by western blot analysis with patient sera. When applied with 14 patients' sera (Figure 3.1-16) 12 of them reacted with at least one antigen, while no reaction was observed in 3 normal uninfected sera. A blot was also developed without sera and was used as a control (Figure 3.18). AMA1 reacted with 11 sera (78.57%), MSP-7 reacted with 10 sera (71.42 %) and WARP reacted with 14 sera (100%). Among the 14 vivax infected individuals, 1 serum (7.14%) reacted with one antigen (Figure 3.10, Lane 2); 5 sera (35.71%) reacted with two antigens (Figure 3.9, Lane 2; Figure 3.11,Lane 2; Figure 3.12, Lane 2; Figure 3.13, Lane 1 and 2; Figure 3.14, Lane 1 and 2) and 8 sera (57.14%) reacted with all the three antigens (Figure 3.1, Lane 1 & 2; Figure 3.2, Lane 1 & 2; Figure 3.3, Lane 1 & 2; Figure 3.4, Lane 1 & 2; Figure 3.5, Lane 1 & 2; Figure 3.6, Lane 1 & 2; Figure 3.7, Lane 1 & 2; Figure 3.8, Lane 1 & 2; Figure 3.15, Lane 1-5; Figure 3.6, Lane 6-9;). Sera from 2 healthy individuals were used as a control (Figure 3.17-18, Lane 2, 3).

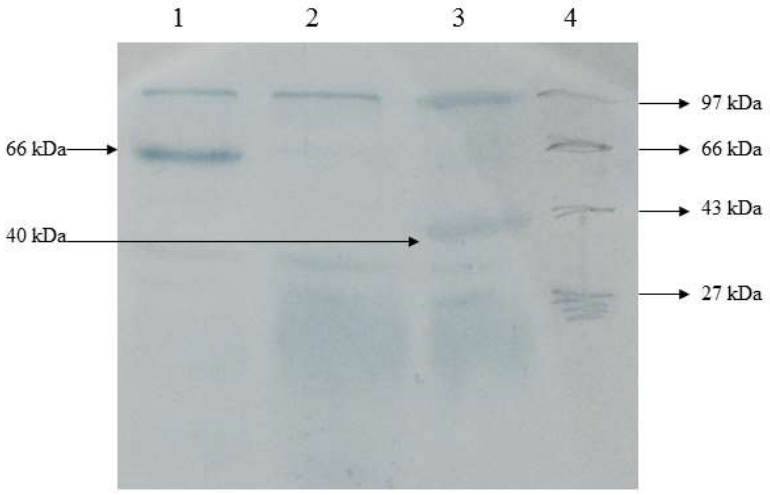
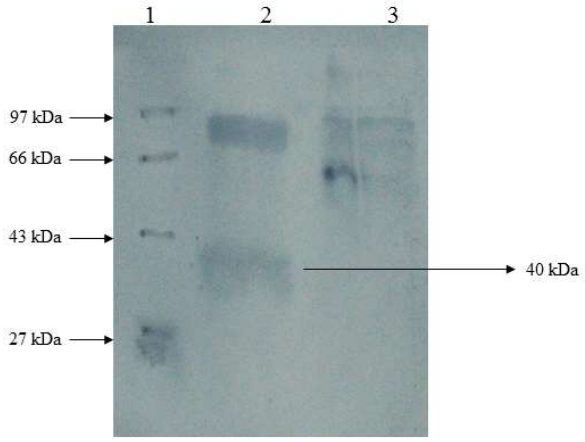


Figure 3. 1 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with serum of patient 1

Lane 1: Purified MSP7 Lane 3: Purified WARP

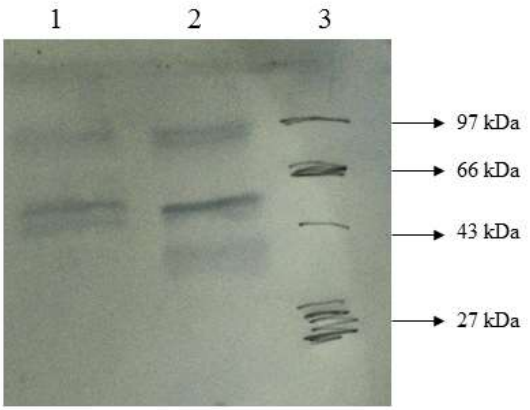
Lane 2: Purified AMA1 Lane 4: High molecular protein marker



Lane 1: High molecular protein marker Lane 3: Purified WARP

Lane 2: Purified AMA1

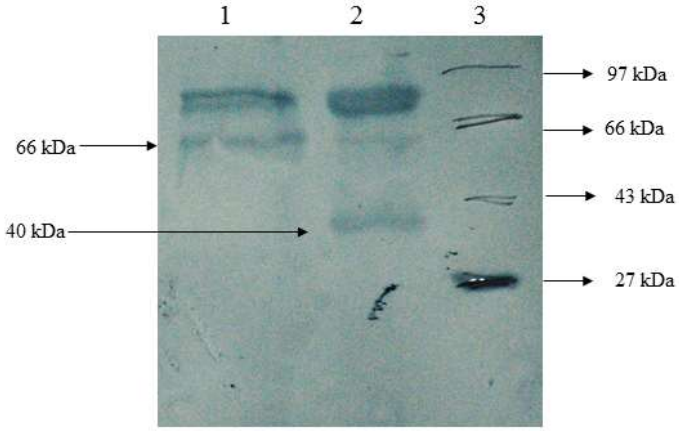
Figure 3. 2 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 2



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

Lane 2: Purified WARP

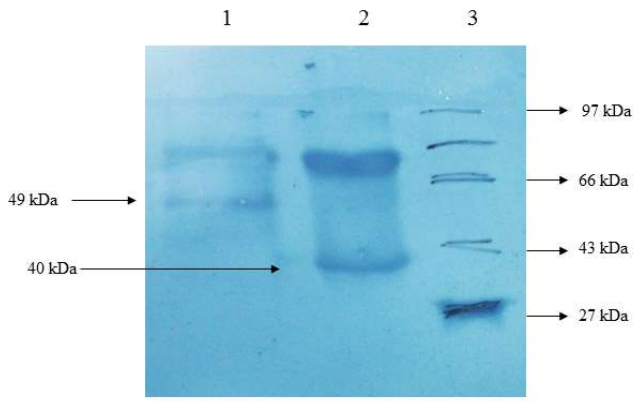
Figure 3. 3 Western blot analysis of recombinant antigen AMA-1 and WARP with serum of patient 3



Lane 1: Purified MSP7 Lane 3: High molecular protein marker

Lane 2: Purified WARP

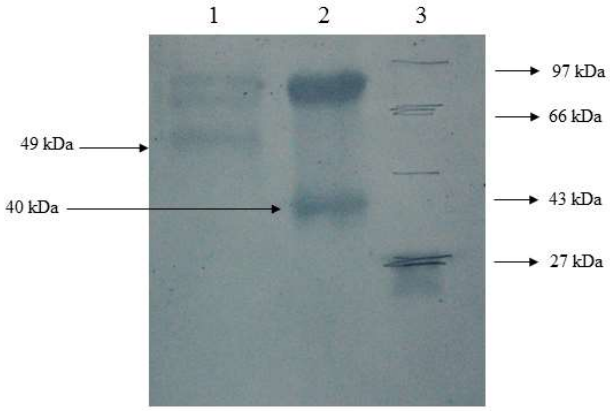
Figure 3. 4 Western blot analysis of recombinant antigen MSP7 and WARP with serum of patient 4



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

Lane 2: Purified WARP

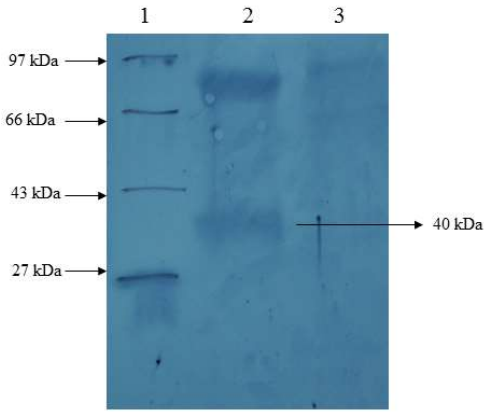
Figure 3. 5 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 5



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

Lane 2: Purified WARP

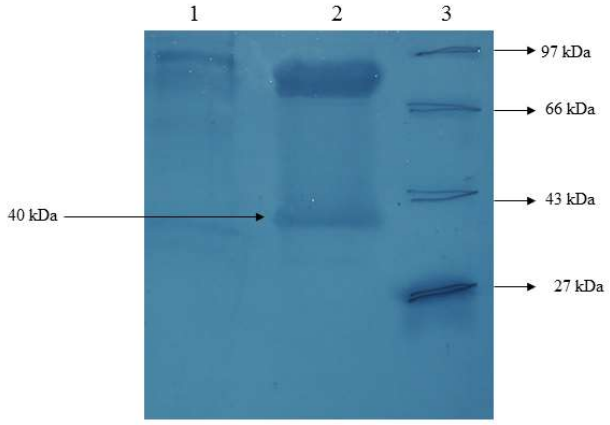
Figure 3. 6 Western blot analysis of recombinant antigen AMA-1 and WARP with serum of patient 6



Lane 1: High molecular protein marker Lane 3: Purified AMA1

Lane 2: Purified WARP

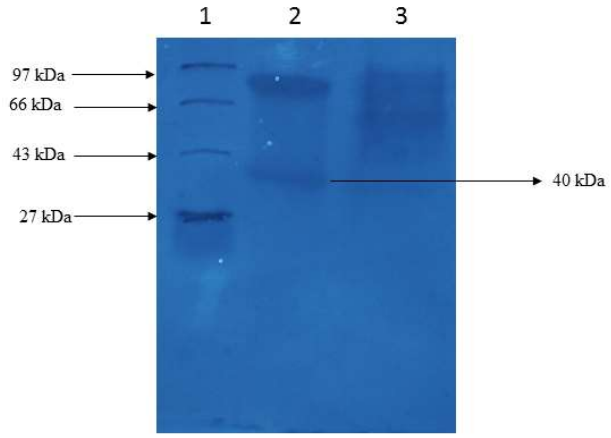
Figure 3. 7 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 7



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

Lane 2: Purified WARP

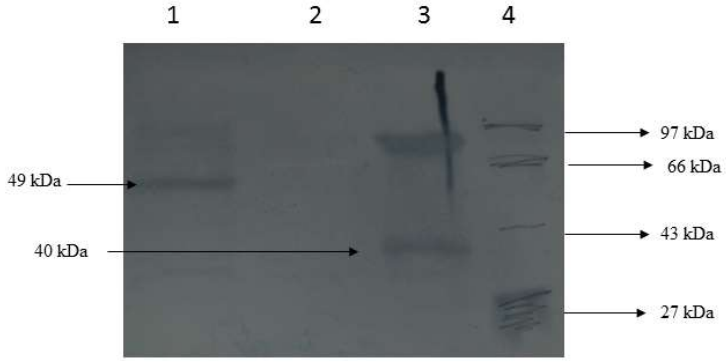
Figure 3. 8 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 8



Lane 1: High molecular protein marker Lane 3: Purified AMA1

Lane 2: Purified WARP

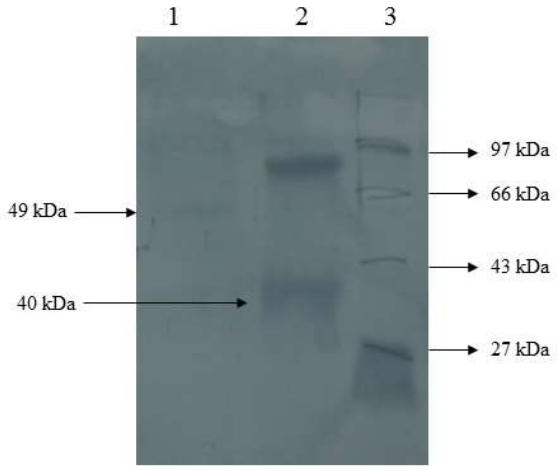
Figure 3. 9 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 9



Lane 1: High molecular protein marker Lane 3: Purified AMA1

Lane 2: Purified WARP

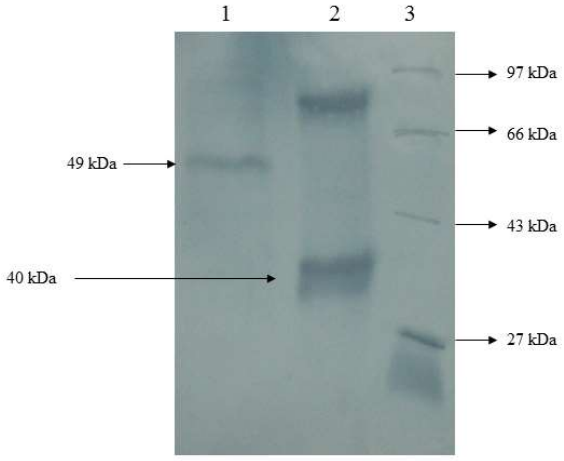
Figure 3. 10 Western blot analysis of recombinant antigen AMA-1 and WARP with serum of patient 10



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

Lane 2: Purified WARP

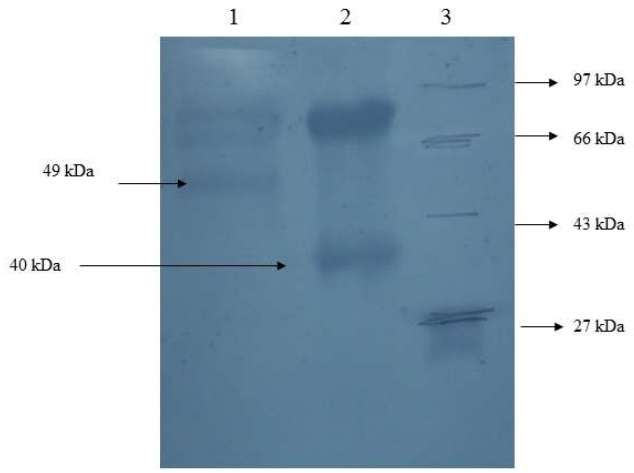
Figure 3. 11 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 11



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

Lane 2: Purified WARP

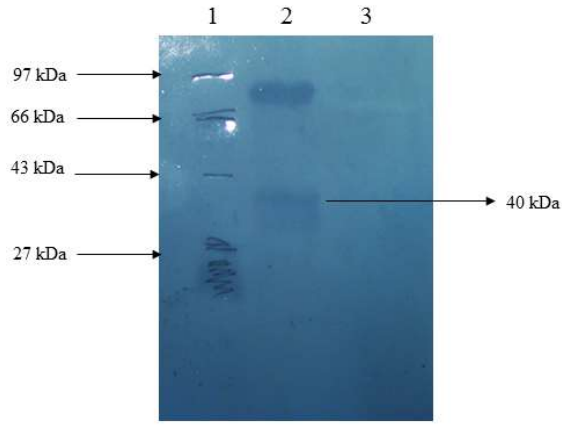
Figure 3. 12 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 12



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

Lane 2: Purified WARP

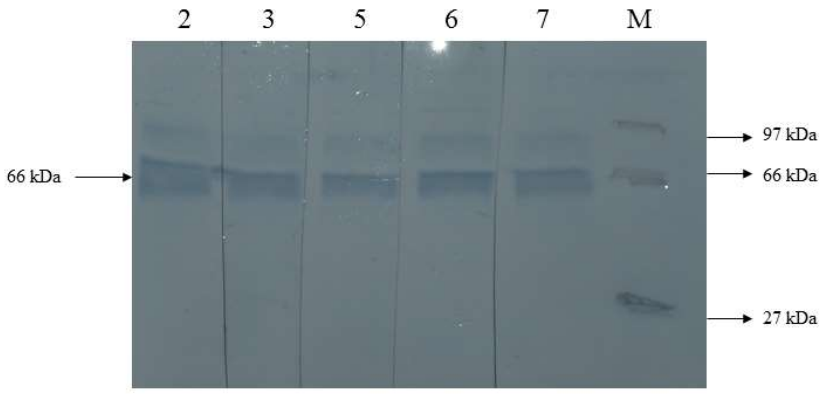
Figure 3. 13 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 13



Lane 1: Purified AMA1 Lane 3: High molecular protein marker

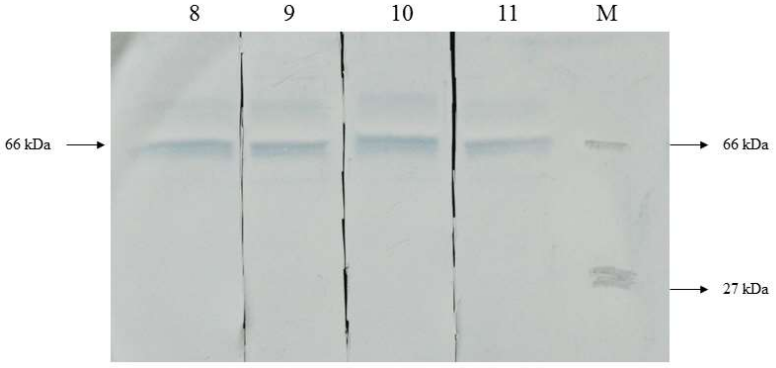
Lane 2: Purified WARP

Figure 3. 14 Western blot analysis of recombinant antigen AMA1 and WARP with serum of patient 14



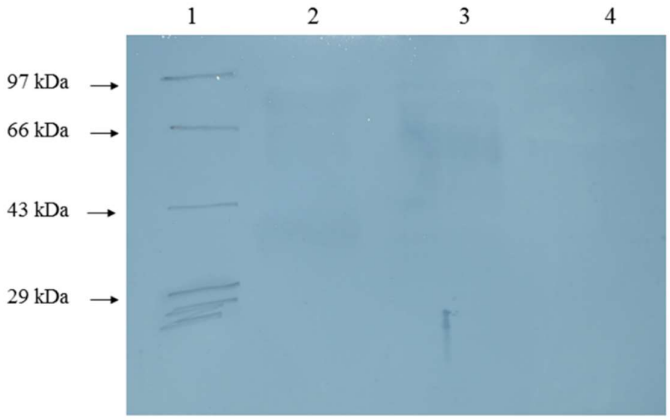
Lane 1-5: Purified MSP7 Lane M: High molecular protein marker

Figure 3. 15 Western blot analysis of recombinant antigen MSP7 with serum of patient 2-3 & 5-7



Lane 6-9: Purified MSP7 Lane M: High molecular protein marker

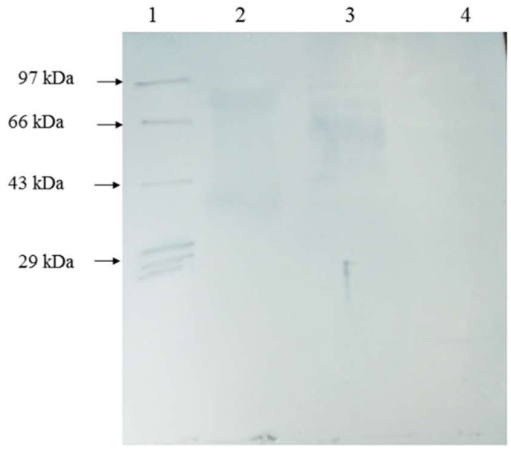
Figure 3. 16 Western blot analysis of recombinant antigen MSP7 with serum of patient 8-11



Lane 1: High molecular protein marker Lane 3: Purified AMA1

Lane 2: Purified WARP Lane 4: Purified MSP7

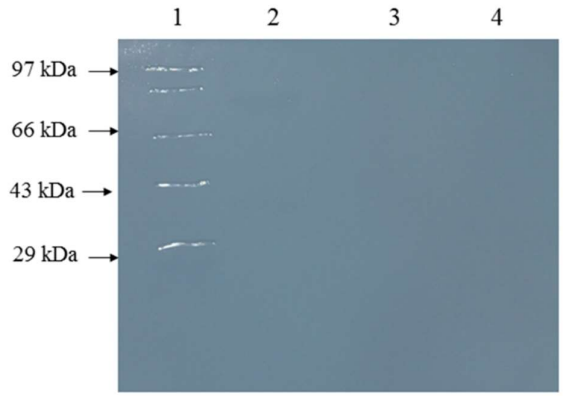
Figure 3. 17 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with serum of control 1



Lane 1: High molecular protein marker Lane 3: Purified AMA1

Lane 2: Purified WARP Lane 4: Purified MSP7

Figure 3. 18 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with serum of control 2



Lane 1: High molecular protein marker Lane 3: Purified AMA1

Lane 2: Purified WARP

Figure 3. 19 Western blot analysis of recombinant antigen AMA-1, MSP7 and WARP with no serum

Table 3. 3 Summary of western blot data.

S. No	NAME	AMA1	MSP7	WARP
1.	No serum	-	-	-
2.	Control 1	Faint bands	Faint bands	Faint bands
3.	Control 2	Faint bands	Faint bands	Faint bands
4.	Patient 1	-	+++	+
5.	Patient 2	+	+++	++
6.	Patient 3	+	+++	+
7.	Patient 4	+	+++	+++
8.	Patient 5	++	+++	+++
9.	Patient 6	+	+++	+++
10.	Patient 7	+	+++	+++
11.	Patient 8	+	+++	+++
12.	Patient 9	-	+++	+
13.	Patient 10	-		+
14.	Patient 11	+		+++
15.	Patient 12	++		++
16.	Patient 13	+++		+++
17.	Patient 14	++		+++

PHAGOCYTOSIS ASSAY

To investigate whether the purified antigens (rPvAMA1, rPvMSP-7 and rPvWARP) have any effect on the rate of phagocytosis by monocytes, they were co-incubated with the monocytes from healthy individuals (n=3) for 72 hours in a chamber slide. Following this latex beads were added to the monocytes and the rate of phagocytosis was studied. Trypan blue was added to quench the presence of any extracellular beads. Monocytes without any beads and antigens were included to account for any non-specific fluorescence. The phagocytosis of the beads was observed in a fluorescence microscope (Figure 3.19-21). To quantify phagocytosis, the percentage of monocytes that had internalized one or more latex beads were scored. Monocytes without antigen served as control. As can be seen from figure 1B, the rate of phagocytosis was reduced for all the three recombinant antigens. Rate of phagocytosis of both, single bead (Figure 3.22) as well as multiple beads (Figure 3.23) in monocytes treated with rPvAMA1 was reduced from 41.92% to 32.67% and from 24.29% to 17.29% respectively. Similarly, for rPvMSP7 the rate of phagocytosis was reduced to 30.17% and 14.03% for single and multiple beads respectively and for rPvWARP to 30.66% and 15.55%. Contrastingly when the respiratory burst in the presence of the antigens was studied by the NBT assay (Figure 2), the blue colour formation due to NBT reduction is more in the case of AMA1 and MSP7 compared to that of the untreated and WARP treated monocytes.

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.

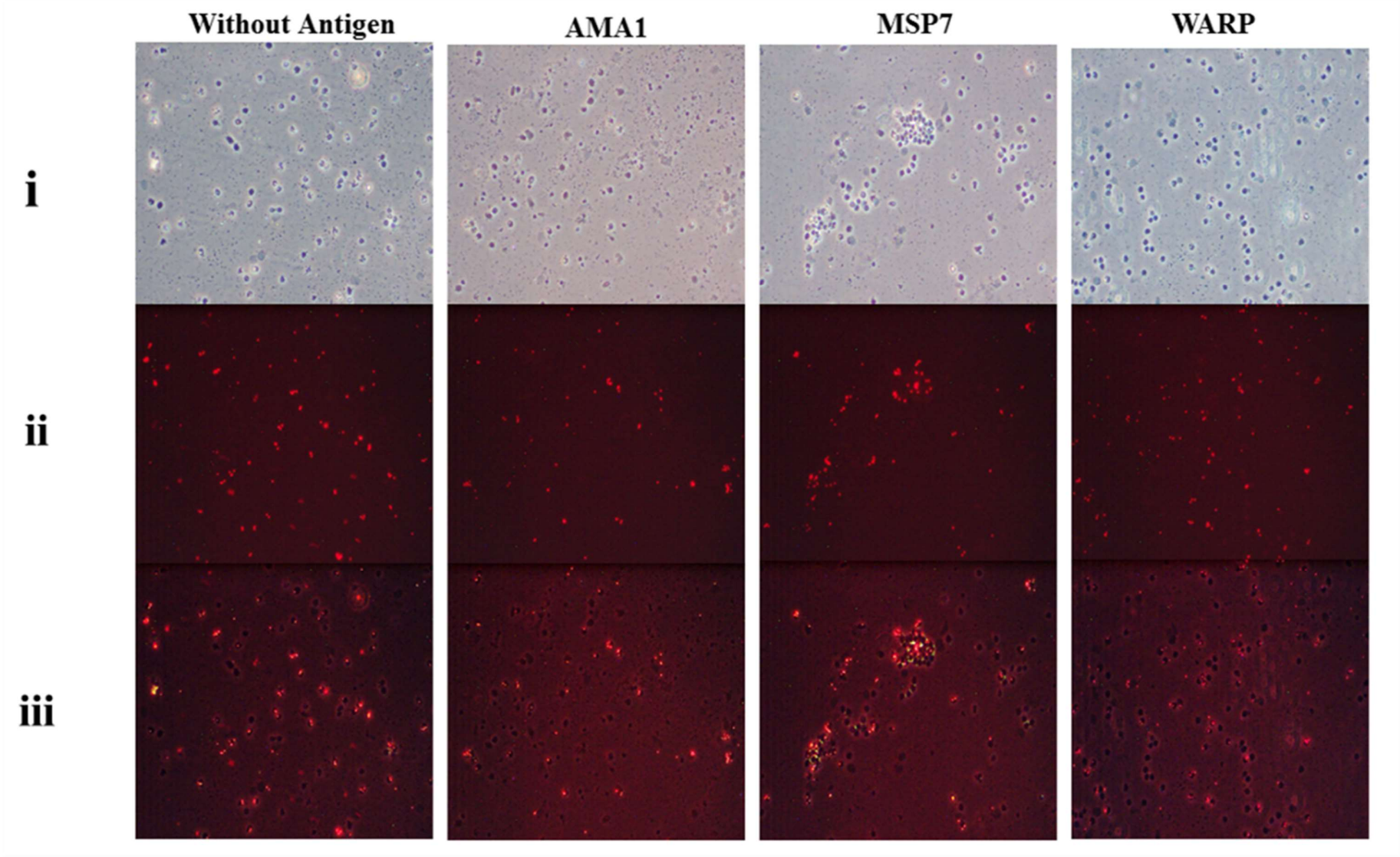

Without Antigen
AMA1
MSP7
WARP
i
ii
iii

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.

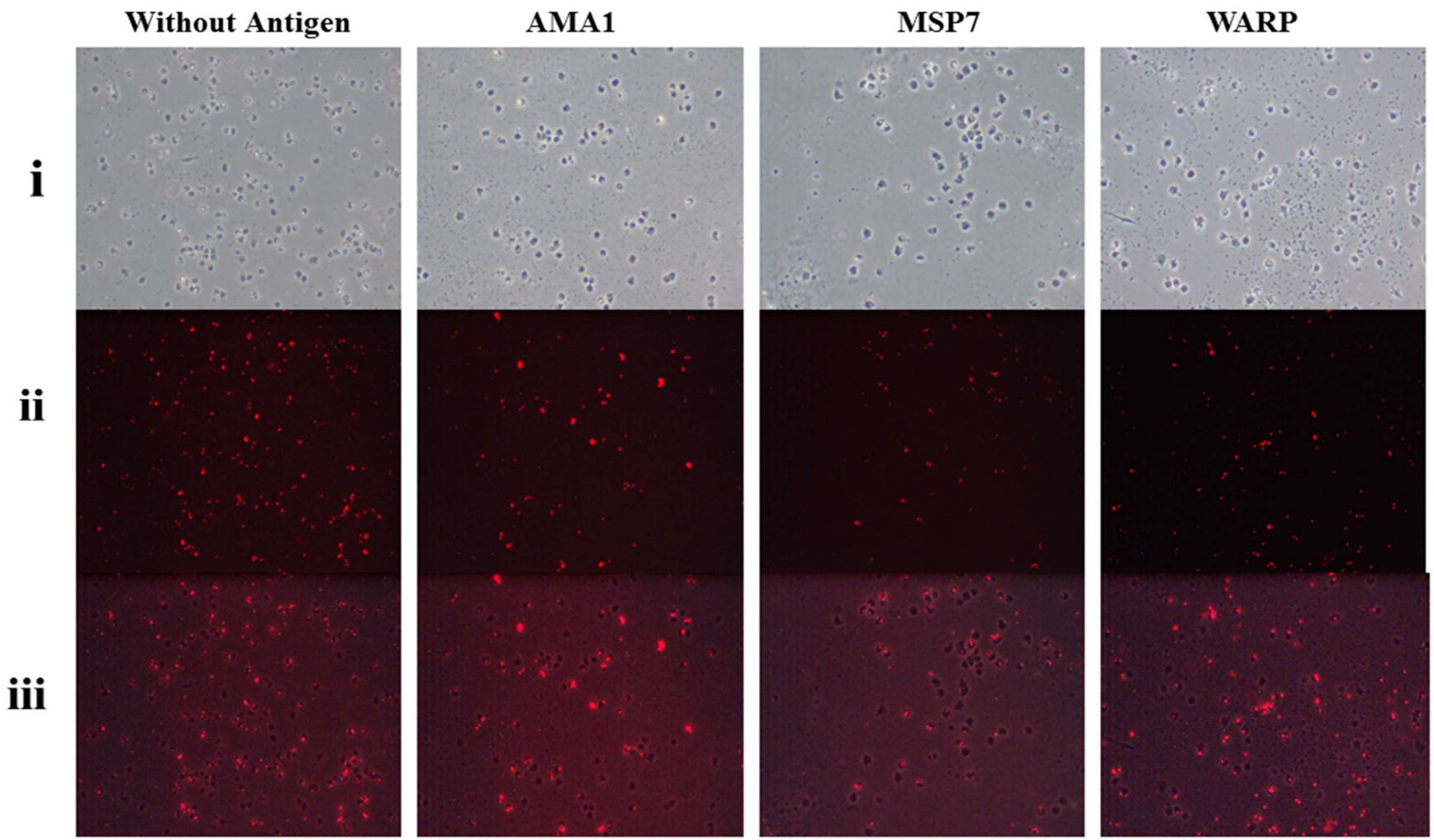
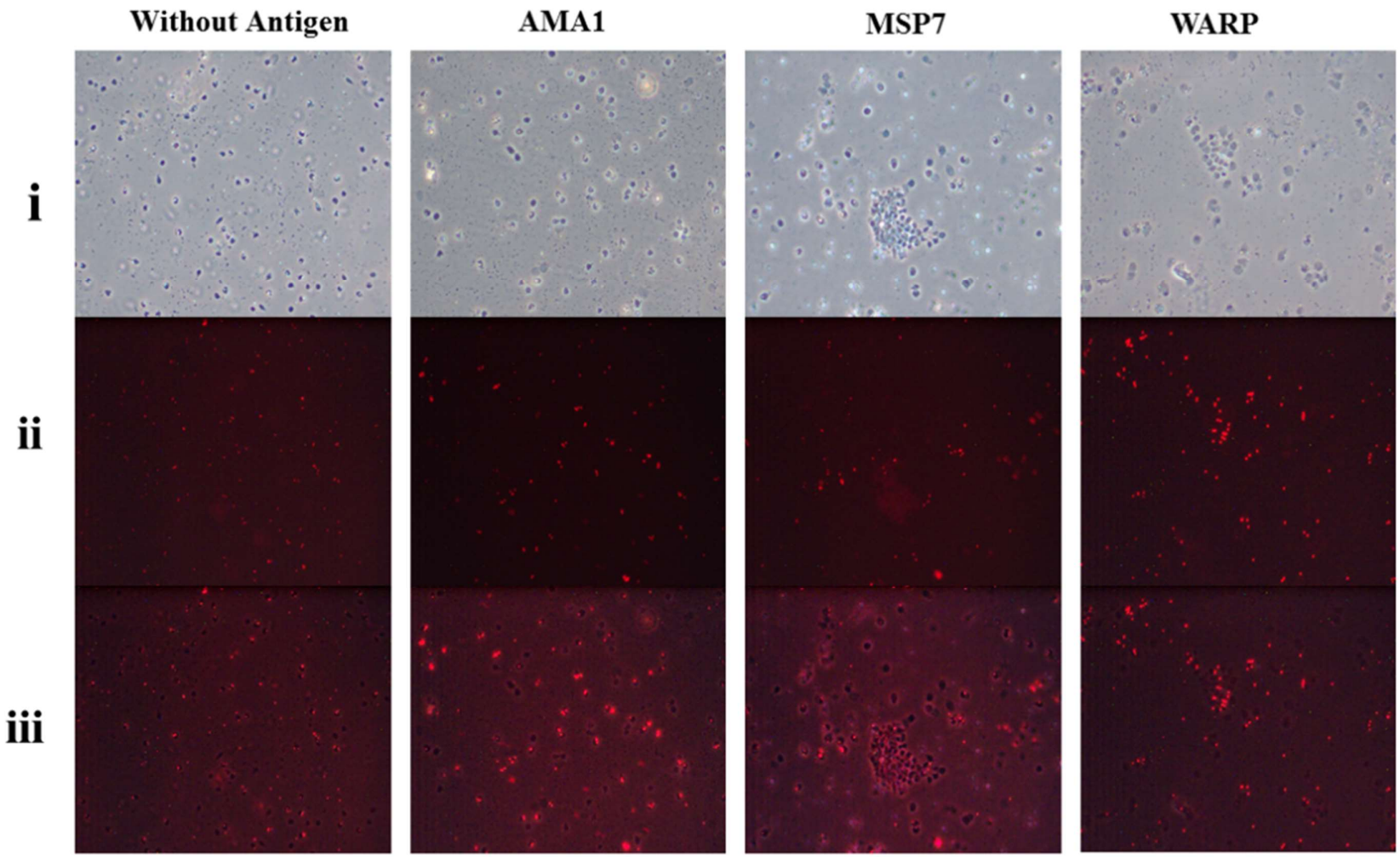

Without Antigen
AMA1
MSP7
WARP
i
ii
iii

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.


Without Antigen
AMA1
MSP7
WARP
i
ii
iii

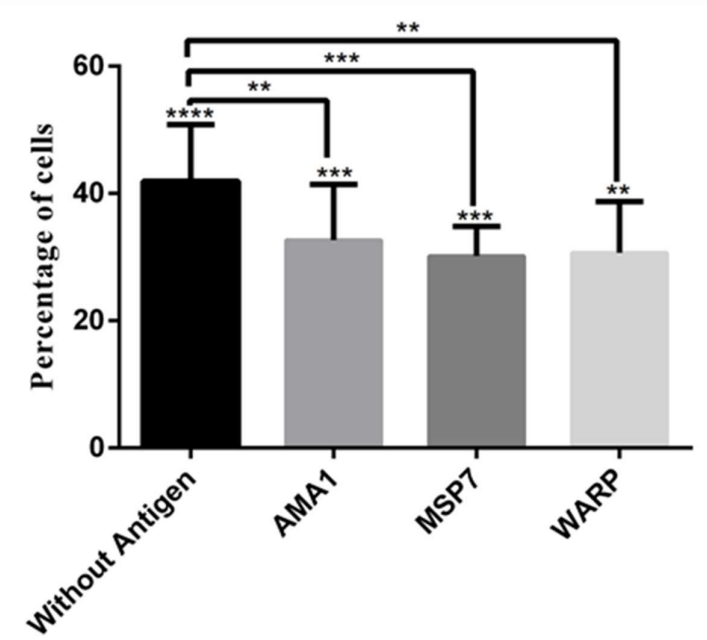


Figure 3. 23 Phagocytosis index: Percentage of macrophages that phagocytosed single latex beads, in the presence and absence of recombinant antigen, were plotted.

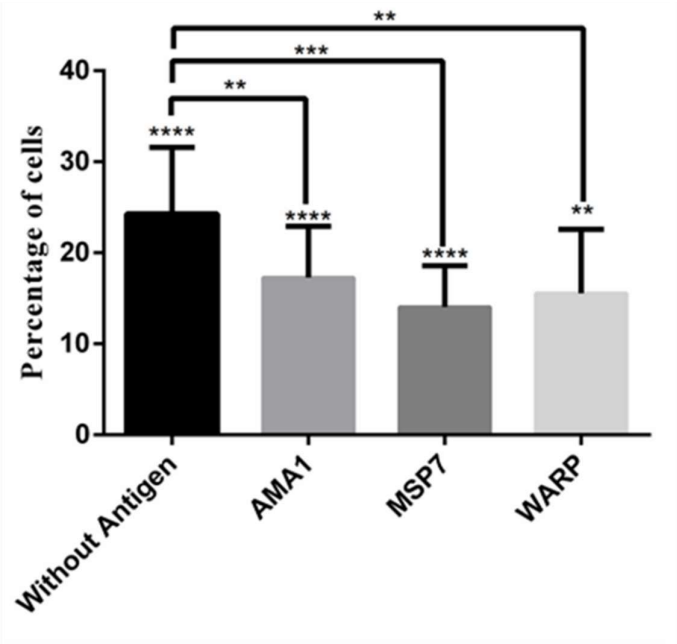


Figure 3. 24 Phagocytosis index: Percentage of macrophages that phagocytosed multiple latex beads, in the presence and absence of recombinant antigen, were plotted

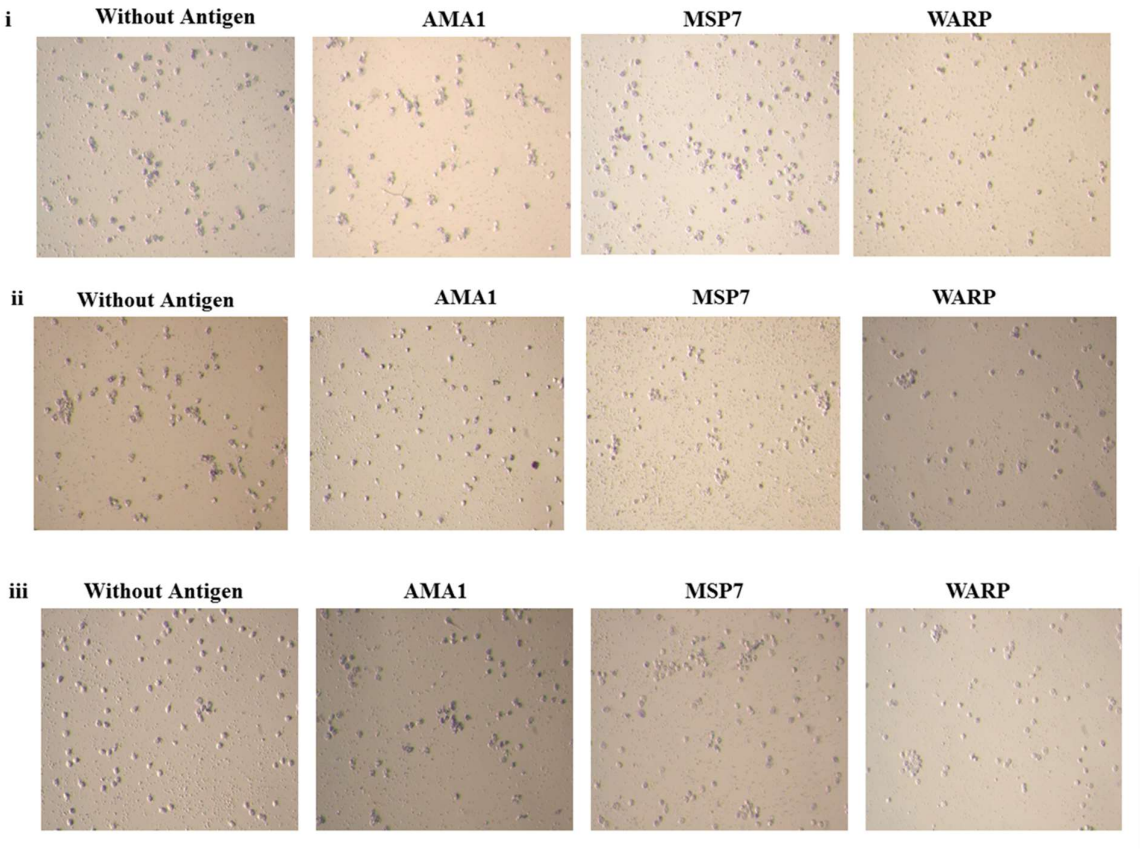


Figure 3. 25 Nitroblue tetrazolium (NBT) reduction by treated and untreated healthy monocytes (i, ii, iii) viewed under 40x magnification.

DISCUSSION

To establish a diagnostic assay, histidine tagged recombinant proteins AMA1, MSP7 and WARP were analysed by western blot using sera from vivax patients. Patient sera used were specific and reactive to each antigen. As explained earlier *P. vivax* sporozoite enters a hepatocyte and begins its exoerythrocytic schizogony stage. This is characterized by multiple rounds of nuclear division without cellular segmentation. After a certain number of nuclear divisions, the parasite cell will segment and merozoites are formed. Under certain conditions, some sporozoites do not immediately start to grow and divide after entering the hepatocyte, but remain in a dormant, hypnozoite stage for weeks or months. The duration of latency is variable from one hypnozoite to another leading to the reoccurrence of parasitaemia or "relapses". So diagnosis with a liver stage antigen will help in controlling the disease. Son et al., (2001) cloned and expressed certain portion of antigenic domain of AMA and used it with 160 patient sera. They reported that 80% of the sera samples reacted with AMA. We observed that our AMA gave comparable results with a reactivity of 78% with patients' sera.

Monocytes are a branch of white blood cells that can differentiate into a range of tissue macrophages and dendritic cells (DCs) (Auffray et al., 2009). Bloodstream monocytes which are derived from bone marrow and are divided as per their size, trafficking and innate immune receptor expression. They also differ in their ability to differentiate following stimulation with cytokines or microbial molecules (Auffray et al., 2009 and van Furth et al., 1968). Monocytes play a key role in host antimicrobial defence and are also involved in many inflammatory diseases (Serbina et al., 2008). Infections with diverse pathogens, like bacteria, parasites, fungi and viruses, may lead to the recruitment of monocytes to sites of infection. It has been reported that during *T. gondii* infection, monocytes are recruited to the lamina propria of the intestine, where they differentiate into major producers of iNOS and TNF (Dunay et al., 2008). Our study demonstrates that when control monocytes are exposed to recombinant malarial antigens their

functions such as phagocytic ability and generation of respiratory burst are compromised. It is widely known that monocytes are important for both phagocytosis and killing of invading pathogens at sites of inflammation, besides their role in antigen presentation after movement into the regional lymph nodes (Janeway et al., 2001 and Shi et al., 2011).

Studies demonstrate that monocytes are capable of phagocytosis of parasites or parasite infected erythrocytes (Silver et al., 2010 and Jafarshad et al., 2007). Protection has been attributed to defective parasite growth or to enhanced removal of the parasitized RBCs. Clearance of parasitized RBCs by monocytes helps in two ways. One, it helps in controlling the infection and second, it limits the excessive inflammation induced by the rupture of parasitized RBCs. This phagocytosis is complement mediated so blocking complement deposition has been shown to prevent ~80–95% of phagocytosis of erythrocytes harbouring immature (ring-stage) parasites in vitro (Turrini et al., 1992). Our study demonstrates clearly that when control monocytes are exposed to recombinant malarial antigens such as AMA1, MSP7 and WARP the phagocytosis of latex beads is drastically reduced. However the ability to generate respiratory burst as seen by the NBT reduction assay varies depending on the antigen used. AMA1 and MSP7 are seen to increase the respiratory burst while the opposite is seen in the case of WARP. This variation in the monocyte functions may be responsible for the diverse outcomes demonstrated by other studies (Chua et al., 2013). Phagocytosis of infected RBCs by monocytes is an effective way of reducing the parasite load in the body. However the efficiency of the clearance of infected RBCs may differ among individuals, resulting in the conflicting degrees of protection and susceptibility to malaria.