Introduction
Malaria parasite is a eukaryotic single-celled microorganism that belongs to Plasmodium (P) genus. There are many species of this genus out of which four species are responsible for infecting humans while a fifth one is P. knowlesi which causes zoonotic infection in humans. These species show variation in their morphology and immunological pattern, geographic distribution and their mode of pathogenesis. P. falciparum is considered the most fatal among all species, and is responsible for malaria deaths happening to young children of Africa.

Malaria is an endemic in 109 countries and prevalent throughout the tropics and sub-tropics. In Africa, Papua New Guinea and Haiti P. falciparum is the predominant species, while the Central and parts of South America, North Africa, Middle East and Indian subcontinent P. vivax malaria is endemic. In Asia, especially in Pakistan and Afghanistan, malaria is still a big public health issue with ongoing transmission of both P. falciparum and P. vivax.

In 2015, it was expected that deaths due to malaria were 50% less compared to 2000, and 22% less compared to the number of malaria-related deaths in 2010. Nearly 13 countries accounted for 75% of the malaria-related deaths that happened in 2015. Sub-Saharan Africa, Congo and Nigeria contribute 36% of the total malaria-related mortality. Pakistan, India, Ethiopia and Indonesia are the four countries that contribute 81% to malaria mortality.

In 2015, global load of malaria was 212 million cases, but among these 90% were from World Health Organisation (WHO) African Region, 7% from South-East Asia and about 2% from Eastern Mediterranean Region. Around 95 million people in Pakistan live in areas with high malaria incidence. It was almost eradicated in 1960s but the sudden rise of malaria took place in 1970s. In the last two decades, rise in malaria cases was attributed to prolonged monsoon season and floods which affected 20 million people in over 60 districts. In these malaria infections P. vivax contributed 65% and the rest of 35% was by P. falciparum, and these two species are the only species present in Pakistan. Even though the Malaria Control Programme has launched many malaria and vector control programmes but every year about 0.5 million new cases of malaria are reported along with many malaria-attributable deaths. Approximately 37% of malaria cases were attributed to the shared border region of Pakistan with Afghanistan and Iran.

Lack of public health facilities, socio-economic conditions, migration of internally displaced peoples (IDPs) and across-border movement are the factors that
have added to malaria incidence. The current study was planned to add valuable information in this regard, because, to our knowledge, it is the first such study done in the border region.

Patients and Methods
The Prospective study on the incidence of malaria parasite infection along the Pak-Afghan bordering area was conducted at Hatmi Medical Centre (HMC), Barmal district, Afghanistan, from March 2014 to February 2016 and comprised patients from Barmal district, Afghanistan, Gorvack village in North Waziristan Agency in the Federally Administration Tribal Area (FATA) of Pakistan, Ser-Zghami, Zawarkari and Laman Adda in Pak-Afghan shared territories, and Barmal district in the Paktika province of Afghanistan were the areas selected (Figure).

The study was designed at the Department of Medical Lab Technology, University of Haripur, Pakistan, in collaboration with the Department of Pathology, Gomal Medical College, Dera Ismail Khan, Pakistan, whose ethics review committee approved the study protocol. Subjects with malaria symptoms regardless of age and gender were enrolled.

Patients having anti-malarial drugs in the preceding four weeks, severely anaemic, history of bleeding, critically ill and those who refused were excluded. Using finger prick method, thick and thin films of blood smear were prepared after taking informed consent from patients/guardians. Giemsa-stained blood film microscopic examination was done under oil immersion objective for malaria parasite. Smears having trophozoite, schizonts and gametocytes were considered positive as per malaria diagnosis standard criteria set by World Health Organisation (WHO).13 Slides were stored in a secure slide box and were reconfirmed by microscopy expert. SPSS 19 was used for data analysis.

Results
Of the 3,840 smears examined by the microscope using oil immersion lens, 1,185(30.9%) were positive for malaria, while 2,655(69.1%) cases tested negative.

Of the positive cases, P. vivax were 1,074(90.6%), P. falciparum 76(6.4%) and mixed infection were 35(3%). No case was found for P. ovale and P. malariae (Table-1).

Table-1: Prevalence of Plasmodium different species (n=1185).

<table>
<thead>
<tr>
<th>Malaria Species</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vivax</td>
<td>1074 (90.6%)</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>76 (6.4%)</td>
</tr>
<tr>
<td>P. malariae</td>
<td>00 %</td>
</tr>
<tr>
<td>P. ovale</td>
<td>00 %</td>
</tr>
<tr>
<td>Mixed (P. vivax + P. falciparum)</td>
<td>35 (3.0%)</td>
</tr>
</tbody>
</table>

P. = Plasmodium.

Table-2: Different pattern of P. falciparum, P. vivax and Plasmodium mixed species infections among gender. (n=1185).

<table>
<thead>
<tr>
<th>P. falciparum</th>
<th>P. vivax</th>
<th>Mixed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>54(72%)</td>
<td>711(66.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>21(28%)</td>
<td>363(33.8%)</td>
</tr>
</tbody>
</table>

*Mix (P. vivax plus P. vivax)

Among the positive cases for malaria, 788(66.5%) individuals were males while 397(33.5%) were females (Table-2).

Discussion
Malaria is said to be one of the leading causes of morbidity and mortality worldwide, especially in tropic and sub-tropic areas. The infecting rate of malaria in Pakistani community is about 1.6 million per annum. Malaria is the second most common disease in Pakistan which accounts for 16.5% disease burden rate across the country. Nearly 0.3 million microscopic examined confirmed cases were reported in 2011. More than 80% causes of malaria were contributed by P. vivax and the remaining by P. falciparum.14 It is difficult to predict trends of malaria incidence in Pakistan, because of fluctuation of malaria confirmed cases on yearly basis.15

This is the first ever malaria prevalence study conducted at Pak-Afghan shared border region with about 31% was ever reported from this area. These areas remained politically unstable for the last few decades, as the military operations have been carried out against terrorists in this
region. Due to unrest in this region, public health infrastructure is greatly affected. Secondly, due to shared border with malaria endemic neighbouring countries the incidence of malaria is quite high in this war-torn region.

A study conducted at the border area of Balochistan also reported high incidence of malaria infection with 28.8% of malarial cases. Another study conducted in Lal Qilla, Lower Dir, revealed 29% malaria incidence, while 27% in Bannu and about 33% in Barkhan and Kohlu districts of Balochistan have also been reported. According to WHO, the border areas of Balochistan, FATA and Khyber Pakhtunkhwa have the highest prevalence of malaria as these areas share borders with malaria-endemic countries, Afghanistan and Iran. A study conducted in Abbottabad reported 7% malaria cases, urban and rural area of Bannu district had 17% malaria endemicity while Buner district had 7% prevalence. A study conducted on clinical isolates from Sindh province reported about 3% malaria endemicity. Within population variation in prevalence and distribution of plasmodium species may exist, unfortunately, there are no simple methodological explanations for this variability. Variation in malaria prevalence reflects the different dynamic of malaria transmission among different areas of Pakistan.

In the current study major contribution in malaria infection was provided by P. vivax (90.6%) than P. falciparum (6.4%). Similarly, a report from Dera Ismail Khan, revealed 95% of malaria cases were of P. vivax, another study reported 96% P. vivax cases from Manshera, 82% P. vivax cases in Quetta and 72% cases in Jacobabad. A comprehensive malarialometric population survey on malarial isolates from all over Pakistan reported P. vivax predominance by 76%. The possible reason of high infection is that relapses frequently occurred in P. vivax not in P. falciparum. The second reason is that P. vivax is commonly present in tropical and subtropical areas.

In contrast, studies reported 58.9% of P. falciparum cases from Larkana, 69.5% from Sanjavi and 65% from Karachi.

In terms of mixed species infection, our study found 3% such infections. Idris et al. also reported similar prevalence (3.4%) in Abbottabad. A study from Quetta reported about 2% and a comprehensive malarial epidemiological survey across Pakistan revealed 7% mixed malaria infection.

Many epidemiological studies have been carried out across Pakistan and they reported that this region is endemic only with P. falciparum and P. vivax. There were no reports of cases P. malariae and P. ovale malaria from Pakistan and our findings are in line with previous evidence.

In the study area the reason for predominance of malaria incidence in male population was perhaps due to their mobility to the areas of high risk during dawn and dusk which is feeding time of mosquitoes. Another reason might be social setup in which female mobility is restricted in these areas. High prevalence of malaria infection in males has also been reported by many researchers.

There are several factors responsible for the high rate of malaria infection. These include limited healthcare facilities, illiteracy, socioeconomic factors like poverty, internal displacement due to seasonal changes or military operation and inadequacy of vector-control measurements.

Conclusion

P. vivax and P. falciparum were more prevalent. High prevalence of malaria is a threat and challenge for public health officials. Political stability, healthcare facilities, vector-control measurements, rapid and exact species detection methods, species-specific malarial treatment, and public awareness against and needful anti-malarial campaign will definitely reduce the malaria burden in this region.

Acknowledgment

We are grateful to all the participants, Khatmi Medical Laboratory, Barmal, Mr. Nasir Khan, Laboratory technician and all other staff at Hatmi Medical Centre for their support.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References
