

PREVALENCE OF MALARIA AT MMC AND AMC HOSPITALS IN DISTRICT
MARDAN KHYBERPUKHTUNKHAWA

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Abstract

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An important public health challenge is the malarial infection in the district Mardan Khyber Pakhtunkhwa. Malaria is highly endemic and the transmission rate is high in the people of district Mardan. The current study's main objective is to explore and identify the frequency of malarial species infection in district Mardan (MMC AND AMC hospitals). This study began in June 2023 and ran through September 2023 and randomly selected 5081 participants for this study by attending two separate laboratories twice a week. Blood films, both thick and thin, were made after the blood samples were obtained, for microscopy and rapid diagnostic testing for both genders (male and female) of different ages who live and work in urban and rural regions. According to the results, the positivity rate of malarial infection is high 7.5%, and found malaria ratio in rural regions is (55%) as compared to urban areas (44%) where males (53.0%) and females (46.9%) are affected by malaria. Apart from that, the high frequency of malaria recorded percentage was among individuals aged 15 and older (45%), while the 0-4 age group had the lowest proportion (20%). Plasmodium vivax was found to be high with the highest endemicity rate of (82.5%) followed by Plasmodium Falciparum (17.2%) and found the highest ratio in September (9.0%). Thus, the prevalence of malarial disease still presents a serious problem. Individuals in the community need to take personal preventive measures to protect themselves, such as using insecticide-treated bed nets, using topical insect repellent, and securing residential areas.

Keywords: Malaria, Mardan, Plasmodium species, Prevalence

1. INTRODUCTION

1.1 Malaria

More than 125 years after discovering the causative parasites malaria is one of the most disastrous and widespread infectious diseases of the parasite in the world and is a major cause of morbidity and mortality among different developing countries (1). The word malaria is derived from two Greek words "mala" and "aria" which means "bad air" and was discovered by French army surgeon Charles Louis Alphonse Laveran in 1880. Malaria is also called black water disease because it causes hemoglobinuria which leads to the dark color of the patient's urine (2)

1.2 Literature Review

The global incidence of malaria of 300 - 500 million cases per year has been reported by studies with an associated two million deaths per annum (3). Every year half a billion people living in 109 countries in Africa, Asia, and Latin America suffer from malaria. In 2010, 219 million cases and 660,000 deaths have been estimated. According to a report, in 2011 about 3.3 billion people were at risk of malaria throughout the world, out of which 80% of cases and 90% deaths occurred in Africa (4). In South East Asia malaria has been considered a big health problem whereas 1256 million people are at risk. (5) World Health Organization (WHO) estimates, 40% of the world's population is at risk of developing malaria. According to the 2013 WHO report, released in December 2013, there were about 13 million cases of malaria in the Eastern Mediterranean Region in 2012 and an estimated 18,000 deaths. (6) According to the World Health Organization (WHO), Pakistan is one of the seven affected countries in the Eastern Mediterranean, accounting for 98% of the total malaria burden in the region. Around 217 million people in Pakistan are at moderate risk of malaria, and 63 million are at high risk. About 0.47 million malaria cases and 800 deaths have been reported in 2020 (7) Malaria is endemic in Pakistan, where it is unstable and spreads seasonally. In Pakistan malaria infection increases in the Autumn season (August, September & October) when mosquitoes experience a groovy atmosphere for breeding and transmitting parasites (Plasmodium) to healthy persons from infected as the highest frequency (protozoal infection, 91.0%) was detected in August and lowest frequency (57.1%) in March. Pakistan is among six Eastern Mediterranean region (WHO countries) having high transmission malaria and almost the whole population of the country is living in danger of infection (8) A total of 374,513 (P. Vivax: 84.0%, P. Falciparum: 14.9%) confirmed malaria cases were reported in Pakistan in 2018. (9) Similarly, malaria spread across the entirety of northern and southern Khyber. Malaria is highly endemic In the regions of Balochistan and KP, notably

encompassing Lower Dir, Quetta, Malakand, Swat, Peshawar, Mardan, Lower Dir, and Buner(10–12). Therefore, the main purpose of this study was to determine the frequency of specialized manifestation of malaria caused by *P. falciparum* and *P. vivax* in patients of both sexes throughout a range of age groups and in varied environments, including both urban and rural locations in Mardan.

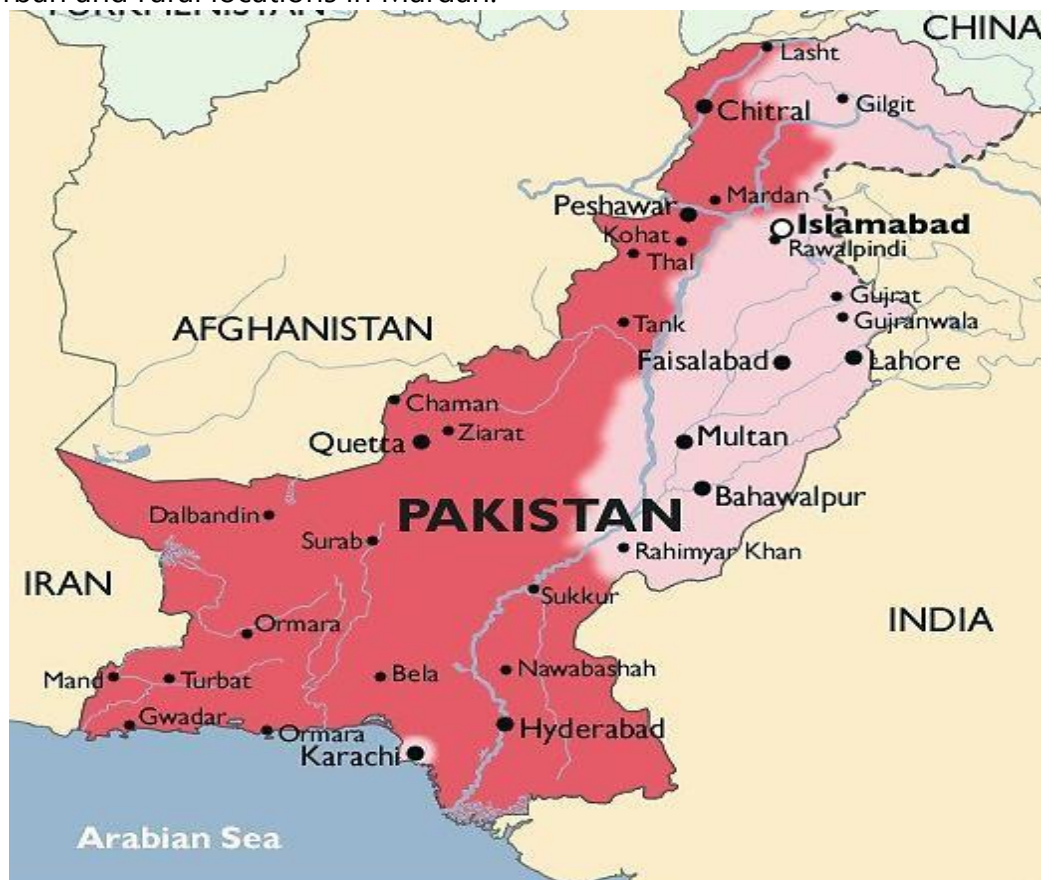


Fig 1.1 Map of Pakistan Where Malaria is at high risk.

(Low malaria risk is indicated by the color pink, The red color is evidence of a greater risk of malaria and The brown color shows countries with malaria risk)

Reference: <https://www.fitfortravel.nhs.uk/destinations/asia-central/pakistan/pakistan-malaria-map>

1.3 Species of Plasmodium parasites

Malaria is caused by a group of parasites known as Plasmodium. Within this group, there are more than 120 different species. However, the five most significant species Plasmodium vivax, The parasites Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi and that's what causes human malaria.. (13) Most deaths and illnesses resulting from malaria can be linked to Plasmodium

falciparum and Plasmodium vivax, two types of parasites that cause the disease. In contrast, Plasmodium ovale and Plasmodium malariae, two other parasite species that can also lead to malaria, are typically not connected with severe or life-threatening cases of the illness. (14). Malaria, specifically

P. falciparum, and P. vivax is Pakistan's main cause of illness and death. Plasmodium falciparum, which causes the deadly form of malaria, is primarily common across Africa; in comparison, P. vivax is worldwide but less harmful. (5)

1.3.1 Plasmodium-vivax

The malaria parasite Plasmodium vivax has a unique ability to form dormant stages called hypnozoites, which allows it to stay hidden in the liver and later reactivate, causing disease relapses. In contrast to Plasmodium falciparum, our knowledge of

P. vivax's population structure and genetic diversity is not as comprehensive. This is largely because we lack suitable Genome-wide genetic markers for P. vivax(15)

1.3.2 Plasmodium-falciparum

Malaria infection in its most severe form can lead to a condition called cerebral malaria, and this is often associated with the malaria parasite P. falciparum(16) Malaria is most commonly caused by the parasite Plasmodium falciparum as compared to Plasmodium ovale or Plasmodium malariae. Plasmodium falciparum is associated with more severe cases of malaria than any other species of Plasmodium. Notably, resistance to chloroquine is a significant characteristic of P. falciparum. This parasite is particularly concerning because it can infect red blood cells at various stages of development, leading to a higher occurrence of parasitemia.

1.4 Signs and symptoms of malaria

Uncommon signs of malaria, in terms of clinical symptoms, include severe headaches, vomiting, high body temperature, excessive perspiration, strong shivering, and fever. Signs and symptoms appear when a person is exposed to malaria parasites. Fever, weakness, fatigue, nausea, vomiting, diarrhea, headache, back pain, chills, and cough are typical malaria symptoms. Due to their similar symptoms, meningitis, the flu, dengue fever, typhoid, blood poisoning, and hemorrhagic fever from viruses can all be mistaken for malaria. Neurologic symptoms like confusion, dizziness, and comas can also occasionally be observed(17)



Fig 1.2 Signs and Symptoms of Malaria

Reference: https://www.freepik.com/free-vector/malaria-transmission-cycle-symptom-information-infographic_13634407.htm

1.5 Transmission of malaria

Malaria is a vector-borne contagious disease. And is caused by the Plasmodium parasite which enters into the human bloodstream through the bite of a female anopheles mosquito(18) Mosquitoes like A. Stephensi, A. Maculetus, A. Fluvilitis, and A. Calicifancis need certain climatic and geographic conditions for reproduction, parasitic transformation, and transmission. Broadly, harsher winters and cold temperatures are not suitable for the prevalence of plasmodium parasites. Transmission rates are the highest above 64°F (18°C) and no parasite incubation occurs below 60°F (16°C). A minimum amount of rainfall is also necessary to provide standing water for vector breeding, but too much rainfall (100 inches or more) can eliminate suitable breeding sites(19). Environmental conditions, including factors like rainfall, climate, and the socioeconomic status of the population, play a substantial role in influencing the transmission of malaria.

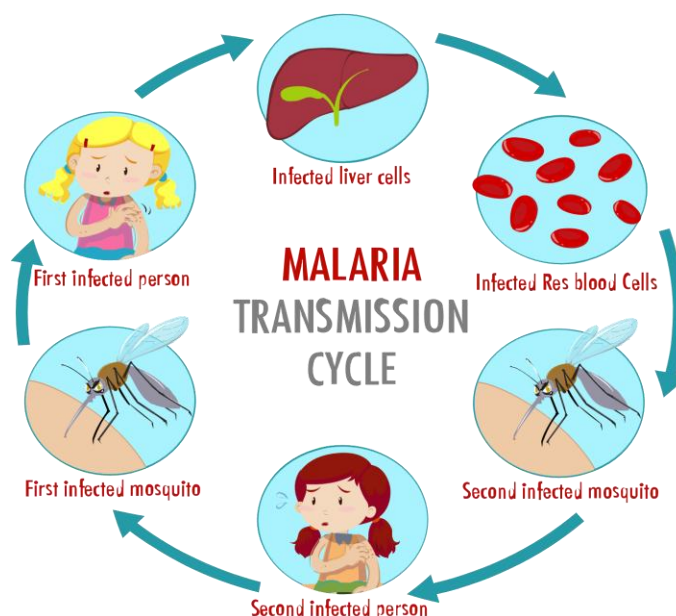


Fig 1.3 Transmission Cycle of Malaria

Reference: https://www.freepik.com/free-vector/malaria-transmission-cycle-symptom-information-infographic_13634407.html

1.6 Lab Diagnosis

In this research, we diagnose malaria by two methods

- 1) Rapid Diagnosing test
- 2) Microscopy

1.6.1 Rapid Diagnostic Test

These tests are conveniently accessible in kit format, complete with all the essential materials needed for the procedures. What makes them particularly user-friendly is that they don't demand extensive training or specialized equipment for either conducting the tests or understanding the outcomes. Rapid diagnostic tests (RDTs) for malaria primarily focus on detecting specific malaria antigens, including

HRP-2, pLDH, and Plasmodium aldolase(20)

A rapid antigen test (RAT) is a quick and easy test also known as a rapid antigen detection test (RADT) is a diagnostic test that instantly identifies the presence or absence of a malaria antigen. It is usually used for the detection of malaria antigen, the plasmodium species that causes malaria. Rapid tests are a type of test that identifies HRP-2, pLDH, and Plasmodium aldolase antigen(20). Rapid tests commonly give a result in 5 to 30 minutes and require minimal training.

1.6.1.1 Sensitivity And Specificity Of The Method

The accuracy and precision of malaria Rapid Diagnostic Tests (RDTs) can vary depending on several factors, including the specific RDT used, the type and strain of malaria parasite prevalent in the region, and the quality of the test kit and its storage. Generally, RDTs for malaria exhibit varying levels of sensitivity and specificity, but they have improved over time as technology and test designs have advanced.

- ***Sensitivity:***

Sensitivity refers to the ability of the RDT to correctly identify individuals who have malaria (true positives). It is computed as the percentage of true positive outcomes (i.e., the number of individuals with malaria correctly identified by the test) divided by the total number of individuals who have malaria (true positives plus false negatives). Sensitivity typically ranges from 85% to 95% for most RDTs used to detect the most prevalent and dangerous malaria parasite *Plasmodium falciparum*. (21)

Sensitivity may vary for RDTs that detect other malaria parasite species, such as *Plasmodium vivax* or *Plasmodium ovale*.

- ***Specificity:***

Specificity refers to the ability of the RDT to correctly identify individuals who do not have malaria (true negatives). It is computed as the percentage of actual negative outcomes. (i.e., the number of individuals without malaria correctly identified by the test) divided by the total number of individuals who do not have malaria (true negatives plus false positives). Specificity for RDTs is generally high, often exceeding 90% and sometimes even 95%. False negatives: RDTs may produce false-negative results, especially when parasite densities are low, which can occur in early-stage infections or cases of low-level parasitemia(22) In such cases, microscopy or molecular testing may be needed for confirmation. False positives: While the specificity of RDTs is generally high, false-positive results can occur due to cross-reactivity with other antigens or non-malarial infections. This is more common in areas where other infectious diseases are prevalent.

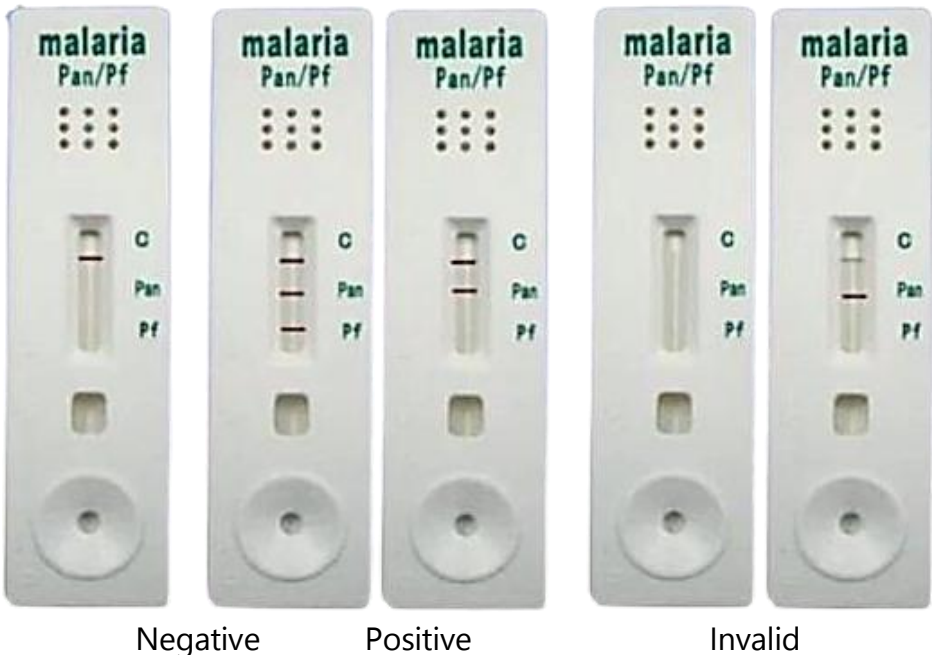


Fig 1.4 Showing Malaria positive, negative, & invalid on strip

Reference: <https://www.graphic.com.gh/entertainment/showbiz-news/noguchi-memorial-institute-calls-for-urgent-need-for-sensitive-rapid-diagnostic-tools.html>

1.6.2 Microscopy

For microscopy, we take venous blood from different malaria patients. The first step is to identify any Plasmodium parasite infection two types of blood smears, thick and thin, were used for analysis. Researchers obtained blood samples from each patient and used them to create two microscope slides. At the MMC and AMC Laboratories, thick or thin blood films were prepared. Skilled microscopists stained the thick blood smears with Giemsa and thoroughly examined them to assess the samples. If no parasites were found in the microscopic fields, a thick smear was classified as negative(23)

2. MATERIALS AND METHODS

2.1 Study design

This study was conducted in the serology and hematology department of pathology at Mardan Medical Complex (MMC) and Ahmad Medical Complex Mardan (AMC). This study design is a descriptive, cross-sectional study. And sampling technique used in this research is convenient sampling.

2.2 Study Area

The study was carried out in District Mardan, Khyber Pakhtunkhwa, Pakistan, from June 2023 to September 2023. The 19th-largest city in Pakistan is Mardan. The second most populated city in Khyber Pakhtunkhwa is situated at an elevation of 283 meters (928 feet)

on the province's southwest edge. We implement several techniques and materials for data collecting within the Mardan Medical Complex and the Ahmed Medical Complex. We collect data using questionnaires, and we confirm species using malaria kits/strips and microscopy.

2.3 Sample selection

This study included patients of all ages who attended MMC and AMC hospitals with signs and symptoms of malaria (fever, chills, headache, sweats, weakness, nausea, and vomiting) and were suggested by doctors to be tested for malaria parasites. At the time of blood collection and final report, each patient filled out a printed questionnaire with information like name, residence, gender, age, etc. Samples that are confirmed by professional technologists by microscopy, RDTs, and other laboratory procedures should be included in this research.

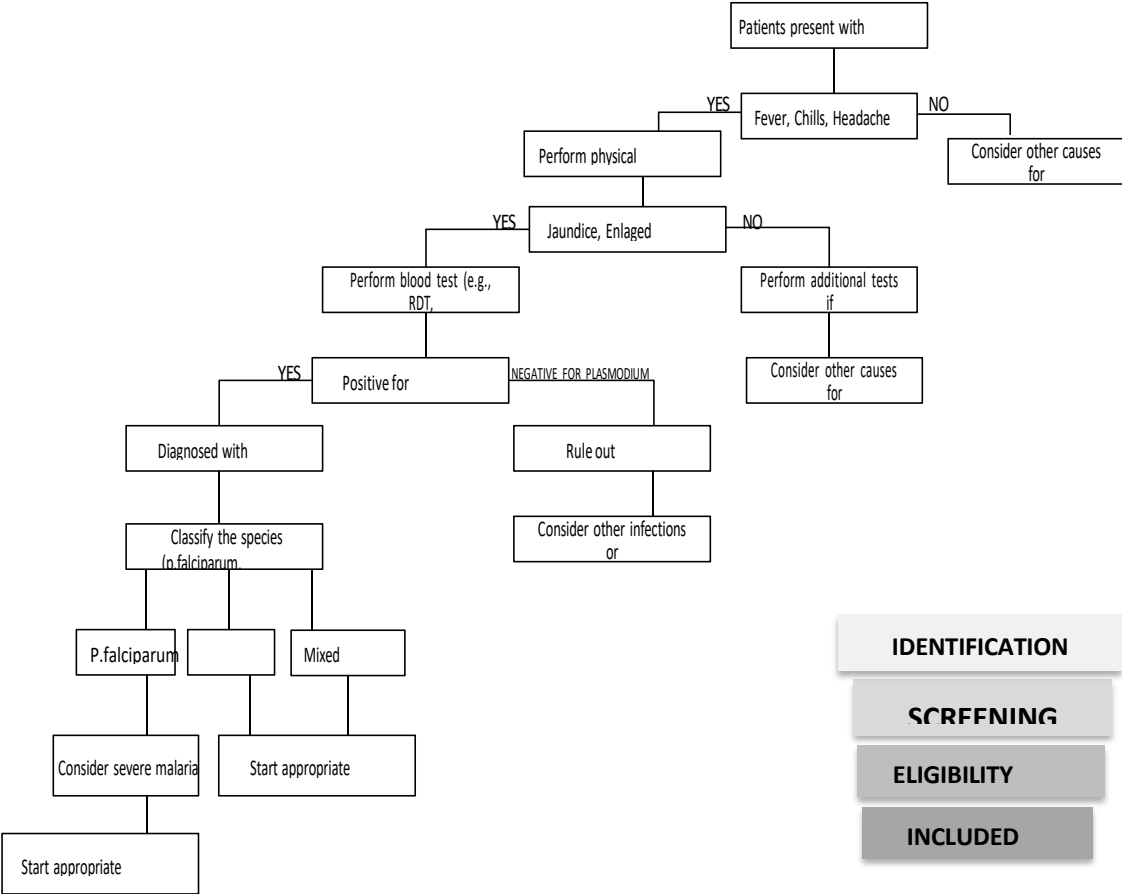


Fig 2.1 Flowchart of data collection

2.4 Ethical considerations

The Prime Institute of Health and Management Science Mardan provided ethical approval for the project. The Mardan Medical Complex and the Ahmed Medical Complex, both in Mardan Pakistan, gave their permission for the research to be done and provided relevant data. The Malaria Patients who responded to the questionnaires and participated in the interviews voluntarily accepted to the study.

2.5 Statistical Analysis

In our statistical analysis, we employed SPSS version 20 and utilized the Chi-Square test as our method. A significance level of less than 0.05 ($P < 0.05$) was deemed as indicative of statistical significance.

2.6 Blood sample collection

Blood samples were taken from the individuals suspected of having malaria. This sample is taken in an EDTA tube to prevent coagulation of the blood. As soon as samples arrived at the laboratory, an automated instrument was used to determine their hemoglobin (Hb) levels and check the existence of *Plasmodium falciparum* and *Plasmodium vivax*.

2.7 Rapid diagnostic test (RDT)

For quick identification, we used the strep method. To test on strep/kit we use a centrifuge to collect plasma from the patient's blood. Then we used plasma in the malaria kit along with their reagents. This test required minimal training. This test gives results within 5 to 30 minutes. The result shows us that the patient's blood has *Plasmodium falciparum* or *Plasmodium vivax* or either none of them.

2.8 Blood film preparation for microscopy

Through Blood film, we can detect malaria species as well as different components of blood. So, we prepared two blood films that are thin and thick blood films for microscopic examination. At MMC and AMC thin blood film is for examination of RBC morphology while thick blood film is for Malaria Parasites.

2.8.1 Thin Film

The primary objective of this film was the clarity of RBC. A single drop of blood is applied to the center of the slide to create a thin film. Place the slide in the horizontal plane, reduce the surface, and place a small drop of blood in its center. Take a second glass slide and use it to gently spread the smaller droplet of blood at the center of the first slide. This action will cause the blood to flow along the edge of the second slide. Maintain a 45-degree angle with the spreader as you carefully and steadily move it along the initial slide to encourage the blood to spread along the border. This technique helps to create a thin, even layer of blood for analysis.



Fig 2.2 Thin blood film

Reference: <https://medicallabtechnology.com/perfect-blood-smear-technique-prepare/>

2.8.2 The Thick Film

By dropping a few tiny droplets of blood in the center of the slide and utilizing the spreader's border to interact with the small drops and stretch to create a smooth thick film, a thick film has been formed. Avoid hitting the blood. The thick spherical layer has a diameter that was approximately 1 cm.

Fig 2.3 thick blood film



Reference: <https://medicallabtechnology.com/perfect-blood-smear-technique-prepare/>

2.9 Blood Film Staining

Giemsa stain is a well-known blood film stain for Plasmodium diagnosis. Each thin covering was fixed by dipping the slide in methanol for a few moments. The slides were arranged back-to-back side in a staining furrow, with the thick layers concentrated at one end of the lowest point. A 3% Giemsa stain solution was made by mixing 3 ml of Giemsa stock solution with 97 ml of buffered distilled water to a pH of 7.2. The trough was filled with stains. After 45-60 minutes of staining, gradually clean water was put into the trough holding slides to let the iridescent 'scum' float off. Pour out the remaining stain gently and wash with clean water slides were carefully retrieved from the trough and placed on a rack for drying with the film side facing down to dry.

2.10 Microscopic Examination Of Blood Film

Microscopic analysis of thin and thick blood films was performed using high power magnification using thin films under a 10x paired eyepiece with an objective of 100x oil immersion for the presence and species identification of parasites(24)

3. RESULTS

3.1 Malaria distribution by species

During the current study period, 5081 suspected blood samples were examined, out of 5081 blood samples 383 were found to be positive for malaria, representing an overall of 7.5%. Out of 383 positive samples, 315 (82.2%) were confirmed to have Plasmodium vivax whereas 66 (17.2%) had Plasmodium falciparum infection. 2 (0.5%) blood samples contained mixed infections.

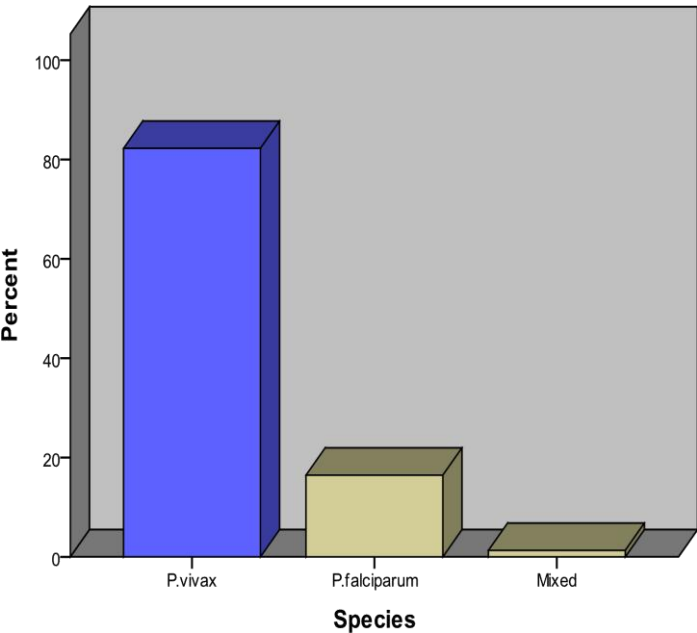


Fig 3.1 Shows malaria species along with their percentage

3.2 Malaria Distribution by Gender

Among all the studied participants, 203 (53.0%) males were found infected while 180 (46.9%) malaria parasites in females were reported. P. vivax affects 145 females and 170 men. The ratio of P. falciparum between genders is Female 28 and Male 38. Comparatively, 2 males were reported for mixed infections.

The chi-square value for gender-wise malaria is 4.338, df is 2 and the p-value is > 0.05

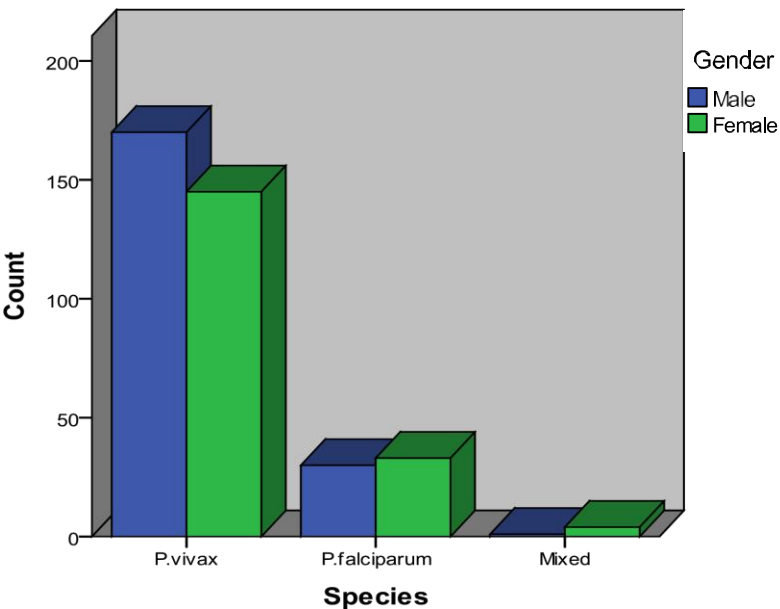


Fig 3.2 Show Malaria species percentage in male and female

3.3 Malaria distribution by age

P. vivax as well as P. falciparum infections affected people of all ages, although the incidence of these infections varied by age group. In our study, participants were categorized into different age groups, and a significant portion, 176 (45%), fell into the 15 years and older category. People aged 5 to 15 are 130 (33%) more affected age group. The lowest percentage of malaria species 77 (20%), is reported in children aged 0 to 4. The chi-square value for age-wise is 14.2, df is 4, and the p-value > 0.07

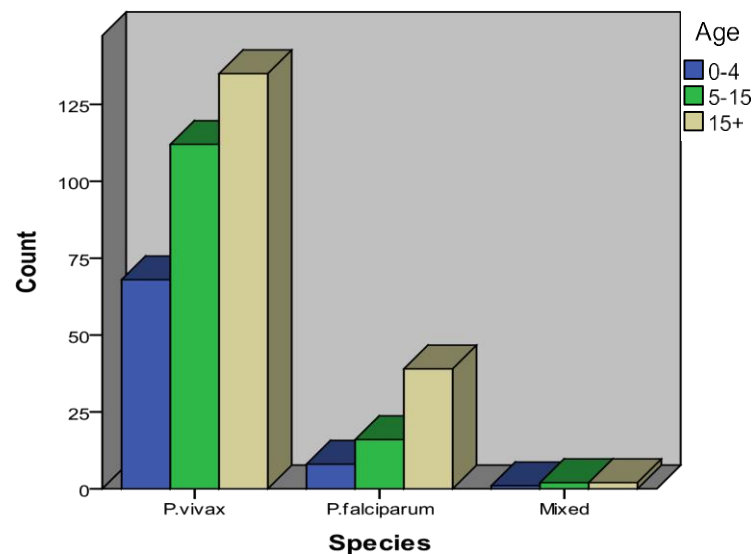


Fig 3.3 shows the distribution of malaria species in different ages

3.4 Monthly-wise distribution of malaria

The endemicity of malaria is at its highest peak in September 9.0% and in August 8.9%. Similarly, 5.9% in July and 5.0% in June shown in Fig 3.4.

Serial no.	Month	Total Enroll	Malaria Species	Total Positive	Male	Female	Urban	Rural
01.	June 2021	717	P. Falciparum	3	1	2	1	2
			P. Vivax	33	16	17	10	23
			Mixed Infection	0	0	0	0	0
			Total	36	17	19	11	25
02.	July 2021	1503	P. Falciparum	4	2	2	1	3
			P. Vivax	85	34	51	36	49
			Mixed Infection	0	0	0	0	0
			Total	89	36	53	37	52
03.	Aug 2021	1091	P. Falciparum	26	15	11	9	17
			P. Vivax	71	45	26	29	42

			Mixed Infection	1	0	1	0	1
			Total	98	60	38	38	60
04.	Sept 2021	1770	P. Falciparum	33	17	16	14	19
			P. Vivax	126	72	54	50	76
			Mixed Infection	1	1	0	0	1
			Total	160	90	70	64	96

Fig 3.4 Show Monthly-wise distribution of malaria

3.5 Area-wise Distribution of Malaria

144 out of 315 P. vivax cases are from urban areas which is 45%, while 171 cases are from rural areas which is 54%. In 66 blood samples that were examined in total, it was determined that P. falciparum, a type of malaria parasite, was present in 41 (62.1%) cases from rural areas and 25 (37.8%) cases from urban locations. This data highlights a P. falciparum is more common in rural settings than in cities., emphasizing the importance of targeted intervention and preventive measures in these regions. The mixed infection ratio in rural areas 2 cases is greater than in urban areas. The chi-square analysis for area-wise malaria is 5.672, df is 2 and the p-value is 0.059

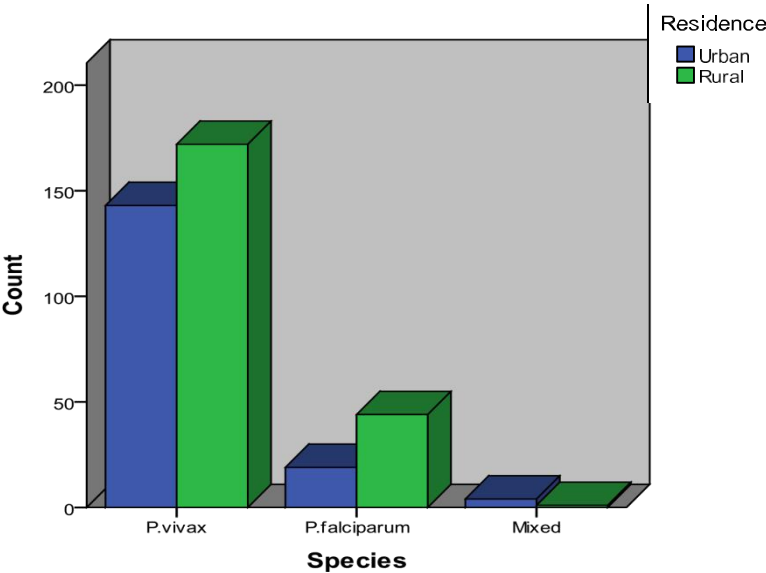


Fig 3.4 Shows species of malaria in urban and rural areas

During this study, most of the participants had malaria for the first time and they did not have a family history. The most common signs and symptoms of malaria found during

this study are fever, chills, headache, muscle aches, tiredness and sometimes vomiting. There is no common disease that is consistently found with malaria. Most participants sleep outside in June and July; indoor sleeping occurs in August and September.

4. DISCUSSION AND RECOMMENDATION

It is important to highlight that 5081 subjects were particularly examined for this study table (3.4). Notably, males (53.0%) have a significantly higher percentage of malaria infection than females (46.9%). Males in the area typically enjoy spending time together, sleeping outside, and actively participating outside, particularly in rural locations where the infection rate is 55.3%, which is noticeably higher than in urban areas where it is 44.2%. The same study(25) shows that males were (59.87%) while females were (40.13%). Another study(26) also showed that Males 56% were predominant as compared to females 44%. The higher representation of males in our study can be attributed to several factors. Males tend to be more actively engaged in the workforce and often have more outdoor activities in our society, which increases their likelihood of being exposed to infected mosquitoes. Additionally, they typically have easier and earlier access to healthcare facilities compared to females, largely due to traditional barriers that can hinder females from seeking prompt medical attention(27). Previous research has shown that several factors play a role in influencing the malaria prevalence rate. These factors include age, the surrounding environment, access to suitable sleeping arrangements, and residing in rural areas, among others.

Similarly, a study in Mardan evidences that *P. vivax* is the predominant malaria parasite. Among the various malaria-causing species, *P. falciparum* was the most commonly identified, with no reported instances of *P. ovale*, *P. malariae*, or mixed infections observed in the cases examined(28) but The current study shows the rate of *P.vivax* is 82.5% while *P.falciparum* is 17.2% and mixed infection is 0.5% at the Mardan. another study shows In the country, the prevalent malaria parasite species are limited to *Falciparum* and *vivax* parasites. Notably, *P. vivax* stands as the dominant species, contributing to more than 70% of the overall malaria cases in the nation(29) *P. vivax* continues to be the predominant species in our region(30) In the Multan district, a combined infection rate of 2.3% was identified(31) *P. vivax* was the main cause of 98% of all malaria cases in the chosen location. (32) Conversely, *P. falciparum* engages in sexual reproduction, a process that necessitates a minimum of 10 days to generate their gametocytes within the host's body.

During this study 15 or above age participant is 45% and 5 to 15 participants age ratio is 33% and the 0-4 age is 20%. Previous studies show *Plasmodium falciparum* exhibited a notably elevated occurrence, with a prevalence of 16.31%, among individuals

aged 21 years and older. and *Plasmodium vivax* was also observed(33) Another study(29) also presents patients aged 15 years or more show 82% prevalence. The age group of 0 to 5 years experienced a notably higher infection rate, with a prevalence rate of 22.8%(34) The prevalence of malaria can be linked to specific factors in our region. Night-time electric power outages, along with hot weather conditions, often lead people to sleep outdoors, particularly for extended periods. Children, in particular, are frequently not dressed in protective clothing like long-sleeved shirts and trousers, nor are they provided with mosquito nets. This situation makes them vulnerable to mosquito bites carrying the infection, which, in turn, contributes to the increased transmission of plasmodium within the population(35). In summary, the most frequent malaria infections are observed in children under the age of ten. This susceptibility can be attributed to their still-developing immune systems and less favorable hygienic conditions. Additionally, accurately diagnosing severe malaria in children displaying danger signs continues to present a significant challenge(22) The current study shows the high endemicity of malaria in September at 9.0%, August at 8.9%, July at 5.9%, and June at 5.0%. Previous studies show that *P.vivax* shows its peak infections between April to September and *P.falciparum* whose peak infections were observed between August to December (36) Another study In Pakistan, there is a higher susceptibility to malaria among women who are pregnant and children who are under age of ten which display noticeable symptoms of the disease from August to September(37) In Khuzdar, the highest infection rate, at 84.84%, was observed, while the lowest rate of infection, at 56.06%, was noted during October. (38) The peak of malaria cases occurred during September, whereas the lowest case numbers were documented in March. Pakistan, being a tropical country with a significant portion of its population engaged in agriculture, experiences a pattern influenced by the seasons. During the rainy season, water accumulates, creating favorable conditions for mosquito breeding, which in turn leads to a higher incidence of malaria cases. (39) One potential explanation for the elevated incidence of malaria cases among men is their greater susceptibility to exposure to malaria parasites compared to women. This heightened risk is often associated with their occupation in fields or outdoor work, which increases their likelihood of contracting the disease(40).

In this study selected participants are from Urban and rural regions with percentages of 44% and 55% it is because of In rural regions, there is a deficiency of medical professionals, compounded by a shortage of trained healthcare personnel and inadequate disease surveillance systems. This combination of factors contributes to a significant increase in the frequency of malaria and its corresponding health

complications in these areas(41) Another study(42) performed in both urban and rural settings of district Quetta observed a high prevalence of *P. falciparum* 55.55% in urban and 65.82% in rural. Another study in Charsadda observed that malaria is more common in illiterate people (54.11%) the reason is that illiterate people are not fully aware of how they can prevent, such infection and once they develop such infection they do not know how to require the treatment properly(43).

During this study fever, chills, and headaches were found more common in malarial patients. A study shows Fever was the most prevalent presenting symptom (98%), then headache, rigors, and chills(44) The fever was mostly associated with rigors (60%) and chills (74%)(45). In the present study, microscopy results are found to be more dependable than rapid diagnostic tests (RDTs). This slight variation in accuracy comparing the two techniques might be influenced by the use and effectiveness of anti-malarial drugs. Specifically, the rapid testing device failed to identify *P. vivax* trophozoites, as they were no longer detectable under the microscope. This was likely due to the administration of anti-malarial drugs, which effectively killed the parasites. As a result, the trophozoites ceased secreting Plasmodium lactate dehydrogenase enzyme (PLDH), a characteristic only exhibited by live forms of the parasite(46).

The current study found 14 pregnant ladies infected by malaria species. So It is very important to study malaria species among pregnant ladies in district Mardan to avoid morbidity and mortality during pregnancy. While Studying the patient's CBC report showed low Hemoglobin and thrombocyte levels. So it is necessary to study anemia and thrombocytopenia in all ages of people during malaria in district Mardan. The association between malaria prevalence is yet another crucial issue that has to be looked further and the hygienic conditions inside schools and homes, to develop effective interventions to reduce the transmission of the disease.

5. CONCLUSIONS

This research highlights the endemicity of malaria in the Mardan district, particularly in healthcare facilities like Mardan Medical Complex and Ahmed Medical Complex in Khyber Pakhtunkhwa. It revealed that there was a higher incidence of malaria in males (53.0%) than in females (46.9%). Additionally, malaria was prevalent in rural regions (55%) compared to urban areas (44%). The study found that the age group of 15 and above had the greatest number of cases of malaria (45%), while the age range 0-4 had the lowest rate (20%). The most prevalent is Plasmodium vivax parasite in the study population (82.5%), followed by Plasmodium falciparum (17.2%), and a small percentage of mixed infections (0.5%) found in the month of June (5.0%) July (5.9%) august (8.9%) and September (9.0%). These findings offer valuable insights into the malaria situation in the

region and similar ecological areas in Khyber Pakhtunkhwa. Increasing awareness and improving healthcare services can be vital steps in managing and reducing malaria cases in rural areas of Pakistan. This research contributes to a better understanding of malaria's epidemiological profile and can aid in more effective control measures.

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