

Immunochromatographic and Demographic Based Evaluation of
Plasmodium Species in Reported Data to Various Clinical Setups in Distract
Karak, Pakistan

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Abstract

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Malaria is a serious public health problem, mainly in the ecological and socioeconomically determined regions. Presently, the area is affected by the occurrence of the disease, though the level of infection in specific tehsils and different age-distribution groups remains elusive. The current research aimed to determine the frequency of malaria-positive cases, identify the prevalent species of

Plasmodium, and establish the correlation of infection with the tehsils, age, and sex in District Karak. The cross-sectional study was conducted in the tertiary care hospitals of Karak, Takht-e-Nasrati, and Banda Daud Shah, which are type-C Tehsil Headquarters. In total, 53,767 patient records were assessed, and rapid diagnostic tests for malaria were conducted using immunochromatographic analysis. The data were analyzed using R software, applying the descriptive statistical procedure. Out of 53,767 patients, 7,983 were diagnosed with malaria. The highest number of positive cases was reported in Tehsil Karak (4,442), followed by Banda Daud Shah (2,005) and Takht-e-Nasrati (1,536). Moreover, *P. vivax* was the prevalent species of Plasmodium in all three tehsils, whereas *P. falciparum* and mixed infections were rare. In addition, more positive cases were detected among the male population than among the female population. The majority of infected patients were aged 21–40, while those aged 61–70 was the least affected. Malaria causes a significant health burden in District Karak, with the highest number of cases reported in Tehsil Karak, whereas *P. vivax* is the prevalent malaria parasite species.

Notably, people aged 21–40 years and the male population are at higher risk of malaria infection. Thus, it is essential to implement proper monitoring and surveillance mechanisms and provide timely treatment for patients. In addition, it is vital to organize the relevant health and administrative authorities to launch the appropriate malaria prevention and control strategies.

Keywords: Malaria; *Plasmodium vivax*; *Plasmodium falciparum*; Rapid diagnostic test

Introduction:

Malaria is an infectious disease caused by the Plasmodium parasite (1) mainly transmitted by infected female Anopheles mosquitoes. While there are numerous species of female Anopheles mosquitoes, only approximately 68 of them are capable of transmitting the malaria parasite (2). Infections can also occur through exposure to contaminated blood (transfusion-associated malaria) and via congenital transmission. Plasmodium encompasses various species, with *P. vivax* and *P. falciparum* being prevalent in human disease. Other species like *P. malariae* and *P. ovale* are less common but are causative agents in specific regions. Malaria can manifest throughout the year, displaying seasonal fluctuations in its prevalence (3). According to WHO (2009), currently malaria is endemic in 109 countries, with 45 of them situated in Africa. Beyond Africa, malaria continues to pose a substantial public health challenge and contributes significantly to global morbidity. Over 90% of malaria related to deaths occur in tropical Africa, disproportionately affecting the most economically (4) disadvantaged nations (5). In European countries, the majority of cases are attributed to *P. malariae* and *P. vivax* (6). Malaria is a significant health concern in Pakistan with *P. falciparum* and *P. vivax* being the predominant species. Over the past 10 years, there has been a substantial six-fold increase in *P. falciparum* associated malaria cases. Currently, *P. falciparum* accounts for 42% of all malaria cases reported by the National Malaria Control Program (MCP). Several factors have been associated with the upsurge in malaria cases. Resistance to Chloroquine, a commonly used antimalarial drug, has become widespread across the country (7). This resistance has made it more challenging to treat malaria effectively. Warmer and autumn seasons have been observed, which favor prolonged transmission of the disease. This extended transmission period allows for a higher likelihood of malaria transmission: There has been a chronic decline in efforts to control the vectors that transmit malaria. Effective vector control is crucial for reducing the spread of the disease. In Punjab province, *Anopheles culicifacies* was traditionally considered the primary vector (8). However, in September it was noted that *A. culicifacies* had disappeared, while *A. stephensi* became more abundant in the North-West Frontier Province (Khyber Pakhtunkhwa) than *A. culicifacies*. In other regions of Pakistan, such as

Duki, Harnai and Sibi, the primary vector species are *A. culicifacies* and *A. stephensi*, as reported by the National Malaria Control Program (MCP) in 1999 and 2006. Malaria is contracted when a mosquito of the *Anopheles* species, which has become infected after feeding on an individual with the disease, bites another person and injects the malaria parasite into their bloodstream. Once inside the mosquito, the parasite undergoes various stages of development in the mosquito's gut. Eventually, the parasite migrates to the mosquito's salivary glands. During a subsequent bite by the mosquito, the parasite is transmitted to another person, leading to the spread of the disease (9). *P. vivax* has developed an increased tolerance to a broader temperature range. *P. malariae* exhibits a similar adaptation to this temperature range, but its developmental period inside the mosquito is prolonged. In contrast, *P. falciparum* requires higher temperatures for development compared to both *P. vivax* and *P. malariae*. Additionally, *P. falciparum* cannot withstand temperature reductions (10). The completion of the *Plasmodium* parasite's life cycle necessitates the involvement of both a human host and a mosquito vector, thus establishing a triadic relationship among the parasites, humans and mosquitoes. Some researchers find these interactions to be highly complex. However, *Plasmodium* parasites exhibit responsiveness to biological and seasonal clues within the human host. In tropical regions where mosquitoes are abundant throughout the year, the formation of gametocytes, a crucial stage in the parasite's life cycle, may be induced at the onset of the transmission season when uninfected mosquitoes bite humans (11). This study primarily investigates the prevalence of malaria caused by *P. falciparum*.

Material and Method:

A retrospective hospital-based study was conducted in the clinical laboratories of Type-C Tehsil Headquarters Hospitals (THQs) of District Karak, Khyber Pakhtunkhwa, Pakistan. Data were collected from three tehsils of the district, namely Karak, Takht-e-Nasrati, and Banda Daud Shah. Prior to data collection, ethical approval was obtained from the Research Ethics Committee of The Professional College of Medical Sciences, Chokara, Karak. Formal permission was also obtained from the concerned hospital administrations and relevant departments to access and collect secondary laboratory data. A total of 53,767 patient records were obtained from the participating hospitals, including 18,917 records from Tehsil Karak, 19,879 from Tehsil Takht-e-Nasrati, and 14,971 from Tehsil Banda Daud Shah. The data comprised records of patients suspected of malaria who were screened at the respective clinical laboratories. Malaria screening was performed using a rapid diagnostic test (RDT) based on an immunochromatographic assay according to the routine procedures followed by the

hospital laboratories. The relevant laboratory and demographic information available in the hospital records was extracted for analysis. The data collected was entered, organized, and analyzed using R. Descriptive statistics, including frequencies and percentages, were used to summarize the findings, and the results were presented using tables and graphical representations. Patient confidentiality and anonymity were maintained throughout the data collection, analysis, and reporting processes.

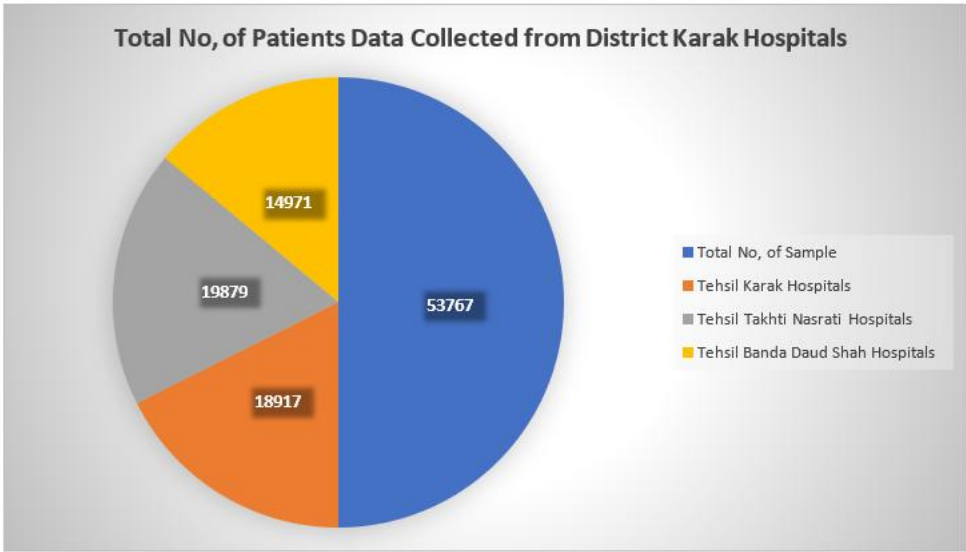


Figure no.1 Demographic distribution of patients in district Karak

Result

Comparative Distribution of Positive and Negative Cases by Tehsil in Karak District:

The given chart presents the cases of total, positive, and negative percentages of the three tehsils of Karak District. First of all, Karak Tehsil has 18,917 total cases (4,442 positive and 14,475 negative). Further, Takhti-e-Nasrati is characterized by the highest total number of cases (19,879) with 1,536 positive and 18,343 negative ones. Finally, Banda Daud Shah reports 14,971 total cases which include 2,005 positive and 12,966 negative cases. It can be concluded that Takhti-e-Nasrati has the largest number of total and negative cases, while Karak Tehsil records the highest number of positive cases.

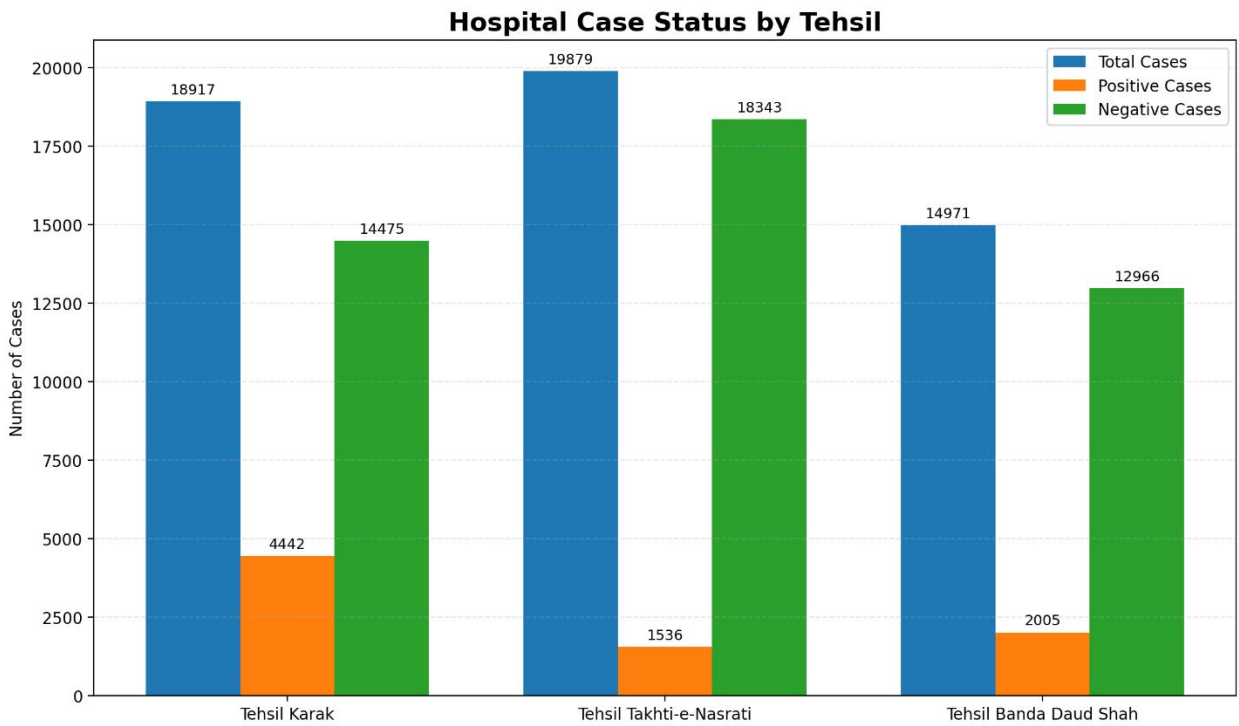


Figure No.2 Shows Tehsil-wise Distribution of Total, Positive, and Negative Cases Distribution of Malaria Species in District Karak:

As per the results, *P. vivax* was the common species, which was predominant in all the tehsils. The tehsil Karak was found to have the highest number of positive cases for malaria infection. Banda Daud Shah and Takhti-e-Nasrati were the second and the third highest infected areas, respectively. Besides that, it was found that *P. falciparum* infection was less prevalent compared to *P. vivax* infection. In addition, the mixed infection was the lowest among the rest, which implies that it had a minimal impact on the overall number of malaria infections as depicted in figure no.3.

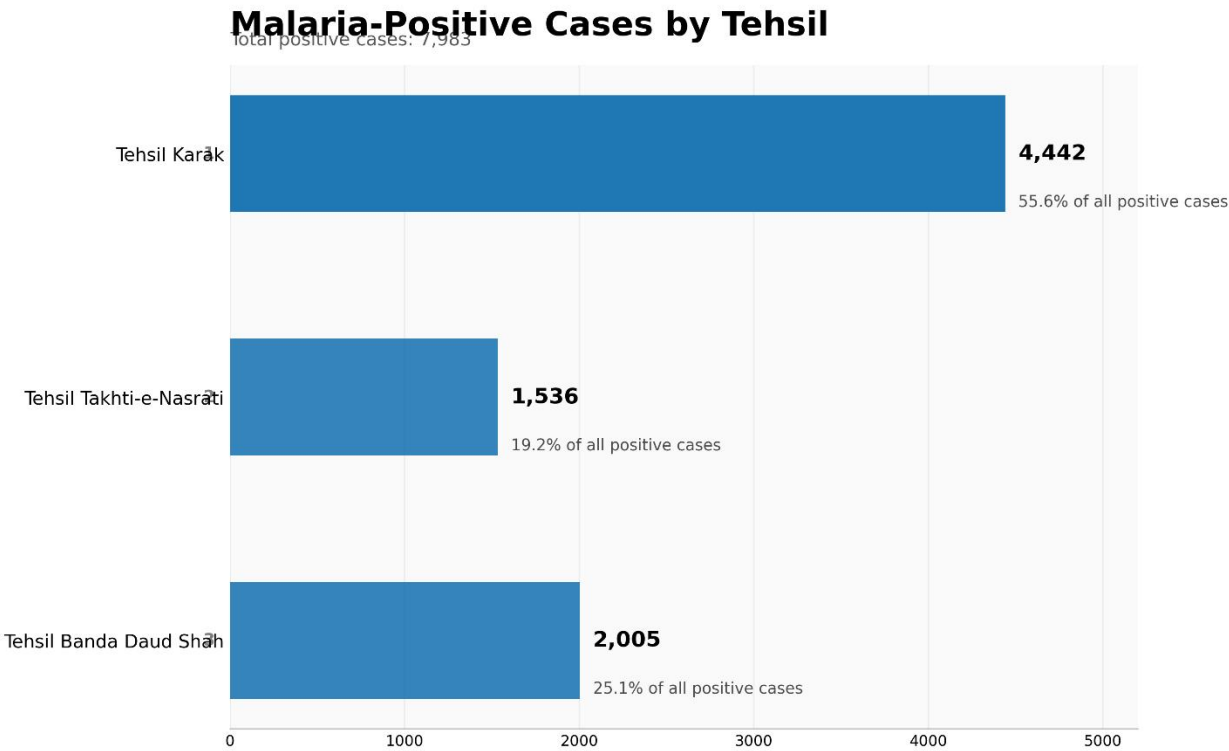


Figure no.3 distribution of malaria-positive cases by parasite species across three tehsils.

Distribution of Malaria-Positive Cases by Age Group and Gender in Tehsil Karak

Figure no. 4 below represents the number of positive cases of malaria in Tehsil Karak according to their age groups and gender. In this particular bar chart, it can be visualized that out of the total number of 4,442 positive cases, 2,863 are male (64.4%) and 1,579 are female (35.6%). Moreover, the chart also clearly shows that the positive malaria patients aged 31-40 years comprises the largest portion (1,478 cases) followed by those patients aged 21-30 years (1,453 cases). In both of the age groups, the majority of the infected patients are male (967) as compared to female (511) for 31-40 years and male (957) versus female (496) for 21-30 years. On the other hand, it can be seen that the lowest number of positive cases is seen among patients aged 61-70 years (14 cases). Overall, based on the graphs, it can be concluded that majority of positive malaria cases are male and the ages of 21-40 years comprise the largest portion of infected people.

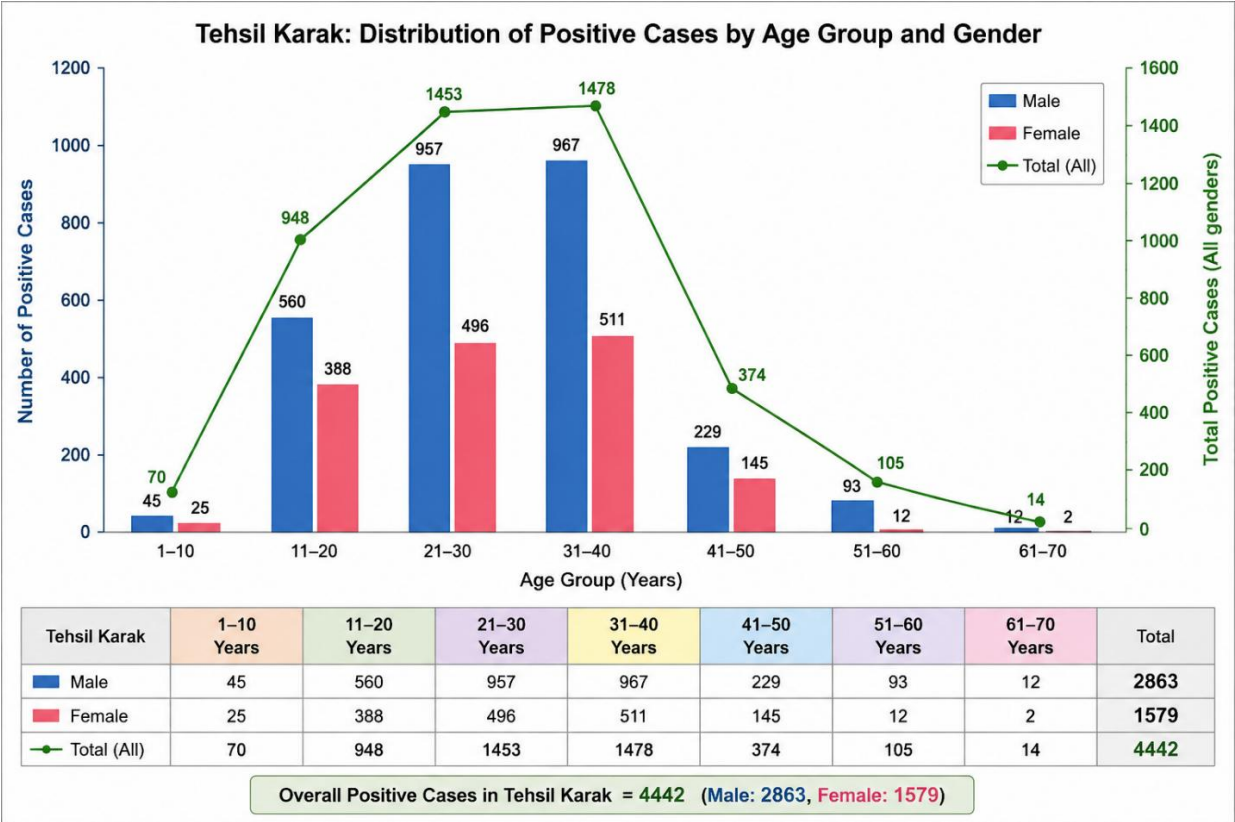


Figure no.4 shows distribution of positive cases by age and gender in Tahil Karak
Distribution of Malaria-Positive Cases by Age Group and Gender in Tehsil Takhti-e-Nasrati:

Figure no.5 illustrates the age- and gender-wise distribution of malaria-positive cases in Tehsil Takhti-e-Nasrati. A total of 1,536 positive cases were recorded, including 850 males (55.3%) and 686 females (44.7%). The highest number of positive cases was found in the 21–30 years age group (528 cases), consisting of 297 males and 231 females, followed by the 31–40 years age group (503 cases), with 271 males and 232 females. The number of positive cases decreased considerably in the older age groups, with only 7 cases recorded among individuals aged 61–70 years. Males had a slightly higher proportion of positive cases than females across all age groups. The findings indicate that malaria-positive cases were predominantly concentrated among individuals aged 21–40 years.

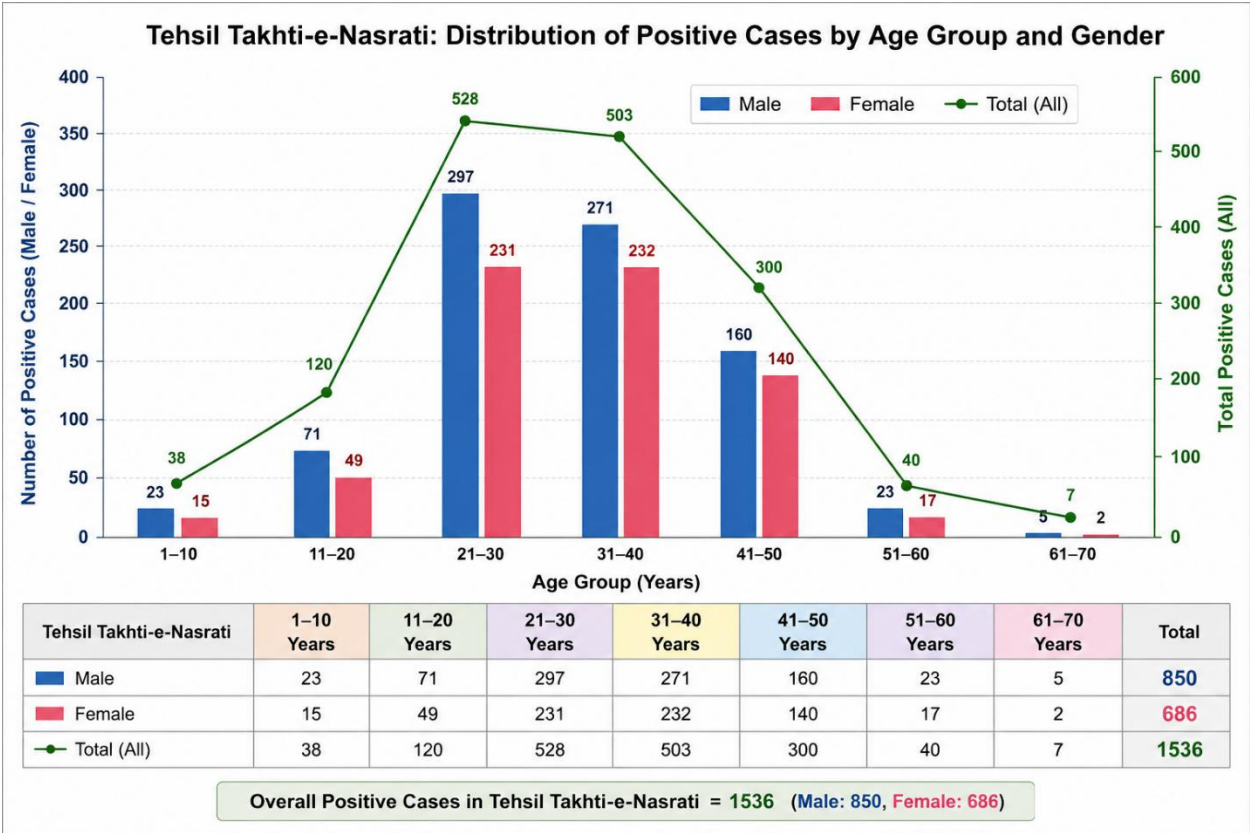


Figure no.5 shows distribution of positive cases by age and gender in Tahil Takhti-e-Nasrati

Distribution of Malaria-Positive Cases by Age Group and Gender in Tehsil Banda Daud Shah:

Figure no.6 presents the distribution of malaria-positive cases by age group and gender in Tehsil Banda Daud Shah. A total of 2,005 positive cases were recorded, comprising 1,120 males (55.9%) and 885 females (44.1%). The highest number of positive cases was observed among individuals aged 21–30 years (653 cases), including 387 males and 266 females, followed by the 31–40 years age group (644 cases), with 339 males and 305 females. The number of cases declined substantially in the older age groups, with 39 cases among those aged 51–60 years and only 4 cases among those aged 61–70 years. Males showed a higher number of positive cases than females in every age group. Overall, the graph indicates that malaria-positive cases in Banda Daud Shah were predominantly concentrated in the 21–40 years age range, with males accounting for the greater proportion of cases.

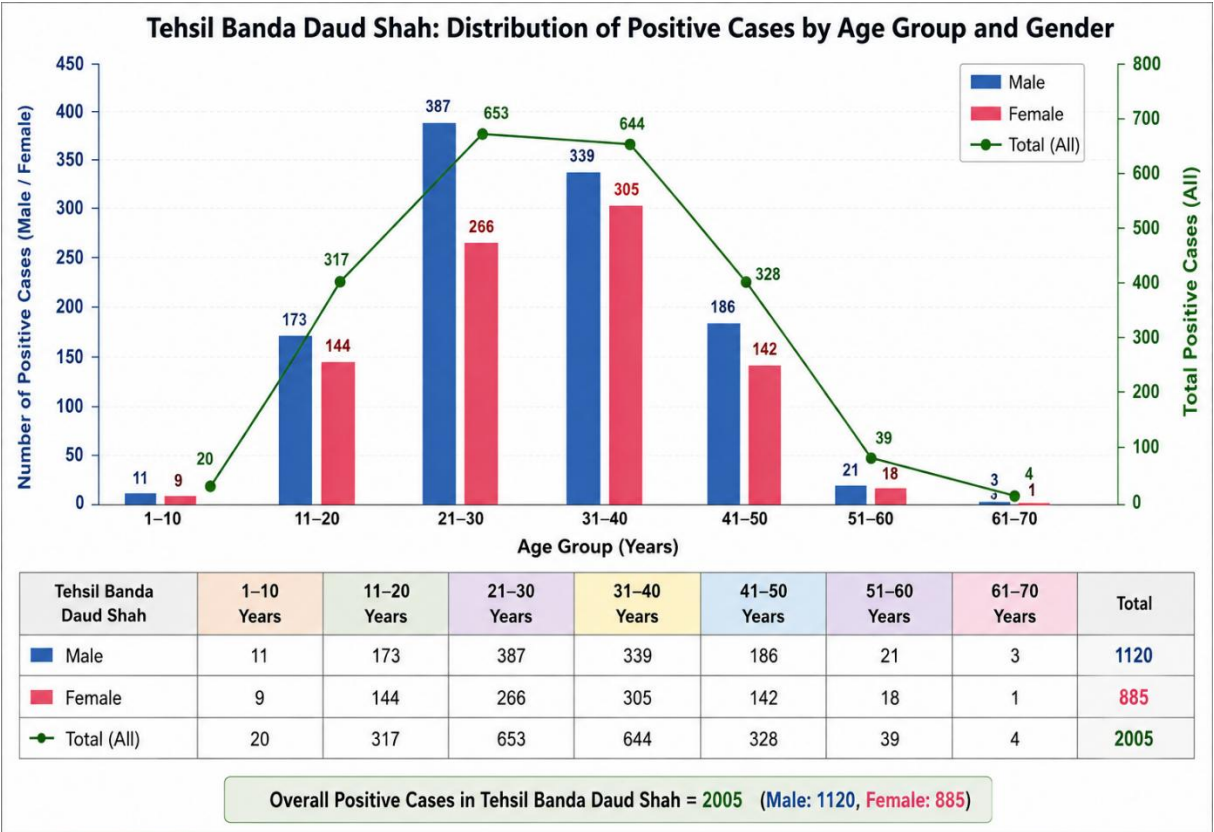


Figure no.6 shows distribution of positive cases by age and gender in Tahil Takhti-e-Nasrati

Discussion:

Malaria is a significant health issue in Pakistan with national wide cases reaching around 2.6 million in 2008 and an estimated annual total death rate are approximately fifty thousand (15). The causative agent of malaria is Plasmodium, an intracellular parasite that can be transmitted from one person to another via mosquito’s bite. Plasmodium comprises various species, with *P. falciparum* being one of the most concerning. Globally, it is estimated that between three to five hundred million people are affected by malaria each year, resulting in approximately 1,520,000 annual fatalities (16). In 2007, the Malaria Case Management Desk Guide (MCMMDG) reported that, most malaria cases in Pakistan were concentrated in Baluchistan and Sindh, followed by FATA and Khyber Pakhtunkhwa (KPK), while Punjab had the lowest number of malaria cases (7). A recent study on malaria cases was Conducted from September 2015 to August 2016, with a particular focus on the incidence of *P. vivax* in various local populations in District Karak. In 2015, a total of 1,491 slides were examined for malaria, with 90.01% of cases

attributed to *P. vivax* and 9.90% to *P. falciparum*. Similar high incidence rates were also reported in district Okara, Punjab (17). In District Karak, the number of cases of *P. vivax* is higher than that of *P. falciparum*. This indicates a greater prevalence rate of *P. vivax* malaria in the various regions. This observation aligns with the findings that emphasize the significant burden of *P. vivax* malaria in District Karak (Age-wise analysis showed the highest proportion of cases in the male age group >14 years (27.9%), followed by the male age group 5-14 years (23.6%). The lowest rate was observed in the male age group <5 years (9.1%). Among females, the >14 years age group showed a relatively lower frequency (9.2%), followed by the 5-14 years age group (16.7%), while the lowest frequency was in the <5 years age group (5%). Similar patterns were observed in previous studies, including those conducted in Mitha khel district Karak (18). The present study was conducted at different tehsil hospitals of district Karak with kind collaboration of The Professional College of Medical Sciences (PCMS) Chokara, Karak affiliated with Khyber Medical University Peshawar. A total number of 53767 patient's data were collected from three tehsil (Karak, Takhti-e-Nasrati & Banda Daud Shah) of district Karak and analyzed via different criteria. Among 53767, only 7983 patient's data show positive while 45784 are negative. Furthermore, patient data were also elaborated via three tehsils of district Karak. In case of tehsil, Karak. A total of 18917 patients' Zdata were collected which shows 4442 positive cases and 1447 noted as negative cases. In Takhti-e-Nasrati, after analyses of 19879 patients' data indicated that 1536 cases positive while 18343 noted for negative cases. While in Tehsil Banda Daud shah, 2005 cases were positive and 12966 are negative. In tehsil Karak, the positive cases more found in male as compared to female patients. While positive cases are more reported at the age of 21 to 40 years. Additionally, only 211 patients were found as *P. falciparum* infected patients among all positive cases. Similarly, tehsil Karak shows that males are frequently infected as compared to females. While the age and species wise data were similar to tehsil Karak reported data. Furthermore, in tehsil Banda Daud Shah all the patient data like tehsil Karak and Takhti-e-Nasrati. Generally, overall positive cases regarding species show that *P. vivax* highly prevalent in all tehsil hospital of district Karak.

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