A COMPARATIVE STUDY ON MALARIA PREVALENCE IN TWO MAJOR HOSPITALS IN HYDERABAD, SINDH, PAKISTAN

Asma Kanwal Thebo[*1], Sajid Siyal[2], Majid Hussain Soomro[3], Khalid Hussain Buriro[4], Shaista Jalbani[5], Attia Bushra[6]

[*1]Department of Zoology, Govt Girls Zubaida College Hyderabad
[2,6]Department of Wildlife Management, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh
[3]Majid Hussain Soomro Department of Veterinary Pathology, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh
[4]Department of Zoology, Govt Girls Degree College Dulatpur, Sindh
[5]Department of Fisheries and Aquaculture, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh

[*1]asmathebo23@gmail.com

Abstract

Malaria was the intended outcome of this investigation of the prevalence of the disease among patients attending two large hospitals in Hyderabad, Sindh, Pakistan. From January 2024 through June 2024, 375 blood samples from patients at two prominent government teaching hospitals the Liaquat University Hospital (also known as Civil Hospital) and the Aga Khan Maternal and Child Care Centre, Hospital of Hyderabad were randomly obtained for the purpose of diagnosing malaria. A thin blood film and a thick blood film were created on a slide in order to identify Plasmodium parasites under a microscope. Plasmodium was found in 66 out of the total samples. Males had a higher prevalence of positive cases (23.5%) than females (10.5%), making up 17.6% of the total. The male age group over 14 years had the highest proportion at 27.5%, while the female age group under 5 years had the lowest frequency at 5%. The urban population had the lowest frequency at 11.3%, while the rural population had the highest at 21.7%. Mixed infections of Plasmodium vivax and Plasmodium falciparum demonstrated the lowest malaria frequency at 0.8%, whereas P. vivax constituted the most frequent malaria infection at 15.5%.

INTRODUCTION

Malaria is categorized as a tropical disease. In underdeveloped countries, malaria is the leading cause of mortality and morbidity worldwide, and among all parasitic diseases, it is the most widespread and deleterious. Malaria constitutes the most significant public health challenge globally. As of 2012, it is endemic in 104 countries, with continuous transmission in 99 nations (Khan, Hussain, Yar, Ullah, & Ahmad, 2024).

The increase of P. falciparum in certain regions of Pakistan may be partially due to ineffective treatment of chloroquine-resistant infections. Chloroquine resistance in P. falciparum was initially reported in Pakistan in 1984 and subsequently verified as prevalent. A recent study revealed that 90% of P.

falciparum samples obtained from the districts of Baluchistan and Sindh have the perfect 76 T allele associated with chloroquine resistance. Chloroquine is primarily indicated for the treatment of P. vivax; nevertheless, P. falciparum infections are frequently treated with chloroquine due to presumptive diagnosis or empirical therapy based on clinical symptoms in resource-constrained nations such as Pakistan (Khattak et al., 2013).

The reproductive cycle of malaria parasites in humans and other primates starts when a female anopheline mosquito injects sporozoites into the host during a blood meal. These parasites are transported by the circulation to the liver, where they enter hepatocytes, undergo asexual reproduction, and mature into schizonts. Ruptured hepatic schizonts release thousands of merozoites that infect red blood cells (RBCs) and encourage their multiplication. Within the erythrocyte, the merozoite develops into a ring or early trophozoite stage. After maturing into an adult, it transforms into a trophozoite, which procreates asexually to generate schizonts that are densely packed with several merozoites. When the erythrocytic schizont explodes, releasing merozoites that infect erythrocytes, the erythrocytic cycle comes to a close. Some merozoites also develop into male and female gametocytes within erythrocytes. These gametocytes are then consumed by female anopheline mosquitoes during a blood meal, where they continue to proliferate. During the hepatic development of malaria parasites, no clinical indications or symptoms are present. These deal with the parasites' life cycle inside the red blood cells. Each Plasmodium species has a different erythrocytic cycle length. P. falciparum, P. vivax, and P. ovale have cycles that last around 48 hours, whereas P. malariae has cycles that last 72 hours. P. knowlesi has the shortest cycle, lasting about 24 hours. Depending on the Plasmodium species involved, parasitemia will increase about every 24, 48, or 72 hours if treatment is not received. When a clone becomes infected with P. knowlesi, P. vivax, P. ovale, or P. malariae simultaneously, fever peaks quartan, tertian, or quotidian fever patterns are caused by the release of merozoites from ruptured schizonts. In the early stages of infection, fever patterns may appear daily and may not follow regular intervals for all Plasmodium species, particularly

when there are mixed species or several parasite broods involved. Global malaria cases grew from 245 million in 2020 to 247 million in 2021, mostly in Africa. The incidence of 59 incidents per 1000 people at risk per year from 2020 to 2021 was constant after rising from 57 in 2019. The 2020 surge was attributed to COVID-19 pandemic-related breakdowns in prevention and control. Malaria-related deaths surged 10% in 2020 but dropped to 619,000 in 2021. The Global Fund's malaria program found that insecticide-treated bed net use decreased in some locations during the pandemic, although home delivery to reduce congestion increased net distribution. However, the number of individuals with suspected malaria who were tested decreased by approximately 4%, which led to a subsequent decrease in treatment. The disruption of malaria control measures in certain regions induced by COVID-19 was estimated to have contributed to an estimated 63,000 malaria fatalities from 2019 to 2021 (González-Sanz, Berzosa, & Norman, 2023).

Despite the fact that a limited number of malaria epidemiological studies have been conducted in various regions of Pakistan, the evaluation of the national malaria burden has been impeded by the absence of sufficient data from a variety of locations. A study on calorimetric surveys conducted between 2005 and 2009 revealed both regional and temporal variations in malaria endemicity. The greatest occurrence of malaria was observed in the Federally Administered Tribal Areas, followed by Baluchistan and Khyber Pakhtunkhwa Provinces, with the highest incidence recorded in 2009 over the five-year period (Qureshi, Fatima, Afzal, Khattak, & Nawaz, 2019).

Malaria continues to be a significant issue for the public health sector in Pakistan, particularly in regions such as Sindh, where the climate and economics make it simpler for the illness to spread. Since Hyderabad is a large city, it experiences a significant number of cases of malaria each year. On the other hand, there is a limited amount of data from various healthcare institutions that compares the total percentage of occurrences. This study looks at how many people with malaria were treated in two big hospitals in Hyderabad. It helps us understand the number of diseases, how they are diagnosed, and where the healthcare system might be falling short. It

can help health workers figure out when the risk is biggest, make better use of resources, and make hospital-based surveillance systems better. By highlighting variations or parallels in how institutions report cases, this study also contributes to the broader effort to enhance malaria control strategies and meet national disease reduction goals.

1. Materials and Methods

The present investigation was conducted at the two principal medical institutions in Hyderabad, specifically the Liaquat University Hospital (also known as Civil Hospital) and the Aga Khan Maternal and Child Care Centre, Hospital of Hyderabad, Pakistan, spanning the period from January 2024 to June 2024. All suspected patients had common symptoms of malaria, including fever, chills, arthralgia, vomiting, hemolytic anemia, and elevated temperature, among others. The descriptive study sought to assess the prevalence of malaria among patients visiting the specified hospital in Hyderabad, Pakistan, encompassing both urban and rural demographics. The evaluation of the data was conducted based on species, sex, and age.

1.1. Blood sample taken from capillaries by fingerstick

The name, date, and time of the patient's collection were written on pre-cleaned slides. After disinfecting with 70–90% alcohol, slides were let to dry. Infants' middle or ring fingers and heels were checked for puncture. The puncture site was disinfected with 70% alcohol and thereafter let to dry. A thin lancet punctured the infant's heel and ball of the foot. The initial blood drop was absorbed with sterile gauze. This procedure was then done with a number of slides, each of which included a minimum of two thick and two thin smears. Blood samples were then put on slides that had been thoroughly cleaned. A careful and careful process was used to make the blood smear.

1.2. Blood was drawn by venipuncture

The name, date, and time of the patient were written on pre-cleaned tubes. 70% alcohol was used to completely clean the blood collection site, which was then allowed to dry. Venous blood was drawn into a vacuum tube that was filled with EDTA, an anticoagulant. Alternatively, the blood might be drawn into a syringe and transferred to a pre-mixed tube containing anticoagulant. At least two thick and two thin streaks were made after the blood was collected.

1.3. Methods for Making Thin and Thick Blood Smears

Different slides were utilized for the thick and thin blood smears.

1.3.1. A slimy film of blood

A sterile spreader slide was placed at a 45° angle to the blood droplet on the specimen slide to ensure uniform distribution throughout the slide's thickness.

1.3.2. Thick blood smear analysis

The blood drop was carefully distributed in a circular pattern using a clean corner of the slide, and the resulting smear was thick enough not to slip off. The thick and thin films were given ample time to cure before being stained. While the thick film remained unfixed, the thin film was secured using absolute methanol and permitted to dry completely.

1.3.3. Diagnosis of Malaria via Microscope

A thin and thick blood smear was made on a slide for the microscopic detection of Plasmodium parasites. A proficient laboratory technician employed a 100X lens, a guide, and 100 small fields to identify malaria following the air-drying of thin blood films.

Following this, the films were preserved in methanol and stained for 10 minutes with a 10% Giemsa solution. Parasitemia was evaluated by enumerating the parasites per 200 white blood cells on a thick blood smear. The determination of parasite density per microliter was conducted based on an assumed total white blood cell count of 8,000/µl in the sample.

In cases where discrepancies arose between the two readers of the Giemsa microscopy, a third expert was consulted to resolve the issues, following the completion of the procedure by two experienced lab workers. The provided parasite density represents the average of the counts obtained from two individuals conducting research in the laboratory. In instances where the discrepancy in parasite count exceeded

10% between the two readers, a third expert was engaged to conduct a blind assessment of the load and provide an accurate report.

2. Result

2.1. Study Area

The investigation took place within the urban and rural communities of Hyderabad, Sindh, spanning from January 2024 to June 2024. The investigation centres on two main government teaching hospitals: the Liaquat University Hospital (also known as Civil Hospital) and the Aga Khan Maternal and Child Care Centre, Hospital of Hyderabad, Pakistan. Data from patients were collected randomly from two major government teaching hospitals. A total of 375 patients were identified as suspected cases of malaria, including males, females, and children.

Table 1. Blood samples collected from two prominent hospitals in Hyderabad

Hospitals	Number of Suspected Samples	MP +iv Samples (%)
Civil Hospital	165	25(15)
Aga Khan Maternal and Child Centre Hospital	210	40 (19)
Total	375	65 (17.3)

Number of Suspected Samples
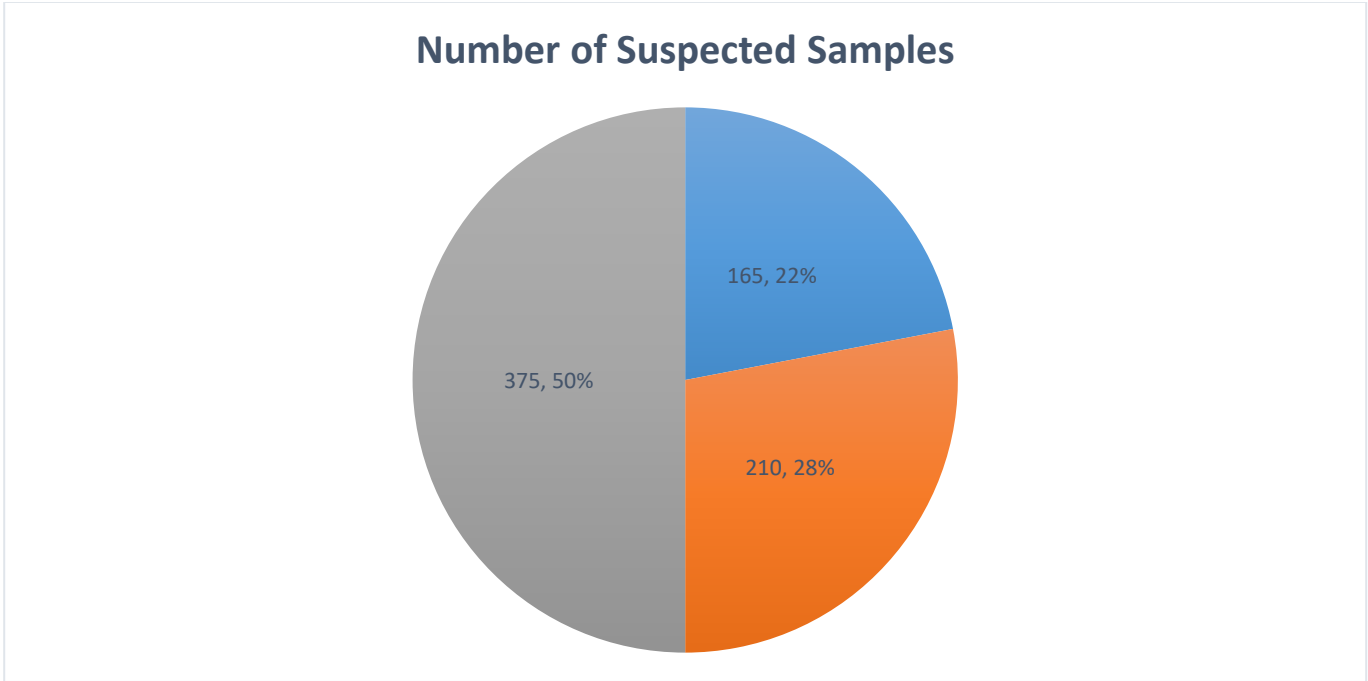
Three hundred seventy-five blood samples, suspected of malaria, were randomly collected from various leading hospitals in Hyderabad, Sindh, Pakistan. The Civil Hospital reported a total of 165, while the Aga Khan Maternal and Child Care Centre Hospital recorded 210. The highest frequency was observed at Aga Khan Maternal and Child Care Centre Hospital, documenting 40 cases (19%), whereas the lowest proportion was identified at Civil Hospital, with 25 cases (15%).

2.2. Hyderabad's leading hospitals' malaria cases by Age

Participants were categorized into six distinct groups <5 years Males, 5-14 years Males, >14 years Males, <5 years Females, 5-14 years Females, and >14 years Females. The male age group over 14 years exhibited the highest percentage, recorded at 31 (26.2%), succeeded by the male age group 5-14 years, which accounted for 13 (23.6%). The male age group under 5 years represented the lowest percentage, standing at

3 (10%). Conversely, the female demographic aged over 14 years demonstrated a notably reduced frequency compared to their male counterparts, with a count of 10 (9.0%). The following group comprised females aged 5-14 years, representing 7

individuals (17.5%). The lowest frequency was recorded in the female group aged under 5 years, amounting to 1 (4.7%). Table 2.

Table 2. Malaria Cases at Selected Hospitals in Hyderabad Organized by Age

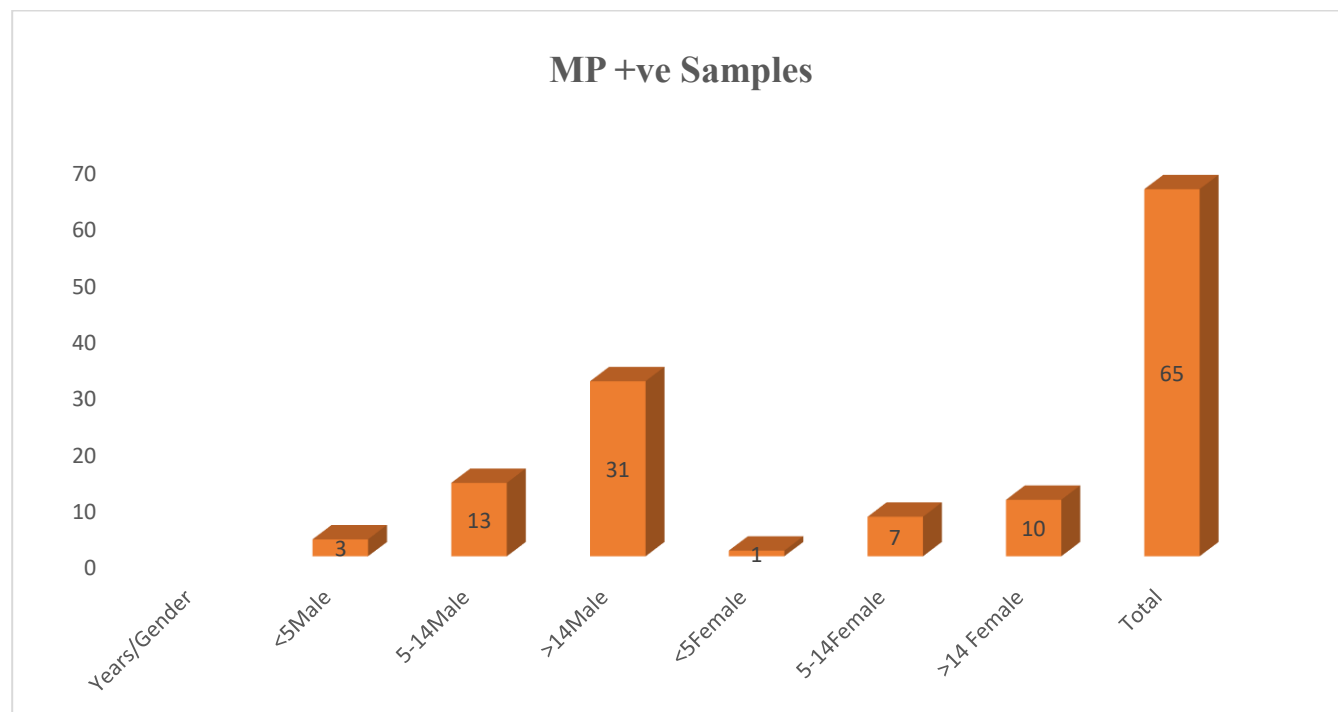| Age Years/Gender	Total Samples	MP +ve Samples (%)
<5Male	30	3 (10)
5-14Male	56	13 (23.2)
>14Male	118	31 (26.2)
<5Female	21	1 (4.7)
5-14Female	40	7 (17.5)
>14 Female	110	10 (9.0)
Total	375	65 (17.6)

Table 2. Provides the male patient age distribution for malaria at two prestigious Pakistani hospitals in Hyderabad. The age categories were delineated as follows: under 5 years, 5 to 14 years, and over 14 years for males. A significant percentage (28%) of malaria-positive cases was observed in males aged over 14 years, while a low percentage (10%) was identified in males aged under 5 years. A significant percentage (17.5%) of positive cases was seen in

females aged 5-14 years, but a low percentage (4.7%) was reported in females under 5 years of age. The compiled data revealed a prevalence of 26.2% among males aged 14 and older, in comparison to their female counterparts. A substantial percentage of patients in the prior study (63.6%) were over the age of 14 years. The trend observed in Karak closely mirrors that of Karachi. The Bannu region of Khyber Pakhtunkhwa has recorded the highest incidence of

malaria, at 20.67%, among individuals aged 5 to 14 years in Pakistan.

2.3. Distribution of malaria cases by Gender-wise in selected hospitals, Hyderabad

In the main region of Hyderabad, Sindh, Pakistan, the incidence rates of malarial infection in males were 205 (24.3%) compared to 170 (8.8%) females. The disproportionate number of male malaria cases is most likely due to greater exposure, as males are less protected than females when working outside, resulting in a higher frequency of Anopheles bites.

Table 3

Malaria cases by gender in notable hospitals, Hyderabad

Gender	Sum of all Samples n=375	Positive MP Samples (%)
Male	205	50 (24.3)
Female	170	15 (8.8)
Total	375	65 (17.3)
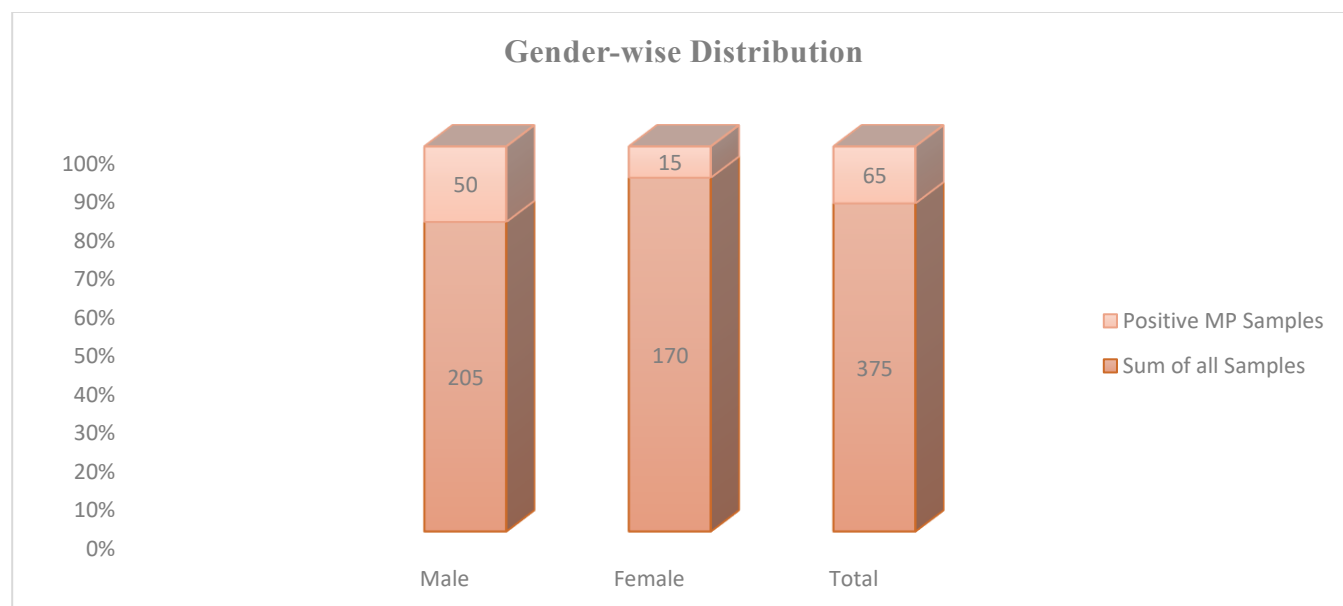
## 2.4. Urban and rural malaria cases in notable hospitals in Hyderabad

To assess the prevalence of malaria in the urban and rural populations of Hyderabad, Sindh. A frequency of 50 (21.7%) was recorded in a rural setting, whereas an urban region documented 15 (10.3%) cases of malaria infection. Compared to urban populations, rural populations are more vulnerable to malaria infection. Table 4

Table 4. Malaria in Hyderabad hospitals' urban and rural populations

Demographics	Whole Sample	MP +vi Sample (%)
Rural	230	50 (21.7)
Urban	145	15 (10.3)
Total	375	65 (17.3)

3.
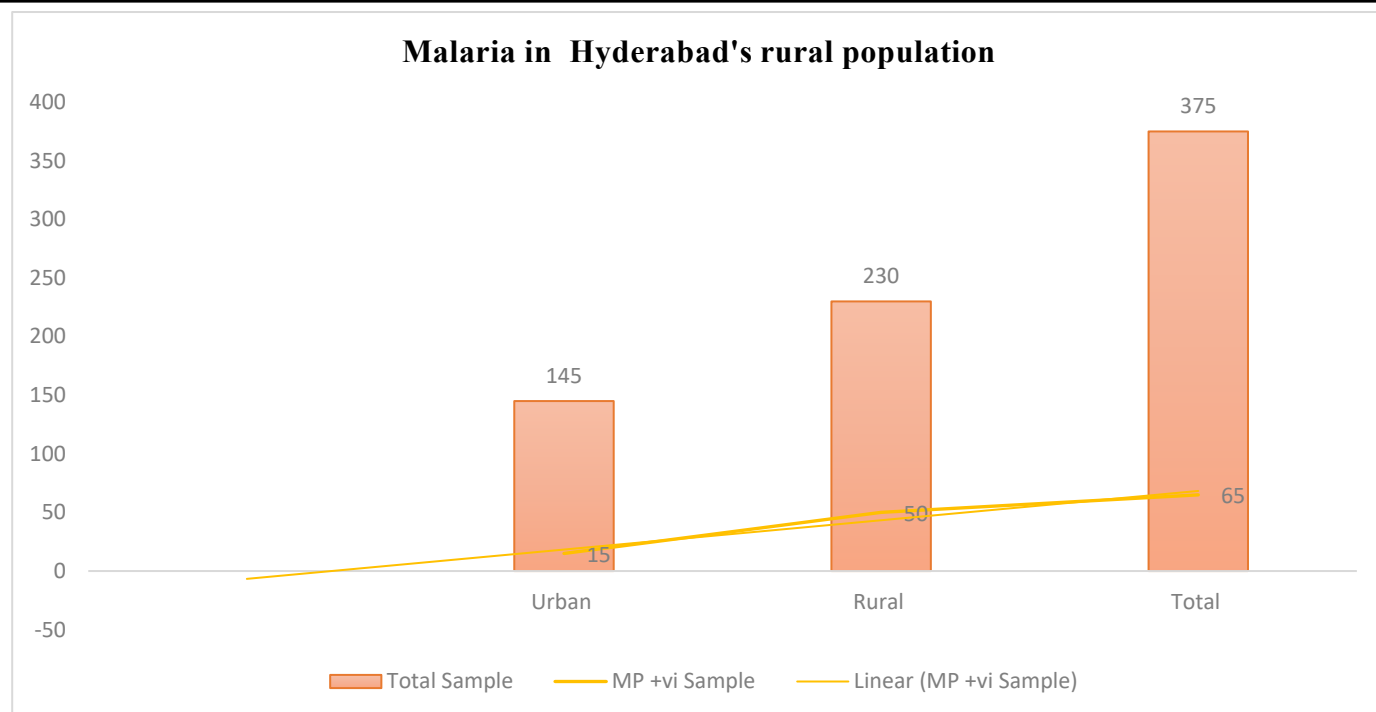
2.5. Selected Hyderabad's hospitals malaria case distribution by species

Every positive malaria sample taken from two big hospitals in Hyderabad, Pakistan, was carefully analysed to find out how often different types of malaria were present. The prevalence rates of malarial infection in Plasmodium species showed a higher proportion for Plasmodium falciparum at 58 (15.4%), followed by Plasmodium vivax at 4 (1.1%), with the lowest prevalence observed in mixed malaria cases at 3 (0.8%) Table 5.

Table 5. Malaria case distribution by species in selected Hyderabad hospitals

Distribution of malaria cases by species						MP Case Confirmation Rate		
Hospitals	Total Samples	Diagnosis of P. Falciparum	Diagnosis of P. vivax	Diagnosis of Mix	MP + ve samples	P.F %	P.V %	Mix %
(DHQ) Hospital	165	24	03	01	28	14.5	1.8	0.6
(MMT) Hospital	210	34	01	02	37	16.1	0.4	0.9
Overall	375	58	04	03	65	15.4	1.1	0.8

3. Discussion

Malaria is seen as a significant health concern in Pakistan. In 2008, the total malaria cases reported nationwide were around 2.6 million, resulting in almost fifty thousand deaths annually. Plasmodium is an internal parasite that spreads from person to person through mosquito bites and is the cause of malaria. There are other species of this internal parasite, but Plasmodium vivax and Plasmodium falciparum are the two most well-known. It is estimated that around three hundred to five hundred million individuals suffer with malaria worldwide, resulting in about 1,520,000 fatalities yearly (Wilson et al., 2020).

The findings of the present study revealed the prevalence of malaria in two prominent hospitals

located in Hyderabad, Sindh, Pakistan. The highest proportion, 19%, was observed at Aga Khan Maternal and Child Care Centre Hospital, whereas the lowest percentage, 15%, was recorded at Civil Hospital. The Quetta district in Pakistan has the greatest incidence of malaria, with a notable prevalence of Plasmodium falciparum. Conversely, the cities of Zhob, East Baluchistan, and Khuzdar, together with the Punjab province and especially Islamabad, have a significantly reduced incidence of malaria. Approximately 2.4% of malaria cases confirmed by microscopy occurred in Punjab province in 1999, according to a door-to-door research (Malik, Hussain, Hassali, & Shafie, 2013). The movement of populations across borders in the specified regions could have played a role in the rise of malaria cases. Between 1979 and 1982, Afghan immigrants pursued refuge in the provinces of Baluchistan and KPK.

The male age group over 14 years demonstrated the greatest percentage at 28%, succeeded by the male age range of 5-14 years at 23.2%. The female age groups demonstrated lower frequencies overall, with the highest at 9.0% in the 14-year age group, followed by 16.75% in the 5–14-year age group, and the lowest frequency recorded in the 14-year age group. The Mithakhel area in Karak also showed similar trends (Gehlot & Vyas, 2023). Infected individuals between the ages of 5 and 14 had a mean incidence of 20.67%. A comparable trend was observed in the Bannu district of Khyber Pakhtunkhwa. Pakistan. The demographic of 14-year-olds in Karachi exhibited a negligible prevalence (Bagchi, Yuan, & Engleman, 2021).

The study revealed that the prevalence of malarial infection in males was significantly higher, at 23.4%, in contrast to females, who exhibited a rate of 10.6%. The sex distribution in our study indicated a predominance of males, totalling 32 individuals (28%), whereas females accounted for 10 (9.0%), and children represented 24 (16%). A significant number of males was also reported.

Males in the field tend to demonstrate greater carelessness compared to females. The rural population demonstrated a higher incidence of malaria infection cases, reporting 49 cases (21.3%), in contrast to the urban population, which recorded 17 cases (11.7%) (Malik et al., 2013).

4. Conclusion
In both rural and urban regions of Hyderabad, Sindh, the research found that malaria is more common in males than in females or children. The largest prevalence of instances was seen in men aged over 14, while the lowest percentage 3%, was noted in the under-5 age group. The examination of the two primary hospitals indicated a prevalence of Plasmodium falciparum at 16.5%, with Plasmodium vivax at 1.1%. Mixed malaria patients exhibited the lowest recorded malaria infection rate at 0.8%.

5. Recommendation
It is important to have a comprehensive understanding of malaria, assist in the early detection of symptoms, and put treatment and preventative measures into action in a timely manner in order to organize an effective malaria awareness workshop.

6. Conflict of interest
The authors have declared no conflict of interest.

7. Acknowledgements

8. Novelty Statement
This work is not previously studied hence the work is novel and would be beneficial for health.

9. REFERENCES
Bagchi, S., Yuan, R., & Engleman, E. G. (2021). Immune checkpoint inhibitors for the treatment of cancer: clinical impact and mechanisms of response and resistance. Annual Review of Pathology: Mechanisms of Disease, 16(1), 223-249.

Gehlot, P., & Vyas, V. K. (2023). Recent advances on patents of Plasmodium falciparum dihydroorotate dehydrogenase (Pf DHODH) inhibitors as antimalarial agents. Expert Opinion on Therapeutic Patents, 33(9), 579-596.

González-Sanz, M., Berzosa, P., & Norman, F. F. (2023). Updates on malaria epidemiology and prevention strategies. Current Infectious Disease Reports, 25(7), 131-139.

Khan, W., Hussain, T., Yar, A., Ullah, H., & Ahmad, S. S. (2024). Epidemiology of malaria and hematological analysis in district Dir (lower), Pakistan. International Journal of Pathology, 55-60.

Khattak, A. A., Venkatesan, M., Nadeem, M. F., Satti, H. S., Yaqoob, A., Strauss, K., . . . Plowe, C. V. (2013). Prevalence and distribution of human Plasmodium infection in Pakistan. Malaria journal, 12, 1-8.

Malik, M., Hussain, A., Hassali, M. A. A., & Shafie, A. A. (2013). Standard treatment guidelines for malaria: Challenges in its implementation in Islamabad (federal capital) and Rawalpindi (twin city), Pakistan. Saudi Pharmaceutical Journal: SPJ, 21(1), 123.

Qureshi, N. A., Fatima, H., Afzal, M., Khattak, A. A., & Nawaz, M. A. (2019). Occurrence and seasonal variation of human Plasmodium infection in Punjab Province, Pakistan. BMC infectious diseases, 19, 1-13.

Wilson, A. L., Courtenay, O., Kelly-Hope, L. A., Scott, T. W., Takken, W., Torr, S. J., & Lindsay, S. W. (2020). The importance of vector control for the control and elimination of vector-borne diseases. PLoS neglected tropical diseases, 14(1), e0007831.