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The frequency of malaria in patients visiting selected hospitals in Peshawar, Khyber Pakhtunkhwa, Pakistan

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Abstract
The aim of this study was to report the incidence of malaria in patients visiting three major hospitals in Peshawar, Khyber Pakhtunkhwa, Pakistan. A total of 375 suspected malaria blood samples were randomly collected from patients in three-main government teaching hospital, Hayatabad Medical Complex, Khyber Teaching Hospital, and Lady Reading Hospital Peshawar, KPK, Pakistan from November 2014 to July 2015. Both thick and thin blood film was prepared on a slide for microscopic detection of *Plasmodium* parasites. Out of the total samples, 66 were reported positive for *Plasmodium*. The results showed 17.6 % positive cases and were more frequent in male, (23.5%) as compared to females (10.5%). The highest percentage was recorded in the age group of >14 male, (27.5%) whereas the lowest frequency was documented in the age group of <5 females (5%). The highest frequency (21.7%) was recorded in the rural population as compared to (11.3%) in urban population. The high percentage of *P. vivax* (15.5%) was recorded, followed by *P. falciparum* (1.3%), while the lowest frequency (0.8%) of malaria infection was recorded in Mix infection (*P. vivax and P. falciparum*).

Keywords: Endemic, Malaria, Pakistan, *Plasmodium*, Peshawar, Rural

Introduction
Malaria is a vector burn disease that affects millions of people around the world, especially developing countries [1]. After every thirty seconds, one child dies from malaria infection, and *Plasmodium* is still very dangerous [2]. It is assumed that one million deaths per year occur from *Plasmodium* infection and about eighty percent of deaths occur in babies and young children of Africa [3]. Annually about 2.5 million malaria cases are recorded from South Asia and approximately seventy sex percent *Plasmodium* infection cases were reported from in India [4]. In the world, many organizations are initiated the malaria control program to control the malaria infection. *Plasmodium* is a specific parasite responsible for causing malaria. Malaria is a parasitic disease of the underdeveloped population that is more widely found in many small villages and urban housing areas in specific areas at specific times. The population of these areas often inhabits a large number of people, causing considerable economic losses. Rural Sindh and Baluchistan are the areas which are having the severe disease effect in Pakistan, and acute malaria is a famous root of morbidity and mortality in these regions [5]. This higher level of death rate is due to the absence of understanding and training of malaria case management instruction in acute and confusion in *Plasmodium vivax* and *Plasmodium falciparum* malaria [6]. Among the sweltering countries, Pakistan has been placed where dependent on a number of factors like atmospheric conditions, increasing human resident's densities, present of huge agricultural area, wide open networks of irrigation and drainage channels, river and some water dams for power production are favorable to the rapid increase in numbers of arthropod vector in country. Total five *Plasmodium* species can infect the human population, and the most severe form of the *Plasmodium* infection are affected by *Plasmodium falciparum. Plasmodium falciparum* and *Plasmodium vivax* have the short duration of the natural incubation period. The prevalence and characterization of *Plasmodium* study have been in different areas of Pakistan. There are 39 districts are classified at higher risk of malaria infection in two southern provinces of Pakistan Sindh and Baluchistan [7]. In Pakistan malaria is endemic and Plasmodium causes most cases of malaria infections *vivax*, while in the southern Punjab, Baluchistan and Sindh provinces the recent malaria infection

caused by *Plasmodium falciparum* [8]. The given study aims to find out the occurrence of malaria among the urban and rural population at govt teaching hospitals Peshawar, Pakistan. It also investigated the species of *Plasmodium* causing malaria Peshawar, KPK, Pakistan.

Materials and Methods

The present study was conducted in three largest hospitals of Peshawar including Hayatabad Medical Complex (HMC), Khyber Teaching Hospital (KTH) and Lady Reading Hospital (LRH), Kpk, Pakistan from, November 2014 to July 2015. All the suspected patients presented with common indications of malaria included temperature, shaking, arthralgia (joint pain), spewing, anemia (caused by hemolysis), and high temperature and worrying etc. were included.

The descriptive study was designed to find out the frequency of malaria in patients who visited the selected hospital in Peshawar, Pakistan among the urban and rural population. Finally, the data was analyzed species-wise, sex-wise and age wise.

Blood Collections

Fingerstick obtained capillary blood

Pre-cleaned slides were labeled with patient's name, date and time of collection. Slides were cleaned with 70 to 90% alcohol and then allow to dry. The finger was checked, usually the middle or ring finger and heel in infant to be punctured. The area to be punctured was made clean with 70% alcohol and then allow to dry. The ball of the figure and heel in the infant was pricked by using the finest lancet. The first drop of the blood was wiped with clean gauze. The next drop of blood was touched with clean slides, and it was repeated with several slides (at least two thick and two thin smears). The blood smear was made carefully.

Venipuncture obtained venous blood

Pre-cleaned slides and collection tubes were labeled with the patients' name, date and time of collection. For blood collection, the site was cleaned well using 70% alcohol and allow drying. The venous blood was collected in a vacuum tube containing anticoagulant (EDTA); alternatively, the blood may also be collected in a syringe and then transfer to a tube having anticoagulant, and the tube was mixed thoroughly. After collection of blood, at least two thick and two thin smears were prepared.

Preparation of Thick and Thin Blood Smears

For thick and thin blood smear the separate slides were used.

Thin blood smear: A hygienic spreader slide was held at a 45° angle towards the drop of blood on the specimen slide and wait so that the blood spread along the whole thickness of the spreader slide.

Thick blood smear: A clean slide corner was used to spread the drop of blood in a circle, and the smear was made too much thick to fall off the slide. Before staining the thick and thin films were allowed to dry completely. The thin film was fixed with methanol (100% or absolute) and let it dry completely and the thick film was not fixed.

Microscopic Diagnosis of Malaria

Both thick and thin blood film was prepared on a slide for microscopic detection of *Plasmodium* parasites. After air-drying, the thin blood films were fixed in methanol and both films were stained with 10% Giemsa solution for 10 min and examined by experienced laboratory expert using 100 microscopic fields with the 100X objective and manual for laboratory diagnosis of malaria. Parasitaemia was estimated by counting the number of parasites per 200 white blood cells in a thick blood film and then calculated as parasite count per microliter by assuming a total specimen WBC count of 8,000/µl. The Giemsa microscopy was performed by two experienced laboratory technicians and verified by a study blinded to a third expert to resolve any inconsistencies between the two readers. The reported parasite density is the average of the number of parasites in two laboratory personnel. when more than 10% discrepancy was observed between the two readers parasite count, the third expert blindly counted parasite load and recorded as correct result.

Results

Study Area

This study was conducted in the urban and rural populations of Peshawar, Pakistan from November 2014 to July 2015. The three leading government teaching hospitals (i.e. Hayatabad Medical Complex, Khyber Teaching Hospital, and Lady Reading Hospital, Peshawar Pakistan) were included. The patients and their data were randomly collected from the three leading government teaching hospitals. The total number of malaria-suspected patients were 375 including males, females, and children. Table 1

Table 1: Collection of blood samples from three major selected hospitals, Peshawar

Hospitals	Total Suspected Samples n=375	MP +iv Samples (%)
Hayatabad Medical Complex	150	30 (20)
Khyber Teaching Hospital	105	15 (14.2)
Leady Reading Hospital	120	21 (17.5)
Total	375	66 (17.6)

A total of 375 malaria suspected blood sample were randomly collected from different major hospitals of Peshawar, Khyber Pakhtunkhwa, Pakistan. Which include Hayatabad Medical Complex was 150, Khyber Teaching Hospital 105 and Lady Ready Hospital 120. The highest frequency was observed in Hayatabad Medical Complex that is 30 (20%), followed by Lady Reading Hospital 21 (17.5%), while the lowest percentage was observed in Khyber Teaching Hospital 15 (14.2%). The prevalence of malaria is very low in Punjab, especially in Islamabad, compared to other parts of Pakistan.

Age wise distribution of malaria cases from selected hospitals, Peshawar

The individuals were categorized into six groups, i.e. <5 M, 5-14 M, >14 M, <5 F, 5-14 F and >14 F years. The highest percentage was recorded in the age group of >14 male, i.e. 32 (27.6%), followed by the age group of 5-14 male, i.e. 13 (23.6%) and the lowest percentage in the age group of <5 male i.e. 3 (9.1%), while >14 age groups of females showed comparatively low frequency than male, i.e. 10 (9.2%), followed by the age group of 5-14 female, i.e. 7 (16.7%) whereas the lowest frequency was recorded in the age group of <5 female, i.e. 1 (5%). Table 2

Table 2: Age wise distribution of malaria cases in selected hospitals, Peshawar

Age	Total Samples	MP +vi Samples (%)
Years/Gender		
<5 Male	33	3 (9.1)
5-14 Male	55	13 (23.6)
>14 Male	116	32 (27.6)
<5 Female	20	1 (5)
5-14 Female	42	7 (16.7)
>14 Female	109	10 (9.2)
Total	375	66 (17.6)

The above results Table 2 showed the age wise distribution of malaria cases in male in three major hospitals of Peshawar, Pakistan. The age groups were categorized, i.e. <5 years, 5-14 years and >14 years male. The high (27.6%) percentage of malaria positive cases were recorded in male in the age group of >14 years while the low (9.1%) percentage of malaria positive were noted in male having age group of <5 years. In the female, the high percentage (16.7%) of positive cases were recorded in a female in the age group of 5-14 years while the low percentage (9.1%) were documented in <5 years age female. The overall results showed high (27.6%) in male in the age of >14 years as compared to female. In the previous study, a majority of patients (63.6%) were >14 years old. The almost similar pattern seen in Karak and Karachi. The highest percentage (20.67%) of malaria in the age group 5-14 years have been reported in the Bannu area of Khyber Pakhtunkhwa. Pakistan.

Sex wise distribution of malaria cases in selected hospitals, Peshawar

The frequency rates of malarial infection in males showed comparatively higher percentage 204 (23.5%) as compared to females 171 (10.5%) population residing in the major region of Peshawar, Khyber Pakhtunkhwa, Pakistan. The probable reason for the unequal number of males' malaria patients comprises a high contact because males are not well covered when it works outside as compared to females, which may lead to a high number of infected Anopheles bites. The similar result is also reported in Karak. Table 3

Table 3: Sex wise distribution of malaria cases in selected hospitals, Peshawar

Sex	Total Samples n=375	MP +ve Samples (%)
Male	204	48 (23.5)
Female	171	18 (10.5)
Total	375	66 (17.6)

Malaria cases among the urban and rural population in selected hospitals, Peshawar

To find the malaria frequency among the urban and rural population in Peshawar, Pakistan. The higher 49 (21.8%) frequency was recorded in a rural area while in an urban area the malaria infection cases frequency was 17 (11.3%). The rural population is more exposed to malaria infection as compared to the urban population. The similar pattern of malaria incidence was also reported in Quetta, Pakistan Table 4

Table 4: Malaria cases among the urban and rural population in selected hospitals, Peshawar

Population	Total Sample n=375	MP +vi Sample (%)
Urban	150	17 (11.3)
Rural	225	49 (21.8)
Total	375	66 (17.6)

Species-wise distribution of malaria cases in selected hospitals, Peshawar

To find out the frequency of species-wise distribution of malaria infection, all the positive malaria samples species were separately screened from selected major three hospitals in Peshawar, Pakistan. The frequency rates of malarial infection in *Plasmodium* species showed comparatively higher percentage of *Plasmodium vivax* 58 (15.5%), followed by *Plasmodium falciparum* 05 (1.3%) and the lowest frequency of malaria infection was in Mix malaria cases 3 (0.8%). The highest incidence of malaria in Pakistan has been found Quetta, the highest proportion of infection was *Plasmodium falciparum*. Table 5

Table 5: Species wise distribution of malaria cases in selected hospitals, Peshawar

Species-wise distribution of malaria cases						% of Confirm MP Cases		
Hospitals	**Total Sample**	**Dx *P. vivax***	**Dx *P. falciparum***	**Dx Mix**	**MP +ve samples**	**P.V %**	**PF %**	**Mix %**
HMC	150	27	02	01	30	18	1.3	0.6
KTH	105	13	00	02	15	12	00	1.9
LRH	120	18	03	00	21	15	2.	0.0
Total	375	58	05	03	66	15.5	1.3	0.8

Discussion

Malaria is regarded as one of the main health problems in Pakistan. National wide the reported malaria cases in 2008 were about 2.6 million and approximately fifty thousand deaths occur annually [9]. The causing agent of malaria is *Plasmodium* which is an intracellular parasite and is spread from one person to other by a mosquito. This intracellular parasite has different species but the most know species are two one is known as *Plasmodium vivax* and the second is known as *Plasmodium falciparum*. It is estimated that approximately from three hundred to five hundred persons are suffered from malaria worldwide, and about 1520000 people expired annually [10]. In 2007, the MCMDG (Malaria Case Management Desk Guide) stated that the majority of malaria cases in Pakistan are contributed by Baluchistan and Sindh which are then followed by FATA and Khyber Pakhtunkhwa, and a very smallest number of cases are recounted in Punjab [10].

The present study results showed the malaria frequency in three majors' hospital of Peshawar Kpk, Pakistan. The highest 30 (20%) percentage was detected in Hayatabad Medical Complex that is followed by Lady Reading Hospital 21 (17.5%), while the lowest percentage was observed in Khyber Teaching Hospital 15 (14.2%). Also, the highest frequency of malaria in Pakistan is found in district Quetta where the highest proportion of *Plasmodium falciparum* occur because *Plasmodium falciparum* is predominant in that area although cities of Zhob, East Baluchistan and Khuzdar in the province

of Baluchistan [11]. In comparison with other regions of Pakistan the province of Punjab and especially in Islamabad city the prevalence of malaria is very less. In 1999, a door to door survey was conducted in the province of Punjab which recorded approximately 2.4% incidence with a positive microscopy malaria [12]. In the areas mentioned above, the migration of cross-border may have contributed the surge of malaria. The Afghanistan immigrants escaped between 1979 and 1982 into provinces of Baluchistan and KPK [13]. The highest proportion was recorded in the age group of >14 male (27.6%), followed by the age group of 5-14 male (23.6%) and the lowest rate in the age group of <5 male (9.1%), while >14 age groups of females showed relatively low frequency than male (9.2%), followed by the age group of 5-14 female (16.7%) whereas the lowest frequency was recorded in the age group of <5 females (5%). In the previous study, out of 375 patient's majority 42 (63.6%) were in the age group of >14 years. Almost similar patterns were seen in Mithakhel district Karak [14], where the medium prevalence of malaria infection 20.67% was reported in the age group of 5-14 years. The almost similar pattern was seen in Bannu district, Khyber Pakhtunkhwa. Pakistan [15]. Low incidence was in the age group of <5 years. Where peak incidence was in the age group of >14 years in Karachi [16]. The frequency rates of malarial infection in males showed the comparatively higher percentage (23.5%) as compared to females (10.5%) population in the given study. Regarding sex distribution there was male predominance, in our study male were 32 (27%), the female was 10 (9.1%), and children were 24 (16%). Male predominance was also reported [14]. In comparison to female in the field, the male is more careless than female. Regarding malaria infection cases among the urban and rural population, the high frequency was recorded in rural population 49 (21.7%), followed by urban population 17 (11.3%). The almost similar pattern was seen in Quetta, Pakistan [17]. Regarding the species-wise distribution of malaria infection cases, the highest frequencies were recorded of *Plasmodium vivax* 58 (15.5%), followed by *Plasmodium falciparum* 05 (1.3%) and the lowest frequency rate 03 (0.8 %) was recorded in Mix infection (*Plasmodium vivax* and *Plasmodium falciparum*). The possible reason for the higher number of malaria infection cases in males because the males work in the field and do not adequately cover their body, so the male has more chance of infection as compared to female [18]. This was also definite former results that in Pakistan there are two major types of *Plasmodium* are present one is *Plasmodium falciparum,* and the other is *Plasmodium vivax,* and in the most region, the *Plasmodium vivax* are predominant [19].

Conclusion

The given study concluded that male has a higher rate of malaria infection than female and children in urban and rural populations in Peshawar, Pakistan. The highest number of cases were reported in males having age group >14 years while the lowest percentage 5% was recorded in the age group of <5 years. Among the three major hospitals the highest frequency was recorded of *Plasmodium vivax* 15.5% followed by *Plasmodium falciparum* 1.3%, and the lowest frequency of malaria infection was recorded in the mix (*Plasmodium vivax* and *Plasmodium falciparum*) 0.8%. To control the malaria infection awareness session, knowledge is needed about the disease, early diagnosis of malaria infection, early treatment and prevention measure is necessary.

Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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