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Epidemiology of Plasmodium Species and Prevalence of Malaria on the Basis of Age, Sex, Area, Seasonality and Clinical Manifestation in the Population of District Lower Dir, Khyber Pakhtunkhwa, Pakistan

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Abstract: The present study was conducted to show plasmodium species burden and malaria related complications among local population of district Lower Dir, Pakistan (Timergara, Munda, Maidan, Samar Bagh and Talash). During this study a total of 3760 blood samples were taken during January to December 2011, from males (2200,58.5%) and females (1460,41.5%). To detect the plasmodium species, microscopy and rapid diagnostic tests (RDTs) were performed. For the collection of information about the health conditionsof malarial symptomatic patients, questionnaires were also designed.Overall result showed that 12.2% samples were infected, with *Plasmodium vivax* (94.3%), *Plasmodium falciparum* (3.9%)and mixed species (*P. vivax*and *P. falciparum*) (1.7%). Seasonal wise prevalence was also checked. Highest infection rate was recorded in autumn (16.5%) followed by summer (13.8%), spring (9.5%) and winter (6.6%). Among people infected with symptoms like severe temperature (9.8%), lower RBCs count (3.7%) and Glucose 6-phosphate dehydrogenase (G-6PD) deficiency (0.6%) were observed. Shortly malaria can be effectively controlled by efforts with an emphasis on improving species diagnosis and treatment availability in district LowerDir, KyberPakhtunkhwa, Pakistan.

Key words: Lower Dir · Microscopy · Prevalence · Plasmodium · Rdts

INTRODUCTION

Influx of refugees and establishment of camps or settlements in malaria endemic areas can affect the distribution and burden of malaria in the host country. Within a decade of the Soviet invasion of Afghanistan and the arrival of 2.3 million Afghan refugees in Pakistan's North West Frontier Province, the annual burden of malaria among refugees had risen tenfold from 11,200 cases in 1981 to 118,000 cases in 1991, a burden greater than the one reported by the Pakistan Ministry of Health for the entire Pakistani population. Political developments in the 1990s led to over half the refugee population repatriating to Afghanistan and the Afghan Refugee Health Programmed (ARHP) was scaled down proportionately. Districts in which the ARHP recorded a reduced incidence of malaria began to show an increased incidence in the statistics of the Pakistan government health program. Over the decade incidence in the refugee camps decreased by 25% as a result of control activities and by 1997 the burden among remaining refugees had fallen to 26,856 cases per annum. These trends indicate that the burden would continue to fall if political conditions in Afghanistan were to improve and more refugees returned to their homeland [1]. Due to terrorism in this belt, Influx of refugees varies and thus no current data is available on the prevalence of malaria.

Malaria is one of the devastating parasitic ailments having high mortality rate in tropical world. It counts for approximately 350 to 500 million clinical infections and deprive 1 to 3 million lives per annum throughout the world [2]. In the developing countries it is still one of the major public health problems [3]. In human, malaria is caused by the four species of *Plasmodium* (*Plasmodium*

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vivax, Plasmodium falciparum, Plasmodium ovale and Plasmodium malariae). Among them, *P. falciparum* and *P. Vivax* are more frequently occurring in most parts of the world [4]. In Pakistan also both *P. falciparum* and *P. vivax*are more common [3]. Plasmodium parasite is responsible for both acute and chronic malaria infections. In human being, the very familiar symptoms of the malaria are high fever and chilliness [5]. Transmissions occur through the biting of female Anopheles mosquito [6].

Climatic condition of Pakistan is tropical with an extensive irrigation system where after monsoon rainfall large ditches are filled with stagnant water, which are suitable for mosquito breeding. Severity of the malaria occurs after raining although its transmission continues year-round [7-9]. The study area (Lower Dir) of Pakistan consists of agricultural bushes where better sites for malaria vectors growth are linked with the crop cultivation, increasing subsequent infection risks. Aim of the current study was to conduct better comprehension of malarial parasites prevalence and malaria related complications in Northwest border between Afghanistan and Pakistan.

MATERIALS AND METHODS

Study Design: The study was carried out in district Lower Dir, Pakistan. This area joined the Pakistan-Afghanistan border where cross border migration of refugees increasing the risk of disease burden. To get information about symptomatic patients, a questionnaire was designed for the household interview which include questions such as sex, age, socio-economic status, previous exposure to malaria, home environmental condition and presence or absence of domestic animals.

Ethical Consideration: Ethical clearances for scientific studies were obtained from the Hazara University, Pakistan. During the current study approved institutional guidelines by local ethical committee were followed. Written permission was also obtained from the hospitals visited for sample collections after discussing the objective of the study.

Collection of Blood Samples: During this study a total of 3760 blood samples of age group rangedfrom 11 to 51 and above years were collected in a 200 µl EDTA tubes from overall five urban and rural areas (Timergara, Munda, Talash, Samar Bagh and Maidan). Out of 3760 total samples, 2200 samples were taken from males and 1460 from females visiting health care centers.

Preparation of Thick and Thin Smears: Fingertips were washed with 70% alcohol followed by pricking with a new sterilized lancet for making blood film. To make a smear, two new slides each for blood film and smear preparation were used. On the same slide both thick and thin smears were prepared. First blood drop was discarded and the second blood drop was collected on the glass slide after pricking. Methyl alcohol was used for thin smear fixation followed by staining with Giemsa stain.Stereomicroscope was used for observing the slides with immersion oil.

Rapid Diagnostic Test (RDTs): From the collected blood samples in anti-coagulant tube,a 0.5 µl blood was taken and then poured into the round sample well of the rapid diagnostic test kit (SD Bioline Malaria kit). Subsequently,according to the manufacturer instructions 4 drops of assay diluents were added. Test checked 15 min were considered positive with the appearance of two lines while the appearance ofsingle control line indicatesnegative result. Mixed infection(*P. vivax*and *P. falciparum*)was observed when all three lines were appeared.

Red Blood Cells Count: The collected blood was taken immediately into a 0.5 ml micro tube. The tube was capped and the contents of the tube were gently mixed by flicking with the anticoagulant to avoid clots formation. Hematological assessment of each sample including red blood cell counts, hemoglobin and platelets was performed using Medonichematology analyzer.

Statistical Analysis: Obtained data was analyzed using Statistix version 9. The independent variables examined include gender, age and seasons while the outcome was the percentage of malarial parasite incidence and its infections. One-way ANOVA test was used to check significance association and$P$ value < 0.05 was considered statistically significant.

RESULTS

The overall findings of the current work show that 460/3760 (12.2%) cases were positive for malarial parasites with species specific burden of 94.3% (*P. vivax*), 3.9% (*P. falciparum*),and 1.7% mixed infection (*P. vivax* and *P. falciparum*). Out of 3760 examined samples, *P. vivax*was found with highest rate (434, 11.5%) followed by *P. falciparum* (18, 0.5%)and mixed infection (8, 0.2%). All the findings are summarized in Table 1.

Table 1: Over all species wise prevalence of malarial parasites

Malarial parasites	Positive cases	Percentage
P.vivax	434	11.5
P. falciparum	18	0.5
Both (P.v andP.f)*	8	0.2
Total	460	12.2

(P.v andP.f)*, *P. vivax*and *P. falciparum*

Table 2: Over all sex, wise distribution of malarial parasites

Mal Malarial parasites	Male (%)	Female (%)
Plasmodium vivax	12.7	9.8
Plasmodium falciparum	0.3	0.4
Both*	0.3	0.1
Total	293	167
Percentage	13.3	10.7

Table 3: Age wise occurrence of malarial parasites among general population in various areas of district Dir Lower

Age Group	*P.vivax*	*P.falciparum*	Mixed*	Total	Percentage
11-20	150	12	3	1183	12.7
21-30	124	3	3	1123	11.0
31-40	68	0	0	593	11.5
41-50	44	1	0	377	11.9
=51	48	2	2	489	10.6

Table 4: Clinical manifestations of affected population

Clinical manifestations	Affected population%
Temperature	9.85
Lower RBC count	3.7
G-6PD deficiency	0.65

Table 5: Seasonal prevalence of malarial parasites

Season	Affected population% *P* values
Autumn	16.5
Summer	13.8
Spring	9.5
Winter	6.6

Overall Sex Wise Distribution of Malarial Parasites: The sex wise distribution of malarial parasites in examined population is represented in Table 2. Among 2200 examined male, 293 (13.3%) were found to be infected with malarial parasites in which *P. vivax*was found in highest rate 280 (12.7%) followed by *P. falciparum* 18 (0.3%) and then mixed (*P. vivax*and *P.falciparum*) infection 6 (0.3%). In case of female out of 1460 samples, 167 (10.7%) blood smears were found positive in which *P. vivax*was highly frequent 154 (9.8%) followed by *P. falciparum* (0.4%) and then mixed (*P. vivax*and *P.falciparum*) infection (0.1%). The data was also analyzed statistically and found least significant ($P \leq 0.01$).

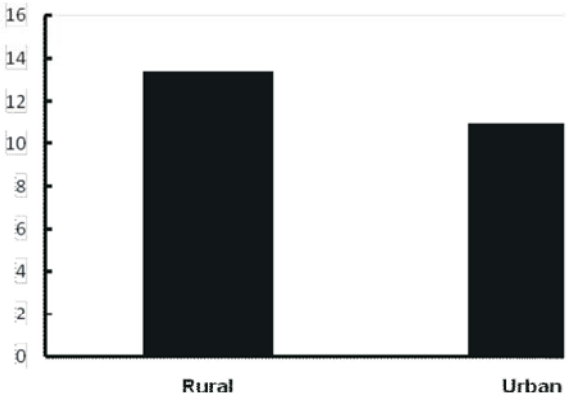

Fig. 1: Area wise prevalence of malarial parasites among various people of Dir Lower

Age Wise Occurrence of Malarial Parasites in Various Areas of District Dir Lower: Prevalence rate was also measured in different age groups as shown in Table 3. Highest infection rate was found within age range between 11 to 20 years were found highly affected (12.7%) followed by 41 to 50 years children (11.9%), whereas lowest number of affected pre-school going children was recorded in the age range between =51 years (10.6%). In case of malarial parasite prevalence, data revealed that highest percentage of people was affected by the*P. vivax*(11.5%) followed by *P. falciparum* (0.5%) and mixed infection (0.2%).

Clinical Manifestations Consistent with Malaria Infections among Different People: High percentage of people both male and female affected with malaria, showed symptoms like severe temperature (9.85%), lower RBS count (3.7%) and Glucose 6-phosphate dehydrogenase (G-6PD) deficiency (0.65%) as shown in Table 4.

Season Wise Prevalence of Malarial Parasites: Highest prevalence of malarial parasites among affected pre-school going children was found in autumn (16.3%) followed by summer (13.8%) and lowest percentage was recorded in winter (Table 5). Statistically significant association was observed between malaria infection and seasons.

Area Wise Prevalence of Malarial Parasites among Female Population of District Dir Lower: Out of 2340 collected samples in urban area, 315 (13.4%) cases were screened positive for malarial parasites. Differently, out of 595 samples from rural areas, 1420 (11.0 %) were screened positive. These are shown in Figure 1.

Comparison of Microscopy and Rapid Diagnostic Test (RDT): The positive cases for *P. vivax*using microscopy and RDTs were 460 (12.3%) and 86 (12.1%) respectively. In case of *P. falciparum* using microscopy and RDTs, positive cases were 18 (0.5%) and 16 (0.0.4%), respectively. Mixed infections were detected equally using both methods 6 (0.2%).

DISCUSSION

The objectiveof the current study was to study the plasmodium infestations and its related complications in the local people of age groups 11 up to 51 and above of District Lower Dir.

In the current study comparative species wise analysis of *Plasmodium* infection showed that, the rate of *P. vivax* infection was higher than that of *P. falciparum* and mixed(*P. vivax*and *P.falciparum*) infection among different age groups. In Pakistan about 80-90% malarial cases are reported due to the *P. Vivax*infection [10].

Comparatively malarial parasites prevalence (12.2.9%, 460/3760) was found higher during current survey than previous reports in endemic regions of Pakistan [11]. In the different regions of the current study variations in malaria prevalence occur which may be due to the different local geographic and climatic conditions [12]. As Afghan refugeesrushed this built and their migration across the border may be the causative agent and help the disease transport resulting infection variations [13]. Similarly, Uzbekistan was also faced the same situation where malaria was eliminated; however reappearance of the disease result due to the cross border migration of the public with Tajikistan [14].

Season wise occurrence of malarial parasites was studied with the highest mean value (2.8700) in autumn (September-November) and lowest (1.7300) was in winter. No significant pairwise differences were found among different seasons. The high prevalence of malaria in autumn may be due to post monsoon period, which may enhance breeding rates of the malaria vectors [15]. Moderate temperature may also be also the reason for this high rate of infection which influencing the flourishing of *Plasmodium* species in the mosquito vector [16]. The lowest rate of infection in winter may be due to low temperature as dry season and fall in temperature slow down the sporogonic development [17]. Irrigation practices in the current study areas may create water stagnant sites for the breeding of malaria vectors [18], resulting in increased prevalence of malarial parasites. Lack of health facilities, rice cultivation, poor sanitation and scattered houses may also be considered as the contributing factors for high prevalence rate [19].

Malaria is a rural friendly disease where the life standard parameters are ignored due to poverty and lack of awareness. Malaria is less frequent in urban areas due to satisfactory life standards [20].

The finding of temperature as the major clinical manifestation for malaria in the current study is in accordance with the WHO case definition for malaria in endemic areas [21]. G-6PD was found as another clinical outcome. The diagnosis and management of glucose-6-phosphate dehydrogenase (G6PD) deficiency is a crucial aspect in the current phases of malaria control and elimination [22]. Coincident to the discovery of G6PD deficiency as the cause of antimalarial induced hemolysis, a strong geographical overlap was noted between the prevalence of G6PD deficiency and malaria endemicity [23, 24]. Based on this observation, it was hypothesized that G6PD deficiency had arisen as a protective factor against lethal malaria.

Similarly the level of RBCs count was found comparatively lower in the malaria infected people than that of non-malarial infected. There are various contributing factors for lowering the level of RBCs in malaria affected people. These factors are increase in the number of merozoites, phagocytosis of RBCs, hiding of the infected RBCs, sequestration and removal of RBCs by the spleen. In addition, release of cytokines may also be considered as the contributing factor for lowering the number of RBCs which decreases the ability of marrow to produce RBCs [25].

In current work, the results of microscopy were more reliable than rapid diagnostic tests (RDTs). In case of *P. falciparum* using microscopy and RDTs, positive cases were 18 (0.5%) and 16 (0.0.4%) respectively. By comparison this minor difference between the two methods may be due to the use and effects of anti-malarial drugs [26]. Rapid device did not detect *P. vivax* trophozoites while seen under microscope were due to the use of anti-malarial drugs that kill the parasite and are no more able to secrete *Plasmodium* lactate dehydrogenase enzyme (PLDH) as secreted by the living forms only [26]. Our findings are parallel with previous reports suggesting microscopy reliability as compared to rapid device for malaria parasite detection [26].

CONCLUSIONS

It was concluded from the present study that both of *Plasmodium* parasite species, *P. vivax*and *P. falciparum* as well as the mixed (*P. vivax*and *P.falciparum*) infections were also observed using RDTs and microscopy. Infection rate due to *P. vivax*was higher as compared to *P. falciparum* infecting both male and female in rural and urban areas. It is strongly recommended that malarial symptomatic patients should be properly treated with anti-malarial drugs to save the lives of people.

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