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ORIGINAL ARTICLE

Prevalent Occurrence of Plasmodium Falciparum Malaria in Pakistan

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ABSTRACT

Background: In Pakistan Plasmodium falciparum has been on the rise over the last few decades.
Objective: This study primarily focused upon finding out the prevalent occurrence of malaria caused by Plasmodium falciparum in Pakistan.
Methodology: This research analyzed different variables related to the respondents (n=209) of the study. The variables included age, occupation, education, patient's visit to the hospital, transmission of malaria, prior treatment of the patient (if any), usage of long lasting insecticidal nets (LLINs), hygienic/unhygienic conditions and insecticidal spraying. Investigative analysis was purely based upon a questionnaire followed by the fundamental microscopic and polymerase chain reaction (PCR) for assessing the disease index.
Results: The results indicated that out of 209 respondents of the study, 100% tested positive for malaria whereas, only 179 (85.6%) cases were confirmed via PCR analysis and were chosen for further investigations. Males (67%) were found to be more prone for malarial infection. Respondents in the age group of 26 to 35 years were the most (43.5%) vulnerable to this infection. 32.9% laborers, as compared to any other occupation affected by plasmodium. About 77.6% of people had already been infected by malaria and were treated, but only 14.5 % people visited the hospital. In addition to this, merely 12.2% respondents had used LLINs and 2.23% of them had utilized insecticidal spray.
Conclusion: Lack of awareness, selfmedication, unhygienic environment, unemployment, and political instability contributed to malarial transmissions.
Keywords: Malaria, Plasmodium falciparum, Pakistan, Microscopy, PCR.

INTRODUCTION

Phylum Apicomplexa is a diverse group that includes many organisms such as parasites including the Plasmodium family responsible for causing malaria. More than 250 types of Plasmodia are known to exist, among which five are known to cause disease in humans: Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae, Plasmodium knowlesi and Plasmodium ovale[1]. P. falciparum is the major factor for most of the deaths caused by malaria. It is the most dominant of all the species in sub Saharan Africa and South East Asia. Spread of Plasmodium falciparum is a major threat to global malaria control. An estimated 500,000 episodes of malaria infection occur in Pakistan annually[1]. Studies indicate that infection with Plasmodium falciparum has been on the rise over the past few decades in Pakistan[2]. Between 1987 and 1990, the proportion of malaria infections caused by P. falciparum increased from 34 to 54% in north west Pakistan[3], and in the provinces of Baluchistan and Sindh. The prevalence of P. falciparum among microscopy positive cases increased from 45% in 1995 to 68% in 2006[4,5], probably due to increasing resistance to commonly used monotherapies. In the year 2010, out of a total of 240,591 reported malaria cases in Pakistan, 73,857 (31%) were P. falciparum[6] which causes the fatal form of malaria. The transmission of Malaria occur through bite of female anopheles mosquito from one individual to another. Symptoms of malaria normally start from day seven to twenty five after bite. The common symptoms of malaria are fever, weakness, headache, vomiting, chills, and cough. Malaria can be confused with many other diseases due to symptom similarities, including flu, dengue, and typhoid. The diagnosis of malarial infections should also be considered in any patient with fever/pyrexia of unknown origin. Treatment of malaria should not be started until the presence of malarial parasites has been confirmed by microscopy[7].

Despite advances in knowledge, malaria continues to cause significant morbidity and mortality worldwide[8]. Over 40 % world population lives in malaria endemic areas which include Southeast Asia, India, Bangladesh, Pakistan, Africa areas of middle east, Central and South America[9]. Pakistan is ranked in endemic belt based on the incidence of one case per thousand. Severe malaria has been a main cause of mortality worldwide, but uncommon in developed countries. It is a significant cause of morbidity and mortality in addition to creating an enormous social and economic burden. Today the most common problems in the management of malaria is drug resistance of P. falciparum to various antimalarial drugs and occurrence of systemic complication[10]. Socioeconomic condition, lifestyle of the people such as sleeping outside, subsequently occupations like farming, fishing, animal rearing and lack of awareness about malaria have a major impact on transmission. Climatic conditions do influence mosquito breeding. The present study was design to see the spreading pattern of Falciparum malaria in different areas of Pakistan in 2018 to 2019 in the demographic, socio economic, and educational context of the patients.

MATERIAL AND METHODS

Study Population: In Punjab, Sindh, Baluchistan, and Khyber Pakhtunkhwa, sixteen public and private hospitals were called to provide routine malaria surveillance blood sample. In which Khyber Pakhtunkhwa (Dera Ismail Khan, FR DI Khan, Bannu, Kohat, Karak, Dir FR Khyber), Punjab (Multan and Dera Ghazi Khan), Baluchistan (Quetta and Zhob), and Sindh (Karachi and Khyerpur) responded positively.
Study Duration: The data from these sites was obtained between January 2018 and February 2019, and this study focused on patients presenting with who attended both government and private hospitals.
Consent Form: After informed consent information was acquired from all patients or guardians before medical examination history and venous blood sample collection.
Inclusion and Exclusion Criteria: Women who were pregnant or nursing and children under two years of age were excluded from the study. However, patients exhibiting relevant signs and symptoms of malaria and those who were confirmed positive through microscopy for P. falciparum malaria were included in the study. Moreover, the patients suffering from malaria that were confirmed through nested PCR were also included[11]. P. falciparum parasites (1000 to 100,000 parasites/ul) in thick films were included in the study. Their auxiliary temperature (> 37.5 ^{O}C) was assessed with a thermometer.

Data Collection Procedure: Information such as gender, age, occupations, education, knowledge about malaria, living conditions, treatment and attitude towards malaria were investigated by correspondents through questionnaires. A total 209 patients of malaria infections were confirmed by microscopy and 3 ml of intravenous blood was collected through vein puncture from the patients. 10% Giemsa solution was used to stain thick and thin films, and the films were seen using oil emersion lens. Thick slides were screened for the diagnosis of malaria while thin slides were analyzed for the identification of species. Laboratory technicians or technologists skilled in malaria diagnosis evaluated smears in accordance with WHO standards[12].

Microscopically confirmed 5 ml of whole blood was collected in EDTA tubes from 209 patients with malaria infections. Samples were shipped to Karolinska Institute, Stockholm, Sweden for subsequent molecular analysis. The QIAamp DNA mini kit (QIAGEN, Inc., Germany) was used to extract DNA from 200 µl of whole blood following the manufacturer's guidelines.The extracted DNA was stored at 20 °C.

PCR was performed for the confirmation of presence of P. falciparum in the patients suffering from malaria. 2% multipurpose agarose gel (Roche Diagnostic Gmbh USA) was electrophoresed in the presence of Tris acetate EDTA (TAE) buffer and stained with ethidium bromide. UV transillumination was carried out after the gel electrophoresis. Using a 100 base pair (bp) ladder, we calculated the length of the difference. 5 mL of the blood was combined with 1 to 2 microliters of a 10× loading dye and separated on 2% agarose gel in TAE, and DNA was detected by ultraviolet transillumination. Using a DNA ladder marker of 100 bp, band fragment sizes were determined. Using the assumption that one band indicated one amplified PCR fragment per gene, the average size of each polymorphism was calculated for each grouping of alleles.

Statistical Analysis: All collected data were entered in Microsoft Excel 2007 and further analysis were through statistical package for social sciences 21. Data presented in tables with mean and percentage.

RESULTS

Total 209 patients (n=209) tested positive for P. falciparum malaria via microscopy. While (179/209) 85% were confirmed positive through PCR for falciparum malaria. Further analyses were carried out on the PCR positive malarial patients. Out of 179 participants, n=120 that is 67% were male and n=59 that is 32% were female (Table 1).

Tables 1: Gender wise distributions of Plasmodium falciparum affected individuals.

Gender	No. of cases	Percentage %
Male	120	67.0
Female	59	32.9
Total	179	100

Among a total of 179 (n=14, 7%) were students, (n=34, 18%) were self employed, (n=33,18%) were drivers, (n=39, 21%), and the majority of the cases reported (n=59, 32%) were laborers (Table 2).

Table 2: Occupation wise distribution of Plasmodium falciparum affected individuals.

Occupations	No. of cases	Percentage
Students	14	7.8
Self Employed	34	18.9
Drivers	33	18.4
Unemployed	39	21.7
Labors	59	32.9
Total	179	100

In total of (n=179), (n=26, 14.5%) had visited the hospital before whereas (n=153, 85.4%) didn't. (Table 3).

Table 3: Distribution of affected individuals based on their prior hospital visit.

Did the patient visit to hospital before	No of affected individuals	Percentage
Yes	26	14.5
No	153	85.4
Total	179	100

Out of (n=179) (n=22, 12.2%) were using the LLINs while (n=157, 87.8%) didn't used the LLINs (Table 4).

Table 4: Percentage of affected individuals using LLINs.

Insecticidal Spray	No of affected individuals	Percentage
Yes	04	2.23
No	175	97.7
Total	179	100

Among (n=179) participants (n=4, 2.2%) stated that insecticidal spray was being done by governments while (n=175, 97.8%) mentioned that there is no spraying done in their areas (Table 5).

Table 5: Distribution of affected individuals based on insecticidal spray.

Usages of LLINs	No affected individuals	Percentage
Yes	22	12.2
No	157	87.7
Total	179	100

DISCUSSION

Malaria is main public health problem in Pakistan ranking at 2nd position after Tuberculosis according to Malaria Control Program Pakistan[13]. It is a disease of tropical and subtropical countries especially Africa, and Asia. Plasmodium has distinct species among which Plasmodium vivax and Plasmodium falciparum are common in Pakistan. Preliminary treatment and prompt diagnosis is critical in resource limited countries like Pakistan. Around 1.6 million people are infected per year in Pakistan. It represents 16.5% of infection weight, and second common disease of the nation. In 2018, 0.3 million cases were found in all districts of Pakistan, among these the causative agent of disease in 33% cases was P. falciparum[14]. Number of cases has varied annually so it is hard to anticipate patterns of occurrence of malaria in Pakistan[15].

Malaria is at its peak during monsoon in Pakistan, consequently infecting many people. In Pakistan, around 95 million people out of the total population survive in the malaria endemic regions. The API (Annual Parasite Incidence) in Pakistan is 0.74/1,000[16]. Pakistan was labelled as an average malaria endemic nation with an acknowledged malaria control strategy[17]. Around 60% people of Pakistan live within malaria endemic areas[18]. In spite of an acknowledged malaria control strategy in Pakistan, 500,000 people are infected with malaria and 50,000 deaths occur because of it per year[19].

Various studies have declared malaria to be predominant in different regions of Pakistan. According to different surveys carried out between 2005 and 2009, the most noteworthy malaria endemic region was FATA, followed by Khyber Pakhtunkhwa and Baluchistan. Infection rate was most elevated in 2009[20]. An investigation led in regions of Baluchistan and Punjab discovered 28.2% suspected cases were caused by P. vivax and 71.7% by P. falciparum[21]. In Sindh however 35% suspected cases were caused by P. falciparum and around 65% by P. vivax according to the surveys conducted in 1997 and between 2004 and 2005[22,23].

WHO revealed that the incidence of malarial infections by P. falciparum raised from 34% recorded in 1984 to 54% cases in 1990. The incidence of malaria due to P.falciprum kept rising from 45% cases in 1995 to approximately 68% cases in 2006 in Jhangara and Quetta[24]. Out of 240,591 cases of malaria country wide, 73,857 were caused by P. falciparum in the year 2010[25].

This study was basically related to incidence of P. falciparum malaria which is severe form of malaria and found second after vivax malaria[13]. It affects all age groups however

males were seen to be affected more as compared to females as reported in previously published studies. According to the studies conducted by Ibrahim et al., 69.79% of the total malarial infected population were of the males[26]. Similarly, the output of the research performed by Hussain et al., demonstrated the higher rate of malarial infection in males (6.3%) than females (3%) by P. falciparum[27]. Likewise, Naqvi et al., investigated the prevalence of malaria in both genders and found out that 4.97% males were affected by malaria whereas infection rate in the females was 3.82%[28]. Recent flooding in Pakistan has contributed significantly to the endemicity of malaria, affecting more than sixty districts and affecting about 20 million people[22]. Pakistan experiences seasonal transmission of malaria, usually occurring during the month of July followed by August[1]. P. falciparum is most prevalent in the late summer and winter (August to December). In Baluchistan, Anopheles fluviatilis and Anopheles annularis were discovered, two novel species also involved as vectors. In KPK, Balochistan, and Sindh, malaria outbreaks are more likely, whereas Punjab has a lower risk[18].

According to the results of our research 43.5% of uneducated people get infected due to malaria, this is because they take the medicine without any proper prescription. This develops resistance in P. falciparum. As depicted by our outcomes, only 14.5% patients visited hospital when they got infected. Self medication or blind treatment can cause drug resistance as previously Chloroquine and Sulfadoxinepyrimethamine (SP) became ineffective[29,31].

In the context of what we found, 77% respondents were living in an unhygienic environment and only 12.2% of them had used LLINs whereas, not more than 2.23% patients had used insecticidal sprays. Poor socioeconomic system, lack of malaria awareness, misconception about transmission, poor drainage system, early treatment, diagnosis and availability of long lasting insecticidal nets (LLINs) and insecticidal spraying[32], are the key challenges to prevent and control malaria[33].

Observing that Plasmodium species are prevalent in Pakistan and many alterations are undergoing in both vector and the host, it is important to make suitable treatment options available[34].

The incredible variety of malarial burden in various topographical areas can be managed by various elements. Malarial infection is the consequence of a complicated exchange between human, parasite, and mosquito. The connection between these components is a significant factor that influences the spread of malaria. Financial variables involve poverty, low quality houses and education, limited access to medical care and the presence of dynamic malaria control strategies[35]. For a nation like Pakistan which suffers from financial imperatives, malaria adds to poverty by diminishing the monetary development and thus also contributes to the burden, bringing about an endless loop. Then again, the pattern could be switched into an ethical cycle by following the proposal that diminishing malarial transmission might be quite possibly the best way to animate monetary improvement in the malaria endemic nations[36]. Although malaria Control Pakistan working in malaria endemic countries with the help of global fund should strengthen the control strategy with advocacy/awareness and stakeholders must show positive attitude and show seriousness as this is the era of Covid-19 which may alter the future scenario which is unpredictable.

CONCLUSION

It is the need of the hour to conduct regular vector control studies to monitor the sensitivity of vectors to insecticides and to implement broad educational programs especially for the uneducated population of Pakistan in order to control the spread of malaria in this resource limited country.

Ethical considerations: The study was ethically approved from Research Ethical Committee (REC) of Kohat University of Science and Technology (KUST).

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