# FREQUENCY AND SEASONAL VARIATION OF PLASMODIUM SPECIES IN SOUTHERN DISTRICTS OF KHYBER PAKHTUNKHWA

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## ABSTRACT

***Objective***: To determine the frequency of malaria and seasonal variation of Plasmodium species in southern districts of Khyber Pakhtunkhwa.

***Study design***: Descriptive study.

***Place and Duration of Study***: Department of Pathology Combined Military Hospital (CMH), Bannu, from 1st January 2010 to 31st December 2011.

***Patients and Methods***: Five thousand eight hundred and seventy eight (5878) patients with symptoms of fever, nausea, malaise and body aches irrespective of age and gender were included in the study. Samples were collected, thin and thick smears of the samples were prepared and stained with Giemsa`s stain. Thick film was used for screening for malarial parasites and species identification was done on thin smears.

***Results***: Out of 5878 patients, 1692 (28.8%) were found to be positive for malaria. Of them 1524 (90%) had *Plasmodium vivax* infection, while 119 (7.0%) patients were infected with *Plasmodium falciparum*, 49 (3.0%) of the patients were infected with both *Plasmodium vivax* and *Plasmodium falciparum*.

*Plasmodium vivax* was most common in the months of August 203 (12.3%) patients, September 235 (14.3%) patients and October 317 (20%), whereas *Plasmodium falciparum* infection was most common in the months of October 34 (28.6%) patients, November 19 (16%) patients and December 30 (25.2%) patients.

***Conclusion***: Malaria is an endemic infectious disease in Pakistan, in the Southern districts of Khyber Pakhtunkhwa and tribal areas of North and South Waziristan. It is prevalent throughout the year and most noticeably from May to November.

**Keywords:** Khyber Pakhtunkhwa, Malaria, *Plasmodium falciparum*, *Plasmodium vivax.*

## INTRODUCTION

Malaria has been declared as the world's worst health problem being responsible for severe illness and deaths in millions of people every year[1]. There were estimated 225 million cases of malaria in 2009 and 216 million cases in 2010. About 655000 deaths were reported due to malaria in 2010 and 781000 in 2009 according to world malaria report 2011[2,3]. In Africa alone, 91% of the deaths occurred, mostly of children under five years. There were an estimated 3.3 billion people at risk in 2010. Malaria was endemic in 106 countries in 2010 including the whole of South and Southeast Asia, Africa, areas of Middle-east, Central and South America[4,5]. Approximately, 40% of the world's population, mostly those living in the world's poorest countries, are at risk of malaria. In some of these countries where the disease burden is very heavy malaria may account for as much as 40% of public health expenditure, 30-50% of inpatient admissions and up to 60% of outpatient visits. Moreover the effects may last for life through increased poverty, impaired learning and decreased attendance in schools and the workplace[6-8].

Malaria continues to be a major public health problem in Pakistan also. The Pakistan Health Management Information System's (HMIS) 2006 report shows malaria as the 2nd most frequently reported disease from public health sector facilities with estimated number of annual malaria episodes of more than 1.6 million[9,10].

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The disease was in the process of elimination in some regions during the 1950s and 1960s but over the past few decades, resurgence is being witnessed with increasing number of cases, rapid spread of drug resistance and increasing insecticide resistance in mosquitoes[11-13]. According to the United Nations World Health Organization (WHO), Pakistan has been classified as a country with moderate malaria prevalence and has relatively well established control programs. Despite this, the disease is estimated to cause at least 50000 deaths out of estimated 500,000 reported malaria cases every year[14].

According to WHO each malarious country must now measure its own burden and progress toward decreasing that burden. Malaria is a serious public health problem in many developing countries but, estimates of the number of malaria cases and deaths frequently have lacked sufficient accuracy for establishing reliable baselines against which to evaluate the success of control measures[15-17].

Four species of the genus Plasmodium cause infections in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*. Virtually all deaths are caused by falciparum malaria. Both *Plasmodium falciparum* and *Plasmodium vivax* are widely distributed in Pakistan with the majority of infections caused by *Plasmodium vivax* in some parts and *Plasmodium falciparum* in others accounting for about 35-40% of cases, the prevalence increasing up to 64% in Sindh[10,11] due to *P. falciparum*. In the last decade there has been a six fold increase in malaria[18] due to *Plasmodium falciparum*. Factors associated with the upsurge include chloroquine resistance across the country, warmer autumns favoring prolonged transmission and a decline in vector control activities[18,19]. *Anopheles culicifacies*, the purported primary vector in the Punjab province was found more or less disappeared by September whereas *Anopheles stephensi* was found more abundant and more common in KPK (Khyber Pakhtunkhwa) than *Anopheles culicifacies*[20].

A study was planned to find out the frequency of malaria and malarial parasites and also their seasonal variation in Southern districts of Khyber Pakhtunkhwa i.e Bannu, Karak, Lakki Marwat and Tank, all but one of them among nineteen highly endemic districts in Pakistan. The Southern districts are lacking proper drainage and irrigation facilities so that sufficient sites are provided for the breeding vectors of plasmodia. This study will help in increasing the awareness among the health community about the most common infectious diseases in Pakistan and in preventing and treating malaria in future.

## PATIENTS AND METHODS

This descriptive study was carried out from 1st January 2010 to 31st December 2011 at CMH Bannu, which serves as tertiary level hospital for the local army and civil population and referral hospital for the adjoining districts of Karak, Lakki Marwat, Tank and tribal areas of North and South Waziristan.

Patients with symptoms of fever, nausea, malaise and body aches and suspected to be suffering from malaria irrespective of age and gender were included in the study. Informed consent was taken and personal histories were obtained. The results of routine laboratory investigation (hemoglobin, TLC and platelets) were endorsed on the proforma. Samples were taken to the laboratory where the blood slides were stained in Giemsa's stain. Malarial parasites were confirmed by thick and thin films of blood smears. The thin film was fixed with methanol and the whole slide was stained for 30 minutes with 3% Giemsa diluted in a phosphate buffer solution, pH 7.2 and examined under a microscope for malaria parasites. The numbers of asexual and sexual stages of *Plasmodium vivax* and *Plasmodium falciparum* were counted against white blood cells (WBCs) in oil immersion field. The thick film was used for screening purpose and the diagnosis was confirmed from the thin film. Parasite index (PI) was estimated on thin

blood film by counting the number of parasitized red blood cells (RBCs) among 1000 RBCs and was expressed as percent of RBCs parasitized. Only asexual forms (ring, trophozoite and schizont) were included for calculating the PI. All blood films were examined for at least 15 min by an experienced laboratory technician under the supervision of pathologist.

**Table-1: Age and gender distribution of patients.**

| | Total | Gender | | Age group (%) | |
|---|---|---|---|---|---|
| | | Male (%) | Female (%) | Pediatric | Adults |
| Cases tested | 5878 | 4297 (73%) | 1581 (27%) | 857 (15%) | 5021 (85%) |
| Cases with malaria | 1692 | 1319 (78%) | 373 (22%) | 221 (13%) | 1471 (87%) |
| *Plasmodium Falciparum* | 119 | 89 (75%) | 30 (25%) | 19 (16%) | 100 (84%) |
| *Plasmodium Vivax* | 1524 | 1194 (78%) | 330 (22%) | 202 (13%) | 1322 (87%) |
| Mixed infection | 49 | 36 (73%) | 13 (27%) | 6 (12%) | 43 (88%) |

**Table-2: Hematological parameters in individuals with and without malaria.**

| | Plasmodium vivax | | Plasmodium falicparum | | No Malaria | |
|---|---|---|---|---|---|---|
| | Mean | Range (mean ± 2SD) | Mean | Range (mean ±2 SD) | Mean | Range (mean ± 2 SD) |
| Hb (g / dl) | 12.7 | 10.8-14.6 | 11.1 | 9.0-13.2 | 13.8 | 11.3-16.3 |
| TLC (109 / L) | 6.0 | 3.7-8.3 | 6.2 | 4.4-8.0 | 8.3 | 4.5-12.1 |
| Platelets (109L) | 122 | 95-149 | 118 | 84-152 | 210 | 125-295 |

**Table-3: Total numbers of patients with malaria in different months.**

| Month | *Plasmodium falciparum* (n=119) | *Plasmodium vivax* (n=1524) | Mixed Infection |
|---|---|---|---|
| January (2010 and 2011) | 5 | 27 | 1 |
| February (2010 and 2011) | 3 | 17 | 2 |
| March (2010 and 2011) | 5 | 31 | 3 |
| April (2010 and 201) | 4 | 67 | - |
| May (2010 and 2011) | 3 | 160 | - |
| June (2010and 2011) | 4 | 182 | 1 |
| July (2010 and 2011) | 2 | 138 | - |
| August (2010 and 2011) | 6 | 203 | 4 |
| September (2010 and 2011) | 4 | 235 | 3 |
| October (2010 and 2011) | 34 | 317 | 21 |
| November (2010 and 2011) | 19 | 103 | 9 |
| December (2010 and 2011) | 30 | 44 | 5 |

### Statistical analysis

The data were compiled for statistical analysis using SPSS version 16. Descriptive statistics were used to find out the incidence of Plasmodium species based on age groups, gender, and month of occurrence (e.g., temporal variation by season). Mean and SD were calculated for quantitative variables. Frequencies and percentages were calculated for qualitative variables.

## RESULTS

Five thousand eight hundred and seventy eight patients were tested for malarial parasites. Out of them 3850 (65.5%) were males, 1171

(19.9%) were females, 447 (7.6%) were male children and 410 (7.0%) were female children. Out of 5878 patients, 1692 (28.8%) were found to be positive for malaria. Of them 1524 (90%) had *Plasmodium vivax* infection, while 119 (7.0%) patients were infected with *Plasmodium falciparum*. Forty nine (3.0%) patients were infected with both *Plasmodium vivax* and *Plasmodium falciparum*. Out of 119 patients infected with *Plasmodium falciparum*, 70 (59%) patients had PI below 5% whereas the remaining 49 (41%) patients had PI between 5%-10%. None of the patients had PI greater than 10%. Out of 1524 patients with *Plasmodium vivax* infection, 1194 (78%) were males whereas out of 119 patients with *Plasmodium falciparum* infection, 89 (75%) were males. Out of 1692 patients diagnosed with malaria, 1471 (87%) were adults whereas 221 (13%) were of paediatric age group. About 5021 male and female while 857 children below the age of 12 were examined for malaria and malarial parasites. Slide positivity was 29.3% for adults and 25.8% for children. (Table-1)

Regarding other haematological parameters, lowest Hb recorded in patients diagnosed with malaria was 6.7 g/dl and highest was 15.1 g/dl. Lowest platelet count recorded was $50x10^9$/l and highest was $267x10^9$/l. Lowest TLC count recorded was $2.3x10^9$/l and highest was $22.9x10^9$/l. Mean and range (mean ±2 SD) for hematological parameters in patients with *Plasmodium falciparum* and *Plasmodium vivax* is given in Table II. However, seasonal variation of Plasmodium species was also noted in the present study. *Plasmodium vivax* was most common in the months of August, 203 cases (12.3%), September 235 cases (14.3%) and October 317 cases (20%). The three months accounting for 46.6% of the load of *Plasmodium vivax* infection as compared to 53.4% for the other nine months. *Plasmodium falciparum* infection was most common in the months of October 34 cases (28.6%), November 19 cases (16%) and December 30 cases (25.2%). The three months accounting for about 70% of the load of *Plasmodium falciparum* as compared to 30% for the remaining nine months (Table III). Highest number of malarial parasites were detected in the month of Oct (372 out of 956 samples) and lowest number was seen in Feb (21 out of 177 samples).

Of them 1524 (90%) had *Plasmodium vivax* infection, while 119 (7.0%) patients were infected with *Plasmodium falciparum*. 49 (3.0%) of the patients were infected with both *Plasmodium vivax* and *Plasmodium falciparum*.

## DISCUSSION

Malaria affects an estimated 500 million people and causes hundreds of thousands of deaths per year worldwide. Falciparum malaria has high mortality as it causes complications like cerebral malaria, renal failure and algid malaria[21]. The incidence of *Plasmodium vivax* was observed to be higher (90%) as compared with that of *Plasmodium falciparum* (7.0%). Yar et al, while studying prevalence of malarial parasite species in Multan district, also observed high incidence of *Plasmodium vivax* (60.5%) and a low incidence of *Plasmodium falciparum* (37.2%)[22].

Similarly, Jan and Kiani, while studying malarial parasites in Kashmiri refugees settled in Muzaffarabad reported high incidence (6.33%) of *Plasmodium vivax* than of *Plasmodium falciparum* (0.67%). Similarly Mohammad and Hussain observed high incidence of *Plasmodium vivax* (5.78%) as compared to *Plasmodium falciparum* (1.08%) among the general population of district Buner. The higher rate of infection was found in age group 1-10 years (11.58%) and the lowest (5.19%) in age group of 51-70 years[24]. Hozhabri et al studied prevalence of plasmodium slide positivity among children treated for malaria in rural health center (RHC) Jhangara, Sindh and observed slide positivity rate of 5.9%[25]. Malaria in paediatric age group of 200 cases was investigated by Jamal et al and found high rate of *Plasmodium vivax* (62.5%) than *Plasmodium falciparum* (36%)[26]. Malaria in Karachi and other areas in Sindh was studied by Mahmood and found that *Plasmodium vivax* was two times more common than *Plasmodium falciparum*[27]. Akbar et al investigated 160 cases of children in Mansehra

and found 140 cases of *Plasmodium vivax* and 12 cases of *Plasmodium falciparum*[28]. Idris et al while studying pattern of malarial infection at Ayub teaching hospital Abbottabad found that out of 1994 patients screened, 145 (7.2%) had malaria. Out of them, *Plasmodium vivax* was seen in the majority (72.4%) than *Plasmodium falciparum* (24.1%)[29].

In current study, 49 cases of mixed infection were noted. Mixed infection (2.3%) was also observed in Multan district by Yar et al[22]. During present study, no case of *Plasmodium malariae* or *Plasmodium ovale* infection was observed, as the same was also not observed by Yar et al in Multan.

Seasonal variation was also noted in the present study. *Plasmodium vivax* was most common in the months of August/September. The three months accounting for 46.6% of the load of *Plasmodium vivax* infection as compared to 53.4% for the other nine months. *Plasmodium falciparum* infection was most common in the months of October 34 cases (28.6%), November 19 cases (16%) and December 30 cases (25.2%). The three months accounting for about 70% of the load of *Plasmodium falciparum* as compared to 30% for the remaining nine months. Bhalli and Samiullah investigated 120 cases of Falciparum malaria at CMH Multan to evaluate seasonal variation and modes of presentation. They observed high incidence of Falciparum malaria among troops in the months of August to November[21]. Mohammed and Hussain studied prevalence of malaria in general population of district Buner and highest rate of infection (11.6%) was recorded in August and lowest rate (3.9%) was observed in March[24].

## CONCLUSION

Malaria is a very common infectious disease in Pakistan and particularly in the Southern districts of Khyber Pakhtunkhwa and tribal areas of North and South Waziristan. It is prevalent throughout the year and most noticeably from May to November.

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