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Prevalence and Rising Burden of Malaria in District Mohmand: A Three-Year Retrospective Study

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ABSTRACT

Background: Malaria is still a serious threat to public health in Pakistan, specifically in tribal districts with limited healthcare access. This study aimed to investigate the prevalence and species distribution of malaria in District Mohmand over a three-year period. **Methods:** A retrospective descriptive study was carried out using data collected from January 2021 to December 2023. Records of 205,557 suspected malaria cases were obtained from the Directorate of Malaria Control, District Mohmand. Diagnostic methods included Rapid Diagnostic Tests (RDT) and microscopy. Data were analyzed by year, species, gender, age group, treatment, and diagnostic method. **Results:** Of the total 205,557 individuals screened, 19,967 (9.27%) were confirmed malaria cases. *Plasmodium vivax* accounted for 95.15% of cases, followed by *P. falciparum* (3.15%) and mixed infections (1.57%). The prevalence increased from 8.15% in 2021 to 12.14% in 2023. Males (54%) were more frequently infected than females (46%). The highest prevalence was found in individuals aged 15+ years (59%). RDT was the primary diagnostic method used (95%). **Interpretation & Conclusion:** The study highlights an increasing trend in malaria prevalence in District Mohmand, with *P. vivax* as the dominant species. Adult males were more affected, likely due to occupational exposure. Enhanced vector control, early detection, and focused public health interventions are recommended to reduce malaria burden in the region.

INTRODUCTION

Malaria is a life-threatening disease transmitted by female Anopheles mosquitoes and caused by parasites of the genus *Plasmodium*. It continues to be one of the most widespread public health challenges globally, contributing significantly to both illness and death. Despite its impact, malaria can be prevented and treated effectively, and it is still very common in many tropical regions (WHO, 2023). In 2022, approximately half of the world's population was at risk of contracting malaria, affecting an estimated 249 million people and around 608,000 deaths occurring across 85 countries (WHO, 2023). Malaria poses the greatest threat to children under five years of age, who are the most vulnerable group. Every two minutes, a child dies from the disease, with the vast majority of these deaths occurring in the most underserved regions of sub-Saharan (UNICEF, 2024). During the 16th century, Spanish explorers in Peru discovered the medicinal potential of the Cinchona tree, later identified as Cinchona succirubra, whose bark was used to treat malaria. The bark yielded quinine, a powerful antimalarial compound. In 1820, French chemists Pierre Joseph Pelletier and Joseph Bienaimé Caventou successfully isolated quinine as the active component of Cinchona succirubra, marking a significant advancement in the fight against malaria. In 1970, Dr. Youyou Tu and her team of Chinese researchers discovered artemisinin, a potent antimalarial compound derived from the plant Artemisia annua. Currently, artemisinin-based therapies are widely used in regions facing chloroquine resistance, reflecting the continuous advancement in malaria treatment strategies (Talapko et al., 2019). Malaria in human is caused by five closely related single-celled parasites: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. Plasmodium falciparum* is the most lethal malaria parasite, causing malignant tertian malaria with an incubation period of 9-14 days. *Plasmodium vivax* is the most commonly found malaria parasite globally, responsible for causing benign

tertian malaria. Its incubation period typically ranges from 18 to 40 days. *Plasmodium ovale*, the least prevalent among human parasites, is also considered the least pathogenic. Primarily, individuals of African descent have demonstrated susceptibility to *Plasmodium ovale*. The incubation period for this species ranges from 18 to 40 days. *Plasmodium malariae* is uncommon in Asia, displaying a slow growth rate throughout all stages of its life cycle. The incubation period for this parasite ranges from 12 to 17 days. *P. falciparum* being the most lethal (WHO, 2011). Malaria diagnosis primarily relies on microscopy and rapid diagnostic tests (RDTs). Microscopic examination of thick and thin blood smears remains the gold standard, showing ~95% sensitivity and 98% specificity (Cordrary & Richard, 2017). RDTs detect malaria antigens such as pHRP-2, pLDH, and aldolase, providing results within 15–20 minutes (Wilson, 2012; Amir et al., 2018). Both methods can detect approximately 50–200 parasites/µL, offering reliable tools for malaria screening (Kolluri et al., 2018; Pham et al., 2018). Only female *Anopheles* mosquitoes can transmit human malaria, yet of the roughly 430 known species, only 30–40 act as natural vectors; the rest rarely bite humans or cannot support parasite development. These mosquitoes feed during the late evening or night to obtain blood for egg production and thrive in warm environments, where higher temperatures accelerate parasite development within the vector. Their life cycle has four stages, egg, larva, pupa, and adult with the first three occurring in water for 7–14 days, depending on species and temperature (CDC, 2020; CDC, 2023). Vector species vary regionally; in Pakistan, *Anopheles culicifacies* and *Anopheles stephensi* are predominant, with *A. stephensi* accounting for an estimated 65% of annual cases (Directorate of Malaria Control, 2015).The management of malaria has evolved considerably over time, progressing from the early use of quinine and chloroquine to the development of artemisinin and its derivatives. Currently, artemisinin-based combination therapies (ACTs) are the World Health Organization's recommended first-line treatment for uncomplicated *Plasmodium falciparum* malaria, owing to their rapid action and efficacy against resistant strains. Chloroquine remains effective in regions where parasite resistance has not yet emerged, while primaquine continues to play an important role in eliminating dormant liver stages of *P. vivax* infections. The continuous refinement of these therapeutic strategies reflects the global commitment to reducing malaria morbidity and mortality (Talapko et al., 2019). Malaria prevention focuses on reducing mosquito bites through insecticide-treated nets (ITNs) and indoor residual spraying (IRS). Environmental management to eliminate mosquito breeding sites and the preventive use of antimalarial drugs in high-risk groups remain essential measures (WHO, 2023). During a mosquito bite, sporozoites enter the skin and move through the bloodstream to the liver, where they avoid host immunity (Amino et al., 2006). After leaving the liver, merozoites invade red blood cells and replicate asexually (schizogony), producing up to 32 new merozoites every 24–72 hours. This cycle causes acute and chronic malaria. *P. vivax* mainly infects reticulocytes, limiting parasitemia, while *P. falciparum* can invade many red blood cells, often leading to more severe disease. A portion of parasites develop into sexual stages called gametocytes, which remain in the bloodstream long enough to be taken up by mosquitoes. In the mosquito midgut, they exit red blood cells, forming microgametes and a macrogamete, which fuse into a zygote (Sologub et al., 2011). The zygote transforms into an ookinete, crosses the midgut wall, and forms an oocyst. New sporozoites develop inside the oocyst, then migrate to the salivary glands to infect a new host during the next bite. Extensive research has been conducted on human malaria and its prevalence in various regions of Pakistan; however, no comprehensive study has yet been undertaken in District Mohmand. Considering the significance of the disease, the current study was initiated to examine the *Plasmodium* species infecting humans in this region, determine their overall prevalence, assess annual fluctuations, and analyze the association of infection with the host's sex and age within the general population of the selected area.

MATERIALS AND METHODS

Description of The Study Area

District Mohmand is the tribal district of Khyber Pakhtunkhwa, Pakistan. It is located on Pak-Afghan border. The area is comprised of rugged mountains with barren slopes. District Mohmand is located in Peshawar Division, Khyber Pakhtunkhwa, Pakistan, bordering Afghanistan to the west. It covers an area of about 2,296 km^2 with its headquarters at Ghalanai. The region is mountainous with rocky hills and settlements mainly along the riverbanks. It shares boundaries with Bajaur, Khyber, Malakand, Charsadda, and Peshawar districts (Aziz et al. 2018). District Mohmand has a semi-arid climate with hot summers reaching up to 40°C and cool winters dropping to around -1°C. The region receives about 93 mm of rainfall annually and remains dry most of the year. The average temperature is 26°C with low humidity (24%) and a mean UV index of 6.

Patients Selection

The data was collected from the head office of the Directorate of Malaria Controle district Mohmand, KP. The data were included patients from all the 8 parts of the district. The study included about 205557 patients which were tested from January 2021 to December 2023. The DMC have provided the malaria diagnostic kits and medicines in each Union Council's public/private hospitals/clinics of the district. The blood sample was taken from from every suspected patient for the investigation of malaria parasite by an authorised laboratory technician. A proforma of data report were also filled in laboratory during field work in which the information about, patient name, sex, age, locality, pregnancy, presence or absence of malaria parasite and type of parasite were mentioned.

Blood Collection

From each susceptible patient the blood was collected by prickling their ring finger and incase of neonate and infant under 6 month the prick was made flat underneath plan of foot. The blood was the investigated by microscope or simply RDT.

Method-I
Microscopy
Materials used for Method-I included: microscope for screening slides, lancet for pricking the finger, slides, Giemsa stain (methylene blue, eosin), oil immersion for clear observation, and cotton and spirit for cleaning the fingertip.
In case of microscopy thick and thin blood smear of blood samples were made on grease free slide and then dries for 4-5 minutes.
a. Thin Blood Preparation: A drop of each blood sample was placed near one end of a clean, grease-free glass slide. Using the edge of a cover slip held at an angle of approximately 45 degrees, the blood was spread evenly across the slide to form a uniform thin smear. Each slide was labeled at the margin using a diamond pencil and left to air-dry horizontally under light for 3–5 minutes. The thin blood films were then fixed by applying a drop of absolute methanol for one minute, followed by air drying for an additional 1–2 minutes.
b. Thick Blood Preparation: A drop of the blood sample was placed at the center of a clean, grease-free glass slide. Using the edge of a second slide, the sample was spread to form a circular thick blood film. The slide was labeled at the margin with a diamond pencil and left to dry in a horizontal position for 4–6 minutes.
c. Staining Technique and Sample Examination: All blood films (thick and thin) were airdried before Giemsa staining. Using a working solution diluted between 1:8 and 1:12, slides were stained for approximately 6–12 minutes, while more concentrated stains (~10%) allowed shorter stain times of about 4–6 minutes. After staining, slides were rinsed in tap water and reairdried before examination under 100× oil immersion. This follows established staining protocols in the histology literature (IHC World, 2024; CDC, 2020).
The thick smear were examined microscopically for the detection of malaria parasite, *Plasmodium* but the thin films were used for the detection of the *Plasmodium* species, *Plasmodium vivax* and *Plasmodium falciparum*.

Method-II
Rapid diagnostic test (RDT)
Materials used for Method-II included: Rapid diagnostic test (RDT) kit, buffer (to carry blood along the length of the RDT), lancet for pricking the finger, dropper to suck blood, cotton swab for cleaning, and alcohol swab for sanitization.
Rapid diagnostic tests (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available.
Malaria RDTs detect specific antigens (proteins) produced by malaria parasites that are present in the blood of infected individuals (WHO, 2021).
In the current study mostly the cases were tested by RDT method.
In this method the blood was taken by the prickling of ring finger of the suspected patient but in case of neonate and children under 6 months the pricked was made on the flat underneath the plan of foot. Then the blood was sucked by the dropper and poured into the S-hole of the RDT device. Then 2 drops of buffer were added into the B-hole. Buffer carries the blood along the length of the RDT. After 20 minutes of blood and buffer dropped, the RDT was showed the result. The RDT devices used in this study were designed for *P. falciparum* and *P. vivax* or both.
The result was showed four possibilities,
If the line made at P.f position, so it was considered *P. falciparum* positive.
If the line made at P.v position, so it was considered *P. vivax* positive.
If the lines were showed both at the P.f and P.v positions, so it was considered mixed positive.
If only one line showed at position C, so it considered malaria negative.

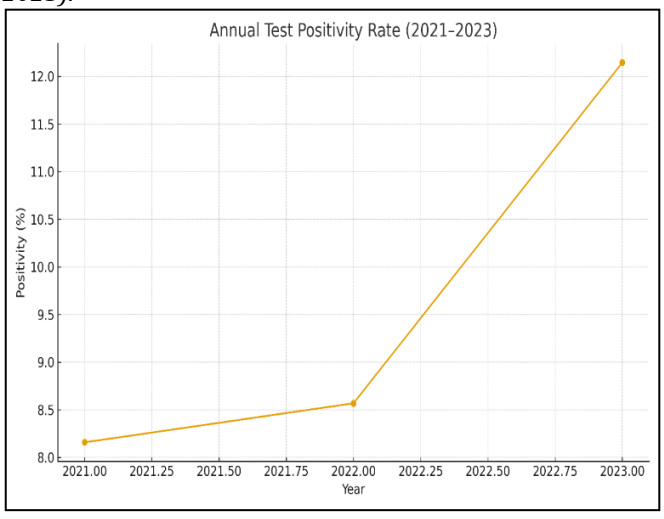
Data Analysis
The data was performed by using %age, ratio, and other mathematical and statistical rules in the annual wise data sheet. From the data sheet, annual wise, overall, species wise, gender wise, age wise, treatment wise and on the basis of testing method prevalence of the malaria infection was calculated.

RESULTS
Overall Burden 2021–2023
From 2021 to 2023, a total of 205,557 suspected patients were screened in District Mohmand, of whom 19,967 were laboratory-confirmed malaria cases. The annual distribution of positives was 5,255 in 2021, 5,820 in 2022, and 8,892 in 2023. Test positivity increased from 8.16% in 2021 to 8.57% in 2022, reaching 12.15% in 2023.

Table 1
Total Tested People and Positivity Rate

Year	Total Tested	Positive	Positivity (%)
2021	64,410	5,255	8.16
2022	67,925	5,820	8.57
2023	73,222	8,892	12.14

Figure 1
Annual Test Positivity Rate in District Mohmand (2021-2023).

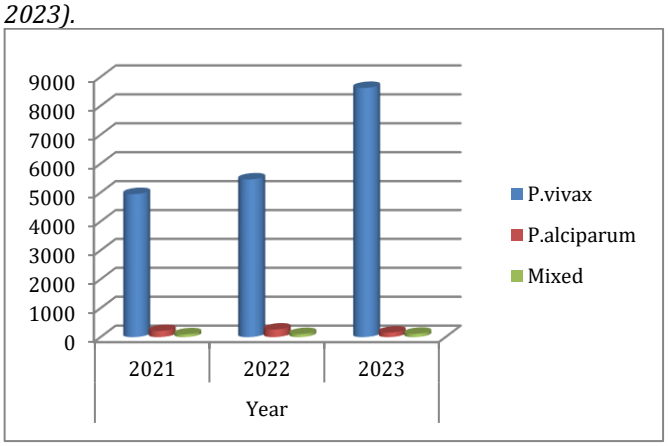


Species Distribution
Across the three-year period, *Plasmodium vivax* accounted for the overwhelming majority of infections (19,023; 95.15%), followed by *P. falciparum* (630; 3.15%) and mixed infections (314; 1.57%).

Table 2
Shows Malarial Cases in Different Species

Year	Cases		Species		
	Total Tested	Positive Cases	*P. falciparum*	*P. vivax*	Mixed species
2021	64410	5255	211	4948	96
2022	67925	5820	261	5455	104
2023	73222	8892	158	8620	114
Total	205557	19967	630	19023	314

Figure 2
Species Distribution of Confirmed Malaria Cases (2021–2023).

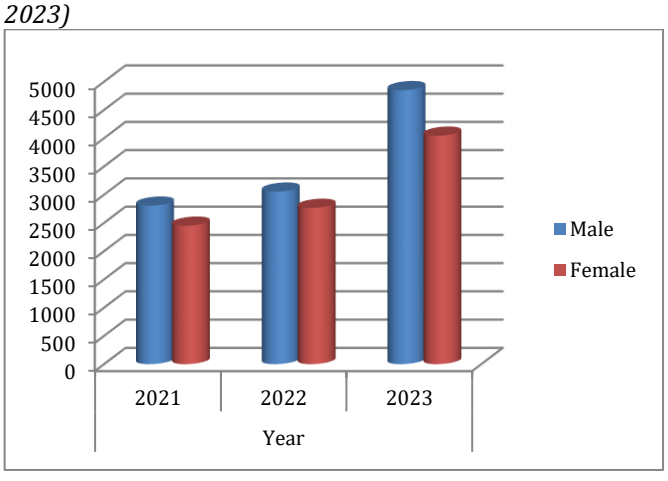


Gender-wise Distribution
During 2021–2023, 10,707 males (54%) and 9,259 females (46%) were confirmed positive. The higher male count likely reflects greater outdoor exposure and mobility.

Table 3
Shows Male and Female Patients in Different Years

Year	Gender	
	Male	Female
2021	2805	2450
2022	3053	2766
2023	4849	4043
Total	10707	9259

Figure 3
Gender-wise Distribution of Confirmed Malaria Cases (2021-2023)

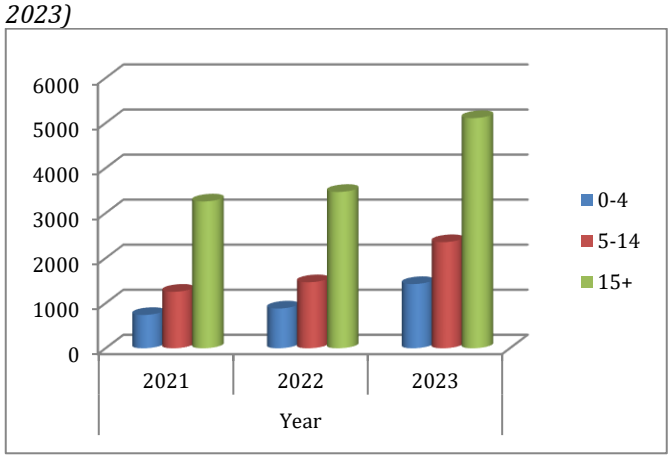


Age-wise Distribution
Age-stratified analyses showed 3,049 cases in children aged 0–4 years (15%), 5,077 cases in the 5–14 years group (25%), and 11,841 cases among individuals aged 15 years and above (59%).

Table 4
Shows Age-wise Patients in Different Years

Year	Age		
	0-4	5-14	15+
2021	740	1255	3260
2022	883	1466	3471
2023	1426	2356	5110
Total	3049	5077	11841

Figure 4
Age-wise Distribution of Confirmed Malaria Cases (2021-2023)

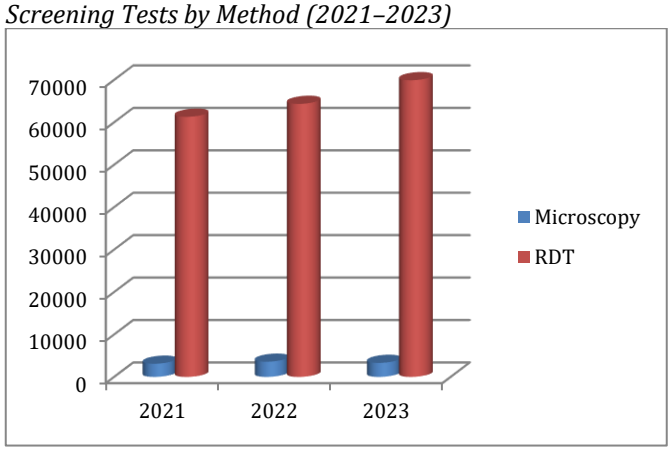


Diagnostic Methods Utilization
Two diagnostic methods were used: microscopy and rapid diagnostic tests (RDTs). Of the 205,557 total screenings, 9,989 (5%) were performed by microscopy and 195,568 (95%) by RDT, indicating near-universal dependence on RDTs in routine screening.

Table 5
Screening Test by Method (2021-2023)

Year	Test Method	
	Microscopy	RDT
2021	3108	61302
2022	3578	64347
2023	3303	69919
Total	9989	195568

Figure 5
Screening Tests by Method (2021–2023)



Treatment Patterns Among Reported Patients

Uncomplicated malaria was treated predominantly with Primaquine (for radical cure in P. vivax) and Chloroquine, alongside ACT (artemether–lumefantrine) and quinine where indicated. No severe malaria cases requiring injectable artesunate or injectable quinine were reported in the dataset.

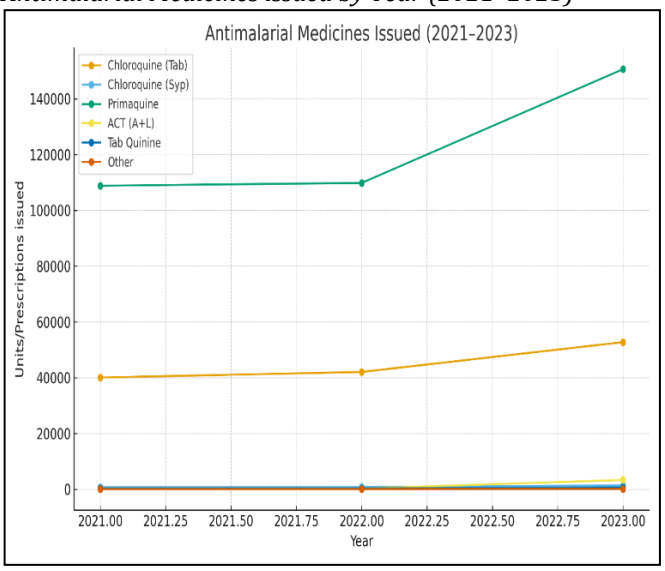
Table 6

Shows Year-wise Data of Different Malarial Drugs

Year	Medicine							
	Uncomplicated						Severe	
	Chloroquine Tab Syp	Primaquin	ACT (A+L)	Tab Quinine	Other	Inj Artesinate	Inj Quinine	
2021	40060	815	108810	285	283	60	0	0
2022	42064	834	109815	299	294	67	0	0
2023	52751	1417	150589	3358	664	130	0	0
Total	134875	3066	369214	3942	1241	257	0	0

Figure 6

Antimalarial Medicines issued by Year (2021–2023)



DISCUSSION

Malaria is a public health problem in Pakistan and is a major factor for impending for socio-economic development of the country (Jahan, 2011). Potentially it is a widely distributed tropical infection and an estimated 3.2 billion people in 95 countries and territories are at risk of being infected with malaria and developing diseass (WHO, 2015).

Pakistan lies in the malaria belt of the world with malaria being a major public health problem. The country's climate, extensive canal irrigation systems, and seasonal rainfall create abundant mosquito breeding habitats. Rural populations, which make up the majority of the country's inhabitants, are especially vulnerable. Malaria transmission is seasonal but intense in many districts, particularly those with irrigated agricultural lands and stagnant water after heavy rainfall (WHO, 2023). Human malaria is caused by four Plasmodium species; however, *Plasmodium vivax* and *Plasmodium falciparum* are common in Pakistan.

The main malaria transmission season is August through November, following moon soon season. There is a brief transmission season during spring (March-April), but most of the spring cases are believed to be delayed expression of infections acquired the moon soon season are relapsing *P. vivax* malaria. Over all *P. vivax* accounts for 75% while *P. falciparum* accounts for 25% of the malaria burden in Pakistan (Ministry of Health Pakistan, 2010). The female *Anopheles*' mosquito is the transmission vehicle for Malaria parasite (Cheesbrough, 1998).

In the present study the overall three years (2021-2023) prevalence of malaria in the general population of district Mohmand was reported 9.71%. In case of individual year the prevalence of malaria is continuously increases from 2021 to 2023. The prevalence of malaria in 2021, 2022 and 2023 was 8.15%, 8.56% and 12.14%.

Many researchers have worked on the prevalence of malaria in different parts of Pakistan.

The highest (81%) prevalence of malaria in Khairpur (Sindh) than that of the present survey. The main reason behind this huge figure was the flood of 2022. Because the blood was completely destroyed the sanitation system as well as the infrastructure of the studied area. Due to the flood the stagnant water was everywhere which is the natural habitat for the breeding and growth of *Plasmodium* vectors (female *Anopheles* mosquitoes) (Buriro et al. 2023).

The highest (46.4%) prevalence of malaria in human population of district Turbat (Baluchistan) as compare to the present study of district Mohmand. The highest prevalence rates may due to poor sanitary condition in Turbat district as comparing to that of our study area. The second reason is may be that of climatic condition the climate and temperature of Mohmand is lower than that of Turbat district, and this lower temperature cause decrease in mosquito population which ultimately decreases the prevalence rate of malaria. On the other hand. the temperature of Turbat is higher which cause a significant prevalence rate of infection (Yasinzai & Kakar sulemankhel, 2012).

The lower (7.83%) prevalence of malaria in the general population of district Swat (KP) compare to the present study of district Mohmand. The lower prevalence rate may be due to the education, well sanitation and mainly due to the lower temperature of district Swat compare to Mohmand. Because lower temperature decreases the metabolism and decelerate the propagation (life cycle) of the vector mosquitoes (Khan et al, 2018).

Regarding malaria species, in these three years study the overall *Plasmodium vivax* was predominant then *Plasmodium* falciparum. In the current study, a higher prevalence of *Plasmodium vivax* was recorded (95.27%) as compared with that of *Plasmodium falciparum* (3.15%) and mixed (1.57%). Numerous researchers have reported the prevalence of malaria in district Khairpur (*P. vivax* 74%, *P. falciparum* 26%), district Kech (*P. vivax* 89.13%, *P. falciparum* 10.41%), district Swat (*P. vivax* 100%, *P. falciparum* 0%)[46-47-64]. In these areas the *P. vivax* were reported to be more prevalent than that of *P. falciparum* but in many other areas the *P. falciparum* was reported dominant as compare to that of *P. vivax* as in Turbat (*P. falciparum* 69.4% and *P. vivax* 30.5%.), Larkana (*P.*

falciparum 58.9%, *P. vivax* 41.1%) (Yasinzai & Kakarsulemankhel, 2012; Junejo et al 2012).

Acording to these researches the *P. falciparum* was dominant as that of *P. vivax* this may be due the climate of this area which is favourable for *P.falciparum* specie as compare to that of

P. vivax.

In the present study, no case of *Plasmodium ovale* and *Plasmodium malariae* infection was founded, and reported. The same were observed in Punjab and in ex.FATA (Qureshi et al., 2019; Karim et al., 2021). These two species were not found in Pakistan because are not founded in temperate and tropical region commonly and Pakistan lies in the tropical region.

A mixed infection of *P. vivax* and *P. falciparum* was reported at 1.57 prevalence rate. The same were observed (9.6%) in Punjab and (4.9%) in ex-FATA (Qureshi et al., 2019; Hussain et al. 2016).

The present study evidence suggests that, males were significantly more likely to catch the disease than that of females. The current study identified the prevalence of malaria in males (54%) was reported more and dominant then that of females (46%). Many research workers reported higher prevalence of male's population then that of female's population, in Tribal Districts (KP) (male 66%, female 34%), Turbat (male 51.84%., female 48.16) (Karim et al., 2021; Baloch et al., 2023).

The higher number of male cases in our study may be due to several factors. In our society, men are more likely to work outside and spend time outdoors, which increases their risk of being bitten by infected mosquitoes. Additionally, women often have quicker and easier access to healthcare facilities compared to men. Because of these important factors there was difference in our study.

Regarding to age of patient, in the present study the percentage ratios between (0-4), (5- 14) and (15+) were 15%, 25% and 59%. Many research workers reported higher prevalence in adult age. A study in Turbat reported in age group 6-20 (30.29%) and age 1-5 (8.47%). (95%) as compare with that of Childs (5%). prevalence of the disease (Baloch et al., 2023). The prevalence was may be due to the fact that the adults are more exposed to the biting of infected mosquito as compare to that of children's. The second reason was may be that the adults are the working population of the society in the fields and the water of irrigation increases the population of mosquito which increases the prevalence of the infection in adults.

CONCLUSION

It is concluded that malaria stands out as a critical public health threat in district Mohmand, Pakistan. The rapid upsurge in malaria cases was observed in 2023 mainly due to the solarisation of the study area which has led to a substantial increase in the surface water level. Males were more infected than females. *P. vivax* was the dominant species reported and individuals over the age of 15 were found to have a higher infection rate, like due to their greater exposure to mosquito bites.

Authorities should spray insecticide and provide nets in endemic areas. Organizations should educate communities. Physicians should use effective drugs. Avoid stagnant water. Conduct a national wide study on malaria awareness.

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