Humera Qureshi,1,2 Muhammad Imran Khan,1,2 Henock Ambachew,3,4 Hai-Feng Pan,1,2 and Dong-Qing Ye
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1Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui, China. 2Anhui Province Key Laboratory of Major Autoimmune Diseases, Hefei, Anhui, China. 3Department of Clinical Laboratory Diagnostics, First Affiliated Hospital, Anhui Medical University, Hefei, Anhui, China. 4School of Medical Laboratory Sciences, College of Medicine and Health Sciences, Hawassa University, Hawassa, Ethiopia. (Correspondence to: Dong-Qing Ye: ydq@ahmu.edu.cn, ydqahmu@126.com)

Abstract

Background: Malaria has remained a significant global public health problem. Both Plasmodium falciparum and Plasmodium vivax are prevalent in Pakistan. Currently, comprehensive data on the epidemiology of Plasmodium infections in different districts of Khyber Pakhtunkhwa (KP) Province are not obtainable.

Aims: This study was done to determine malaria prevalence and assess health facilities for malaria services and to provide the baseline information for malaria control in these areas.

Methods: A cross-sectional study was conducted using cluster sampling technique in three districts of KP province. A total of 31 041 individuals, 864 households and 98 healthcare centres were selected for a malaria prevalence survey, parasite net-owning survey and malaria services respectively. The data were analyzed using SPSS version 23.

Results: Among all individuals screened for malaria using rapid diagnostic tests (RDT) 4297 (13.8%) were found positive for RDT. The prevalence of P. vivax, P. falciparum and mixed infection was 92.4%, 4.7%, and 2.9%, respectively. The average number of individuals positive
for malaria among the three districts was 238.72 (95% CI: 82.67–394.78). There were significant differences in the prevalence of malaria infection among the districts (P < 0.05). The average number of households owned (44.10%) more than two long-lasting insecticidal bed nets (LLINs). The ratio of Chloroquine and Primaquine usage was high (0.58) compared to the other antimalarial drugs.

Conclusions: The prevalence of malaria infection was high in the three endemic districts of KP province. Healthcare facilities of malaria services in these districts were weak. Additionally, the rate of LLINs household ownership was low. Malaria control or elimination strategies should be strengthened in these districts.

Keywords: Prevalence; malaria; rapid diagnostic tests; Khyber Pakhtunkhwa; Pakistan


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Introduction

Malaria has remained significant global public health problem and it is estimated that around 3 billion people are at risk of acquiring malaria, with 212 million cases and 429 000 related deaths occurring in 2015 (1). Malaria is one of the most devastating parasitic diseases in Pakistan, with a higher malaria death rate compared to any other country in Asia. Plasmodium falciparum (P. falciparum) and Plasmodium vivax (P. vivax) are the two widespread and dangerous species, causing the high rate of morbidity and mortality (2). In Pakistan, the prevalence of P. vivax and P. falciparum was 88% and 12%, respectively, based on the World Health Organization’s (WHO) 2013 Malaria Report (3). An estimated 500 000 incidences of malaria and 50 000 malaria-attributable mortalities occurs annually (4,5), with a 37% malaria incidence burden reported along the borders of Afghanistan and the Islamic Republic of Iran. In the past few
decades, malaria transmission was highest in the northern part of Pakistan, especially in Khyber Pakhtunkhwa province.

Malaria transmission is seasonal and Pakistan is susceptible to epidemic outbreaks in particular geographical parts, predominantly Khyber Pakhtunkhwa province, Sindh province and Balochistan province. In Khyber Pakhtunkhwa province the three malaria-endemic districts include Bannu, Dera Ismail Khan and Lakki Marwat (6). The peak season of P. vivax transmission is from June to September and again in April to June when relapses of Plasmodium infections developed from the previous season is observed (7). The P. falciparum dominant transmission period in Pakistan is between August and December (8).

The malirometric cross-sectional surveys were conducted in five special Afghan refugee camps to evaluate malaria infection. Prevalence of malaria infection in the five camps ranged from (0–0.2 to 0.4–9 %) by RDT and (0–1.39 and 5–15%) by PCR for P. vivax and P. falciparum, respectively. Anti-malarial antibodies prevalence to P. falciparum antigens and P. vivax antigens was 3–11% and 17–45%, respectively (9). Another cross-sectional study was conducted in four geographically and socio-demographically distinct areas around Mumbai, India, to measure malaria-related information and control practices. A stratified random sample of 119 households was selected for the study. Households were mostly familiar about bed nets as an essential control strategy, but only 30% of households used them and only 4% used insecticide-treated bed nets. Prevention practices and knowledge differ across the four areas of Mumbai (10). A study done in the federally administrated tribal areas of Pakistan reported out of 691 patients, covering all age groups and genders, 626 were positive for malaria using polymerase chain reaction. Among these positive cases P. vivax (81.1%), P. falciparum (13.8%) and mixed infections (4.9%) containing both P. falciparum and P. vivax were reported (11).

An epidemiological study has been conducted in some areas of Pakistan, but the investigation of malaria transmission on a nationwide scale has been hindered by the shortage of data from several areas (26). Surveillance and reporting methods for Plasmodium infections incidence are relatively weak and epidemiological data are inadequate. It is most important to analyze the burden of malaria in a specific local area for obtaining utilizable information for different stakeholders involved in prevention and control of malaria. Therefore, the present study was conducted to determine the baseline malaria burden and health facilities for malaria services in three endemic districts of Khyber Pakhtunkhwa, Pakistan.

**Methods**

**Study design, area and period**

This cross-sectional study was conducted in three endemic districts of Khyber Pakhtunkhwa
namely: Bannu (Bnu), Dera Ismail Khan (DIK), and Lakki Marwat (LM) districts. The total population estimated in the entire study area was 3,634,186 (1,218,416 in Bnu, 1,239,247 in DIK, and 1,176,523 in LM). Most of the residents in the three districts of Khyber Pakhtunkhwa are Muslims, but ethnic minorities include Hindus, Sikhs and Christians. The study was done between August and October 2015, which is the peak season for malaria transmission. The endemic districts have all the characteristics of deserts and plains due to its dunes, hot temperatures and dry weather. Winters are moderately cold, while summers are very hot. The summer season starts from early April and continues until late October. The cold wave begins in early November, and the winter ranges from December up to February. The temperature in DIK is very high in summer and reaches 50 °C.

Before the survey, the prevalence of malaria had peaked due to several reasons, mainly socioeconomic, due to an unstable political environment and internally displaced peoples (IDPs) from North Waziristan to neighbouring districts in Khyber Pakhtunkhwa province. In total, 950,000 people were displaced (73% women and children), and who were resettled in local communities and camps (11).

A total of 98 healthcare centres with malaria Rapid Diagnostic Test (RDT) services were surveyed in the three endemic districts (36 in Bnu, 37 in DIK, and 25 in LM). The epidemiological data were obtained using multi-stage cluster sampling technique, by selection of healthcare centres as the first sampling unit followed by selection of households as the second sampling unit. A total of 31,041 individuals and 864 households were included in this study. The strata were urban and rural locations. The data were organized according to sex, age, and species of Plasmodium infections.

**Rapid diagnostic test (RDT)**

Malaria rapid diagnostic tests (RDTs) contribute to malaria diagnosis by detecting evidence of malaria species in human blood. RDTs allow reliable detection of malaria infections especially in remote areas with limited access to good quality microscopy services. RDTs are comparatively easy to execute and interpret; they speedily provide outcomes, limited training requires, and allow malaria diagnosis at the public level. RDTs detect specific antigens produced by malaria species that are existing in the infected individual's blood. Some RDTs kits detect a single species either P. vivax or P. falciparum, some detect multiple species (P. vivax, P. falciparum, P. ovale and P. malariae) and some additional differentiate between P. falciparum and non-P. falciparum infections. For RDTs, blood is commonly obtained from a finger-prick and results are presented within 15–30 minutes.

**Cluster sampling**
Cluster sampling is a method of sampling whereby the whole population is divided into clusters, and a random sample of this clusters are selected. All observations in the chosen cluster are included in the sample. It can be understood as a simple random sampling as described, except that the primary sampling unit is a cluster or group of people instead of the individual. Cluster sampling is typically used when the researcher cannot get a complete breakdown of a population. It also used when a random sample would produce a list of subjects so widely scattered that surveying them would prove to be far too expensive.

**Multi-stage sampling**

A sample is named a multi-stage sample when it is chosen in stages, the sampling units at each stage being subsampled from the larger units selected at the previous stage. Here a population is separated into a number of units, called first-stage units, which are subsampled. Each of the selected second-stage units are further divided into third-stage units, from which a subsample is chosen again and repeated. In a multistage sample, the sample size is the number of units included in the sample at the final stage in the sampling. The advantage of multistage sampling is that only the parts of the population chosen at any stage need to be listed for sampling at the next stage.

**Data Collection**

**Survey questionnaires**

The major data collection tool utilized for this study was a survey questionnaire, which consisted of three types: Registration form for the rapid diagnostic test (RDT); malaria facility service form for the healthcare centres; and long-lasting insecticidal nets (LLINs) form for households. Demographic data including age, sex, place of residence, the name of healthcare centre, individual code and ID number were incorporated in the RDT examination database. The long-lasting insecticidal bed nets form comprised the family size and the total number of LLINs. The malaria facility service form primarily comprised information on malaria diagnosis, treatment, and LLINs dissemination at the healthcare centre.

**Quality assurance**

To assure the quality of data collected for this study, a variety of quality control activities were implemented such as training of data collectors, re-checking of RDT (10% positive and 20% negative), supervision by experienced professionals, and monitoring the integrity and completeness of data.

**Data analysis**

SPSS version 23 (SPSS Inc. Chicago: IL, USA) was used for data analysis. Descriptive statistics was given as means (± SD) for continuous variables and as frequency and percentage.
for categorical variables. Chi-square ($\chi^2$) test was used to explore the relationship between categorical variables. The level of significance was considered at probability level ($P \leq 0.05$).

**Ethical considerations**

Ethical clearance was obtained from the Institutional Review Board of Anhui Medical University, Pakistan. Permission to conduct the research and relevant information was obtained by the relevant authorities at the Directorate of the Malaria Control Programme, Pakistan.

**Results**

**Basic characteristics**

In this study, a total of 31,041 individuals (9,117 individuals from Bnu, 13,659 from DIK, and 8,265 from LM) were surveyed, with the ratio of male to female at 1.17. The basic characteristic of the study population is summarized in Table 1. A total of 864 households (286 households from Bnu, 312 from DIK, and 266 from LM) were interviewed on LLINs ownership. Information regarding malaria facilities such as diagnosis, reporting, treatment and distribution of LLINs were obtained from 98 healthcare centres, which was provided by governments and international non-government organizations (NGOs).

**Prevalence of malaria infection**

A total of 4,297 (13.8%) individuals were found positive for malaria RDT, of which 3,970 (92.4%) were positive for P. vivax, 204 (4.7%) positive for P. falciparum, and 123 (2.9%) showed mixed infection (P. vivax/P. falciparum). The average malaria prevalence in the three districts was 238.72 (95% CI: 82.67–394.78), while the average ratio of P. vivax/P. falciparum in three districts was 0.05. The prevalence of malaria in Bnu district was higher than LM district and DIK district, and the difference in malaria prevalence among the three districts was statistically significant ($\chi^2 = 594.74$, $P < 0.05$). Table 2 shows the prevalence of malaria infection in three districts with respect to the distribution of Plasmodium species.

The prevalence of malaria in children < 5 years, 5–14 years, and > 14 years were 5.2%, 10.5%, and 22.6% respectively. The prevalence was found higher in individuals > 14 years compared to the other age groups ($\chi^2 = 1670.01$, $P < 0.05$) (Table 3). The prevalence of malaria was higher among females than males (P. falciparum 0.72% vs 0.60%, P. vivax 14.25% vs 11.5%, and mixed 0.41% vs 0.38%). The significant values of Plasmodium infection are P. falciparum ($\chi^2 = 11.87$, $P < 0.05$), P. vivax ($\chi^2 = 39.71$, $P < 0.05$) and mixed infections ($\chi^2 = 1.18$, $P > 0.05$) respectively.

**Malaria treatment in healthcare centres**
The treatment used for malaria infection in Bnu district was Artemisinin Combination Therapy (Artesunate + Sulfadoxine-Pyrimethamine) (ACT (AS+SP): 0.32%, Chloroquine(CQ): 37.25%, Primaquine (PQ): 60.68%, Artemisinin Combination Therapy (Artemether + Lumefantrine) ACT (ART+LF): 0.00%, and Tab Quinine: 1.74%), in DIK district (ACT (AS+SP): 0.48%, CQ: 36.17%, PQ: 62.69%, ACT (ART+LF): 0.00%, and Tab Quinine: 0.66%), and in LM district (ACT (AS+SP): 0.06%, CQ: 34.68%, PQ: 64.24%, ACT (ART+LF): 0.01%, and Tab Quinine: 1.02%). The Chloroquine and Primaquine usage ratio was (0.58) high compared to the other malaria treatments in the three endemic districts (Table 4).

Household LLINs ownership

No mosquito nets were re-treated with insecticide in the three endemic districts. The average (44.10%) of households owned one or more LLINs based on family size, and all LLINs were distributed by The Global Fund. There was a slight deviation among the three districts: the LLINs ownership in Bnu was higher than DIK and LM ($\chi^2 = 7.11, P < 0.05$) (Table 5). The LLINs was introduced during 2004–2005 by the Global Fund in Pakistan.

Discussion

Malaria incidence and prevalence can be efficiently reduced through active and passive diagnosis (12). The accurate and actual evaluation of malaria infection can also be useful in scaling up control interventions and malaria surveillance in Pakistan (13). Thus, to accomplish and continue the malaria eradication operation in Pakistan, the prevalence and occurrence of Plasmodium parasites were determined in three endemic districts of Khyber Pakhtunkhwa, Pakistan, for the first time through the current study, by using rapid diagnostic tests.

Since the late 1970s, malaria annual incidence cases described by health departments of the four provinces have gradually increased. Punjab province described a rapid increase until the mid-1980s, a slow decrease in the late 1980s and a further rise in the 1990s. The current malaria prevalence reported in Punjab province is very low, while the prevalence ranged from 1.7% in Lahore to 5.5% in Bhakkar districts (8). Khyber Pakhtunkhwa and Sindh provinces reported a stable increase all areas,, while Baluchistan province reported an increase in the 1990s (14). In the 1980s this malaria burden moved from the northern and southern regions of Khyber Pakhtunkhwa province to the western areas in the 1990s. By the end of the decade, a relatively high malaria prevalence axis was noticed extending from Swat and Chitral in the north, through to Mardan, Malakand, Swabi, and Khyber and Mohmmand along the western border with Afghanistan (15).

This study provided the baseline information for malaria control in the three endemic districts of Khyber Pakhtunkhwa province. Our study has demonstrated that in a population of individuals...
with malaria symptoms, the overall prevalence of malaria was 13.8%, of which P. vivax and P. falciparum accounts for 92.4% and 4.7%, respectively. The predominance of P. vivax in our study is consistent with studies conducted in other parts of Pakistan (8,16–18), but our finding were found to differ from other studies (19). In this region, a number of studies have also described a high prevalence (10.8%) of Plasmodium infections and a high proportion of cases attributed to P. falciparum in Bannu, Hangu and Thall Districts of KP province (8). In other high endemic districts of KP province (but not part of this study) there have been a comparatively high number of cases with fluctuating P. falciparum incidences, from 16% of Plasmodium infections in Buner (20) to 25% in Bannu and Abbottabad (21,22) to over 90% of cases in Manshera (23). Cross-border migration may have contributed to the surge or maintenance of Plasmodium infections in these areas. From 1979 to 1982, refugees from Afghanistan fled across the border into Balochistan and KP provinces (24).

The present study also depicts a high prevalence of malaria in the age group > 14 years, which is consistent with other studies in Pakistan and India (25,26). Our study showed a higher prevalence of malaria among females than males, which is congruent with another study (27), yet contradicts other studies (28,29). The risk factor and reason for the sex difference in prevalence among individuals could not be explained due to the shortage of behaviour data on study subjects. In future, further study should be conducted with the incorporation of behaviour data as a study parameter. The average ratio of P. falciparum/P. vivax in all three districts was 0.05.

LLINs have played a significant role in the outstanding success in reducing malaria transmission over the past few decades. In the initial stage LLINs were introduced and implemented by The Global Fund in Pakistan. There were different approaches used for LLINs distribution including community health workers, campaigns, and private outlets. In 2012 the procedure was reformed, and for the first time a comprehensive LLINs distribution strategy was improved and implemented in Pakistan through the support of The Global Fund in endemic districts (30,31).

Household LLINs ownership rate in the three endemic districts (44.10%) was very low; a large number of LLINs should be disseminated freely among the residents to protect from Plasmodium infection. During the household survey, it was noted that some people did not use the LLINs distributed three years ago. Moreover, it is recommended that malaria health schooling should be performed to encourage the use of LLINs (30–32). In three endemic districts, comprehensive interventions should be provided, such as anti-malarial drugs, bed nets, and health schooling.

**Limitations**
The current study was conducted in the three endemic districts of Khyber Pakhtunkhwa province (Bannu, Dera Ismail Khan, and Lakki Marwat); thus, the findings may not be generalized to the whole country. Furthermore, due to the cross-sectional nature of the study, it does not permit examining causality in the association between risk factors and malaria prevalence. To determine the seasonal variation and other risk factors for malaria prevalence in these three districts another study should be done for a prolonged period.

**Conclusions**

Findings of this study indicate that there is a high prevalence of malaria in the three districts of Khyber Pakhtunkhwa Province, Pakistan. In Bannu district the highest prevalence of malaria was observed. Individuals in the age group >14 years are highly affected by malaria. In all districts, the healthcare facilities of malaria services were weak; additionally, household LLINs ownership rate was also low. Therefore, special attention should be given to those living in the districts and malaria control or elimination strategies should be strengthened.

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