Malaria is one of the most prevalent parasitic diseases found in 97 countries with estimated 198 million clinical cases and 584,000 deaths, representing a major global public health problem. Approximately 90% of deaths occur in the African region mostly in children under five years of age. A total of 1.07 million cases and 535 deaths were recorded due to malaria in India (WHO 2014). Majority of malaria morbidity and mortality is caused by *Plasmodium falciparum*. It has been observed that *P. vivax* is also moving towards malignancy which may prove fatal in severe cases. Recent reports have indicated life threatening complications and even death in *P. vivax* infection. This disease is managed by antimalarial drugs as no effective vaccine is yet available. However, the parasite has developed resistance against most commonly used antimalarials, chloroquine and sulfadoxine-pyrimethamine (SP) posing a major hurdle in malaria control programme. Malaria has been a serious problem in some parts of India due to widespread resistance against sulfadoxine-pyrimethamine.

Prevalence of malaria was studied by observing thick and thin blood smears stained with Giemsa and JSB stains under Nikon Eclipse-600 research microscope at 100x. Quantitative Buffy Coat test was also performed to see the positivity of malaria. *P. vivax* patients were treated with chloroquine and primaquine and were observed for 12 months to study relapse pattern. For recrudescence in *P. falciparum*, patients were treated with standard doses of sulfadoxine-pyrimethamine, artesunate plus SP, artemether and artemether plus lumefantrine, and observed for a month after the medication was over. Positivity rates for *P. falciparum*, *P. vivax* and mixed infection during this period were 28.30, 69.39 and 2.29%, respectively. Malaria transmission was high in the months of August, September and October, reaching a peak in September and October. During peak transmission season, mean temperature and relative humidity ranged somewhere around 26-28°C and 77-88%, respectively, which favoured vector population. 38.43% *P. falciparum* cases were uncomplicated type with parasitaemia less than 10%, whereas 61.57% were severe having more than 10% parasitaemia. In *P. vivax* 58.69% patients were uncomplicated type with parasitaemia less than 6%, while rest 41.31% were severe complicated type with more than 6% parasitaemia. In mixed infection, majority (70.91%) were of complicated type with parasitaemia more than 10%, while 29.09% were uncomplicated type. Male predominance was observed in the patients with 59% positivity for malaria.
P. vivax patients treated with chloroquine alone showed relapse in 31.48% cases, of which 25.92% were of short term and 5.55% long term relapse. But those who were treated with 1500 mg chloroquine along with 75 mg primaquine showed 15% short term and 5% long term relapse. Patients who were administered with 210 mg primaquine showed only short term relapse in 6.66% patients. Drug resistance in P. falciparum against sulfadoxine-pyrimethamine (SP) was recorded in 24.44% cases. RI, RII and RIII levels of resistance were recorded in 15.35, 7.50 and 1.58% cases, respectively.

Recent drugs used for the treatment of falciparum malaria were artemisinin based combination therapy (ACT). Artemisinin derivatives which are used in Aligarh are artesunate plus sulfadoxine-pyrimethamine, arteether and arteether plus lumefantrine. Cure rate in P. falciparum cases was 100% with artemisinin plus lumefantrine while recrudescence was observed in 2.04% patients who were treated with arteether alone. In patients treated with artesunate plus sulfadoxine-pyrimethamine, recrudescence was observed in 1.25% patients. The rate of recrudescence is negligible but it appeared more quickly and therefore, it is predicted that its efficacy will decrease in the years to come, if not used judiciously. Among the ACTs, arteether-lumifantrine combination was proved the most effective drug showing no resistance.

For mutation studies in dhfr gene, DNA was extracted from the blood of the patients who were infected with P. falciparum and P. vivax. The extracted DNA was eluted and the aliquot was used for PCR amplification. After diluting, priming PCR product was used as a DNA template for nesting PCR to amplify the 784-bp region, which covered the entire dhfr domain. Semi nested PCR was carried out under the same conditions and the PCR products were purified and subjected to sequencing. The nucleotide sequences were translated into amino acids using the edit sequence program. Finally, the amino acid sequences were aligned using Gen Doc Multiple Sequence Alignment Editor and Shading Utility.

Sequencing study of pfdhfr and pvdhfr gene showed mutations in DNA isolates. pfdhfr mutations were observed at codons S108N, C59R and N51I, maximum being at codon S108N. Double mutations at codons C59R and S108N were observed in 55% isolates, while mutations at N51I and S108N were observed in only 4% isolates.
Single mutation at codon S108\text{N} was detected in 33 isolates, whereas 5\% isolates had wild amino acids with genotype A16, N51, C59, S108, I164.

In \textit{P. vivax}, majority of the isolates (60.87\%) contained the wild type amino acids having genotype F57, S58, T61, S93 and S117. Mutations were observed at codons S117\text{N}, S93\text{H} and S58\text{R}. Frequency of mutation was highest at codon S117\text{N} followed by S93\text{H}. Double mutations (S58\text{R} and S117\text{N}) were noticed in \textit{pvdhfr} gene in 26.09\% isolates, while mutations at single locus were detected at codon S93\text{H} or S117\text{N} in 13\% isolates (6.52\% each). During the present study variations in size, shape and thickness of cytoplasm of the rings were more prominent in \textit{P. falciparum} which had mutations at two loci. This change may be due to the alteration of amino acids in \textit{dhfr} enzyme or a mere coincidence.

Liver enzymes and kidney markers were analysed from the blood of malaria positive patients. These samples were collected, allowed to clot and centrifuged to remove any cell contamination. The sera were then analysed for liver enzymes and kidney markers with the help of standard techniques. Clinical symptoms such as high fever, chills, vomiting, anaemia, splenomegaly, jaundice, respiratory distress, convulsions and neurological sequelae were recorded in \textit{P. falciparum}, \textit{P. vivax} and mixed infections. As far as symptoms are concerned, all the patients had fever but chills were observed in only 80\% patients. Other symptoms such as sweating, headache, nausea, dizziness were more prominent in \textit{P. falciparum}. Maximum numbers of malaria patients belonged to the age group of 21-30 years, followed by 10-20 years. Male patients dominated over females in almost all age groups except 21-30 years where females were more in number.

As far as clinical features are concerned, thrombocytopenia, jaundice, respiratory distress, convulsions, neurological sequelae and acute renal failures were much higher in \textit{P. falciparum} infections compared to \textit{P. vivax}. Splenomegaly was observed in 40\% \textit{P. falciparum} infections compared to 15\% in \textit{P. vivax}. In \textit{P. falciparum} patients, splenomegaly, jaundice and neurological sequelae were more pronounced in children compared to adults. In \textit{P. vivax} these symptoms were present but in a comparatively less number of patients. Both in \textit{P. falciparum} and \textit{P. vivax} infections, splenomegaly, jaundice and neurological sequelae were almost twice among children under the age of ten years compared to the older group.
Haemoglobinaemia was more prominent in *P. falciparum* patients (35%) than in *P. vivax* malaria patients (22%). Liver enzymes and kidney markers were elevated in severe complicated cases of *P. falciparum, P. vivax* and mixed infections where parasitaemia was more than 10% compared to patients having low and mild infections. Elevated liver enzymes clearly indicated liver and kidney damage in a few severe malaria patients having high parasitaemia. Serum bilirubin was also raised in severe malaria cases irrespective of the species. Elderly people between 40-60 years showed higher elevated levels of AST, ALT and ALP compared to younger patients. Serum bilirubin was also raised in older patients compared to younger ones, the values being almost in normal range in the latter. In most of the patients creatinine and urea were found to be in normal ranges except in the older patients where these were at a slightly higher side. Almost similar trend was seen in the liver enzymes and kidney markers in *P. vivax* patients, where elevated values were recorded in severely affected elderly. Creatinine and urea were also slightly raised in older patients. Generally, male patients showed slightly higher levels of liver enzymes as well as kidney markers in almost all age groups.

From the results obtained during this study, it has become evident that prevalence of *P. vivax* has increased from 65.40 to 76.95% if 2011 data is compared with that of 2013. In the mean time, prevalence of *P. falciparum* has decreased from 32.26 to 21.24% during the same years which may be correlated with creation of more breeding places by developmental project in and around Aligarh which increased the vector population and thereby increased transmission of vivax malaria. Decrease in the prevalence of *P. falciparum* may be correlated with the introduction of artemunate and sulfadoxine-pyrimethamine which contained the *P. falciparum* infection to some extent. In *P. falciparum* and mixed infections, severe cases ranged between 62-71%, while in *P. vivax* situation was just reverse and only 41% cases were severe with a few complicated cases. The probable reason for more complications in *P. falciparum* may be its higher rate of multiplication during pre-erythrocytic schizogony and its ability to infect all the RBCs whether young or old which results in higher parasitaemia and more damage to the vital organs such as liver, spleen and brain. In the present study, resistance was observed in 2.04 and 1.28% patients treated with artemether and artemunate plus sulfadoxine-pyrimethamine combination, respectively. The low percentage in the resistance rate of these drugs is indicative of the fact that artemether alone as well as (AS+SP) combination are still quite effective in my study.
area and therefore, may be considered as safe and comparatively much effective drug for the treatment of acute uncomplicated as well as severe resistant falciparum malaria as a viable alternative of sulfadoxine-pyrimethamine.

In *P. falciparum* infection, all the erythrocytic stages, except early ring stage develop in the capillaries of internal organs which probably damage them. As a result of this, serum bilirubin, liver enzymes and kidney markers get increased which might have resulted in liver dysfunctions and kidney failures in some severe cases. Anaemia and thrombocytopenia were other prominent features of malaria which were recorded in more patients of falciparum malaria compared to those of *P. vivax*. Neurological deficits which were more prominent in *P. falciparum* infection were also recorded from *P. vivax* patients, but in a comparatively less number. *P. vivax* produces less number of merozoites while undergoing pre-erythrocytic schizogony in the liver and infects only younger RBCs. This species completes its erythrocytic schizogony in peripheral blood only and generally does not migrate to inner vital organs and results in less pathogenicity. But probably some patients receive higher inoculums of sporozoites which results in higher rates of parasitaemia and damage of the vital organs like that of *P. falciparum*. In such heavy infections almost similar comparable pathological conditions such as splenomegaly, liver dysfunction and neurological deficits are observed in *P. vivax* infection. It has also become clear that the efficacy of both curative as well as anti-relapse drugs has decreased, as recrudescence and treatment failures were observed along with relapse in *P. vivax* in the patients who received chloroquine and primaquine. Relapse cases were more in the patients who were given 75 mg primaquine for 5 days compared to those received 210 mg primaquine for 14 days. Those given primaquine for 14 days showed 6.66% short term relapse, indicating its decreasing efficacy.

As far as mutation in *pfldhfr* gene is concerned, in *P. falciparum* double mutations were recorded in 59% compared to 26% in *P. vivax*. Probable reason for higher mutations in falciparum malaria is continuous long exposure of *P. falciparum* to sulfadoxine-pyrimethamine which was given for about last two decades either alone or in combination with amodiaquine or artesunate which made this drug susceptible and resulted in a higher level of resistance due to mutations in *pfldhfr* gene. Higher rates of double and single mutations in *P. falciparum* were due to the reason that SP was directed against this species and mutations which were reported in a few *P. vivax*
patients were probably due to the reason that clinicians generally do not wait for the species diagnosis and give a medicine which covers both *P. falciparum* and *P. vivax* infections to cure the patients. This resulted in mutation of *P. vivax* also as both *P. falciparum* and *P. vivax* co-exist in the same area and have the same biochemical target in the form of dihydrofolate reductase (dhfr) enzyme.

From the results obtained during this study, it may be concluded that peak transmission of malaria occurs in Aligarh during the months of September and October. Cases of resistance and relapse are increasing due to decreased efficacy of curative and antirelapse drugs. Single and double mutations were observed in *dhfr* genes of both *P. falciparum* and *P. vivax* which were exposed to sulfadoxine-pyrimethamine for many years. This long exposure of parasite to antifolates resulted in mutations which caused recrudescence and a few treatment failures especially in *P. falciparum* which is a malignant species causing damage to vital organs such as brain, liver and spleen etc. and may prove fatal in severe cases. *P. vivax* is also moving towards malignancy in severe infections showing pathology in aforesaid organs. It is therefore, need of the hour that SP should not be given alone in Aligarh as it may cause more mutations and treatment failures. In the meantime, ACT such as artemether plus lumefantrine should not be given to every falciparum patient in this area as artesunate plus SP is still quite effective with recrudescence in only 1.28% cases. Artemether plus lumefantrine should be treated as emergency drug and may only be given in severe complicated cases as it has 100% efficacy rate in this area.